Supplementary data

燃石医学
Burning Rock Dx

Specimen ID：RS19058620FFP

# BurningRock OncoScreen Plus™ Report

Report No: 32034501903190　　Report Date: 2019/11/19 PM

# CONTENT

## PATIENT INFORMATION

| | | | | | |
| --- | --- | --- | --- | --- | --- |
| **Name** | 吴成云 | **Specimen ID** | RS19058620FFP | **Accession #** | A00144591 |
| **Gender** | Male | **Specimen Type** | FFPE slides | **Accession Date** | 2019/11/11 |
| **Age** | 67 | **Biopsy Type** | Puncture | **Specimen Received** | 2019/11/13 AM |
| **ID** | 3209211952****11 | **Specimen Site** | lung | **Report Date** | 2019/11/19 PM |
| **Tumor Type*** | Other | **Hospital** | 江苏省人民医院 | | |
| **Additional Information *** | not provided | | | | |

***Note :** Tumor Type and Additional Information are not from the test, but provided by the patient.

## ABOUT THE TEST

BurningRock OncoScreen Plus™ is a NGS based assay that identifies genomic alterations within 520 cancer-related genes.

## SUMMARY OF RESULTS

| CATEGORIES | RESULTS |
| --- | --- |
| **Somatic Variants** | 12 variants in total, 3 of which with clinical significance |
| **Significant Results** | MAP2K1: p.I103_K104del PIK3CA: p.H1047L<br>TP53: p.G187S |
| **TMB** | 11.1 muts/Mbp |
| **MSI** | Microsatellite Stable (MSS) |
| **Germline Variants** | None |
| **Quality Control** | Qualified |

## 1. Somatic Variants and Interpretation

### Tier I: Variants with Strong Clinical Significance

| Variant | Allelic Fraction | Interpretation | Relevant Therapies (Responsiveness , Evidence) |
| --- | --- | --- | --- |
| No Tier I mutation detected | | | |

### Tier II: Variants of Potential Clinical Significance

| Variant | Allelic Fraction | Interpretation | Relevant Therapies (Responsiveness , Evidence) |
| --- | --- | --- | --- |
| MAP2K1<br>Exon 3 p.I103_K104del<br>disruptive_inframe_deletion<br>c.306_311del<br>p.Ile103_Lys104del | 17.03% | MAP2K1 has the deletion between position 103 and position 104.The variant lies within protein kinase domain(UniProt.org: Q02750). | Trametinib(Resistance, Level D)<br>RO5126766(Resistance, Level D) |
| PIK3CA<br>Exon 21 p.H1047L<br>missense_variant<br>c.3140A>T<br>p.His1047Leu | 7.38% | PIK3CA has the substitution of Histidine with Leucine at position 1047.The variant lies within PI3K/PI4K domain(UniProt.org: P42336). The variant causes Gain-of-function[PMID: 20593314, 26627007, 22120714]. | Alpelisib(Response, Level C) |
| TP53<br>Exon 5 splice_region_variant<br>c.559G>A<br>p.Gly187Ser | 14.5% | TP53 has the substitution of the G nucleotide at c.559 by a A. The variant is located at the boundary of an exon, which may affect alternative splicing by prediction. | Adavosertib+Olaparib (Response, Level C) |

### Tier III: Variants of Unknown Clinical Significance

| Gene | Variant Type | Exon | Nucleotide Change | Amino Acid Change | Allelic Fraction |
| --- | --- | --- | --- | --- | --- |
| ARID2 | splice_acceptor_variant | 3 | c.187-2A>G | - | 15.72% |
| ARID2 | frameshift_variant | 15 | c.4709_4710del | p.Ile1570fs | 6.87% |
| DICER1 | missense_variant | 22 | c.4061G>T | p.Cys1354Phe | 6.23% |
| HGF | missense_variant | 5 | c.610C>T | p.Pro204Ser | 16.32% |
| MCL1 | missense_variant | 2 | c.734C>T | p.Ser245Leu | 6.73% |
| NOTCH3 | missense_variant | 3 | c.304G>T | p.Ala102Ser | 12.56% |

| | | | | | |
|---|---|---|---|---|---|
| PIK3CG | stop_gained | 2 | c.1229G>A | p.Trp410* | 16.28% |
| TET2 | frameshift_variant | 6 | c.3733_3737del | p.Tyr1245fs | 2.05% |
| TSC1 | disruptive_inframe_deletion | 23 | c.3459_3461del | p.Ile1153del | 6.56% |

**Note :** 1. The responsiveness to the relevant therapies corresponding to the genetic variation is derived from OncoDB, which is an internal database of Burning Rock Dx, with reference to public data such as NCCN guidelines. This data is for reference purpose by clinicians only. With the continuous improvement of the database and the update of clinical data, the grade of variants may change.

2. The evidence levels are grouped into four levels according to the AMP / ASCO / CAP guidelines [PMID: 27993330]: level A (FDA-approved therapies or have been included in professional guidelines), level B (based on well-powered studies with consensus from experts), level C (level A evidence in a different tumor type, or serve as inclusion criteria for clinical trials, or supported by multiple small studies), level D (preclinical studies , or supported by case reports). The variants are categorized into four categories based on their clinical impact: tier I, variants with strong clinical significance (level A and B evidence); tier II, variants with potential clinical significance (level C or D evidence); tier III, variants with unknown clinical significance; and tier IV, variants that are benign or likely benign.

## 2. Germline Variants and Interpretation

| Gene | Variant Type | Exon | Nucleotide Change | Amino Acid Change | Zygosity | Interpretation |
| --- | --- | --- | --- | --- | --- | --- |
| No pathogenic or likely pathogenic germline variant detected in this sample | | | | | | |

**Note :** 1. Only pathogenic or likely pathogenic variants of hereditary cancer related genes will be reported.

2.Hereditary cancer related genes are defined by ACMG secondary finding guideline v2.0 [PMID: 27854360] and NCCN guidelines.This product tests 62 genes: APC、ATM、AXIN2、BARD1、BMPR1A、BRCA1、BRCA2、BRIP1、CDH1、CDK12、CDK4、CDKN2A、CHEK1、CHEK2、EGFR、EPCAM、FANCA、FANCI、FANCL、FH、FLCN、GREM1、HOXB13、KIT、MEN1、MET、MLH1、MSH2、MSH3、MSH6、MUTYH、NBN、NF1、NF2、NTHL1、PALB2、PDGFRA、PMS2、POLD1、POLE、PPP2R2A、PRKAR1A、PTCH1、PTEN、RAD51B、RAD51C、RAD51D、RAD54L、RB1、RET、SDHA、SDHAF2、SDHB、SDHC、SDHD、SMAD4、STK11、TP53、TSC1、TSC2、VHL、WT1.

3. According to ACMG guidelines[PMID:25741868], the pathogenicity of germline variant is classified into five categories : 1-benign; 2-likely benign; 3-variants with uncertain significance; 4-likely pathogenic; 5-pathogenic. The interpretation of variant is based on available literature and relevant databases and may change as research progresses.

## 3. Summary of Immunotherapy Biomarkers

### TMB

| | |
| --- | --- |
| • **RESULT** | **11.1 muts/Mbp** |
| • **TMB Introduction** | Tumor mutation burden (TMB) represents the number of somatic mutations that occur per million bases in the genome. This test evaluates the mutation load of the genome based on non-silent mutations (excluding tumor hotspot mutations) within regions of the PANEL captured. |
| • **Clinical Significance** | Previous retrospective studies have shown that a higher mutational burden in the tumor genome is correlated with a better efficacy of anti-PD-1/PD-L1 (± anti-CTLA-4) treatments. The TMB obtained from large NGS panels is highly correlated with that from whole exome sequencing (WES), confirming that selective sequencing of the specific genomic region is sufficient to obtain full understanding of a patient's TMB and can help predict the efficacy of immune checkpoint inhibitors. The CheckMate-227 study further confirmed that EGFR/ALK-negative advanced NSCLC patients with TMB≥10 have significant benefits in both objective response rate (ORR) and progression-free survival (PFS) when they were treated with first-line immune checkpoint inhibitor (Navurizumab plus Ipilimumab) regardless of their PD-L1 expression level compared to standard chemotherapy [PMID: 29658845]. |

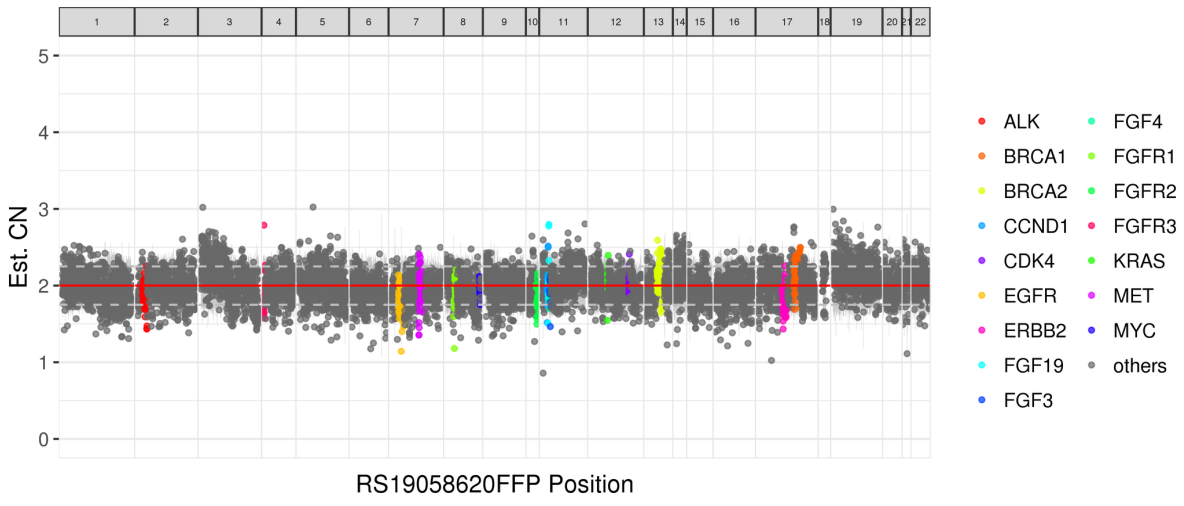
### MSI

| | |
| --- | --- |
| • **RESULT** | **Microsatellite Stable (MSS)** |
| • **MSI Introduction** | Microsatellite instability (MSI) refers to insertions and deletions of simple repetitive sequences in a microsatellite locus, resulting in genomic instability. Microsatellite instability (MSI) status is evaluated by the MSI detection algorithm independently developed by Burning Rock Dx. |
| • **Clinical Significance** | No immunotherapy drugs are recommended for solid tumors with microsatellite stable (MSS). |

## 4. SNPs in Drug Metabolism Enzymes

| SNP | Genotype | Zygosity | Clinical Significance |
|---|---|---|---|
| CYP2D6*10 rs1065852 | G/A | Wild Type | Cytochrome P450 2D6 (CYP2D6) is involved in the metabolism of multiple drugs. Genetic polymorphisms cause substantial variation in CYP2D6 activity and serve as biomarkers guiding drug therapy.The *10 heterozygous variant alleles have partial activity and the exact effect on enzymatic activity is difficult to determine. |
| DPYD*13 rs55886062 | A/A | Wild Type | DPD deficiency is associated with increased toxicity of fluorouracil (5-FU, capecitabine, or tegafur). Individuals with wild type DPD have normal DPD expression. |
| DPYD*2846A>T rs67376798 | T/T | Wild Type | DPD deficiency is associated with increased toxicity of fluorouracil (5-FU, capecitabine, or tegafur). Individuals with wild type DPD have normal DPD expression. |
| DPYD*2A rs3918290 | C/C | Wild Type | DPD deficiency is associated with increased toxicity of fluorouracil (5-FU, capecitabine, or tegafur). Individuals with wild type DPD have normal DPD expression. |
| UGT1A1*28 rs8175347 | $(TA)_6/(TA)_7$ | Wild Type | UGT1A1-specific polymorphisms are associated with increased irinotecan toxicity.The precise dose reduction in heterozygous carriers of the variants UGT1A1*28 is not known and subsequent dose modifications should be considered based on the tolerance of individual patient. |
| UGT1A1*6 rs4148323 | G/G | Wild Type | UGT1A1-specific polymorphisms are associated with increased irinotecan toxicity. It is less likely for individuals with wild-type UGT1A1 to have irinotecan-related toxicity. |

## 5. Gene Copy Number Distribution




**Note :** The figure above shows the copy number distribution for all detected genes. Each dot represents an interval from captured gene regions, and the highlighted dots represent those genes focused on copy number alterations. The horizontal axis represents the chromosome where a gene is located, and the vertical axis represents the copy number calculated based on the NGS data (the red horizontal line represents the normal copy number of a gene. Copy number

obtained by this test cannot completely represent the gene copy number in tumor cells due to the dilution of normal cells.

## APPENDIX1：QUALITY CONTROL

| Quality Parameters | | Value | Criteria |
|---|---|---|---|
| **Pathology** | Tumor Purity (%)[1] | - | ≥10% |
| **DNA Quality** | DNA Quantity(ng)[2] | 838 | ≥30 |
| | DNA Degradation[3] | A | A-B-C |
| | Pre-library Quantity (ng)[4] | 4490 | ≥300 |
| **Data Quality** | Mean Depth[5] | 1249 | ≥500 |
| | Library Diversity[6] | 78% | ≥20% |
| | Insert Length (bp)[7] | 242 | ≥150 |
| | Coverage Uniformity[8] | 97% | ≥90% |
| | Mapping Ratio[9] | 100% | ≥95% |
| | Q30 Percentage[10] | 92% | ≥80% |
| | SNP Concordance[11] | 99% | ≥90% |
| **Overall QC[12]** | | **Qualified** | |

**Note：** 1. Tumor Purity: Tumor purity was assessed by Burning Rock using HE staining. This step will be omitted if the sample does not meet the requirement for tumor content assessment. This assessment is not performed on cfDNA samples.

2. DNA Amount: the amount of DNA extracted from the submitted specimen.

3. DNA Degradation: the level of DNA degradation assessed by gel electrophoresis; A-D indicate different levels of DNA degradation, A indicates the lowest degradation and D indicates the highest degradation. This step will be omitted if a sample does not need such assessment. This assessment is not performed on cfDNA samples.

4. Pre-library Amount: the amount of libraries for sequencing after adapter-ligated DNA fragments are amplified and purified.

5. Mean Sequencing Depth: the mean and median number of unique reads being mapped to a given nucleotide of a targeted gene

6. Library Diversity: the proportion of DNA libraries from the original DNA fragments.

7. Insert Length: the median length of the sequenced DNA fragments. For tissue samples, if the median insert length is less than 170bp, it indicates DNA degradation. This may introduce DNA degradation induced false positives. The insert length for cfDNA is 170bp.

8. Coverage Uniformity: the percentage of targeted base positions in which the read depth is greater than 0.2 times the mean coverage depth.

9. Mapping Ratio: the percentage of the number of sequences successfully aligned to the reference genome.

10. Q30 Percentage: the percentage of bases that have a Q-score above or equal to 30(below a probability of incorrect base calling of 1 in 1000).

11. SNP Concordance: the fraction of the genotype calls for SNPs from paired specimen.

12. Overall QC: the overall quality control based on multiple parameters; overall QC is divided into three levels: qualified, warning and fail. When the overall quality is warning or fail, the accuracy and sensitivity of this test may be affected.

## APPENDIX2：METHODOLOGY AND LIMITATIONS

### METHODOLOGY

This test performed hybrid capture-based next-generation sequencing (NGS) to detect genomic alterations. The detection method was independently developed, analyzed and validated by Burning Rock.Dx. Burning Rock has established CLIA-certified and CAP-accredited NGS laboratory, and passed the relevant quality assessment of National Center for Clinical Laboratories of China.

This test covers up to 20bp on either side of the intron/exon boundaries of the targeted genes. Variant types that this test can detect single nucleotide variation (SNV),small insertion or deletion, and some rearrangements.

### LIMITATIONS

1. This report only provides reference for clinical diagnosis and treatment decisions-making. Clinical diagnosis and treatment decisions should be made by the clinician in combination with the clinical information of patient.

2. The analysis and interpretation of results are based on available literature and databases. With the advancements of relevant research and updates of databases, the interpretation of mutations may change.

3. This test is only applicable to the detection of DNA mutations. DNA methylation, RNA-level or protien-level modifications are not inferred by this test.

4. If no muation detected (negative reult), we cannot rule out the possibility of the presence of mutations which are below the limit of detection.

5. Due to the complexity of the tumorigenic mechanism, the results of this test alone cannot confirm or exclude the presence of malignant tumors.

6. Acquired mutations may occur during treatment and tumor development, resulting in the change of mutation spectrum. Tumors also have spataial and temporal heterogeneitytest. Therefore, results from different specimens of the same patient may differ. This test is only responsible for the specimen submitted.

7. This test cannot determine chromosomal polyploidy which may result in copy number variations (CNV). The sensitivity of CNV detection is affected by the proportion of tumor cells in a specimen. If the proportion of tumor cells is <20%, the detection sensitivity is limited.

8. The sensitivity of TMB detection is affected by the proportion of tumor cells in a specimen which is correlated with the maximum allelic fraction (maxAF). If the proportion of tumor cells is <10% or maxAF <5%, the detection sensitivity is limited.

9. The sensitivity of MSI detection is affected by the proportion of tumor cells in a specimen which is correlated with the maximum allelic fraction (maxAF). If the proportion of tumor cells is <20% or maxAF <10%, the detection sensitivity is limited.

## APPENDIX3：GENE LIST

| | | | | |
|---|---|---|---|---|
| **ABL1** NM_005157.5 | **ABL2** NM_007314.3 | **ABRAXAS1** NM_139076.2 | **ACVR1** NM_001105.4 | **ACVR1B** NM_020328.3 |
| **AKT1** NM_001014432.1 | **AKT2** NM_001626.5 | **AKT3** NM_005465.4 | **ALK** NM_004304.4 | **ALOX12B** NM_001139.2 |
| **AMER1** NM_152424.3 | **APC** NM_000038.5 | **AR** NM_000044.3 | **ARAF** NM_001256196.1 | **ARFRP1** NM_001267547.2 |
| **ARID1A** NM_006015.4 | **ARID1B** NM_020732.3 | **ARID2** NM_152641.2 | **ARID5B** NM_032199.2 | **ASXL1** NM_015338.5 |
| **ASXL2** NM_018263.4 | **ATM** NM_000051.3 | **ATR** NM_001184.3 | **ATRX** NM_000489.4 | **AURKA** NM_001323303.1 |
| **AURKB** NM_001284526.1 | **AXIN1** NM_003502.3 | **AXIN2** NM_004655.3 | **AXL** NM_021913.4 | **B2M** NM_004048.2 |
| **BAP1** NM_004656.3 | **BARD1** NM_000465.3 | **BBC3** NM_001127240.2 | **BCL10** NM_003921.4 | **BCL2** NM_000633.2 |
| **BCL2L1** NM_001317919.1 | **BCL2L11** NM_001204107.1 | **BCL2L2** NM_001199839.1 | **BCL6** NM_001130845.1 | **BCOR** NM_001123383.1 |
| **BCORL1** NM_021946.4 | **BIRC3** NM_001165.4 | **BLM** NM_000057.3 | **BMPR1A** NM_004329.2 | **BRAF** NM_004333.4 |
| **BRCA1** NM_007294.3 | **BRCA2** NM_000059.3 | **BRD4** NM_058243.2 | **BRD7** NM_001173984.2 | **BRINP3** NM_199051.2 |
| **BRIP1** NM_032043.2 | **BTG1** NM_001731.2 | **BTG2** NM_006763.2 | **BTK** NM_000061.2 | **CALR** NM_004343.3 |
| **CARD11** NM_032415.5 | **CASP8** NM_001228.4 | **CBFB** NM_022845.2 | **CBL** NM_005188.3 | **CCND1** NM_053056.2 |
| **CCND2** NM_001759.3 | **CCND3** NM_001760.4 | **CCNE1** NM_001238.3 | **CD274** NM_014143.3 | **CD74** NM_001025159.2 |
| **CD79A** NM_001783.3 | **CD79B** NM_000626.3 | **CDC73** NM_024529.4 | **CDH1** NM_004360.4 | **CDH18** NM_001291956.1 |
| **CDK12** NM_016507.3 | **CDK4** NM_000075.3 | **CDK6** NM_001145306.1 | **CDK8** NM_001260.2 | **CDKN1A** NM_001291549.1 |
| **CDKN1B** NM_004064.4 | **CDKN1C** NM_000076.2 | **CDKN2A** NM_000077.4 | **CDKN2B** NM_004936.3 | **CDKN2C** NM_001262.2 |
| **CEBPA** NM_004364.4 | **CENPA** NM_001809.3 | **CHD1** NM_001270.2 | **CHD2** NM_001271.3 | **CHD4** NM_001273.3 |
| **CHEK1** NM_001274.5 | **CHEK2** NM_007194.3 | **CIC** NM_015125.4 | **CREBBP** NM_004380.2 | **CRKL** NM_005207.3 |
| **CRLF2** NM_022148.3 | **CSF1R** NM_001288705.1 | **CSF3R** NM_156039.3 | **CSMD1** NM_033225.5 | **CSMD3** NM_198123.1 |
| **CTCF** NM_006565.3 | **CTLA4** NM_005214.4 | **CTNNA1** NM_001323982.1 | **CTNNB1** NM_001904.3 | **CUL3** NM_001257198.1 |
| **CUL4A** NM_001008895.2 | **CXCR4** NM_003467.2 | **CYLD** NM_015247.2 | **CYP17A1** NM_000102.3 | **CYP2D6** NM_000106.5 |
| **DAXX** NM_001141970.1 | **DCUN1D1** NM_020640.3 | **DDR1** NM_013994.2 | **DDR2** NM_001014796.1 | **DICER1** NM_177438.2 |
| **DIS3** NM_014953.4 | **DNAJB1** NM_006145.2 | **DNMT1** NM_001130823.2 | **DNMT3A** NM_022552.4 | **DNMT3B** NM_006892.3 |
| **DOT1L** NM_032482.2 | **DPYD** NM_000110.3 | **EED** NM_001308007.1 | **EGFR** NM_005228.3 | **EIF1AX** NM_001412.3 |
| **EIF4E** NM_001130679.1 | **EMSY** NM_001300942.1 | **EP300** NM_001429.3 | **EPCAM** NM_002354.2 | **EPHA2** NM_004431.4 |
| **EPHA3** NM_005233.5 | **EPHA5** NM_001281765.2 | **EPHA7** NM_004440.3 | **EPHB1** NM_004441.4 | **EPHB4** NM_004444.4 |
| **ERBB2** NM_004448.3 | **ERBB3** NM_001982.3 | **ERBB4** NM_005235.2 | **ERCC1** NM_202001.2 | **ERCC2** NM_000400.3 |
| **ERCC3** NM_000122.1 | **ERCC4** NM_005236.2 | **ERCC5** NM_000123.3 | **ERG** NM_001136154.1 | **ERRFI1** NM_018948.3 |
| **ESR1** NM_000125.3 | **ETV4** NM_001079675.2 | **ETV5** NM_004454.2 | **ETV6** NM_001987.4 | **EWSR1** NM_013986.3 |
| **EZH2** NM_004456.4 | **EZR** NM_001111077.1 | **FANCA** NM_000135.2 | **FANCC** NM_000136.2 | **FANCD2** NM_001018115.2 |
| **FANCE** NM_021922.2 | **FANCF** NM_022725.3 | **FANCG** NM_004629.1 | **FANCI** NM_001113378.1 | **FANCL** NM_018062.3 |
| **FANCM** NM_020937.3 | **FAS** NM_000043.5 | **FAT1** NM_005245.3 | **FBXW7** NM_033632.3 | **FGF10** NM_004465.1 |
| **FGF12** NM_021032.4 | **FGF14** NM_175929.2 | **FGF19** NM_005117.2 | **FGF23** NM_020638.2 | **FGF3** NM_005247.2 |
| **FGF4** NM_002007.2 | **FGF6** NM_020996.2 | **FGF7** NM_002009.3 | **FGFR1** NM_023110.2 | **FGFR2** NM_000141.4 |
| **FGFR3** NM_000142.4 | **FGFR4** NM_002011.4 | **FH** NM_000143.3 | **FLCN** NM_144997.5 | **FLT1** NM_002019.4 |
| **FLT3** NM_004119.2 | **FLT4** NM_182925.4 | **FOXA1** NM_004496.3 | **FOXL2** NM_023067.3 | **FOXO1** NM_002015.3 |
| **FOXP1** NM_001244810.1 | **FRS2** NM_001042555.2 | **FUBP1** NM_003902.4 | **FYN** NM_002037.5 | **GABRA6** NM_000811.2 |
| **GATA1** NM_002049.3 | **GATA2** NM_001145661.1 | **GATA3** NM_001002295.1 | **GATA4** NM_001308093.1 | **GATA6** NM_005257.5 |
| **GEN1** NM_001130009.2 | **GID4** NM_024052.4 | **GLI1** NM_005269.2 | **GNA11** NM_002067.4 | **GNA13** NM_006572.5 |
| **GNAQ** NM_002072.4 | **GNAS** NM_080425.3 | **GPS2** NM_004489.4 | **GREM1** NM_013372.6 | **GRIN2A** NM_000833.4 |
| **GRM3** NM_000840.2 | **GSK3B** NM_002093.3 | **H3F3A** NM_002107.4 | **H3F3B** NM_005324.4 | **H3F3C** NM_001013699.2 |
| **HDAC1** NM_004964.2 | **HDAC2** NM_001527.3 | **HGF** NM_000601.5 | **HIST1H1C** NM_005319.3 | **HIST1H2BD** NM_021063.3 |
| **HIST1H3A** NM_003529.2 | **HIST1H3B** NM_003537.3 | **HIST1H3C** NM_003531.2 | **HIST1H3D** NM_003530.4 | **HIST1H3E** NM_003532.2 |
| **HIST1H3G** NM_003534.2 | **HIST1H3H** NM_003536.2 | **HIST1H3I** NM_003533.2 | **HIST1H3J** NM_003535.2 | **HIST2H3D** NM_001123375.2 |
| **HIST3H3** NM_003493.2 | **HLA-A** NM_001242758.1 | **HLA-B** NM_005514.7 | **HLA-C** NM_001243042.1 | **HNF1A** NM_000545.6 |
| **HOXB13** NM_006361.5 | **HRAS** NM_005343.3 | **HSD3B1** NM_000862.2 | **HSP90AA1** NM_001017963.2 | **ICOSLG** NM_001283050.1 |
| **ID3** NM_002167.4 | **IDH1** NM_005896.3 | **IDH2** NM_002168.3 | **IFNGR1** NM_000416.2 | **IGF1** NM_001111285.2 |
| **IGF1R** NM_000875.4 | **IGF2** NM_000612.5 | **IKBKE** NM_014002.3 | **IKZF1** NM_006060.5 | **IL10** NM_000572.2 |
| **IL7R** NM_002185.3 | **INHA** NM_002191.3 | **INHBA** NM_002192.3 | **INPP4A** NM_001134224.1 | **INPP4B** NM_001101669.1 |
| **INSR** NM_000208.3 | **IRF2** NM_002199.3 | **IRF4** NM_002460.3 | **IRS1** NM_005544.2 | **IRS2** NM_003749.2 |
| **JAK1** NM_001320923.1 | **JAK2** NM_004972.3 | **JAK3** NM_000215.3 | **JUN** NM_002228.3 | **KAT6A** NM_006766.4 |
| **KDM5A** NM_001042603.2 | **KDM5C** NM_004187.3 | **KDM6A** NM_001291415.1 | **KDR** NM_002253.2 | **KEAP1** NM_012289.3 |
| **KEL** NM_000420.2 | **KIT** NM_000222.2 | **KLF4** NM_001314052.1 | **KLHL6** NM_130446.2 | **KMT2A** NM_001197104.1 |
| **KMT2C** NM_170606.2 | **KMT2D** NM_003482.3 | **KRAS** NM_033360.3 | **LATS1** NM_004690.3 | **LATS2** NM_014572.2 |

| | | | | |
| --- | --- | --- | --- | --- |
| **LMO1** NM_002315.2 | **LRP1B** NM_018557.2 | **LTK** NM_002344.5 | **LYN** NM_002350.3 | **MAF** NM_005360.4 |
| **MAGI2** NM_012301.3 | **MALT1** NM_006785.3 | **MAP2K1** NM_002755.3 | **MAP2K2** NM_030662.3 | **MAP2K4** NM_001281435.1 |
| **MAP3K1** NM_005921.1 | **MAP3K13** NM_001242314.1 | **MAPK1** NM_002745.4 | **MAPK3** NM_002746.2 | **MAX** NM_002382.4 |
| **MCL1** NM_021960.4 | **MDC1** NM_014641.2 | **MDM2** NM_002392.5 | **MDM4** NM_002393.4 | **MED12** NM_005120.2 |
| **MEF2B** NM_001145785.1 | **MEN1** NM_000244.3 | **MERTK** NM_006343.2 | **MET** NM_000245.3 | **MGA** NM_001164273.1 |
| **MIR21** NR_029493.1 | **MITF** NM_000248.3 | **MKNK1** NM_003684.5 | **MLH1** NM_000249.3 | **MLH3** NM_001040108.1 |
| **MPL** NM_005373.2 | **MRE11** NM_005591.3 | **MSH2** NM_000251.2 | **MSH3** NM_002439.4 | **MSH6** NM_000179.2 |
| **MST1** NM_020998.3 | **MST1R** NM_002447.3 | **MTAP** NM_002451.3 | **MTOR** NM_004958.3 | **MUTYH** NM_001128425.1 |
| **MYC** NM_002467.4 | **MYCL** NM_001033082.2 | **MYCN** NM_001293228.1 | **MYD88** NM_002468.4 | **MYOD1** NM_002478.4 |
| **NAV3** NM_001024383.1 | **NBN** NM_002485.4 | **NCOA3** NM_181659.2 | **NCOR1** NM_006311.3 | **NCOR2** NM_006312.5 |
| **NEGR1** NM_173808.2 | **NF1** NM_000267.3 | **NF2** NM_000268.3 | **NFE2L2** NM_006164.4 | **NFKBIA** NM_020529.2 |
| **NKX2-1** NM_001079668.2 | **NKX3-1** NM_006167.3 | **NOTCH1** NM_017617.4 | **NOTCH2** NM_024408.3 | **NOTCH3** NM_000435.2 |
| **NOTCH4** NM_004557.3 | **NPM1** NM_002520.6 | **NRAS** NM_002524.4 | **NRG1** NM_001322205.1 | **NSD1** NM_022455.4 |
| **NSD2** NM_001042424.2 | **NSD3** NM_023034.1 | **NT5C2** NM_001134373.2 | **NTHL1** NM_002528.6 | **NTRK1** NM_001007792.1 |
| **NTRK2** NM_006180.4 | **NTRK3** NM_001012338.2 | **NUP93** NM_014669.4 | **NUTM1** NM_001284292.1 | **P2RY8** NM_178129.4 |
| **PAK1** NM_001128620.1 | **PAK3** NM_001128168.2 | **PAK5** NM_020341.3 | **PALB2** NM_024675.3 | **PARP1** NM_001618.3 |
| **PARP2** NM_005484.3 | **PARP3** NM_001003931.3 | **PAX5** NM_016734.2 | **PBRM1** NM_018313.4 | **PCDH11X** NM_032968.4 |
| **PDCD1** NM_005018.2 | **PDCD1LG2** NM_025239.3 | **PDGFRA** NM_006206.4 | **PDGFRB** NM_002609.3 | **PDK1** NM_001278549.1 |
| **PGR** NM_000926.4 | **PHOX2B** NM_003924.3 | **PIK3C2B** NM_002646.3 | **PIK3C2G** NM_001288772.1 | **PIK3C3** NM_002647.3 |
| **PIK3CA** NM_006218.3 | **PIK3CB** NM_006219.2 | **PIK3CD** NM_005026.3 | **PIK3CG** NM_001282426.1 | **PIK3R1** NM_181523.2 |
| **PIK3R2** NM_005027.3 | **PIK3R3** NM_001303427.1 | **PIM1** NM_001243186.1 | **PLCG2** NM_002661.4 | **PLK2** NM_006622.3 |
| **PMS1** NM_000534.4 | **PMS2** NM_000535.6 | **PNRC1** NM_006813.2 | **POLD1** NM_001256849.1 | **POLE** NM_006231.3 |
| **PPARG** NM_015869.4 | **PPM1D** NM_003620.3 | **PPP2R1A** NM_014225.5 | **PPP2R2A** NM_002717.3 | **PPP6C** NM_001123355.1 |
| **PRDM1** NM_001198.3 | **PREX2** NM_024870.3 | **PRKAR1A** NM_002734.4 | **PRKCI** NM_002740.5 | **PRKDC** NM_006904.6 |
| **PRKN** NM_004562.2 | **PTCH1** NM_000264.3 | **PTEN** NM_000314.6 | **PTPN11** NM_002834.3 | **PTPRD** NM_002839.3 |
| **PTPRO** NM_030667.2 | **PTPRS** NM_002850.3 | **PTPRT** NM_133170.3 | **QKI** NM_006775.2 | **RAB35** NM_006861.6 |
| **RAC1** NM_018890.3 | **RAD21** NM_006265.2 | **RAD50** NM_005732.3 | **RAD51** NM_001164269.1 | **RAD51B** NM_133509.3 |
| **RAD51C** NM_058216.2 | **RAD51D** NM_002878.3 | **RAD52** NM_001297419.1 | **RAD54L** NM_003579.3 | **RAF1** NM_002880.3 |
| **RARA** NM_000964.3 | **RASA1** NM_002890.2 | **RB1** NM_000321.2 | **RBM10** NM_001204468.1 | **RECQL4** NM_004260.3 |
| **REL** NM_002908.3 | **RET** NM_020975.4 | **RHEB** NM_005614.3 | **RHOA** NM_001664.3 | **RICTOR** NM_001285439.1 |
| **RIT1** NM_001256821.1 | **RNF43** NM_017763.5 | **ROS1** NM_002944.2 | **RPA1** NM_002945.3 | **RPS6KA4** NM_003942.2 |
| **RPS6KB2** NM_003952.2 | **RPTOR** NM_020761.2 | **RSPO2** NM_178565.4 | **RUNX1** NM_001754.4 | **RUNX1T1** NM_001198679.1 |
| **SDC4** NM_002999.3 | **SDHA** NM_004168.3 | **SDHAF2** NM_017841.2 | **SDHB** NM_003000.2 | **SDHC** NM_003001.3 |
| **SDHD** NM_003002.3 | **SETD2** NM_014159.6 | **SF3B1** NM_012433.3 | **SGK1** NM_001143676.1 | **SH2B3** NM_005475.2 |
| **SH2D1A** NM_002351.4 | **SHQ1** NM_018130.2 | **SLC34A2** NM_006424.2 | **SLIT2** NM_004787.3 | **SLX4** NM_032444.2 |
| **SMAD2** NM_001003652.3 | **SMAD3** NM_005902.3 | **SMAD4** NM_005359.5 | **SMARCA4** NM_001128849.1 | **SMARCB1** NM_003073.4 |
| **SMARCD1** NM_003076.4 | **SMO** NM_005631.4 | **SNCAIP** NM_001308100.1 | **SOCS1** NM_003745.1 | **SOX10** NM_006941.3 |
| **SOX17** NM_022454.3 | **SOX2** NM_003106.3 | **SOX9** NM_000346.3 | **SPEN** NM_015001.2 | **SPOP** NM_001007226.1 |
| **SPTA1** NM_003126.2 | **SRC** NM_198291.2 | **SRSF2** NM_003016.4 | **STAG2** NM_001042749.2 | **STAT3** NM_139276.2 |
| **STAT4** NM_001243835.1 | **STAT5A** NM_001288718.1 | **STAT5B** NM_012448.3 | **STK11** NM_000455.4 | **STK40** NM_001282546.1 |
| **SUFU** NM_016169.3 | **SYK** NM_001174167.2 | **TAF1** NM_001286074.1 | **TBX3** NM_016569.3 | **TCF3** NM_003200.3 |
| **TCF7L2** NM_001146274.1 | **TEK** NM_000459.4 | **TENT5C** NM_017709.3 | **TERC** NR_001566.1 | **TERT** NM_198253.2 |
| **TET1** NM_030625.2 | **TET2** NM_001127208.2 | **TGFBR1** NM_001306210.1 | **TGFBR2** NM_001024847.2 | **TIPARP** NM_001184717.1 |
| **TMEM127** NM_017849.3 | **TMPRSS2** NM_001135099.1 | **TNFAIP3** NM_001270507.1 | **TNFRSF14** NM_003820.3 | **TOP1** NM_003286.2 |
| **TOP2A** NM_001067.3 | **TP53** NM_000546.5 | **TP63** NM_003722.4 | **TRAF2** NM_021138.3 | **TRAF7** NM_032271.2 |
| **TRIM58** NM_015431.3 | **TRPC5** NM_012471.2 | **TSC1** NM_000368.4 | **TSC2** NM_000548.4 | **TSHR** NM_000369.2 |
| **TYRO3** NM_006293.3 | **U2AF1** NM_001025203.1 | **UGT1A1** NM_000463.2 | **VEGFA** NM_001025366.2 | **VEGFB** NM_003377.4 |
| **VHL** NM_000551.3 | **WISP3** NM_198239.1 | **WRN** NM_000553.4 | **WT1** NM_024426.4 | **XIAP** NM_001167.3 |
| **XPO1** NM_003400.3 | **XRCC2** NM_005431.1 | **XRCC3** NM_001100118.1 | **YAP1** NM_001282101.1 | **YES1** NM_005433.3 |
| **ZBTB16** NM_001018011.1 | **ZBTB2** NM_020861.2 | **ZNF217** NM_006526.2 | **ZNF703** NM_025069.2 | **ZNRF3** NM_001206998.1 |

**Note :** The NM number after the gene name is the transcript number (RefSeq) used when analyzing.

## Gene List Focused on Rearrangements

| | | | | | |
| --- | --- | --- | --- | --- | --- |
| **ALK** | **BRAF** | **CD274** | **CD74** | **ETV4** | **ETV5** |
| **ETV6** | **EWSR1** | **EZR** | **FGFR1** | **FGFR2** | **FGFR3** |
| **NRG1** | **NTRK1** | **NTRK2** | **NTRK3** | **RAF1** | **RET** |

## APPENDIX4：REFERENCE

1. NCCN Clinical Practice Guidelines in Oncology ( NCCN Guidelines® )
2. Li MM et al. (2017) 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 Diagn [PMID: 27993330]
3. Kalia SS et al. (2017) Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet. Med. [PMID: 27854360]
4. Richards S et al. (2016) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. [PMID: 25741868]
5. Ng KP et al. (2012) A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat. Med. [PMID: 22426421]

燃石医学
Burning Rock Dx

Specimen ID：RS19058622FFP

# PD-L1（Antibody Clone 22C3）Report

Burning Rock Dx | a CLIA certified lab

Report Serial Number 3131901668 Dat2019/11/15 AM

Anti-Forgery Port

## Patient Information

| | | | | | |
|---|---|---|---|---|---|
| **Name** | 吴成云 | **Specimen ID** | RS19058622FFP | **Application Form** | A00144592 |
| **Gender** | Male | **Specimen Type** | Section | **Accession Date** | 2019/11/11 |
| **Age** | 67 | **Biopsy Type** | Puncture | **Specimen Received** | 2019/11/13 AM |
| **Patient ID** | 3209211952****11 | **Specimen Site** | Lung | **Report Date** | 2019/11/15 AM |
| **Tumor Type** | Unknown | **Hospital** | 江苏省人民医院 | | |
| **Additional Information** | Not provided | | | | |

*NOTE： Patient information was provided by the patient upon sample receiving. Above information is not conferred from this report therefore, this report is not responsible for the accuracy of the above information.

## Introduction of Products

| Protein | Clone number | Method | Reagent | Clinical interpretation |
|---|---|---|---|---|
| PD-L1 | 22C3 | IHC | Mouse anti-Human PD-L1 Monoclonal Antibody | Keytruda (Pembrolizumab) Antibody for Companion Diagnostics |

**This report is only responsible for the detection of this specimen, and the results are only for medical reference.**

**Thechnician:** 梁丽仪

**Doctor:**徐婷

广州燃石医学检验所有限公司 医学检验部

## Quality Control (QC)

| Quality Parameter | | Value | QC Standard |
|---|---|---|---|
| **Pathological Assessment** | Number of malignant cells | ⩾100 | ⩾100 |
| **Overall Quality Assessment** | Qualified | | |

## Result

| Evaluation / Protein | TPS | CPS |
|---|---|---|
| **PD-L1** | **90%** | / |

NOTE Please refer to the results of PD-L1 protein expression according to the corresponding instructions of immunotherapy treatments and the evaluation criteria of different types of tumors.

**Result Analytical Reference:**

**Methods:**

TPS：PD-L1 stained tumor cell /total tumor cell (PD-L1 stained and non-stained) x100%

CPS：PD-L1 stained cell (tumor cell, lymphocyte, macrophage)/total tumor cell (PD-L1 stained and non-stained) x100

**Limitations:**

The following samples are not suitable for CPS evaluation

a. Cytological samples
b. Shredded tissue samples
c. Decalcification samples of bone metastases

**Definition of boundary value:**

Negative: < 1%

Low expression: 1-49%

High expression: ⩾ 50%

NOTE This threshold is the definition of TPS test results for NSCLC samples.

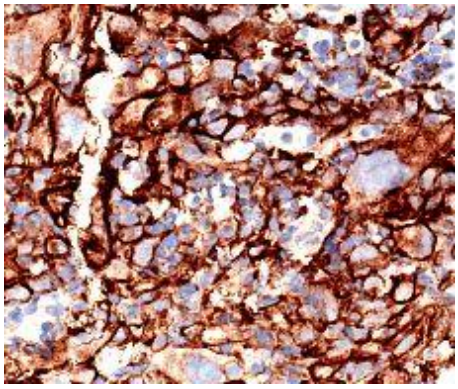
NOTE 1. Due to tumor heterogeneity (temporal and spatial), and the way of sample preservation, the results only reflect the specimen sent for examination.

2. If the number of malignant tumor cells is less than 100, the accuracy of the detection may be affected.

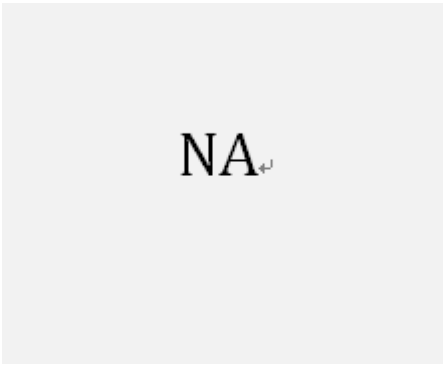
3. The pharmacokinetics and the effect of its in vivo process are complex, and there are many factors affecting the efficacy and toxicity of the drug. Thus, doctors should consider the following factors prior to treatment decision-making, including but not limited to the pathophysiological characteristics of the patient, clinical manifestations, adjuvant therapy that is being used or to be used. This report serves as a reference for treatment-guidance based on the results of this test. Do not use this result as the sole basis for treatment-guidance.

# Result chart

Sample results:

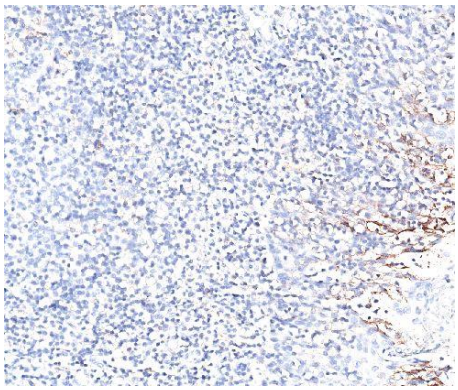


TPS

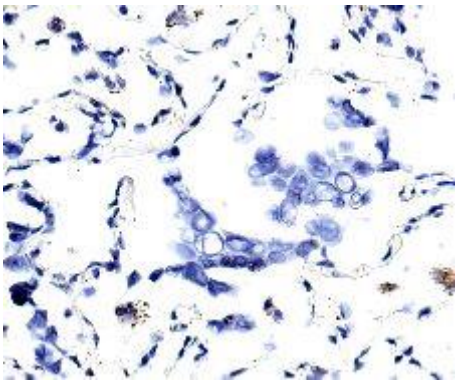



CPS

QC sample results:



Positive Control



Negative Control

## PD-L1 Introduction

PD-L1 (Programmed death-ligand 1, B7-H1 or CD274) protein, a type I transmembrane protein with a molecular weight of 40 kDa, is the ligand of PD-1 (programmed death-1). The encoding gene of PD-L1 is located on human chromosome 9p24.2 and its extracellular region of PD-L1 contains Ig-V and Ig-C-like domains. PD-1 is expressed in a wide range of cells, including T cells, B cells, dendritic cells, macrophages, mast cells, fibroblasts, and mesenchymal stem cells. PD-L1 is also widely expressed on the cell surface of tumor cells, and is proposed to be responsible for promoting tumor immune escape.

When binding to its ligand PD-L1, PD-1 can activate intracellular signaling pathways and inhibit the activity of killer T lymphocytes, thereby reducing the secretion of cytokines by immune cells. During infection and inflammation, interaction of PD-1 with PD-L1 helps to prevent autoimmunity to maintain immune homeostasis. In the tumor microenvironment, after binding to PD-L1 on the cell surface of tumor cells, PD-1 on the surface of tumor specific cytolytic T lymphocytes (CTL) can inhibit the activation of CTLs, and even promote the apoptosis of CTLs, ultimately leading to tumor immune escape.

Since the PD-L1/PD-1 signaling pathway plays a vital role in tumor immune escape, it becomes an important target of immunotherapy. Anti-PD-1 monoclonal antibodies (such as nivolumab and pembrolizumab) and anti-PD-L1 monoclonal antibodies (such as atezolizumab, durvalumab, and avelumab) could potentially restore the immune response of cytolytic T lymphocytes to tumor cells by blocking the PD-L1/PD-1 signaling pathway so as to prevent tumors from achieving immune evasion, and thus improve the prognosis of patients. Several antibodies targeting the PD-L1-PD-1 axis have been approved by the FDA for multiple cancers, including malignant melanoma(MM), non-small cell lung cancer (NSCLC), renal cell carcinoma(RCC), urothelial carcinoma, head and neck carcinoma, Hodgkin lymphoma, gastric cancer, hepatocellular carcinoma(HCC), small cell lung cancer and solid tumors with microsatellite instability-high(MSI-H). In addition, a large number of antibodies targeting the PD-L1-PD-1 axis are in clinical development.

Expression of PD-L1 is one of the more promising prognostic biomarker for predicting response to monotherapies blocking the PD-L1-PD-1 axis. In several clinical trials of antagonistic drugs designed to block PD-L1/PD-1, PD-L1 expression in tumor cells+/- immune cells has been used for stratifying patients. In addition, PD-L1 IHC is used as either the companion or the complementary diagnostic assay for several approved indications of PD-1/PD-L1 monoclonal antibodies.

Monoclonal antibody PD-L1 IHC 22C3 is the companion diagnostic antibody used in multiple registered clinical trials of pembrolizumab（KEYTRUDA）. It is also the first PD-L1 antibody approved for companion diagnosis of PD-1/PD-L1 immunotherapy in following indications: NSCLC, gastric or esophagogastric junction adenocarcinoma (GEJ), esophageal squamous cell carcinomas, cervical cancer, urothelial carcinoma, and head and neck cancers. In NSCLC, PD-L1 protein expression is accessed by tumor proportion score (TPS), which refers to percentage of complete or partial cell membrane staining of tumor cells with any intensity over total tumor cells in the denominator. PD-L1 positive is defined as TPS ⩾ 1% and high PD-L1 expression as TPS defin. While in

gastric or esophagogastric junction adenocarcinoma(GEJ), esophageal squamous cell carcinomas, cervical cancer, urothelial carcinoma, and head and neck cancers, PD-L1 expression is categorized by combined proportion score (CPS), which is defined as the sum of PD-L1 stained tumor cell and surrounding lymphocytes and macrophages divided by the total number of viable tumor cells multiplied by 100. In gastric or esophagogastric junction adenocarcinoma(GEJ), cervical cancer, and head and neck cancers, PD-L1 positive is defined as CPS ⩾ 1, and in esophageal squamous cell carcinomas and urothelial carcinoma, positive threshold is CPS ⩾ 10.

There is a lack of consensus on the correlation of the level of PD-L1 and efficacy of PD-1/PL-D1 inhibitors in solid tumors due to the presence of various anti-PD-L1 antibodies and multiple platforms for PD-L1 detection. Multiple clinical trials show that high expression of PD-L1 is a favorable prognostic biomarker, but regardless of how cut-off is defined, there is no definitive indication of whether the patient will benefit from the therapy. Thus, selecting threshold of positive PD-L1 expression for different cancer patients should be based on expected clinical benefit, patients ‘ability to shoulder economically, and other factors, particularly for therapies that PD-L1 detection has not been approved as companion diagnostics.

Clinical trials suggested that expression of PD-L1 is not an optimal prognostic biomarker for predicting response to PD-L1/PD-1 immunotherapy. Patients with positive or high PD-L1 expression have a higher objective response rate compared with those with negative or low PD-L1 expression. Many PD-L1-positive tumors, however, do not respond, and a few PD-L1-negative tumors do respond to PD-L1/PD-1 immunotherapy. Thus, detection and assessment of PD-L1 expression in the ongoing clinical trials is just the first step in exploring the prognostic marker for predicting response of PD-1/PD-L1 inhibitors and it should not be the prerequisite and the only standard for selecting candidate patients receiving PD-1/PD-L1 immunotherapy.

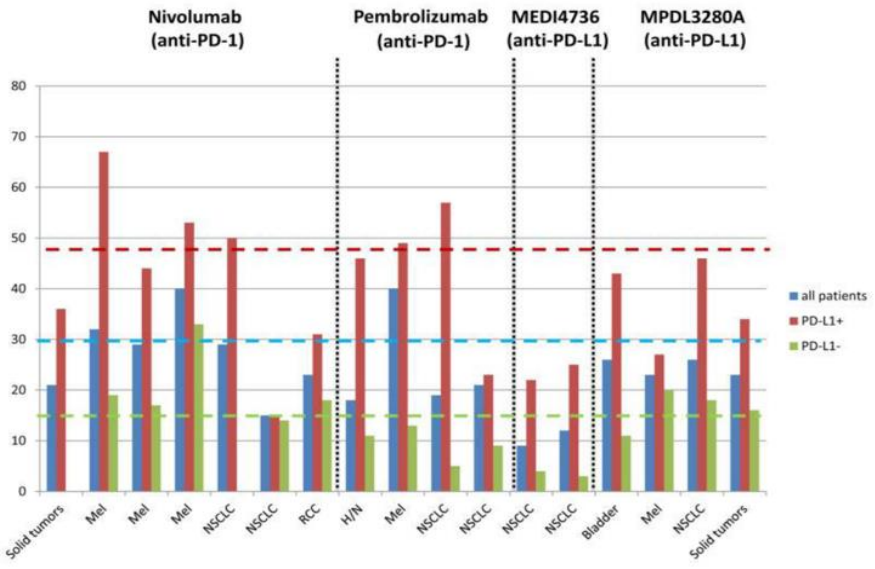



Figure 1. PD-L1 positive patients had better objective response to anti-PD-1/PD-L1 therapy than PD-L1 negative patients.（Graph was cited from Joel Sunshine, Janis M Taube. PD-1/PD-L1 inhibitors. Current Opinion in Pharmacology 2015,23:32-38）