VENTANA PD-L1 (SP263) Assay

REF 740-4907

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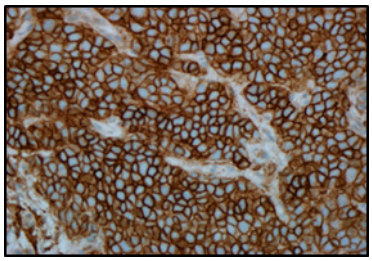

Figure 1. Non-small cell lung cancer stained with VENTANA PD-L1 (SP263) Assay.

INTENDED USE

VENTANA PD-L1 (SP263) Assay is a qualitative immunohistochemistry assay using rabbit monoclonal anti-PD-L1 clone SP263 intended for use in the assessment of the programmed death ligand-1 (PD-L1) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue specimens by light microscopy. The VENTANA PD-L1 (SP263) Assay is used with the OptiView DAB IHC Detection Kit for staining on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

PD-L1 protein expression in NSCLC is determined by the percentage of tumor cells (% TC) with any membrane staining above background.

VENTANA PD-L1 (SP263) Assay is indicated as an aid in identifying patients eligible for treatment with the therapies listed in Table 1 for the indication and PD-L1 status in accordance with the approved therapeutic product labeling.

Table 1. VENTANA PD-L1 (SP263) Assay Companion Diagnostic Indication.

Indication for Use	PD-L1 Cutoff	Therapy
NSCLC	≥ 1% TC	TECENTRIQ (atezolizumab)
	≥ 50% TC	LIBTAYO (cemiplimab-rwlc)

Results of the VENTANA PD-L1 (SP263) Assay should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA PD-L1 (SP263) Assay is an immunohistochemical (IHC) assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody (VENTANA PD-L1 (SP263) antibody) to recognize the programmed death ligand-1 (PD-L1) protein.

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors, programmed death-1 (PD-1) and B7-1.[1] PD-1 is an inhibitory receptor expressed on T-cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[2] Binding of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T-cells.[2] B7.1 is a molecule expressed on antigen presenting cells and activated T-cells. PD-L1 binding to B7.1 on T-cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.[3] PD-L1 expression has been observed in immune cells and malignant cells[4,5] and aberrant expression of PD-L1 on malignant cells has been reported to impede anti-tumor immunity, resulting in immune evasion.[2,5] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment.

The association between PD-L1 expression in TC or tumor-infiltrating immune cells (IC) and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported across multiple cancers.

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP263) Assay is a rabbit monoclonal primary antibody which binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies ULTRA LCS (Predilute) reagent (Cat. No. 650-210 / 05424534001), which minimizes evaporation of the aqueous reagents from the specimen slide.

MATERIAL PROVIDED

VENTANA PD-L1 (SP263) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PD-L1 (SP263) Assay contains approximately 8 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris-HCl with carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 1.6 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA PD-L1 (SP263) Assay is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
8. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
9. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
10. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
11. Permanent mounting medium
12. Cover glass
13. Automated coverslipper
14. General purpose laboratory equipment
15. BenchMark ULTRA or BenchMark ULTRA PLUS instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with OptiView DAB IHC Detection Kit and BenchMark ULTRA or BenchMark ULTRA PLUS instruments.

Based on testing of placenta and tonsil tissues that express PD-L1, the recommended tissue fixative is 10% neutral buffered formalin[6] (NBF) for a period of at least 6 hours up to 72 hours. Acceptable fixatives for use with VENTANA PD-L1 (SP263) Assay are Zinc Formalin and Z-5 fixatives when used with at least 6 hours of fixation time. Other fixatives, including 95% alcohol, AFA and PREFER fixative, are unacceptable for use with VENTANA PD-L1 (SP263) Assay. The amount of fixative used is 15 to 20 times the

volume of tissue. Fixation can be performed at 15-25°C. Refer to VENTANA PD-L1 (SP263) Assay Interpretation Guide for Non-Small Cell Lung Cancer (P/N 1020383US) for further discussion of the impact of specimen preparation on PD-L1 staining with VENTANA PD-L1 (SP263) Assay. Sections should be cut at approximately 4-5 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[7,8]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark ULTRA or BenchMark ULTRA PLUS instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 2. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

STAINING PROCEDURE

VENTANA PD-L1 (SP263) Assay has been developed for use on BenchMark ULTRA and BenchMark ULTRA PLUS instruments in combination with VENTANA detection kits and accessories. Refer to the table below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

Table 3. Recommended staining protocol for VENTANA PD-L1 (SP263) Assay with OptiView DAB IHC Detection Kit on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

Staining Procedure: U VENTANA PD-L1 (SP263) Assay	
Procedure Parameter	**Selection**
Deparaffinization	Selected
Baking	Optional, 60ºC, 12 minutes
Cell Conditioning	CC1 Cell Conditioning, 64 minutes
Pre-primary Antibody Peroxidase	Selected
Antibody (Primary)	VENTANA PD-L1 (SP263) Selected, 16 minutes, 36ºC or Negative Control Selected, 16 minutes, 36ºC
OptiView HQ Linker	8 minutes (default)
OptiView HQ Multimer	8 minutes (default)
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

NEGATIVE REAGENT CONTROL

A matched negative reagent control slide must be run for every specimen to aid in the interpretation of results. Rabbit Monoclonal Negative Control Ig is a matched negative reagent control antibody for this assay and is used in place of the primary antibody to evaluate non-specific staining. The staining procedure for the negative reagent control should be identical to the primary antibody. Use of a different negative control reagent, or failure to use the recommended negative control reagent, may result in false interpretation of the assay-stained slide.

POSITIVE TISSUE CONTROL

A tissue control must be included with each staining run. This helps identify any failures applying reagents to the slide. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections. Such tissue may monitor all steps of the analysis, from tissue preparation through staining.

Qualified normal human term placental tissue can be used as a tissue control for VENTANA PD-L1 (SP263) Assay. A placenta sample used as a tissue control must exhibit the staining pattern described as acceptable in Table 4. Placenta tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. Appropriate staining of placental tissue components is described in Table 4 and in the interpretation guide (P/N 1020383US).

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimens included in the same staining run should be considered invalid.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by VENTANA PD-L1 (SP263) Assay. The cellular staining pattern for VENTANA PD-L1 (SP263) Assay is membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membrane, diffuse cytoplasmic, and/or punctate staining. The stained slide(s) are interpreted using light microscopy. A qualified pathologist experienced in IHC staining interpretation must evaluate tissue controls before interpreting patient results.

Refer to VENTANA PD-L1 (SP263) Assay Interpretation Guide for Non-Small Cell Lung Cancer (P/N 1020383US) for specifics and images.

Placenta Tissue Control

Placenta tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. The positive and negative staining elements should be examined to ascertain that all reagents are functioning properly. If these elements fail to demonstrate appropriate staining, any results with the test specimens included in the same staining run should be considered invalid.

Placenta tissue stained with VENTANA PD-L1 (SP263) Assay shows moderate to strong uniform staining of the membrane and weak to strong uniform staining of the cytoplasm of trophoblast-lineage cells. Placental stromal tissue and vasculature can be used for assessment of any background staining (Table 4).

Table 4. Placenta tissue control evaluation criteria for VENTANA PD-L1 (SP263) Assay.

Interpretation	Staining Description
Acceptable	Moderate to strong uniform membrane staining of trophoblast-lineage cells, and placental stroma and vasculature with no staining.
Unacceptable	No to weak uniform membrane staining of trophoblast-lineage cells and/or specific staining within placental stromal and vascular tissue.

Negative Reagent Control

Non-specific staining, if present, may have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Examples of background staining for this assay can be found in the interpretation guide (P/N 1020383US).

Patient Tissue

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 5 and Table 6. Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of Non-Small Cell Lung Cancer (P/N 1020383US) for representative images and instructions for scoring.

The cellular staining pattern for VENTANA PD-L1 (SP263) Assay is membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membrane, diffuse cytoplasmic, and/or punctate staining. Tumor cell cytoplasmic staining, if present, is not considered positive for scoring purposes.

Tumor cells are scored as the percentage of tumor cells with PD-L1 membrane staining at any intensity above background staining as noted on the corresponding negative control.

It can be particularly challenging to estimate the percentage of tumor cells with membrane staining for cases that fall near the PD-L1 expression level cutoffs relevant to the diagnostic indication. For these borderline cases, it is recommended to also view the case at a magnification that enables the entire tumor and/or tumor area to be assessed. In addition, consultation with a second pathologist is recommended per standard practice.

Scoring Algorithm - NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 5) and the non-specific background scoring criteria (Table 6). Refer to the interpretation guide (P/N 1020383US) for additional instructions and representative images.

Table 5. VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC.

PD-L1 Interpretation	Staining Description
≥ 1%	≥ 1% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
< 1%	< 1% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
≥ 50%	≥ 50% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
< 50%	< 50% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.

For cases scored around the 1% cutoff (0% to 9%TC), consultation with a second pathologist is recommended per standard medical practice. Reporting of the final results based on a consensus score should be considered.

For cases scored around the 50% cutoff (40% to 59% TC), consultation with a second pathologist is recommended per standard medical practice. Reporting of the final results based on a consensus score should be considered.

Table 6. Non-specific background scoring criteria for VENTANA PD-L1 (SP263) Assay.

Interpretation	Staining Description
Acceptable	Non-specific staining that is not obtrusive to interpretation of specific staining.
Unacceptable	Non-specific staining that is obtrusive to interpretation of specific staining.

SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP263) Assay has been developed for BenchMark ULTRA and BenchMark ULTRA PLUS instruments with the OptiView DAB IHC Detection Kit and is not approved with any other detection or instrument.
2. A patient specimen slide should be stained with Rabbit Monoclonal Negative Control Ig. Other negative control reagents are not suitable for this assay.
3. This assay has not been validated for use with cytology samples or decalcified bone specimens.
4. Cold ischemia testing of VENTANA PD-L1 (SP263) Assay using a xenograft tissue model did not establish any conditions from zero hours to up to 24 hours that were not favorable with the assay.
5. Cut-slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slides stability within their own environment beyond 45 days, when desired.
6. For optimal results, consultation with a second pathologist with reporting on the consensus result is recommended for cases scored near the cutoffs for companion diagnostic indications.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, precision, and method comparison were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity was evaluated by characterizing PD-L1 prevalence in the intended use NSCLC tissue samples. The overall prevalence of PD-L1 positive cases based on the tumor cell expression ≥ 1% cutoff was 60% and for ≥ 50% cutoff was 19%.

For the evaluation of analytical specificity, arrays containing a variety of normal tissues were stained with VENTANA PD-L1 (SP263) Assay and evaluated for presence of membranous PD-L1 staining as listed in Table 7.

In addition, an array of neoplastic tissues was evaluated for tumor cell and immune cell staining with VENTANA PD-L1 (SP263) Assay as described in Table 8.

Table 7. Specificity of VENTANA PD-L1 (SP263) Assay was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Esophagus [a,b]	1/3
Cerebellum	0/3	Stomach [a,b]	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Adrenal gland [a]	0/3	Small intestine [b]	0/3
Ovary	0/3	Colon [b]	0/3
Pancreas [a]	0/3	Liver	0/3
Lymph node [b]	0/3	Salivary gland [b]	0/3
Parathyroid gland	0/4	Kidney [b]	0/3
Hypophysis [a,b]	0/3	Prostate	0/3
Testis	0/3	Bladder	0/3
Thyroid [a,b]	0/3	Endometrium	0/3
Breast	0/3	Cervix	0/3
Spleen [b]	0/3	Skeletal muscle	0/3
Tonsil [b]	3/3	Skin [c]	0/4
Thymus gland [b]	0/3	Nerve (sparse)	0/3
Myeloid (bone marrow) [a,b]	0/4	Mesothelium [b]	0/3
Lung [b]	0/3	Larynx [b]	0/3
Heart	0/3		

Additional staining observed: [a] Cytoplasmic staining, [b] Immune cell staining, [c] Melanocyte staining.

Percent of immune cells present above background cannot be evaluated in this study because there is no tumor area for which to score tumor infiltrating immune cells.

Table 8. Specificity of VENTANA PD-L1 (SP263) Assay was determined by testing a variety of FFPE neoplastic tissues for any tumor cell membranous and immune cell staining.

Pathology	# positive / total cases	
	Tumor Cells	Immune Cells
Glioblastoma (Cerebrum)	0/1	1/1
Meningioma (Cerebrum)	0/1	0/1
Ependymoma (Cerebrum)	0/1	1/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Serous adenocarcinoma (Ovary)	0/1	1/1
Adenocarcinoma (Ovary)	1/1	0/1
Neuroendocrine neoplasm (Pancreas)	0/1	0/1
Adenocarcinoma (Pancreas)	0/1	1/1
Seminoma (Testis)	0/1	0/1
Embryonal carcinoma (Testis)	0/1	0/1
Medullary carcinoma (Thyroid)	0/1	0/1
Papillary carcinoma (Thyroid)	1/1	0/1
Ductal carcinoma in situ (Breast)	0/1	1/1
Invasive ductal carcinoma (Breast)	0/2	0/2
B-cell lymphoma; NOS (Spleen)	0/1	1/1
Small cell carcinoma (Lung)	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	1/1
Adenocarcinoma (Lung)	0/1	0/1
Neuroendocrine carcinoma (Esophagus)	0/1	0/1
Adenocarcinoma (Esophagus)	0/1	0/1
Signet-ring cell carcinoma (Stomach)	0/1	0/1
Adenocarcinoma (Small intestine)	0/1	0/1
Stromal sarcoma (Small intestine)	0/1	0/1
Adenocarcinoma (Colon)	0/1	1/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1	0/1
Adenocarcinoma (Rectum)	0/1	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1	0/1
Hepatocellular carcinoma (Liver)	0/1	0/1
Hepatoblastoma (Liver)	0/1	0/1
Clear cell carcinoma (Kidney)	0/1	0/1
Adenocarcinoma (Prostate)	0/2	0/2
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	0/1	0/1
Clear cell carcinoma (Uterus)	1/1	0/1
Squamous cell carcinoma (Cervix)	0/2	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1	0/1
Melanoma (Rectum)	0/1	0/1
Basal cell carcinoma (Skin)	0/1	0/1
Squamous cell carcinoma (Skin)	0/1	0/1
Neurofibroma (Lumbar)	0/1	1/1
Neuroblastoma (Retroperitoneum)	0/1	0/1
Mesothelioma (Abdominal cavity)	0/1	0/1
B-cell lymphoma; NOS (Mediastinum)	1/1	1/1
Hodgkin lymphoma (Lymph node)	1/1	1/1
B-cell lymphoma; NOS (Lymph node)	1/1	1/1
Anaplastic large cell lymphoma (Lymph node)	1/1	1/1
Leiomyosarcoma (Bladder)	0/1	0/1
Osteosarcoma	0/1	1/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1	0/1
Leiomyosarcoma (Smooth muscle)	0/1	0/1
Urothelial carcinoma (Bladder)	1/1	1/1

ANALYTICAL PERFORMANCE IN NON-SMALL CELL LUNG CANCER TISSUE

Tissue Thickness

Tissue thickness was evaluated using 7 unique NSCLC specimens (2 PD-L1 < 1% and 5 PD-L1 ≥ 1%; 5 PD-L1 < 50% and 2 PD-L1 ≥ 50%). Duplicate sections at 2, 3, 5, 6, and 7 microns were tested for each case. Four microns thickness was used as reference. 2 and 5 microns thickness demonstrated concordant PD-L1 protein expression and acceptable background levels for VENTANA PD-L1 (SP263) Assay staining when compared to the reference of 4 microns. 3, 6, and 7 microns exhibited a change in PD-L1 protein

expression compared to the reference. Ventana recommends that specimens be cut at 4-5 microns for staining with VENTANA PD-L1 (SP263) Assay.

Repeatability and Intermediate Precision

The repeatability and intermediate precision of VENTANA PD-L1 (SP263) Assay was evaluated on the BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit by staining 24 unique cases of human NSCLC.

For within-day repeatability, 5 replicate slides from each of the NSCLC specimens (11 PD-L1 < 1% and 13 PD-L1 ≥ 1%; 11 PD-L1 < 50% and 13 PD-L1 ≥ 50%) were stained on a single BenchMark ULTRA instrument within one day.

For between-day precision, 2 replicate slides from each of the NSCLC specimens (11 PD-L1 < 1% and 13 PD-L1 ≥ 1%; 11 PD-L1 < 50% and 13 PD-L1 ≥ 50%) were stained with VENTANA PD-L1 (SP263) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days in a span of at least 20 days.

For between-instrument and between-lot precision, 27 slides each from 24 unique NSCLC specimens (10 PD-L1 < 1% and 14 PD-L1 ≥ 1%; 12 PD-L1 < 50% and 12 PD-L1 ≥ 50%) were stained with VENTANA PD-L1 (SP263) Assay using three lots of VENTANA PD-L1 (SP263) Antibody and three lots of OptiView DAB IHC Detection Kit on three BenchMark ULTRA instruments.

All slides were blinded, randomized, and evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm (Table 5).

A summary of the results can be found in Table 9.

Table 9. Repeatability and intermediate precision study of VENTANA PD-L1 (SP263) Assay on individual NSCLC specimens.

Cutoff	Repeatability / Intermediate Precision Parameter	Agreement % (n/N), (95% CI) [a]
≥ 1%	Within-day Repeatability (within a single day)	PPA: 100.0 (65/65), (94.4-100.0) NPA: 100.0 (55/55), (93.5-100.0) OPA: 100.0 (120/120), (96.9-100.0)
	Between-day Precision (5 non-consecutive days)	PPA: 100.0 (130/130), (97.1-100.0) NPA: 100.0 (110/110), (96.6-100.0) OPA: 100.0 (240/240), (98.4-100.0)
	Between-instrument and Between-lot precision (3 instruments, 3 antibody lots and 3 detection kit lots)	PPA: 100.0 (378/378), (99.0-100.0) NPA: 100.0 (270/270), (98.6-100.0) OPA: 100.0 (648/648), (99.4-100.0)
≥ 50%	Within-day Repeatability (within a single day)	PPA: 100.0 (65/65), (94.4-100.0) NPA: 100.0 (55/55), (93.5-100.0) OPA: 100.0 (120/120), (96.9-100.0)
	Between-day Precision (5 non-consecutive days)	PPA: 100.0 (130/130), (97.1-100.0) NPA: 100.0 (110/110), (96.6-100.0) OPA: 100.0 (240/240), (98.4-100.0)
	Between-instrument and Between-lot precision (3 instruments, 3 antibody lots and 3 detection kit lots)	PPA: 97.2 (315/324), (92.6-100.0) NPA: 97.5 (316/324), (94.7-99.4) OPA: 97.4 (631/648), (94.9-99.2)

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, OPA = Overall Percent Agreement.

[a] 2-sided 95% confidence intervals (CI) were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

Reader Precision Studies

To assess between- and within-reader precision, three pathologists evaluated a minimum of 110 unique cases. The cases were blinded and randomized prior to evaluation for PD-L1 IHC staining per the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 5. The results provided in Table 10 reflect the between-reader and within -reader precision rates for unique cases from the study cohort.

Table 10. Between- and within-reader precision of VENTANA PD-L1 (SP263) Assay staining NSCLC specimens.

Cutoff	Reader Precision	Agreement % (n/N), (95% CI) [a]
≥ 1%	Between-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 94.3 (362/384), (90.5-97.4) ANA: 92.6 (274/296), (87.8-96.5) OPA: 93.5 (318/340), (89.9-97.1)
	Within-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 96.7 (376/389), (94.7-98.3) ANA: 95.6 (280/293), (92.9-97.8) OPA: 96.2 (328/341), (94.1-98.0)
≥ 50%	Between-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 94.6 (298/315), (90.6-97.8) ANA: 95.0 (320/337), (91.1-97.9) OPA: 94.8 (309/326), (91.2-97.8)
	Within-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 97.2 (310/319), (95.2-98.8) ANA: 97.3 (326/335), (95.2-98.9) OPA: 97.2 (318/327), (95.4-98.8)

APA = Average Positive Agreement, ANA = Average Negative Agreement, OPA = Overall Percent Agreement.

[a] 2-sided 95% confidence intervals (CI) were calculated using the percentile bootstrap method from 2000 bootstrap samples.

Inter-Laboratory Reproducibility Study - BenchMark ULTRA

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP263) Assay staining was conducted to demonstrate reproducibility of the assay in determining PD-L1 protein expression in NSCLC tissue specimens on BenchMark ULTRA instruments. Twenty-eight unique NSCLC specimens with a range of PD-L1 expression were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. The specimens were randomized before evaluation by 6 readers (2 readers/site) blinded to the sample identity. At each site, the stained slides were independently evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 5). Results are summarized in Table 11.

Table 11. Inter-laboratory reproducibility of VENTANA PD-L1 (SP263) Assay staining of NSCLC specimens.

Cutoff	Inter-laboratory Reproducibility	Agreement % (n/N), (95% CI) [e]
≥ 1% [a]	Overall agreement [c] (compared to a consensus score, across sites, days and readers)	PPA: 99.5 (418/420), (98.6-100.0) NPA: 100.0 (419/419), (99.1-100.0) OPA: 99.8 (837/839), (99.3-100.0)
	Between-site agreement [d] (average of site-to-site pairwise comparisons)	APA: 99.5 (8320/8360), (98.6-100.0) ANA: 99.5 (8360/8400), (98.6-100.0) OPA: 99.5 (8340/8380), (98.6-100.0)
	Between-reader agreement [d] (average of reader-to-reader pairwise comparisons within each site)	APA: 100.0 (418/418), (99.1-100.0) ANA: 100.0 (420/420), (99.1-100.0) OPA: 100.0 (419/419), (99.1-100.0)
≥ 50% [b]	Overall agreement [c] (compared to a consensus score, across sites, days and readers)	PPA: 94.3 (395/419), (90.2-98.1) NPA: 90.1 (374/415), (85.1-94.7) OPA: 92.2 (769/834), (89.0-95.2)
	Between-site agreement [d] (average of site-to-site pairwise comparisons)	APA: 87.5 (7610/8698), (82.2-92.0) ANA: 86.2 (6774/7862), (80.5-91.3) OPA: 86.9 (7192/8280), (81.5-91.7)

Cutoff	Inter-laboratory Reproducibility	Agreement % (n/N), (95% CI) [e]
	Between-reader agreement [d] (average of reader-to-reader pairwise comparisons within each site)	APA: 88.5 (386/436), (83.6-92.9) ANA: 87.4 (346/396), (81.5-92.5) OPA: 88.0 (366/416), (82.7-92.7)

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, APA = Average Positive Agreement, ANA = Average Negative Agreement, OPA = Overall Percent Agreement.

[a] n = 839 PD-L1 slide observations

[b] n = 834 PD-L1 slide observations

[c] Agreement of study results with the case-level modal PD-L1 status.

[d] Pairwise agreement rates

[e] 95% CI = Confidence interval

Note: For PPA/NPA/OPA, 95% CIs were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

For APA/ANA, 95% CIs were calculated using the transformed Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

Method Comparison Study on BenchMark ULTRA PLUS vs BenchMark ULTRA instrument

Three laboratories, from separate institutions in the United States, participated in a concordance study between the BenchMark ULTRA PLUS instrument and the BenchMark ULTRA instrument. A total of 196 unique commercially acquired, anonymized NSCLC tissue specimens representing the staining range of the VENTANA PD-L1 (SP263) Assay at 1% and 50% cutoffs were used in the study. The tissue cohort contained 2 overlapping 140-case analysis population with a balanced distribution between PD-L1 positive and PD-L1 negative cases for each cutoff as determined by Roche Tissue Diagnostics (RTD) pathologists' consensus review. For each case, two tissue slides were stained with VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig on a BenchMark ULTRA instrument using the recommended staining protocol at RTD, and two additional tissue slides were stained with VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig on a BenchMark ULTRA PLUS instrument using the recommended staining protocol at one of the three external laboratories. Each site stained approximately 1/3 of the study cases on a BenchMark ULTRA PLUS instrument. Case slides were randomized prior to staining on BenchMark ULTRA or BenchMark ULTRA PLUS instruments. Two pathologists from each external laboratory and one RTD pathologist, blinded to the sample identity, independently evaluated all BenchMark ULTRA slides and all BenchMark ULTRA PLUS slides using the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 5) with at least two week washout period between the reads. The performance equivalence of VENTANA PD-L1 (SP263) Assay on the investigational instrument (BenchMark ULTRA PLUS) relative to the reference instrument (BenchMark ULTRA) was assessed separately at the 1% and 50% cutoffs by pooling the agreement of all seven pathologists per cutoff. Results are summarized in Table 12.

Table 12. Pooled Agreement of PD-L1 Status Between BenchMark ULTRA PLUS and BenchMark ULTRA at 1% and 50 % Cutoffs.

1% Cutoff [a]	BenchMark ULTRA		
BenchMark ULTRA PLUS	≥ 1%	< 1%	Total
≥ 1%	474	12	486
< 1%	6	473	479
Total	480	485	965
Agreement [c]	**% (n/N) (95% CI [d])**		
Positive percent agreement	98.8 (474/480) (97.5-99.8)		
Negative percent agreement	97.5 (473/485) (96.2-98.7)		
Overall percent agreement	98.1 (947/965) (97.2-99.0)		
50% Cutoff [b]	**BenchMark ULTRA**		
BenchMark ULTRA PLUS	≥ 50%	< 50%	Total
≥ 50%	478	21	499
< 50%	5	464	469
Total	483	485	968
Agreement [c]	**% (n/N) (95% CI [d])**		
Positive percent agreement	99.0 (478/483) (97.5-100.0)		
Negative percent agreement	95.7 (464/485) (93.4-97.9)		
Overall percent agreement	97.3 (942/968) (96.0-98.6)		

[a] n = 965 evaluable observations

[b] n = 968 evaluable observations

[c] The pooled agreement pools all cases and readers.

[d] 95% CI = Confidence interval. Two-sided 95% CI were calculated using the percentile bootstrap method with 2000 replicates stratified by diagnostic score bin (positive, negative, borderline positive, borderline negative).

Inter-Laboratory Reproducibility Study - BenchMark ULTRA PLUS

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP263) Assay staining was conducted on BenchMark ULTRA PLUS instruments to demonstrate reproducibility of the assay in determining PD-L1 expression in NSCLC tissue specimens at 1% and 50% cutoffs. Thirty-eight unique commercially acquired NSCLC specimens with a range of PD-L1 expression were used in the study. The tissue cohort contained 2 over-lapping 28-case analysis populations with a balanced distribution between PD-L1 positive and PD-L1 negative cases for each cutoff as determined by RTD pathologists' consensus review. Each case was stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Case slides were randomized prior to staining on a BenchMark ULTRA PLUS instrument. At each site, the stained slides were independently evaluated by 2 pathologists, blinded to the sample identity, using the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 5). Results are summarized in Table 13. Please note that, due to an observed decrease in precision for cases with borderline expression, consultation with a second pathologist is recommended per standard medical practice for cases with borderline expression levels for both the 1% and 50% cutoff. Please refer to the limitation in the Scoring Algorithm and the Specific Limitations section.

Table 13. Inter-laboratory reproducibility of VENTANA PD-L1 (SP263) Assay staining of NSCLC specimens on BenchMark ULTRA PLUS instruments.

Cutoff	Inter-laboratory Reproducibility	Agreement % (n/N), (95% CI) [d]
≥ 1% [a]	Overall agreement [b] (compared to a consensus score, across sites, days and readers)	PPA: 96.2 (404/420), (91.6-100.0) NPA: 100.0 (420/420), (99.1-100.0) OPA: 98.1 (824/840), (95.6-100.0)

Cutoff	Inter-laboratory Reproducibility	Agreement % (n/N), (95% CI) [d]
	Between-site agreement [c] (average of site-to-site pairwise comparisons)	APA: 96.8 (7822/8080), (92.5-100.0) ANA: 97.0 (8462/8720), (93.0-100.0) OPA: 96.9 (8142/8400), (92.9-100.0)
	Between-reader agreement [c] (average of reader-to-reader pairwise comparisons within each site)	APA: 97.0 (392/404), (93.2-100.0) ANA: 97.2 (424/436), (93.5-100.0) OPA: 97.1 (408/420), (93.6-100.0)
≥ 50% [a]	Overall agreement [b] (compared to a consensus score, across sites, days and readers)	PPA: 95.9 (374/390), (93.1-98.6) NPA: 98.9 (445/450), (96.9-100.0) OPA: 97.5 (819/840), (95.8-99.0)
	Between-site agreement [c] (average of site-to-site pairwise comparisons)	APA: 94.8 (7188/7580), (91.8-98.0) ANA: 95.7 (8828/9220), (92.6-98.4) OPA: 95.3 (8008/8400), (92.2-98.2)
	Between-reader agreement [c] (average of reader-to-reader pairwise comparisons within each site)	APA: 94.5 (358/379), (91.1-97.8) ANA: 95.4 (440/461), (92.0-98.4) OPA: 95.0 (399/420), (91.7-98.1)

APA = Average Positive Agreement, ANA = Average Negative Agreement, PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, OPA = Overall Percent Agreement.

[a] n = 840 PD-L1 slide observations

b Agreement of study results with the case-level modal PD-L1 status.

[c] Pairwise agreement rates

[d] 95% CI = Confidence interval

Note: For PPA/NPA/OPA, 95% CIs were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

For APA/ANA, 95% CIs were calculated using the transformed Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

CLINICAL PERFORMANCE IN NON-SMALL CELL LUNG CANCER

Clinical Performance of TECENTRIQ (atezolizumab) in IMpower010 Study

The clinical performance of VENTANA PD-L1 (SP263) Assay was evaluated in IMpower010 (NCT02486718), a Phase III, open-label, randomized study to investigate the efficacy and safety of TECENTRIQ (atezolizumab) (anti−PD L1 antibody) compared with best supportive care following adjuvant cisplatin-based chemotherapy in patients with completely resected stage IB-IIIA NSCLC.

A total of 1280 enrolled patients had complete tumor resection and were eligible to receive up to 4 cycles of cisplatin-based chemotherapy. A total of 1005 patients were randomized (1:1) to receive TECENTRIQ 1200 mg by intravenous infusion every 3 weeks for 16 cycles unless disease recurrence or unacceptable toxicity, or Best Supportive Care (BSC), following recovery from surgery. Randomization was stratified by sex, stage of disease, histology, and PD-L1 expression. Among randomized patients, 12% of patients had stage IB, 47% had stage II and 41% had stage IIIA disease.

Tumor specimens from 1169 of the 1280 enrolled patients (including 985 of the 1005 randomized patients) were tested with VENTANA PD-L1 (SP263) Assay to determine their PD-L1 expression level. The biomarker evaluable population for the VENTANA PD-L1 (SP263) Assay in intent-to-treat (ITT) population was comprised of 979 patients. The percentage of randomized patients who had tumors with PD-L1 expression on ≥ 1% of tumor cells (TC) as determined by VENTANA PD-L1 (SP263) Assay was 53%. The final staining acceptability rate among patients in the intended use population of the VENTANA PD-L1 (SP263) Assay was 99.3%.

The primary efficacy outcome measure of IMpower010 was disease-free survival (DFS) as assessed by the investigator. The primary efficacy analysis population (n = 476) was patients with Stage II – IIIA NSCLC with PD-L1 expression on ≥ 1% of tumor cells (PD-L1 ≥ 1% TC). DFS was defined as the time from the date of randomization to the date of occurrence of any of the following: first documented recurrence of disease, new primary NSCLC, or death due to any cause, whichever occurred first. A key secondary efficacy outcome measure was overall survival (OS) in the ITT population.

At the time of the interim DFS analysis (clinical data cutoff date: 21-Jan-2021), the study demonstrated a statistically significant improvement in DFS in the TECENTRIQ arm compared with the BSC arm in the PD-L1 ≥ 1% TC, stage II - IIIA patient population (stratified HR: 0.66, 95% CI (0.50, 0.88), p-value 0.004). Efficacy results are presented in Table 14.

At the time of the DFS interim analysis 19% of patients in the PD-L1 ≥1% TC stage II - IIIA patient population had died. An exploratory analysis of OS in this population resulted in a stratified HR of 0.77 (95% CI: 0.51, 1.17).

Table 14. Efficacy Results from IMpower010 in Patients Stage II - IIIA NSCLC with PD-L1 expression ≥ 1% TC.

	Arm A (TECENTRIQ) n = 248	Arm B (Best Supportive Care) n = 228
DFS events (%)	88 (35.5)	105 (46.1)
Median DFS, months (95% CI)	NR (36.1, NE)	35.3 (29.0, NE)
Hazard ratio [a] (95% CI)	0.66 (0.50, 0.88)	
p-value	0.004	

DFS = Disease-Free Survival, CI = Confidence Interval, NE = Not Estimable, NR = Not Reached.

[a] Stratified by stage, sex, and histology.

Clinical Performance of LIBTAYO (cemiplimab-rwlc) in EMPOWER-Lung 1 Study

EMPOWER-Lung 1 Clinical Study Results

Safety and efficacy of LIBTAYO (cemiplimab-rwlc) was evaluated in EMPOWER-Lung 1 (NCT03088540), a randomized, multi-center, open-label, active-controlled trial in patients with locally advanced NSCLC who were not candidates for surgical resection, or definitive chemoradiation, or with metastatic NSCLC.

The trial was designed to enroll patients whose tumors had high PD-L1 expression (Tumor Proportion Score (TPS) ≥ 50%) as determined by an FDA approved immunohistochemistry assay (referred to hereafter as Clinical Trial Assay or CTA), and who had not received prior systemic treatment for metastatic NSCLC. A total of 710 patients (Intent-To-Treat (ITT) population) were enrolled, and an analysis was performed on a population (n=563) who had PD-L1 expression of TPS ≥ 50%.

Patients were randomized (1:1) to receive LIBTAYO 350 mg intravenously (IV) every 3 weeks for up to 108 weeks or a platinum doublet chemotherapy regimen for 4 to 6 cycles followed by optional pemetrexed maintenance for patients with non-squamous histology who received a pemetrexed containing regimen. Randomization was stratified by histology (non-squamous vs squamous) and geographic region (Europe vs Asia vs rest of the world).

The major efficacy endpoints of the study were overall survival (OS) and progression-free survival (PFS). In the population with TPS ≥ 50%, the trial demonstrated statistically significant improvement in OS and PFS for patients randomized to LIBTAYO as compared with chemotherapy (Table 16). Similar efficacy was observed in the ITT population (clinical data cutoff date: 01-Mar-2020).

VENTANA PD-L1 (SP263) Assay Clinical Performance for EMPOWER-Lung 1 PD-L1 ≥ 50% TC NSCLC population – Clinical Bridging Study

Clinical performance of the VENTANA PD-L1 (SP263) Assay was evaluated using archived clinical study samples from EMPOWER-Lung 1. A total of 871 clinical trial specimens were retrospectively tested with VENTANA PD-L1 (SP263) Assay, 481 from randomized patients and 390 from a random subset of the screen-failed patients. Staining acceptability rates for VENTANA PD-L1 (SP263) Assay were evaluated at the subject level. The final staining acceptability rate in the Intended Use (IU) population was 92.8% (95% CI: 90.9, 94.4).

Agreement of PD-L1 status between VENTANA PD-L1 (SP263) Assay and CTA results was calculated using the CTA results as the reference. The concordance analysis results are shown in Table 15.

Table 15. PD-L1 Status Concordance between the VENTANA PD-L1 (SP263) Assay Results and EMPOWER-Lung 1 Study CTA Results [a]

PD-L1 (SP263) Status (CDx) [c]	PD-L1 Status (CTA) [b]		
	Positive	Negative	Total
Positive	324	24	348
Negative	68	352	420
Total	392	376	768
Agreement rates [d]	PPA = 82.7% (324/392) (95% CI: 78.6-86.1)	NPA = 93.6% (352/376) (95% CI: 90.7-95.7)	OPA = 88.0% (676/768) (95% CI: 85.5-90.1)

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, OPA = Overall Percent Agreement.

[a] All patients who had evaluable CTA and VENTANA PD-L1 (SP263) Assay results, excluding patients whose final VENTANA PD-L1 (SP263) Assay result was associated with a diagnostic protocol deviation.

[b] Performed according to the approved labeling. For the purpose of the analyses, a PD-L1 (CTA) TPS ≥ 50% result was considered positive and a PD-L1 (CTA) TPS < 50% result was considered negative.

[c] For the purpose of the analyses, a PD-L1 (SP263) expression ≥ 50% TC result was considered positive and a PD-L1 (SP263) expression < 50% TC result was considered negative.

[d] Two-sided 95% CI were calculated using the Wilson score method.

LIBTAYO efficacy was evaluated in the population with PD-L1 expression in tumor cells (TC) ≥ 50% identified by the VENTANA PD-L1 (SP263) Assay (SP263+) using the method described by Li 2015.[9] For the subset of SP263+ (PD-L1 (SP263) expression ≥ 50% TC)/ CTA+ (TPS ≥ 50%) patients, efficacy was estimated based on EMPOWER-Lung 1 study data. For the subset of SP263+/CTA- (TPS < 50%) patients, a range of scenarios were considered where efficacy was assumed to be the same (best-case scenario) or attenuated with respect to the SP263+/CTA+ subset (a hazard ratio (HR) = 1 was assumed under the worst-case scenario). Efficacy for the SP263+ patients was determined as the weighted average of the SP263+/CTA+ and SP263+/CTA - groups as described in Li 2015.[9] Sensitivity analyses were performed to account for the impact of missing VENTANA PD-L1 (SP263) Assay results via multiple imputation. The drug efficacy including both available and imputed PD-L1 (SP263) test results was estimated using the same statistical methods used in the EMPOWER-Lung 1 study efficacy analysis. Observed efficacy in SP263+/CTA+ patients were similar to efficacy observed in CTA+ patients in the EMPOWER-Lung 1 Study (Table 16).

Table 16. Efficacy Results from EMPOWER-Lung 1 Study in CTA+ Patients

Endpoints	CTA+ [a,b] (N=563)	
	LIBTAYO n=283	Chemotherapy n=280
Overall Survival		
Number of deaths (%)	70 (24.7)	105 (37.5)
Median in months (95% CI) [c]	NR (17.9, NE)	14.2 (11.2, 17.5)
Hazard ratio (95% CI) [d]	0.57 (0.42, 0.77)	
p-Value	0.0002	
Progression-free Survival per BICR		
Number of events (%)	147 (51.9)	197 (70.4)
Median in months (95% CI) [c]	8.2 (6.1, 8.8)	5.7 (4.5, 6.2)
Hazard ratio (95% CI) [d]	0.54 (0.43, 0.68)	
p-Value	< 0.0001	

BICR: blinded independent central review; CI: confidence interval; NE: Not evaluable; NR: Not reached; LIBTAYO: cemiplimab-rwlc

[a] CTA+ refers to subset of randomized patients with PD-L1 expression of TPS ≥ 50% tumor cell (TC) for CTA according to the approved labeling

[b] From EMPOWER-Lung 1 Study

[c] Based on Kaplan-Meier method

[d] Based on stratified proportional hazards model

TROUBLESHOOTING

Troubleshooting guidance is provided in the table below. If a problem cannot be attributed to any of these causes, or if the suggested corrective action fails to resolve the problem, consult your local support representative.

Table 17. Troubleshooting Guidance for VENTANA PD-L1 (SP263) Assay.

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that the recommended staining procedure was used.
		Verify that VENTANA PD-L1 (SP263) was selected for Primary Antibody.
	Degradation of tissue	Verify tissue was stained within the recommended time frame following sectioning.
	Dispenser malfunction	Verify nozzle cap is removed.
		Ensure dispenser is primed.
		Check the priming chamber for foreign materials or particulates, such as fibers or precipitate.
		Refer to inline dispenser method sheet associated with P/N 741-4905 located at navifyportal.roche.com.

Problem	Probable Cause	Suggested Action
		Ensure that only recommended fixatives and fixation times are used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that the recommended staining procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

REFERENCES

1. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26:677-704.
2. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 2007; 56(5):739-745.
3. Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T-cell responses. Immunity 2007; 27(1):111-122.
4. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 1999; 5(12):1365-1369.
5. Massard C, Gordon MS, Sharma S, et al. Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer. J Clin Oncol 2016; 34(26):3119-3125.
6. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
7. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
8. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
9. Li M. Statistical consideration and challenges in bridging study of personalized medicine. J Biopharm Stat 2015; 25(3):397-407.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
C	Updates to Materials Required But Not Provided, Warnings and Precautions, Staining Interpretation / Expected Results, Performance Characteristics, Symbols, and Intellectual Property sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

For USA: Rx only

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