CeGaT

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Name	Doe, Jane (*DD.MM.YYYY)
Sex	Female
Patient-ID	######
Report date	DD.MM.YYYY
Report-ID	R#

CancerPrecision® - Report of Somatic Tumor Variants
Doe, Jane (*DD.MM.YYYY)

Indication **Breast cancer (ID MM/YYYY)**

Result Overview

Tumor Tissue & Tumor Content (TC)	Germline Variants	Tumor Drivers	Fusions, Structural Variants	Pharmacogenetics
Biopsy sample from MM/YYYY 50% diagnostically Diag-TC min 20%	Detection of a pathogenic germline variant in gene *BRCA2*.	Identified tumor drivers: *BRCA2*, *PIK3CA*, *FGFR1*, *TP53* Relevant genes without oncogenic alterations: *BRCA1*, *ERBB2*	No evidence for therapeutically relevant structural variants (on DNA level)	Detection of a germline variant in gene *UGT1A1*

Tumor Mutational Burden (TMB)	Microsatellite Instability (MSI)	Homologous Recombination Deficiency (HRD)	Viral Infection	CHIP
1.8 Var/Mb High ≥ 10	No evidence for MSI (NGS prediction) Score 0.14 Indication of MSI ≥ 0.33	Evidence for HRD Score 58 Indication of HRD ≥ 30	No evidence for an infection with HPV/EBV/CMV/MCV in the tumor sample	No evidence for CHIP

Variants with Potential Therapeutic Relevance

Gene	Functional category	Variant	NAF (tumor)	Effect on protein function	Therapeutic option for discussion in the MTB	Approved by EMA/FDA	Approved for current entity
HRD	N/A	N/A	N/A	N/A	PARP inhibitor	EMA* & FDA*	EMA* & FDA*
BRCA2 (germline)	frameshift[1]	c.3847_3848delGT; p.Val1283Lys*fs**2 chr13:32912337-32912339 CTG>C (hg19) and loss of wildtype allele in tumor tissue	0.66	inactivating	PARP inhibitor	EMA* & FDA*	EMA* & FDA*
PIK3CA	missense	c.3140A>G; p.His1047Arg chr3:178952085 A>G (hg19)	0.25	activating	AKT inhibitor	EMA* & FDA*	EMA* & FDA*
					mTOR inhibitor	EMA* & FDA*	EMA* & FDA*
					PI3Kα inhibitor	EMA* & FDA*	EMA* & FDA*
					Possible resistance to HER2 inhibitor	N/A	N/A
					Possible resistance to BRAF inhibitor	N/A	N/A
					Possible resistance to EGFR inhibitor	N/A	N/A
					Possible resistance to MEK inhibitor	N/A	N/A
FGFR1	amplification	complete gene, non focal (10 copies)	N/A	activating	FGFR inhibitor	EMA* & FDA*	no
					Possible resistance to CDK4/6 inhibitor	N/A	N/A
					Possible resistance to EGFR inhibitor	N/A	N/A
					Possible resistance to Endocrine therapy	N/A	N/A
TP53	missense	c.817C>T; p.Arg273Cys chr17:7577121 G>A (hg19) and loss of wildtype allele	0.46	function changed	CHK1 inhibitor	no	no
					Wee inhibitor	no	no

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 as well as copy number alterations and do not directly correlate with the variant's frequency in the tumor. **1:** Heterozygous in germline.
Protein function: The somatic alterations were classified with respect to their effect on protein function with the following categories: inactivating/activating/function changed, likely inactivating/activating/function changed, unknown, and benign (details in the methods section).
Approval: Only those organisations having approved the respective therapeutical option are listed here. An asterisk indicates approval restrictions (for details regarding targeted therapeutical options please refer to the appendix).

Please refer to the table in the appendix for more information regarding targeted approved drug therapies (EMA/FDA), including information on approval requirements and potential drug resistance.

Variants with Pharmacogenetic Relevance

Gene	Functional category	Variant	Transcript-ID	Zygosity	Effect on protein function	Therapeutic option	Phenotype
UGT1A1	5_prime_UTR	c.-41_-40dup (*28/*28) chr2:234668879 C>CAT (hg19)	NM_000463.3	homozygous	inactivating	Topoisomerase inhibitor	Poor Metabolizer

The variants were classified with respect to their effect on protein function with the following categories: inactivating/activating/function changed, likely inactivating/activating/function changed, unknown, and benign (please refer to the method section for further details regarding variant classification).

Complete List of Automatically Detected Somatic Variants

The table below includes all somatic variants (single nucleotide variants and small deletions/insertions (≤ 40bp)) detected automatically within the sequenced regions (tumor panel V.8).

Gene	Functional category	Variant	Transcript-ID	NAF
GRM3	missense	c.145G>C; p.Glu49Gln chr7:86394606 G>C (hg19)	NM_000840.3	0.23
JAK1	synonymous	c.120C>T; p.= chr1:65349045 G>A (hg19)	NM_002227.4	0.14
PIK3CA	missense	c.3140A>G; p.His1047Arg chr3:178952085 A>G (hg19)	NM_006218.4	0.25
PTPRC	missense	c.1352C>T; p.Pro451Leu chr1:198685871 C>T (hg19)	NM_002838.5	0.22
TP53	missense	c.817C>T; p.Arg273Cys chr17:7577121 G>A (hg19)	NM_000546.6	0.46

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 as well as copy number alterations and do not correlate directly with the variant frequency in the tumor.

Based on the DNA sequencing analysis of the EDTA blood sample (normal tissue) the HLA genotype was determined to be:
HLA-A*##:##, HLA-A*##:##, HLA-B*##:##, HLA-B*##:##, HLA-C*##:##, HLA-C*##:##, HLA-DPA1*##:##, HLA-DPA1*##:##, HLA-DPB1*##:##, HLA-DPB1*##:##, HLA-DQA1*##:##, HLA-DQA1*##:##, HLA-DQB1*##:##, HLA-DQB1*##:##, HLA-DRB1*##:##, HLA-DRB1*##:##, HLA-DRB3*##:##

Copy Number Alterations

Our sequencing data provide evidence for the presence of potentially relevant copy number alterations of large genomic segments as well as single therapeutically relevant genes (see tables above and below).

Overall, there are indications for genomic instability in the tumor.

Chromosomal region	Functional category	Variant	Copy number	Affected genes with potential therapeutic relevance
chr8 37553300-39786309	amplification	p-arm, partial	10	*FGFR1*

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.

Recommendation

The detected variant c.3847_3848delGT; p.Val1283Lys*fs2 in gene *BRCA2* is a pathogenic germline variant.** Therefore, we strongly recommend genetic counseling.

The detected variant *28/*28 in gene *UGT1A1* is a homozygous germline variant. Potential increased toxicity has been described for this genotype (also known as (TA)7/(TA)7, rs8175347 or rs3064744) when treated with irinotecan-based chemotherapeutic agents (Steventon, 2020, PMID: 31092094; PharmGKB Level of Evidence 1A; Whirl-Carrillo et al., 2012, PMID: 22992668; Dean, Medical Genetics Summaries, updated 2018, PMID: 28520360). In addition, when using the TROP2 inhibitor sacituzumab govitecan in patients with known reduced UGT1A1 activity, close monitoring of side effects is recommended as they may be at increased risk of neutropenia, febrile neutropenia and anemia (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761115s023lbl.pdf).
Drug dosing adjustments should exclusively be performed following consultation with the attending clinician.

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.

Medical report written by: ###

Proofread by: ###

Validated by: ###

With kind regards,

Dr. B. Kerner

Dr. med. Berit Kerner

Consultant for Human Genetics

Additional Information

Order

Somatic molecular genetic analysis of a tumor tissue sample:
Tumor panel analysis TUM01, evaluation of somatic variants of potential clinical relevance

Sample material

Tumor tissue: Biopsy sample
Sample collection MM/YYYY
DNA isolation from tumor in FFPE (FFPE-ID: #####/##) with estimated tumor content of 60% (HE staining)
Diagnostically estimated tumor content 50%

Normal tissue: EDTA blood

Sample receipt

DD.MM.YYYY (Normal-DNA: EDTA blood, ID P######_1)
DD.MM.YYYY (Tumor-DNA: FFPE material, ID P######_2)

Requested Regions

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

CACNA1S, *DPYD*, *G6PD*, *NUDT15*, *RYR1*, *TPMT*, *UGT1A1* (Pharmacogenetics)

ABCB1, *ABCG2*, *ABL1*, *ABL2*, *ABRAXAS1*, *ACD*, *ACVR1*, *ACVR2A*, *ADGRA2*, *ADRB1*, *ADRB2*, *AIP*, *AIRE*, *AJUBA*, *AKT1*, *AKT2*, *AKT3*, *ALK*, *ALOX12B*, *AMER1*, *ANKRD26*, *APC*, *APLNR*, *APOBEC3A*, *APOBEC3B*, *AR*, *ARAF*, *ARFRP1*, *ARHGAP35*, *ARID1A*, *ARID1B*, *ARID2*, *ARID5B*, *ASXL1*, *ASXL2*, *ATM*, *ATR*, *ATRX*, *AURKA*, *AURKB*, *AURKC*, *AXIN1*, *AXIN2*, *AXL*, *B2M*, *B4GALNT1*, *BAP1*, *BARD1*, *BAX*, *BCHE*, *BCL10*, *BCL11A*, *BCL11B*, *BCL2*, *BCL2L1*, *BCL2L11*, *BCL3*, *BCL6*, *BCL9*, *BCOR*, *BCORL1*, *BCR*, *BIRC2*, *BIRC3*, *BIRC5*, *BLM*, *BMI1*, *BMPR1A*, *BRAF*, *BRCA1*, *BRCA2*, *BRD3*, *BRD4*, *BRD7*, *BRIP1*, *BTK*, *BTN3A1*, *BUB1B*, *CACNA1S*, *CALR*, *CARD11*, *CASP8*, *CBFB*, *CBL*, *CBLB*, *CBLC*, *CCDC6*, *CCND1*, *CCND2*, *CCND3*, *CCNE1*, *CD274*, *CD276*, *CD70*, *CD79A*, *CD79B*, *CD82*, *CDC42*, *CDC73*, *CDH1*, *CDH11*, *CDH2*, *CDH3*, *CDH5*, *CDK1*, *CDK12*, *CDK2*, *CDK4*, *CDK5*, *CDK6*, *CDK8*, *CDKN1A*, *CDKN1B*, *CDKN1C*, *CDKN2A*, *CDKN2B*, *CDKN2C*, *CEACAM5*, *CEBPA*, *CENPA*, *CEP57*, *CFTR*, *CHD1*, *CHD2*, *CHD4*, *CHEK1*, *CHEK2*, *CIC*, *CIITA*, *CLDN18*, *CNKSR1*, *COL1A1*, *COMT*, *COQ2*, *CREB1*, *CREBBP*, *CRKL*, *CRLF2*, *CRTC1*, *CSF1R*, *CSF3R*, *CSMD1*, *CSNK1A1*, *CTAG1B*, *CTCF*, *CTLA4*, *CTNNA1*, *CTNNB1*, *CTR9*, *CTRC*, *CUL3*, *CUX1*, *CXCR4*, *CYLD*, *CYP1A2*, *CYP2A7*, *CYP2B6*, *CYP2C19*, *CYP2C8*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP4F2*, *DAXX*, *DCC*, *DDB2*, *DDR1*, *DDR2*, *DDX11*, *DDX3X*, *DDX41*, *DHFR*, *DICER1*, *DIS3L2*, *DLL3*, *DNMT1*, *DNMT3A*, *DOT1L*, *DPYD*, *E2F3*, *EED*, *EFL1*, *EGFR*, *EGLN1*, *EGLN2*, *EIF1AX*, *ELAC2*, *ELF3*, *EME1*, *EML4*, *EMSY*, *EP300*, *EPAS1*, *EPCAM*, *EPHA2*, *EPHA3*, *EPHB4*, *EPHB6*, *ERBB2*, *ERBB3*, *ERBB4*, *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERG*, *ERRFI1*, *ESR1*, *ESR2*, *ETNK1*, *ETV1*, *ETV4*, *ETV5*, *ETV6*, *EWSR1*, *EXO1*, *EXT1*, *EXT2*, *EZH1*, *EZH2*, *EZHIP*, *F3*, *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*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FH*, *FLCN*, *FLI1*, *FLT1*, *FLT3*, *FLT4*, *FOLH1*, *FOLR1*, *FOXA1*, *FOXE1*, *FOXL2*, *FOXO1*, *FOXQ1*, *FRK*, *FRS2*, *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*, *H3C1*, *H3C2*, *H3C3*, *HABP2*, *HAVCR2*, *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*, *ICOSLG*, *ID2*, *ID3*, *IDH1*, *IDH2*, *IDO1*, *IFNGR1*, *IFNGR2*, *IFNL3*, *IGF1*, *IGF1R*, *IGF2*, *IGF2R*, *IKBKB*, *IKBKE*, *IKZF1*, *IKZF3*, *IL1B*, *IL1RN*, *IL7R*, *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*, *KRAS*, *KSR1*, *LAG3*, *LAMP1*, *LATS1*, *LATS2*, *LCK*, *LIG4*, *LIMK2*, *LRP1B*, *LRRK2*, *LTK*, *LYN*, *LZTR1*, *MAD2L2*, *MAF*, *MAGEA1*, *MAGEA12*, *MAGEA3*, *MAGEA4*, *MAGEA8*, *MAGI1*, *MAGI2*, *MAML1*, *MAP2K1*, *MAP2K2*, *MAP2K3*, *MAP2K4*, *MAP2K5*, *MAP2K6*, *MAP2K7*, *MAP3K1*, *MAP3K13*, *MAP3K14*, *MAP3K3*, *MAP3K4*, *MAP3K6*, *MAP3K8*, *MAPK1*, *MAPK11*, *MAPK12*, *MAPK14*, *MAPK3*, *MAX*, *MBD4*, *MC1R*, *MCL1*, *MDC1*, *MDH2*, *MDM2*, *MDM4*, *MECOM*, *MED12*, *MEF2B*, *MEN1*, *MERTK*, *MET*, *MGA*, *MGMT*, *MITF*, *MLH1*, *MLH3*, *MLLT10*, *MLLT3*, *MMP2*, *MMS22L*, *MN1*, *MPL*, *MRE11*, *MS4A1*, *MSH2*, *MSH3*, *MSH4*, *MSH5*, *MSH6*, *MSLN*, *MSR1*, *MST1R*, *MT-RNR1*, *MTAP*, *MTHFR*, *MTOR*, *MTRR*, *MUC1*, *MUTYH*, *MXI1*, *MYB*, *MYC*, *MYCL*, *MYCN*, *MYD88*, *MYH11*, *MYH9*, *MYOD1*, *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*, *NSD1*, *NSD2*, *NSD3*, *NT5C2*, *NTHL1*, *NTRK1*, *NTRK2*, *NTRK3*, *NUDT15*, *NUMA1*, *NUP98*, *NUTM1*, *OBSCN*, *OPRM1*, *PAK1*, *PAK3*, *PAK4*, *PAK5*, *PALB2*, *PALLD*, *PARP1*, *PARP2*, *PARP4*, *PAX3*, *PAX5*, *PAX7*, *PBK*, *PBRM1*, *PBX1*, *PDCD1*, *PDCD1LG2*, *PDGFA*, *PDGFB*, *PDGFC*, *PDGFD*, *PDGFRA*, *PDGFRB*, *PDK1*, *PDPK1*, *PGR*, *PHF6*, *PHOX2B*, *PIAS4*, *PIGA*, *PIK3C2A*, *PIK3C2B*, *PIK3C2G*, *PIK3CA*, *PIK3CB*, *PIK3CD*, *PIK3CG*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PIM1*, *PLCG1*, *PLCG2*, *PLK1*, *PMEL*, *PML*, *PMS1*, *PMS2*, *POLB*, *POLD1*, *POLE*, *POLH*, *POLQ*, *POR*, *POT1*, *PPARG*, *PPM1D*, *PPP2R1A*, *PPP2R2A*, *PRAME*, *PREX2*, *PRKAR1A*, *PRKCA*, *PRKCI*, *PRKDC*, *PRKN*, *PRMT5*, *PRR4*, *PSMB1*, *PSMB10*, *PSMB2*,

PSMB5, *PSMB8*, *PSMB9*, *PSMC3IP*, *PSME1*, *PSME2*, *PSME3*, *PTCH1*, *PTCH2*, *PTEN*, *PTGS2*, *PTK2*, *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*, *REST*, *RET*, *RFWD3*, *RFX5*, *RFXANK*, *RFXAP*, *RHBDF2*, *RHEB*, *RHOA*, *RICTOR*, *RIF1*, *RINT1*, *RIPK1*, *RIT1*, *RNASEL*, *RNF43*, *ROS1*, *RPS20*, *RPS6KB1*, *RPS6KB2*, *RPTOR*, *RSF1*, *RSPO1*, *RSPO2*, *RSPO3*, *RSPO4*, *RUNX1*, *RYR1*, *SAMHD1*, *SAV1*, *SBDS*, *SCG5*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SEC23B*, *SERPINB9*, *SETBP1*, *SETD2*, *SETDB1*, *SF3B1*, *SGK1*, *SH2B3*, *SHH*, *SHLD2*, *SIK2*, *SKP2*, *SLC19A1*, *SLC26A3*, *SLC45A2*, *SLCO1B1*, *SLFN11*, *SLIT2*, *SLX4*, *SMAD3*, *SMAD4*, *SMARCA2*, *SMARCA4*, *SMARCB1*, *SMARCE1*, *SMC1A*, *SMC3*, *SMO*, *SOCS1*, *SOS1*, *SOX11*, *SOX2*, *SOX9*, *SPEN*, *SPINK1*, *SPOP*, *SPRED1*, *SRC*, *SRD5A2*, *SRGAP1*, *SRSF2*, *SSTR2*, *SSX1*, *STAG2*, *STAT1*, *STAT3*, *STAT5A*, *STAT5B*, *STK11*, *SUCLG2*, *SUFU*, *SUZ12*, *SYK*, *TACSTD2*, *TAF1*, *TAF15*, *TAP1*, *TAP2*, *TAPBP*, *TBK1*, *TBX3*, *TCF3*, *TCF4*, *TCL1A*, *TEK*, *TERC*, *TERF2IP*, *TERT*, *TET1*, *TET2*, *TFE3*, *TGFB1*, *TGFBR2*, *TMEM127*, *TMPRSS2*, *TNFAIP3*, *TNFRSF13B*, *TNFRSF14*, *TNFRSF8*, *TNFSF11*, *TOP1*, *TOP2A*, *TP53*, *TP53BP1*, *TP63*, *TPMT*, *TPX2*, *TRAF2*, *TRAF3*, *TRAF5*, *TRAF7*, *TRIM28*, *TRRAP*, *TSC1*, *TSC2*, *TSHR*, *TTK*, *TYMS*, *U2AF1*, *UBE2T*, *UBR5*, *UGT1A1*, *UGT2B15*, *UGT2B7*, *UIMC1*, *USP9X*, *VEGFA*, *VEGFB*, *VHL*, *VKORC1*, *VTCN1*, *WRN*, *WT1*, *XIAP*, *XPA*, *XPC*, *XPO1*, *XRCC1*, *XRCC2*, *XRCC3*, *XRCC5*, *XRCC6*, *YAP1*, *YES1*, *ZFHX3*, *ZNF217*, *ZNF703*, *ZNRF3*, *ZRSR2* (somatic tumor panel version 8)

Methods

DNA isolation: The isolation of tumor and normal DNA was performed at CeGaT GmbH. Macrodissection prior to tumor and normal DNA isolation was performed, if necessary. 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; Jones et al., 2019, PMID: 31061401). In cases with low material quality the detection of aberrations (variant calling, copy number variation, structural variants) as well as mutational burden, microsatellite instability (MSI), viral infection in the tumor, and HRD-score determination may be impaired or even impossible.

NGS-laboratory: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus 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. Typing of HLA class I/II was performed using sequencing data from patient's normal tissue using OptiType (Szolek et al., 2014, PMID: 25143287).

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. Known hotspot variants may also be reported up to a NAF of ≥ 2%. 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, 98.94% of the targeted regions were covered by a minimum of 70 high-quality sequencing reads per base. The diagnostic tumor content (expert estimate) was 50%. A theoretical sensitivity of >99% can be obtained for variants with a NAF ≥25% when a coverage of 43 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. cBioPortal, My Cancer Genome, Clinical Interpretations of Variants in Cancer (CIVIC), MD Anderson Personalized Medicine Center Database, *TP53* database (tp53.cancer.gov/), CKB, OncoKB, PubMed research) and/or using *in silico* predictions (MetaLR, PrimateAI, and SpliceAI). 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.

A variant is classified as a driver mutation if it represents a disease-causing germline variant, or a somatic mutation known to define a specific cancer entity. Additionally, recurring and well described somatic mutations known to "drive" tumor development/progression in the analyzed tumor entity, or across multiple cancer entities, are classified as driver mutations.

The relevance of germline variants in genes belonging to our pharmacogenetic subpanel (PGX-01) were assessed using the PharmGKB and CPIC databases and guidelines.

In the context of the pharmacogenetic evaluation (PGX-01), not all detected variants in a gene are taken into account; only variants with therapeutic relevance, variants for which "dosing guidelines" are published, or variants which have an evident influence on drug administration.

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 (only applicable for nuclear encoded genes). 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. Aberrations on the Y chromosome and in the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

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. 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).

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. A loss of one allele does not necessarily result in reduced protein expression and likewise, low grade amplification does not necessarily lead to an increase of protein expression. Therefore, only strong amplifications (≥ 5 copies) and homozygous deletions are reported. 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.

Structural variants 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*

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 exome 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 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): A probable MSI status is predicted from sequencing data (step-wise difference (DIF); threshold 0.33; Kautto et al., 2017, PMID: 27980218). Please be aware that bioinformatics MSI prediction cannot replace a validated diagnostic test for MSI.

Viral Infection: Viral coding sequences are enriched using probes specifically designed for the genomes of EBV (Epstein-Barr virus), CMV (Cytomegalovirus), MCV (Merkel cell polyomavirus) and HPV (human papilloma virus) types 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. Reads that cannot be mapped to the human genome are compared with these genomes and hits are counted.

Therapeutic options: The placement of drugs into different drug classes is done by cross referencing information from FDA, EMA, and PubChem. Approval status and limitations are taken from drugs.com (FDA) and ema.europa.eu (EMA).

In case of evidence (NCCN and/or ESMO guidelines) of a respective biomarker causing non-response, decreased response, or resistance to the specified medication class in the given entity, or in case of evidence in current literature suggesting non-response, decreased response, or resistance, the affected drugs will be marked with a warning sign in appendix.

Clonal hematopoiesis of indeterminate potential (CHIP): CHIP is defined by low frequency (~10%) somatic mutations found in peripheral blood in the absence of hematopoietic dysplasia. Such variants are considered to be of uncertain disease relevance with a low risk (0.5-1% per year) of transformation into myeloid or lymphoid neoplasms (Heuser et al., 2016, PMID: 27215596). As CHIP variants can have allele frequencies <5%, the diagnosis in our reports is considered to be an incidental finding.

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.

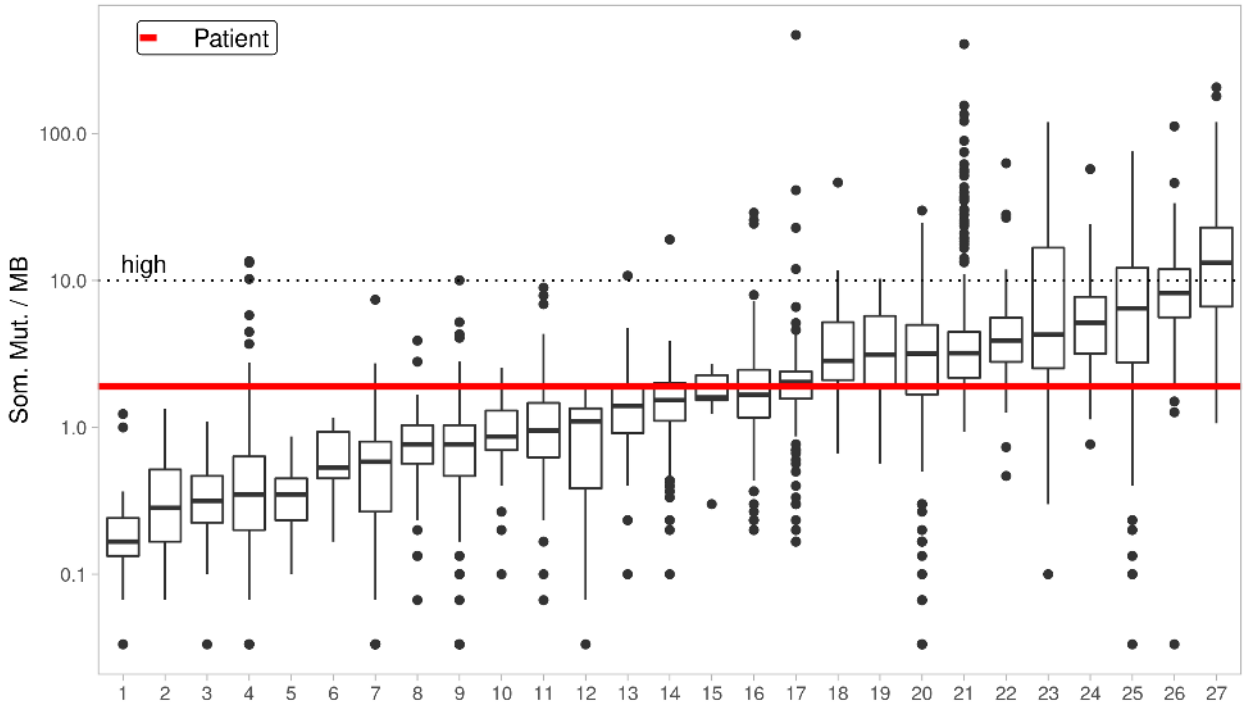
Genetic Counseling

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, Ellard et al., 2020, Association for Clinical Genomic Science).

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 approximated 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). TMB on exome level is extrapolated, taking the results of panel data analysis as a basis. 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).

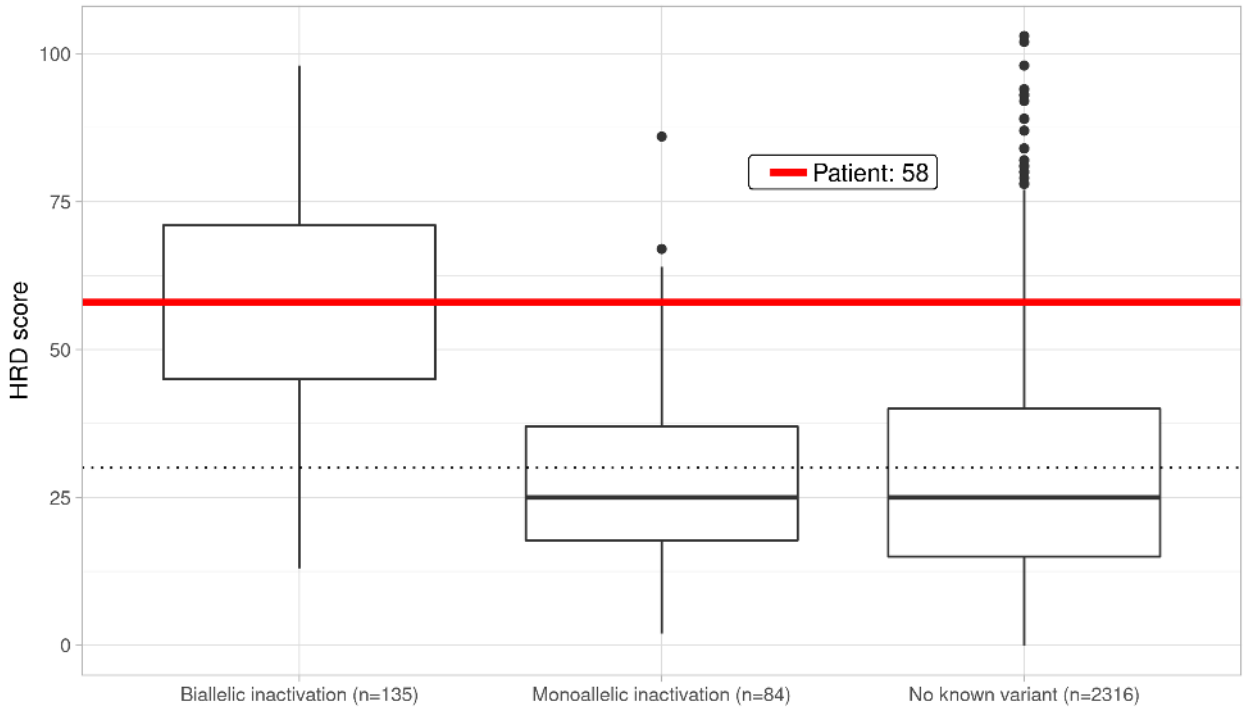


Distribution of tumor mutational burden in 27 tumor entities

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 1.8 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.

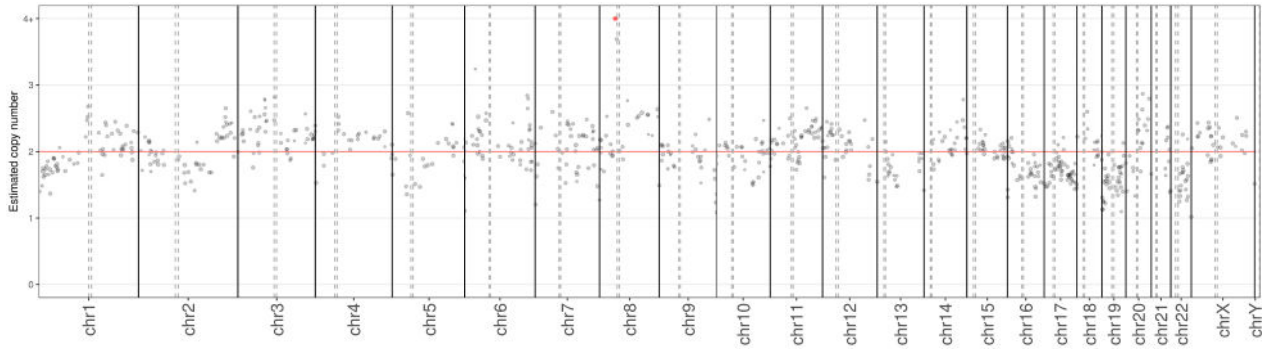
Entities are: (1) Rhabdoid tumor, (2) Ewing Sarcoma, (4) Acute myeloid leukemia, (5) Medulloblastoma, (6) Carcinoid, (7) Neuroblastoma, (8) Prostate cancer, (9) Chronic lymphocytic leukemia, (10) Low-grade glioma, **(11) Breast cancer**, (12) Pancreatic cancer, (13) Multiple myeloma, (14) Kidney clear 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).

Supplement - Homologous Recombination Deficiency (HRD)



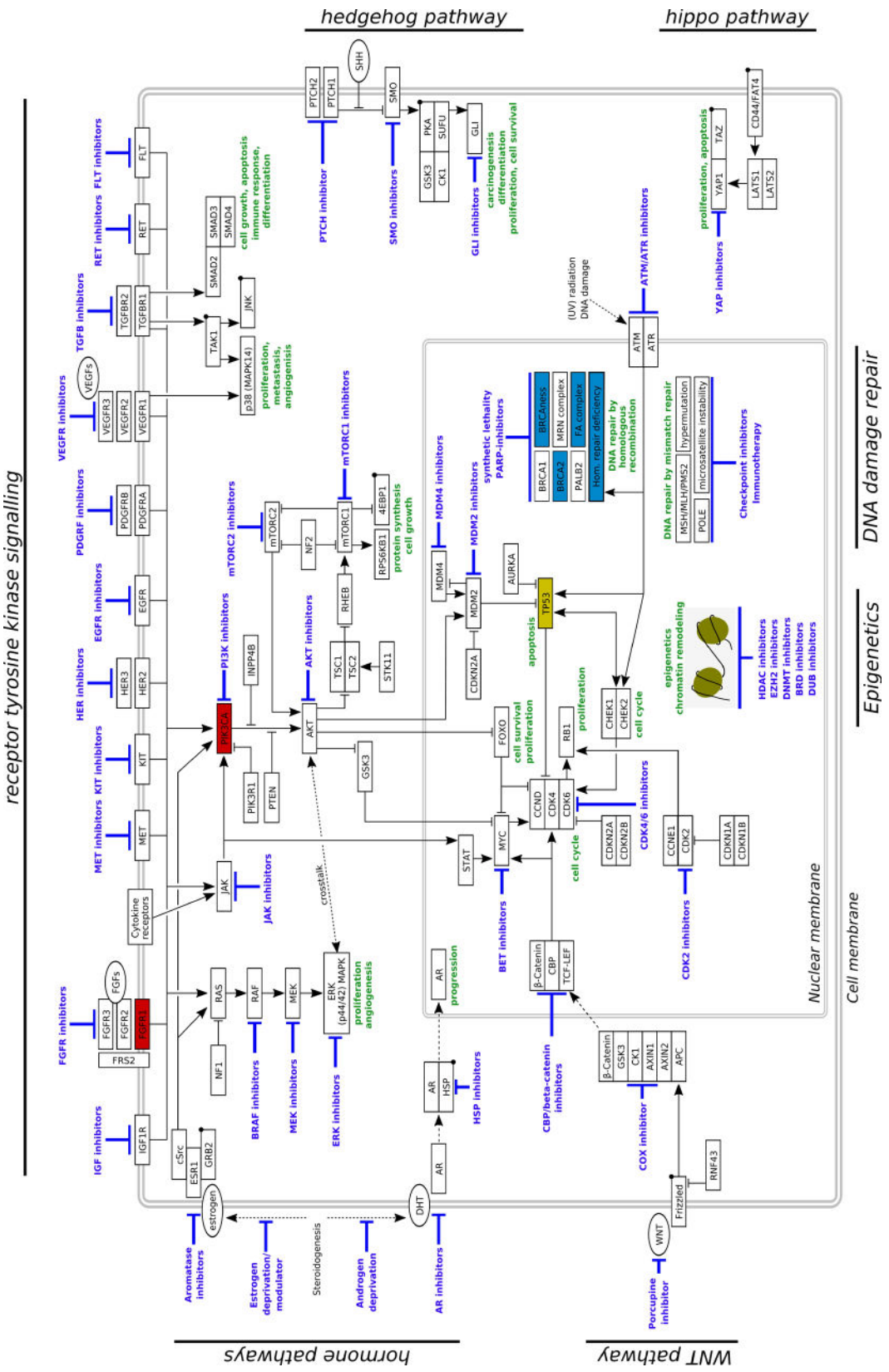
Homologous recombination deficiency (HRD) score of this sample compared to a cohort of patients with biallelic inactivation of HRD-related genes (*ATM*, *BRCA1/2*, *BRIP1*, *PALB2*, *RAD51C*), monoallelic inactivation of HRD-related genes (or second hit not found in available data), and controls with no detectable inactivation of HRD-related genes. Score is calculated as the sum of the markers described in Birkbak et al., 2012, PMID: 22576213; Abkevich et al., 2012, PMID: 23047548; Popova et al., 2012, PMID: 22933060. Higher scores mean higher likelihood of HRD.

Supplement - Copy Number Profile



The genome of a tumor often shows many large copy number variations (CNV). The figure shows each chromosome on the X-axis. The space per chromosome corresponds to its length in base pairs. The coverage profile of the sequenced tumor sample is plotted on Y-axis. Every dot contains binned coverage data of 1 Mb of DNA. Copy numbers from zero (homozygous deletion) to 4+ copies are pictured. CNVs equal to or above 4 copies are indicated by a red colour. Please note that tumor content, as well as subclonal composition of a given tumor sample, may affect copy number estimation. Thus, the plot doesn't show copy number variation of an isolated clonal cell population but provides average measures of the CNV profile of the entire sequenced sample.

Supplement - Pathways



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 - Possible Therapeutic Strategies

Please note that the provided information on potential drugs is only a specific selection and makes no claim of completeness. Furthermore, the listing is limited to targeted therapies and does not include common chemotherapies.

Approvals affecting your patient's tumor entity are highlighted in blue.

BRCA2, c.3847_3848delGT; p.Val1283Lys_fs_*2 (germline) and loss of wildtype allele in tumor tissue, NM_000059.4:

Relevant therapeutics for gene _BRCA2_
Relevant therapeutics due to the homologous recombination deficiency

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Olaparib PARP inhibitor	Breast cancer	EMA	**germline BRCA1/2 variant, HER2-negative** -adults, monotherapy or in combination with endocrine therapy, adjuvant, high risk early breast cancer, prior (neo)adjuvant chemotherapy -adults, locally advanced or metastatic disease, prior anthracycline and taxane ; HR-pos: progressed after/considered unsuitable for endocrine therapy	
		FDA	**deleterious or suspected deleterious germline BRCA mutation, HER2-negative** - adjuvant treatment, adult patients, high-risk early breast cancer, treated with neoadjuvant or adjuvant chemotherapy - adult patients, metastatic cancer, treated with chemotherapy in the neoadjuvant, adjuvant or metastatic setting; HR-positive cancer: treated with a prior endocrine therapy or considered inappropriate for endocrine therapy	
Rucaparib PARP inhibitor	Fallopian tube carcinoma	EMA	adult patients, maintenance treatment, platinum-sensitive relapsed cancer, in response to platinum-based chemotherapy	
		FDA	**deleterious BRCA mutation (germline and/or somatic)** adult patients, maintenance treatment, recurrent cancer, in response to platinum-based chemotherapy	
	Ovarian cancer	EMA	adult patients, maintenance treatment, platinum-sensitive relapsed or advanced high-grade epithelial ovarian cancer, in response to platinum-based chemotherapy	
		FDA	**deleterious BRCA mutation (germline and/or somatic)** adult patients, maintenance treatment, recurrent epithelial ovarian cancer, in response to platinum-based chemotherapy	
	Primary peritoneal carcinoma	EMA	adult patients, maintenance treatment, advanced or relapsed disease, in response to platinum-based chemotherapy	
		FDA	**deleterious BRCA mutation (germline and/or somatic)** adult patients, maintenance treatment, recurrent cancer, in complete or partial response to platinum-based chemotherapy	
	Prostate cancer	FDA	**deleterious germline or somatic BRCA mutation** adult patients, metastatic disease, prior androgen receptor-directed therapy and taxane-based chemotherapy	
Talazoparib PARP inhibitor	Breast cancer	EMA	**deleterious or suspected deleterious germline BRCA1/2 mutation, HER2-negative** metastatic or locally advanced cancer, prior treatments (anthracycline and/or a taxane (neo)adjuvant; HR positive: endocrine-based therapy) or considered unsuitable for these treatments	
		FDA	**deleterious or suspected deleterious germline BRCA mutation, HER2-negative** adult patients, metastatic or locally advanced cancer	

PIK3CA, c.3140A>G; p.His1047Arg, NM_006218.4:

Relevant therapeutics for gene *PIK3CA*

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Capivasertib AKT inhibitor	Breast cancer	EMA	**PIK3CA/AKT1/PTEN-alteration; HR pos. HER2 neg.** adults, locally advanced or metastatic following recurrence or progression on or after an endocrine-based regimen.	Fulvestrant
		FDA	**PIK3CA/AKT1/PTEN-alteration; HR pos. HER2 neg.** adults, following progression on at least one endocrine-based regimen in the metastatic setting or recurrence on or within 12 months of completing adjuvant therapy	Fulvestrant
Alpelisib PI3Kα inhibitor PI3K inhibitor	Breast cancer	EMA	**HR-positive, HER2-negative, PIK3CA mutated** postmenopausal women, and men, locally advanced or metastatic breast cancer, prior endocrine therapy	Fulvestrant
		FDA	**HR positive, HER2-negative, PIK3CA-mutated** adults, advanced or metastatic breast cancer, prior endocrine-based therapy	Fulvestrant
Inavolisib PI3Kα inhibitor PI3K inhibitor	Breast cancer	FDA	**HR pos./HER2 neg. + PIK3CA mut** adults, endocrine-resistant, locally advanced or metastatic following recurrence on or after completing adjuvant endocrine therapy.	Palbociclib, Fulvestrant
Everolimus mTOR inhibitor	Breast cancer	EMA	**HR positive, HER2 negative** advanced breast cancer, postmenopausal women without symptomatic visceral disease after recurrence or progression following a non-steroidal aromatase inhibitor	Exemestane
		FDA	**HR positive, HER2 negative** advanced disease, postmenopausal, recurrence or progression after letrozole or anastrozole	Exemestane
Sirolimus mTOR inhibitor	Soft tissue neoplasm	FDA	adult patients with locally advanced unresectable or metastatic malignant perivascular epithelioid cell tumor (PEComa)	
Temsirolimus mTOR inhibitor	B-cell lymphoma (BCL)	EMA	adult patients with relapsed and/or refractory mantle-cell lymphoma (MCL)	
	Renal cell carcinoma	EMA	first-line treatment, adult, advanced renal-cell carcinoma (RCC) who have at least three of six prognostic risk factors	
		FDA	advanced renal cell carcinoma	

FGFR1, amplification, complete gene, non focal (10 copies), NM_023110.3:

Relevant therapeutics for gene *FGFR1*

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Ponatinib FGFR inhibitor BCR-ABL inhibitor KIT inhibitor PDGFR inhibitor SRC inhibitor VEGFR inhibitor	Chronic myelogenous leukemia (CML)	EMA	**(ABL T315I)** - chronic phase, accelerated phase, or blast phase CML, resistant to dasatinib or nilotinib - who are intoloerant to dasatinib or nilotinib and for whom subsequent treatment with imatinib is not clinically appropriate - or who have the T315I mutation	
		FDA	**(ABL T315I)** adult patients, - chronic-phase CML, resistant or intolerant to at least two prior kinase inhibitors - accelerated phase or blast phase CML if no other kinase inhibitors are indicated - or T315I-positive CML (in all CML phases)	
	Ph-positive acute lymphoblastic leukemia (Ph+ ALL)	EMA	**(ABL T315I)** - resistant to dasatinib - intolerant to dasatinib and for whom subsequent treatment with imatinib is not clinically appropriate - or who have the T315I mutation	
		FDA	**(ABL T315I)** adult patients if no other kinase inhibitor is indicated or *ABL* T315I-positive	
		FDA	newly diagnosed	Vincristin, Methotrexat, Dexamethasone, Cytarabine, Prednisone

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Erdafitinib FGFR inhibitor	Bladder carcinoma	EMA	**FGFR3 alteration** adults, unresectable or metastatic disease, at least one prior line of therapy containing a PD-1 or PD-L1 inhibitor in the unresectable or metastatic treatment setting	
		FDA	**FGFR3 or FGFR2 alteration** adult, locally advanced or metastatic urothelial carcinoma, progressed during/following at least one line of prior platinum-containing chemotherapy including within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy	
Futibatinib FGFR inhibitor	Cholangiocellular carcinoma	EMA	**FGFR2 gene fusion or other rearrangements** adults, locally advanced or metastatic that have progressed after at least one prior line of systemic therapy	
		FDA	**FGFR2 gene fusion or other rearrangements** adult patients with previously treated, unresectable, locally advanced or metastatic intrahepatic CCC	
Lenvatinib FGFR inhibitor KIT inhibitor PDGFR inhibitor RET inhibitor VEGFR inhibitor	Endometrial carcinoma	EMA	adult, advanced or recurrent, previous platinum-based treatment, surgery or radiation impossible	Pembrolizumab
		FDA	**not MSI-high or dMMR** advanced endometrial carcinoma, disease progression following prior systemic therapy but curative surgery or radiation ineligible.	Pembrolizumab
	Hepatocellular carcinoma	EMA	adult patients, no prior systemic therapy, advanced or unresectable hepatocellular carcinoma (HCC)	
		FDA	unresectable hepatocellular carcinoma	
	Renal cell carcinoma	EMA	adults, advanced renal cell carcinoma, first line treatment	Pembrolizumab
		EMA	adults, advanced renal cell carcinoma, following one prior vascular endothelial growth factor (VEGF)-targeted therapy	Everolimus
		FDA	advanced renal cell carcinoma, previously treated with an anti-angiogenic therapy	Everolimus
	Thyroid carcinoma	EMA	adult, differentiated (papillary/follicular/Hürthle cell), progressive or locally advanced or metastatic and refractory to radioactive iodine	
		FDA	locally recurrent or metastatic, progressive, radioactive iodine-refractory differentiated thyroid cancer	
Pazopanib FGFR inhibitor KIT inhibitor PDGFR inhibitor VEGFR inhibitor	Renal cell carcinoma	EMA	adults, advanced renal cell carcinoma, no previous treatment or in patients who have already been treated with cytokines	
		FDA	adults, advanced renal cell carcinoma	
	Soft tissue sarcoma	EMA	adults, previously treated with chemotherapy for metastatic disease or who have progressed within 12months after (neo) adjuvant therapy	
		FDA	adults, advanced disease, prior chemotherapy	
Nintedanib FGFR inhibitor PDGFR inhibitor VEGFR inhibitor	Non-small cell lung carcinoma	EMA	adults, locally advanced, metastatic or locally recurrent, NSCLC of adenocarcinoma tumor histology, previous chemotherapy	Docetaxel
Pemigatinib FGFR inhibitor selective FGFR-Inhibitor	Cholangiocellular carcinoma	EMA	**FGFR2 fusions or rearrangements** adult patients, locally advanced or metastatic, progressed after at least one prior line of systemic therapy	
		FDA	**FGFR2 fusions or rearrangements** adults, previously treated, unresectable locally advanced or metastatic cholangiocarcinoma	
	Leukemia	FDA	**FGFR1 rearrangement** adults, relapsed or refractory myeloid/lymphoid neoplasms	