CeGaT

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Dr. John Doe
Paul-Ehrlich-Str. 23
72076 Tübingen
GERMANY

Name	Doe, Jane (*01.01.19xx)
Sex	Female
Patient-ID	xxxxx
Report date	01.07.2022

Report of somatic tumor variants – Doe, Jane (*01.01.19xx)

Indication **Breast cancer**

RESULTS OVERVIEW

Tumor tissue & tumor content	Tumor mutational burden (TMB)	Microsatellite instability (MSI)	Homologous recombination deficiency (HRD)	Fusions, structural variants
FFPE material Sample collection 03/2022 30% (histologically) 25% (bioinformatically)	7.6 Var/Mb	No evidence for MSI Score 0.14	Evidence for HRD Score 58.0	No evidence for structural variants
Required min 20%	High ≥ 10	Indication of MSI ≥ 0.33	Indication of HRD ≥ 30	

Tumor Drivers	Viral infection	Germline variants	Pharmacogenetics	CHIP
Identified tumor drivers: *PIK3CA* Relevant genes without oncogenic alterations: *BRCA1*, *ERBB2*	No evidence for an infection with HPV/EBV in the tumor sample	Detection of a pathogenic germline variant in gene *BRCA2*	No evidence of germline variants that are likely to affect drug tolerance	No evidence for CHIP

VARIANTS WITH POTENTIAL THERAPEUTIC RELEVANCE:

Gene	Functional category	Variant	NAF	Effect on protein function	Therapeutic option for discussion in the MTB	Approved by EMA/FDA	Approved for current entity
BRCA2 (germline)	frameshift	c.3847_3848delGT; p.Val1283Lysfs*2	het. loss of wt-allele in the tumor	inactivating	PARP inhibitor	EMA* & FDA*	EMA* & FDA*
HRD	N/A	N/A	N/A	N/A	PARP inhibitor	EMA* & FDA*	EMA* & FDA*
PIK3CA	missense	c.3140A>G; p.His1047Arg	0.15	activating	PI3K inhibitor	EMA & FDA	EMA* & FDA*
					mTOR inhibitor	EMA* & FDA*	EMA* & FDA*
					possible resistance to EGFR/HER inhibitor	N/A	N/A
					possible resistance to Endocrine therapy	N/A	N/A
					AKT inhibitor	no	no
FGFR1	amplification	complete gene, non focal (10 copies)	N/A	activating	FGFR inhibitor	EMA* & FDA	no
					possible resistance to Endocrine therapy	N/A	N/A

NAF: *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content and do not directly correlate with the variant's frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein levels in the following categories: inactivating/activating/function altered, likely inactivating/activating/function altered, unknown and benign (details in the methods section).
Approval: Only those organisations having approved the respective therapeutical option are listed here. An asterisk indicates approval restrictions (please refer to the appendix for details).

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

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.6).

Gene	Functional category	Variant	Transcript-ID	NAF
ARID5B	synonymous	c.537C>G; p.=	NM_032199.3	0.14
CSMD1	synonymous	c.6021G>A; p.=	NM_033225.6	0.09
DPYD	stop_gained	c.697C>T; p.Gln233*	NM_000110.4	0.15
PIK3CA	missense	c.3140A>G; p.His1047Arg	NM_006218.4	0,15

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

The medical report of relevant somatic variants, and their assessment with regard to functional relevance, is limited to the requested genes.

COPY NUMBER ALTERATIONS:

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

Chromosomal region	Functional category	Variant	Copy number	Affected genes with potential therapeutic relevance
chr8 37553498-41906820	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.Val1283Lysfs*2 in gene BRCA2 is a germline variant. Therefore, we strongly recommend genetic counseling.

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:

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup
Dr. med. Friedmar Kreuz, M.A.

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. RNA fusions panel analysis STR.

Sample material

Tumor tissue: mastectomy sample
Sample collection 03/2022
DNA and RNA isolation from tumor in FFPE (FFPE-ID: 22/xxxxx) after macrodissection with estimated tumor content of 30% (HE staining)
Bioinformatically estimated tumor content 25% (min 16%, max 28%)

Normal tissue: EDTA blood

Sample receipt

06.06.2022 (EDTA blood)
19.06.2022 (Tumor-DNA)
19.06.2022 (Tumor-RNA)

Requested Regions

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

AAK1, ABCB1, ABCG2, ABL1, ABL2, ABRAXAS1, ACD, ACVR1, ADGRA2, ADRB1, ADRB2, AIP, AIRE, AJUBA, AKT1, AKT2, AKT3, ALK, ALOX12B, AMER1, ANKRD26, APC, APLNR, APOBEC3A, APOBEC3B, AR, ARAF, ARHGAP35, ARID1A, ARID1B, ARID2, ARID5B, ASXL1, ASXL2, ATM, ATP1A1, ATR, ATRX, AURKA, AURKB, AURKC, AXIN1, AXIN2, AXL, B2M, BAP1, BARD1, BAX, BCHE, BCL10, BCL11A, BCL11B, BCL2, BCL3, BCL6, BCL9, BCL9L, BCOR, BCORL1, BCR, BIRC2, BIRC3, BIRC5, BLM, BMI1, BMPR1A, BRAF, BRCA1, BRCA2, BRD3, BRD4, BRD7, BRIP1, BTK, BUB1B, CALR, CAMK2G, CARD11, CASP8, CBFB, CBL, CBLB, CBLC, CCDC6, CCND1, CCND2, CCND3, CCNE1, CD274, CD79A, CD79B, CD82, CDC73, CDH1, CDH11, CDH2, CDH5, CDK1, CDK12, CDK4, CDK5, CDK6, CDK8, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CENPA, CEP57, CFTR, CHD1, CHD2, CHD4, CHEK1, CHEK2, CIC, CIITA, CKS1B, CNKSR1, COL1A1, COMT, COQ2, CREB1, CREBBP, CRKL, CRLF2, CRTC1, CRTC2, CSF1R, CSF3R, CSMD1, CSNK1A1, CTCF, CTLA4, CTNNA1, CTNNB1, CTRC, CUX1, CXCR4, CYLD, CYP1A2, CYP2A7, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4, CYP3A5, CYP4F2, DAXX, DCC, DDB2, DDR1, DDR2, DDX11, DDX3X, DDX41, DEK, DHFR, DICER1, DIS3L2, DNMT1, DNMT3A, DOT1L, DPYD, E2F3, EBP, EED, EFL1, EGFR, EGLN1, EGLN2, EIF1AX, ELAC2, ELF3, EME1, EML4, EMSY, EP300, EPAS1, EPCAM, EPHA2, EPHA3, EPHA4, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ERRFI1, ESR1, ESR2, ETNK1, ETS1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXO1, EXT1, EXT2, EZH1, EZH2, FAN1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FAT1, FBXO11, FBXW7, FEN1, FES, FGF10, FGF14, FGF19, FGF2, FGF23, FGF3, FGF4, FGF5, FGF6, FGF9, FGFBP1, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLI1, FLT1, FLT3, FLT4, FOXA1, FOXA2, FOXE1, FOXL2, FOXO1, FOXO3, FOXP1, FOXQ1, FRK, FRS2, FUBP1, FUS, FYN, G6PD, GALNT12, GATA1, GATA2, GATA3, GATA4, GATA6, GGT1, GLI1, GLI2, GLI3, GNA11, GNA13, GNAQ, GNAS, GNB3, GPC3, GPER1, GREM1, GRIN2A, GRM3, GSK3A, GSK3B, GSTP1, H3-3A, H3-3B, H3C2, HABP2, HCK, HDAC1, HDAC2, HDAC6, HGF, HIF1A, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, HMGA2, HMGCR, HMGN1, HNF1A, HNF1B, HOXB13, HRAS, HSD3B1, HSP90AA1, HSP90AB1, HTR2A, ID3, IDH1, IDH2, IDO1, IFNGR1, IFNGR2, IGF1R, IGF2, IGF2R, IKBKB, IKBKE, IKZF1, IKZF3, IL1B, IL1RN, ING4, INPP4A, INPP4B, INPPL1, INSR, IRF1, IRF2, IRS1, IRS2, IRS4, ITPA, JAK1, JAK2, JAK3, JUN, KAT6A, KDM5A, KDM5C, KDM6A, KDR, KEAP1, KIAA1549, KIF1B, KIT, KLF2, KLF4, KLHL6, KLLN, KMT2A, KMT2B, KMT2C, KMT2D, KNSTRN, KRAS, KSR1, LATS1, LATS2, LCK, LIG4, LIMK2, LRP1B, LRRK2, LTK, LYN, LZTR1, MAD2L2, MAF, MAGI1, MAGI2, MAML1, MAP2K1, MAP2K2, MAP2K3, MAP2K4, MAP2K5, MAP2K6, MAP2K7, MAP3K1, MAP3K13, MAP3K14, MAP3K3, MAP3K4, MAP3K6, MAP3K8, MAPK1, MAPK11, MAPK12, MAPK14, MAPK3, MAX, MBD1, MC1R, MCL1, MDC1, MDH2, MDM2, MDM4, MECOM, MED12, MEF2B, MEN1, MERTK, MET, MGA, MGMT, MITF, MLH1, MLH3, MLLT10, MLLT3, MN1, MPL, MRE11, MS4A1, MSH2, MSH3, MSH4, MSH5, MSH6, MSR1, MST1R, MT-RNR1, MTAP, MTHFR, MTOR, MTRR, MUC1, MUTYH, MXI1, MYB, MYC, MYCL, MYCN, MYD88, MYH11, MYH9, NAT2, NBN, NCOA1, NCOA3, NCOR1, NF1, NF2, NFE2L2, NFKB1, NFKB2, NFKBIA, NFKBIE, NIN, NKX2-1, NLRC5, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NPM1, NQO1, NR1I3, NRAS, NRG1, NRG2, NSD1, NSD2, NSD3, NT5C2, NT5E, NTHL1, NTRK1, NTRK2, NTRK3, NUMA1, NUP98, NUTM1, OPRM1, PAK1, PAK3, PAK4, PAK5, PALB2, PALLD, PARP1, PARP2, PARP4, PAX3, PAX5, PAX7, PBK, PBRM1, PBX1, PDCD1, PDCD1LG2, PDGFA, PDGFB, PDGFC, PDGFD, PDGFRA, PDGFRB, PDIA3, PDK1, PDPK1, PGR, PHF6, PHOX2B, PIGA, PIK3C2A, PIK3C2B, PIK3C2G, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIK3R3, PIM1, PKHD1, PLCG1, PLCG2, PLK1, PML, PMS1, PMS2, POLD1, POLE, POLH, POLQ, POT1, PPM1D, PPP2R1A, PPP2R2A, PRDM1, PREX2, PRKAR1A, PRKCA, PRKCI, PRKD1, PRKDC, PRKN, PRMT5, PRSS1, PSMB1, PSMB10, PSMB2, PSMB5, PSMB8, PSMB9, PSMC3IP, PSME1, PSME2, PSME3, PSPH, PTCH1, PTCH2, PTEN, PTGS2, PTK2, PTK6, PTK7, PTPN11, PTPN12, PTPRC, PTPRD, PTPRS, PTPRT, RABL3, RAC1, RAC2, RAD21, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD54B, RAD54L, RAF1, RALGDS, RARA, RASA1, RASAL1, RB1, RBM10, RECQL4, RET, RFC2, RFWD3, RFX5, RFXANK, RFXAP, RHBDF2, RHEB, RHOA, RICTOR, RINT1, RIPK1, RIT1, RNASEL, RNF43, ROS1, RPS20, RPS6KB1, RPS6KB2, RPTOR,

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RSF1, RUNX1, RYR1, SAMHD1, SAV1, SBDS, SCG5, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEC23B, SERPINB9, SETBP1, SETD2, SETDB1, SF3B1, SGK1, SH2B1, SH2B3, SHH, SIK2, SIN3A, SKP2, SLC19A1, SLC26A3, SLCO1B1, SLIT2, SLX4, SMAD3, SMAD4, SMARCA4, SMARCB1, SMARCD1, SMARCE1, SMC1A, SMC3, SMO, SOCS1, SOX11, SOX2, SOX9, SPEN, SPINK1, SPOP, SPRED1, SPTA1, SRC, SRD5A2, SRGAP1, SRSF2, SSTR1, SSTR2, SSX1, STAG1, STAG2, STAT1, STAT3, STAT5A, STAT5B, STK11, SUFU, SUZ12, SYK, TAF1, TAF15, TAP1, TAP2, TAPBP, TBK1, TBL1XR1, TBX3, TCF3, TCF4, TCF7L2, TCL1A, TEK, TENT5C, TERC, TERF2IP, TERT, TET1, TET2, TFE3, TGFB1, TGFBR2, TLR4, TLX1, TMEM127, TMPRSS2, TNFAIP3, TNFRSF11A, TNFRSF13B, TNFRSF14, TNFRSF8, TNFSF11, TNK2, TOP1, TOP2A, TP53, TP53BP1, TP63, TPMT, TPX2, TRAF2, TRAF3, TRAF5, TRAF6, TRAF7, TRRAP, TSC1, TSC2, TSHR, TTK, TUBB, TYMS, U2AF1, UBE2T, UBR5, UGT1A1, UGT2B15, UGT2B7, UIMC1, UNG, USP34, USP9X, VEGFA, VEGFB, VHL, VKORC1, WRN, WT1, XIAP, XPA, XPC, XPO1, XRCC1, XRCC2, XRCC3, XRCC5, XRCC6, YAP1, YES1, ZFHX3, ZNF217, ZNF703, ZNRF3, ZRSR2 (somatic tumor panel version 6)

Methods

DNA and RNA isolation: The isolation of tumor DNA and RNA was performed following macrodissection at CeGaT. 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.

Bioinformatic tumor content: Tumor content is inferred from sequencing data (cellularity value based on Favero et al., 2015 PMID: 25319062).

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 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 NovaSeq6000 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 NovaSeq6000 systems.

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.

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* and *MET* were extracted from STAR output.

Genetic data evaluation DNA: Only variants (SNVs/small indels) with a novel allele frequency (NAF) of ≥ 5% in the tumor sample within the coding regions and their adjacent intronic regions (-/+ 8 base pairs) were evaluated. A list of all the variants with an allele frequency of 5% considered in the genetic data evaluation can be requested at any time. The clinical interpretation of variants is based on different external and internal databases and on information from scientific literature. The sensitivity of the test is dependent on the tumor content of the analyzed material, the sample quality, and the sequencing depth. In this case, 98.46% of the targeted regions were covered by a minimum of 70 high-quality sequencing reads per base. The tumor content estimated by the pathologist was 50%. Therefore, somatic variants occur at a calculated NAF of around 25%. 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.

Genetic data evaluation RNA: The sensitivity of the 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 11.17 gigabases RNA was sequenced. Therefore, this analysis is appropriate to detect structural variants on RNA level.

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, IARC *TP53* database, 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.

Copy Number Analysis: (only applicable for nuclear encoded genes) 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. 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. 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 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 analysed 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*

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

ABL1, *AFAP1*, *AGK*, *AKAP12*, *AKAP4*, *AKAP9*, *AKT2*, *AKT3*, *ALK*, *ASPSCR1*, *BAG4*, *BCL2*, *BCORL1*, *BCR*, *BICC1*, *BRAF*, *BRD3*, *BRD4*, *CCAR2*, *CCDC6*, *CD74*, *CIC*, *CLTC*, *CNTRL*, *COL1A1*, *CRTC1*, *DDIT3*, *EGFR*, *EML4*, *ERBB2*, *ERBB4*, *ERG*, *ESR1*, *ETV1*, *ETV4*, *ETV5*, *ETV6*, *EWSR1*, *EZR*, *FGFR1*, *FGFR2*, *FGFR3*, *FLI1*, *FN1*, *FUS*, *GOPC*, *JAZF1*, *KIAA1549*, *KIF5B*, *MAGI3*, *MAML1*, *MET*, *MGA*, *MYB*, *MYC*, *NAB2*, *NCOA4*, *NFIB*, *NOTCH2*, *NPM1*, *NRG1*, *NSD3*, *NTRK1*, *NTRK2*, *NTRK3*, *NUTM1*, *PAX3*, *PAX7*, *PAX8*, *PDGFB*, *PDGFRB*, *PIK3CA*, *PLAG1*, *PML*, *POU5F1*, *PRKAR1A*, *QKI*, *RAF1*, *RARA*, *RET*, *ROS1*, *SDC4*, *SHTN1*, *SLC34A2*, *SND1*, *SQSTM1*, *SS18*, *SSX1*, *STAT6*, *STRN*, *SUZ12*, *TACC1*, *TACC3*, *TAF15*, *TFE3*, *TFG*, *THADA*, *TMPRSS2*, *TPM3*, *TPR*, *TRIM24*, *TRIM33*, *WT1*, *YAP1*, *ZMYM2*, *ZNF703* (Structural Variants Panel version 6)

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-NTKR2, 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

Specific transcript variants:

EGFR del ex2-3, *EGFR* del ex2-4, *EGFR* del ex2-14, *EGFR* del ex2-22 (mLEEK), *EGFR* del ex5-6, *EGFR* del ex6-7, *EGFR* del ex9, *EGFR* del ex9-10, *EGFR* del ex10, *EGFR* del ex12, *EGFR* del ex25-26, *EGFR* del ex25-27, *EGFR* del ex26-27, *EGFR* VII, *EGFR* VIII, *MET* ex14 skipping

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 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 sequences are captured with custom probes designed to bind to the genomic sequence of EBV (Epstein-Barr virus and HPV (human papilloma virus) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Sequencing reads not mapping to the human genome are mapped against these viral 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, https://www.acgs.uk.com/quality/best-practice-guidelines/).

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

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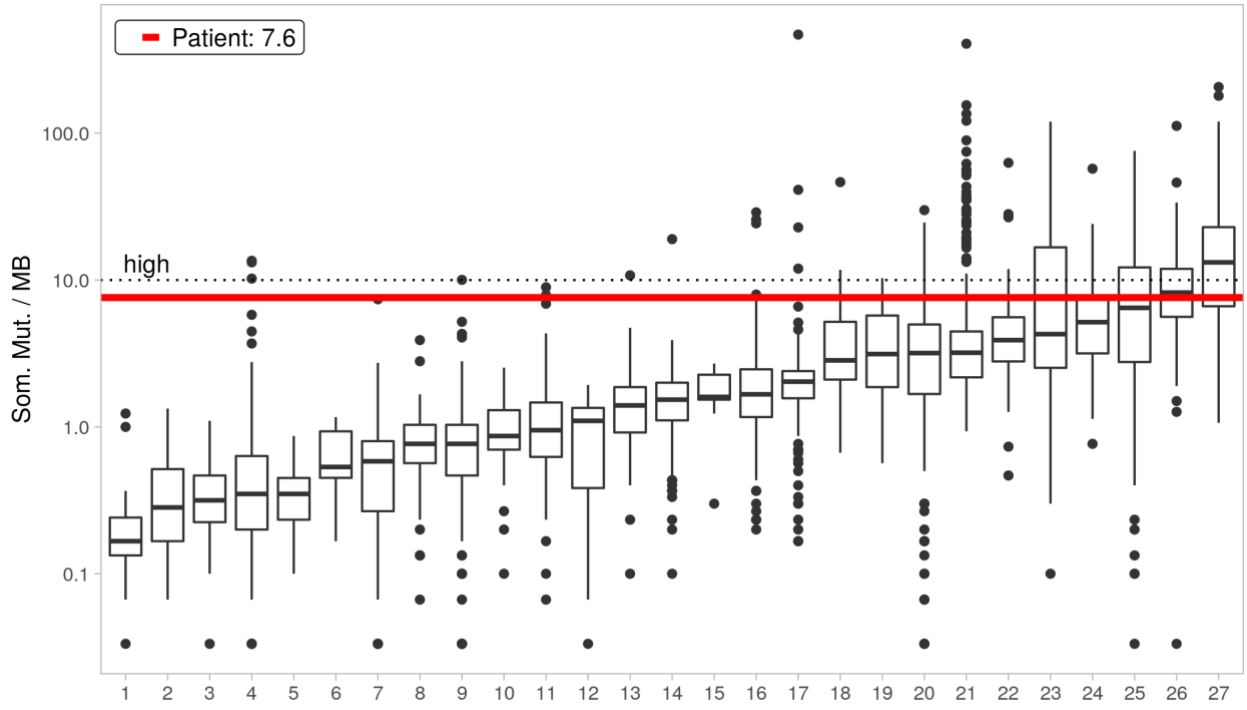
SUPPLEMENT - TUMOR MUTATIONAL BURDEN

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).

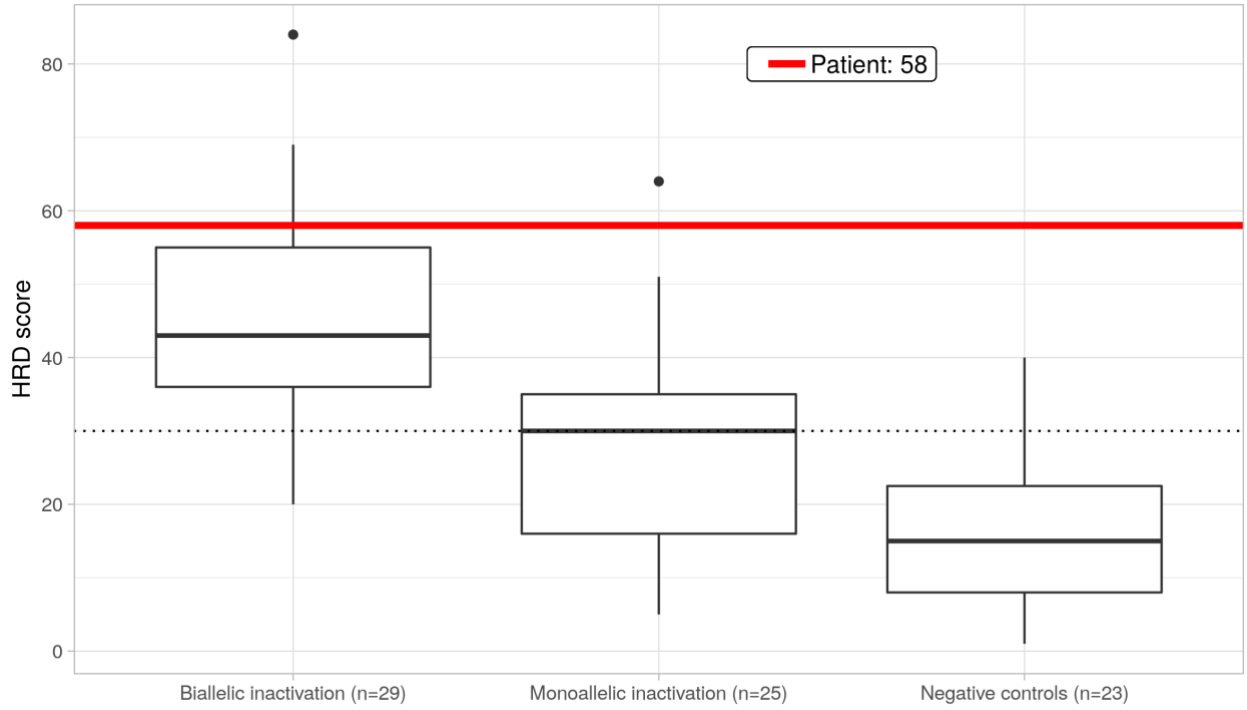


Distribution of tumor mutational burden in 27 tumor entities

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 7.6 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.

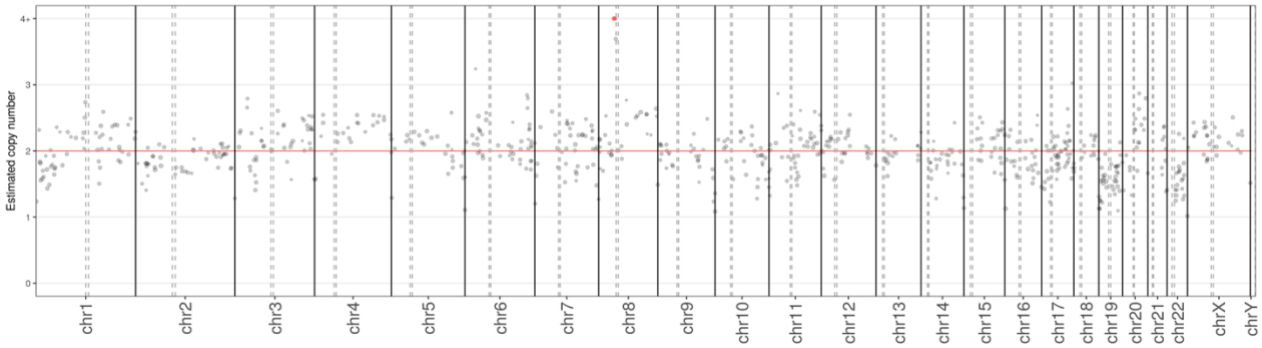
Entities are: (1) Rhabdoid tumor, (2) Ewing Sarcoma, (4) Acute myeloid leukemia, (5) Medulloblastoma, (6) Carcinoid, (7) Neuroblastoma, (8) Prostate cancer, (9) Chronic lymphocytic leukemia, (10) Low-grade glioma, **(11) Breast cancer**, (12) Pancreatic cancer, (13) Multiple myeloma, (14) Kidney clear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



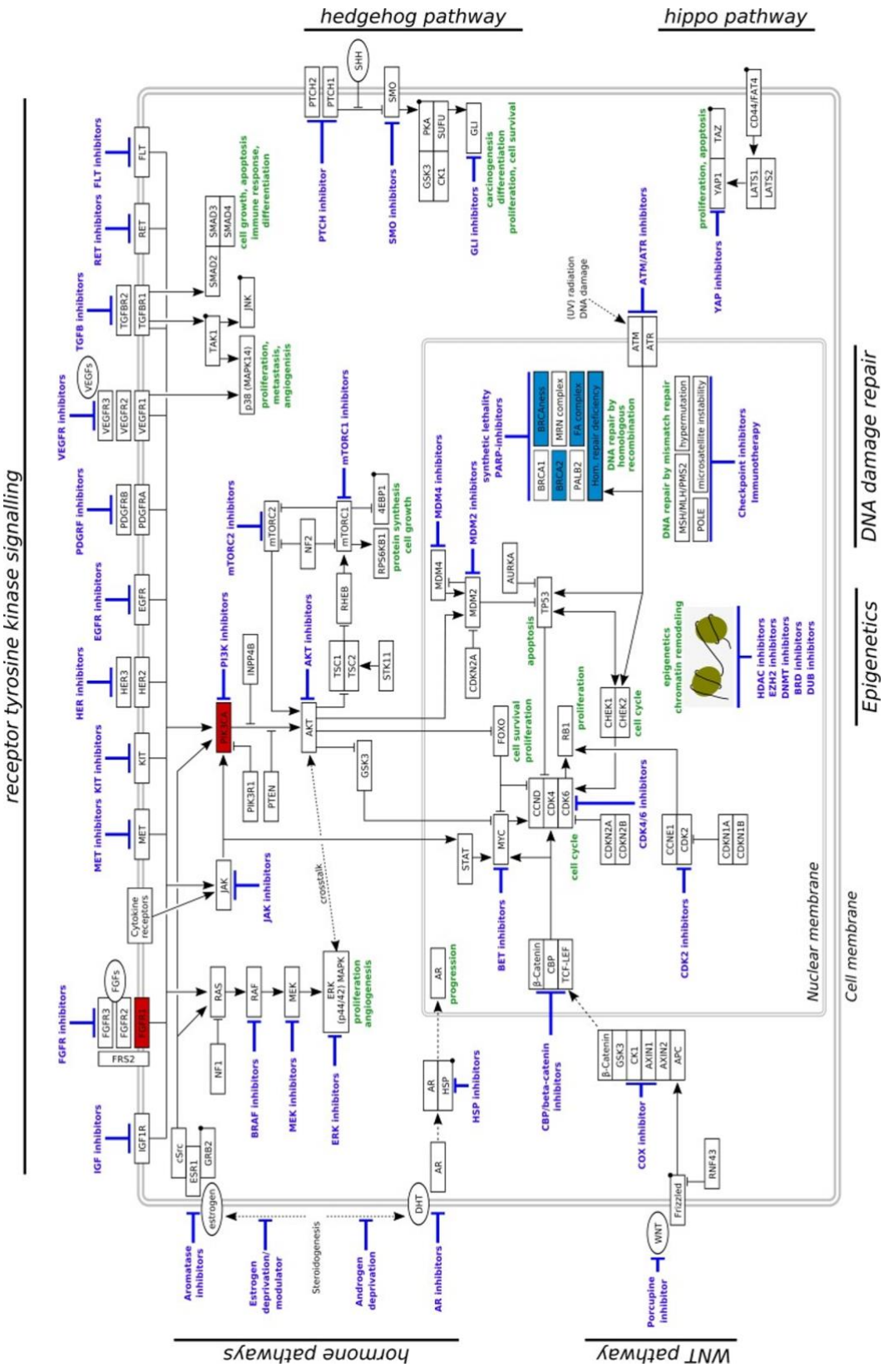
Homologous recombination deficiency (HRD) score of this sample compared to a cohort of patients with biallelic inactivation of HRD-related genes, monoallelic inactivation of HRD-related genes (or second hit not found in available data), and negative controls with no detectable inactivation of HRD-related 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. The continuous black line indicates a simplified CNV profile following normalization.

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.

POSSIBLE THERAPEUTIC STRATEGIES

Please note that the provided information on potential drugs is only a specific selection and makes no claim of completeness.

Relevant therapeutics due to the homologous recombination deficiency and *BRCA2* (germline), c.3847_3848delGT; p.Val1283Lysfs*2, NM_000059.4:

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Niraparib PARP inhibitor	fallopian tube carcinoma	FDA	adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy, cancer associated with homologous recombination deficiency (HRD)	
		EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy	
	ovarian cancer	FDA	adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy, cancer associated with homologous recombination deficiency (HRD)	
		EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy	
	primary peritoneal carcinoma	FDA	adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy, cancer associated with homologous recombination deficiency (HRD)	
		EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy	
Olaparib PARP inhibitor	fallopian tube carcinoma	FDA	adult patients, deleterious or suspected deleterious g or s BRCA-mutation, prior response to platinum-based chemotherapy	
		EMA	FDA (BRCA), EMA (BRCA)	
	prostate cancer	FDA	adult patients, mCRPC, deleterious or suspected deleterious g or s homologous recombination repair (HRR) gene-mutated, progress following prior treatment with enzalutamide or abiraterone.	
		EMA	HRD, BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L	
	ovarian cancer	FDA	s or g BRCAmut adult patients, prior response to platinum-based chemotherapy	
		EMA	FDA (BRCA), EMA (BRCA)	
		FDA	adult patients, advanced cancer, prior response to platinum-based chemotherapy, cancer associated with homologous recombination deficiency (HRD)	Bevacizumab
	primary peritoneal carcinoma	FDA	adult patients, deleterious or suspected deleterious g or s BRCA-mutation, prior response to platinum-based chemotherapy	
		EMA	FDA (BRCA), EMA (BRCA)	
	breast cancer	**FDA**	**adult patients, metastatic cancer, deleterious or suspected deleterious gBRCAm, HER2-negative, prior adjuvant or neo-adjuvant therapy**	
		EMA	**FDA (BRCA), EMA (BRCA)**	
	neoplasm of the pancreas	FDA	adult patients, deleterious or suspected deleterious gBRCA mutation	
		EMA	FDA (BRCA), EMA (BRCA)	
Rucaparib PARP inhibitor	fallopian tube carcinoma	FDA	adult patients, deleterious or suspected deleterious g or s BRCA-mutation, prior response to platinum-based chemotherapy, Select patients for therapy based on an FDA-approved companion diagnostic for Rubraca.	
		EMA	recurrent cancer, prior with at least one platinum-based chemotherapy, test for BRCA mutation	
	prostate cancer	FDA	adult patients, mCRPC, deleterious g or s BRCA mutation, prior AR-directed therapy and taxane-based chemotherapy	
	ovarian cancer	FDA	adult patients, deleterious or suspected deleterious g or s BRCA-mutation, prior response to platinum-based chemotherapy, Select patients for therapy based on an FDA-approved companion diagnostic for Rubraca.	
		EMA	recurrent cancer, prior with at least one platinum-based chemotherapy, test for BRCA mutation	
	primary peritoneal carcinoma	FDA	adult patients, deleterious or suspected deleterious g or s BRCA-mutation, prior response to platinum-based chemotherapy, Select patients for therapy based on an FDA-approved companion diagnostic for Rubraca.	
		EMA	recurrent cancer, prior with at least one platinum-based chemotherapy, test for BRCA mutation	

Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Talazoparib PARP inhibitor	**breast cancer**	**FDA**	**metastatic or locally advanced cancer, deleterious or suspected deleterious gBRCAm, HER2-negative**	
		EMA	**metastatic or locally advanced cancer, deleterious or suspected deleterious gBRCAm, HER2-negative, prior treatments**	

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
Alpelisib PI3K inhibitor	**breast cancer**	**FDA**	**postmenopausal women, and men, HR positive, HER2-negative, PIK3CA-mutated**	**Fulvestrant**
		EMA	**postmenopausal women, and men, HR-positive, HER2-negative, PIK3CA mutated**	**Fulvestrant**
Copanlisib PI3K inhibitor	non-Hodgkin lymphoma (NHL)	FDA	adult patients, relapsed FL	
Duvelisib PI3K inhibitor	leukemia	FDA	adult patients, third line therapy for CLL	
		EMA	third line treatment for CLL	
	non-Hodgkin lymphoma (NHL)	FDA	adult patients, third line therapy SLL	
		EMA	third line treatment for FL	
Idelalisib PI3K inhibitor	leukemia	FDA	relapsed CLL	
		EMA	17p deletion, or TP53 mutation, CLL, at least one prior treatment	Rituximab or Ofatumumab
	non-Hodgkin lymphoma (NHL)	FDA	relapsed FL or SLL	
		EMA	third line therapy for FL	
Umbralisib PI3K inhibitor	non-Hodgkin lymphoma (NHL)	FDA	relapsed or refractory MZL, at least one prion CD-20-based therapy relapsed or refractory FL, fourth line treatment	
Everolimus mTOR inhibitor	neuroendocrine neoplasm	FDA	well differentiated NET (GI or lung), unresectable, locally advanced or metastatic	
		EMA	adult patients, progressive disease, well or moderately differentiated NET (pancreatic, GI or lung)	
	breast cancer	**FDA**	**HR positive. HER2 negative, advanced breast cancer, post-menopausal women**	**Exemestane**
		EMA	**HR positive. HER2 negative, advanced breast cancer, post-menopausal women, prior treatment with non-steroidal aromatase inhibitor**	**Exemestane**
	glioma	FDA	TSC-associated subependymal giant cell astrocytoma (SEGA)	
	renal cell carcinoma	FDA	advanced RCC, second line treatment adult patients with renal angiomyolipomas or TSC	
		EMA	Advanced RCC, progress after VEGF-therapy	
Sirolimus mTOR inhibitor	soft tissue neoplasm	FDA	PEComa	
	neoplasm of head and neck	FDA	facial angiofibroma associated with tuberous sclerosis, adults or pediatric patients aged 6 and older	
Temsirolimus mTOR inhibitor	non-Hodgkin lymphoma (NHL)	EMA	adult patients, relapsed or refractory MCL	
	renal cell carcinoma	FDA	advanced RCC	
		EMA	adult patients, advanced RCC	

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 BCR-ABL inhibitor FGFR inhibitor PDGFR inhibitor VEGF/VEGFR inhibitor	lymphoid leukemia	FDA	T315I-positive Ph+ ALL	
		EMA	Ph+ ALL, resistant to dasatinib or T315I-positive Ph+ ALL	
	myeloid leukemia	FDA	T315I-positive CML and CP, AP or BP CML, resistant to prior treatment with kinase inhibitors	
		EMA	CML, resistant to dasatinib or nilotinib or T315I-positive CML	
Erdafitinib FGFR inhibitor	bladder carcinoma	FDA	FGFR2 or FGFR3 alteration, adult patients, locally advanced or metastatic bladder cancer, prior platinum- based chemotherapy	
Infigratinib FGFR inhibitor	cholangiocellular carcinoma	FDA	FGFR2 fusion or rearrangement, adult patients, unresectable locally advanced or metastatic CCC	
Lenvatinib FGFR inhibitor KIT inhibitor PDGFR inhibitor RET inhibitor VEGF/VEGFR inhibitor	thyroid carcinoma	FDA	progressive, differentiated thyroid cancer, radioactive iodine refractory	
		EMA	adult patients, progressed or metastatic differentiated thyroid cancer, radioactive iodine refractory	
	renal cell carcinoma	FDA	advanced RCC, following VEGF-therapy	Everolimus
		EMA	adult patients, advanced RCC, a) first line, b) following VEGF-therapy	a) Pembrolizumab b) Everolimus
	endometrial carcinoma	FDA	advanced endometrial carcinoma, <u>not</u> MSI-H or dMMR, prior systemic therapy	Pembrolizumab
	hepatocellular carcinoma	FDA	unresectable HCC, first line therapy	
		EMA	adult patients, advanced or unresectable HCC, first line therapy	
Pazopanib FGFR inhibitor KIT inhibitor PDGFR inhibitor VEGF/VEGFR inhibitor	sarcoma	FDA	advanced STS, prior chemotherapy	
		EMA	adult patients, advanced or metastatic STS, prior chemotherapy	
	renal cell carcinoma	FDA	advanced RCC	
		EMA	adult patients, advanced RCC, first-line therapy	
Nintedanib FGFR inhibitor PDGFR inhibitor VEGF/VEGFR inhibitor	non-small cell lung carcinoma	EMA	Adult patients, locally advanced, metastatic or locally recurrent NSCLC (adenocarcinoma), second-line therapy	Docetaxel
Pemigatinib FGFR inhibitor selective FGFR-Inhibitor	cholangiocellular carcinoma	FDA	FGFR2 fusion or rearrangement, advanced or metastatic CCC, previous treatment	
		EMA	FGFR2 fusion or rearrangement, adult patients, locally advanced or metastatic CCC, previous treatment	