# VENTANA PD-L1 (SP263) Assay

REF 740-4907

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IVD Σ 50 **Rx Only**

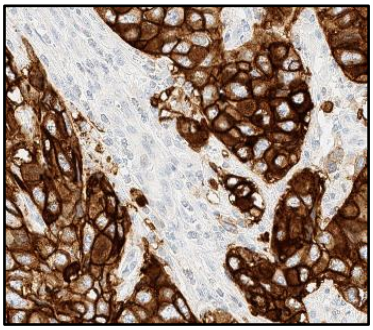

**Figure 1. PD-L1 expression in urothelial carcinoma.**

## INTENDED USE

VENTANA PD-L1 (SP263) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP263 intended for use in the assessment of the PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma tissue stained with OptiView DAB IHC Detection Kit on a VENTANA BenchMark ULTRA instrument.

PD-L1 status is determined by the percentage of tumor cells with any membrane staining above background or by the percentage of tumor-associated immune cells with staining (IC+) at any intensity above background. The percent of tumor area occupied by any tumor-associated immune cells (Immune Cells Present, ICP) is used to determine IC+, which is the percent area of ICP exhibiting PD-L1 positive immune cell staining. PD-L1 status is considered High if any of the following are met:

- ≥ 25% of tumor cells exhibit membrane staining; or,
- ICP > 1% and IC+ ≥ 25%; or,
- ICP = 1% and IC+ = 100%.

PD-L1 High status as determined by VENTANA PD-L1 (SP263) Assay was associated with increased objective response rate (ORR) in a single arm study of IMFINZI™ (durvalumab).

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

## SUMMARY AND EXPLANATION

VENTANA PD-L1 (SP263) Assay is an immunohistochemical assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody (VENTANA PD-L1 (SP263)) to recognize the programmed death ligand 1 (PD-L1). It recognizes a transmembrane bound glycoprotein that has a molecular mass of 45-55 kDa. This antibody produces membranous and/or cytoplasmic staining.

Urothelial carcinoma (also known as urothelial cell carcinoma, transitional cell carcinoma of the urinary tract, or urothelial bladder cancer) is the most common cancer of the urinary system worldwide. The majority of urothelial tumors arise in the bladder with the remainder originating in the renal pelvis, urethra, or ureter. Transitional cell carcinoma (TCC) is the most common histologic subtype associated with bladder cancer and accounts for greater than 90% of all urothelial carcinoma cases in the industrialized world; non-urothelial subtypes (e.g., squamous cell carcinoma, adenocarcinoma, small cell carcinoma) are more frequent in other areas of the world.[1]

Globally, there were an estimated 429,793 new cases of bladder cancer and 165,084 deaths in 2012.[2] In Europe alone, for 2012, there were an estimated 151,297 new cases of bladder cancer and 52,411 deaths. In 2015, it was estimated that there would be 74,000 new cases of bladder cancer and 16,000 deaths in the United States.[3] Urothelial carcinoma presents as non-muscle-invasive, muscle-invasive, or metastatic disease. The overall 5-year survival rate for metastatic urothelial carcinoma (mUC) is approximately 5.4%.[4]

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors programmed death-1 (PD-1) and B7-1 (CD80).[5] 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.[6] 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. CD80 is a molecule expressed on antigen presenting cells and activated T cells. PD-L1 binding to CD80 on T cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.[7] PD-L1 expression has been observed in immune cells and tumor cells.[8,9] Aberrant expression of PD-L1 on tumor cells and tumor associated immune cells has been reported to impede anti-tumor immunity, resulting in immune evasion.[6,9] 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.

PD-L1 is expressed in a broad range of cancers including lung, melanoma, urothelial, ovarian, and colorectal cancer. Prevalence of PD-L1 expression has been reported from 12% to 100% depending on the tumor type, anti PD-L1 clone and cutoff for positivity.[10]

IMFINZI (durvalumab) is a human immunoglobulin G1 (IgG1) kappa mAb with immune checkpoint inhibitory and antineoplastic activities that blocks the interaction of PD-L1 (but not programmed cell death ligand-2) with PD-1 on T cells and CD80 on immune cells (IC). IMFINZI (durvalumab) is engineered to reduce antibody dependent cell mediated cytotoxicity and has a calculated molar mass of 146.3 kg/mol.

## PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP263) 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 VENTANA BenchMark 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 (Cat. No. 650-210 / 05424534001), which minimizes evaporation of the aqueous reagents from the specimen slide.

In addition to staining with VENTANA PD-L1 (SP263), a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001). The negative reagent control is used to assess background staining.

## REAGENT PROVIDED

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

One 5 mL dispenser of VENTANA PD-L1 (SP263) contains approximately 8.05 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. 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) is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General 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.

The following reagents and materials may be required for staining:

1. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
2. Microscope slides, positively charged
3. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
4. Xylene (Histological grade)
5. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
6. Deionized or distilled water
7. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)

8. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
15. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
16. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
17. Light microscope
18. Absorbent wipes

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

## STORAGE

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

To ensure proper reagent delivery and 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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with the OptiView DAB IHC detection kit and VENTANA BenchMark ULTRA instrument. Based on placenta and tonsil tissues which express PD-L1, the recommended tissue fixative is 10% neutral buffered formalin (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 the VENTANA PD-L1 (SP263) Assay. The amount used is 15 to 20 times the volume of tissue. Fixation can be performed at room temperature (15-25°C).[11] For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[12]

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. 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.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

## STAINING PROCEDURE

VENTANA PD-L1 (SP263) Assay has been developed for use on a VENTANA BenchMark ULTRA automated slide stainer in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, and ancillary reagents. An assay-specific staining procedure must be used with VENTANA PD-L1 (SP263) Assay. Refer to Table 1 for the recommended staining protocol. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

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 instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

**Table 1.** Recommended staining protocol for VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit on a BenchMark ULTRA instrument.

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

* user-selectable

## QUALITY CONTROL PROCEDURES

### Rabbit Monoclonal Negative Control Ig

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

### Placental Tissue Control

A tissue control must be included with each staining run. Qualified normal human term placental tissue is to be used as the control. Control tissue should be fixed as soon as possible and processed in a manner identical to patient tissues. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Placental 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 tissue components are used to confirm that the assay functioned properly.

Placental tissue 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.

### Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known IHC performance characteristics representing PD-L1 positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[13] or the CLSI Guideline[14]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Urothelial carcinoma tissues with known PD-L1 status, and normal human term placental tissue samples, are suitable for assay verification.

## STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the VENTANA PD-L1 (SP263) Assay. The stained slide(s) are interpreted by a qualified pathologist using light

microscopy. A qualified pathologist experienced in IHC procedures must evaluate tissue controls and qualify the stained product before interpreting results.

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.

### Positive/Negative System-Level Tissue Controls

The stained positive and negative tissue controls should be examined to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product on the PD-L1 positive urothelial tissue consists of membranous and/or cytoplasmic staining of tumor cells, and linear membrane, diffused cytoplasmic, and /or punctate staining of immune cells, and is indicative of positive reactivity.

If the positive or negative tissue controls fail to demonstrate appropriate staining or demonstrate a change in interpretation, any results with the test specimens should be considered invalid.

Placental Tissue Control Evaluation Criteria are described in Table 2. Representative images are provided in the Interpretation Guide for VENTANA PD-L1 (SP263) Assay for Urothelial Carcinoma P/N 1014738US.

**Table 2.** Placenta Tissue Control Evaluation Criteria for the 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 1014738US).

### Patient Tissue

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Tables 3 and 4. Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of Urothelial Carcinoma P/N 1014738US for representative images and instructions for scoring.

**Table 3.** VENTANA PD-L1 (SP263) Assay Scoring Algorithm for Urothelial Carcinoma

| PD-L1 Interpretation | Staining Description |
|---|---|
| PD-L1 status is determined by the percentage of tumor cells with any membrane staining above background or by the percentage of tumor-associated immune cells with staining (IC+) at any intensity above background. The percent of tumor area occupied by any tumor-associated immune cells (Immune Cells Present, ICP) is used to determine IC+, which is the percent area of ICP exhibiting PD-L1 positive immune cell staining is also evaluated. | |
| High | PD-L1 Status is considered High if any of the following are met:<br>• ≥ 25% of tumor cells exhibit membrane staining; or,<br>• ICP > 1% and IC+ ≥ 25%; or,<br>• ICP = 1% and IC+ = 100%. |
| Low/negative | PD-L1 Status is considered Low/negative if:<br>• none of the criteria for PD-L1 High Status are met. |

**Table 4.** 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 |

## GENERAL LIMITATIONS

1. IHC is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and system-level controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[15,16]
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[17]
9. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.[18]
10. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

## SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP263) Assay has been solely approved on the BenchMark ULTRA instrument 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 stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised 6 months after cutting from the paraffin block for urothelial carcinoma specimens and 9 months for

placenta specimens (see the interpretation guide (P/N 1014738US) and the Performance Characteristics section below).

5. The clinical associations between VENTANA PD-L1(SP263) Assay and objective response rate have been evaluated in a single arm study of IMFINZI™ (durvalumab) where all patients were treated with durvalumab. The associations observed between PD-L1 status and ORR may be predictive or prognostic.
6. There are extremely limited clinical samples which contained ICP = 1% and had 100% of the ICP area stain above background; there were no analytical samples which contained this criteria. Therefore, there is limited analytical or clinical data to support scoring immune cells when ICP = 1%.

## PERFORMANCE CHARACTERISTICS

Tests for staining specificity, sensitivity, impact of tissue thickness, repeatability, and intermediate precision, as well as tests for reader precision, inter-laboratory reproducibility, and clinical outcome were conducted and the results are listed in the following section.

### 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 5. Additional staining, such as cytoplasmic or immune cell staining, is also noted (see Table 5 footnote).

**Table 5.** VENTANA PD-L1 (SP263) Assay staining of formalin-fixed, paraffin-embedded normal tissues.

| Tissue | # positive / total cases | Tissue | # positive / total cases |
|---|---|---|---|
| Adrenal gland | 0/3* | Mesothelium | 0/3† |
| Bladder | 0/3 | Myeloid (bone marrow) | 0/4*,† |
| Breast | 0/3 | Nerve (sparse) | 0/3 |
| Cerebellum | 0/3 | Ovary | 0/3 |
| Cerebrum | 0/3 | Pancreas | 0/3* |
| Cervix | 0/3 | Parathyroid gland | 0/4 |
| Colon | 0/3† | Prostate | 0/3 |
| Endometrium | 0/3 | Salivary gland | 0/3† |
| Esophagus | 1/3*,† | Skeletal muscle | 0/3 |
| Heart | 0/3 | Skin | 0/4§ |
| Hypophysis | 0/3*,† | Spleen | 0/3† |
| Intestine, small | 0/3† | Stomach | 0/3*,† |
| Kidney | 0/3† | Testis | 0/3 |
| Larynx | 0/3† | Thymus gland | 0/3† |
| Liver | 0/3 | Thyroid | 0/3*,† |
| Lung | 0/3† | Tonsil | 3/3† |
| Lymph node | 0/3† | | |

Additional staining observed: * Cytoplasmic staining, † Immune cell staining, § Melanocyte staining. Percent of IC present above background cannot be evaluated in this study because there is no tumor area for which to score tumor infiltrating immune cells.

### Sensitivity

Analytical sensitivity was tested on a total of 211 commercially sourced unique cases of urothelial carcinoma FFPE specimens that were tested with manufactured lots. 169/211 (80.09%) tissues were classified as PD-L1 High using the VENTANA PD-L1(SP263) Assay scoring criteria for urothelial carcinoma.

An array of neoplastic tissues was evaluated for TC and IC staining with VENTANA PD-L1 (SP263) Assay as described in Table 6.

**Table 6.** VENTANA PD-L1 (SP263) Assay staining of formalin-fixed, paraffin-embedded neoplastic tissues for any tumor cell or immune cell staining.

| Origin | Pathology | # positive / total cases | |
|---|---|---|---|
| | | Tumor Cells | Immune Cells |
| Cerebrum | Glioblastoma | 0/1 | 1/1 |
| Cerebrum | Atypical meningioma | 0/1 | 0/1 |
| Cerebrum | Malignant ependymoma | 0/1 | 1/1 |
| Cerebrum | Oligodendroglioma | 0/1 | 0/1 |
| Ovary | Serous adenocarcinoma | 0/1 | 1/1 |
| Ovary | Adenocarcinoma | 1/1 | 0/1 |
| Pancreas | Islet cell carcinoma | 0/1 | 0/1 |
| Pancreas | Adenocarcinoma | 0/1 | 1/1 |
| Testis | Seminoma | 0/1 | 0/1 |
| Testis | Embryonal carcinoma | 0/1 | 0/1 |
| Thyroid | Medullary carcinoma | 0/1 | 0/1 |
| Thyroid | Papillary carcinoma | 1/1 | 0/1 |
| Breast | Intraductal carcinoma | 0/1 | 1/1 |
| Breast | Invasive ductal carcinoma | 0/2 | 0/2 |
| Spleen | Diffuse B-cell lymphoma | 0/1 | 1/1 |
| Lung | Small cell undifferentiated carcinoma | 1/1 | 1/1 |
| Lung | Squamous cell carcinoma | 1/1 | 1/1 |
| Lung | Adenocarcinoma | 0/1 | 0/1 |
| Esophagus | Neuroendocrine carcinoma | 0/1 | 0/1 |
| Esophagus | Adenocarcinoma | 0/1 | 0/1 |
| Stomach | Signet-ring cell carcinoma | 0/1 | 0/1 |
| Intestine | Adenocarcinoma | 0/1 | 0/1 |
| Intestine | Stromal sarcoma | 0/1 | 0/1 |
| Colon | Adenocarcinoma | 0/1 | 1/1 |
| Colon | Interstitialoma | 0/1 | 0/1 |
| Rectum | Adenocarcinoma | 0/1 | 0/1 |
| Rectum | Moderate malignant interstitialoma | 0/1 | 0/1 |
| Liver | Hepatocellular carcinoma | 0/1 | 0/1 |
| Liver | Hepatoblastoma | 0/1 | 0/1 |
| Kidney | Clear cell carcinoma | 0/1 | 0/1 |
| Prostate | Adenocarcinoma | 0/2 | 0/2 |
| Uterus | Leiomyoma | 0/1 | 0/1 |
| Uterus | Adenocarcinoma | 0/1 | 0/1 |
| Uterus | Clear cell carcinoma of endometrium | 1/1 | 0/1 |

| Origin | Pathology | # positive / total cases | |
|---|---|---|---|
| | | Tumor Cells | Immune Cells |
| Uterine cervix | Squamous cell carcinoma | 0/2 | 2/2 |
| Striated muscle | Embryonal rhabdomyosarcoma | 0/1 | 0/1 |
| Rectum | Malignant melanoma | 0/1 | 0/1 |
| Skin | Basal cell carcinoma | 0/1 | 0/1 |
| Skin | Squamous cell carcinoma | 0/1 | 0/1 |
| Back | Neurofibroma | 0/1 | 1/1 |
| Retroperitoneum | Neuroblastoma | 0/1 | 0/1 |
| Abdominal cavity | Malignant mesothelioma | 0/1 | 0/1 |
| Mediastinum | Diffuse B-cell lymphoma | 1/1 | 1/1 |
| Lymph node | Hodgkin's lymphoma | 1/1 | 1/1 |
| Lymph node | Diffuse B-cell lymphoma | 1/1 | 1/1 |
| Pelvic cavity | Anaplastic large cell lymphoma | 1/1 | 1/1 |
| Bladder | Low grade malignant leiomyosarcoma | 0/1 | 0/1 |
| Bone | Osteosarcoma | 0/1 | 1/1 |
| Retroperitoneum | Spindle cell rhabdomyosarcoma | 0/1 | 0/1 |
| Smooth muscle | Moderate malignant leiomyosarcoma | 0/1 | 0/1 |
| Bladder | Transitional cell carcinoma (bladder) | 1/1 | 1/1 |

### Tissue Thickness

Tissue thickness was evaluated using 7 unique cases of human urothelial carcinoma (4 with tumor positivity ≥ 25% and 3 with tumor positivity <25%; 4 with IC+ ≥ 25% and 3 with IC+ <25% for IC). Tissues were sectioned and tested in duplicate at 2, 3, 4, 5, 6, and 7 microns. All tissue thicknesses from 3 to 7 microns demonstrated appropriate specific staining and acceptable background levels with VENTANA PD-L1 (SP263) Assay. Ventana recommends that specimens be cut at 4-5 microns for the assay.

### Repeatability and Intermediate Precision Studies

Repeatability studies for VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens were completed to demonstrate:

- Intra-day Repeatability – Five replicate slides each from 24 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay on a single BenchMark ULTRA instrument within one day.
- Inter-day Precision – Two replicate slides each from 24 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay on a single Benchmark ULTRA instrument across 5 non-consecutive days spanning at least a 20 day period.
- Inter-instrument and Inter-lot Precision – 27 replicate slides each from 26 unique urothelial carcinoma specimens 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.

The 24 unique cases used for the Intra-day Repeatability and Inter-day Precision Studies had an overlapping case distribution of 12 PD-L1 High and 12 PD-L1 Low/negative.

The 26 unique cases used for the Inter-instrument and Inter-lot Precision Study had an overlapping case distribution of 13 PD-L1 High and 13 PD-L1 Low/negative.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm as it pertains to tumor cells and tumor associated immune cells (Tables 3 and 4). Results are summarized in Table 7.

**Table 7.** Repeatability and Intermediate Precision of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

| Repeatability/Intermediate precision parameter | Positive Percent Agreement (95% CI) | Negative Percent Agreement (95% CI) | Overall Percent Agreement (95% CI) |
|---|---|---|---|
| Intra-day Repeatability (within a single day) n = 120 observations | 100.0% (60/60) (94.0 – 100%)* | 98.3.0% (59/60) (91.1 – 99.7%)* | 99.2% (119/120) (95.4 - 99.9%)* |
| Inter-day Precision (5 non-consecutive days) n = 240 observations | 100.0% (120/120) (96.9 – 100%)* | 100.0% (120/120) (96.9– 100.0%)* | 100.0% (240/240) (98.4– 100.0%)* |
| Inter-instrument and Inter-lot Precision (3 instruments, 3 antibody lots, and 3 detection kit lots) n = 702 observations | 98.3% (345/351) (96.7 – 99.7%)** | 99.7% (350/351) (99.1 – 100.0%)** | 99.0% (695/702) (98.1 – 99.7%)** |

* 2-sided 95% confidence intervals were calculated using the Wilson Score method.
** 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2,000 bootstrap samples

### Inter- and Intra-Reader Precision Studies

To assess Inter- and Intra-reader Precision, three trained pathologists evaluated an overlapping subset of approximately 50 unique urothelial carcinoma specimens representing the dynamic range of the VENTANA PD-L1 (SP263) Assay with case distribution of 22 PD-L1 High and 28 PD-L1 Low/negative.

Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP263) Assay scoring algorithm as it pertains to tumor cells as well as tumor associated immune cells (Tables 3 and 4). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates are summarized in Table 8.

**Table 8.** Inter- and Intra-reader Precision of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

| Reader Precision | Average Positive Agreement (95% CI)* | Average Negative Agreement (95% CI)* | Overall Percent Agreement (95% CI)* |
|---|---|---|---|
| Inter-reader Precision (average of all three reader pairwise comparison for the first read) n =143 pairs | 92.8% (128/138) (85.5-98.3%) | 93.2% (138/148) (86.5-98.5%) | 93.0% (133/143) (87.1-98.6%) |
| Intra-reader Precision (average of all three readers' agreement rates between first and second reads) n =145 pairs | 92.1% (128/139) (85.4-96.7%) | 92.7% (140/151) (87.1-96.8%) | 92.4% (134/145) (87.2-96.6%) |

* 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2,000 bootstrap samples.

### Inter-laboratory Reproducibility Study

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP263) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 expression in urothelial carcinoma cases, using tissue specimens run across 5 non-consecutive days over a 20-day period at three external laboratories.

The Inter-laboratory Reproducibility Study analyzed 35 urothelial carcinoma specimens with a case distribution of 21 PD-L1 High and 14 PD-L1 Low/negative.

The specimens were blinded, randomized and evaluated by a total of 6 readers (2 readers/site). Results are summarized in Table 9.

**Table 9.** Inter-laboratory Reproducibility of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

| Inter-laboratory Reproducibility | Positive Agreement (95% CI)** | Negative Agreement (95% CI)** | Overall Percent Agreement (95% CI)** |
|---|---|---|---|
| Overall agreement * (across sites, days and readers) n =1048 observations*** | 95.4% (599/628) (91.3-98.4%) | 88.3% (371/420) (81.6-94.6%) | 92.6 (970/1048) (88.9-95.8%) |
| Inter-site agreement ^ (average of site-to-site pairwise comparisons) n =10460 pairs*** | 90.0% (11632/12920) (83.1-94.5%) | 83.9% (6712/8000) (75.0-90.6%) | 87.7% (9172/10460) (81.9-92.7%) |
| Inter-reader agreement ^ (average of reader-to-reader pairwise comparisons within each site) n =524 pairs*** | 89.2% (578/648) (81.5-94.6%) | 82.5% (330/400) (71.5-90.0%) | 86.6% (454/524) (80.2-92.7%) |

* Agreement of study results with the case-level modal PD-L1 status. PPA and NPA were used for overall agreement.

** 2-sided 95% confidence interval calculated using the percentile bootstrap method from 2,000 bootstrap samples.

*** One PD-L1 slide was found to be unevaluable by two readers at one site due to broken negative reagent control slide.

^ APA and ANA agreements were used for inter-site and inter–reader agreements.

### Clinical Outcome Study

The clinical performance of VENTANA PD-L1(SP263) Assay was evaluated in Study 1, a single arm, multicenter, open-label clinical trial of IMFINZI (durvalumab) on patients with locally advanced or metastatic urothelial carcinoma. Tumor specimens from 332 patients were evaluated prospectively for PD-L1 expression at a central laboratory using the VENTANA PD-L1 (SP263) Assay. PD-L1 status was not evaluable for 31 patients due to insufficient tumor cells present in sample (n=25), inappropriate tissue being submitted (n=3), tissue folding (n=1), dye trapping (n=1) or tissue washing of the slide (n=3). There were no instances where a sample was non-evaluable for PD-L1 status due to assay failure.

In Study 1, 182 patients with locally advanced or metastatic urothelial carcinoma were enrolled. The patients that were enrolled had progressed while on or after a platinum-based therapy, including those who progressed within 12 months of receiving therapy in a neo-adjuvant or adjuvant setting. These patients had initiated durvalumab therapy at least 13 weeks prior to the data cut-off date. The trial excluded patients with a history of immunodeficiency; medical conditions that required systemic immunosuppression (not to exceed 10 mg/day of prednisone or equivalent); history of severe autoimmune disease; untreated CNS metastases; HIV; active tuberculosis, or hepatitis B or C infection. All patients received IMFINZI 10 mg/kg via intravenous infusion every 2 weeks for up to 12 months or until unacceptable toxicity or disease progression. Tumor assessments were performed at Weeks 6, 12 and 16, then every 8 weeks for the first year and every 12 weeks thereafter. The major efficacy outcome measures were confirmed Objective Response Rate (ORR) according to RECIST v1.1 as assessed by Blinded Independent Central Review (BICR), and duration of response (DoR).

In Study 1, the median age was 67 years (range: 34 to 88), 72% were male, 64% were Caucasian. Sixty-six percent (66%) had visceral metastasis (bone, liver, or lung), including 34% with liver metastasis. Lymph node only metastasis were present in 13% of patients. Sixty-six percent (66%) of patients had ECOG score of 1 and 41% of patients had a baseline creatinine clearance of <60 mL/min. The Bellmunt risk score (which includes ECOG score, baseline hemoglobin, and liver metastases) was 0 in 23%, 1 in 38%, 2 in 29%, and 3 in 9% of patients. Twenty percent (20%) of patients had disease progression following platinum-containing neo-adjuvant or adjuvant chemotherapy as their only prior line of therapy. Seventy percent (70%) of patients received prior cisplatin, 30% prior carboplatin and 35% received ≥2 prior lines of systemic therapy. The median follow-up time was 5.6 months. A partial or complete response was observed in 31/182 patients (17.0%; 95% CI = 11.9 – 23.3%) following treatment with IMFINZI.

Of the 182 patients that were enrolled in Study 1, 128 were enrolled without regard to PD-L1 status and after the VENTANA PD-L1 (SP263) Assay scoring algorithm was finalized. Of these 128 patients, 58 were classified as PD-L1 High, 56 as PD-L1 Low/negative, and samples for 14 patients were non-evaluable.The efficacy results are summarized in Table 10. The median follow-up time for these patients was 4.9 months.

**Table 10.** Efficacy Results for a 128 patient subset from Study 1; PD-L1 expression in patients with urothelial carcinoma.

| Efficacy Parameter* | PD-L1 High (N=58) | PD-L1 Low/Negative (N=56) | PD-L1 Non-Evaluable (N=14) |
|---|---|---|---|
| Number of confirmed responders by BICR | 11 | 2 | 3 |
| Objective Response Rate (95% CI) | 19.0% (9.9% - 31.4%) | 3.6% (0.4% - 12.3%) | 21.4% (4.7% - 50.8%) |
| CR, n (%) | 2 (3.4%) | 0 | 1 (7.1%) |
| PR, n (%) | 9 (15.5%) | 2 (3.6%) | 2 (14.3%) |
| Median (DoR), months, (range) | 4.24 (0.9+ - 4.2) | NR (1.9+ - 4.2+) | NR (2.3+ - 2.6+) |

* Objective Response Rate and Duration of Response determined by RECIST v1.1

BICR=Blinded Independent Central Review

CR=Complete Response

CI=Confidence Interval

DoR=Duration of Response

NR=Not Reached

PR=Partial Response

(refer to Drugs@FDA for the most recent therapeutic product labeling)

## TROUBLESHOOTING

Troubleshooting guidance is provided in Table11. 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 11.** 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 U VENTANA PD-L1 (SP263) Assay 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 package insert associated with P/N 740-4907 / 07208162001 located at www.ventana.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 U VENTANA PD-L1 (SP263) Assay 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. Chalasani V, Chin JL, Izawa JI. Histologic variants of urothelial bladder cancer and nonurothelial histology in bladder cancer. Can Urol Assoc J. 2009;3(6 Suppl 4):S193-198.
2. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. http://globocan.iarc.fr. Published 2013-12-12. Updated 2014-01-09. Accessed 2014-02-02.
3. American Cancer Society. Cancer Facts & Figures 2015. Atlanta: American Cancer Society; 2015.
4. Howlader N, Noone AM, Krapcho M, et al. (eds). SEER Cancer Statistics Review (CSR),1975-2012. National Cancer Institute. http://seer.cancer.gov/csr/1975_2012/. Published 2015-04-23. Updated 2015-11-18. Accessed 2016-04-19.
5. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677-704.
6. 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.
7. 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.
8. 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.
9. 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):3119-25.
10. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther. 2015;14(4) 847-856.
11. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
12. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
13. Anatomic Pathology Checklist. College of American Pathologists. Jul 28, 2015.
14. CLSI. Quality assurance for design control and implementation of immunohistochemistry assay: approved guidelines. 2nd edition Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011
15. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
16. Hautzer NW, Wittkuhn JF, McCaughey WT. Trypsin digestion in immunoperoxidase staining. J Histochem Cytochem. 1980;28(1):52-53.
17. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. 1980;73(5):626-632.
18. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.


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