

<b>Name</b>	Doe, Jane (*DD.MM.YYYY)
<b>Sex</b>	Female
<b>Patient-ID</b>	XXXXXX
<b>Report date</b>	DD.MM.YYYY
<b>Report-ID</b>	R999999999

**Indication**      **Breast cancer**

## Previously Reported Findings

External ID <i>receipt date</i>	Institution	Tumor / Tissue	Marker
XXXXXX MM/YYYY	Model Institution, Model City	<b>Tissue:</b> FFPE XXXX, breast, collected: MM/YYYY	<b>IHC Results:</b> ER: 90 %, HER2neu/ERBB2: 0, Ki-67/Mib-1: 31 %, PR: 90 %
XXXXXX MM/YYYY	Model Institution, Model City	<b>Tissue:</b> DNA XXXX, germline, collected: MM/YYYY	<b>Wild-type Genes:</b> <i>ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53</i>
XXXXXX MM/YYYY	Model Institution, Model City	<b>Tissue:</b> FFPE XXXX, brain, left cerebellar, collected: MM/YYYY	<b>IHC Results:</b> ER: 100 %, HER2neu/ERBB2: 1+, Ki-67/Mib-1: 32 %, PR: 30-40 % <b>ISH Results:</b> HER2neu/ERBB2: no amp

Information in table above makes no claim to completeness.

## Medical History

Date	Treatment / Staging
MM/YYYY	tumor resection (primary in breast)
MM/YYYY	breast conserving surgery (primary in breast - right side - with result R0) pT2, pN1a, cM0, G3
MM/YYYY- MM/YYYY	cyclophosphamide + epirubicin (4 cycles)
MM/YYYY- MM/YYYY	docetaxel (4 cycles)
MM/YYYY- MM/YYYY	radiotherapy
MM/YYYY- MM/YYYY	tamoxifen
MM/YYYY	new metastases: liver, bone
MM/YYYY- MM/YYYY	letrozole, ribociclib



MM/YYYY

PD: liver

new metastases: brain (MRI of brain)

MM/YYYY

tumor resection (metastasis in brain - left cerebellar)

**Information in table above makes no claim to completeness. Stated staging results do not comply with RECIST or RANO rules as applied in clinical studies but aim to generally describe the course of the patient's disease.**



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## CancerPrecision® - Report of Somatic Tumor Variants

### Doe, Jane (\*DD.MM.YYYY)

Indication **Breast cancer**

### Result Overview

Tumor Tissue & Tumor Content (TC)	Germline Variants	Tumor Drivers	Fusions, Structural Variants	Pharmacogenetics (PGx)
<p>brain metastasis from MM/YYYY (FFPE-ID XXXXX)</p> <p>60% diagnostically</p> <p>Diag. TC min 20%</p>	<p>No evidence for pathogenic or likely pathogenic alterations</p>	<p>Identified tumor drivers: <i>PIK3CA, BRCA1, FOLR1, TP53</i></p> <p>Relevant genes without oncogenic alterations: <i>BRCA2, ERBB2, ESR1, CD274, TACSTD2</i></p>	<p>No evidence for therapeutically relevant structural variants (on DNA and RNA level)</p>	<p>Detection of a germline variant in gene <i>UGT1A1</i></p>
Tumor Mutational Burden (TMB)	Microsatellite Instability (MSI)	Homologous Recombination Deficiency (HRD)	Viral Infection	CHIP
<p>1.7 Var/Mb</p> <p>High ≥ 10</p>	<p>No evidence for MSI (NGS prediction)</p> <p>Score 0.16</p> <p>Indication of MSI ≥ 0.33</p>	<p>Evidence for HRD</p> <p>Score 48</p> <p>Indication of HRD ≥ 30</p>	<p>No evidence for an infection with HPV/EBV/CMV/MCV in the tumor sample</p> <p>Indication of infection ≥ 50 reads</p>	<p>No evidence for CHIP</p>



## Variants with Potential Therapeutic Relevance and Recurrent Tumor Drivers

Gene	Functional category	Variant	NAF	Effect on protein function	Therapeutic option for discussion in the MTB	Approved for current entity (EMA/FDA)
<b>HRD</b>	N/A	N/A	N/A	N/A	PARP inhibitor	EMA & FDA
<b>PIK3CA</b> NM_006218.4	missense	c.1633G>A; p.Glu545Lys, exon 10 chr3:178936091 G>A (hg19)	0.29	activating OS2:VS, OS3, OP4	<b>AKT inhibitor</b> <b>mTOR inhibitor</b> <b>PI3Kα inhibitor</b>  Possible resistance to HER2 inhibitor, BRAF inhibitor, EGFR inhibitor	<b>EMA &amp; FDA</b> <b>EMA &amp; FDA</b> <b>EMA &amp; FDA</b>  -
<b>BRCA1</b> NM_007294.4	stop_gained	c.2079_2082delinsGT; p.Asp693Glufs*2, exon 10 chr17:41245465 CGCTG>CAC (hg19)  and loss of heterozygosity	0.61	probably inactivating OVS1, OP4	PARP inhibitor	EMA & FDA
<b>FOLR1</b> (coding for Folate receptor alpha)	overexpression	complete gene	N/A	activating	FRα antibody-drug conjugate	no
<b>TP53</b> NM_000546.6	missense	c.844C>T; p.Arg282Trp, exon 8 chr17:7577094 G>A (hg19)  and heterozygous deletion	0.58	function changed OS2:VS, OS3, OP4	N/A	N/A

**NAF:** *Novel allele frequency*, the frequency with which the altered allele occurs in the sequencing data (1 is 100%). The observed frequencies in the tumor sample 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.

**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.

Therapy options highlighted in blue are approved for your patient's entity and meet all approval restrictions that are verifiable with our methods.

**Gene expression:** An altered gene expression does not necessarily lead to a (therapeutically) relevant, altered protein biosynthesis. We recommend using an independent method such as direct staining of the target structure at the protein level to support therapeutic decisions based on gene expression data only.

**N/A:** Not applicable.

**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.**

You will find an additional table listing approved drugs for other entities based on the detected changes in the appendix. This may serve as a guide for possible off-label treatment options.



## Variants with Pharmacogenetic Relevance

Gene	Functional category	Variant	Transcript-ID	Zygoty	Effect on protein function	Affected therapeutic option	Phenotype
<b>UGT1A1</b>	5_prime_UTR	c.-41_-40dup (*28/*28) chr2:234668879 C>CAT (hg19)	NM_000463.3	homozygous	risk factor	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).

## Further Automatically Detected Somatic Variants

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

Gene	Functional category	Variant	Transcript-ID	NAF
<b>CTNNB1</b>	missense	c.110C>A; p.Ser37Tyr, exon 3 chr3:41266113 C>A (hg19)	NM_001904.4	0.41
<b>CTR9</b>	missense	c.2369A>G; p.His790Arg, exon 18 chr11:10792176 A>G (hg19)	NM_014633.5	0.25
<b>NOTCH3</b>	missense	c.421C>T; p.Arg141Cys, exon 4 chr19:15303029 G>A (hg19)	NM_000435.3	0.30

**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\*##:##, HLA-DRB4\*##:##

## Copy Number Alterations

Our sequencing data do not provide evidence for the presence of potentially relevant copy number alterations of large genomic segments. There is no evidence for the presence of homozygous deletions or strong amplifications of single therapeutically relevant genes.

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

## Additional gene expression data of requested genes

The following gene expression data were additionally analyzed in the tumor sample.

Gene	Functional category	Variant	FPKM
<b>CD274 (coding for PD-L1)</b>	reduced expression	complete gene	0.73
<b>ERBB2 (coding for HER2)</b>	normal expression	complete gene	30.61
<b>TACSTD2 (coding for TROP2)</b>	normal expression	complete gene	66.71

**FPKM:** Fragments Per Kilobase Million, expression level metric.

An altered gene expression does not necessarily lead to a (therapeutically) relevant, altered protein biosynthesis. We recommend using an independent method such as direct staining of the target structure at the protein level to support therapeutic decisions based on gene expression data only.

## Recommendation

The detected variant \*28/\*28 in gene *UGT1A1* is a homozygous germline variant. For this genotype (also known as (TA)<sub>7</sub>/(TA)<sub>7</sub>, rs8175347 or rs3064744), a possible increased toxicity upon treatment with irinotecan-based chemotherapeutic agents has been described (Steventon, 2020, PMID: 31092094; ClinPGx: Level of evidence 1A; Whirl-Carrillo et al., 2012, PMID: 22992668; Dean et al., updated 2018, Medical Genetics Summaries, 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](https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761115s023lbl.pdf)).

**Drug dosing adjustments should exclusively be performed following consultation with the attending clinician.**

We recommend immunohistochemical staining (IHC) of the Folate receptor 1 due to the RNA expression of *FOLR1*.

**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: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,

*Dr. B. Kerner*  
Dr. med. Berit Kerner

Consultant for Human Genetics

## Additional Information

<b>Order</b>	<b>1. Somatic molecular genetic analysis of a tumor tissue sample:</b> Tumor panel analysis TUM01, evaluation of somatic variants of potential clinical relevance, gene expression analysis <b>2. RNA fusions panel analysis STR</b>
<b>Sample material</b>	<b>Normal tissue: EDTA blood</b> <b>Tumor tissue: brain metastasis</b> Sample collection MM/YYYY DNA and RNA isolation from tumor in FFPE (FFPE-ID: XXXX) with estimated tumor content of 80% (HE staining) Diagnostically estimated tumor content 60%
<b>Sample receipt</b>	DD.MM.YYYY (Normal-DNA: EDTA blood, ID PXXXXXX_1) DD.MM.YYYY (Tumor DNA: FFPE material, ID PXXXXXX_2) DD.MM.YYYY (Tumor DNA: FFPE material, ID PXXXXXX_3) DD.MM.YYYY (Tumor RNA: FFPE material, ID PXXXXXX_4)
<b>Requested Regions</b>	Somatic tumor panel (TUM01) contains interpretation of the following cancer-relevant genes: <i>CACNA1S, DPYD, G6PD, NUDT15, RYR1, TPMT, UGT1A1</i> (Pharmacogenetics) <i>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,</i>



## Methods

**DNA and RNA isolation:** The isolation of tumor and normal DNA as well as tumor RNA was performed at CeGaT GmbH. Macrodissection prior to tumor and normal DNA as well as tumor RNA 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 DNA:** 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 X Plus system.

**NGS-laboratory RNA:** RNA from tumor tissue was sequenced. Fusion transcripts were enriched using in-solution hybridization technology. For fusion transcripts with known breakpoints, breakpoint spanning probes were used. For genes with unknown breakpoints or a large number of possible fusion partners, the coding sequence was used for enrichment. Sequencing was performed on Illumina NovaSeq X Plus systems.

Library preparation for gene expression analysis was performed using the TruSeq Total RNA (RiboZero rRNA removal Kit) or- SMARTer Stranded Total RNA Library Kit and subsequently analyzed using high-throughput sequencing on the HiSeq/NovaSeq system (Illumina). Mapping of sequencing reads to the hg19 reference genome was performed with STAR.

**Computational Analysis DNA:** 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).

**Computational Analysis RNA:** Sequencing data was demultiplexed using bcl2fastq2. Adapter sequences were removed using Skewer and the resulting reads were mapped to the human reference genome hg19 using STAR aligner. Fusions were detected using the software STAR-Fusion (Haas et al., 2017). Additional intragene structural events in genes *EGFR*, *MET*, *NFE2L2* and *PDGFRA* were extracted from STAR output. Gene expression analysis (counting of aligned reads per gene, calculation of normalized read counts and calculation of FPKM values) was done with DESeq2 (Love et al., 2014, PMID: 25516281) in R (R Core Team 2015).

**Genetic data evaluation DNA:** Only variants (single-nucleotide variants (SNVs)/small indels) with a novel allele frequency (NAF) of  $\geq 5\%$  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 down to a NAF of  $\geq 2\%$ . 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, 98.93% of the targeted regions were covered by a minimum of 70 high-quality sequencing reads per base. The diagnostic tumor content (expert estimate) was 60%. A theoretical sensitivity of  $>99\%$  can be obtained for variants with a NAF  $\geq 30\%$  when a coverage of 35 reads per base is achieved.

**Genetic data evaluation RNA:** The sensitivity of the CancerFusionRx<sup>®</sup> test is dependent on the tumor content of the analyzed material, the sample quality, and the amount of transcripts sequenced. In this case, an amount of 20.26 gigabases RNA was sequenced. Therefore, this analysis is appropriate to detect structural variants on RNA level.

**Variant classification (SNVs/small indels):** Somatic alterations were classified according to ClinGen/CGC/ICC guidelines (Horak et al., 2022, PMID: 35101336). Variants assessed as (likely) oncogenic based on these guidelines were assigned to the categories (likely) inactivating/activating/function changed according to their impact on protein function of the altered gene. The classification is based on the available datasets (e.g. cBioPortal, My Cancer Genome, Clinical Interpretations of Variants in Cancer (CIVIC), MD Anderson Personalized Medicine Center Database, TP53 database (tp53.cancer.gov), Clinical Knowledge Database (CKB), OncoKB, PubMed research) and/or using *in silico* predictions (MetaLR, PrimateAI, and

SpliceAI). Variants classified as unclear significance or (likely) benign according to ClinGen/CGC/VICC guidelines were not functionally categorized.

The clinical relevance of germline variants in genes within our pharmacogenetic subpanel (PGX-01) was assessed using the ClinPGx and Clinical Pharmacogenetics Implementation Consortium (CPIC) databases and guidelines.

For pharmacogenetic evaluation (PGX-01) only variants with therapeutic relevance, variants for which "dosing guidelines" are published, and/or variants which would strongly influence drug administration decisions were considered.

**Variant classification (structural variants):** The relevance of detected structural variants was determined based on the possible altered function of the resulting fusion protein, according to available databases (e.g. FASMIC, PubMed research). The functional categories assigned were: activating/inactivating/function changed, likely activating/inactivating/function changed, and unknown. "Activating" and "function changed": known activating/function changing structural variants. The functional evidence of structural variants classified as activating and function changed is highly reliable, indicated by *in vivo/in vitro* analyses. "Inactivating": known inactivating structural variants as well as structural variants which are expected to lead to a loss of protein product (*nonsense-mediated decay*) due to their location. "Likely activating/inactivating/function changed": an impact of the structural variant on protein function is considered likely with respect to the genes/breakpoints within the described regions, the currently available literature, and frequency in tumor samples, but there is insufficient functional data available to confirm a functional impact. "Unknown": based upon the currently available data, we are not able to conclusively confirm or exclude the possible functional relevance of the structural variant.

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.

**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.

Copy number variants from tumor tissue 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 ( $\geq 5$  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 detected in DNA:** 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, SXX1, SUZ12, TAF15, TCF3,*

## TFE3, TMPRSS2

**Prediction of structural variants detected in RNA:** RNA fusions panel (CancerFusionRx®) contains interpretation of translocations/fusions of the following cancer-relevant genes:

*ABL1, ACTB, ADGRG7, AFAP1, AGK, AKAP12, AKAP4, AKAP9, AKT1, AKT2, AKT3, ALK, ARHGAP26, ARHGAP6, ASPSCR1, ATF1, ATP1B1, ATRX, AVIL, AXL, BAG4, BCL2, BCOR, BCORL1, BCR, BEND2, BICC1, BRAF, BRD3, BRD4, CAMTA1, CCAR2, CCDC170, CCDC6, CCDC88A, CCNB3, CCND1, CD44, CD74, CEP85L, CIC, CLDN18, CLIP1, CLTC, CNTRL, COL1A1, CREB1, CREB3L1, CREB3L2, CRTC1, CTNBN1, DDIT3, DNAJB1, EGFR, EML4, EPC1, EPCAM, ERBB2, ERBB4, ERG, ESR1, ESRRA, ETV1, ETV4, ETV5, ETV6, EWSR1, EZR, FEV, FGFR1, FGFR2, FGFR3, FLI1, FN1, FOXO1, FOXO4, FOXR2, FUS, GLI1, GOPC, HEY1, HMGA2, HTRA1, IGF1R, INSR, JAK2, JAZF1, KIAA1549, KIF5B, KIT, LEUTX, LMNA, LPP, LTK, MAGI3, MAML1, MAML2, MAML3, MAMLD1, MAP3K8, MARS1, MAST1, MAST2, MEAF6, MET, MGA, MGMT, MITF, MN1, MRTFB, MSH2, MYB, MYBL1, MYC, NAB2, NCOA1, NCOA2, NCOA3, NCOA4, NFATC2, NFIB, NOTCH2, NPM1, NR4A3, NRG1, NRG2, NSD3, NTRK1, NTRK2, NTRK3, NUTM1, PAX3, PAX7, PAX8, PBX1, PDGFB, PDGFD, PDGFRA, PDGFRB, PHF1, PIK3CA, PLAG1, PML, POU5F1, PPARG, PPARGC1A, PPP1CB, PRKACA, PRKAR1A, PRKCA, PRKCB, PRKD1, PRKD2, PRKD3, PTPRZ1, QKI, RAD51B, RAF1, RANBP2, RARA, REL, RELCH, RET, ROS1, RPS6KB1, RREB1, RSPO2, RSPO3, SDC1, SDC4, SH3PXD2A, SHTN1, SLC1A2, SLC34A2, SLC44A1, SLC45A3, SND1, SQSTM1, SS18, SSX1, SSX2, SSX4, STAT6, STRN, SUZ12, TACC1, TACC2, TACC3, TAF15, TCF12, TCF3, TERT, TFE3, TFE6, TFG, THADA, TMPRSS2, TPM3, TPR, TRIM24, TRIM33, TRIO, TTYH1, VGLL2, VGLL3, VMP1, WT1, WWTR1, YAP1, YWHAE, ZC3H7B, ZFTA, ZMYM2, ZNF703* (Structural Variants Panel version 8)

Selected break points within the mentioned fusion genes:

*TRIM24-BRAF, KIAA1549-BRAF, SND1-BRAF, EML4-ALK, CLTC-ALK, NPM1-ALK, TPM3-ALK, KIF5B-ALK, ETV6-NTRK3, EWSR1-ERG, EWSR1-FLI1, FGFR3-TACC3, FGFR2-BICC1, FGFR2-TACC3, FGFR1-TACC1, TMPRSS2-ERG, TPM3-NTRK1, TPR-NTRK1, TRIM24-NTRK2, AFAP1-NTRK2, QKI-NTRK2, ETV6-NTRK2, KIF5B-RET, CCDC6-RET, NCOA4-RET, PRKAR1A-RET, TRIM33-RET, CD74-ROS1, EZR-ROS1, SLC34A2-ROS1, TPM3-ROS1, SDC4-ROS1, BRD4-NUTM1, BRD3-NUTM1, MGA-NUTM1, NSD3-NUTM1, NAB2-STAT6, CD74-NRG1, SDC4-NRG1, ATP1B1-NRG1, BCOR-CCNB3, DNAJB1-PRKACA, EGFR-PPARGC1A, CCDC88A-ALK, PPP1CB-ALK, PAX3-FOXO1, PAX7-FOXO1, SS18-SSX1, SS18-SSX2, EWSR1-WT1, EWSR1-ATF1, TRIO-TERT*

Specific transcript variants:

*EGFR del ex2-22 (mLEEK), EGFR del ex25-26 (EGFRvIvB), EGFR del ex25-27 (EGFRvIVa), EGFR del ex26-27, EGFR del ex14-15 (vII), EGFR del ex2-7 (vIII), FGFR2IIIb, MET ex14 skipping, NFE2L2 ex2 skipping, PDGFRA del ex8-9*

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

**Microsatellite instability (MSI) in tumor tissue:** 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 (RNA and DNA):** 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 [www.accessdata.fda.gov/scripts/cder/daf/](http://www.accessdata.fda.gov/scripts/cder/daf/) (FDA - Food and Drug Administration) and [ema.europa.eu](http://ema.europa.eu) (EMA - European Medicines Agency).

Drugs listed in the appendix as "Approved drugs for your patient's tumor entity" may also include drugs that are approved for solid tumors in general regardless of a specific entity.

In case of evidence (NCCN - National Comprehensive Cancer Network) 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**

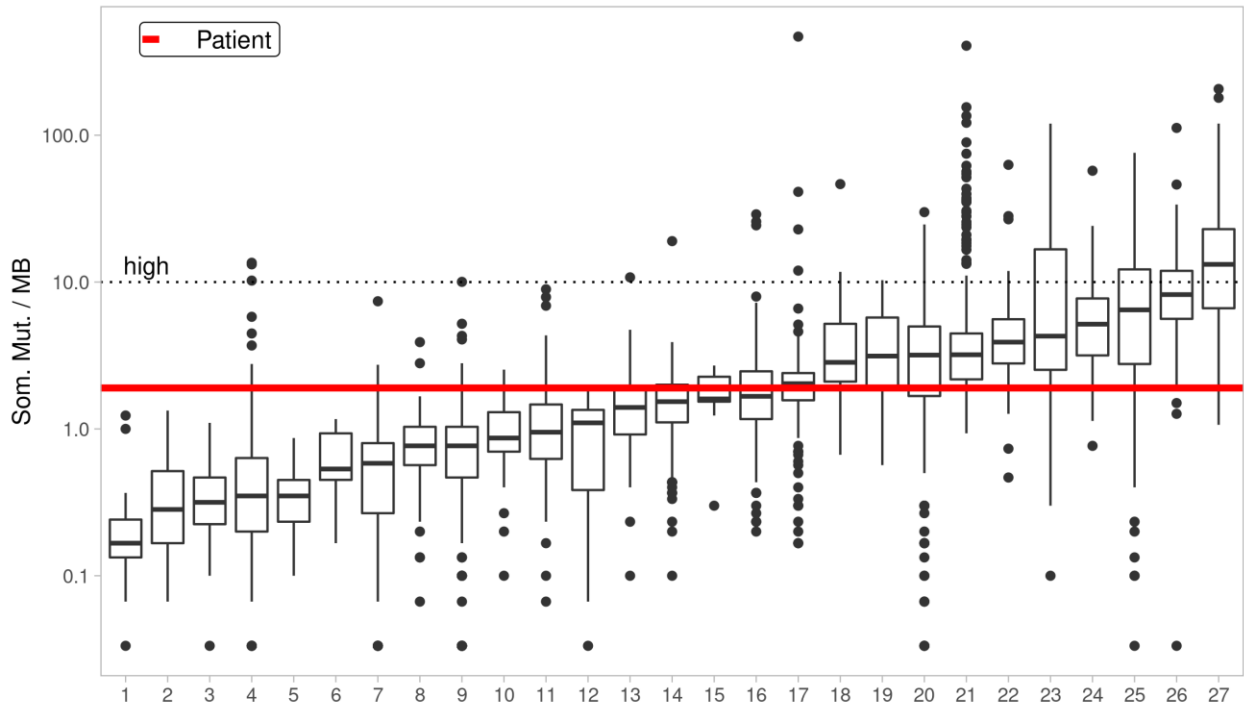
Please be aware that this somatic report cannot replace conventional germline diagnostics. A lack of evidence for therapy relevant or likely disease causing germline variants does not exclude the presence of disease relevant germline mutations. In cases where a relevant germline mutation has been detected, genetic counseling should be considered. Variants were classified and reported based on ACMG/ACGS-2024v1.2 guidelines (Richards et al., 2015, PMID: 25741868, Durkie et al., 2024, 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).



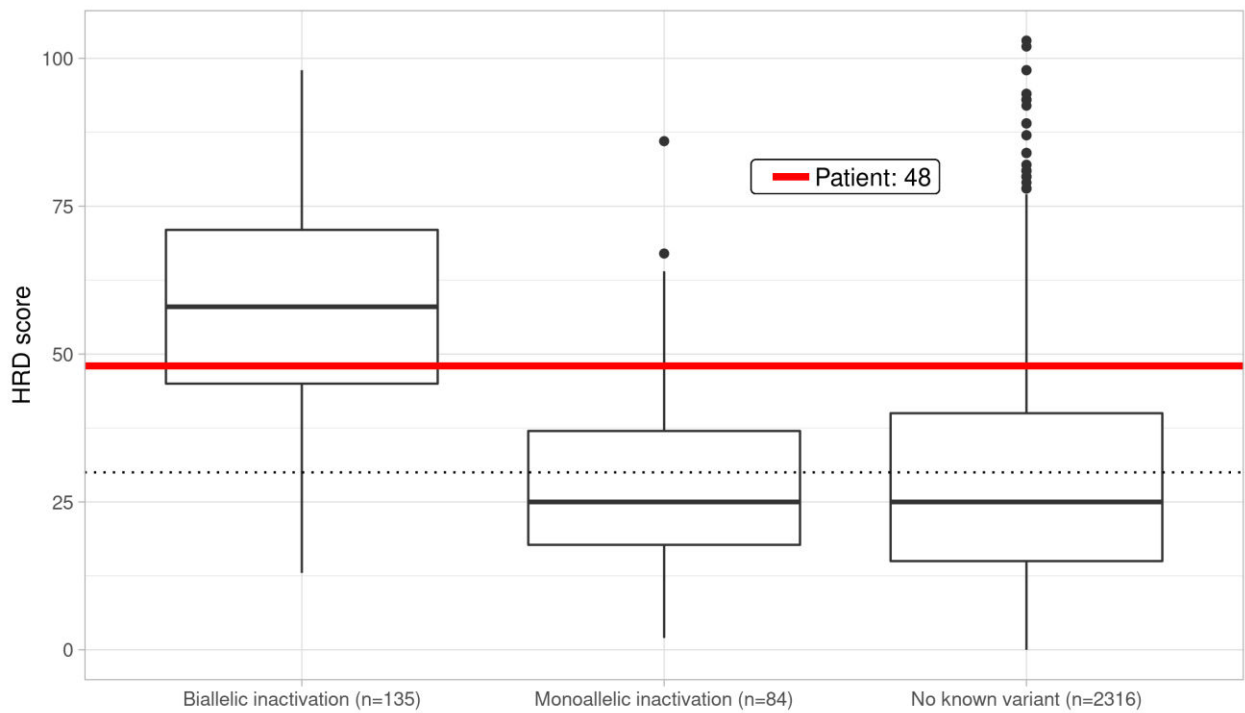
### Distribution of tumor mutational burden in 27 tumor entities

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 1.9 mut/Mbp determined for the current case is shown for comparison (solid red line). Y-axis is log scaled. A high mutational burden ( $\geq 10$  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).



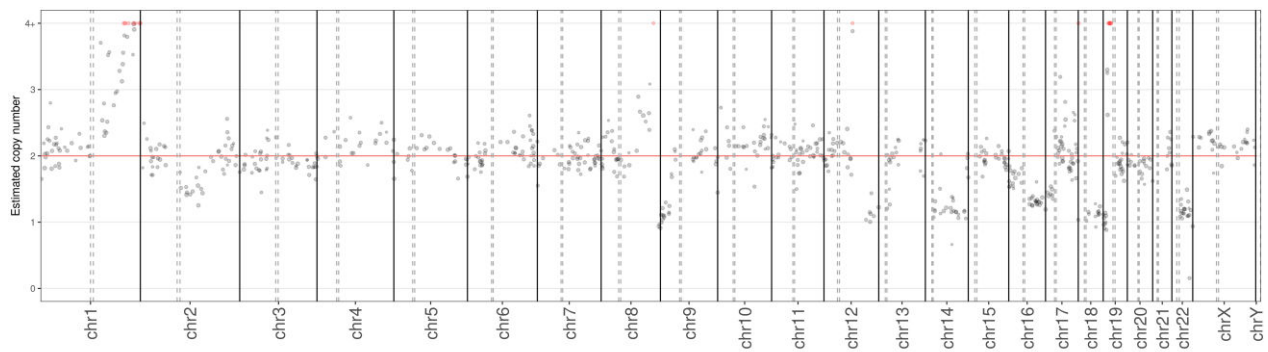
## Supplement - Homologous Recombination Deficiency (HRD)



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.

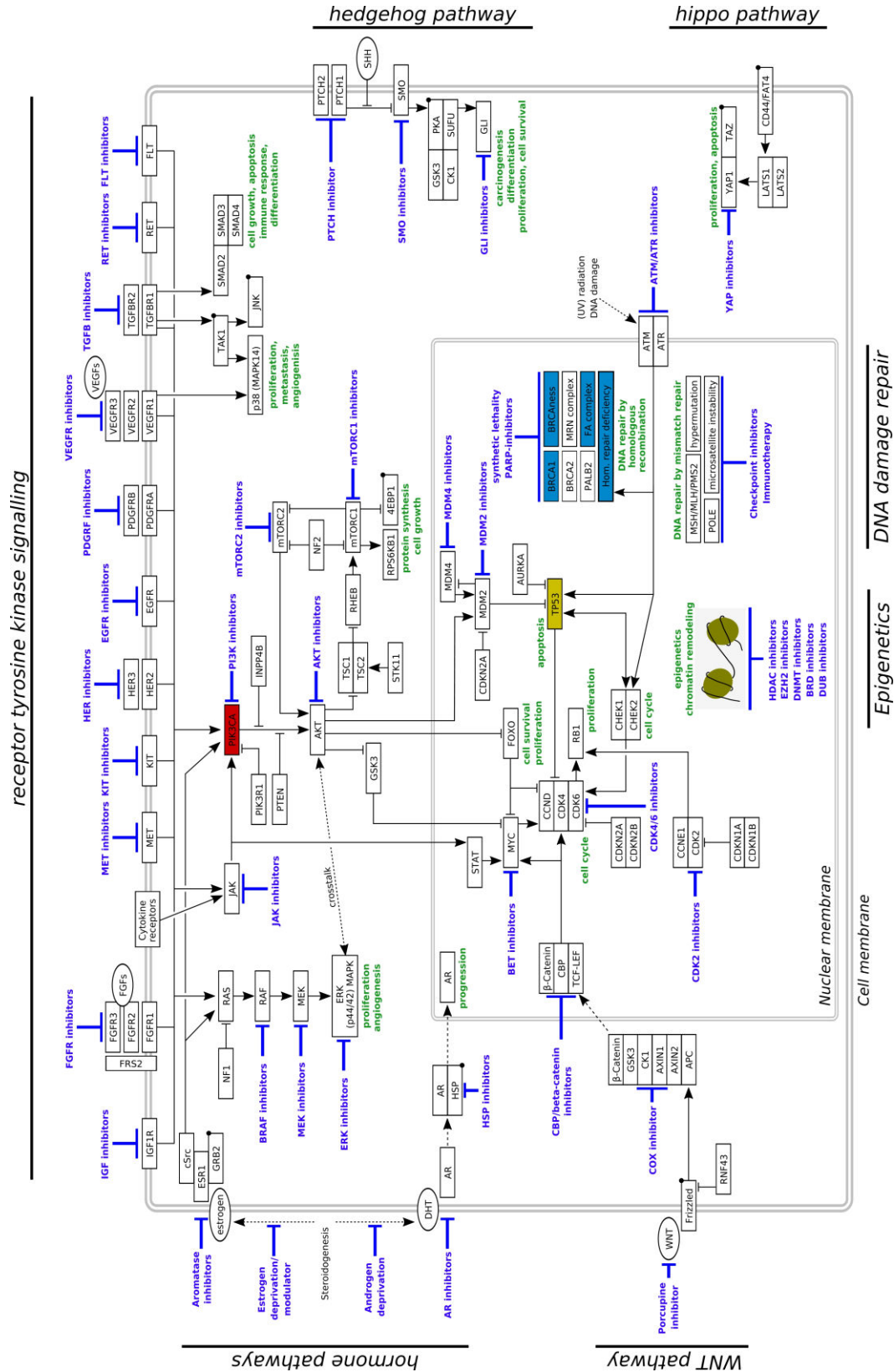


## Supplement - Copy Number Profile



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.





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 - Analysis of Tumor Gene Expression

The following table lists expression values of all therapy-relevant genes and their interpretation (if available). The distribution of the gene-specific FPKM values within all analyzed samples is depicted in the subsequent figures below.

Gene	Interpretation	FPKM	Median	Ratio	Percentile
<i>FOLR1</i> (coding for Folate receptor alpha)	overexpression	13	0.3 (0.28)	↑43x (↑46x)	96 (97)
<i>CD274</i> (coding for PD-L1)	reduced expression	0.73	1.2 (1.3)	↓1.6x (↓1.7x)	24 (27)
<i>ERBB2</i> (coding for HER2)	normal expression	31	15 (4.1)	-	78 (99)
<i>TACSTD2</i> (coding for TROP2)	normal expression	67	61 (0.41)	-	55 (87)

If two values are given, the first value refers to a tumor reference cohort consisting of analyzed samples whose tumor entity matches the current analysis. Values in brackets refer to a tumor reference cohort consisting of analyzed samples whose tumor entity deviates from the current analysis.

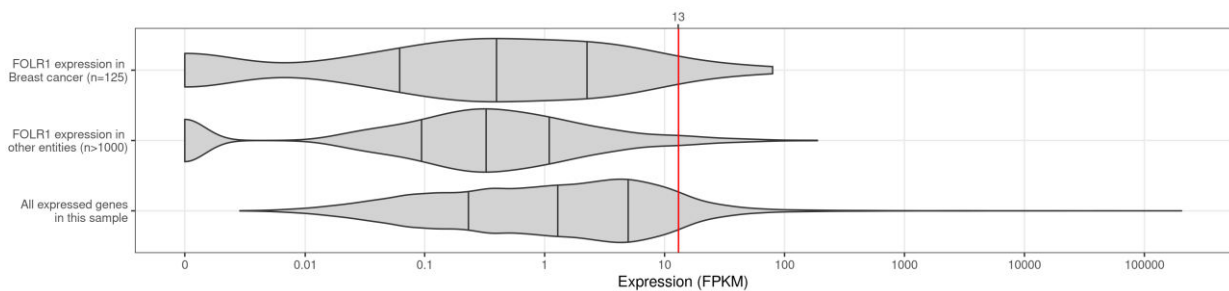
**FPKM:** The expression level of the given gene in the tumor sample under investigation.

**Median:** The median expression level (FPKM) of the given gene in the respective tumor reference cohort.

**Ratio:** The ratio of the expression level of the given gene in the examined tumor sample compared to the median expression in the respective tumor reference cohort. The direction of the expression difference relative to the analyzed tumor sample is indicated by arrows.

**Percentile:** The percentage of analyzed samples in the respective tumor reference cohort whose gene-specific FPKM value is lower than the one of the current analysis.

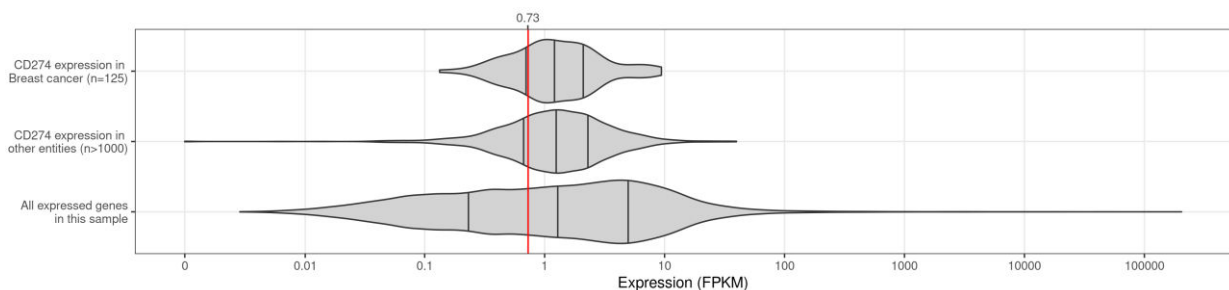
### *FOLR1* Expression



The X-axis shows the gene expression in FPKM in logarithmic scale. The red bar marks the level of gene expression of the examined gene of the analyzed sample. The subdivision of the violin plots shown by longitudinal bars represents the percentiles in the respective distribution (percentiles from left to right: 0-25, 25-50, 50-75, and 75-100).

Each figure shows from top to bottom: 1) The distribution of gene expression of the indicated gene in a cohort of tumor samples matching the patient's tumor entity. 2) The distribution of gene expression of the indicated gene in a cohort of tumor samples of other tumor entities. 3) The distribution of gene expression of all expressed genes in the analyzed tumor RNA of the patient.

### *CD274* Expression

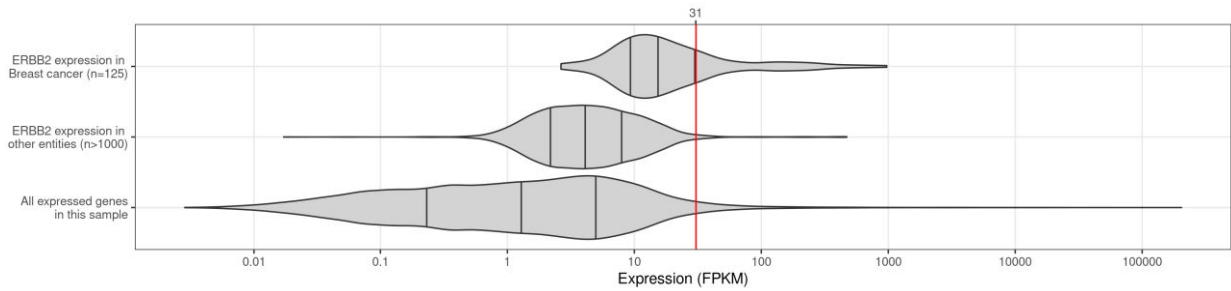


The X-axis shows the gene expression in FPKM in logarithmic scale. The red bar marks the level of gene expression of the examined gene of the analyzed sample. The subdivision of the violin plots shown by longitudinal bars represents the percentiles in the respective distribution (percentiles from left to right: 0-25, 25-50, 50-75, and 75-100).

Each figure shows from top to bottom: 1) The distribution of gene expression of the indicated gene in a cohort of tumor samples matching the patient's tumor entity. 2) The distribution of gene expression of the indicated gene in a cohort of tumor samples of other tumor entities. 3) The distribution of gene expression of all expressed genes in the analyzed tumor RNA of the patient.



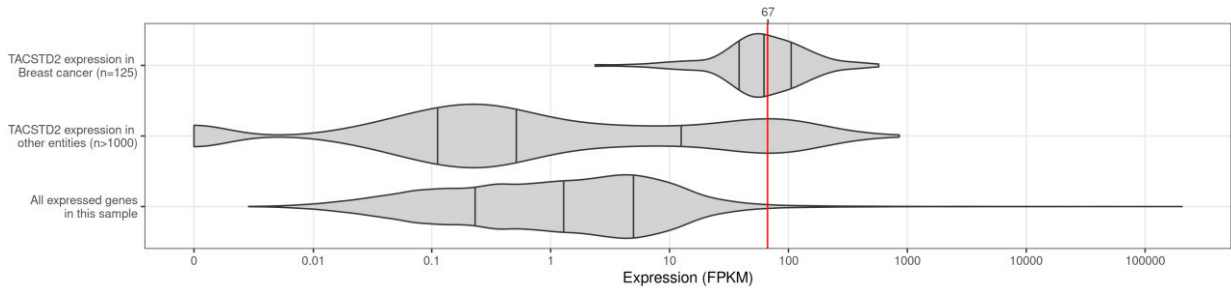
## ERBB2 Expression



The X-axis shows the gene expression in FPKM in logarithmic scale. The red bar marks the level of gene expression of the examined gene of the analyzed sample. The subdivision of the violin plots shown by longitudinal bars represents the percentiles in the respective distribution (percentiles from left to right: 0-25, 25-50, 50-75, and 75-100).

Each figure shows from top to bottom: 1) The distribution of gene expression of the indicated gene in a cohort of tumor samples matching the patient's tumor entity. 2) The distribution of gene expression of the indicated gene in a cohort of tumor samples of other tumor entities. 3) The distribution of gene expression of all expressed genes in the analyzed tumor RNA of the patient.

## TACSTD2 Expression



The X-axis shows the gene expression in FPKM in logarithmic scale. The red bar marks the level of gene expression of the examined gene of the analyzed sample. The subdivision of the violin plots shown by longitudinal bars represents the percentiles in the respective distribution (percentiles from left to right: 0-25, 25-50, 50-75, and 75-100).

Each figure shows from top to bottom: 1) The distribution of gene expression of the indicated gene in a cohort of tumor samples matching the patient's tumor entity. 2) The distribution of gene expression of the indicated gene in a cohort of tumor samples of other tumor entities. 3) The distribution of gene expression of all expressed genes in the analyzed tumor RNA of the patient.

**The genes shown represent a manual selection. Please note that the tumor content of the sample has a significant influence on the measured gene expression. In particular, a loss of expression (heterozygous, homozygous) in combination with a low tumor content may not be reliably detected. An altered gene expression does not necessarily lead to a (therapeutically) relevant, altered protein biosynthesis. We recommend using an independent method such as direct staining of the target structure at the protein level to support therapeutic decisions based on gene expression data. The RNA sequencing method used cannot detect post-translational protein modifications.**



## 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.

For a comprehensive assessment of the listed therapeutic options, please also consider your patient's previously reported findings and medical history (an overview can be found in section „Oncological Course“ on page 1). Furthermore, select biomarkers may require verification via a method approved by the FDA/EMA.

### Approved drugs for your patient's tumor entity

The following list includes drugs for your patient's tumor entity and age with **completely fulfilled** approval restrictions due to the detected alterations and your patient's age. The list also comprises drugs with approvals based on criteria independent of our methods.

Drug name	Affected genes	Approval	Fulfilled approval restrictions	Further approval restrictions to be checked	Approval in combination with other drugs
Alpelisib PI3K $\alpha$ inhibitor PI3K inhibitor	PIK3CA	EMA	adult, PIK3CA mutation	<b>HER2-negative (via IHC/ISH), HR-positive</b> locally advanced or metastatic, postmenopausal women, and men, after disease progression following endocrine therapy as monotherapy	Fulvestrant
		FDA	adult, PIK3CA-mutated	<b>HER2-negative (via IHC/ISH), HR-positive</b> advanced or metastatic, following progression on or after an endocrine-based regimen	Fulvestrant
Capivasertib AKT inhibitor	PIK3CA	EMA	adult, one or more PIK3CA/AKT1/PTEN-alterations	<b>HER2-negative (via IHC/ISH), ER-positive</b> locally advanced or metastatic, following recurrence or progression on or after an endocrine-based regimen	Fulvestrant
		FDA	adult, one or more PIK3CA/AKT1/PTEN-alterations	<b>HER2-negative (via IHC/ISH), HR-positive</b> locally advanced or metastatic, 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
Everolimus mTOR inhibitor	PIK3CA	EMA	adult, female	<b>HER2-negative (via IHC/ISH), HR positive</b> advanced, postmenopausal, no symptomatic visceral disease after recurrence or progression following a non-steroidal aromatase inhibitor	Exemestane
		FDA	adult, female	<b>HER2-negative (via IHC/ISH), HR positive</b> advanced, postmenopausal, after failure of treatment with letrozole or anastrozole	Exemestane
Inavolisib PI3K $\alpha$ inhibitor PI3K inhibitor	PIK3CA	EMA	adult, PIK3CA-mutated	<b>HER2-negative (via IHC/ISH), ER-positive</b> locally advanced or metastatic, recurrence on or within 12 months of completing adjuvant endocrine treatment; after CDK 4/6 inhibitor in the (neo)adjuvant setting: interval of at least 12 months between termination of CDK 4/6 inhibitor treatment and the detection of recurrence	Palbociclib, Fulvestrant
		FDA	adult, PIK3CA-mutated	<b>HER2-negative (via IHC/ISH), HR-positive</b> endocrine-resistance, locally advanced or metastatic, recurrence on or after completing adjuvant endocrine therapy	Palbociclib, Fulvestrant



The following list includes drugs for your patient's tumor entity with **unmet or only partially met** approval restrictions due the detected alterations and your patient's age.

Drug name	Affected genes/biomarker	Approval	Checked approval restrictions	Further approval restrictions to be checked	Approval in combination with other drugs
Olaparib PARP inhibitor	HRD, <i>BRCA1</i>	EMA	<b>Fulfilled:</b> adult <b>Not fulfilled:</b> germline <i>BRCA1/2</i> -mutation	<b>HER2-negative (via IHC/ISH)</b> - monotherapy or in combination with endocrine therapy, adjuvant, high risk early breast cancer, after neo- or adjuvant chemotherapy - locally advanced or metastatic, after prior anthracycline and taxane in the (neo)adjuvant or metastatic setting unless patient was not suitable for these treatments; HR-positive: progress on or after or ineligible for endocrine therapy	
		FDA	<b>Fulfilled:</b> adult <b>Not fulfilled:</b> deleterious or suspected deleterious germline <i>BRCA</i> mutation	<b>HER2-negative (via IHC/ISH)</b> - adjuvant treatment, high-risk early breast cancer, after neoadjuvant or adjuvant chemotherapy - metastatic, after chemotherapy in the neoadjuvant, adjuvant or metastatic setting; HR-positive cancer: after a prior endocrine therapy or ineligible for endocrine therapy	
Talazoparib PARP inhibitor	HRD, <i>BRCA1</i>	EMA	<b>Fulfilled:</b> adult <b>Not fulfilled:</b> germline <i>BRCA1/2</i> -mutation	<b>HER2-negative (via IHC/ISH)</b> metastatic or locally advanced, previously treated with an anthracycline and/or a taxane in the (neo)adjuvant, locally advanced or metastatic setting unless patients were not suitable for these treatments; HR positive should have been treated with a prior endocrine-based therapy, or be considered unsuitable for endocrine-based therapy	
		FDA	<b>Fulfilled:</b> adult <b>Not fulfilled:</b> deleterious or suspected deleterious germline <i>BRCA</i> -mutated	<b>HER2-negative (via IHC/ISH)</b> metastatic or locally advanced	



## Further approved drugs for other entities

The following list includes all drugs based on the detected alterations that are approved for other entities.

Drug name	Affected genes/biomarker	Tumor entity	Approval
<b>Niraparib</b> PARP inhibitor	<b>HRD, BRCA1</b>	Fallopian tube carcinoma	EMA, FDA
		Ovarian cancer	EMA, FDA
		Primary peritoneal carcinoma	EMA, FDA
		Prostate cancer	EMA, FDA
<b>Rucaparib</b> PARP inhibitor	<b>HRD, BRCA1</b>	Fallopian tube carcinoma	EMA, FDA
		Ovarian cancer	EMA, FDA
		Primary peritoneal carcinoma	EMA, FDA
		Prostate cancer	FDA
<b>ONC-201 (dordaviprone)</b> AKT inhibitor DRD2 antagonist ERK inhibitor	<b>PIK3CA</b>	Glioma	FDA
<b>Sirolimus</b> mTOR inhibitor	<b>PIK3CA</b>	Soft tissue neoplasm	FDA
<b>Temsirolimus</b> mTOR inhibitor	<b>PIK3CA</b>	B-cell lymphoma (BCL)	EMA
		Renal cell carcinoma	EMA, FDA
<b>Mirvetuximab soravtansine-gynx</b> FR $\alpha$ antibody-drug conjugate	<b>FOLR1</b>	Fallopian tube carcinoma	EMA, FDA
		Ovarian cancer	EMA, FDA
		Primary peritoneal carcinoma	EMA, FDA

