CeGaT

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Calle Vilana 12
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SPAIN

Name	Banat, Mohammed Hasan Mousa (*27.01.1978)
Sex	Male
Patient-ID	81273
Sample receipt	13.01.2021 (EDTA blood) 13.01.2021 (Tumor-FFPE)
Report date	09.02.2021

Report of somatic tumor variants – Banat, Mohammed Hasan Mousa (*27.01.1978)

Indication

Left temporal astrocytoma, WHO grade II (ID 02/2009)
02/2009: Left temporal craniotomy and excision of tumor
morphological 01/2021: oligodendroglioma
11/2020: Progressive disease
01/2021: second brain surgery

Previously reported genetic findings

Methylation specific PCR (report from 05.03.2009): MGMT promoter gene is methylated

IHC (report from 01/2021; FFPE-ID TB 2021 53): ATRX positive in 30% of tumor cells; P53 negative

Material

Tumor tissue: Brain biopsy with neoplastic glial proliferation
Sample collection 01/2021
DNA isolation from tumor in FFPE (FFPE-ID TB 2021 53 A1) after macrodissection with estimated tumor content of 90% (HE staining)
Bioinformatically estimated tumor content 78% (min 74%, max 81%)

Normal tissue: EDTA blood

Order

1. **Somatic molecular genetic analysis of a tumor tissue sample:**
   Tumor exome-analysis TUM02, evaluation of somatic variants of potential clinical relevance in 766 tumor-related genes (TUM01)
2. Tumor transcriptome analysis, neoepitope prediction and peptide selection

RESULTS

- **We detected variants with potential therapeutic relevance in the current sample.**
- **No evidence of therapeutically relevant variants in genes *CDKN2A*, *CDKN2B*, *EGFR* (amplifications, activating mutations).**
- **No detection of a pathogenic/likely pathogenic germline variant.**

Tumor mutational burden (TMB): 1.2 variants/megabase

Microsatellite instability: no evidence for MSI (based on a prediction from NGS data)

Homologous recombination deficiency (HRD): not evaluable

Copy number alterations: not evaluable

Germline variants: No evidence of potentially disease causing or therapeutically relevant germline variants.

Variants with potential therapeutic relevance:

Gene	Functional category	Variant	NAF	Effect on protein function	Related pathway	Therapeutic option	Predicted response	Level of evidence
IDH1	missense	c.395G>A; p.Arg132His	0.38	function changed	-	IDH1 inhibitor	sensitive	2A
						DNMT inhibitor	sensitive	2B
						PARP inhibitor	sensitive	2B
						Immune checkpoint inhibitor	unclear	R2
KRAS	missense	c.351A>T; p.Lys117Asn	0.12	activating	RAS/RAF/MEK, EGFR/HER, MAPK	MEK/ERK inhibitor	unclear	2B
						Hydroxychloroquine#	sensitive	3
						PLK inhibitor	sensitive	3
						SHP2 inhibitors	sensitive	3
						pan-KRAS/SOS1 inhibitor	sensitive	3
						MNK1/2 inhibitor	sensitive	4
						eIF4-Inhibitor	unclear	5
						EGFR/HER inhibitor	unclear	R2
TERT	upstream_gene	c.-124C>T; (C228T)	0.46	activating	-	Telomerase inhibitor	sensitive	4
CIC	stop_gained	c.3640C>T; p.Arg1214*	0.27	inactivating	-	MEK/ERK inhibitor	unclear	R2

NAF: *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content and do not directly correlate with the variant's frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein levels in the following categories: inactivating/activating/function altered, likely inactivating/activating/function altered, unknown and benign (details in the methods section). **Predicted response:** represents the predicted response considering known interferences and pathway crosstalks. Please note that the predicted drug-response is made based on the identified biomarkers only and does not take clinical (or tumor entity specific) features into consideration. **Level of evidence:** for legend see supplement. **#:** In combination with a MEK inhibitor.

ADDITIONAL SOMATIC VARIANTS

The following somatic variants were classified as having no current therapeutic relevance.

Gene	Functional category	Variant	Transcript-ID	NAF
ETV4	inframe	c.926_928delATG; p.Asp309del	NM_001986.4	0.07
HLA-DQB1	missense	c.604G>A; p.Val202Ile	NM_002123.5	0.13
PHOX2B	missense	c.544G>C; p.Asp182His	NM_003924.4	0.22
PIK3R1	inframe	c.1365_1370dupGTTTCA; p.Phe456_Gln457dup	NM_181523.3	0.10
PKHD1	missense	c.2378G>A; p.Arg793His	NM_138694.4	0.15
PKHD1	missense	c.56G>A; p.Arg19His	NM_138694.4	0.14

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NAF: *Novel allele frequency*, the frequency with which the mutated allele was detected in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content and do not correlate directly with the variant frequency in the tumor.

RECOMMENDATION

The results of this report should be evaluated against this patient's current clinical status and should be reviewed by an interdisciplinary tumor board.

Please do not hesitate to contact us if you have any questions.

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup	Dr. rer. nat. Christian Grosser	Dr. sc. hum. Marion Klaumünzer	Dr. rer. nat. Tabea Riedlinger
Consultant for Human Genetics	Diagnostics	Diagnostics	Diagnostics

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ADDITIONAL INFORMATION

Investigated genes

Somatic tumor exome (TUM02) contains interpretation of the following cancer-relevant genes from whole exome sequencing:

AAK1, ABCB1, ABCG2, ABL1, ABL2, ABRAXAS1, ACD, ACVR1, ADGRA2, ADRB1, ADRB2, AIP, AIRE, AJUBA, AKT1, AKT2, AKT3, ALK, ALOX12B, AMER1, ANKRD26, APC, APLNR, APOBEC3A, APOBEC3B, AR, ARAF, ARHGAP35, ARID1A, ARID1B, ARID2, ARID5B, ASXL1, ASXL2, ATM, ATP1A1, ATR, ATRX, AURKA, AURKB, AURKC, AXIN1, AXIN2, AXL, B2M, BAP1, BARD1, BAX, BCHE, BCL10, BCL11A, BCL11B, BCL2, BCL3, BCL6, BCL9, BCL9L, BCOR, BCORL1, BCR, BIRC2, BIRC3, BIRC5, BLM, BMI1, BMPR1A, BRAF, BRCA1, BRCA2, BRD3, BRD4, BRD7, BRIP1, BTK, BUB1B, CALR, CAMK2G, CARD11, CASP8, CBFB, CBL, CBLB, CBLC, CCDC6, CCND1, CCND2, CCND3, CCNE1, CD274, CD79A, CD79B, CD82, CDC73, CDH1, CDH11, CDH2, CDH5, CDK1, CDK12, CDK4, CDK5, CDK6, CDK8, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CENPA, CEP57, CFTR, CHD1, CHD2, CHD4, CHEK1, CHEK2, CIC, CIITA, CKS1B, CNKSR1, COL1A1, COMT, COQ2, CREB1, CREBBP, CRKL, CRLF2, CRTC1, CRTC2, CSF1R, CSF3R, CSMD1, CSNK1A1, CTCF, CTLA4, CTNNA1, CTNNB1, CTRC, CUX1, CXCR4, CYLD, CYP1A2, CYP2A7, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4, CYP3A5, CYP4F2, DAXX, DCC, DDB2, DDR1, DDR2, DDX11, DDX3X, DDX41, DEK, DHFR, DICER1, DIS3L2, DNMT1, DNMT3A, DOT1L, DPYD, E2F3, EBP, EED, EFL1, EGFR, EGLN1, EGLN2, EIF1AX, ELAC2, ELF3, EME1, EML4, EMSY, EP300, EPAS1, EPCAM, EPHA2, EPHA3, EPHA4, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ERRFI1, ESR1, ESR2, ETNK1, ETS1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXO1, EXT1, EXT2, EZH1, EZH2, FAN1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FAT1, FBXO11, FBXW7, FEN1, FES, FGF10, FGF14, FGF19, FGF2, FGF23, FGF3, FGF4, FGF5, FGF6, FGF9, FGFBP1, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLI1, FLT1, FLT3, FLT4, FOXA1, FOXA2, FOXE1, FOXL2, FOXO1, FOXO3, FOXP1, FOXQ1, FRK, FRS2, FUBP1, FUS, FYN, G6PD, GALNT12, GATA1, GATA2, GATA3, GATA4, GATA6, GGT1, GLI1, GLI2, GLI3, GNA11, GNA13, GNAQ, GNAS, GNB3, GPC3, GPER1, GREM1, GRIN2A, GRM3, GSK3A, GSK3B, GSTP1, H3-3A, H3-3B, H3C2, HABP2, HCK, HDAC1, HDAC2, HDAC6, HGF, HIF1A, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, HMGA2, HMGCR, HMGN1, HNF1A, HNF1B, HOXB13, HRAS, HSD3B1, HSP90AA1, HSP90AB1, HTR2A, ID3, IDH1, IDH2, IDO1, IFNGR1, IFNGR2, IGF1R, IGF2, IGF2R, IKBKB, IKBKE, IKZF1, IKZF3, IL1B, IL1RN, ING4, INPP4A, INPP4B, INPPL1, INSR, IRF1, IRF2, IRS1, IRS2, IRS4, ITPA, JAK1, JAK2, JAK3, JUN, KAT6A, KDM5A, KDM5C, KDM6A, KDR, KEAP1, KIAA1549, KIF1B, KIT, KLF2, KLF4, KLHL6, KLLN, KMT2A, KMT2B, KMT2C, KMT2D, KNSTRN, KRAS, KSR1, LATS1, LATS2, LCK, LIG4, LIMK2, LRP1B, LRRK2, LTK, LYN, LZTR1, MAD2L2, MAF, MAGI1, MAGI2, MAML1, MAP2K1, MAP2K2, MAP2K3, MAP2K4, MAP2K5, MAP2K6, MAP2K7, MAP3K1, MAP3K13, MAP3K14, MAP3K3, MAP3K4, MAP3K6, MAP3K8, MAPK1, MAPK11, MAPK12, MAPK14, MAPK3, MAX, MBD1, MC1R, MCL1, MDC1, MDH2, MDM2, MDM4, MECOM, MED12, MEF2B, MEN1, MERTK, MET, MGA, MGMT, MITF, MLH1, MLH3, MLLT10, MLLT3, MN1, MPL, MRE11, MS4A1, MSH2, MSH3, MSH4, MSH5, MSH6, MSR1, MST1R, MT-RNR1, MTAP, MTHFR, MTOR, MTRR, MUC1, MUTYH, MXI1, MYB, MYC, MYCL, MYCN, MYD88, MYH11, MYH9, NAT2, NBN, NCOA1, NCOA3, NCOR1, NF1, NF2, NFE2L2, NFKB1, NFKB2, NFKBIA, NFKBIE, NIN, NKX2-1, NLRC5, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NPM1, NQO1, NR1I3, NRAS, NRG1, NRG2, NSD1, NSD2, NSD3, NT5C2, NT5E, NTHL1, NTRK1, NTRK2, NTRK3, NUMA1, NUP98, NUTM1, OPRM1, PAK1, PAK3, PAK4, PAK5, PALB2, PALLD, PARP1, PARP2, PARP4, PAX3, PAX5, PAX7, PBK, PBRM1, PBX1, PDCD1, PDCD1LG2, PDGFA, PDGFB, PDGFC, PDGFD, PDGFRA, PDGFRB, PDIA3, PDK1, PDPK1, PGR, PHF6, PHOX2B, PIGA, PIK3C2A, PIK3C2B, PIK3C2G, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIK3R3, PIM1, PKHD1, PLCG1, PLCG2, PLK1, PML, PMS1, PMS2, POLD1, POLE, POLH, POLQ, POT1, PPM1D, PPP2R1A, PPP2R2A, PRDM1, PREX2, PRKAR1A, PRKCA, PRKCI, PRKD1, PRKDC, PRKN, PRMT5, PRSS1, PSMB1, PSMB10, PSMB2, PSMB5, PSMB8, PSMB9, PSMC3IP, PSME1, PSME2, PSME3, PSPH, PTCH1, PTCH2, PTEN, PTGS2, PTK2, PTK6, PTK7, PTPN11, PTPN12, PTPRC, PTPRD, PTPRS, PTPRT, RABL3, RAC1, RAC2, RAD21, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD54B, RAD54L, RAF1, RALGDS, RARA, RASA1, RASAL1, RB1, RBM10, RECQL4, RET, RFC2, RFWD3, RFX5, RFXANK, RFXAP, RHBDF2, RHEB, RHOA, RICTOR, RINT1, RIPK1, RIT1, RNASEL, RNF43, ROS1, RPS20, RPS6KB1, RPS6KB2, RPTOR, RSF1, RUNX1, RYR1, SAMHD1, SAV1, SBDS, SCG5, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEC23B, SERPINB9, SETBP1, SETD2, SETDB1, SF3B1, SGK1, SH2B1, SH2B3, SHH, SIK2, SIN3A, SKP2, SLC19A1, SLC26A3, SLCO1B1, SLIT2, SLX4, SMAD3, SMAD4, SMARCA4, SMARCB1, SMARCD1, SMARCE1, SMC1A, SMC3, SMO, SOCS1, SOX11, SOX2, SOX9, SPEN, SPINK1, SPOP, SPRED1, SPTA1, SRC, SRD5A2, SRGAP1, SRSF2, SSTR1, SSTR2, SSX1, STAG1, STAG2, STAT1, STAT3, STAT5A, STAT5B, STK11, SUFU, SUZ12, SYK, TAF1, TAF15, TAP1, TAP2, TAPBP, TBK1, TBL1XR1, TBX3, TCF3, TCF4, TCF7L2, TCL1A, TEK, TENT5C, TERC, TERF2IP, TERT, TET1, TET2, TFE3, TGFB1, TGFBR2, TLR4, TLX1, TMEM127, TMPRSS2, TNFAIP3, TNFRSF11A, TNFRSF13B, TNFRSF14, TNFRSF8, TNFSF11, TNK2, TOP1, TOP2A, TP53, TP53BP1, TP63, TPMT, TPX2, TRAF2, TRAF3, TRAF5, TRAF6, TRAF7, TRRAP, TSC1, TSC2, TSHR, TTK, TUBB, TYMS, U2AF1, UBE2T, UBR5, UGT1A1, UGT2B15, UGT2B7, UIMC1, UNG, USP34, USP9X, VEGFA, VEGFB, VHL, VKORC1, WRN, WT1, XIAP, XPA, XPC, XPO1, XRCC1, XRCC2, XRCC3, XRCC5, XRCC6, YAP1, YES1, ZFHX3, ZNF217, ZNF703, ZNRF3, ZRSR2 (somatic tumor panel version 6)

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Methods

DNA isolation: The isolation of tumor DNA was performed following macrodissection at the CeGaT Tübingen. The tumor material was assessed by a pathology specialist.

The pathological services (confirmation of the histological diagnosis and determination of the tumor content) were carried out on our behalf by a specialist in pathology. Pathology services are not within the scope of the ISO 15189 accreditation.

Sample quality: The suitability of a sample for molecular genetic analysis depends on the tumor content as well as on the overall material quality (e.g. impairment of quality by chemical or physical stress due to fixation, Arreaza et al., 2016 PMID: 27657050; Einaga et al., 2017, PMID: 28498833). In cases with low material quality the detection of aberrations (variant calling, copy number variation, structural variants) as well as mutational burden and HRD-score determination may be impaired or even impossible.

NGS-laboratory: The coding and flanking intronic regions were enriched using in solution hybridization technology and were sequenced using the Illumina HiSeq/NovaSeq system.

Computational analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Genetic data evaluation: Only variants (SNVs/small indels) with a novel allele frequency (NAF) of ≥ 5% in the tumor sample within the coding regions and their adjacent intronic regions (-/+ 8 base pairs) were evaluated. A list of all the variants with an allele frequency of 5% considered in the genetic data evaluation can be requested at any time. The clinical interpretation of variants is based on different external and internal databases and on information from scientific literature. The sensitivity of the test is dependent on the tumor content of the analyzed material, the sample quality, and the sequencing depth. In this case, 87.6% of the targeted regions were covered by a minimum of 60 high-quality sequencing reads per base. The tumor content estimated by the pathologist was 90%. Therefore, somatic variants occur at a calculated NAF of around 45%. A theoretical sensitivity of >99% can be obtained for variants with a NAF ≥45% when a coverage of 22 reads per base is achieved. Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

Variant classification: The somatic alterations were assessed with respect to their possible impact on protein function based upon the available data (i.e. Catalogue of somatic mutations in cancer (COSMIC), cBioPortal, My Cancer Genome, Clinical Interpretations of Variants in Cancer (CIVIC), MD Anderson Personalized Medicine Center Datenbank, IARC *TP53* database, CKB, OnkoKB, PubMed research) and/or using *in silico* predictions (Mutation Taster, fathmm, Mutation Assessor, SIFT, fathmm-MKL coding, LRT and PROVEAN). The functional categories assigned are: inactivating, activating, function altered, likely inactivating/activating/function altered, unknown or benign. "Inactivating": known inactivating variants as well as frameshift, nonsense and essential splice site variants, unless they are described as activating or benign. "Activating" and "function altered": known activating/function changing variants. The functional evidence of variants classified as inactivating, activating and function altered is highly reliable (i.e. ClinVar/ClinGen data with a review status of at least two stars, databases of specific consortia and/or *in vivo/in vitro* analyses). "Likely inactivating/activating/function altered": an impact of the variant on protein function is considered as likely with respect to the affected amino acid position (e.g. known hot spot, pathogenic variant in the same codon, high conservation, *in silico* predictions), but there are insufficient functional data available. "Unknown": based upon the available data, we are not able to conclusively confirm or exclude a possible functional relevance of the variant. "Benign": the variant is described as benign and does not impair protein function.

Classification criteria for theoretical response:

sensitive: We expect a favorable response to the specified medication class, in the presence of this biomarker. This prediction is based on the affected signal transduction pathway and is made considering all therapeutically relevant biomarkers. However, the potential interference of other mutated genes with the effectiveness of this class of medication cannot be ruled out. A level of evidence (LoE) of 1-4 must be indicated to assign a sensitive theoretical response.

unclear: An unclear theoretical response is given if there is another mutated biomarker in the pathway of this biomarker, or downstream of the medication target, that could reduce the efficacy of the specified drug class. Also, where evidence of non-response (see resistance) has been described in the scientific literature. Moreover, an LoE of 5 is always given an "unclear" theoretical response, unless the corresponding drug class has already been provided with an LoE of 1-4 with a "sensitive" theoretical response due to another biomarker. In this case, a "sensitive" theoretical response is also assigned for the biomarker with an LoE of 5.

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resistant: If there is evidence that the current biomarker (NCCN and/or ESMO) will have a non-response, decreased response, or resistance to the specified medication class in the given entity, then it will be given an LoE of R1 with a "resistant" theoretical response.

However, if the resistance of the biomarker towards the medication class is not indicated in the NCCN- or ESMO-guidelines, but there is data in the current literature suggesting non-response, decreased response, or resistance, then an LoE of R2 is given with an "unclear" theoretical response.

Copy Number Analysis: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Copy number variants as well as breakpoints were estimated on the basis of the NGS data and should be treated as estimated values. CNVs are assigned to be therapeutically relevant when both 1: a focal or cluster amplification of 4 or more copies or a homozygous deletion is detected, containing known druggable genes, and 2: the detected gain or loss of DNA is consistent with the underlying pathomechanism of the affected druggable gene (e.g. amplification of oncogenes and deletion of tumor suppressor genes).

The list of genes additionally reported in the copy number alterations table represents a selection of therapeutically relevant genes potentially affected by CNVs and makes no claim of completeness. Please be aware that a loss of one allele does not necessarily result in reduced protein expression. Likewise, low grade amplification does not necessarily lead to an increase of protein expression. Gross deletions and amplifications likely cover a large number of genes. The evaluation of CNV effects on relevant oncogenes or tumor suppressor genes may therefore remain speculative.

Tumor mutational burden (TMB): Tumor mutational burden is defined as the number of somatic SNV-, InDel- and essential splice site variants (NAF ≥ 0.1) per megabase of coding DNA. Somatic variants with an inhouse frequency of ≥ 1% are not accounted. Tumor mutational burden is classified as high, when ≥ 10 Mut/Mb are present in the tumor (Hellmann et al., 2018, PMID: 29658845; Reck et al., 2019, PMID: 31195357).

Microsatellite instability (MSI): The prediction was carried out analogous to Kautto et al., 2017, PMID: 27980218.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT). A minimal tumor content of 20% was taken as a basis.

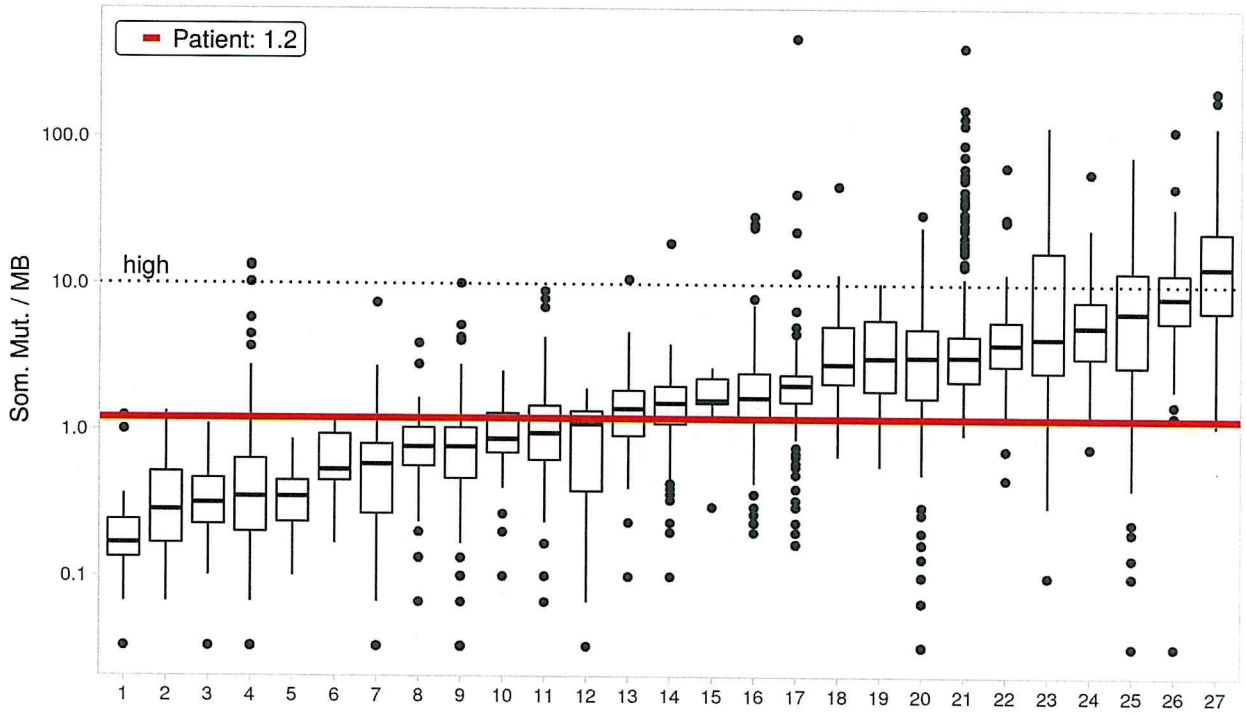
Genetic Counseling

Please be aware that this somatic report cannot replace conventional germline diagnostics. A lack of evidence for therapy relevant or likely disease causing germline variants does not exclude the presence of disease relevant germline mutations. In cases where a relevant germline mutation has been detected, genetic counseling should be considered. Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

SUPPLEMENT - TUMOR MUTATIONAL BURDEN

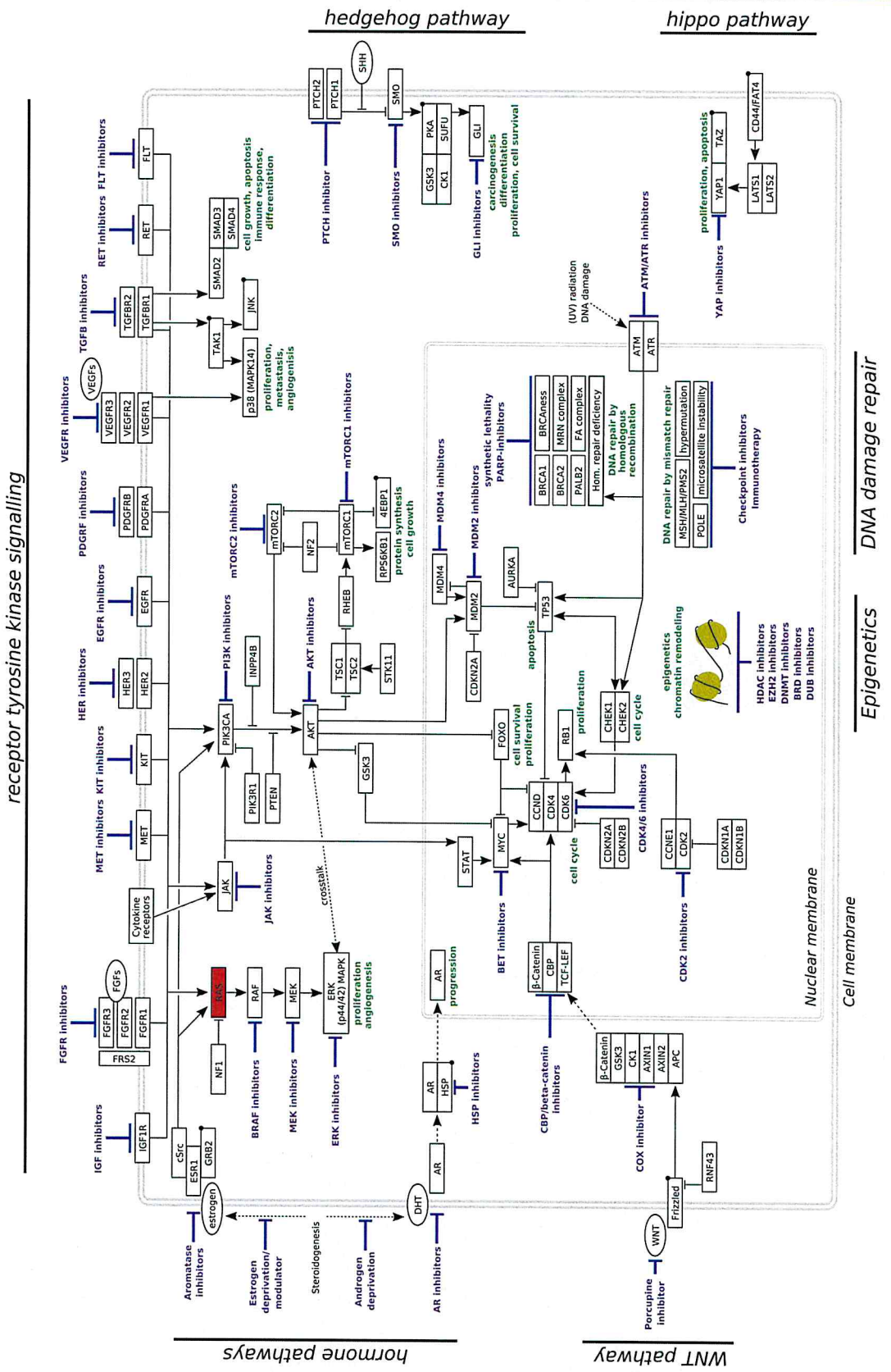
The figure shows the tumor mutational burden (TMB) of the previously described tumor sample (red bar) in relation to TMB published for different tumor entities (Lawrence et al., 2013, PMID: 23770567). A high TMB has been associated with a superior response to immune therapy approaches in different tumor entities (Johnson et al., 2016, PMID: 27671167; Rizvi et al., 2015, PMID: 25765070; Snyder et al., 2014, PMID: 25409260; Le et al., 2015, PMID: 26028255; Bouffet et al., 2016, PMID: 27001570; Hellmann et al., 2018, PMID: 29658845; Reck et al., 2019, PMID: 31195357).



Distribution of tumor mutational burden in 27 tumor entities

The distribution of tumor mutational burden (somatic variants per megabase of coding DNA) is shown for 27 different tumor entities (n=3083). Boxplots show the range containing 50% of all values (interquartile range, IQR, between percentile 75 and 25) as boxes, medians as solid horizontal lines. Outliers (circles) are shown for values deviating by more than 1.5 times the IQR (indicated by vertical lines). Tumor mutational burden of 1.2 mut/Mbp determined for the current case is shown for comparison (solid red line). Y-axis is log scaled. A high mutational burden (≥ 10 Mut/Mb) is indicated with a dashed line.

Entities are: (1) Rhabdoid tumor, (2) Ewing Sarcoma, (3) Thyroid cancer, (4) Acute myeloid leukemia, (5) Medulloblastoma, (6) Carcinoid, (7) Neuroblastoma, (8) Prostate cancer, (9) Chronic lymphocytic leukemia, **(10) Low-grade glioma**, (11) Breast cancer, (12) Pancreatic cancer, (13) Multiple myeloma, (14) Kidney clear cell, (15) Kidney papillary cell, (16) Ovarian cancer, (17) Glioblastoma multiforme, (18) Cervical cancer, (19) Diffuse large B-cell lymphoma, (20) Head and neck carcinoma, (21) Colorectal cancer, (22) Esophageal adenocarcinoma, (23) Gastric cancer, (24) Bladder carcinoma, (25) Lung adenocarcinoma, (26) Lung squamous cell carcinoma, (27) Melanoma (Figure modified referring to Lawrence et al., 2013, PMID: 23770567).



The figure illustrates the most important cancer biomarkers in relation to their associated cancer pathways. In addition, potential drug classes are provided. Circles: ligands; rectangular boxes: biomarkers covered in current analyses; rectangular boxes with dot: biomarkers not covered in current analyses; —|: repression, →: activation, —|: inhibiting drugs, ⇢: transport. **Biomarkers affected in your patient's tumor are highlighted.** Blue: biomarker probably inactivated; Red: biomarker probably activated; Brown: biomarker function probably changed. Please note that crosstalks, feedback regulations, interfering pathways and drug resistances are not illustrated.

SUPPLEMENT - LEVEL OF THERAPEUTIC EVIDENCE

LoE	
1A	**Approved drug, specific to the biomarker and entity** Drug is approved for the biomarker within the same entity (FDA and/or EMA)
1B	**Approved drug, specific to entity but not specific to the biomarker OR specific to biomarker, but only in organ related entities** Drug is approved independently of the biomarker within the same entity OR drug is approved for the biomarker in an organ related entity, e. g. benign tumor (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
2A	**Approved drug, specific to the biomarker for a different entity** Drug is approved for the biomarker in a different entity (FDA and/or EMA)
2B	**Approved drug, not specific to the biomarker for a different entity** Drug is approved independently of the biomarker in a different entity (FDA and/or EMA). The reported biomarker must have significant clinical relevance, despite biomarker-independent approval of the indicated drug.
3	**Efficacy of the drug is currently being/was analyzed in clinical trials**
4	**Efficacy of the drug is based on preclinical analyses and/or case reports**
5	**Hypothetical response** The biomarker could hypothetically induce response to the drug
R1	**The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)** The variant and/or biomarker is associated with a non-response, decreased response, or resistance to a specific drug or drug class in the same entity. The information is based on high impact guidelines (NCCN and/or ESMO)
R2	**Biomarker might be associated with a non-response or a resistance** The biomarker might be associated with a non-response, reduced response, or resistance to the stated drug class in this or another tumor entity (based on current literature)