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OncoRx - for Precision Oncology

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Title: OncoRx - for Precision Oncology

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Objective: Technical Specifications of OncoRx.

Intended Use:

Tumor is a hub for genetic mutations and hence though the cancers known to date are the same, every tumor is genetically diverse. We decipher the FDA approved molecular biomarkers of the tumors to personalize cancer treatment and increase the survival times and QoL of cancer patients through targeted therapies. One such step forward is OncoRx for precision oncology services which identifies molecular targets and recommends tailored therapies for patients with cancer.

OncoRx services are for prescription use only. The gene targets tested play an important role in the pathogenesis of the disease and aid in patient selection. This test is intended for use as a directional test for drug decision making of approved therapeutic product labeling and helps in choosing the right drug to prevent treatment failure and side effects. It also assists in predicting outcomes of a drug - response and resistance, immunotherapy selection and management.

Test description:

This Next-Generation Sequencing (NGS) based invitro diagnostic genomic test targets DNA variants from FFPE tumor tissue sample, will decipher the genomic information of 353 cancer genes. (comprehensive footprint of many genes including the regulatory regions, SNPs of complete coding regions, CNVs, Selective rearrangements such as Translocations, genomic signatures with microsatellite instability (MSI) and tumor mutational burden (TMB) etc.). This cancer panel at spans 174 high confidence onco genes related to response, resistance to therapies & 179 candidate cancer biomarkers genes with proven and emerging clinical utilities (for drugs in clinical trials).

Test principle:

The panel works on a principle of probe hybridization using probes of around 120mer length providing high and specific interactions with target DNA molecules along with - dual molecular barcodes with dual sample indexing for library preparation. The usage of these dual molecular barcodes reduces or eliminates the index hopping and false positives thus making it more reliable.

Precision:

High performance standards, regulatory compliance, and quality control are ensured with automated protocols and pipelines. The test results are represented in two parts: companion diagnostic biomarker results with associated therapy indication and other biomarkers with full proof scientific evidences / biomarker results. Accuracy in targeted drug response prediction is an exclusive feature of *OncoRx for Precision Oncology*.

Methodology and Limitations:

A targeted Hybrid capture-based technology was performed to enable profiling cancer specific loci using The Next Generation Sequence (NGS) analyzer – Illumina's NovaSeq 6000. This NGS Analyzer delivers a high yield of error-free reads, and a high percentage of base calls above Q30. DNA is extracted from a fresh biopsy or Formalin-Fixed Paraffin-Embedded (FFPE) tissue which is positive for tumor. It is quantified, fragmented and library is constructed using Agilent Sureselect XT HS2 Library Preparation kit. Gene targets (regions covering 353 cancer specific genes) are captured using Sureselect XT HS2 target Enrichment kit, the library pool is quality checked with tape station and sequenced at depth of 2000x-3000x.

The raw data is checked for quality by fastQC and aligned to human reference genome GRCh38 considering the targeted regions from the panel. Various well validated tools such as FASTQC, Trimmomatic & Cutadapt, BOWTIE2, SAM Tools (with various utilities), IGV, PICARDS along with a few In-house programs are used to process, visualize and analyze the raw data. GATK is employed for structural variant identification and validation. CNV KIT and GeneFusion tools were utilized for identification of amplifications and rearrangements.

GenepoweRx uses standardardized and well validated pipelines with annotations from FDA approved database. GenepoweRx collaborated with Memorial Sloan Kettering Cancer Center (MSK), the world's oldest and largest private cancer centre to utilize MSK's clinical genomics and research insights. OncoKB is a precision cancer database powered by the clinical expertise of Memorial Sloan Kettering Cancer Center. The licensed version of the OncoKB database is adopted for FDA-approved therapeutic guidance and decision support. The test results are then carefully reviewed and manually curated by our team of highly trained and experienced individuals. MSKs clinical information along with GenepoweRx proprietary database information is utilized to provide accurate recommendations for Indian cancer populace.

Who needs to be tested/ Patient group:

- ✓ OncoRx is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies mentioned below (in accordance with the approved therapeutic product labeling).
- ✓ Oncologists can also identify ideal candidates who could benefit from other established targeted therapies of other cancers, immunotherapies and personalized pharmacogenomics.
- ✓ For patients with malignant solid tumors of the Breast / Ovary / NSCLC / Colorectal cancer / Prostate /Melanoma / Cholangiocarcinoma / Esophagogastric / Head and Neck cancer/ Cervical/ Hepatobiliary / Pancreatic / Gliomas / Cancers of Unknown Primary Origin.

Genomic region Coverage: The NGS test is performed with >95% coverage at a depth of 2000x to 3000x

Cancer Type, Gene markers and therapeutic responses:

BREAST CANCER

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
ERBB2 (HER2)	Amplification	Copy count-xx	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumabemtansine), or Perjeta® (pertuzumab)
PIK3CA >40 pathogenic genomic alterations	Substitutions	Mutation positive / Mutation negative	Piqray® (alpelisib) Alpelisib + Fulvestrant

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Biomarkers from other scientific evidences of clinical significance

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
HRR mutations (Germline/somatic) – BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L alterations	Substitutions	HRR mutations- High, Intermediate , Low	1. Sensitive to PARP inhibitor therapy. 2. In Triple Negative Breast Cancer - predictive of complete pathological response in these tumors
Mutation analysis and ESR1 fusions . Ligand binding domain (Exon 6-10) - E380Q, Y537C/D/N/S, L536H, D538G Mutations.	Substitutions & Rearrangement	ESR1 mutation positive tumor/ ESR1 mutation deficit tumor	Fulvestrant, newer generation SERDs, or combinations with biologic agents such as CDK4/6 inhibitors
FGFR1 Amplification or TP53 mutation profile	Copy Number & Substitutions	FGFR1 Amplification - Positive / Negative	Risk of early relapse, aggressive phenotype and worse Progression Free Survival for both fulvestrant alone and fulvestrant plus palbociclib treatments (from PALOMA-3 trial). (Ben O'Leary et.al. 2021).
PIK3CA - mutation hot spots in the C2, helical, and kinase domains of PI3K. (corresponding to exons 7, 9, and 20, respectively)	Substitutions	PIK3CA mutations- High, Intermediate , Low	Prolonged progression-free survival with alpelisib–fulvestrant among patients with PIK3CA-mutated, HR-positive, HER2-negative advanced breast cancer who had received endocrine therapy previously (SOLAR-1 ClinicalTrials.gov number, NCT02437318), (Criscitiello, C et.al. 2021)
PIK3CA - mutation hot spots in the C2, helical, and kinase domains of PI3K (corresponding to exons 7, 9, and 20, respectively)	Substitutions	Mutation positive /Mutation negative	Sensitivity to Everolimus

Breast cancer risk assessment profile for patient relatives

High Risk genes	Moderate Risk genes	Low Risk genes
BRCA1 - *CN ,FFP	ATM - *CE,CN	AKT - *CE,CN
BRCA2 - *CN ,FFP	BRIP1 - *CE,CN	RAD50 -* CE
CDH1 - *CE,CN	CHEK2 - *CE	NF1 - *CE,CN
MUTYH - *CE	PALB2 - *CE,CN	PIK3CA - *CE
PMS2 - *CE	BARD1 - *CE,CN	PMS1 -* CE,CN
PTEN - *CN, FFP	FANCC - *CE,CN	
STK11 - *CE,CN	NBN - *CE	
TP53 - *CN, FFP	RAD51C - *CE,CN	
	RAD51D - *CE,CN	

22 Gene markers from various study cohorts (PMID: 29988077).

*variant types CN - copy number, CE - coding exons, FFP - full footprint.

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OVARIAN CANCER

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
BRCA1/2 alterations	Substitutions & CNVs	Mutations – Consequence / Position / Other details	Lynparza® (olaparib) or Rubraca® (rucaparib) Niraparib, Olaparib + Bevacizumab

Biomarkers from other scientific evidences of clinical significance

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
HRR mutations (Germline/somatic) – BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L alterations	Substitutions	HRR mutations-High/Intermediate / Low	1. Sensitive to PARP inhibitor therapy.
ERCC2: Potent pathogenic genomic variants	Substitutions and InDels	Genotype status	Drug response and Toxicity to Platinum compounds (cisplatin ;oxaliplatin ;platinum)

NON-SMALL CELL LUNG CANCER (NSCLC)

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
EGFR exon 19 deletions and EGFR exon 21 L858R alterations (L861Q, G719X, or S768I)	InDels & Substitution	InDel status	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
EGFR exon 20 T790M alterations	Substitution	Mutation positive / Negative	Tagrisso® (osimertinib)
ALK rearrangements	Rearrangements	Partner gene and its associated mutation status.	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
BRAF V600E	Substitution	Mutation positive / Negative	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
BRAF rearrangements	Rearrangements	Partner gene and its associated mutation status.	Dabrafenib + Trametinib
MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping	Substitution & InDels	MET mutation positive / Negative MET exon 14 status-	Tabrecta® (capmatinib) Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
BRAF V600K	Substitutions	Mutation positive / Negative	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
ROS1	Rearrangements	Partner gene and its associated mutation status.	Crizotinib, Entrectinib

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MELANOMA

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
BRAF V600E	Substitution	Mutation positive / Negative	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
BRAF V600E and V600K	Substitution	Mutation positive / Negative	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
BRAF	Rearrangements, Oncogenic mutations	Fusion Positive/ Fusion Negative	Vemurafenib + Atezolizumab + Cobimetinib, Dabrafenib, Vemurafenib, Dabrafenib + Trametinib, Encorafenib + Binimetinib, Trametinib, Vemurafenib + Cobimetinib
PDGFB COL1A1-PDGFB Fusion			Imatinib

COLORECTAL CANCER

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
KRAS wild-type (absence of mutations in codons 12 and 13)	Rearrangement	Fusion Positive/ Fusion Negative	Erbitux® (cetuximab)
KRAS wild-type (absence of mutations in exons 2, 3, and 4)	-	Mutation positive / Negative	Vectibix® (panitumumab) Cetuximab, Cetuximab + Chemotherapy, Panitumumab, Panitumumab + Chemotherapy
BRAF	Rearrangement	Fusion Positive/ Fusion Negative	Encorafenib + Cetuximab
NRAS (absence of mutations in exons 2, 3, and 4)	Oncogenic mutations	Mutation positive / Negative	Panitumumab, Panitumumab + Chemotherapy

Biomarkers from other scientific evidences of clinical significance

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
TMB ≥ 10 mutations per megabase Or MSI-H	Substitutions	TMB-Ultrahigh/ High / Intermediate/ Low MSS/ MSI-H/ MSI-Low/ MS-Instable	Keytruda® (pembrolizumab)
MMR genes (MLH1, MSH2, MSH6 and PMS2).	Substitutions	deficient (dMMR) or MSI-H	Improved prognosis, identifying patients unlikely to benefit from adjuvant chemotherapy.
ERCC2 (rs13181)	Substitution	ERCC2 genotype GG/GT/TT	fluorouracil, leucovorin, oxaliplatin efficacy and Toxicity

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PROSTATE CANCER

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
Homologous Recombination Repair (HRR) gene (BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L) alterations	Substitutions, truncating mutations	HRR alterations	Lynparza® (olaparib), Rucaparib.

BRAIN TUMORS

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
KIT	Oncogenic Mutations	Mutation positive / Negative	Avapritinib
NF1	Oncogenic Mutations	Mutation positive / Negative	Selumetinib

THYROID TUMORS

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
BRAF	Rearrangements	Fusion Positive/ Fusion Negative	Dabrafenib + Trametinib
RET	Rearrangements	Fusion Positive/ Fusion Negative	Pralsetinib, Selpercatinib

BLADDER TUMORS

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
FGFR2, FGFR3	Rearrangements	Fusion Positive/ Fusion Negative	Erdafitinib

SOLID TUMORS

From companion diagnostic biomarker findings

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
TMB ≥ 10 mutations per megabase Or MSI-H	Substitutions	TMB-Ultrahigh/ High / Intermediate/ Low MSS/ MSI-H/ MSI-Low/ MS-Instable	Keytruda® (pembrolizumab)
NTRK1/2/3 fusions	Rearrangements	Fusion Positive / Negative	Vitrakvi® (larotrectinib), TRK inhibitors.

Biomarkers from other scientific evidences of clinical significance

Biomarkers	Variant type	Test Result	Associated Targeted Therapy
TMB ≥ 10 mutations per mega base	Substitutions	TMB-Ultrahigh/ High / Intermediate/ Low	favorable response to PD1/PD-L1 or CTLA-4 blockade

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Selected Cancer Panel genes:

AKT1	CTNNB1	KIT	POLE
AKT2	DAXX	KLF4	POLQ
AKT3	DICER1	KMT2A	PPP2R1A
ALK	DNMT3A	KRAS	PTCH1
AMER1	EGFR	MAP2K1	PTEN
APC	EP300	MAP2K2	PTPN11
APLNR	EPHA3	MAP2K4	RAC1
AR	ERBB2	MAP3K1	RAD21
ARAF	ERBB3	MAPK1	RAD50
ARID1A	ERBB4	MAX	RAF1
ARID1B	ERG	MCL1	RB1
ARID2	ESR1	MDM2	RET
ASXL1	ETV6	MED12	RHOA
ATM	EZH2	MEN1	RNF43
ATR	FAS	MET	ROS1
ATRX	FBXW7	MLH1	RPL5
AURKA	FGF19	MSH2	RUNX1
AXL	FGFR1	MSH6	SETBP1
B2M	FGFR2	MTOR	SETD2
BAP1	FGFR3	MUTYH	SF3B1
BCL2	FGFR4	MYB	SMAD4
BLM	GATA3	MYC	SMARCA4
BRAF	GNA11	MYCN	SMARCB1
BRCA1	GNAQ	NBN	SMO
BRCA2	GNAS	NF1	SOCS1
CBL	H3F3A	NF2	SPOP
CCND1	H3F3B	NFE2L2	SRC
CCND2	HGF	NOTCH1	STAG1
CCND3	HIST1H3B	NOTCH2	STAG2
CCNE1	HIST1H3C	NOTCH3	STAT3
CD274	HIST2H3C	NOTCH4	STAT5B
CDH8	HLA-A	NPM1	STK11
CDK12	HLA-B	NRAS	SYK
CDK2	HLA-C	NTRK1	TERT
CDK4	HNF1A	PALB2	TGFBR2
CDK6	HRAS	PBRM1	TP53
CDKN1A	IDH1	PDCD1LG2	TSC1
CDKN1B	IDH2	PDGFRA	TSC2
CDKN2A	IGF1R	PDGFRB	U2AF1
CDKN2B	JAK1	PHF6	VHL
CHEK2	JAK2	PIK3CA	WT1
CIITA	JAK3	PIK3CB	YAP1
CREBBP	JUN	PIK3R1	
CTCF	KDR	PMS2	

ABL1	EIF4A2	MDM4	RFXAP
ABL2	ELF3	MECOM	RHEB
ABR	ELOC	MGA	RICTOR
ABRAXAS1	EPHA2	MGMT	RIT1
ACVR1B	ERCC2	MRE11	RPL22
ACVR2A	ERCC3	MSH3	SERPINB3
AJUBA	ERCC4	MYCL	SERPINB4
AKAP9	ERCC5	MYH9	SLC34A2
ALOX12B	ETV1	NAB2	SMAD2
ALOX15B	ETV4	NCOA2	SMAD3
ARHGAP35	ETV5	NCOR1	SMC1A
ARID5B	FANCA	NLRC5	SMC3
ASXL2	FANCC	NRG1	SMC1
AURKB	FANCD2	NSD1	SOS1
AURKC	FANCE	NSD3	SOX17
AXIN1	FANCF	NTRK2	SOX9
AXIN2	FANCG	NTRK3	SPEN
BARD1	FANCL	PARP1	SRSF2
BCOR	FANCM	PAX5	STAT1
BIRC3	FAT1	PCBP1	TAF1
BRIP1	FLT3	PIAS3	TAF3
CARD11	FOXA1	PIAS4	TAP1
CASP8	FOXA2	PIK3CD	TAP2
CBFB	FOXL2	PIK3R2	TAPBP
CD74	FOXO1	PIM1	TBL1XR1
CDC73	FOXP1	PLCG1	TBX3
CDH1	FUBP1	PMS1	TCF12
CDK8	GATA6	POLQ	TCF7L2
CDKN1C	GNA13	PPM1D	TET2
CDKN2C	GPS2	PPP2R2A	TGFBRN
CHD4	HIF1A	PPP4R2	TMPRSS2
CHD8	HIST1H1C	PPP6C	TP53BP1
CHEK1	IDO1	PRKAR1A	TP73
CIC	IDO2	PSIP1	TRAF7
CKS1B	IFNGR1	PTK2	UVRAG
CTLA4	IFNGR2	PTPRD	WRN
CUL3	IL6ST	QSER1	XBP1
CUX1	IRF1	RAD51B	XPO1
CYLD	KDM5C	RAD51C	ZFHX3
DDR2	KDM6A	RAD51D	ZFP36L1
DDX3X	KEAP1	RAD52	ZMYM2
DDX5	KMT2B	RAD54L	ZMYM3
DEFB134	KMT2C	RASA1	ZNF703
DHX9	KMT2D	RBM10	ZNF750
DNMT3A	LZTR1	RFX5	

Targeted variant types: • coding exons ♦ copy number ★ rearrangement ■ regulatory region ✚ full footprint

Sensitivity and specificity:

- GenepoweRx pipeline for the targeted panel has been validated for repeatability and reproducibility of alterations with 50 datasets covering various cancers. The raw files for the validation and their related information have been considered from the European Nucleotide Archive (ENA).
- Repeatability and reproducibility in terms of variant calling has been tested with in-house pipeline and validated commercial pipeline.
- Across all samples, the pre-sequencing process failure rate is found to be ≤ 2%

Sample and Sequence data guidelines:

DNA and RNA variants are screened from FFPE tumor tissue sample. Recommended read length of 2 x 150 bps are considered for targeted panel sequencing and paired end reads are considered for analysis.

TMB Status:

TMB status is highly accurate considering large targeted panel size of 3.96 MB and high confidence cancer genes covered at GenepoweRx Precision Oncology panel. Synonymous and non-synonymous variants with allele frequency ≥5%, are considered for TMB analysis. TMB result interpretation is as follows.

- TMB Ultrahigh - (≥ 100 mutations per megabase)
- TMB High - (≥ 10 mutations per MB)
- TMB Intermediate (5-10 mutations per MB)
- TMB Low (<5 mutations per MB)

MSI assessment:

Microsatellite refers to a short DNA segment, usually from 1 to 6 or more base pairs. These are generally stable. However, microsatellite instability (MSI) can be caused by errors that are uncorrected when DNA is copied into a cell. Typically, these errors are corrected by the DNA mismatch repair (MMR) system, but when deficient, microsatellite replication errors accumulate in the genome and can result in tumorogenesis. Though MSI is found most often in colorectal cancer, gastric cancer, and endometrial cancer, it has lately been shown to prevail in most kinds of cancer with varying frequencies. In 2017 with FDA's approval of KEYTRUDA® (pembrolizumab), MSI marked a paradigm shift in biomarker-guided therapy research. MSI is known to have a positive correlation with survival outcome and predicting response to immune checkpoint blockade with pembrolizumab (NCT01876511) .

MSI analysis:

To determine the microsatellite instability of the tumor, we analyze the genotypes using 13 MSI markers, 8 of which are recommended by the National Cancer Institute, USA (also called as Bethesda panel), a gold standard for MSI detection and 5 additional monomorphic markers of high sensitivity in multiple cancer types. This is performed by a fluorescent multiplex PCR fragment analysis assay through a standard capillary electrophoresis workflow. Overall call of stable and unstable markers will be reported.

MSI markers:

MSI Marker	Gene	Chromosome Location	Dye
BAT-25	C-KIT	4q12	6-FAM™
BAT-26	hMSH2	2p21	SID™
BAT-40	HSD3B2	1p12	VIC™
CAT-25	CASP2	7q34	VIC™
NR-21	SLC7A8	14q11.2	6-FAM™
NR-22	transmembrane protein precursor B5	11q24.2	NED™
NR-24	Zinc finger 2	2q11.1	6-FAM™
NR-27	MIHC: Inhibitor of apoptosis protein-1	11q22.2	NED™
ABI-16	Monomorphic markers of high sensitivity, proprietary to Applied Biosystems(AB)	17p13.2	SID™
ABI-17	Monomorphic markers of high sensitivity, proprietary to AB	17p12	SID™
ABI-19	Monomorphic markers of high sensitivity, proprietary to AB	1q42.3	NED™
ABI-20A	Monomorphic markers of high sensitivity, proprietary to AB	12q24.1	SID™
ABI-20B	Monomorphic markers of high sensitivity, proprietary to AB	1q21.3	NED™

Microsatellite (MS) Instability Interpretation	
MS-Stable	MS stable at all sites
MS-Low	The instability of one site (Minimum Unstable Rate ~5%)
MS-High	The instability of two or more sites (Minimum Unstable Rate~30%)
MS-Instable	The instability of all sites.

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Sample requirements:

FFPE tumor tissue sample or Fresh/frozen tumor tissue in normal saline are considered for OncoRx Analysis.

Limitations and Disclaimer:

- The genomic variations reported may include somatic and germline variations; but does not distinguish between the two alterations.
- The NGS test is performed with >95% coverage. For any minor alleles or rare alleles, a negative result does not rule out the presence of a mutation due to rare cases of tumor cell purity, intra tumor heterogeneity.
- For in vitro diagnostic use and for prescription use only. This test must be ordered by a qualified medical professional in accordance with required medical regulations in oncology for patients with solid malignant neoplasms.
- Patient care treatment decisions must be based on the self-determining medical judgment of the respective physician. Do consider complete information of the patient such as patient preferences, medical history and family history, physical examination profiles, other lab results in accordance with the standard of care medical practice.
- In case of ERBB2/ HER2 amplification with copy number 4 (baseline ploidy of tumor +2) and FGFR1 in breast cancer FFPE sample, dual analyte reflex testing with FISH or RNA profiling is recommended for confirmation of amplification.
- HER2 overexpression needs to be studied in samples identified with HER2 Copy count 4 for validation and reporting.
- Clinical performance of Tagrisso® (osimertinib) in patients with an EGFR exon 20 T790M mutation detected with an allele fraction <5% is ongoing and hence is not an established companion diagnostics biomarker.
- Genetic variations at allele frequencies below the established limit of detection may not be detected consistently.
- Tumor cell purity, intra-tumor heterogeneity might at times lead to fewer mutations.
- For confirmed germline predisposition of Homologous Recombination Repair (HRR) mutations if any, it is generally recommended to get a re-test with blood sample.
- TMB can vary by ≤2 mutations/ Mb as the following factors can affect the TMB score – Site of biopsy (primary or metastatic lesion), Specimen tumor content, variant filtering of alterations, tumor heterogeneity, and read depth.

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Contraindication

There are no known contraindications.

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