VENTANA PD-L1 (SP142) Assay

REF 740-4859

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IVD Σ 50

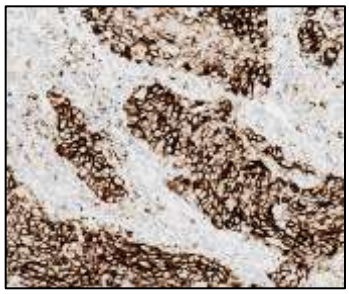

Figure 1. PD-L1 expression in non-small cell lung cancer.

INTENDED USE

VENTANA PD-L1 (SP142) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP142 intended for use in the assessment of the PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma and non-small cell lung cancer (NSCLC) tissue stained with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a VENTANA BenchMark ULTRA instrument. Determination of PD-L1 status is indication-specific, and evaluation is based on either the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity.

PD-L1 expression in ≥ 5% IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is associated with increased objective response rate (ORR) in a non-randomized study of TECENTRIQ (atezolizumab).

PD-L1 expression in ≥ 50% TC or ≥ 10% IC determined by VENTANA PD-L1 (SP142) Assay in NSCLC tissue may be associated with enhanced overall survival from TECENTRIQ (atezolizumab).

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

SUMMARY AND EXPLANATION

VENTANA PD-L1 (SP142) Assay is an immunohistochemical assay that utilizes a rabbit monoclonal primary antibody (SP142) produced against the PD-L1 protein. This assay was co-developed by Roche/Ventana Medical Systems, Inc. (Ventana) and Roche/Genentech to identify patients who are most likely to respond to treatment with TECENTRIQ®.

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors programmed death-1 (PD-1) and B7.1. 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.[1] Ligation 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. 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.[2] PD-L1 expression has been observed in immune cells and malignant cells.[3] Aberrant expression of PD-L1 on tumor cells (TC) has been reported to impede anti-tumor immunity, resulting in immune evasion.[1] 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.

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors. Atezolizumab is a non-glycosylated IgG1 kappa immunoglobulin that has a calculated molecular mass of 145 kDa.

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP142) Assay utilizes a rabbit monoclonal primary antibody that binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be visualized using VENTANA OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) followed by OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test)). Refer to the appropriate OptiView DAB IHC Detection Kit and OptiView Amplification Kit package inserts for further information.

REAGENT PROVIDED

VENTANA PD-L1 (SP142) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PD-L1 (SP142) Assay contains approximately 36 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 7 µg/mL.

VENTANA PD-L1 (SP142) Assay contains 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. Benign human tonsil tissues for use as control tissue
2. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
3. Microscope slides, positively charged
4. Barcode labels
5. Xylene (Histological grade)
6. 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
7. Deionized or distilled water
8. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
9. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))
10. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
11. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
12. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
15. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
16. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
17. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
18. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
19. Light microscope
20. 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 VENTANA detection kits and BenchMark ULTRA instruments. Ventana recommends tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours. Fixation times of less than 6 hours may result in a loss of staining for PD-L1. The amount of NBF used should be 15 to 20

times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[4-5]

Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative, and other alcohol-containing fixatives have demonstrated a loss of specific staining for PD-L1 at all fixation times tested (1-72 hours); they are not recommended for use with this assay. See the interpretation guide (P/N 1015243US) for further discussion of the impact of specimen preparation on PD-L1 staining intensity.

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised 2 months after cutting from the paraffin block for NSCLC and tonsil specimens (see the interpretation guide (P/N 1015243US) and the Performance Characteristics section below).

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. 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 (SP142) Assay has been developed for use on a BenchMark ULTRA instrument in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, OptiView Amplification Kit, and ancillary reagents. An assay-specific staining procedure must be used with VENTANA PD-L1 (SP142) Assay. Refer to 0 for the recommended staining protocol and required staining procedure. 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 (SP142) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark ULTRA instrument.

Staining Procedure: U OptiV DAB VENTANA PD-L1 (SP142)	
Protocol Parameter	**Selection**
Deparaffinization	Selected
Cell Conditioning	CC1 Cell Conditioning 48 minutes
Pre-primary antibody peroxidase	Selected
Primary Antibody	VENTANA PD-L1 (SP142) Selected or Negative Control Selected 16 minutes, 36°C
OptiView HQ Linker	8 minutes (default)
OptiView Multimer	8 minutes (default)
Amplifier and Amplification	Selected
Amplifier and Amplification H2O2	8 minutes
Amplification Multimer	8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

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.

Tonsil Tissue Control

A tissue control must be included with each staining run. Qualified benign human tonsil 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. Tonsil 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.

Appropriate staining of tonsil tissue components is described in Table 2 and in the interpretation guide (P/N 1015243US).

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[6] or the CLSI Approved Guideline[7]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. NSCLC tissues with known PD-L1 status, and tonsil samples, are suitable for assay verification.

INTERPRETATION OF 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 (SP142) Assay antibody. 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.

Tonsil Tissue Control Interpretation

The stained tonsil tissue control should be examined for appropriate staining. The presence of PD-L1 staining within the macrophages and lymphocytes in germinal centers and reticulated crypt epithelium of tonsil serve as positive tissue elements. Absence of staining in superficial squamous epithelium and negative immune cells in interfollicular regions of tonsil serve as negative tissue elements. Acceptability criteria are listed in Table 2. (Refer to the interpretation guide (P/N 1015243US)).

If the tissue control fails to demonstrate appropriate staining, any results with the patient specimens should be considered unevaluable and repeat staining should be performed.

Table 2. Tonsil tissue control evaluation criteria.

Acceptable	Unacceptable
Positive tissue elements: Moderate to strong PD-L1 staining noted in lymphocytes and macrophages in germinal centers, with diffuse staining in reticulated crypt epithelial cells.	Excessive non-specific background staining obscuring the identification of PD-L1 positive cells.
Negative tissue elements: PD-L1 negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Weak to no PD-L1 staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.

Negative Reagent Control

Non-specific staining, if present, will 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 1015243US).

Patient Tissue

Tumor cells (TC) are scored as the percentage of tumor cells with the presence of discernible PD-L1 membrane staining of any intensity. Tumor-infiltrating immune cells (IC) are scored as the proportion of tumor area, including associated intratumoral and contiguous peritumoral stroma, occupied by PD-L1 staining IC of any intensity. NSCLC patient tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm provided in Table 3. High PD-L1 expression is defined as having PD-L1 expression on ≥ 50% of TC or ≥ 10% of IC. Refer to the interpretation guide (P/N 1015243US) for additional instructions and representative images.

Table 3. VENTANA PD-L1 (SP142) Assay Scoring Algorithm for NSCLC.

STEP 1 Tumor Cell (TC) Staining Assessment	PD-L1 Expression
Presence of discernible PD-L1 membrane staining of any intensity in ≥ 50% of tumor cells	≥ 50% TC
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 membrane staining of any intensity in < 50% of tumor cells	Proceed to Step 2
STEP 2 Tumor Infiltrating Immune Cell (IC) Staining Assessment	**PD-L1 Expression**
Presence of discernible PD-L1 staining of any intensity in tumor infiltrating immune cells covering ≥ 10% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 10% IC
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor infiltrating immune cells covering < 10% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 50% TC and < 10% IC

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.[8-9] Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[10]
8. 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.[11] 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 (SP142) Assay has been solely approved on the BenchMark ULTRA instrument with the OptiView DAB IHC Detection Kit and the OptiView Amplification 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. VENTANA PD-L1 (SP142) Assay antibody is stable for up to eight days at 30°C. Assay performance beyond these limits has not been established.
4. This assay has not been validated for use with cytology smears or decalcified specimens.
5. NSCLC patient tissue should be stained within 2 months of sectioning from the tissue block. Loss of staining performance has been observed with VENTANA PD-L1 (SP142) Assay staining of NSCLC tissue sections that have been stored at room temperature for longer than 2 months.
6. Ventana recommends that samples be fixed between 6 and 72 hours in 10% NBF. Use of fixation times or fixative types other than those recommended can lead to false negative results. Fixatives such as AFA, PREFER fixative, and other alcohol-containing fixatives have demonstrated a loss of specific PD-L1 protein staining. Refer to the interpretation guide (P/N 1015243US) for further discussion.
7. Artifacts such as DAB spots, Blank spots, DAB dots, Speckling, and/or may require repeat staining if they interfere with the interpretation of VENTANA PD-L1 (SP142) Assay. Always compare the PD-L1 stained slide to the negative reagent control to ensure that background is acceptable. Refer to the interpretation guide (P/N 1015243US) for further discussion.
8. Occasional DAB dots have been observed in normal tonsil control, cerebellum and testicular tissues and focal nuclear staining has been observed in normal pancreatic (acinar cells) and hypophyseal tissue (Table 4); however nuclear staining is not included in scoring of VENTANA PD-L1 (SP142) Assay staining.

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 (SP142) Assay and evaluated for the presence of immune cell staining (any immune cell staining, of any intensity) as described in Table 4.

Table 4. Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive*/ total cases	Tissue	# positive*/ total cases
Adrenal gland	1/3	Muscle, cardiac	0/3
Bladder	3/36**	Muscle, skeletal	0/2
Breast	1/3	Myeloid	0/2
Cerebellum	0/3***	Nerve, peripheral	0/3
Cerebrum	0/3	Ovary	0/3
Cervix	2/2	Pancreas	0/3****
Colon	2/3	Parathyroid	0/2
Endometrium	1/3	Prostate	0/3
Esophagus	0/3	Salivary gland	2/3
Hypophysis	0/3****	Skin	0/3
Intestine, small	1/3	Spleen	3/3
Kidney	2/3	Stomach	0/3
Lingual gland	0/1	Testis	0/3***
Liver	0/3	Thymus gland	3/3
Lung	1/3	Thyroid gland	1/3
Lymph node	3/3	Tonsil	3/3
Mesothelium	0/3		

* Immune cell staining of any intensity ** Focal immune cell staining

*** Focal DAB dots were observed in 1/3 cerebellum and 1/3 testis tissues

**** Nuclear staining was observed in 1/3 pancreas and 1/3 hypophysis tissues

Sensitivity

3750 urothelial carcinoma specimens (including 55 metastases) were evaluated with VENTANA PD-L1 (SP142) Assay. Of these, 2545 (67.9%) showed immune cell staining of any percentage; 466 (12.4%) showed ≥ 5% immune cell staining; 512 (13.7%) showed tumor cell staining of any percentage. In addition, an array of neoplastic tissues was evaluated for immune cell and tumor cell staining with VENTANA PD-L1 (SP142) Assay as described in Table 5.

Table 5. Sensitivity of VENTANA PD-L1 (SP142) Assay staining was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Origin	Pathology	# positive*/total cases	
		Immune cells	Tumor cells
Abdomen	Malignant mesothelioma	1/1	0/1
Back	Neurofibroma	1/1	0/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bladder	Transitional cell carcinoma	1/1	0/1
Bone	Osteosarcoma	0/1	0/1
Breast	Invasive ductal carcinoma	1/1	0/1
Breast	Intraductal carcinoma with early infiltrate	1/1	0/1
Cerebrum	Glioblastoma	0/1	0/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	0/1
Cerebrum	Oligodendroglioma	0/1	0/1
Colon	Adenocarcinoma	1/1	0/1
Colon	Interstitialoma	0/1	0/1
Esophagus	Squamous cell carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	1/1	0/1
Intestine	Adenocarcinoma	1/1	0/1
Intestine	Stromal sarcoma	1/1	0/1
Kidney	Clear cell carcinoma	1/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	1/1	0/1
Lung	Adenocarcinoma	0/1	0/1
Lung	Small cell undifferentiated carcinoma	1/1	0/1
Lung	Squamous cell carcinoma	1/1	0/1
Lymph node	Diffuse B-cell lymphoma	1/1**	1/1**
Lymph node	Hodgkin's lymphoma	1/1	0/1
Mediastinum	Diffuse B-cell lymphoma	1/1**	1/1**
Muscle, smooth	Moderate malignant leiomyosarcoma	1/1	0/1
Muscle, striated	Embryonal rhabdomyosarcoma	0/1	0/1
Ovary	Serous adenocarcinoma	1/1	0/1
Ovary	Adenocarcinoma	1/1	0/1
Pancreas	Islet cell tumor	0/1	0/1
Pancreas	Adenocarcinoma	1/1	0/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1**	1/1**
Prostate	Adenocarcinoma	0/2	0/2

Origin	Pathology	# positive*/total cases	
		Immune cells	Tumor cells
Rectum	Adenocarcinoma	1/1	1/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Rectum	Malignant melanoma	1/1	0/1
Retroperitoneum	Neuroblastoma	1/1	0/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Skin	Basal cell carcinoma	1/1	0/1
Skin	Squamous cell carcinoma	1/1	0/1
Spleen	Diffuse B-cell lymphoma	1/1*	1/1*
Stomach	Signet-ring cell carcinoma	1/1	0/1
Testis	Seminoma	1/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	0/1	1/1
Uterine cervix	Squamous cell carcinoma	2/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	1/1	0/1
Uterus	Clear cell carcinoma of endometrium	1/1	1/1

* Immune cell or tumor cell staining of any intensity

** Tumor cell and immune cell staining could not be differentiated

Tissue Thickness

Tissue thickness was evaluated using NSCLC specimens. Duplicate sections at 3, 4, 5, 6, and 7 microns were stained with VENTANA PD-L1 (SP142) Assay and evaluated for PD-L1 TC and IC expression. Sample sets consisted of a minimum of 8 NSCLC specimens with a range of PD-L1 expression for each IC and TC level tested.

All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 TC or IC level within the range of thickness tested. Ventana recommends that NSCLC specimens be cut at 4 microns for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision

Repeatability studies for VENTANA PD-L1 Assay staining of NSCLC specimens were completed to demonstrate:

Intra-day Repeatability - A minimum of 5 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument in a single day and evaluated for PD-L1 TC and IC expression. Sample sets consisted of a minimum of 8 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.

Inter-day Precision - A minimum of 10 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days. Sample sets consisted of a minimum of 8 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level tested.

Instrument, Antibody and Detection Lot Precision - a minimum of 9 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments. Sample sets consisted of a minimum of 18 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.

All slides were blinded and randomized and then evaluated for PD-L1 TC or IC expression level. Repeatability and Intermediate Precision results are summarized in Table 6 and Table 7.

Table 6. Repeatability and Intermediate Precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 Expression ≥ 50% TC)

Repeatability/ Intermediate Precision Parameter	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Intra-day Repeatability (within a single day)	100.0% (88.6-100.0%)*	100.0% (83.9-100.0%)**	100.0% (92.9-100.0%)***
Inter-day Precision (5 non-consecutive days)	100.0% (94.0-100.0%)*	100.0% (90.4-100.0%)**	100.0% (96.2-100.0%)***
Inter-instrument and Inter-lot Precision (compared to case-level mode, across instruments and lots)	99.7% (98.1-99.9%)*	95.2% (91.2-97.5%)**	97.9% (96.2-98.9%)***

CI = Confidence Interval

* Positive Percent Agreement, Two-sided Wilson score method CI

** Negative Percent Agreement, Two-sided Wilson score method CI

*** Overall Percent Agreement, Two-sided Wilson score method CI

Table 7. Repeatability and Intermediate Precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 Expression ≥ 10% IC)

Repeatability/ Intermediate Precision Parameter	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Intra-day Repeatability (within a single day)	100.0% (91.2-100.0%)*	100.0% (91.2-100.0%)**	100.0% (95.4-100.0%)***
Inter-day Precision (5 non-consecutive days)	100.0% (96.3-100.0%)*	100.0% (96.3-100.0%)**	100.0% (98.1-100.0%)***
Inter-antibody and Inter-detection Agreement (pairwise-comparison)	95.1% (91.1-98.1%)†	90.2% (82.3-96.2%)††	93.4% (88.7-97.5%)***
Inter-instrument and Inter-detection lots Agreement (pairwise-comparison)	96.3% (93.2-98.8%)†	92.7% (86.0-97.7%)††	95.1% (91.2-98.4%)***
Inter-instrument and Inter-antibody Agreement (pairwise-comparison)	96.3% (93.1-98.8%)†	92.6% (85.9-97.8%)††	95.1% (91.1-98.4%)***

CI = Confidence Interval

* Positive Percent Agreement, Two-sided Wilson score method CI

** Negative Percent Agreement, Two-sided Wilson score method CI

*** Overall Percent Agreement, Two-sided Wilson score method CI

† Average Positive Agreement, Bootstrapping method CI

†† Average Negative Agreement, Bootstrapping method CI

Reader Precision Study

To assess Inter- and Intra-Reader Precision, three pathologists evaluated 80 unique NSCLC cases, with a range of PD-L1 expression, that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 3). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers, compared to a consensus score, across two reads, and between each pathologist's reads are summarized in Table 8.

Table 8. Reader Precision of VENTANA PD-L1 (SP142) Assay Staining of NSCLC Specimens

Reader Precision	Agreement %*(95% CI)
Inter-reader Precision (average of all three readers compared to a consensus score and across two reads)	PPA: 89.2% (84.1-92.7%) NPA: 99.0% (96.3-99.8%) OPA: 93.5% (90.4-95.6%)
Intra-reader Precision (average of all three readers' agreement rates between first and second reads)	PPA: 90.1% (84.0-94.1%) NPA: 98.1% (92.8-99.5%) OPA: 93.6% (89.7-96.1%)

* NPA = Negative Percent Agreement, OPA = Overall Percent Agreement, PPA = Positive Percent agreement

Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility Study for VENTANA PD-L1 (SP142) Assay staining was conducted to demonstrate reproducibility of the assay in determining PD-L1 status 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. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers) using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 3). Results are summarized in Table 9.

Table 9. Inter-Laboratory Reproducibility of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens.

Inter-laboratory Reproducibility	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 86.6% (83.0-89.5%) NPA: 99.8% (98.7-100.0%) OPA: 93.2% (91.3-94.7%)
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 89.5% (80.9-95.5%) ANA: 92.1% (84.4-97.1%) OPA: 91.0% (90.3-91.6%)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 93.9% (89.3-97.4%) ANA: 95.4% (90.6-98.2%) OPA: 94.7% (92.2-96.5%)

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

CLINICAL OUTCOME STUDY - NSCLC

The efficacy of TECENTRIQ was investigated in a separate Phase III multi-center, international, randomized, open-label trial in patients with metastatic NSCLC who progressed during or following a platinum-containing regimen. This trial enrolled 1225 patients with the primary analysis population consisting of the first 850 randomized patients; eligible patients were stratified by PD-L1 expression status in IC, by the number of prior chemotherapy regimens, and by histology. Patients were randomized (1:1) to receive either TECENTRIQ administered intravenously at 1200 mg every 3 weeks until unacceptable toxicity or either radiographic or clinical progression or docetaxel administered intravenously at 75 mg/m^2 every 3 weeks until unacceptable toxicity or disease progression. Tumor specimens were evaluated prospectively for PD-L1 expression on TC and IC using VENTANA PD-L1 (SP142) Assay and the results were used to define the PD-L1 expression subgroups for pre-specified analyses described below.

In the Phase III study, among patients in the primary analysis population, the median age was 64 years (range: 33 to 85), and 61% of patients were male. The majority of patients were white (70%). Approximately three-fourths of patients had non-squamous disease (74%), 10% had known EGFR mutation, 0.2% had known ALK rearrangements, and most patients were current or previous smokers (82%). Baseline ECOG performance status was 0 (37%) or 1 (63%). Seventy five percent of patients received only one prior platinum-based therapeutic regimen.

The major efficacy outcome measure of the Phase III study was overall survival (OS) in the primary analysis population (first 850 randomized patients). The results of the Phase III study with a median follow up of 21 months are presented in Table 10 and Figure 2.

Tumor specimens were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used to define the PD-L1 expression subgroups for pre-specified analyses. Of the 850 patients, 16% were classified as having high PD-L1 expression, defined as having PD-L1 expression on ≥ 50% of TC or ≥ 10% of IC. In an exploratory efficacy subgroup analysis of OS based on PD-L1 expression, the hazard ratio was 0.41 (95% CI: 0.27, 0.64) in the high PD-L1 expression subgroup and 0.82 (95% CI: 0.68, 0.98) in patients who did not have high PD-L1 expression.

Table 10. Efficacy Results in the Primary Analysis Population from the Phase III Study

Overall Survival	TECENTRIQ (n=425)	Docetaxel (n=425)
Deaths (%)	271 (64%)	298 (70%)
Median, months (95% CI)	13.8 (11.8, 15.7)	9.6 (8.6, 11.2)
Hazard ratio* (95% CI)	0.74 (0.63, 0.87)	
p-value**	0.0004	

* Stratified by PD-L1 expression in tumor-infiltrating immune cells, the number of prior chemotherapy regimens, and histology

** Based on the stratified log-rank test

CI = confidence interval

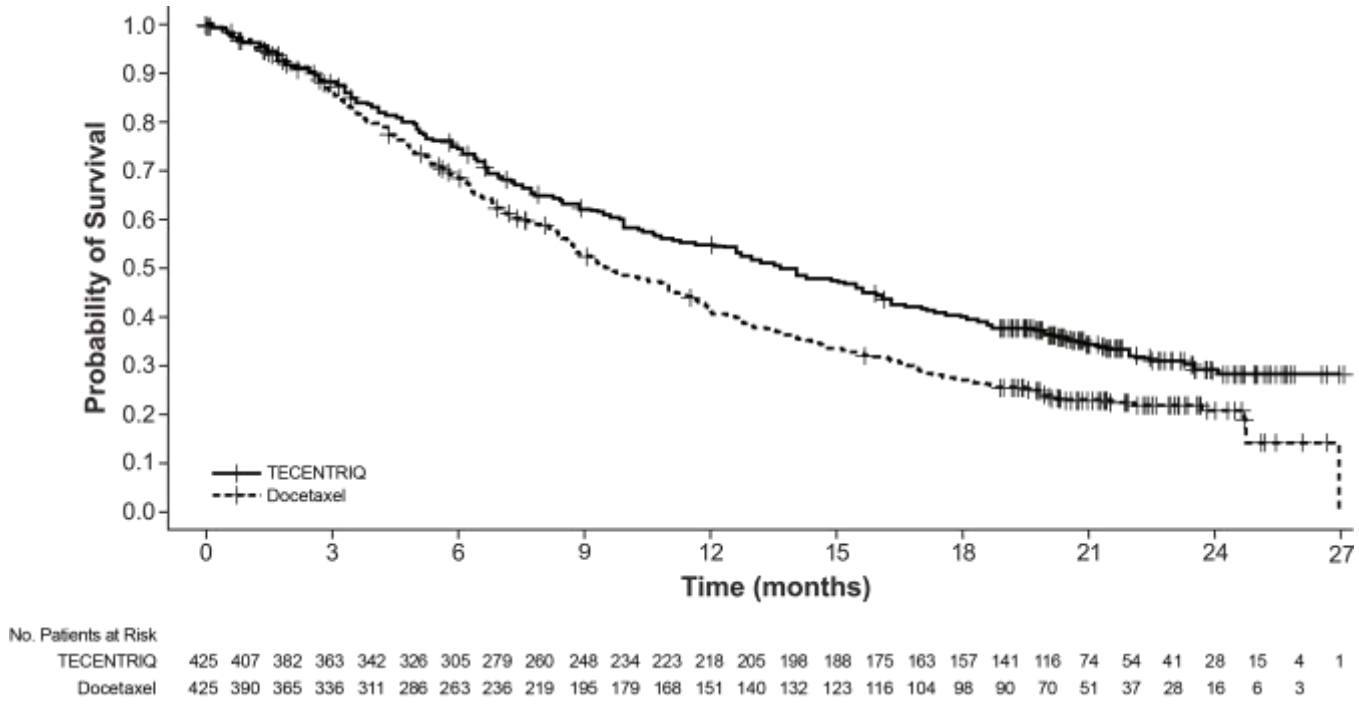

Figure 2. Kaplan-Meier Plot of Overall Survival in the Primary Analysis Population of the Phase III Study

REFERENCES

1. 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):739-45.
2. 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):111-22.
3. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563-7.
4. Carson FL, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
5. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
6. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2011.
7. CLSI. Quality assurance for design control and implementation of immunohistochemistry assay: approved guidelines. 2nd edition Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011.
8. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
9. Hautzer NW, Wittkuhn JF, Elliott-McCaughey WT. Trypsin Digestion in Immunoperoxidase Staining. Jour of Histochem and Cytochem. 1980;28:52-53.
10. 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 32.
11. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.

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CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)
www.ventana.com