VENTANA PD-L1 (SP263) Assay

REF 741-7139

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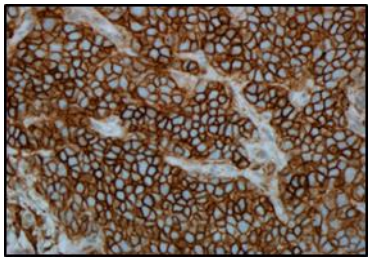


Figure 1. Non-small cell lung cancer stained with VENTANA PD-L1 (SP263) Assay.

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) tissue stained with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument.

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 IMFINZI™ (durvalumab).

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).

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 TECENTRIQ® (atezolizumab).

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. Please refer to the respective drug labeling for clinical recommendations pertaining to PD-L1 expression.

Table 1. Tumor indications and intended uses.

Indication for use	Therapy	PD-L1 Expression-Therapeutic Line
NSCLC	IMFINZI™	≥ 1% TC –Post chemoradiation therapy (CRT)
	KEYTRUDA®	≥ 50% TC – First Line
		≥ 1% TC – Second Line
	OPDIVO®	≥ 1%, ≥ 5% and ≥ 10% TC – Second Line
	TECENTRIQ®	≥ 1% TC – adjuvant treatment following resection and platinum-based chemotherapy

SUMMARY AND EXPLANATION

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

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

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]

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

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP263) Assay is a rabbit monoclonal primary antibody which binds to PD-L1 in FFPE tissue sections. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

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

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

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

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

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

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

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

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

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass or tape
15. Automated or manual coverslipper
16. General purpose laboratory equipment
17. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

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

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

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

SPECIMEN PREPARATION

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

Based on testing of placenta and tonsil tissues that 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]. Refer to VENTANA PD-L1 (SP263) Assay Interpretation Guide Staining of NSCLC (P/N 1020535) 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 desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slides stability within their own environment beyond 45 days, when desired.

WARNINGS AND PRECAUTIONS

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

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

Table 2. Hazard information.

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

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

STAINING PROCEDURE

VENTANA PD-L1 (SP263) Assay has been developed for use on BenchMark 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 User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

Table 3. Recommended staining protocol for VENTANA PD-L1 (SP263) Assay with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	**U VENTANA PD-L1 (SP263) IVD W XT VENTANA PD-L1 (SP263) IVD W GX VENTANA PD-L1 (SP263) IVD W**
Protocol Step	**Parameter Input**
Baking	Optional
Antibody (Primary)	VENTANA PD-L1 (SP263) IVD W Ab Selected or Negative Control Selected
Counterstain	Hematoxylin II, 4 or 8 minutes

NEGATIVE REAGENT CONTROL

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

POSITIVE TISSUE CONTROL

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

Qualified normal human term placental tissue can be used as a tissue control for VENTANA PD-L1 (SP263) Assay. A placenta sample used as a tissue control must exhibit

the staining pattern described as acceptable in Table 4. Placenta tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. Appropriate staining of placental tissue components is described in Table 4 and in the Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining for NSCLC (P/N 1020535).

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

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by VENTANA PD-L1 (SP263) Assay. The stained slide is interpreted by a qualified pathologist using light microscopy. A qualified pathologist experienced in immunohistochemistry (IHC) procedures must evaluate tissue controls and qualify the stained product before interpreting results.

The cellular staining pattern for VENTANA PD-L1 (SP263) Assay is membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membrane, diffuse cytoplasmic, and/or punctate staining.

Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC (P/N 1020535) for specifics and images.

Placenta Tissue Control

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

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

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

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

Negative Reagent Control

Non-specific staining, if present, may have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Refer to Table 6 for the acceptability criteria for non-specific staining. Examples of background staining for this assay can be found in the Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC.

Patient Tissue

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 5 and Table 6. Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC for representative images and instructions for scoring.

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

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

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

Scoring Algorithm

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 Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC 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.
≥ 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	Non-specific staining that is not obtrusive to interpretation of specific staining.
Unacceptable	Non-specific staining that is obtrusive to interpretation of specific staining.

SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP263) Assay has been developed for BenchMark IHC/ISH 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. Cold ischemia testing of VENTANA PD-L1 (SP263) Assay using a xenograft tissue model did not establish any conditions from zero hours to up to 24 hours that were not favorable with the assay.
4. This assay has not been validated for use with other cytology sample types (smears, brushings, washings, lavages, and effusions), or decalcified bone specimens.
5. Slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories

should validate cut slides stability within their own environment beyond 45 days, when desired.

6. This assay has not been validated for use with FNA cell blocks fixed in cytology preservatives.
7. NSCLC FFPE FNA cell blocks were not included in the analytical method comparison or clinical outcome studies described in this method sheet.
8. This assay might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

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

Sensitivity and Specificity

Arrays containing a variety of normal tissues were stained with VENTANA PD-L1 (SP263) Assay and evaluated for presence of membranous PD-L1 staining as listed in Table 7. Additional staining, such as cytoplasmic or immune cell staining, is also noted (see Table 7 footnote).

An array containing a variety of neoplastic tissues was stained with VENTANA PD-L1 (SP263) Assay evaluated for tumor cell and immune cell staining as listed in Table 8.

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

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Myeloid (bone marrow) [a,b]	0/4
Cerebellum	0/3	Lung [b]	0/3
Adrenal gland [a]	0/3	Heart	0/3
Ovary	0/3	Esophagus [a,b]	1/3
Pancreas [a]	0/3	Stomach [a,b]	0/3
Parathyroid gland	0/4	Small intestine [b]	0/3
Pituitary gland [a,b]	0/3	Colon [b]	0/3
Testis	0/3	Liver	0/3
Thyroid [a,b]	0/3	Salivary gland [b]	0/3
Breast	0/3	Lymph node [b]	0/3
Spleen [b]	0/3	Kidney [b]	0/3
Larynx [b]	0/3	Prostate	0/3
Tonsil [b]	3/3	Cervix	0/3
Endometrium	0/3	Bladder	0/3
Skeletal muscle	0/3	Skin [c]	0/4
Nerve (sparse)	0/3	Mesothelium [b]	0/3
Thymus gland [b]	0/3		

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

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

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

Pathology	# positive / total cases	
	Tumor Cells	Immune Cells
Glioblastoma (Cerebrum)	0/1	1/1
Meningioma (Cerebrum)	0/1	0/1
Ependymoma (Cerebrum)	0/1	1/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Serous adenocarcinoma (Ovary)	0/1	1/1
Adenocarcinoma (Ovary)	1/1	0/1
Neuroendocrine neoplasm (Pancreas)	0/1	0/1
Adenocarcinoma (Pancreas)	0/1	1/1
Seminoma (Testis)	0/1	0/1
Embryonal carcinoma (Testis)	0/1	0/1
Medullary carcinoma (Thyroid)	0/1	0/1
Papillary carcinoma (Thyroid)	1/1	0/1
Ductal carcinoma in situ (Breast)	0/1	1/1
Invasive ductal carcinoma (Breast)	0/2	0/2
B-cell lymphoma, NOS (Spleen)	0/1	1/1
Small cell undifferentiated carcinoma (Lung)	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	1/1
Adenocarcinoma (Lung)	0/1	0/1
Neuroendocrine carcinoma (Esophagus)	0/1	0/1
Adenocarcinoma (Esophagus)	0/1	0/1
Signet-ring cell carcinoma (Stomach)	0/1	0/1
Adenocarcinoma (Small Intestine)	0/1	0/1
Stromal sarcoma (Small Intestine)	0/1	0/1
Adenocarcinoma (Colon)	0/1	1/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1	0/1
Adenocarcinoma (Rectum)	0/1	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1	0/1
Hepatocellular carcinoma (Liver)	0/1	0/1
Hepatoblastoma (Liver)	0/1	0/1
Clear cell carcinoma (Kidney)	0/1	0/1
Adenocarcinoma (Prostate)	0/2	0/2
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	0/1	0/1
Clear cell carcinoma (Uterus)	1/1	0/1
Squamous cell carcinoma (Cervix)	0/2	2/2

Pathology	# positive / total cases	
	Tumor Cells	Immune Cells
Embryonal rhabdomyosarcoma (Striated muscle)	0/1	0/1
Melanoma (Rectum)	0/1	0/1
Basal cell carcinoma (Skin)	0/1	0/1
Squamous cell carcinoma (Skin)	0/1	0/1
Neurofibroma (Back)	0/1	1/1
Neuroblastoma (Retroperitoneum)	0/1	0/1
Mesothelioma (Abdominal cavity)	0/1	0/1
B-cell lymphoma, NOS (Mediastinum)	1/1	1/1
Hodgkin lymphoma (Lymph node)	1/1	1/1
B-cell lymphoma, NOS (Lymph node)	1/1	1/1
Anaplastic large cell lymphoma (Pelvic cavity)	1/1	1/1
Leiomyosarcoma (Bladder)	0/1	0/1
Osteosarcoma (Bone)	0/1	1/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1	0/1
Leiomyosarcoma (Smooth muscle)	0/1	0/1
Urothelial carcinoma (Bladder)	1/1	1/1

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 within-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 within one day.

For between-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) Assay. The overall percent agreement rate for inter-antibody lot was 98.8%.

ANALYTICAL PERFORMANCE IN NON-SMALL CELL LUNG CANCER

Sensitivity NSCLC

Sensitivity of VENTANA PD-L1 (SP263) Assay was tested on 733 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 within one day.

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 20 days.

For inter-instrument precision testing, 2 replicate slides from each of the 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 algorithms (provided in Table 5).

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

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

PD-L1 Expression Level	≥ 1% Expression	≥ 5% Expression	≥ 10% Expression	≥ 50% Expression
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)**

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

** 2-sided 95% confidence intervals (CI) were calculated using the percentile bootstrap method from 2000 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) Assay. 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 10 as overall percent agreement, positive percent agreement, and negative percent agreement rates for each expression level.

Table 10. Lot-to-lot reproducibility agreement rates across NSCLC tissue 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 ≥ 1% Expression	100% (99.0-100.0)*	100% (98.6-100.0)*	100% (99.4-100.0)*

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 ≥ 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)*
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)**

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

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

Reader Precision Studies- NSCLC

To assess inter- and intra-reader precision, three pathologists evaluated a minimum of 110 unique cases. The cases were blinded and randomized prior to evaluation for PD-L1 IHC staining per the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 5. The results provided in Table 11 reflect the inter-reader and intra-reader precision rates for unique cases from the study cohort.

Table 11. Summary of the inter- and intra-reader precision study of VENTANA PD-L1 (SP263) Assay on individual NSCLC tissue specimens.

Reader Precision	Average Positive Agreement (95% CI)*	Average Negative Agreement (95% CI)*	Overall Percent Agreement (95% CI)*
Inter-Reader Precision Average of all three 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)

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

Inter-Laboratory Reproducibility Study - NSCLC

An inter-laboratory reproducibility study for VENTANA PD-L1 (SP263) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 protein expression in NSCLC tissue 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 six readers (2 readers/site). See Table 12 for results.

Table 12. Inter-laboratory reproducibility: agreement rates for VENTANA PD-L1 (SP263) Assay.

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	89.9% (87.0-92.3)*	85.8% (81.6-89.2)*	88.3% (85.9-90.3)*
Across all Cases ≥ 5% Expression	89.2% (85.9-91.9)*	93.7% (90.9-95.7)*	91.4% (89.3-93.2)*
Across all Cases ≥ 10% Expression	95.4% (92.6-97.2)*	94.0% (91.6-95.8)*	94.6% (92.8-95.9)*
Across all Cases ≥ 50% Expression	94.3% (89.3-98.3)**	90.1% (82.6-96.1)**	92.2% (87.8-96.0)**

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

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

Method Comparison Study - NSCLC

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.[11]

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, negative percent agreement, and overall percent agreement using the Dako assay as the comparator. See 0 and Table 14 for results.

Table 13. 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)	

CI = Confidence Interval

Table 14. 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)	

CI = Confidence Interval

CLINICAL PERFORMANCE - NSCLC

IMFINZI

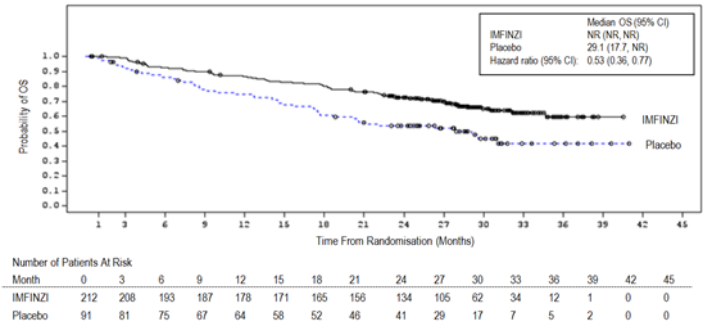
The efficacy of IMFINZI was evaluated in the PACIFIC Study, a randomized, double blind, placebo controlled, multicenter study in 713 patients with locally advanced, unresectable NSCLC. Patients had completed at least 2 cycles of definitive platinum based chemotherapy with radiation therapy within 1 to 42 days prior to initiation of the study and had a ECOG performance status of 0 or 1. Ninety-two percent of patients had received a total dose of 54 to 66 Gy of radiation. The study excluded patients who had progressed following chemoradiation therapy, patients with prior exposure to any anti-PD-1 or anti-PD-L1 antibody, patients with active or prior documented autoimmune disease within 2 years of initiation of the study; a history of immunodeficiency; a history of severe immune mediated adverse reactions; medical conditions that required systemic immunosuppression, except physiological dose of systemic corticosteroids; active tuberculosis or hepatitis B or C or HIV infection or patients receiving live attenuated vaccine within 30 days before or after the start of IMFINZI. Patients were randomized 2:1 to receive 10 mg/kg IMFINZI (n = 476) or 10 mg/kg placebo (n = 237) via intravenous infusion every 2 weeks for up to 12 months or until unacceptable toxicity or confirmed disease progression. Randomization was stratified by gender, age (< 65 years vs. 65 years) and smoking status (smoker vs. non-smoker). Patients with disease control at 12 months were given the option to be re treated upon disease progression. Tumor assessments were conducted every 8 weeks for the first 12 months and then every 12 weeks thereafter. Patients were enrolled regardless of their tumor PD-L1 expression level. Where available, archival tumor tissue specimens taken prior to chemoradiation therapy were retrospectively tested for PD-L1 expression on tumor cells using the VENTANA PD-L1 (SP263) Assay. Of the 713 patients randomized, 63% of patients provided a tissue sample of sufficient quality and quantity to determine PD-L1 expression and 37% were unknown.

The demographics and baseline disease characteristics were well balanced between study arms. Baseline demographics of the overall study population were as follows: male (70%), age ≥ 65 years (45%), age ≥ 75 years (8%), White (69%), Asian (27%), other (4%), current smoker (16%), past smoker (75%), never smoker (9%), ECOG Performance Status 0 (49%), ECOG Performance Status 1 (51%). Disease characteristics were as follows: Stage IIIA (53%), Stage IIIB (45%), histological sub groups of squamous (46%), non-squamous (54%). Of 451 patients with PD-L1 expression available, 67% were tumor cell ≥ 1% and 33% were tumor cell < 1%.

The two primary endpoints of the study were progression free survival (PFS) and overall survival (OS) of IMFINZI vs. placebo. Secondary efficacy endpoints included PFS at 12 months (PFS 12) and 18 months (PFS 18) from randomization and Time from Randomization to Second Progression (PFS2). PFS was assessed by Blinded Independent Central Review (BICR) according to RECIST v1.1.

The study demonstrated a statistically significant improvement in PFS in the IMFINZI treated group compared with the placebo group [hazard ratio (HR) = 0.52 (95% CI: 0.42, 0.65), p < 0.0001]. The study demonstrated a statistically significant improvement in overall survival in the IMFINZI-treated group compared with the placebo group [HR = 0.68 (95% CI: 0.53, 0.87), p = 0.00251]. The improvements in PFS and overall survival in favor of patients receiving IMFINZI compared to those receiving placebo were consistently observed in all predefined subgroups analyzed, including ethnicity, age, gender, smoking history, EGFR mutation status and histology.

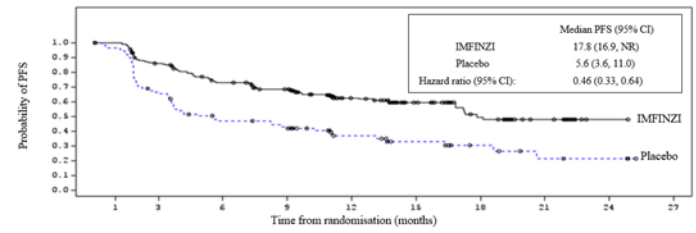
Additional post-hoc exploratory subgroup analyses were conducted to evaluate the efficacy by tumor PD-L1 expression ≥ 1%, < 1% and for patients whose PD-L1 status cannot be established (PD-L1 unknown). PFS and overall survival results are summarized in Figures 2, 3, 4 and 5.



Number of Patients At Risk

Month	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45
IMFINZI	212	208	193	187	178	171	165	156	134	105	62	34	12	1	0	0
Placebo	91	81	75	67	64	58	52	46	41	29	17	7	5	2	0	0

Figure 2. Kaplan Meier curve of OS for PD-L1 tumor cell ≥ 1 %.



Number of patients at risk

Month	0	3	6	9	12	15	18	21	24	27
IMFINZI	212	174	143	127	82	52	30	14	1	0
Placebo	91	59	39	34	20	13	8	4	3	0

Figure 3. Kaplan Meier curve of PFS for PD-L1 tumor cell ≥1%

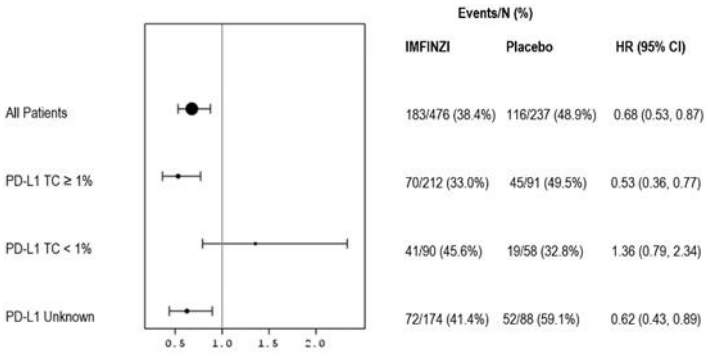


Figure 4. Forest Plot of OS by PD-L1 expression.

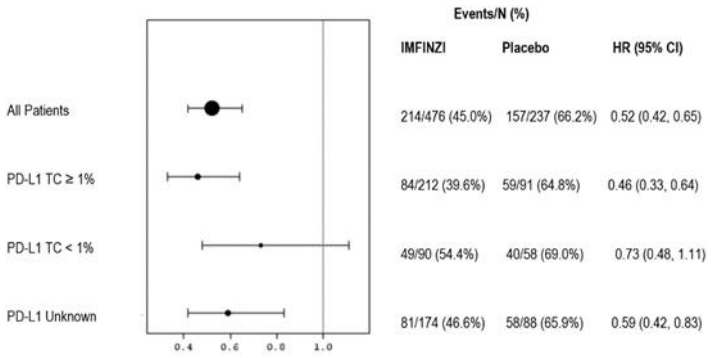


Figure 5. Forest Plot of PFS by PD-L1 expression.

KEYTRUDA

KEYNOTE-024: Controlled Trial of NSCLC Patients Naive 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.[12] 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 maintainance). 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 v1.1). Secondary efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST v1.1. Table 15 summarizes key efficacy measures for the entire intent to treat (ITT) population. The Kaplan-Meier Curve for overall survival is provided in Figure 6.

Table 15. 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 [a] (95% CI)	0.50 (0.37, 0.68)	—
p-Value [b]	< 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 [a] (95% CI)	0.60 (0.41, 0.89)	—
p-Value [b]	0.005 [a]	—
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 [c]		
Median in months (range)	Not reached (1.9+, 14.5+)	6.3 (2.1+, 12.6+)
% with duration > 6 months	88% [d]	59% [e]

CI = Confidence Interval; * Assessed by BICR using RECIST v1.1

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

[b] Based on stratified Log rank test

[c] Based on patients with a best overall response as confirmed complete or partial response

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

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

NA = not available

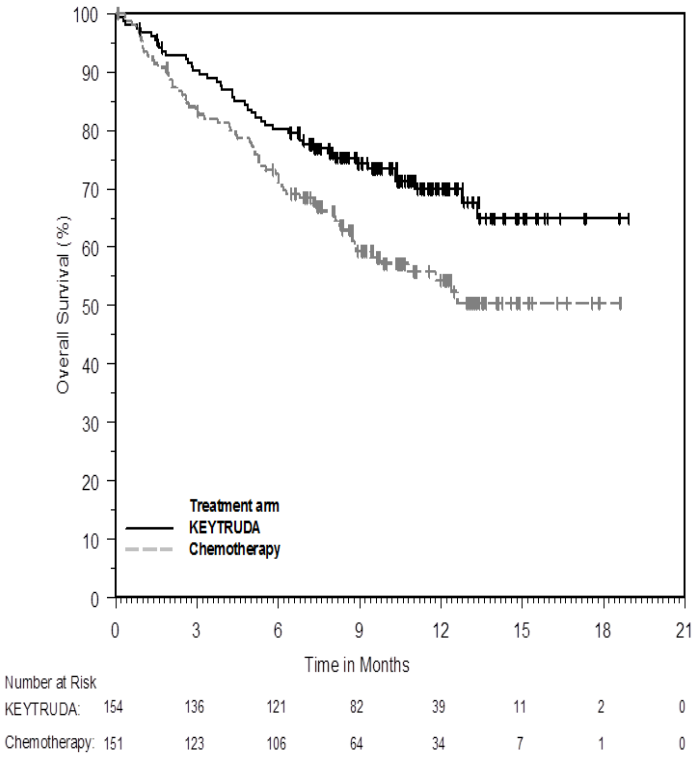


Figure 6. Kaplan-Meier Curve for Overall Survival in KEYNOTE-024.

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.[13] Patients had PD-L1 expression with a ≥ 1% TPS based on a clinical trial assay 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/m^2 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 overall survival and progression free survival as assessed by BICR using RECIST v1.1.

Based on the clinical trial assay, a total of 1033 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 1033 patients, tumor tissue from 529 patients was retrospectively tested with PD-L1 IHC 22C3 pharmDx test. Specimen from 413 patients had PD-L1 expression (≥ 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 (≥ 50% of viable tumor cells exhibiting membrane staining at any intensity).

The level of agreement achieved between the clinical trial assay (CTA) and PD-L1 IHC 22C3 pharmDx is shown in Table 16.

Table 16. Clinical trial assay (CTA) vs. PD-L1 IHC 22C3 pharmDx agreement.

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

CI = Confidence Interval

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 17 and Table 18. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS ≥ 1%), which was enhanced in patients with high PD-L1 expression (TPS ≥ 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 ≥ 1%) and in the high PD-L1 expression (TPS ≥ 50%) subset for the overall clinical study (TPS ≥ 1% by clinical trial assay) and in the population with PD-L1 expression by PD-L1 IHC 22C3 pharmDx. The Kaplan-Meier curve for overall survival (TPS ≥ 1%), as determined by PD-L1 IHC 22C3 pharmDx is shown in Figure 7. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 17. 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 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 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 [a]	< 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 [b]						
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 [a]	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 [b]						
ORR % [c] (95% CI)	18% (14, 23)	24% (17, 32)	18% (15, 23)	20% (14, 28)	9% (7, 13)	5% (2, 11)

CI = Confidence Interval

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

[a] Based on stratified Log rank test; [b] Assessed by BICR using RECIST v1.1

[c] All responses were partial responses

Table 18. Response to KEYTRUDA in previously treated patients: overall clinical study and PD-L1 IHC 22C3 pharmDx positive patients: PD-L1 ≥ 50%.

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 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 [a]	< 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 [b]						
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 [a]	< 0.001	0.00221	< 0.001	< 0.001	—	—

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
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 [b]						
ORR % [c] (95% CI)	30% (23, 39)	37% (25, 52)	29% (22, 37)	28% (18, 41)	8% (4, 13)	4% (1, 15)

CI = Confidence Interval

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

[a] Based on stratified Log rank test; [b] Assessed by BICR using RECIST v1.1

[c] All responses were partial responses

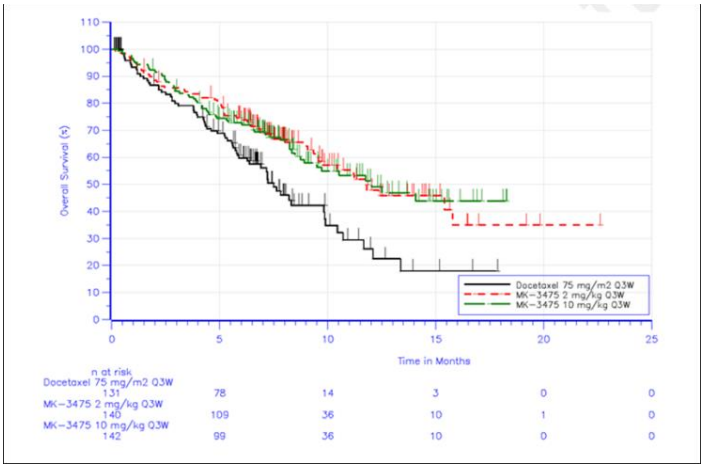


Figure 7. Kaplan-Meier Curve for Overall Survival in all Randomized Patients in KEYNOTE-010.

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with PD-L1 expression (TPS ≥ 1%) by PD-L1 IHC 22C3 pharmDx, but who may have had no PD-L1 expression (TPS < 1%) by the clinical trial assay. 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 clinical trial assay screening. To account for these missing data, a sensitivity analysis was conducted to understand the plausible range for the hazard ratio estimated based on PD-L1 IHC 22C3 pharmDx in the TPS ≥ 1% and TPS ≥ 50% subpopulations under an ITD framework to verify the consistency with the observed hazard ratio based on enrolment with the clinical trial assay. The hazard ratio sensitivity analysis results showed that the hazard ratio 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.[13] 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. Other secondary endpoints were objective response rate, 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 overall survival were observed in nivolumab over docetaxel subgroups when analyzed by PD-L1 expression level. Median overall survival 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 ≥ 1%, ≥ 5%, and ≥ 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 8. The Kaplan-Meier plot for subgroups by PD-L1 expression level is shown in Figure 9 and Figure 10.

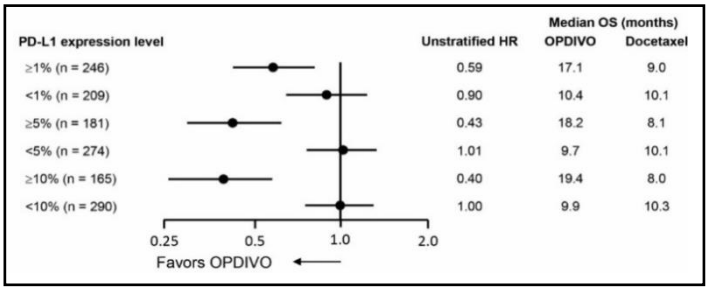


Figure 8. Forest Plot - OS based on PD-L1 expression in non-squamous NSCLC patients - CA209057.

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.

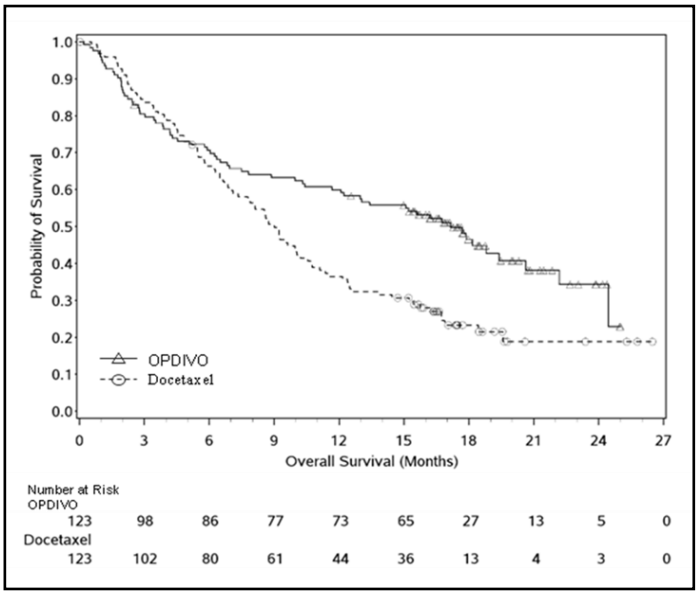


Figure 9. Overall Survival - patients with ≥ 1% PD-L1 expression-CA209057.

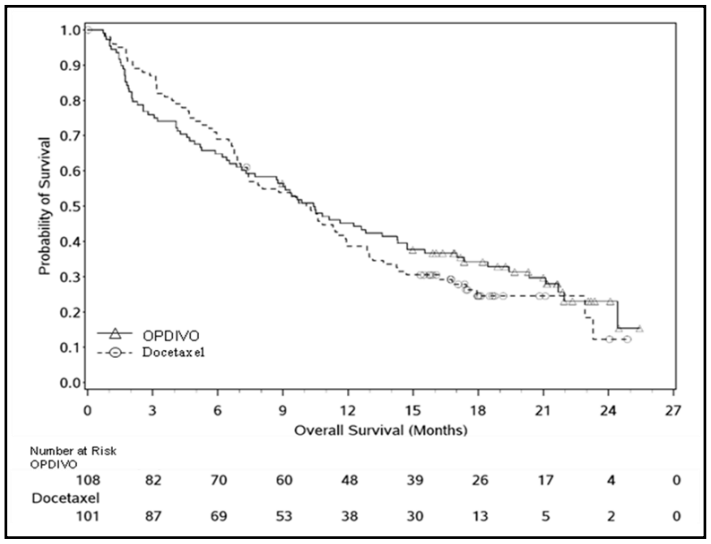


Figure 10. Overall Survival - non-squamous NSCLC patients with < 1% PD-L1 expression - CA209057.

TECENTRIQ-IMpower010

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

A total of 1280 enrolled patients had complete tumor resection and were eligible to receive up to 4 cycles of cisplatin-based chemotherapy. A total of 1005 patients were randomized (1:1) to receive TECENTRIQ 1200 mg by intravenous infusion every 3 weeks for 16 cycles unless disease recurrence or unacceptable toxicity, or Best Supportive Care (BSC), following recovery from surgery. Randomization was stratified by sex, stage of disease, histology, and PD-L1 expression. Among randomized patients, 10% of patients had stage IB, 48% had stage II and 43% had stage IIIA disease.

Tumor specimens from 1169 of the 1280 enrolled patients (including 985 of the 1005 randomized patients) were tested with VENTANA PD-L1 (SP263) Assay to determine their PD-L1 expression level. The percentage of patients who had tumors with PD-L1 expression on ≥ 1% of tumor cells as determined by VENTANA PD-L1 (SP263) Assay was 55%. The final staining acceptability rate among patients in the intended use population of the VENTANA PD-L1 (SP263) Assay was 99.3%.

The primary efficacy outcome measure of IMpower010 was disease-free survival (DFS) as assessed by the investigator. DFS was defined as the time from the date of randomization to the date of occurrence of any of the following: first documented recurrence of disease, new primary NSCLC, or death due to any cause, whichever occurred first. A key secondary efficacy outcome measure was overall survival (OS).

At the time of the interim DFS analysis (clinical data cutoff date: 21-Jan-2021), the study met its primary endpoint and demonstrated a statistically significant and clinically meaningful improvement in DFS in the TECENTRIQ arm compared with the BSC arm in the PD-L1 ≥ 1% TC, stage II - IIIA patient population (stratified HR: 0.66, 95% CI (0.50, 0.88), p-value 0.004). Efficacy results are presented in Table 19.

The median follow-up time was approximately 32 months. The overall survival (OS) data were immature at the time of the DFS interim analysis with 18.9% death events for both arms in the PD-L1 ≥ 1% TC stage II - IIIA patient population. An exploratory analysis of OS suggested a trend in favor of TECENTRIQ over BSC (stratified HR = 0.77; 95% CI: (0.51, 1.17) in this patient population.

Table 19. Efficacy Results from IMpower010 in Patients Stage II - IIIA NSCLC with PD-L1 expression (TC ≥ 1%).

	Arm A (TECENTRIQ) n = 248	Arm B (Best Supportive Care) n = 228
DFS events (%)	88 (35.5)	105 (46.1)
Median DFS, months [1] (95% CI)	NE (36.1, NE)	35.3 (29.0, NE)
Hazard ratio [2] (95% CI)	0.66 (0.50, 0.88)	
p-value	0.004	
3 year DFS Rate	60.0	48.2

DFS = Disease-free survival; CI = confidence interval

[1] The median follow-up time was approximately 32 months.

[2] Stratified by stage, sex, and histology

TROUBLESHOOTING

Troubleshooting guidance is provided in Table 20. 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 20. Troubleshooting guidance for VENTANA PD-L1 (SP263) Assay.

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that the recommended staining procedure was used.
		Verify that VENTANA PD-L1 (SP263) was selected for Primary Antibody.
	Degradation of tissue	Verify tissue was stained within the recommended time frame following sectioning.
	Dispenser malfunction	Verify nozzle cap is removed.
		Ensure dispenser is primed.
		Check the priming chamber for foreign materials or particulates, such as fibers or precipitate.
		Refer to inline dispenser method sheet associated with P/N 741-7139 located at dialog.roche.com.
		Ensure that only recommended fixatives and fixation times are used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that the recommended staining procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.

Problem	Probable Cause	Suggested Action
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

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

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
C	Updates to Intended Use, Warnings and Precautions, Symbols, and Revision History sections.

INTELLECTUAL PROPERTY

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.roche.com