VENTANA PD-L1 (SP263) Assay

REF 740-4907

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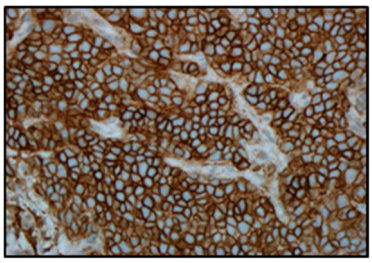

Figure 1. Non-small cell lung cancer stained with VENTANA PD-L1 (SP263) Assay.

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 the BenchMark ULTRA 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 therapy 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 Cut-off	Therapy
NSCLC	≥ 1% TC	TECENTRIQ (atezolizumab)

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]) 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 BenchMark ULTRA 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 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 1.61 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. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
16. General purpose laboratory equipment
17. BenchMark IHC/ISH 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 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 NSCLC (P/N 1020383US) for further discussion of the impact of specimen preparation on PD-L1 staining with VENTANA PD-L1 (SP263) Assay. 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. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. 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.
7. 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]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. For further information on the use of this device, refer to the BenchMark ULTRA instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
12. Consult local and/or state authorities with regard to recommended method of disposal. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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.

STAINING PROCEDURE

VENTANA PD-L1 (SP263) Assay has been developed for use on BenchMark IHC/ISH 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 2. Recommended staining protocol for VENTANA PD-L1 (SP263) Assay with OptiView DAB IHC Detection Kit on BenchMark ULTRA instruments.

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 2. 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 2 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 NSCLC (P/N1020383US) 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 3).

Table 3. 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. Refer to Table 5 for the acceptability criteria for non-specific staining. 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 4 and Table 5. Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC 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.

Scoring Algorithm - NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 4) and the non-specific background scoring criteria (Table 5). Refer to the interpretation guide (P/N 1020383US) for additional instructions and representative images.

Table 4. 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.

Table 5. 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 instruments with the OptiView DAB IHC Detection Kit and is not approved with any other detection or instruments
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. 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.
4. Sections approximately 4-5 µm in thickness should be cut and mounted on positively charged slides. 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.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE – GENERAL

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%.

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 6.

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 7.

Table 6. 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
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
Hyphophysis [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		

Tissue	# positive / total cases	Tissue	# positive / total cases

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 7. 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 – NSCLC

Tissue Thickness: NSCLC 1% TC

Tissue thickness was evaluated using 7 unique NSCLC specimens (2 PD-L1 ≥ 1% and 5 PD-L1 < 1%). 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 were stained on a single BenchMark ULTRA instrument within one day.

For between-day precision, 2 replicate slides from each of the NSCLC specimens 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.

Inter-instrument and Inter-lot Precision – 27 slides each from 24 unique NSCLC specimens (11 PD-L1 ≥ 1% and 13 PD-L1 < 1%) 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 4).

A summary of the results can be found in Table 8.

Table 8. Repeatability and intermediate precision study of VENTANA PD-L1 (SP263) Assay on individual NSCLC specimens at 1% TC.

Repeatability / Intermediate Precision Parameter	Agreement % (n/N), (95% CI)*
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)

* 2-sided 95% confidence intervals (CI) were calculated using the Wilson Score method.

Reader Precision Studies- NSCLC

To assess inter- and intra-reader precision, three pathologists evaluated a total of 114 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 4. The results provided in Table 9 reflect the inter-reader and intra-reader precision rates for unique cases from the study cohort.

Table 9. NSCLC 1% - Inter- and intra-reader precision of VENTANA PD-L1 (SP263) Assay staining NSCLC specimens.

Reader Precision	Agreement % (n/N), (95% CI)
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)

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

Inter-Laboratory Reproducibility Study - NSCLC

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. 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 4). Results are summarized in Table 10.

Table 10. Inter-laboratory reproducibility of VENTANA PD-L1 (SP263) Assay staining of NSCLC specimens at 1% TC.

Inter-laboratory Reproducibility [a]	Agreement % (n/N), (95% CI) [b]
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)
Inter-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)
Inter-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)

[a] n = 839 PD-L1 slide observations

[b] Note: 95% CI = Confidence interval

For PPA/NPA/OPA 95% CIs were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method 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 for agreements less than 100%

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

[d] Pairwise agreement rates.

CLINICAL PERFORMANCE - NSCLC

TECENTRIQ - IMpower010

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 11.

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 11. 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)

	Arm A (TECENTRIQ) n = 248	Arm B (Best Supportive Care) n = 228
Hazard ratio [1] (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

[1] Stratified by stage, sex, and histology

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 12. 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 dialog.roche.com.
		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 F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
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 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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 dialog.roche.com for definition of symbols used):

GTIN Global Trade Item Number

UDI Unique Device Identifier

INTELLECTUAL PROPERTY

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