

VENTANA PD-L1 (SP263) Assay

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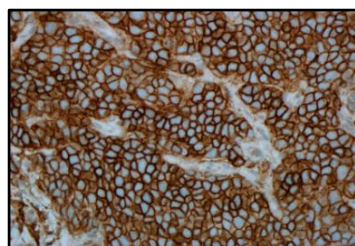


Figure 1. Non-small cell lung cancer stained with VENTANA PD-L1 (SP263) Assay.

INTENDED USE

VENTANA PD-L1 (SP263) Assay is intended for the qualitative detection of the programmed death ligand 1 (PD-L1) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC), urothelial carcinoma (UC) and other tumor tissues stained with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument.

NSCLC:

PD-L1 expression in tumor cell (TC)

membrane as detected by VENTANA PD-L1 (SP263) Assay in NSCLC is indicated as an aid in identifying patients for treatment with KEYTRUDA® (pembrolizumab).

PD-L1 expression in tumor cell (TC) membrane as detected by VENTANA PD-L1 (SP263) Assay in NSCLC may be associated with enhanced survival from OPDIVO® (nivolumab).

Urothelial Carcinoma:

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). 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.

This product 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.

The assay is intended for identifying patients who may benefit from therapy as shown in Table 1.

Table 1. Therapy and associated indication.

Indication for use	Therapy	PD-L1 Expression-Therapeutic Line
NSCLC	KEYTRUDA®	≥ 50% TC – First Line
	OPDIVO®	≥ 1% TC – Second Line
UC	IMFINZI™	≥ 1%, ≥ 5% and ≥ 10% TC – Second Line
		PD-L1 status is considered High if any of the following are met: <ul style="list-style-type: none"> • ≥ 25% TC membrane staining; or, • ICP > 1% and IC+ ≥ 25%; or, • ICP = 1% and IC+ = 100%. – Second Line

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) antibody) to recognize the programmed death-ligand 1 (PD-L1) also known as B7 homolog 1 (B7-H1) or CD274.

PD-L1 is a transmembrane protein that down regulates immune responses through binding to its two receptors programmed death-1 (PD-1) and B7-1 (CD80).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 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.³ PD-L1 expression has been observed in immune cells and tumor cells.^{4,5} Aberrant expression of PD-L1 on tumor 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.

PD-L1 is expressed in a broad range of cancers including lung, melanoma, urothelial, ovarian, and colorectal. 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.⁶

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP263) Assay utilizes a rabbit monoclonal primary antibody that binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the appropriate OptiView DAB IHC Detection Kit package inserts for further information.

REAGENT PROVIDED

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

One 5 mL dispenser of VENTANA PD-L1 (SP263) Assay contains approximately 8.05 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) Assay 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 but are not provided:

1. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
2. Microscope slides, positively charged
3. Bar code labels
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. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
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 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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with OptiView DAB IHC Detection Kit and BenchMark IHC/ISH instruments. Based on testing of 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 VENTANA PD-L1 (SP263) Assay. The amount of fixative used is 15 to 20 times the volume of tissue. Fixation can be performed at room temperature (15-25°C).⁷ See the interpretation guide (P/N 1015317EN for NSCLC and 1015882EN for UC) for further discussion of the impact of specimen preparation on PD-L1 staining with VENTANA PD-L1 (SP263) Assay.

Sections should be cut at 4-5 µm in thickness and mounted on positively charged slides. Slides should be stained within the maximum storage duration as stipulated in Table 2. Antigenicity of cut tissue sections may diminish over time and may be compromised after cutting from the paraffin block.

Table 2. Stability of cut tissue sections.

Tissue type	Maximum Storage (at 2-8°C or 15-30°C)
NSCLC	12 months
Urothelial carcinoma	6 months
Placenta Specimens	9 months

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.
9. KEYTRUDA®, OPDIVO® or IMFINZI™ therapies may not be available in all geographies.

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 Table 3 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 Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 3. Recommended Staining Protocol for VENTANA PD-L1 (SP263) Assay with OptiView DAB IHC Detection Kit and BenchMark IHC/ISH instruments.

Procedure Type	ULTRA VENTANA PD-L1 (SP263) Assay XT VENTANA PD-L1 (SP263) Assay GX VENTANA PD-L1 (SP263) Assay
Protocol Step	Parameter Input
Baking	Optional
Antibody (Primary)	VENTANA PD-L1 (SP263) Selected or Negative Control Selected
Counterstain	Hematoxylin II, 4 to 8 minutes

QUALITY CONTROL PROCEDURES

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.

Tissue Control

A tissue control must be included with each staining run. 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. A tissue section fixed or processed differently from the patient tissues can be used as a control for reagents and staining but not for fixation or tissue preparation.

Qualified normal human term placental tissue can be used as a tissue control for VENTANA PD-L1 (SP263) Assay. Placenta tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. Appropriate staining of placental tissue components is described in Table 4 and in the interpretation guide (P/N 1015317EN for NSCLC and 1015882EN for UC).

Additionally, a NSCLC or UC tissue that shows heterogeneous staining intensity with overall moderate PD-L1 expression can also be used as a tissue control for VENTANA PD-L1 (SP263) Assay.

If the tissue controls fail to demonstrate appropriate staining or demonstrate a change in interpretation, any results with the test specimens should be considered unevaluable and repeat staining should be performed.

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 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.

Refer to VENTANA PD-L1 (SP263) Assay Interpretation Guide for NSCLC (P/N 1015317EN) and UC (1015882EN) for specifics and images.

Placenta Tissue Control

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

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

Interpretation	Staining Description
Acceptable	Moderate to strong uniform membrane staining of trophoblast-lineage cells, and placental stroma and vasculature with no staining.
Not Acceptable	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.

Patient Tissue

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 (TC) 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.

Immune cells (IC) are scored as the percentage of tumor-associated immune cells (IC+) at any intensity above background staining as noted on the corresponding negative control.

Patient tissue must be evaluated according to the indication-specific VENTANA PD-L1 (SP263) Assay scoring algorithm.

Scoring Algorithm - NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 5) and the non-specific background scoring criteria (Table 6). Refer to the interpretation guide (P/N 1015317EN) for additional instructions and representative images.

Table 5. VENTANA PD-L1 (SP263) Assay Scoring Algorithm for NSCLC.

PD-L1 Interpretation	Staining Description
≥ 1%	≥ 1% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
< 1%	< 1% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
≥ 5%	≥ 5% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
< 5%	< 5% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
≥ 10%	≥ 10% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
< 10%	< 10% of tumor cells with membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.

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

Table 6. Non-Specific Background Scoring Criteria for VENTANA PD-L1 (SP263) Assay.

Interpretation	Staining Description
Acceptable	Background does not interfere with interpretation of PD-L1 expression level
Unacceptable	Background interferes with interpretation of PD-L1 expression level

Scoring Algorithm - Urothelial Carcinoma

UC tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm for urothelial carcinoma (Table 7) and the non-specific background scoring criteria (Table 6). Refer to the interpretation guide (P/N 1015882EN) for additional instructions and representative images.

Table 7. 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: • ≥ 25% of tumor cells exhibit membrane staining; or, • ICP > 1% and IC+ ≥ 25%; or, • ICP = 1% and IC+ = 100%.
Low/negative	PD-L1 Status is considered Low/negative if: • none of the criteria for PD-L1 High Status are met.

SPECIFIC LIMITATIONS - GENERAL

1. VENTANA PD-L1 (SP263) Assay has been developed for BenchMark series automated staining 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.
4. Cold ischemia testing of VENTANA PD-L1 (SP263) Assay using a xenograft tissue model did not establish any conditions from zero hours to up to 24 hours that were not favorable with the assay.

SPECIFIC LIMITATIONS – UROTHELIAL CARCINOMA

1. 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.
2. 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 - GENERAL

Specificity - General

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 8. Additional staining, such as cytoplasmic or immune cell staining, is also noted (see Table 8 footnote)

Table 8. Specificity of VENTANA PD-L1 (SP263) Assay was determined by testing 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 - General

An array of neoplastic tissues was evaluated for tumor cell and immune cell staining with VENTANA PD-L1 (SP263) Assay as described in Table 9.

Table 9. Sensitivity of VENTANA PD-L1 (SP263) Assay was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues for any tumor cell and 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

Origin	Pathology	# positive / total cases	
		Tumor Cells	Immune Cells
Cerebrum	Oligodendroglioma	0/1	0/1
Ovary	Serous adenocarcinoma	0/1	1/1
Ovary	Adenocarcinoma	0/1	0/1
Pancreas	Islet cell carcinoma	1/1	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	0/1	0/1
Uterine cervix	Squamous cell carcinoma	0/2	2/2
Striated muscle	Embryonal rhabdomyosarcoma	0/1	0/1
Rectum	Malignant melanoma	0/1	0/1

Origin	Pathology	# positive / total cases	
		Tumor Cells	Immune Cells
Skin	Basal cell carcinoma	0/1	0/1
Skin	Squamous cell carcinoma	0/1	0/1
Back	Neurofibroma	0/1	1/1
Retroperitoneal	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

Repeatability and Intermediate Precision – Placenta Tissue Control

The repeatability and intermediate precision of VENTANA PD-L1 (SP263) Assay for human placenta tissue was evaluated on the BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit.

For Intra-day Repeatability, 5 replicate slides from each of 8 unique placenta specimens were stained with VENTANA PD-L1 (SP263) Assay on a single BenchMark ULTRA instrument.

For Inter-day Precision, 2 replicate slides from each of 8 unique placenta 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 twenty days.

For Inter-instrument Precision, each of 12 unique placenta specimens was stained with VENTANA PD-L1 (SP263) Assay across three BenchMark ULTRA instruments.

All slides were evaluated using the VENTANA PD-L1 (SP263) Assay scoring guide for placenta control tissue (provided in Table 4).

The overall percent agreement for Intra-day, Inter-day, and Inter-instrument Reproducibility was 100%, 100% and 98.8%, respectively.

Lot-to-Lot Reproducibility – Placenta Tissue Control

Lot-to-lot reproducibility of VENTANA PD-L1 (SP263) Assay for control tissue was evaluated on 12 unique human placenta tissue specimens using three lots of VENTANA PD-L1 (SP263) antibody. The overall percent agreement rate for inter-antibody lot was 98.8%.

PERFORMANCE CHARACTERISTICS - NSCLC

Sensitivity - NSCLC

Sensitivity of VENTANA PD-L1 (SP263) Assay was tested on 245 unique cases of NSCLC FFPE specimens using manufactured production lots of VENTANA PD-L1 (SP263) Assay. Assessment of PD-L1 expression demonstrated staining across a range of 0-100% positive tumor cell staining.

Repeatability and Intermediate Precision - NSCLC

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 Intra-day Repeatability, 5 replicate slides from each of the NSCLC specimens were stained on a single BenchMark ULTRA instrument.

For Inter-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 twenty days.

For Inter-instrument Precision testing each of NSCLC specimens were stained with VENTANA PD-L1 (SP263) Assay across three BenchMark ULTRA instruments. All slides were blinded, randomized, and evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm (provided in Table 5).

A summary of the results can be found in Table 10.

Table 10. Repeatability and Intermediate Precision Study of VENTANA PD-L1 (SP263) Assay on Individual NSCLC Specimens.

PD-L1 Expression Level	≥ 1% Expression	≥ 5% Expression	≥ 10% Expression	≥ 50% Expression
NSCLC Tissue Repeatability/ Precision	Overall Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Intra-Day Repeatability (within a single day)	100.0% (96.9-100.0)*	99.2% (95.4-99.9)*	98.3% (94.1-99.5)*	100.0% (96.9-100.0)*
Inter-Day Precision (5 non-consecutive days)	100.0% (98.4-100.0)*	97.9% (95.2-99.1)*	98.8% (96.4-99.6)*	100.0% (98.4-100)*
Inter-Instrument Precision (across 3 instruments)	100% (99.4-100.0)*	96.5% (94.7-97.6)*	95.2% (93.3-96.6)*	97.2% (94.6-99.2)**

* Confidence intervals were calculated using the Wilson Score method.

** Confidence intervals were calculated using the percentile bootstrap method from 2,000 bootstrap samples.

Lot-to-Lot Reproducibility - NSCLC

Lot-to-lot Reproducibility of VENTANA PD-L1 (SP263) Assay was determined by testing three lots of VENTANA PD-L1 (SP263) Assay across 24 unique NSCLC cases on BenchMark ULTRA instruments using OptiView DAB IHC Detection Kit. All cases were stained with each of the three lots of VENTANA PD-L1 (SP263) antibody. Slides were blinded and randomized prior to evaluation for PD-L1 expression as determined by the VENTANA PD-L1 (SP263) Assay scoring algorithm (provided in Table 5). Results are reported in Table 11 as overall percent agreement, positive percent agreement, and negative percent agreement rates for each expression level.

Table 11. Lot-to-Lot Reproducibility Agreement Rates across NSCLC Tissue Specimens.

Lot -to-Lot Reproducibility Agreement Rates	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three lot-to-lot comparisons ≥ 1% Expression	100% (99.0-100.0)*	100% (98.6-100.0)*	100% (99.4-100.0)*
Average of all three lot-to-lot comparisons ≥ 5% Expression	94.4% (91.4-96.5)*	98.5% (96.4-99.3)*	96.5% (94.7-97.6)*
Average of all three lot-to-lot comparisons ≥ 10% Expression	97.5% (94.7-98.9)*	93.8% (91.0-95.8)*	95.2% (93.3-96.6)*

Lot –to-Lot Reproducibility Agreement Rates	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Average of all three lot-to-lot comparisons ≥ 50% Expression	96.9% (91.9-99.7)**	97.5% (94.9-99.5)**	97.2% (94.4-99.1)**

* Confidence intervals were calculated using the Wilson Score method.

** Confidence intervals were calculated using the percentile bootstrap method from 2,000 bootstrap samples.

Inter- and Intra-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 5. The results provided in Table 12 below reflect the inter-reader and intra-reader precision rates for unique cases from the study cohort.

Table 12. Summary of the Inter- and Intra-Reader Precision Study.

Reader Precision	Average Positive Agreement (95% CI)*	Average Negative Agreement (95% CI)*	Overall Percent Agreement (95% CI)*
Inter-Reader Precision Average of all three readers ≥ 1% Expression	94.3% (90.5-97.4)	92.6% (87.8-96.5)	93.5% (89.9-97.1)
Inter-Reader Precision Average of all three readers ≥ 5% Expression	94.7% (90.7-97.8)	94.7% (90.6-97.7)	94.7% (91.1-97.7)
Inter-Reader Precision Average of all three readers ≥ 10% Expression	93.0% (88.4-96.6)	94.0% (90.0-97.1)	93.5% (89.5-97.0)
Inter-Reader Precision Average of all three readers ≥ 50% Expression	94.6% (90.6-97.8)	95.0% (91.1-97.9)	94.8% (91.2-97.8)
Intra-Reader Precision Average of all three readers ≥ 1% Expression	96.7% (94.7-98.3)	95.6% (92.9-97.8)	96.2% (94.1-98.0)
Intra-Reader Precision Average of all three readers ≥ 5% Expression	95.8% (92.7-98.2)	96.0% (93.2-98.3)	95.9% (93.3-98.2)
Intra-Reader Precision Average of all three readers ≥ 10% Expression	97.7% (95.9-99.2)	98.1% (96.4-99.4)	97.9% (96.2-99.4)
Intra-Reader Precision Average of all three readers ≥ 50% Expression	97.2% (95.2-98.8)	97.3% (95.2-98.9)	97.2% (95.4-98.8)

* Confidence intervals were calculated using the percentile bootstrap method from 2,000 bootstrap samples. CIs for 100% average agreement were calculated using the Clopper-Pearson based method.

Inter-Laboratory Reproducibility Study - NSCLC

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 NSCLC cases, using 28 tissue specimens run across 5 non-consecutive days over a 20-day period at three external laboratories. The specimens were blinded, randomized and evaluated by a total of 6 readers (2 readers/site). See Table 13 for results.

Table 13. Inter-Laboratory Reproducibility: Agreement Rates for VENTANA PD-L1 (SP263) Assay.

Agreement Rates for Inter-Laboratory Reproducibility (PD-L1 Expression)	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)	Overall Percent Agreement (95% CI)
Across all Cases ≥ 1% Expression	94.0% (91.3-95.9)*	89.8% (85.5-92.9)*	92.4% (90.1-94.1)*
Across all Cases ≥ 5% Expression	94.7% (91.9-96.6)*	94.9% (92.1-96.8)*	94.8% (92.9-96.2)*
Across all Cases ≥ 10% Expression	95.4% (92.6-97.2)*	96.8% (94.8-98.1)*	96.2% (94.6-97.4)*
Across all Cases ≥ 50% Expression	96.1% (91.3-99.6)**	93.8% (88.5-98.5)**	95.0% (91.6-97.9)**

* Confidence intervals were calculated using the Wilson Score method.

** 95% CI are calculated using the percentile bootstrap method from 2,000 bootstrap samples.

Method Comparison Study - NSCLC

For a CE mark, Ventana relied on a method comparison study carried out by AstraZeneca, which compares data from currently available PD-L1 assays, PD-L1 IHC 22C3 pharmDx (used in the clinical studies of KEYTRUDA), PD-L1 IHC 28-8 pharmDx (used in the clinical studies of OPDIVO), and VENTANA PD-L1 (SP263) Assay.⁸

A method comparison study using approximately 500 commercially acquired NSCLC biopsy specimens that have been de-identified and unlinked from patient information were compared for staining performance of VENTANA PD-L1 (SP263) Assay on the BenchMark ULTRA instruments to that of PD-L1 IHC 22C3 pharmDx and PD-L1 IHC 28-8 pharmDx on the Autostainer Link 48. The study included NSCLC cases representing a dynamic range of PD-L1 expression. A single central laboratory stained all cases using each method. The stained slides were evaluated and assigned PD-L1 expression level by a pathologist trained to both the Ventana and Dako PD-L1 assays. One pathologist participated in the study.

The primary endpoint was a point estimate of 85% or higher for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) using the Dako assay as the comparator. See Tables 14 and 15 for results.

Table 14. Method Comparison: Agreement Rates for VENTANA PD-L1 (SP263) Assay vs. PD-L1 IHC 22C3 pharmDx.

Assay ≥ 1% Expression	PD-L1 IHC 22C3 pharmDx		
VENTANA PD-L1 (SP263) Assay	Positive	Negative	Total
Positive	256	21	277
Negative	24	199	223
Total	280	220	500
	n/N	% (95% CI)	
Positive percent agreement	256/280	91.4 (87.6-94.2)	
Negative percent agreement	199/220	90.5 (85.8-93.7)	
Overall percent agreement	455/500	91.0 (88.2-93.2)	
Assay ≥ 50% Expression	PD-L1 IHC 22C3 pharmDx		
VENTANA PD-L1 (SP263) Assay	Positive	Negative	Total
Positive	111	22	133
Negative	10	357	367
Total	121	379	500
	n/N	% (95% CI)	

Positive percent agreement	111/121	91.7 (85.5-95.4)
Negative percent agreement	357/379	94.2 (91.4-96.1)
Overall percent agreement	468/500	93.6 (91.1-95.4)

Table 15. Method Comparison: Agreement Rates for VENTANA PD-L1 (SP263) Assay vs. PD-L1 IHC 28-8 pharmDx.

Assay ≥ 1% Expression	PD-L1 IHC 28-8 pharmDx		
VENTANA PD-L1 (SP263) Assay	Positive	Negative	Total
Positive	264	13	277
Negative	29	194	223
Total	293	207	500
	n/N	% (95% CI)	
Positive percent agreement	264/293	90.1 (86.1-93.0)	
Negative percent agreement	194/207	93.7 (89.6-96.3)	
Overall percent agreement	458/500	91.6 (88.8-93.7)	
Assay ≥ 5% Expression	PD-L1 IHC 28-8 pharmDx		
VENTANA PD-L1 (SP263) Assay	Positive	Negative	Total
Positive	225	9	234
Negative	21	245	266
Total	246	254	500
	n/N	% (95% CI)	
Positive percent agreement	225/246	91.5 (87.3-94.3)	
Negative percent agreement	245/254	96.5 (93.4-98.1)	
Overall percent agreement	470/500	94.0 (91.6-95.8)	
Assay ≥ 10% Expression	PD-L1 IHC 28-8 pharmDx		
VENTANA PD-L1 (SP263) Assay	Positive	Negative	Total
Positive	192	17	209
Negative	19	272	291
Total	211	289	500
	n/N	% (95% CI)	
Positive percent agreement	192/211	91.0 (86.4-94.2)	
Negative percent agreement	272/289	94.1 (90.8-96.3)	
Overall percent agreement	464/500	92.8 (90.2-94.8)	

CLINICAL OUTCOME STUDY - NSCLC

KEYTRUDA

KEYNOTE-024: Controlled trial of NSCLC patients naïve to treatment

The safety and efficacy of pembrolizumab were investigated in KEYNOTE-024, multicenter, controlled study for the treatment of previously untreated metastatic NSCLC. Patients had PD-L1 expression with a ≥ 50% Tumor Proportion Score (TPS) based on PD-L1 IHC 22C3 pharmDx.⁹ Patients were randomized (1:1) to receive pembrolizumab at a dose of 200 mg every 3 weeks (n=154) or investigator's choice platinum-containing chemotherapy (n=151; including pemetrexed+carboplatin, pemetrexed+cisplatin, gemcitabine+cisplatin, gemcitabine+carboplatin, or paclitaxel+carboplatin. Non-squamous patients could receive pemetrexed maintenance). Patients were treated with pembrolizumab until unacceptable toxicity or disease progression. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving clinical benefit by the investigator. The study excluded patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required

systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks. Assessment of tumor status was performed every 9 weeks. Patients on chemotherapy who experienced independently-verified progression of disease were able to crossover and receive pembrolizumab.

Among the 305 patients in KEYNOTE-024, baseline characteristics were: median age 65 years (54% age 65 or older); 61% male; 82% White and 15% Asian; and ECOG performance status 0 and 1 in 35% and 65%, respectively. Disease characteristics were squamous (18%) and non-squamous (82%); M1 (99%); and brain metastases (9%).

The primary efficacy outcome measure was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST 1.1). Secondary efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST 1.1. Table 16 summarizes key efficacy measures for the entire intent to treat (ITT) population. The Kaplan-Meier Curve for overall survival is provided in Figure 2.

Table 16. Efficacy Results in KEYNOTE-024.

Endpoint	KEYTRUDA 200 mg every 3 weeks n=154	Chemotherapy n=151
PFS*		
Number (%) of patients with event	73 (47%)	116 (77%)
Hazard ratio† (95% CI)	0.50 (0.37, 0.68)	—
p-Value ‡	<0.001	—
Median in months (95% CI)	10.3 (6.7, NA)	6.0 (4.2, 6.2)
OS		
Number (%) of patients with event	44 (29%)	64 (42%)
Hazard ratio† (95% CI)	0.60 (0.41, 0.89)	—
p-Value‡	0.005†	—
Median in months (95% CI)	Not Reached (NA, NA)	Not Reached (9.4, NR)
Objective Response Rate*		
ORR% (95% CI)	45% (37,53)	28% (21, 36)
Complete Response %	4%	1%
Partial Response %	41%	27%
Response Duration§		
Median in months (range)	Not reached (1.9+, 14.5+)	6.3 (2.1+, 12.6+)
% with duration > 6 months	88%¶	59%#

* Assessed by BICR using RECIST 1.1

† Hazard ratio (KEYTRUDA compared to chemotherapy) based on the stratified Cox proportion hazard model

‡ Based on stratified Log rank test

§ Based on patients with a best overall response as confirmed complete or partial response

¶ Based on Kaplan-Meier estimates; includes 43 patients with response of 6 months or longer

Based on Kaplan-Meier estimates; includes 16 patients with response of 6 months or longer

NA = not available

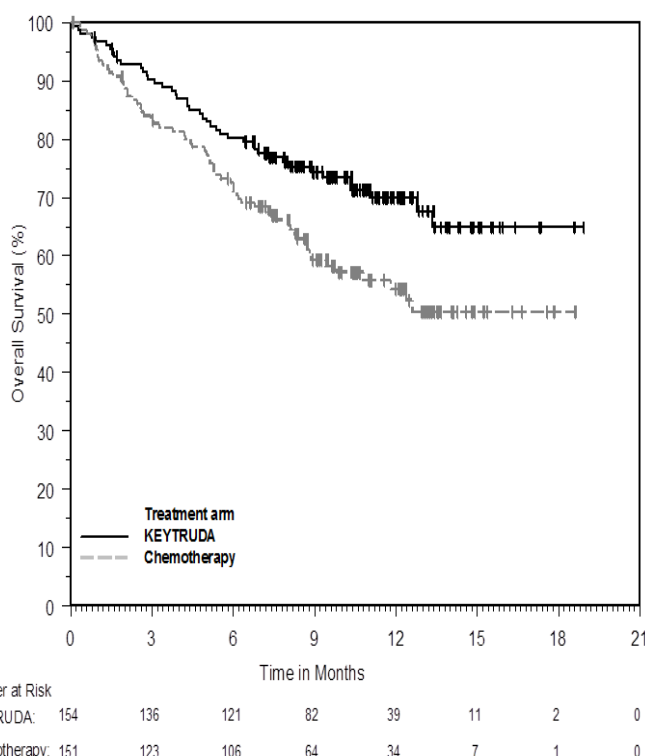


Figure 2. Kaplan-Meier Curve for Overall Survival in KEYNOTE-024.

KEYNOTE-010: Controlled trial of NSCLC patients previously treated with chemotherapy

The clinical benefit of PD-L1 IHC 22C3 pharmDx was investigated in KEYNOTE-010, a multicenter, open-label, randomized clinical study conducted to assess the safety and efficacy of KEYTRUDA in patients with advanced NSCLC previously treated with platinum-containing chemotherapy.¹⁰ Patients had PD-L1 expression with a $\geq 1\%$ TPS based on a clinical trial assay (CTA) version of PD-L1 IHC 22C3 pharmDx. Patients with EGFR activation mutation or ALK translocation also had disease progression on approved therapy for these mutations prior to receiving pembrolizumab. Patients were randomized (1:1:1) to receive pembrolizumab at a dose of 2 (n=344) or 10 mg/kg (n=346) every 3 weeks or docetaxel at a dose of 75 mg/m² every 3 weeks (n=343) until disease progression or unacceptable toxicity. The trial excluded patients with autoimmune disease; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks. Assessment of tumor status was performed every 9 weeks. The primary efficacy outcome measures were OS and PFS as assessed by BICR using RECIST 1.1.

Based on the CTA, a total of 1,033 NSCLC patients were randomized in the study. To evaluate the clinical utility of PD-L1 IHC 22C3 pharmDx, archived clinical study samples were retrospectively tested at a US based reference laboratory with PD-L1 IHC 22C3 pharmDx. Out of the 1,033 patients, tumor tissue from 529 patients was retrospectively tested with PD-L1 IHC 22C3 pharmDx test. Specimen from 413 patients had PD-L1 expression ($\geq 1\%$ of viable tumor cells exhibiting membrane staining at any intensity) and samples from 94 patients did not have PD-L1 expression ($< 1\%$ of viable tumor cells exhibiting membrane staining at any intensity). Within these 413 patients with PD-L1 expression, specimens from 163 patients had high PD-L1 expression ($\geq 50\%$ of viable tumor cells exhibiting membrane staining at any intensity).

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 17.

Table 17. Efficacy Results of the Subgroup of Patients with TPS $\geq 50\%$ in KEYNOTE-010.

Agreement Rates	PD-L1 Cut-off	Negative Percent Agreement (95% CI)	Positive Percent Agreement (95% CI)
CTA vs. PD-L1 IHC 22C3	TPS $\geq 1\%$	94.5% (91.4%-96.6%)	80.0% (76.9%-82.8%)
	TPS $\geq 50\%$	98.3% (97.1%-99.0%)	73.2% (67.9%-77.9%)

Among randomized patients having PD-L1 expression by PD-L1 IHC 22C3 pharmDx, the demographic and other baseline characteristics were well balanced between the treatment arms. The median age was 63 years (44% age 65 or older). The majority of patients were white (77%) and male (58%); baseline ECOG performance status was 0 (29%) or 1 (71%). Seventy-eight percent (78%) of patients were former/current smokers. Twenty-two percent (22%) of patients had squamous histology and 69% had non-squamous histology. The baseline and demographic characteristics were similarly well balanced across pembrolizumab and docetaxel arms in the overall study.

Efficacy results are summarized in Table 18 and 19. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS $\geq 1\%$), which was enhanced in patients with high PD-L1 expression (TPS $\geq 50\%$) as determined by PD-L1 IHC 22C3 pharmDx. The magnitude of benefit was comparable to that in the overall clinical trial. The tables below summarize the key efficacy measures in the overall population with PD-L1 expression (TPS $\geq 1\%$) and in the high PD-L1 expression (TPS $\geq 50\%$) subset for the overall clinical study (TPS $\geq 1\%$ by CTA) and in the population with PD-L1 expression by PD-L1 IHC 22C3 pharmDx. The Kaplan-Meier curve for OS (TPS $\geq 1\%$), as determined by PD-L1 IHC 22C3 pharmDx is shown in Figure 3. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 18. Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Study and PD-L1 IHC 22C3 pharmDx Positive Patients: PD-L1 TPS ≥ 1%.

Endpoint	KEYTRUDA 2mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of Patients	344	140	346	142	343	131
OS						
Deaths (%)	172 (50%)	59 (42%)	156 (45%)	59 (42%)	193 (56%)	67 (51%)
Hazard Ratio* (95% CI)	0.71 (0.58, 0.88)	0.54 (0.37, 0.78)	0.61 (0.49, 0.75)	0.57 (0.39, 0.82)	—	—
p-Value†	< 0.001	< 0.001	< 0.001	0.00115	—	—
Median in months (95% CI)	10.4 (9.4, 11.9)	11.8 (9.6, NA)	12.7 (10.0, 17.3)	12.0 (8.7, NA)	8.5 (7.5, 9.8)	7.5 (6.3, 9.9)
PFS‡						
Events (%)	266 (77%)	97 (63%)	255 (74%)	103 (73%)	257 (75%)	94 (72%)
Hazard Ratio* (95% CI)	0.88 (0.73, 1.04)	0.68 (0.50, 0.92)	0.79 (0.66, 0.94)	0.79 (0.59, 1.06)	—	—
p-Value†	0.068	0.00578	0.005	0.05767	—	—
Median in months (95% CI)	3.9 (3.1, 4.1)	4.9 (4.1, 6.2)	4.0 (2.6, 4.3)	4.0 (2.2, 4.6)	4.0 (3.1, 4.2)	3.8 (2.2, 4.2)
Overall response rate‡						
ORR %§ (95% CI)	18% (14, 23)	24% (17, 32)	18% (15, 23)	20% (14, 28)	9% (7, 13)	5% (2, 11)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

† Based on stratified Log rank test

‡ Assessed by BICR using RECIST 1.1

§ All responses were partial responses

Table 19. Response to KEYTRUDA in Previously Treated Patients: Overall Clinical Study and PD-L1 IHC 22C3 pharmDx Positive Patients: PD-L1 ≥ 50%.

Endpoint	KEYTRUDA 2mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of Patients	139	56	151	60	152	47
OS						
Deaths (%)	58 (42%)	18 (32%)	60 (40%)	19 (32%)	86 (57%)	25 (53%)
Hazard Ratio* (95% CI)	0.54 (0.38, 0.77)	0.45 (0.24, 0.84)	0.50 (0.36, 0.70)	0.29 (0.15, 0.56)	—	—
p-Value†	< 0.001	0.00541	< 0.001	< 0.001	—	—
Median in months (95% CI)	14.9 (10.4, NA)	Not reached (9.3, NA)	17.3 (11.8, NA)	Not reached (8.3, NA)	8.2 (6.4, 10.7)	7.2 (4.4, 8.3)
PFS‡						
Events (%)	89 (64%)	33 (59%)	97 (64%)	34 (57%)	118 (78%)	33 (70%)
Hazard ratio* (95% CI)	0.58 (0.43, 0.77)	0.47 (0.28, 0.80)	0.59 (0.45, 0.78)	0.41 (0.24, 0.70)	—	—
p-Value†	< 0.001	0.00221	< 0.001	< 0.001	—	—
Median in months (95% CI)	5.2 (4.0, 6.5)	5.9 (4.2, 9.0)	5.2 (4.1, 8.1)	4.8 (2.8, NA)	4.1 (3.6, 4.3)	3.9 (2.0, 4.3)
Overall response rate‡						
ORR %§ (95% CI)	30% (23, 39)	37% (25, 52)	29% (22, 37)	28% (18, 41)	8% (4, 13)	4% (1, 15)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

† Based on stratified Log rank test

‡ Assessed by BICR using RECIST 1.1

§ All responses were partial responses

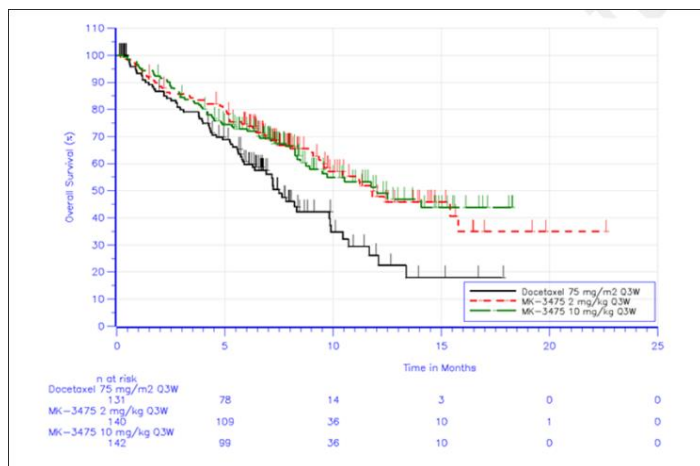


Figure 3. Kaplan-Meier Curve for Overall Survival in all Randomized Patients in KEYNOTE-010.

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with PD-L1 expression (TPS $\geq 1\%$) by PD-L1 IHC 22C3 pharmDx, but who may have had no PD-L1 expression (TPS $< 1\%$) by the CTA. Patients with such test results are part of the intended use/ intent to diagnose (ITD) population of PD-L1 IHC 22C3 pharmDx; however, they were excluded from the clinical trial due to no PD-L1 expression upon CTA screening. To account for these missing data, a sensitivity analysis was conducted to understand the plausible range for the hazard ratio (HR) estimated based on PD-L1 IHC 22C3 pharmDx in the TPS $\geq 1\%$ and TPS $\geq 50\%$ subpopulations under an ITD framework to verify the consistency with the observed HR based on enrolment with the CTA. The HR sensitivity analysis results showed that the HR estimates are robust to any assumed attenuation of the treatment effect under the ITD framework.

OPDIVO

CA209057 Phase 3 study in patients with advanced or metastatic non-squamous cell NSCLC

Clinical utility was evaluated in CA209057, a Phase 3, randomized, open-label study of nivolumab vs docetaxel in adult (≥ 18 years) subjects with advanced or metastatic non-squamous cell NSCLC after failure of prior platinum doublet -based chemotherapy.¹¹ Subjects were randomized 1:1 and stratified according to 1) prior use of maintenance therapy vs. no use of maintenance therapy and 2) second-line vs. third-line therapy. Pre-study (baseline) tumor tissue specimens were collected prior to randomization and prior to first treatment to conduct pre-planned analyses of efficacy according to predefined baseline PD-L1 expression levels (secondary objective). The primary endpoint was overall survival (OS). Other secondary endpoints were objective response rate (ORR), progression-free survival (PFS), and disease-related symptom improvement by 12 weeks, as measured by the Lung Symptom Cancer Scale (LCSS).

The baseline demographic and disease characteristics were generally balanced between randomized subjects in the nivolumab and docetaxel groups. The mean age was 62 years (range: 21 to 85) with 34% ≥ 65 years of age and 7% ≥ 75 years of age. The majority of patients were white (92%) and male (55%); baseline ECOG performance status was 0 (31%) or 1 (69%). Seventy-nine percent of patients were former/current smokers.

Patients with PD-L1 expression as determined by the Dako PD-L1 IHC 28-8 pharmDx by all predefined expression levels in the OPDIVO group were associated with enhanced survival compared to docetaxel, whereas survival was similar to docetaxel in patients with no PD-L1 expression. Meaningful differences in median OS were observed in nivolumab over docetaxel subgroups when analyzed by PD-L1 expression level. Median OS was 17.1, 18.2, and 19.4 months for nivolumab subjects compared to 9.0, 8.1, and 8.0 months for docetaxel subjects with $\geq 1\%$, $\geq 5\%$, and $\geq 10\%$ PD-L1 expression levels, respectively. There were no differences in OS between the treatment groups in subjects with $< 1\%$, $< 5\%$, and $< 10\%$ expression levels, with ranges of median OS of 9.7 to 10.4 months for nivolumab and 10.1 to 10.3 months for docetaxel. The unstratified hazard ratios (HR) and median overall survival (OS) are presented in Figure 4. The Kaplan-Meier plot for subgroups by PD-L1 expression level is shown in Figure 5 and Figure 6.

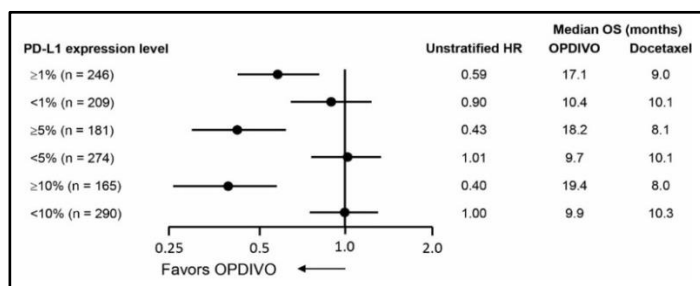


Figure 4. Forest Plot - OS Based on PD-L1 Expression in non-squamous NSCLC Patients - CA209057.

Note: The unstratified hazard ratio and the corresponding 95% CI were estimated in a Cox proportional hazards model using the randomized arm as a single covariate.

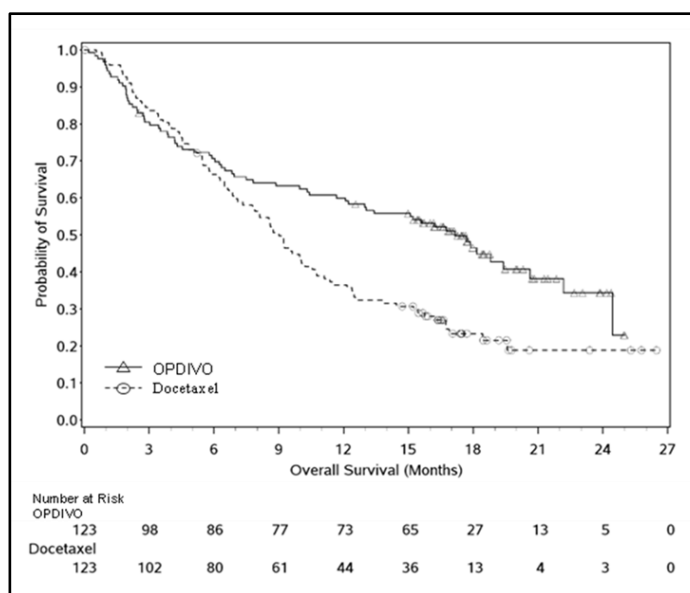


Figure 5. Overall Survival - Patients with $\geq 1\%$ PD-L1 Expression-CA209057.

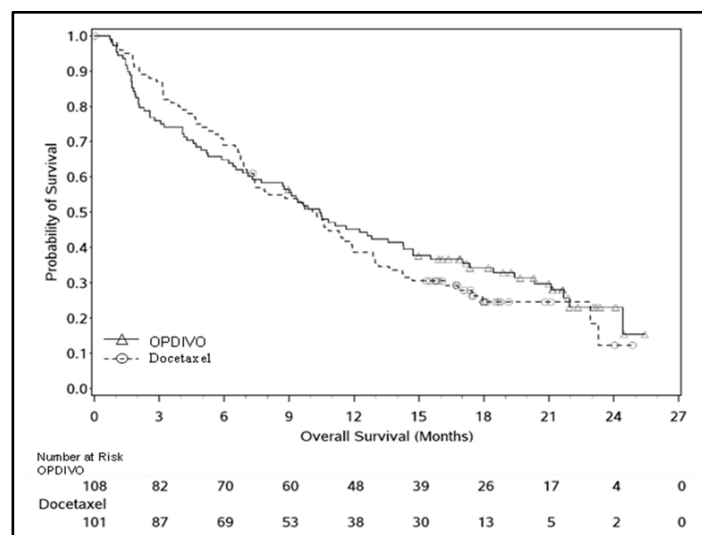


Figure 6. Overall Survival - non-squamous NSCLC Patients with < 1% PD-L1 Expression - CA209057.

PERFORMANCE CHARACTERISTICS - UROTHELIAL CARCINOMA

Sensitivity - Urothelial Carcinoma

Sensitivity of VENTANA PD-L1 (SP263) Assay was tested on 211 unique cases of urothelial carcinoma FFPE specimens using manufactured production lots of VENTANA PD-L1 (SP263) Assay. 169/211 tissues were classified as PD-L1 High using the VENTANA PD-L1 (SP263) Assay scoring criteria for urothelial carcinoma

Repeatability and Intermediate Precision - Urothelial Carcinoma

The repeatability and intermediate precision of VENTANA PD-L1 (SP263) Assay for urothelial carcinoma was evaluated on the BenchMark ULTRA instruments using OptiView DAB IHC Detection Kit.

For intra-day repeatability, 5 replicate slides from each of 13 unique urothelial carcinoma specimens were stained on a single BenchMark ULTRA instrument.

For inter-day precision, 2 replicate slides from each of 13 unique urothelial carcinoma 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 twenty days.

For Inter-instrument precision testing, 22 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay across three BenchMark ULTRA instruments. All slides were blinded, randomized, and evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm (provided in Table 7).

A summary of results can be found in Table 20.

Table 20. Repeatability and Intermediate Precision of VENTANA PD-L1 (SP263) Assay staining on Urothelial Carcinoma Specimens.

Repeatability/Precision	Overall Percent Agreement (95% CI)*
Intra-Day Repeatability (within a single day)	98.5% (91.8 - 99.7%)
Inter-Day Precision (5 non-consecutive days)	100.0% (97.1 - 100.0%)
Inter-Instrument Precision (across 3 instruments)	98.8% (97.6 - 99.4%)

* Confidence intervals were calculated using the Wilson Score method.

Lot-to-Lot Reproducibility – Urothelial Carcinoma

Lot-to-lot reproducibility of VENTANA PD-L1 (SP263) Assay was determined by testing three lots of VENTANA PD-L1 (SP263) Assay across 22 unique urothelial carcinoma

cases on ULTRA instruments using OptiView DAB IHC Detection Kit. All cases were stained with each of the three lots of primary antibody. Slides were blinded and randomized prior to evaluation for PD-L1 expression as determined by the VENTANA PD-L1 (SP263) Assay scoring algorithm (provided in Table 7). The overall percent agreement, positive percent agreement, and negative percent agreement rates were summarized in Table 21.

Table 21. Lot-to-Lot Reproducibility of VENTANA PD-L1 (SP263) Assay on Urothelial Carcinoma Specimens.

Lot –to-Lot Reproducibility	Positive Percent Agreement (95% CI)*	Negative Percent Agreement (95% CI)*	Overall Percent Agreement (95% CI)*
Average of all three lot to lot comparisons	99.4 % (97.9 – 99.8)	98.8% (96.5 – 99.6)	99.2% (98.0 – 99.6)

*Confidence intervals were calculated using the Wilson Score method.

Inter- and Intra-Reader Precision Studies – Urothelial Carcinoma

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 PD-L1 expression level. The cases were blinded and randomized prior to evaluation for PD-L1 staining per the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 7. Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates are summarized in Table 22.

Table 22. Inter- and Intra-Reader Precision of VENTANA PD-L1 (SP263) Assay on Urothelial Carcinoma Specimens.

Reader Precision	Average Positive Agreement (95% CI)*	Average Negative Agreement (95% CI)*	Average Overall Agreement (95% CI)*
Inter-reader Precision (average of all three reader pairwise comparison for the first read)	92.8% (85.5-98.3%)	93.2% (86.5-98.5%)	93.0% (87.1-98.6%)
Intra-reader Precision (average of all three readers' agreement rates between first and second reads)	92.1% (85.4-96.7%)	92.7% (87.1-96.8%)	92.4% (87.2-96.6%)

* Percentile Bootstrap confidence intervals were constructed from the 2.5th and 97.5th percentiles using 2,000 replicates when sampling at the case level.

Inter-laboratory Reproducibility Study – Urothelial Carcinoma

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 26 tissue specimens run across 5 non-consecutive days over a 20-day period at three external laboratories. The specimens were blinded, randomized and evaluated by a total of 6 readers (2 readers/site). Results are summarized in Table 23.

Table 23. Inter-laboratory Reproducibility of VENTANA PD-L1 (SP263) Assay staining on urothelial carcinoma specimens.

Agreement Rates for Inter-Laboratory Reproducibility (PD-L1 Expression)	Positive Percent Agreement (95% CI)*	Negative Percent Agreement (95% CI)*	Overall Percent Agreement (95% CI)*
Across all Cases	95.2% (93.0-96.7%)	85.8% (80.9-89.7%)	92.3% (90.2-94.0%)

* Confidence intervals were calculated using the Wilson Score method.

CLINICAL OUTCOME STUDY – UROTHELIAL CARCINOMA IMFINZI

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 24. The median follow-up time for these patients was 4.9 months.

Table 24. 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

TROUBLESHOOTING

Troubleshooting guidance is provided in Table 25. 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 25. 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 package insert associated with P/N 741-4905 located at www.ventana.com .
	Incorrect or missing bulk reagent	Ensure that only recommended fixatives and fixation times are used.
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.

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