INTENDED USE

OmniSeq INSIGHT is a next generation sequencing-based in vitro diagnostic device for the detection of genomic variants, HLA Class I genotypes, signatures, and immune gene expression in formalin-fixed paraffin-embedded (FFPE) tumor tissue. DNA is sequenced to detect small variants in the full exonic coding region of 523 genes (single and multinucleotide substitutions, insertions, deletions and indels), including genes leading to homologous recombination repair defects (HRR/HRD), copy number alterations in 59 genes (gains and losses), as well as analysis of microsatellite instability (MSI) and tumor mutational burden (TMB) genomic signatures. RNA is sequenced to detect fusions and splice variants in 55 genes, in addition to mRNA expression in 64 immune genes. The resultant information, along with PD-L1 protein expression by immunohistochemistry (IHC), is intended for use by qualified health care professionals in accordance with professional guidelines in oncology for management of patients with solid neoplasms, and is not conclusive or prescriptive for labeled use of any specific therapeutic product. *(See last page of report for a complete list of markers included in OmniSeq INSIGHT.)*

TEST PRINCIPLE

OmniSeq INSIGHT is performed as a laboratory service using DNA and RNA co-extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples. The assay employs a single nucleic acid extraction method from routine FFPE biopsy or surgical resection specimens; 40 - 100 ng of DNA and 30 - 100 ng RNA undergo library construction and hybridization-based capture of all coding exons from 523 cancer-related genes and select regions from 55 commonly rearranged genes. Using the Illumina® NovaSeq 6000 platform, hybrid capture–selected libraries are sequenced to high uniform depth (targeting >150X median coverage with >90% of exons at coverage >50X), and the sequence data is analyzed to detect genomic variants and signatures. Amplicon-based targeted next generation RNA-sequencing for digital gene expression is used to assess mRNA expression in 64 immune genes, and immunohistochemistry (IHC) is used to measure PD-L1 protein expression (22C3 antibody) based on the tumor type tested.

Small Variants

DNA-sequencing of the full exonic coding region for 523 genes is performed to detect single nucleotide variants (SNV), multinucleotide variants (MNV), insertions, deletions and indels. Detected small variants are not reportable if present in the gnomAD database at a prevalence of 1% or greater, are benign or likely benign in the ClinVar database, synonymous, or intronic (outside of splice sites). Select variants with strong clinical significance are considered detected at a minimum of 2% variant allele frequency (VAF). All other variants are considered detected at a minimum of 5% VAF. Clinically significant variants detected below 2% VAF are considered “Indeterminate” when testing for the variant position was performed but did not meet minimum coverage criteria for reporting the variant as a pertinent negative finding, or, when evidence of a sequence mutation is observed in an area of low coverage, but results do not meet acceptance criteria for reporting as a positive finding.

Copy Number Alterations

DNA-sequencing is performed to detect and report gene copy number alterations (CNA), including gain (amplification) in 59 genes, and loss (deletion) in 4 genes. For accurate detection and reporting of copy gain, specimens must have at least 30% tumor purity. A fold change (FC) ≥3.2 is considered a copy “gain” and a FC=2.2-<3.2 as copy “gain–indeterminate”. A 2.2x FC is equivalent to 10 copies in a tumor at 30% tumor purity. Copy gain is fully validated for *AR, CCND1, CCNE1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, MDM2, MYC, MYCN, PDGFRA,* and *PIK3CA* genes. For accurate detection and reporting of copy loss, specimens must have at least 50% tumor purity. A FC ≤0.5 is considered as copy “loss” and a FC >0.5–0.7 as copy “loss-indeterminate”. A 0.5x FC is equivalent to 0 copies (somatic homozygous deletion) in a tumor at 50% tumor purity. Copy loss is fully validated and reported for *ATM, BRCA1, BRCA2* and *PTEN* genes.

Fusions and Splice Variants

RNA-sequencing of 55 commonly rearranged genes is performed for fusion analysis and 2 genes for splice variants. Fusion calling uses unique gene fusion reads to score variants, with a minimum number of unique candidate reads required for detection. Only fusions for *ALK, FGFR2, FGFR3, NRG1, NTRK1, NTRK3, RET,* and *ROS1* are fully validated. Fusion donor and acceptor genes are annotated as GeneA-GeneB fusion for reporting. Splice variant calling is performed for *EGFR* and *MET* using an algorithm to identify reads in these genes that span candidate splice junctions. Only splice variants that do not match a database of non-tumor junctions from normal FFPE samples and that align with MET exon 14 and EGFR exons 2-7 are reported as skipping mutations.

Tumor Mutational Burden (TMB)

Tumor mutational burden (TMB) is determined using the small variant DNA-sequencing output from 523 genes, excluding HLA, and dynamically adjusted per sample based on sequencing depth. Non-germline synonymous and nonsynonymous variants >5% VAF are included in the TMB score after application of filters. The TMB is calculated as follows: TMB = (Eligible Variants / Effective panel size). The resulting TMB result is reported as mutations per megabase units (mut/Mb) and interpreted as “High” (≥10 mut/Mb) or “Not High” (<10 mut/Mb). This cutoff was determined in non-small cell lung cancer (NSCLC) patients. Tumor-specific cutoffs have not been established in other tumor types.

Microsatellite Instability (MSI)

Microsatellite instability (MSI) status is determined by analyzing microsatellite sites for evidence of instability relative to a baseline derived from an independent cohort of normal samples from the manufacturer. There are 130 potential sites assessed for MSI, however, the total number of assessed sites varies between samples and depends on the number of sites that are assessable. To be assessable, a site must have a minimum of 60 reads spanning the at least 40 sites. The proportion of unstable MSI sites to total evaluable MSI sites is reported as a sample-level microsatellite score. The score is then evaluated against a pre-defined threshold to determine whether the sample is reported as MSI-High (>20% MSI unstable sites) or MS-Stable (≤20% MSI unstable sites).

PD-L1 Immunohistochemistry (IHC)

PD-L1 by IHC is measured based on the tumor type tested. The Dako PD-L1 IHC 22C3 FDA approved assay follows scoring guidelines for reporting either tumor proportion score (TPS) for non-small cell lung cancer or combined positive score (CPS) for other indicated tumor types with interpretation. The Dako PD-L1 IHC 22C3 assay is also used in non-indicated tumor types or tumors of unknown origin, however no interpretation is provided. Scoring information can be found at the link https://www.fda.gov/media/119249/download

Immune Gene Expression

Amplicon-based targeted next generation sequencing (NGS) for digital gene expression detection (RNA-Seq) is used to interrogate 50 T-cell receptor signaling (TCRS) genes including PD-L1, and 8 tumor infiltrating lymphocytes (TILs) genes including CD8, that have functions across the cycle of immunity, and 6 cancer testis antigen (CT antigens) genes frequently expressed in various types of cancer making them promising candidate targets for cancer immunotherapy, including cancer vaccination and adoptive T-cell transfer with chimeric T-cell receptors. Interpretation of TCRS and TILs gene expression by RNA-Seq: each gene is

compared to a reference population derived from 735 unique tumors, normalized to a value between 1 and 100, and scored as the percentile relative rank (% Rank). TCRS and TILS gene expression ranks ≥75 are considered "highly expressed" and may have immunotherapy targets in clinical trials. CT antigen genes are interpreted as "Positive" for markers with normalized reads per million (nRPM) ≥20, and "Negative" for markers with nRPM <20.

Human Leukocyte Antigen (HLA)

DNA-sequencing is performed on the tumor to identify HLA Class I alleles at HLA-A, HLA-B, and HLA-C genes. The HLA genotype is determined by aligning the sequenced nucleic acids to an HLA-specific reference genome and is reported as HLA-A*, HLA-B* and HLA-C* to the two-field, four-digit level using standard HLA nomenclature.

MARKER CLINICAL SIGNIFICANCE

The criteria used to classify the clinical significance of reported genomic variants relative to the tested tumor type is reported in accordance with recommendations described in *Li MM, et al., Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagnostics. 2017;19(1):4-23.* OmniSeq INSIGHT does not report marker-based evidence from non-human studies.

Tier I: Variants with strong clinical significance

- Level A: FDA-approved or professional guideline-indicated therapies in the tumor type tested
- Level B: Well-powered clinical studies with consensus from experts in the field including trials and meta-analyses for therapies in the tumor type tested.

Tier II: Variants with potential clinical significance

- Level C: FDA-approved therapies in other tumor types, investigational therapies, or multiple small studies with some consensus.
- Level D: Plausible therapeutic significance based on retrospective clinical studies or multiple case reports without consensus.

Tier III: Variants of unknown clinical significance (VUS)

Variants not observed at a significant allele frequency in general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases. No convincing published evidence of cancer association.

Potential Germline Variants

OmniSeq INSIGHT genomic profiling evaluates 34 genes (*APC, ATM, BAP1, BMPR1A, BRCA1, BRCA2, CDH1, CDK4, CDKN2A, CHEK2, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NF1, NF2, PMS2, PTCH1, PTEN, RB1, RET, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL,* and *WT1*) considered to be associated with causing or increasing susceptibility to hereditary diseases and syndromes, including those designated by the American College of Medical Genetics and Genomics (ACMG). OmniSeq INSIGHT results do not distinguish between somatic and germline variants as only tumor tissue is tested. Genetic counseling may be appropriate if an inherited syndrome associated with a reported possible germline variant is suspected.

MATCHING & PRIORITIZATION OF THERAPY CONSIDERATIONS

Genomic variants and immune markers from OmniSeq INSIGHT are matched to therapies based on the tested patient's tumor type, FDA regulatory approval status, National Comprehensive Cancer Center (NCCN) professional guideline indications, published emerging efficacy data to support unmet clinical need, including FDA breakthrough and fast track designations (see https://www.fda.gov/patients/learn-about-drug-and-device-approvals/fast-track-breakthrough-therapy-accelerated-approval-priority-review), potential expanded access /compassionate use (https://www.fda.gov/news-events/public-health-focus/expanded-access), and other peer-reviewed human clinical studies as described in the OmniSeq Knowledgebase® on the report date. Therapy Considerations are prioritized as follows: Markers associated with clinical benefit or resistance/decreased response in the patient's tumor type, ordered by approval status and variant clinical significance (if applicable); markers associated with clinical benefit in other tumor types (ordered alphabetically by marker and ranked by variant clinical significance, if applicable); and markers associated with clinical trials (ordered by proximity to the patient and later trial phase). Genomic variants with potential clinical significance but no therapy considerations identified on the report date, are also provided.

PERFORMANCE CHARACTERISTICS

Performance characteristics were established using DNA and RNA derived from a wide range of FFPE tissue specimens harboring variants with both strong and potential clinical significance, including resections, needle core biopsies and cell blocks from fine needle aspirations. For genomic profiling, each performance study included representative variant types from each alteration class (substitutions, insertions, insertions and deletions, copy number alterations, and fusions/splice variants), in various genomic contexts across a broad selection of genes, in addition to analysis of TMB and MSI genomic signatures. The detection of genomic variants by OmniSeq INSIGHT was compared to results of other validated next generation sequencing assays to assess concordance with orthogonal methods. For immune gene expression, sequencing analytical validation studies were performed to confirm standard measurements including accuracy, sensitivity and specificity. Additional studies addressed variability in nucleic acid input amounts, genomic DNA contamination of RNA, batch size and linearity of detection across all genes within a wide distribution of signal on the overall immune response signature.

Table 1. OmniSeq INSIGHT Performance Characteristics

NGS	Passing Criteria	Genes/ Loci	Marker	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
DNA-Seq	Tier I hotspots: ≥ 2% VAF Non-hotspots: ≥ 5% VAF	523	Substitutions	99%	>99%
			Insertions	96%	>99%
			Deletions	99%	>99%
	≥ 2.2x fold change; 30% tumor purity	59	Copy gain	99%	99%
	≤ 0.7x fold change; 50% tumor purity	4	Copy loss	77%	97%
	≥ 20% tumor purity	521	TMB ≥ 10 mut/Mb	85%	88%
		130	MSI	88%	>99%
		3	HLA	100%	>99%
RNA-Seq		55	Fusions	93%	>99%
		2	Splice variants	89%	>99%
	≥ 20 reads	64	Immune gene expression	Not applicable	

LIMITATIONS OF PROCEDURE

1. OmniSeq INSIGHT is not conclusive or prescriptive for labeled use of any specific therapeutic product.
2. OmniSeq INSIGHT has been validated using genomic DNA and RNA from formalin fixed paraffin-embedded tumor samples.
3. OmniSeq INSIGHT is designed to report somatic variants and is not intended to report germline variants. To remove likely germline mutations, the test filters variants occurring at ≥1% in the gnomAD database (https://gnomad.broadinstitute.org/) and/or considered

"benign or "likely benign" in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

4. Clinical validity performance of this test for predicting treatment effect has not been established.
5. The assay has been validated using samples with a minimum of 20% tumor purity in the tissue area to be extracted.
6. For the detection of copy number alterations (CNA), tumor purity above 30% yields consistent detection of fold change (FC) ≥2.2 for gain, and tumor purity above 50% yields consistent detection of fold change (FC) ≤0.7 for loss.
7. Concordance with other validated methods for the detection of copy number alterations (CNA), fusions and splice variants has been demonstrated for copy gain genes *AR, CCND1, CCNE1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, MDM2, MYC, MYCN, PDGFRA,* and *PIK3CA*, copy loss genes *ATM, BRCA1, BRCA2,* and *PTEN*, fusion genes *ALK, FGFR2, FGFR3, NRG1, NTRK1, NTRK3, RET,* and *ROS1*, and splice variant genes *EGFR* and *MET*. If clinically indicated, copy alterations and fusions/splice variants identified in other genes tested by OmniSeq INSIGHT should be confirmed by additional testing.
8. The MSI-High/MS-Stable designation by the OmniSeq INSIGHT test is based on genome-wide analysis of 130 potential microsatellite loci. The threshold for MSI-High/MS-Stable was determined by analytical concordance to a validated comparator NGS assay using colorectal, uterine and other cancer FFPE tissues. Samples with >20% MSI unstable sites are consider MSI-High, while samples with ≤20% unstable sites are considered MS-Stable. The clinical validity of the qualitative MSI designation has not been established.
9. TMB is reported as mutations per megabase (mut/Mb). TMB may differ across specimens (e.g., primary versus metastatic, tumor content) and targeted panels. The TMB calculation will increase or decrease depending on:
   - Size and region used to calculate TMB
   - Percentage of tumor in the sample
   - Germline variant filtering method
   - Read depth and other bioinformatic test specifications

   The clinical significance of TMB measurement has not been fully established.
10. Performance of OmniSeq INSIGHT has not been established for the detection of insertions and deletions larger than 25 base pairs.
11. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
12. The variant allele frequency (VAF) associated with each alteration is for information use only and should not be used to make any quantitative clinical assessment.
13. The assay does not genotype HLA class II molecules, HLA class I genes HLA-E, HLA-F, or HLA-G, nor provide HLA class I resolution greater than four-digits, copy number, somatic, or loss of heterozygosity (LOH) calls.
14. OmniSeq INSIGHT is not validated for use in samples with extensive necrosis nor regular decalcification

DISCLAIMER

The selection of any, all or none of the matched therapies reported by OmniSeq INSIGHT resides solely with the treating physician. Associated therapies may or may not be suitable for administration to a specific patient. OmniSeq, Inc., does not promise or guarantee that a specific drug may be effective in the treatment of the tested patient's disease, nor that a drug with potential lack of benefit may not provide clinical benefit to the tested patient. Decisions about patient care and treatment must be based on the independent medical judgment of the treating physician, accounting for all information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the community standard of care. A treating physician's decisions should not be solely based on the OmniSeq INSIGHT test, or the information contained in this report.

OmniSeq INSIGHT was developed, and its performance characteristics determined by the OmniSeq, Inc. in Buffalo, NY. OmniSeq® is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) and by the New York State Clinical Laboratory Evaluation Program as qualified to perform high complexity clinical laboratory testing, including all components of OmniSeq INSIGHT. OmniSeq's CLIA certification number is located at the bottom of each report, and all registered marks are the property of OmniSeq, Inc. The genomic and immune NGS components of OmniSeq INSIGHT are laboratory developed tests that have not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that clearance or approval is not currently necessary for certain laboratory developed tests. The FDA has approved the PD-L1 IHC components of OmniSeq INSIGHT for in vitro diagnostic use. OmniSeq INSIGHT is for clinical purposes and should not be regarded as investigational or for research.

APPENDIX All Markers Assayed by OmniSeq INSIGHT

DNA-Sequencing of 523 genes (full coding exonic regions) for the detection of substitutions, indels, MSI and TMB

ABL1	BMPR1A	CSF1R	ERCC5	FLI1	HIST1H3I	KDR	MRE11A	PAX3	PTCH1	SDHD	TCF7L2
ABL2	BRAF	CSF3R	ERG	FLT1	HIST1H3J	KEAP1	MSH2	PAX5	PTEN	SETBP1	TERC
ACVR1	BRCA1	CSNK1A1	ERRFI1	FLT3	HIST2H3A	KEL	MSH3	PAX7	PTPN11	SETD2	TERT
ACVR1B	BRCA2	CTCF	ESR1	FLT4	HIST2H3C	KIF5B	MSH6	PAX8	PTPRD	SF3B1	TET1
AKT1	BRD4	CTLA4	ETS1	FOXA1	HIST2H3D	KIT	MST1	PBRM1	PTPRS	SH2B3	TET2
AKT2	BRIP1	CTNNA1	ETV1	FOXL2	HIST3H3	KLF4	MST1R	PDCD1	PTPRT	SH2D1A	TFE3
AKT3	BTG1	CTNNB1	ETV4	FOXO1	HLA-A	KLHL6	MTOR	PDCD1LG2	QKI	SHQ1	TFRC
ALK	BTK	CUL3	ETV5	FOXP1	HLA-B	KMT2A	MUTYH	PDGFRA	RAB35	SLIT2	TGFBR1
ALOX12B	C11orf30	CUX1	ETV6	FRS2	HLA-C	KMT2B	MYB	PDGFRB	RAC1	SLX4	TGFBR2
ANKRD11	CALR	CXCR4	EWSR1	FUBP1	HNF1A	KMT2C	MYC	PDK1	RAD21	SMAD2	TMEM127
ANKRD26	CARD11	CYLD	EZH2	FYN	HNRNPK	KMT2D	MYCL1	PDPK1	RAD50	SMAD3	TMPRSS2
APC	CASP8	DAXX	FAM123B	GABRA6	HOXB13	KRAS	MYCN	PGR	RAD51	SMAD4	TNFAIP3
AR	CBFB	DCUN1D1	FAM175A	GATA1	HRAS	LAMP1	MYD88	PHF6	RAD51B	SMARCA4	TNFRSF14
ARAF	CBL	DDR2	FAM46C	GATA2	HSD3B1	LATS1	MYOD1	PHOX2B	RAD51C	SMARCB1	TOP1
ARFRP1	CCND1	DDX41	FANCA	GATA3	HSP90AA1	LATS2	NAB2	PIK3C2B	RAD51D	SMARCD1	TOP2A
ARID1A	CCND2	DHX15	FANCC	GATA4	ICOSLG	LMO1	NBN	PIK3C2G	RAD52	SMC1A	TP53
ARID1B	CCND3	DICER1	FANCD2	GATA6	ID3	LRP1B	NCOA3	PIK3C3	RAD54L	SMC3	TP63
ARID2	CCNE1	DIS3	FANCE	GEN1	IDH1	LYN	NCOR1	PIK3CA	RAF1	SMO	TRAF2
ARID5B	CD274	DNAJB1	FANCF	GID4	IDH2	LZTR1	NEGR1	PIK3CB	RANBP2	SNCAIP	TRAF7
ASXL1	CD276	DNMT1	FANCG	GLI1	IFNGR1	MAGI2	NF1	PIK3CD	RARA	SOCS1	TSC1
ASXL2	CD74	DNMT3A	FANCI	GNA11	IGF1	MALT1	NF2	PIK3CG	RASA1	SOX10	TSC2
ATM	CD79A	DNMT3B	FANCL	GNA13	IGF1R	MAP2K1	NFE2L2	PIK3R1	RB1	SOX17	TSHR
ATR	CD79B	DOT1L	FAS	GNAQ	IGF2	MAP2K2	NFKBIA	PIK3R2	RBM10	SOX2	U2AF1
ATRX	CDC73	E2F3	FAT1	GNAS	IKBKE	MAP2K4	NKX2-1	PIK3R3	RECQL4	SOX9	VEGFA
AURKA	CDH1	EED	FBXW7	GPR124	IKZF1	MAP3K1	NKX3-1	PIM1	REL	SPEN	VHL
AURKB	CDK12	EGFL7	FGF1	GPS2	IL10	MAP3K13	NOTCH1	PLCG2	RET	SPOP	VTCN1
AXIN1	CDK4	EGFR	FGF10	GREM1	IL7R	MAP3K14	NOTCH2	PLK2	RFWD2	SPTA1	WISP3
AXIN2	CDK6	EIF1AX	FGF14	GRIN2A	INHA	MAP3K4	NOTCH3	PMAIP1	RHEB	SRC	WT1
AXL	CDK8	EIF4A2	FGF19	GRM3	INHBA	MAPK1	NOTCH4	PMS1	RHOA	SRSF2	XIAP
B2M	CDKN1A	EIF4E	FGF2	GSK3B	INPP4A	MAPK3	NPM1	PMS2	RICTOR	STAG1	XPO1
BAP1	CDKN1B	EML4	FGF23	H3F3A	INPP4B	MAX	NRAS	PNRC1	RIT1	STAG2	XRCC2
BARD1	CDKN2A	EP300	FGF3	H3F3B	INSR	MCL1	NRG1	POLD1	RNF43	STAT3	YAP1
BBC3	CDKN2B	EPCAM	FGF4	H3F3C	IRF2	MDC1	NSD1	POLE	ROS1	STAT4	YES1
BCL10	CDKN2C	EPHA3	FGF5	HGF	IRF4	MDM2	NTRK1	PPARG	RPS6KA4	STAT5A	ZBTB2
BCL2	CEBPA	EPHA5	FGF6	HIST1H1C	IRS1	MDM4	NTRK2	PPM1D	RPS6KB1	STAT5B	ZBTB7A
BCL2L1	CENPA	EPHA7	FGF7	HIST1H2BD	IRS2	MED12	NTRK3	PPP2R1A	RPS6KB2	STK11	ZFHX3
BCL2L11	CHD2	EPHB1	FGF8	HIST1H3A	JAK1	MEF2B	NUP93	PPP2R2A	RPTOR	STK40	ZNF217
BCL2L2	CHD4	ERBB2	FGF9	HIST1H3B	JAK2	MEN1	NUTM1	PPP6C	RUNX1	SUFU	ZNF703
BCL6	CHEK1	ERBB3	FGFR1	HIST1H3C	JAK3	MET	PAK1	PRDM1	RUNX1T1	SUZ12	ZRSR2
BCOR	CHEK2	ERBB4	FGFR2	HIST1H3D	JUN	MGA	PAK3	PREX2	RYBP	SYK	
BCORL1	CIC	ERCC1	FGFR3	HIST1H3E	KAT6A	MITF	PAK7	PRKAR1A	SDHA	TAF1	
BCR	CREBBP	ERCC2	FGFR4	HIST1H3F	KDM5A	MLH1	PALB2	PRKCI	SDHAF2	TBX3	
BIRC3	CRKL	ERCC3	FH	HIST1H3G	KDM5C	MLLT3	PARK2	PRKDC	SDHB	TCEB1	
BLM	CRLF2	ERCC4	FLCN	HIST1H3H	KDM6A	MPL	PARP1	PRSS8	SDHC	TCF3	

DNA-Sequencing of 59 genes for the detection of copy gain and 4 genes for copy loss *(ATM, BRCA1, BRCA2, PTEN)*

AKT2	BRCA1	CDK4	ERBB2	FGF1	FGF23	FGF7	FGFR3	LAMP1	MYCL1	PDGFRB	RET
ALK	BRCA2	CDK6	ERBB3	FGF10	FGF3	FGF8	FGFR4	MDM2	MYCN	PIK3CA	RICTOR
AR	CCND1	CHEK1	ERCC1	FGF14	FGF4	FGF9	JAK2	MDM4	NRAS	PIK3CB	RPS6KB1
ATM	CCND3	CHEK2	ERCC2	FGF19	FGF5	FGFR1	KIT	MET	NRG1	PTEN	TFRC
BRAF	CCNE1	EGFR	ESR1	FGF2	FGF6	FGFR2	KRAS	MYC	PDGFRA	RAF1	

RNA-Sequencing of 55 genes for the detection of fusions and 2 genes for splice variants *(EGFR, MET)*

ABL1	BCL2	CSF1R	ESR1	EWSR1	FLI1	KIF5B	MSH2	NRG1	PAX7	RAF1
AKT3	BRAF	EGFR	ETS1	FGFR1	FLT1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA1	EML4	ETV1	FGFR2	FLT3	KMT2A	NOTCH1	NTRK2	PDGFRB	ROS1
AR	BRCA2	ERBB2	ETV4	FGFR3	JAK2	MET	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CDK4	ERG	ETV5	FGFR4	KDR	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2

RNA-sequencing of 64 immune genes

ADORA2A	CD2	CD39	CD80	CXCR6	ICOS	KLRD1	MX1	PD-L1	TBX21	TLR9
BTLA	CD20	CD4	CD86	DDX58	ICOSLG	LAG3	NECTIN2	PD-L2	TGFB1	TNF
CCL2	CD27	CD40	CSF1R	FOXP3	IDO1	LAGE1A	NY-ESO-1	PVR	TIGIT	TNFRSF14
CCR2	CD28	CD40LG	CTLA4	GATA3	IFNG	MAGEA1	OX40	SLAMF4	TIM3	VISTA
CD137	CD3	CD68	CXCL10	GITR	IL10	MAGEA3	OX-40L	SSX2	TLR7	
CD163	CD38	CD8	CXCR2	GZMB	IL1B	MAGEA4	PD-1	STAT1	TLR8	

Immunohistochemistry for expression of PD-L1

PD-L1 IHC (22C3)