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Dr. Physician
University hospital of capital
Physician street 1
1234 capital
Country

Name	Doe
Forename	Jane
Date of birth	01.01.1901
Sex	female
Patient-ID	12345
Sample receipt	DD.MM.YYYY (EDTA blood) DD.MM.YYYY (Tumor-FFPE)
Your ID	8765432
Report date	DD.MM.YYYY

cc. encrypted via email to Dr. physician, physician@clinic.com

Report of somatic tumor variants – Doe, Jane | 01.01.1901

Indication	**Glioblastoma WHO Grade IV, left temporal**
Previous analysis	IDH1 wildtype
Material	**Tumor tissue: brain tumor sample** Sample collection 01/2020, DNA-Isolation from Tumor in FFPE (FFPE-ID: XX1234) after macrodissection with estimated tumor content of 90% **Normal tissue: EDTA blood**
Order	**Somatic molecular genetic analysis of a tumor tissue sample:** Tumor panel-analysis TUM01, evaluation of somatic variants of potential clinical relevance

RESULTS

KEY SUMMARY:

We detected variants with potential therapeutic relevance in the current sample.

Tumor mutational burden (TMB): high (122 variants/megabase)

Microsatellite instability: MSI-high (based on a prediction from NGS data)

Copy number alterations: detection of chromosomal aberrations, evidence of genomic instability

Germline variants: no evidence of likely disease causing or therapy 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
CDKN2A	stop_gained	c.387C>G; p.Tyr129*	0.40	inactivating	cell cycle	CDK4/6 inhibitor	sensitive	2B
PTEN	stop_gained	c.675T>G; p.Tyr225*	0.35	inactivating	PI3K/AKT /mTOR	PI3K inhibitor	sensitive	2B
						mTOR inhibitor	sensitive	2B
						Akt inhibitor	sensitive	3
						immunotherapy	unclear	R2

NAF: *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing (1 is 100%). The observed frequencies are influenced by the tumor content and do not correlate directly to the variant frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein level in the 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. **Level of evidence**: for legend see supplement.

HIGH TUMOR MUTATIONAL BURDEN:

A high tumor mutational burden 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). Immune checkpoint inhibitors are approved drugs for the treatment of melanoma, lung-cancer, kidney cancer, Hodgkin's lymphoma, urothelial carcinoma and head and neck cancer. In cases with high mutational burden, a broad applicability of immune checkpoint inhibitors is generally assumed for different tumor entities (Colli et al., 2016 and 2017, PMID: 27197178, 28446466).

MICROSATELLITE INSTABILITY:

The predicted microsatellite instability is based on NGS data. We recommend validating the MSI high status by a validated diagnostic laboratory test. Therapeutically, patients with an MSI-high tumor could benefit from treatment with immune checkpoint inhibitors (Passardi et al., 2017; PMID: 28635639). The PD-1 inhibitor Keytruda® (pembrolizumab) was approved by the FDA for the treatment of unresectable or metastatic solid tumors that are MSI-high or mismatch repair deficient (dMMR) and have progressed following prior treatment.

The highest level of therapeutic evidence is: 1A (FDA)

CHROMOSOMAL ABBERATIONS:

Our sequencing data do provide evidence for the presence of copy number alteration (deletions and/or amplifications) of large genomic segments:

Chromosomal region	Functional category	Variant	Copy number	Affected genes with potential therapeutic relevance
chr3 39329-3191317	heterozygous deletion	p-arm, partial	1	-
chr4	duplication	p-arm	3	-
chr6 13456-17131603	heterozygous deletion	p-arm, partial	1	-
chr7	duplication		3	*MET*
chr8	duplication	q-arm	3	*MYC*
chr9	duplication		3	-

chr10	duplication	p-arm	3	-
chr10	heterozygous deletion	q-arm	1	*PTEN*
chr11	heterozygous deletion	p-arm	1	-
chr12	amplification	q-arm, partial	3	*CDK4*
chr13	heterozygous deletion	q-arm	1	*BRCA2*, *RB1*
chr15 64881813-65426174	amplification	q-arm, partial	>5	-
chr16	duplication	p-arm	4	-
chr17 70588479-81009686	heterozygous deletion	q-arm, partial	1	-

The sensitivity of copy number detection depends on the sample's tumor content and the sample's overall quality. Copy numbers as well as breakpoints are estimated on the basis of the NGS data and should be treated as estimated values. The set of candidate genes represents a selection only and makes no claim of completeness. Please be aware that copy number variants likely cover a large number of genes. Possible interactions between these genes may impair reliable prediction of single gene effects on the analyzed tumor.

However, please note that next generation sequencing is not the gold standard for detection of copy number variation. **If a precision medicine approach targeting the detected copy number alterations is going to be considered for further treatment, these findings should be validated by a second method (e.g. FISH, IHC).**

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. Diagnostic 3	Dr. rer. nat. Diagnostic 2	Dr. rer. nat. Diagnostic 1
Consultant for Human Genetics	Diagnostics	Diagnostics	Diagnostics

ADDITIONAL INFORMATION

Investigated genes

Somatic tumor panel (TUM01) contains interpretation of the following cancer-relevant genes:

ABL1, *ABL2*, *ABRAXAS1*, *ACD*, *ACVR1*, *ADGRA2*, *AIP*, *AIRE*, *AJUBA*, *AKT1*, *AKT2*, *AKT3*, *ALK*, *AMER1*, *ANKRD26*, *APC*, *APLNR*, *AR*, *ARAF*, *ARHGAP35*, *ARID1A*, *ARID1B*, *ARID2*, *ARID5B*, *ASXL1*, *ASXL2*, *ATG2B*, *ATM*, *ATP1A1*, *ATR*, *ATRX*, *AURKA*, *AURKB*, *AURKC*, *AXIN1*, *AXIN2*, *AXL*, *B2M*, *BAP1*, *BARD1*, *BCL10*, *BCL11A*, *BCL11B*, *BCL2*, *BCL3*, *BCL6*, *BCL9*, *BCOR*, *BCORL1*, *BCR*, *BIRC2*, *BIRC3*, *BIRC5*, *BLM*, *BMPR1A*, *BRAF*, *BRCA1*, *BRCA2*, *BRD3*, *BRD4*, *BRIP1*, *BTK*, *BTNL2*, *BUB1B*, *CALR*, *CAMK2G*, *CANX*, *CARD11*, *CASP8*, *CBFB*, *CBL*, *CBLB*, *CBLC*, *CCDC6*, *CCND1*, *CCND2*, *CCND3*, *CCNE1*, *CD274*, *CD38*, *CD52*, *CD58*, *CD74*, *CD79A*, *CD79B*, *CD82*, *CDC73*, *CDH1*, *CDH11*, *CDH2*, *CDK12*, *CDK4*, *CDK6*, *CDK8*, *CDKN1A*, *CDKN1B*, *CDKN1C*, *CDKN2A*, *CDKN2B*, *CDKN2C*, *CEBPA*, *CEP57*, *CHD1*, *CHD2*, *CHD4*, *CHEK1*, *CHEK2*, *CIC*, *CIITA*, *CKS1B*, *CNOT3*, *COL1A1*, *COMMD1*, *CREB1*, *CREBBP*, *CRKL*, *CRTC1*, *CRTC2*, *CSF1R*, *CSF2*, *CSF3R*, *CSMD1*, *CSNK1A1*, *CTCF*, *CTLA4*, *CTNNA1*, *CTNNB1*, *CTSB*, *CTSL*, *CTSS*, *CUL4B*, *CUX1*, *CXCR4*, *CYLD*, *CYP2A7*, *DAXX*, *DCC*, *DDB2*, *DDR1*, *DDR2*, *DDX11*, *DDX3X*, *DDX41*, *DEK*, *DHFR*, *DICER1*, *DIS3*, *DIS3L2*, *DKC1*, *DNMT1*, *DNMT3A*, *DOT1L*, *DPYD*, *E2F3*, *EBP*, *EGFR*, *EGLN1*, *EGR2*, *EGR3*, *ELAC2*, *ELANE*, *ELF3*, *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*, *FBXW7*, *FES*, *FGF10*, *FGF14*, *FGF19*, *FGF2*, *FGF23*, *FGF3*, *FGF4*, *FGF5*, *FGF6*, *FGFBP1*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FH*, *FKBP1A*, *FLCN*, *FLI1*, *FLT1*, *FLT3*, *FLT4*, *FOXA1*, *FOXA2*, *FOXE1*, *FOXL2*, *FOXO1*, *FOXO3*, *FOXP1*, *FOXQ1*, *FRK*, *FRS2*, *FUBP1*, *FUS*, *FYN*, *G6PD*, *GABRA6*, *GALNT12*, *GATA1*, *GATA2*, *GATA3*, *GATA4*, *GATA6*, *GLDN*, *GLI1*, *GLI2*, *GNA11*, *GNA13*, *GNAQ*, *GNAS*, *GPC3*, *GPER1*, *GREM1*, *GRIN2A*, *GRM3*, *GSK3A*, *H3F3A*, *HCK*, *HGF*, *HIF1A*, *HIST1H3B*, *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA*, *HLA-DRB1*, *HLF*, *HMGA2*, *HMGN1*, *HMOX2*, *HNF1A*, *HNF1B*, *HOXB13*, *HOXD8*, *HRAS*, *HSD3B1*, *HSP90AA1*, *HSP90AB1*, *HSPA4*, *ID3*, *IDH1*, *IDH2*, *IFI30*, *IFNGR1*, *IFNGR2*, *IGF1R*, *IGF2*, *IGF2R*, *IKBKB*, *IKBKE*, *IKZF1*, *IKZF3*, *IL1B*, *IL1RN*, *IL2*, *IL21R*, *IL6*, *IL6ST*, *IL7R*, *IL8*, *ING4*, *INPP4B*, *INPPL1*, *IRF1*, *IRS2*, *ITK*, *JAK1*, *JAK2*, *JAK3*, *JUN*, *KAT6A*, *KDM5A*, *KDM5C*, *KDM6A*, *KDR*, *KEAP1*, *KIAA1549*, *KIT*, *KLF2*, *KLF4*, *KLHDC8B*, *KLHL6*, *KMT2A*, *KMT2B*, *KMT2C*, *KMT2D*, *KRAS*, *LATS1*, *LATS2*, *LCK*, *LGMN*, *LIG4*, *LIMK2*, *LMO1*, *LRP1B*, *LRRK2*, *LTK*, *LYN*, *LZTR1*, *MAD2L2*, *MAFB*, *MAGEA1*, *MAGI1*, *MAGI2*, *MAML1*, *MAP2K1*, *MAP2K2*, *MAP2K3*, *MAP2K4*, *MAP2K5*, *MAP2K6*, *MAP2K7*, *MAP3K1*, *MAP3K14*, *MAP3K3*, *MAP3K4*, *MAP3K6*, *MAPK1*, *MAPK11*, *MAPK12*, *MAPK14*, *MAPK3*, *MAPK8IP1*, *MAX*, *MBD1*, *MC1R*, *MCL1*, *MDC1*, *MDM2*, *MDM4*, *MECOM*, *MED12*, *MEF2B*, *MEN1*, *MET*, *MGA*, *MGMT*, *MITF*, *MLH1*, *MLH3*, *MLLT10*, *MLLT3*, *MN1*, *MPL*, *MRE11*, *MS4A1*, *MSH2*, *MSH3*, *MSH4*, *MSH5*, *MSH6*, *MSR1*, *MST1R*, *MTHFR*, *MTOR*, *MTRR*, *MUC1*, *MUTYH*, *MXI1*, *MYB*, *MYC*, *MYCL*, *MYCN*, *MYD88*, *MYH11*, *MYH9*, *NBN*, *NCOA1*, *NCOA3*, *NCOR1*, *NF1*, *NF2*, *NFE2L2*, *NFKB1*, *NFKB2*, *NFKBIA*, *NFKBIE*, *NFYA*, *NFYB*, *NFYC*, *NIN*, *NLRC5*, *NOP10*, *NOTCH1*, *NOTCH2*, *NOTCH3*, *NOTCH4*, *NPM1*, *NQO1*, *NR1I3*, *NRAS*, *NRG2*, *NSD1*, *NSD2*, *NT5C2*, *NT5E*, *NTHL1*, *NTRK1*, *NTRK2*, *NTRK3*, *NUMA1*, *NUP98*, *PAK1*, *PAK3*, *PALB2*, *PALLD*, *PARP1*, *PARP2*, *PARP4*, *PAX3*, *PAX5*, *PAX7*, *PBK*, *PBRM1*, *PBX1*, *PDCD1*, *PDCD1LG2*, *PDF*, *PDGFA*, *PDGFB*, *PDGFC*, *PDGFD*, *PDGFRA*, *PDGFRB*, *PDIA3*, *PDK1*, *PGR*, *PHF6*, *PHOX2B*, *PIAS4*, *PIGA*, *PIK3C2A*, *PIK3C2B*, *PIK3C2G*, *PIK3CA*, *PIK3CB*, *PIK3CD*, *PIK3CG*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PIM1*, *PKHD1*, *PLCG1*, *PLCG2*, *PML*, *PMS1*, *PMS2*, *POLD1*, *POLE*, *POLH*, *POLQ*, *POT1*, *PPM1D*, *PPP2R2A*, *PRDM1*, *PRDM16*, *PREX2*, *PRF1*, *PRKAR1A*, *PRKCA*, *PRKD1*, *PRKDC*, *PRKN*, *PROM2*, *PRSS1*, *PRX*, *PSIP1*, *PSMB1*, *PSMB10*, *PSMB2*, *PSMB5*, *PSMB8*, *PSMB9*, *PSMC3IP*, *PSME1*, *PSME2*, *PSME3*, *PSPH*, *PTCH1*, *PTCH2*, *PTEN*, *PTGS2*, *PTK2*, *PTK7*, *PTPN11*, *PTPN12*, *PTPRC*, *PTPRD*, *PTPRT*, *RAC1*, *RAC2*, *RAD21*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54B*, *RAD54L*, *RAF1*, *RALGDS*, *RARA*, *RARB*, *RARG*, *RASA1*, *RASAL1*, *RB1*, *RBM10*, *RECQL4*, *REL*, *RET*, *RFC2*, *RFX5*, *RFXANK*, *RFXAP*, *RHBDF2*, *RHEB*, *RHOA*, *RICTOR*, *RINT1*, *RIPK1*, *RIT1*, *RNASEL*, *RNF2*, *RNF43*, *ROS1*, *RPL22*, *RPS20*, *RPS6KB1*, *RPTOR*, *RSF1*, *RUNX1*, *RYR1*, *SACS*, *SAMHD1*, *SAV1*, *SBDS*, *SCG5*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SEC23B*, *SEM1*, *SEMA4A*, *SETBP1*, *SETD2*, *SETDB1*, *SF3B1*, *SGK1*, *SH2B1*, *SH2B3*, *SH2D1A*, *SHH*, *SIK2*, *SIN3A*, *SIRT1*, *SKP2*, *SLC26A3*, *SLIT2*, *SLX4*, *SMAD3*, *SMAD4*, *SMARCA4*, *SMARCB1*, *SMARCE1*, *SMC1A*, *SMC3*, *SMO*, *SOCS1*, *SOX11*, *SOX2*, *SOX9*, *SPEN*, *SPINK1*, *SPOP*, *SPRED1*, *SPTA1*, *SRC*, *SRD5A2*, *SRGAP1*, *SRP72*, *SRSF2*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR5*, *SSX1*, *STAG1*, *STAG2*, *STAT1*, *STAT3*, *STAT5A*, *STAT5B*, *STK11*, *SUFU*, *SUZ12*, *SYK*, *TAF1*, *TAF15*, *TAP1*, *TAP2*, *TAPBP*, *TBK1*, *TBL1XR1*, *TBX3*, *TCF3*, *TCF4*, *TCL1A*, *TEK*, *TENT5C*, *TERC*, *TERF2IP*, *TERT*, *TET1*, *TET2*, *TFE3*, *TGFB1*, *TGFBR2*, *TLR4*, *TLX1*, *TMEM127*, *TNF*, *TNFAIP3*, *TNFRSF11A*, *TNFRSF13B*, *TNFRSF14*, *TNFRSF1A*, *TNFRSF1B*, *TNFRSF25*, *TNFRSF8*, *TNFSF11*, *TNK2*, *TOP1*, *TOP2A*, *TP53*, *TP53BP1*, *TP63*, *TPX2*, *TRAF2*, *TRAF3*, *TRAF5*, *TRAF6*, *TRAF7*, *TRRAP*, *TSC1*, *TSC2*, *TSHR*, *TTK*, *TUBA4A*, *TUBB*, *TYMS*, *U2AF1*, *UBE2T*, *UBR5*, *UGT2B15*, *UGT2B7*, *UIMC1*, *UNG*, *USP34*, *USP9X*, *VEGFA*, *VEGFB*, *VHL*, *VKORC1*, *WAS*, *WASF3*, *WISP3*, *WRN*, *WT1*, *XIAP*, *XPA*, *XPC*, *XPO1*, *XRCC1*, *XRCC2*, *XRCC3*, *XRCC5*, *XRCC6*, *YAP1*, *YES1*, *ZFHX3*, *ZHX3*, *ZNF217*, *ZNRF3*, *ZRSR2* (somatic tumor panel version 5)

Translocations

Genomic regions known to be frequently involved in translocation events are additionally enriched using in solution technology. The alignment is bioinformatically checked for potential translocations apparent by discordant read pairs and split reads. Regions of interest are visually reviewed and possible translocations are manually annotated.

Translocations potentially affecting the following genes are being assessed:

ALK, *BCL2*, *BCR*, *BRAF*, *BRD4*, *EGFR*, *ERG*, *ETV4*, *ETV6*, *EWSR1*, *FGFR1*, *FGFR2*, *FGFR3*, *FUS*, *MET*, *MYB*, *MYC*, *NOTCH2*, *NTRK1*, *PAX3*, *PDGFB*, *RAF1*, *RARA*, *RET*, *ROS1*, *SSX1*, *SUZ12*, *TAF15*, *TCF3*, *TFE3*, *TMPRSS2*

Methods

DNA isolation: DNA from tumor tissue was isolated after macrodissection at the CeGaT, Tübingen after evaluation by a pathologist.

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 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 depending on the tumor load, the sample quality and sequencing depth. A coverage of 120 reads per base achieves a theoretical sensitivity of 99% for the detection of variants with a ≥ 10% NAF. In this case, 99.99% of the targeted regions were covered by a minimum of 120 high-quality sequencing reads per base. 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, 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.

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.

Prediction of structural variants: Genomic regions known to be involved in translocation, gene fusion or large insertion/deletion events are additionally enriched during the sequencing process. The alignment data is bioinformatically analyzed for potential structural variants by identifying discordant read pairs and split reads (Chen et al., 2016, PMID: 26647377). Regions of interest are visually reviewed and possible structural variants are manually annotated. Please note that targets evaluated for the occurrence of relevant structural variants only represent a selection of hot spots frequently mutated. The absence of reported structural variants therefore does not ultimately guarantee the absence of structural variants.

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. On exom level it is extrapolated, taking the results of panel data analysis as a basis. Truncating variants in tumor suppressor genes and known driver mutations as well as somatic variants with an inhouse frequency of ≥ 1% are not accounted. Tumor mutational burden is classified low (0-3.3 mut/Mbp), intermediate (3.3-23.1 mut/Mbp) or high (≥ 23.1 mut/Mbp) (Chalmers et al., 2017, PMID: 28420421; Johnson et al., 2016, PMID: 27671167).

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.

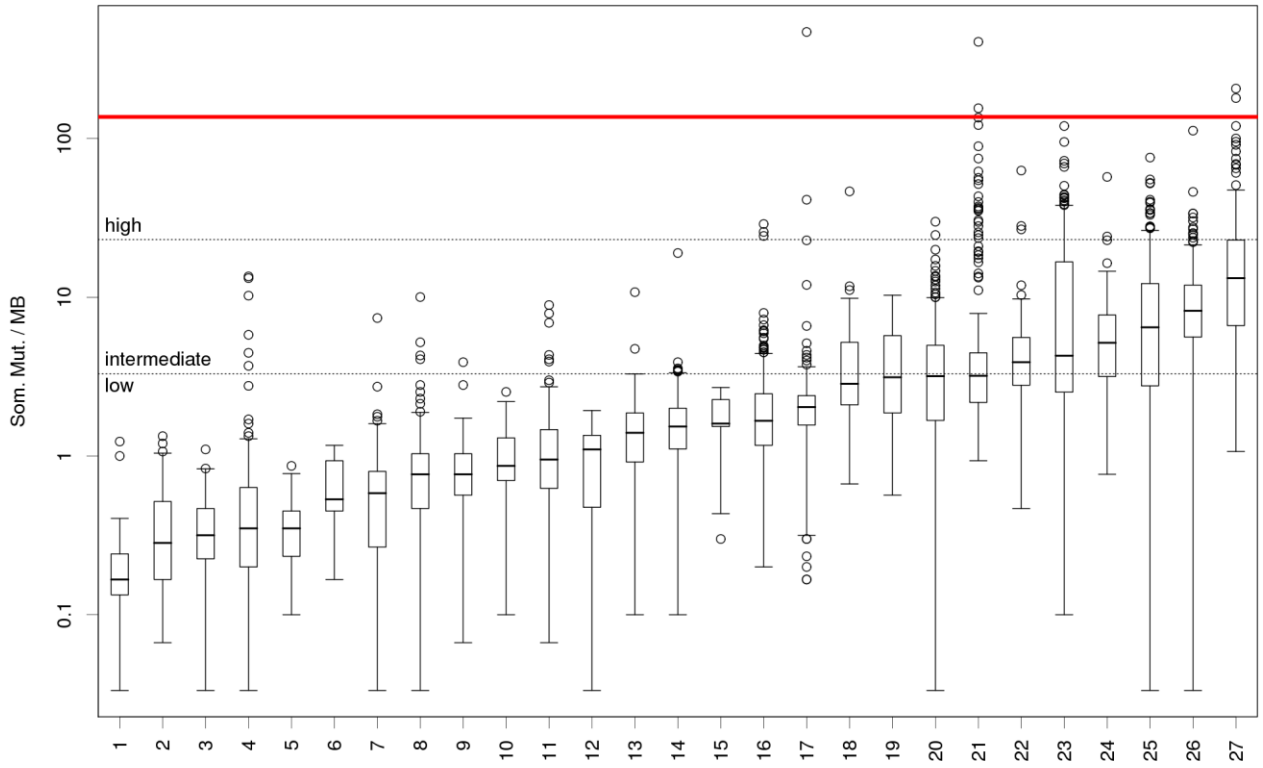
Genetic Counseling

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.

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance to applicable law (GenDG).

SUPPLEMENTARY INFORMATION – MUTATIONAL LOAD

The figure shows the approximated mutational load of the previously described tumor sample (red bar) in relation to the mutational load published for different tumor entities (Lawrence et al., 2013, PMID: 23770567). The mutational load on exome level is extrapolated, taking the results of panel data analysis as a basis. The mutational load is classified low (0-3.3 mut/Mbp), intermediate (3.3-23.1 mut/Mbp) or high (≥23.1 mut/Mbp) according to Johnson et al. (2016, PMID: 27671167). A high mutational load 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).



Distribution of mutational load in 27 tumor entities

The distribution of mutational load (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). The mutational load of 2.7 mut/Mbp determined for the current case is shown for comparison (solid red line). Y-axis is log scaled. The classification into low (<3.3 mut/Mbp), intermediate (3.3-23.1 mut/Mbp), and high (>23.1 mut/Mbp) mutational loads as described by Johnson et al. (2016, PMID: 27671167) is indicated with dashed lines.

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).

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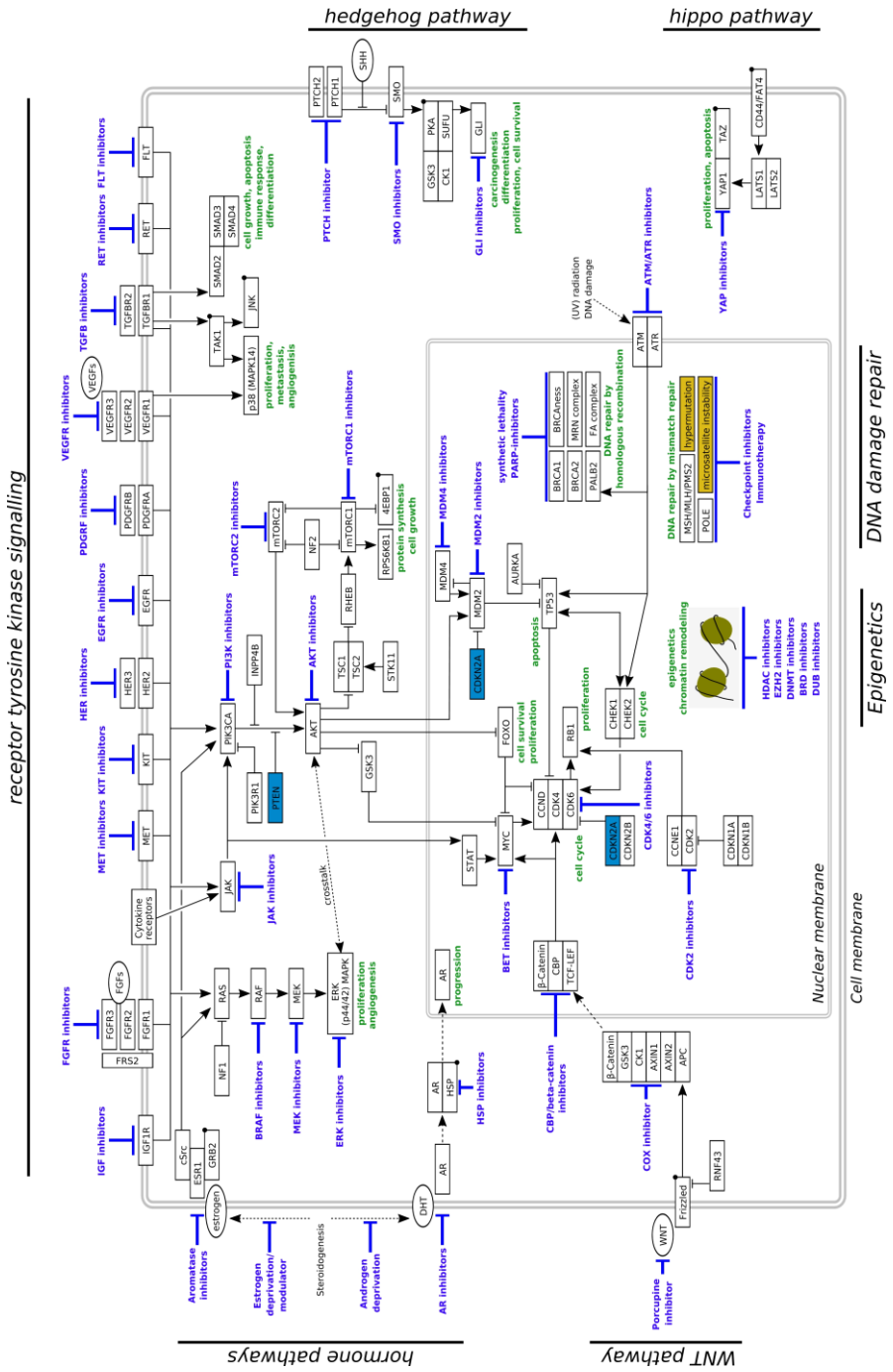
SUPPLEMENTARY INFORMATION – ADDITIONAL SOMATIC VARIANTS

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

Gene	Functional category	Variant	Transcript-ID	NAF
AR	missense	p.Ala412Val	NM_000044.3	0.44
DNMT3A	missense	p.Arg181His	NM_022552.4	0.23
EPHA5	synonymous	p.=	NM_004439.6	0.30
FGFR1	missense	p.Asp132Val	NM_015850.3	0.26
NTRK3	missense	p.Leu238Ile	NM_002530.3	0.39
PRKACA	splice_region	p.?	NM_002730.3	0.26
PRKD1	missense	p.Asn195Ser	NM_002742.2	0.27
PRKD1	splice_region	p.?	NM_002742.2	0.32
REL	synonymous	p.=	NM_002908.3	0.21
RUNX1T1	missense	p.Arg367Gln	NM_004349.3	0.39
RYR1	missense	p.Arg1583His	NM_000540.2	0.32
SMAD4	missense	p.Cys363Tyr	NM_005359.5	0.32
SMAD4	missense	p.Trp524Gly	NM_005359.5	0.32
SPTA1	missense	p.Arg1694Cys	NM_003126.2	0.14
SRSF2	missense	p.Pro46Gln	NM_003016.4	0.26
TAL1	synonymous	p.=	NM_003189.5	0.15

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.



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

SUPPLEMENTARY INFORMATION - 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)
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)
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)