VENTANA®

Roche

VENTANA PD-L1 (SP263) Assay

REF 741-4905

07419821001

IVD Σ 50

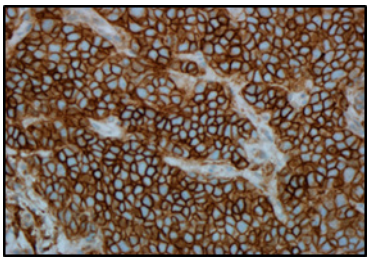

Figure 1. Non-small cell lung cancer stained with VENTANA PD-L1 (SP263) Assay.

INTENDED USE

VENTANA PD-L1 (SP263) Assay is intended for laboratory use in the qualitative immunohistochemical detection of the programmed death ligand 1 (PD-L1) by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissues as indicated in the tables below stained with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument.

PD-L1 expression in non-small cell lung cancer (NSCLC) is determined by the percentage of tumor cells (% TC) with any membrane staining above background.

Refer to the tables below for the specific tumor types and clinical applications. Refer to the respective medicinal product labeling for clinical recommendations pertaining to PD-L1 expression.

Table 1. CDx Indications for Use.

Tumor Type	PD-L1 Expression Level	Clinical Application
NSCLC	≥ 1% TC	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).
	≥ 50% TC ≥ 1% TC	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).
	≥ 50% TC ≥ 1% TC	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 LIBTAYO® (cemiplimab).
	≥ 50% TC	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 with early stage NSCLC for adjuvant treatment with TECENTRIQ® (atezolizumab).

For details on staining interpretation, refer to the Interpretation Guide.

Table 2. Additional Indications for Use.

Tumor Type	PD-L1 Expression Level	Clinical Application
Non-squamous NSCLC	≥ 1% TC, ≥ 5% TC, and ≥ 10% TC	PD-L1 expression in tumor cell (TC) membrane as detected by VENTANA PD-L1 (SP263) Assay in non-squamous NSCLC may be associated with enhanced survival from OPDIVO® (nivolumab).

For details on staining interpretation, refer to the Interpretation Guide.

The results of the VENTANA PD-L1 (SP263) Assay should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

SUMMARY AND EXPLANATION

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

PD-L1 is a transmembrane protein that 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.

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

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

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

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)

11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass 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 VENTANA detection kits 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[6] (NBF) for a period of at least 6 hours up to 72 hours. Acceptable fixatives for use with VENTANA PD-L1 (SP263) Assay are Zinc Formalin and Z-5 fixatives when used with at least 6 hours of fixation time. Other fixatives, including 95% alcohol, AFA and PREFER fixative, are unacceptable for use with VENTANA PD-L1 (SP263) Assay. The amount of fixative used is 15 to 20 times the volume of tissue. Fixation can be performed at room temperature (15-25°C).

Refer to VENTANA PD-L1 (SP263) Assay Interpretation Guide (P/N 1015317) 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 immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. 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.
5. Clinical trials described in this method sheet have not validated NSCLC FNA cell blocks in the context of prescribing a specific therapy.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[7,8]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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 navifyportal.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 established.
14. KEYTRUDA®, OPDIVO®, IMFINZI™, LIBTAYO® 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 3. 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 mist or vapours.
	P273	Avoid release to 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, 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 4 and Table 5 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 4. Staining procedures for VENTANA PD-L1 (SP263) Assay on BenchMark IHC/ISH instruments.

Instrument Platform	Staining Procedure
BenchMark GX	GX VENTANA PD-L1 (SP263) Assay
BenchMark XT	XT VENTANA PD-L1 (SP263) Assay
BenchMark ULTRA or BenchMark ULTRA PLUS	ULTRA VENTANA PD-L1 (SP263) Assay

Table 5. Recommended staining protocol for VENTANA PD-L1 (SP263) Assay with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Protocol Step	Parameter Input
Baking	Optional
Antibody (Primary)	VENTANA PD-L1 (SP263) Selected or Negative Control Selected
Counterstain	Hematoxylin II, 4 minutes

NEGATIVE REAGENT CONTROL

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

POSITIVE TISSUE CONTROL

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

Qualified normal human term placental tissue can be used as a tissue control for VENTANA PD-L1 (SP263) Assay. A placenta sample used as a tissue control must exhibit the staining pattern described as acceptable in Table 6. 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 6 and in the interpretation guide (P/N 1015317).

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimens 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 of 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 VENTANA PD-L1 (SP263) Assay Interpretation Guide (1015317) for specifics and images.

Placenta Tissue Control

Placenta tissue control contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as 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 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 6).

Table 6. 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 8 for the acceptability criteria for non-specific staining. Examples of background staining for this assay can be found in the interpretation guide (P/N 1015317).

Patient Tissue

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 7 and Table 8. Refer to Interpretation Guide for VENTANA PD-L1 (SP263) Assay Staining of NSCLC P/N 1015317 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 VENTANA PD-L1 (SP263) Assay scoring algorithm.

Scoring Algorithm - NSCLC

NSCLC samples must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 7) and the non-specific background scoring criteria (Table 8). Refer to the interpretation guide for additional instructions and representative images.

Table 7. 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 8. 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, and precision 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 9. Additional staining, such as cytoplasmic or immune cell staining, is also noted (see Table 9 footnote).

In addition, an array of neoplastic tissues was evaluated for tumor cell and immune cell staining with VENTANA PD-L1 (SP263) Assay as described in Table 10.

Table 9. 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 10. 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 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

Pathology	# positive / total cases	
	Tumor Cells	Immune Cells
Adenocarcinoma (Uterus)	0/1	0/1
Clear cell carcinoma (Uterus)	1/1	0/1
Squamous cell carcinoma (Cervix)	0/2	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1	0/1
Melanoma (Rectum)	0/1	0/1
Basal cell carcinoma (Skin)	0/1	0/1
Squamous cell carcinoma (Skin)	0/1	0/1
Neurofibroma (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 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 within one day.

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 20 days.

For inter-instrument precision, each of 12 unique placenta specimens were 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 6).

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%.

ANALYTICAL PERFORMANCE IN NON-SMALL CELL LUNG CANCER

Sensitivity - NSCLC Tissue

Sensitivity of VENTANA PD-L1 (SP263) Assay was tested on 733 unique cases of NSCLC specimens using manufactured production lots of VENTANA PD-L1 (SP263) Assay. Assessment of PD-L1 expression demonstrated staining across a range of 0-100% tumor cell staining.

Repeatability and Intermediate Precision - NSCLC Tissue

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, each of NSCLC specimens were stained with VENTANA PD-L1 (SP263) Assay across three BenchMark ULTRA, three BenchMark XT, and three BenchMark GX instruments. All slides were blinded, randomized, and evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm (provided in Table 7).

A summary of the results can be found in Table 11.

Table 11. Repeatability and intermediate precision study of VENTANA PD-L1 (SP263) Assay on individual NSCLC tissue specimens – BenchMark ULTRA, BenchMark XT, and BenchMark GX.

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)*
Intra-platform precision (3 Benchmark ULTRA instruments)	100% (99.4-100.0)*	96.5% (94.7-97.6)*	95.2% (93.3-96.6)*	97.2% (94.6-99.2)**
Intra-platform precision (3 Benchmark XT instruments)	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*
Intra-platform precision (3 Benchmark GX instruments)	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*	100.0% (94.0-100.0)*

* 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 Tissue

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 7). Results are reported in Table 12 as overall percent agreement, positive percent agreement, and negative percent agreement rates for each expression level.

Table 12. Lot-to-lot reproducibility agreement rates across individual 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)*
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.

Inter-Platform Concordance

An inter-platform study was conducted which included four indications, 10 NSCLC cases, 10 SCCHN cases, 10 urothelial cases, 10 melanoma cases and 11 placenta cases. For this study, slides were re-evaluated from previously executed intermediate precision studies. Staining consistency was collectively assessed by evaluating concordance staining intensity as well as PD-L1 percent staining in tumor cells of various tissue types from each of the XT and GX platforms to respective reference samples stained on the Benchmark ULTRA platform. See Table 13 for results.

Table 13. Inter-platform concordance of VENTANA PD-L1 (SP263) Assay

	Observation Analyzed	Overall Percent Agreement (95% CI)
BenchMark ULTRA: BenchMark XT	PD-L1 Expression Level	99.7 (99.1-100.0)[a]
	PL-L1 Staining Intensity	100.0 (98.5-10-0.0)[b]
BenchMark ULTRA: BenchMark GX	PD-L1 Expression Level	99.4 (98.4-100.0)[a]
	PL-L1 Staining Intensity	98.4 (95.9- 99.4)[b]

[a]: 2-sided 95% confidence intervals (CI) were calculated using the percentile bootstrap method from 2000 bootstrap samples

[b]: 2-sided 95% confidence intervals (CI) were calculated using the Wilson Score method

Reader Precision Studies - NSCLC Tissue

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 7. The results provided in Table 14 reflect the inter-reader and intra-reader precision rates for unique cases from the study cohort.

Table 14. 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 on BenchMark ULTRA Instrument - NSCLC Tissue

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 six readers (2 readers/site). See Table 15 for results.

Table 15. Inter-laboratory reproducibility: Agreement rates for VENTANA PD-L1 (SP263) Assay on individual NSCLC tissue specimens.

Inter-Laboratory Reproducibility	Positive Percent Agreement (95% CI)*	Negative Percent Agreement (95% CI)*	Overall Percent Agreement (95% CI)*
Across all Cases ≥ 1% Expression	99.5% (98.6-100.0)	100.0% (99.1-100.0)	99.8% (99.3-100.0)
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% (90.2-98.1)	90.1% (85.1-94.7)	92.2% (89.0-95.2)

* Note: 95% CI = Confidence interval

For PPA/NPA/OPA 95% CIs were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

Method Comparison Study on BenchMark ULTRA PLUS vs BenchMark ULTRA instrument - NSCLC Tissue

Three laboratories, from separate institutions in the United States, participated in a concordance study between the BenchMark ULTRA PLUS instrument and the BenchMark ULTRA instrument. A total of 196 unique commercially acquired, anonymized NSCLC tissue specimens representing the staining range of the VENTANA PD-L1 (SP263) Assay at 1% and 50% cutoffs were used in the study. The tissue cohort contained 2 overlapping 140-case analysis population with a balanced distribution between PD-L1 positive and PD-L1 negative cases for each cutoff as determined by Roche Tissue Diagnostics (RTD) pathologists' consensus review. For each case, two tissue slides were stained with VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig on a BenchMark ULTRA instrument using the recommended staining protocol at RTD, and two additional tissue slides were stained with VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig on a BenchMark ULTRA PLUS instrument using the recommended staining protocol at one of the three external laboratories. Each site stained approximately 1/3 of the study cases on a BenchMark ULTRA PLUS instrument. Case slides were randomized prior to staining on BenchMark ULTRA or BenchMark ULTRA PLUS instruments. Two pathologists from each external laboratory and one RTD pathologist, blinded to the sample identity, independently evaluated all BenchMark ULTRA slides and all BenchMark ULTRA PLUS slides using the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 7) with at least two week washout period between the reads. The performance equivalence of VENTANA PD-L1 (SP263) Assay on the investigational instrument (BenchMark ULTRA PLUS) relative to the reference instrument (BenchMark ULTRA) was assessed separately at the 1% and 50% cutoffs. Results are summarized in Table 16.

Table 16. Pooled Agreement of PD-L1 Status Between BenchMark ULTRA PLUS and BenchMark ULTRA at 1% and 50 % Cutoffs.

1% Cutoff [a]	BenchMark ULTRA		
BenchMark ULTRA PLUS	≥1%	<1%	Total
≥1%	474	12	486
<1%	6	473	479
Total	480	485	965
Agreement [c]	**% (n/N) (95% CI [d])**		
Positive percent agreement	98.8 (474/480) (97.5-99.8)		
Negative percent agreement	97.5 (473/485) (96.2-98.7)		
Overall percent agreement	98.1 (947/965) (97.2-99.0)		
50% Cutoff [b]	**BenchMark ULTRA**		
BenchMark ULTRA PLUS	≥50%	<50%	Total
≥50%	478	21	499
<50%	5	464	469
Total	483	485	968
Agreement [c]	**% (n/N) (95% CI [d])**		
Positive percent agreement	99.0 (478/483) (97.5-100.0)		
Negative percent agreement	95.7 (464/485) (93.4-97.9)		
Overall percent agreement	97.3 (942/968) (96.0-98.6)		

[a] n = 965 evaluable observations

[b] n = 968 evaluable observations

[c] The pooled agreement pools all cases and readers.

[d] 95% CI = Confidence interval. Two-sided 95% CI calculated using the percentile bootstrap method with 2000 replicates stratified by diagnostic score bin (positive, negative, borderline positive, borderline negative).

Inter-Laboratory Reproducibility Study on BenchMark ULTRA PLUS Instrument - NSCLC Tissue

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP263) Assay staining was conducted on BenchMark ULTRA PLUS instruments to demonstrate reproducibility of the assay in determining PD-L1 expression in NSCLC tissue specimens at 1% and 50% cutoffs. Thirty-eight unique commercially acquired NSCLC specimens with a range of PD-L1 expression were used in the study. The tissue cohort contained 2 over-lapping 28-case analysis populations with a balanced distribution between PD-L1 positive and PD-L1 negative cases for each cutoff as determined by RTD pathologists' consensus review. Each case was stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Case slides were randomized prior to staining on a BenchMark ULTRA PLUS instrument. At each site, the stained slides were independently evaluated by 2 pathologists, blinded to the sample identity, using the VENTANA PD-L1 (SP263) Assay scoring algorithm for NSCLC (Table 7). Results are summarized in Table 17.

Table 17. Inter-laboratory reproducibility of VENTANA PD-L1 (SP263) Assay staining of NSCLC specimens on BenchMark ULTRA PLUS instruments.

Cutoff	Inter-laboratory Reproducibility	Agreement % (n/N), (95% CI) [b]
≥ 1% [a]	Overall agreement [c] (compared to a consensus score, across sites, days and readers)	PPA: 96.2 (404/420), (91.6-100.0) NPA: 100.0 (420/420), (99.1-100.0) OPA: 98.1 (824/840), (95.6-100.0)
	Between-site agreement [d] (average of site-to-site pairwise comparisons)	APA: 96.8 (7822/8080), (92.5-100.0) ANA: 97.0 (8462/8720), (93.0-100.0) OPA: 96.9 (8142/8400), (92.9-100.0)
	Between-reader agreement [d] (average of reader-to-reader pairwise comparisons within each site)	APA: 97.0 (392/404), (93.2-100.0) ANA: 97.2 (424/436), (93.5-100.0) OPA: 97.1 (408/420), (93.6-100.0)
≥ 50% [a]	Overall agreement [c] (compared to a consensus score, across sites, days and readers)	PPA: 95.9 (374/390), (93.1-98.6) NPA: 98.9 (445/450), (96.9-100.0) OPA: 97.5 (819/840), (95.8-99.0)
	Between-site agreement [d] (average of site-to-site pairwise comparisons)	APA: 94.8 (7188/7580), (91.8-98.0) ANA: 95.7 (8828/9220), (92.6-98.4) OPA: 95.3 (8008/8400), (92.2-98.2)
	Between-reader agreement [d] (average of reader-to-reader pairwise comparisons within each site)	APA: 94.5 (358/379), (91.1-97.8) ANA: 95.4 (440/461), (92.0-98.4) OPA: 95.0 (399/420), (91.7-98.1)

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

[a] n = 840 PD-L1 slide observations

[b] Note: 95% CI = Confidence interval

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

[d] Pairwise agreement rates.

Note: For PPA/NPA/OPA, 95% CIs were calculated using the Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

For APA/ANA, 95% CIs were calculated using the transformed Wilson Score method for agreements of 100% or using the percentile bootstrap method from 2000 bootstrap samples for agreements less than 100%.

CLINICAL PERFORMANCE IN NON-SMALL CELL LUNG CANCER

Clinical Performance of IMFINZI (durvalumab) in PACIFIC Study

The clinical utility of VENTANA PD-L1 (SP263) Assay was investigated in the PACIFIC Study (NCT02125461), a randomized, double blind, placebo controlled, multicenter study that evaluated the efficacy and safety of IMFINZI (durvalumab) vs placebo in patients with locally advanced, unresectable NSCLC. Patients were randomized 2:1 to receive 10 mg/kg IMFINZI (n = 476) or 10 mg/kg placebo (n = 237) after having completed at least 2 cycles of definitive platinum based chemotherapy with radiation therapy and having stable disease, partial response or complete response. Randomization was stratified by gender, age (< 65 years vs. 65 years), and smoking status (smoker vs. non-smoker). 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% had unknown PD-L1 status. Of 451 patients with PD-L1 expression available, 67% had PD-L1 expression ≥ 1% and 33% had PD-L1 expression < 1%.

The two primary efficacy endpoints of the study were progression free survival (PFS), assessed by Blinded Independent Central Review (BICR) according to Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST v1.1), and overall survival (OS) of IMFINZI vs. placebo.

The study demonstrated a statistically significant improvement in PFS [hazard ratio (HR) = 0.52 (95% CI: 0.42, 0.65), p < 0.0001] and OS [HR = 0.68 (95% CI: 0.53, 0.87), p = 0.00251] in the IMFINZI treated group compared with the placebo group among all randomized patients]. The improvements in PFS and OS 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 could not be established (PD-L1 unknown). PFS and OS results are summarized in Figure 2 and Figure 3.

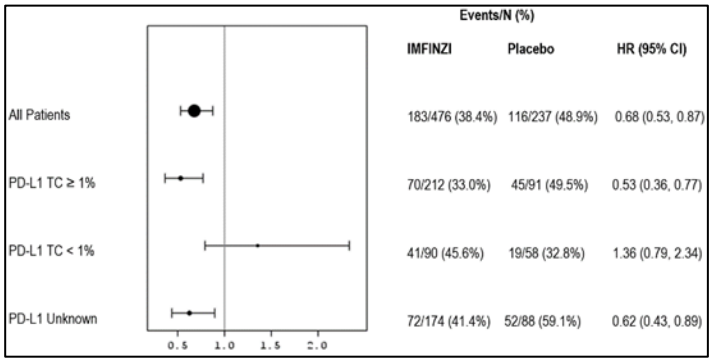

Figure 2. Forest Plot of OS by PD-L1 expression.

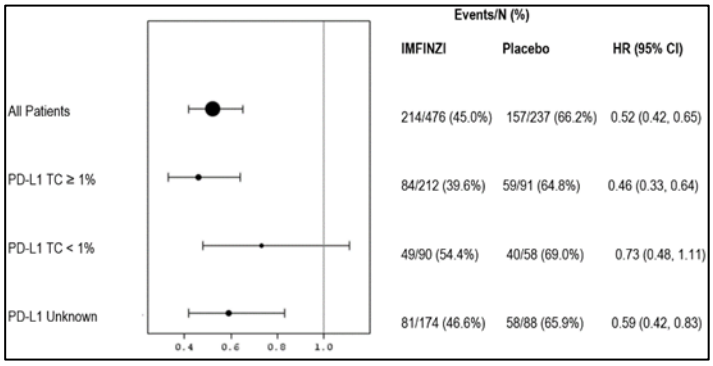

Figure 3. Forest Plot of PFS by PD-L1 expression.

Clinical Performance of KEYTRUDA (pembrolizumab) in KEYNOTE-024, KEYNOTE-042, and KEYNOTE-010 Studies

KEYNOTE-024 Clinical Study Results

The safety and efficacy of KEYTRUDA (pembrolizumab) were evaluated in KEYNOTE-024 (NCT02142738), a multicenter, controlled study for the treatment of previously untreated metastatic NSCLC with no EGFR or ALK genomic tumor aberrations. 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 KEYTRUDA 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).

The primary efficacy endpoint of the study was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using RECIST v1.1. Secondary efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST v1.1. The trial demonstrated a statistically significant

improvement in both PFS and OS for patients randomized to KEYTRUDA as compared with chemotherapy. Refer to Table 18 for a summary of key efficacy measures for the entire intent to treat (ITT) population. PFS and ORR results were reported from an interim analysis at a median follow-up of 11 months. OS results were reported from the final analysis at a median follow-up of 25 months.

Table 18. 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	73 (47%)	96 (64%)
Hazard ratio [a] (95% CI)	0.63 (0.47, 0.86)	—
p-Value [b]	0.002 [a]	—
Median in months (95% CI)	30.0 (18.3, NA)	14.2 (9.8, 19.0)
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

KEYNOTE-042 Clinical Study Results

The safety and efficacy of KEYTRUDA were also evaluated in KEYNOTE-042 (NCT02220894), a multicentre, controlled study for the treatment of previously untreated locally advanced or metastatic NSCLC. The study design was similar to that of KEYNOTE-024, except that patients had PD-L1 expression with a ≥ 1% TPS based on the PD-L1 IHC 22C3 pharmDxTM Kit. Patients were randomized (1:1) to receive KEYTRUDA at a dose of 200 mg every 3 weeks (n=637) or investigator's choice platinum-containing chemotherapy (n=637; including pemetrexed maintenance, pemetrexed+carboplatin or paclitaxel+carboplatin). Among the 1274 patients in KEYNOTE-042, 599 (47%) had tumors that expressed PD-L1 with TPS ≥ 50% based on the PD-L1 IHC 22C3 pharmDxTM Kit.

The primary efficacy endpoint of the study was OS. Secondary efficacy outcome measures were PFS and ORR (as assessed by BICR using RECIST v1.1). The study demonstrated a statistically significant improvement in OS for patients whose tumors expressed PD-L1 TPS ≥ 1% randomized to KEYTRUDA monotherapy compared to chemotherapy (HR 0.82; 95% CI 0.71, 0.93 at the final analysis) and in patients whose tumors expressed PD-L1 TPS ≥ 50% randomized to KEYTRUDA monotherapy compared to chemotherapy. Table 19 summarizes key efficacy measures for the TPS ≥ 50% population at the final analysis performed at a median follow-up of 15.4 months.

Table 19. Efficacy results (PD-L1 TPS ≥ 50%) in KEYNOTE-042.

Endpoint	KEYTRUDA 200 mg every 3 weeks n=299	Chemotherapy n=300
OS		
Number (%) of patients with event	180 (60%)	220 (73%)
Hazard ratio (95% CI) [a]	0.70 (0.58, 0.86)	
p-Value [b]	0.0003	
Median in months (95% CI)	20.0 (15.9, 24.2)	12.2 (10.4, 14.6)
PFS		
Number (%) of patients with event	238 (80%)	250 (83%)
Hazard ratio (95% CI) [a]	0.84 (0.70, 1.01)	
Median in months (95% CI)	6.5 (5.9, 8.5)	6.4 (6.2, 7.2)
Objective response rate		
ORR % (95% CI)	39% (34, 45)	32% (27, 38)
Complete response %	1%	0.3%
Partial response %	38%	32%
Response duration [c]		
Median in months (range)	22.0 (2.1+, 36.5+)	10.8 (1.8+, 30.4+)
% with duration ≥ 18 months	57%	34%

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

[b] Based on stratified log-rank test

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

KEYNOTE-010 Clinical Study Results

The safety and efficacy of KEYTRUDA were evaluated in KEYNOTE-010 (NCT01905657), a multicenter, open-label, randomized clinical study in patients with advanced NSCLC previously treated with platinum-containing chemotherapy.[10] 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 KEYTRUDA. Patients were randomized (1:1:1) to receive KEYTRUDA 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 primary efficacy endpoints were OS and PFS 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. Archived clinical study samples from 529 patients were retrospectively tested with PD-L1 IHC 22C3 pharmDx. Of those, specimens from 94 patients had PD-L1 expression < 1%, specimens from 413 patients had PD-L1 expression ≥ 1% and specimens from 163 patients had PD-L1 expression ≥ 50%.

The negative and positive percent agreement between the clinical trial assay and PD-L1 IHC 22C3 pharmDx were: NPA= 94.5% (91.4%-96.6%); PPA=80.0% (76.9%-82.8%), at the 1% threshold, and NPA=98.3% (97.1%-99.0%); PPA=73.2% (67.9%-77.9%) at the 50% threshold.

KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS ≥ 1%), which was enhanced in patients in the TPS ≥ 50% subgroup as determined by PD-L1 IHC 22C3 pharmDx. The magnitude of benefit was comparable to that in the overall clinical trial. Table 20 summarizes the key efficacy measures in the overall population with PD-L1 expression (TPS ≥ 1%) by CTA, and among patients with PD-L1 expression (TPS ≥ 1%) by PD-L1 IHC 22C3 pharmDx. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms. Sensitivity analyses showed that the hazard ratio estimates were robust to 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.

Table 20. Efficacy results in KEYNOTE-010: Overall clinical study and PD-L1 IHC 22C3 pharmDx positive patients: PD-L1 TPS ≥ 1%.

Endpoint	KEYTRUDA 2 mg/kg bw every 3 weeks		KEYTRUDA 10 mg/kg bw 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 (%)	284 (83%)	109 (78%)	264 (76%)	106 (75%)	295 (86%)	111 (85%)
Hazard Ratio* (95% CI)	0.77 (0.66, 0.91)	0.61 (0.46, 0.81)	0.61 (0.52, 0.73)	0.57 (0.43, 0.76)	—	—
p-Value [a]	0.00128	< 0.001	< 0.001	<0.001	—	—
Median in months (95% CI)	10.4 (9.5, 11.9)	12.5 (10.0, 17.1)	13.2 (11.2, 16.7)	12.3 (9.4, 20.5)	8.4 (7.6, 9.5)	7.6 (5.8, 9.6)
PFS [b]						
Events (%)	305 (89%)	118 (84%)	292 (84%)	121 (85%)	314 (92%)	118 (90%)
Hazard Ratio* (95% CI)	0.88 (0.75, 1.04)	0.62 (0.47, 0.82)	0.75 (0.63, 0.89)	0.75 (0.57, 0.98)	—	—
p-Value [a]	0.065	<0.001	<0.001	0.01776	—	—
Median in months (95% CI)	3.9 (3.1, 4.1)	5.2 (4.1, 6.3)	4.0 (2.7, 4.5)	4.0 (2.2, 4.8)	4.1 (3.8, 4.5)	4.0 (2.3, 4.3)
Overall response rate (ORR) [b]						
ORR % (95% CI)	20% (16, 25)	27% (20, 35)	21% (17, 26)	23% (17, 31)	9% (6, 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

VENTANA PD-L1 (SP263) Assay Clinical Performance for KEYNOTE-024, KEYNOTE-042, and KEYNOTE-010 PD-L1 positive NSCLC Populations - Published Analytical Method Comparison and In-Silico Bridging Analysis

Tissue samples from patients screened for the KEYNOTE-010, KEYNOTE-024 and KEYNOTE-042 trials were not available to be tested with the VENTANA PD-L1 (SP263) Assay. Therefore, the clinical performance of the VENTANA PD-L1 (SP263) Assay was indirectly evaluated via a published analytical performance study and retrospective in-silico bridging studies.

Published Analytical Performance Study

A method comparison study carried out by AstraZeneca, compared VENTANA PD-L1 (SP263) Assay and PD-L1 IHC 22C3 pharmDx (used in the clinical studies of KEYTRUDA).[11]

Approximately 500 commercially acquired NSCLC biopsy specimens representing the dynamic range of PD-L1 expression were stained with VENTANA PD-L1 (SP263) Assay on the BenchMark ULTRA instrument and with PD-L1 IHC 22C3 pharmDx on the Autostainer Link 48, at a single central laboratory. The stained slides were evaluated by one pathologist trained to both the VENTANA and Dako PD-L1 assays. PD-L1 expression data from this method comparison was subsequently analyzed by Ventana to calculate the PPA and NPA at the 1% and 50% thresholds. The primary endpoint of the study was a point estimate of 85% or higher for PPA, and NPA, using PD-L1 IHC 22C3 pharmDx as the reference. Both PPA and NPA were > 90% at both expression thresholds (see Table 21).

Table 21. Method Comparison: Agreement rates for VENTANA PD-L1 (SP263) Assay vs. PD-L1 IHC 22C3 pharmDx.

1% Threshold	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)	
50% Threshold	**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

In-silico Bridging Analysis

To further assess the efficacy of KEYTRUDA in PD-L1 ≥ 1% NSCLC patients in the second line (2L) setting (KEYNOTE-010) and PD-L1 ≥ 50% NSCLC patients in the first line (1L) setting (KEYNOTE-024 and KEYNOTE-042) as identified by the VENTANA PD-L1 (SP263) Assay in-silico bridging studies were performed.

Data from a Roche-sponsored method comparison study including a commercial cohort of 850 NSCLC samples, which assessed the analytical concordance between VENTANA PD-L1 (SP263) Assay and PD-L1 IHC 22C3 pharmDx, was used to develop a predictive model for the imputation of PD-L1 SP263 status. PD-L1 SP263 status was imputed for all the subjects tested with PD-L1 IHC 22C3 pharmDx in these three trials. KEYTRUDA efficacy was then evaluated in the PD-L1 ≥ 1% (KEYNOTE-010), PD-L1 ≥ 50% (KEYNOTE-024 and KEYNOTE-042) populations identified by the VENTANA PD-L1 (SP263) Assay determined using the method described by Li (2015).[14] For the subset of SP263+/ 22C3+ patients, efficacy was directly estimated from patients randomized in the trials. For the subset of SP263+/ 22C3- patients (not randomized in the trials), a range of scenarios was modeled where efficacy was assumed to be the same (best-case scenario) or attenuated with respect to the SP263+/22C3+ subset (worst-case scenario assumed a HR=1). Efficacy for the SP263 selected patients was then determined by combining the SP263+/22C3+ and SP263+/ 22C3- groups via weighting as described in Li (2015).[14] For each study, this calculation was done over multiple simulated trial outcomes using the imputation models and then summarized across trial simulations to understand the robustness of the conclusions.

The results of the in-silico bridging analyses show that the clinical benefit of KEYTRUDA vs chemotherapy is maintained in the PD-L1 (SP263) selected populations. In particular, the estimated treatment effects are robust even to full attenuation of the treatment effect among patients who are 22C3- and SP263+, providing support for use of KEYTRUDA monotherapy in the 1L setting for patients with PD-L1 ≥ 50% TC expression and 2L setting for patients with PD-L1 ≥ 1% TC expression, in the NSCLC patient population identified by VENTANA PD-L1 (SP263) Assay.

Clinical Performance of OPDIVO (nivolumab) in CHECKMATE-057 Study

CHECKMATE-057 Clinical Study Results

The safety and efficacy of OPDIVO was evaluated in CHECKMATE-057 (NCT01673867), a Phase 3, randomized, open-label study 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 to OPDIVO vs docetaxel 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).

Archival tumor specimens were retrospectively evaluated for PD-L1 expression using the PD-L1 IHC 28-8 pharmDx assay. Across the trial population, 22% of 582 patients had non-quantifiable results. Of the remaining 455 patients, 46% were PD-L1 negative, defined as < 1% of tumor cells expressing PD-L1 and 54% had PD-L1 expression, defined as ≥ 1% of tumor cells expressing PD-L1. Among the 246 patients with tumors expressing PD-L1, 26% had ≥ 1% but < 5% tumor cells with positive staining, 7% had ≥ 5% but < 10% tumor cells with positive staining, and 67% had ≥10% tumor cells with positive staining.

The primary efficacy endpoint of the study was OS. 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 OPDIVO over docetaxel subgroups when analyzed by PD-L1 expression level. Median OS overall survival was 17.1, 18.2, and 19.4 months for subjects treated with OPDIVO compared to 9.0, 8.1, and 8.0 months for subjects treated with docetaxel 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 OPDIVO and 10.1 to 10.3 months for docetaxel. The unstratified hazard ratios (HR) and median OS are presented in Figure 4.

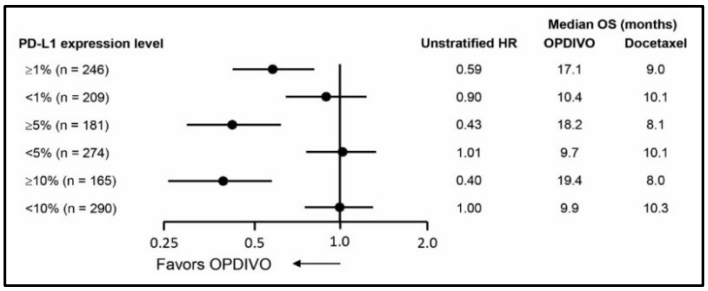

Figure 4. Forest Plot - Overall Survival (OS) based on PD-L1 expression in non-squamous NSCLC patients – CHECKMATE-057.

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.

VENTANA PD-L1 (SP263) Assay Clinical Performance for CHECKMATE-057 NSCLC Population - Published Analytical Method Comparison and External Method Comparison Study

Tissue samples from patients screened for the CHECKMATE-057 trial were not available to be tested with the VENTANA PD-L1 (SP263) Assay. Therefore, the clinical performance of the VENTANA PD-L1 (SP263) Assay was indirectly evaluated via a published analytical performance study and an external concordance study between VENTANA PD-L1 (SP263) Assay and Dako PD-L1 IHC 28-8 pharmDx.

Published Analytical Performance Study

A method comparison study carried out by AstraZeneca, compared VENTANA PD-L1 (SP263) Assay and PD-L1 IHC 28-8 pharmDx (used in the clinical studies of OPDIVO).[11] Approximately 500 commercially acquired NSCLC biopsy specimens representing the dynamic range of PD-L1 expression were stained with VENTANA PD-L1 (SP263) Assay on the BenchMark ULTRA instrument and with PD-L1 IHC 28-8 pharmDx on the Autostainer Link 48, at a single central laboratory. The stained slides were evaluated by one pathologist trained to both the VENTANA and Dako PD-L1 assays. PD-L1 expression data from this method comparison was subsequently analyzed by Ventana to calculate the PPA and NPA at the 1%, 5%, and 10% cutoffs. The primary endpoint of the study was a point estimate of 85% or higher for PPA, and NPA, using PD-L1 IHC 28-8 pharmDx as the reference. Both PPA and NPA were >90% at the three expression thresholds (see Table 22).

Table 22. Method Comparison: Agreement rates for VENTANA PD-L1 (SP263) Assay vs. PD-L1 IHC 28-8 pharmDx.

1% Threshold	**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)	
5% Threshold	**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)	
10% Threshold	**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

External Method Comparison Study

The study included a commercial cohort of 850 samples and assessed the analytical concordance between the assays, following a non-inferiority design using Dako PD-L1 IHC 28-8 pharmDx as the reference (Li 2016).[12] Each case was tested twice with the Dako PD-L1 IHC 28-8 pharmDx and once with the VENTANA PD-L1 (SP263) Assay. The endpoints of the study were the positive and negative percent agreement (PPA/NPA) between the assays, deducted from the PPA and NPA between the two replicates of the Dako PD-L1 IHC 28-8 pharmDx assay, used as the baseline. The primary analyses of the study were based on the entire NSCLC cohort including both squamous and non-squamous histological subtypes. Given that the CHECKMATE-057 study only included patients with non-squamous NSCLC, a post-hoc analysis was performed to assess concordance between the assays in the non-squamous NSCLC subset (N=239) at the same PD-L1 expression thresholds evaluated in the CHECKMATE-057 trial. PPA and NPA differences for the non-squamous NSCLC subset at the 1%, 5%, and 10% PD-L1 expression thresholds were below the 15% non-inferiority threshold pre-specified for the primary analyses (Table 23), providing support for the use of VENTANA PD-L1 (SP263) Assay as a follow-on device for OPDIVO in the same target patient population.

Table 23. Concordance between VENTANA PD-L1 (SP263) Assay and Dako PD-L1 IHC 28-8 pharmDx using a commercial cohort of non-squamous NSCLC cases.

Prevalence of PD-L1 Positive	Expression Threshold	Measure	Point Estimate	2-sided 95% CI
0.541	1% TC	Diff PPA1	0.0%	(-4.3, 4.3)
		Diff PPA2	-5.5%	(-10.9, 0.0)
		Diff NPA1	8.4%	(2.8, 14.5)
		Diff NPA2	4.0%	(-3.1, 11.4)
0.398	5% TC	Diff PPA1	-2.8%	(-7.7, 1.9)
		Diff PPA2	-5.1%	(-10.7, 0.4)
		Diff NPA1	4.7%	(-0.8, 10.6)
		Diff NPA2	6.2%	(1.2, 11.8)
0.363	10% TC	Diff PPA1	-2.0%	(-7.0, 2.8)
		Diff PPA2	-6.5%	(-12.8, -0.4)
		Diff NPA1	3.6%	(-1.5, 8.9)
		Diff NPA2	5.0%	(0.5, 10.1)

Notes:

Diff PPA1 and Diff PPA2 = intra (28-8 replicate 1 vs 28-8 replicate 2) PPA minus inter (SP263 vs 28-8 replicate 1 or 2) PPA;

Diff NPA1 and Diff NPA2 = intra (28-8 replicate 1 vs 28-8 replicate 2) NPA minus inter (SP263 vs 28-8 replicate 1 or 2) NPA;

CIs are calculated using bootstrap with 100000 resamples.

Calculation is based on 236 evaluable non-squamous NSCLC cases.

Clinical Performance of LIBTAYO (cemiplimab) in Study 1624

Study 1624 Results

Safety and efficacy of LIBTAYO (cemiplimab) was evaluated in Study 1624 (NCT03088540), a randomized, multi-center, open-label, active-controlled trial in patients with locally advanced NSCLC who were not candidates for surgical resection, or definitive chemoradiation, or with metastatic NSCLC.

The trial was designed to enroll patients whose tumors had high PD-L1 expression (Tumor Proportion Score [TPS] ≥ 50% as determined by an immunohistochemistry assay using the PD-L1 IHC 22C3 pharmDx kit) and who had not received prior systemic treatment for metastatic NSCLC. A total of 710 patients (Intent-To-Treat [mITT] population) were enrolled, and an analysis was performed on a population (n=563) who had PD-L1 expression of TPS ≥ 50% using PD-L1 IHC 22C3 pharmDx according to approved labeling (22C3+ population).

Patients were randomized (1:1) to receive LIBTAYO 350 mg intravenously (IV) every 3 weeks for up to 108 weeks or a platinum doublet chemotherapy regimen for 4 to 6 cycles followed by optional pemetrexed maintenance for patients with non-squamous histology who received a pemetrexed containing regimen. Randomization was stratified by histology (non-squamous vs squamous) and geographic region (Europe vs Asia vs rest of the world).

The major efficacy endpoints of the study were overall survival (OS) and progression-free survival (PFS). In the population with TPS ≥ 50%, the trial demonstrated statistically significant improvement in OS and PFS for patients randomized to LIBTAYO as compared with chemotherapy. Similar efficacy was observed in the mITT population (clinical data cutoff date: 01-Mar-2020).

VENTANA PD-L1 (SP263) Assay Clinical Performance for Study 1624 PD-L1 ≥ 50% TC NSCLC Population – Clinical Bridging Study

Clinical performance of the VENTANA PD-L1 (SP263) Assay was evaluated using archived clinical study samples from Study 1624. A total of 871 clinical trial specimens were retrospectively tested with VENTANA PD-L1 (SP263) Assay, 481 from randomized patients and 390 from a random subset of the screen-failed patients at the 50% PD-L1 expression threshold in tumor cells (% TC). Staining acceptability rates for VENTANA PD-L1 (SP263) Assay were evaluated at the subject level. The final staining acceptability rate in the Intended Use (IU) population was 92.8% (95% CI: 90.9, 94.4).

Agreement of PD-L1 status between VENTANA PD-L1 (SP263) Assay and PD-L1 IHC 22C3 pharmDx results was calculated using the PD-L1 IHC 22C3 pharmDx results as the reference. The concordance analysis results are shown in Table 24.

Table 24. PD-L1 Status Concordance between the VENTANA PD-L1 (SP263) Assay Results and Study 1624 PD-L1 IHC 22C3 pharmDx Results.[a]

PD-L1 (SP263) Status [c]	PD-L1 (22C3) Status [b]		
	Positive	Negative	Total
Positive	324	24	348
Negative	68	352	420
Total	392	376	768
Agreement rates [d,e]	PPA = 82.7% (324/392) (95% CI: 78.6-86.1)	NPA = 93.6% (352/376) (95% CI: 90.7-95.7)	OPA = 88.0% (676/768) (95% CI: 85.5-90.1)

[a] All patients who had evaluable PD-L1 IHC 22C3 pharmDx and VENTANA PD-L1 (SP263) Assay results, excluding patients whose final VENTANA PD-L1 (SP263) Assay result was associated with a diagnostic protocol deviation.

[b] Performed according to the approved labeling. For the purpose of the analyses, a PD-L1 (22C3) TPS ≥ 50% result was considered positive and a PD-L1 (22C3) TPS < 50% result was considered negative.

[c] For the purpose of the analyses, a PD-L1 (SP263) expression ≥ 50% TC result was considered positive and a PD-L1 (SP263) expression < 50% TC result was considered negative.

[d] PPA = positive percent agreement; NPA = negative percent agreement; OPA = overall percent agreement.

[e] Two-sided 95% CI were calculated using the Wilson score method.

LIBTAYO efficacy was evaluated in the population with PD-L1 expression in tumor cells (TC) ≥ 50% identified by the VENTANA PD-L1 (SP263) Assay (SP263+) using the method described by Li 2015.[14] For the subset of SP263+ (PD-L1 [SP263] expression ≥ 50% TC)/ 22C3+ (TPS ≥ 50%) patients, efficacy was estimated based on Study 1624 data. For the subset of SP263+/22C3- (TPS < 50%) patients, a range of scenarios were considered where efficacy was assumed to be the same (best-case scenario) or attenuated with respect to the SP263+/22C3+ subset (a hazard ratio (HR) = 1 was assumed under the worst-case scenario). Efficacy for the SP263+ patients was determined as the weighted average of the SP263+/22C3+ and SP263+/ 22C3- groups as described in Li 2015.[14] Sensitivity analyses were performed to account for the impact of missing VENTANA PD-L1 (SP263) Assay results via multiple imputation. The drug efficacy including both available and imputed PD-L1 (SP263) test results was estimated using the same statistical methods used in the Study 1624 efficacy analysis.

Baseline and demographic characteristics were similar between SP263+ patients, 22C3+ patients, and patients in the mITT population.

Observed efficacy in SP263+/22C3+ patients was similar to efficacy in 22C3+ patients (Table 25). Results of the bridging analyses, with and without imputation, and across all the scenarios considered demonstrate that the clinical benefit of LIBTAYO vs chemotherapy is maintained in the SP263+ population relative to the 22C3+ population, providing support for use of LIBTAYO monotherapy in the 1L setting for patients with PD-L1 ≥ 50% TC expression, in the NSCLC patient population identified by the VENTANA PD-L1 (SP263) Assay.

Table 25. Clinical Efficacy of Cemiplimab in Patients with PD-L1 ≥ 50% as Determined by PD-L1 IHC 22C3 PharmDx and VENTANA PD-L1 (SP263) Assay.

Endpoints	22C3+ [a,b] (N=563)		22C3+, SP263+ [c] (N=324)	
	LIBTAYO n=283	Chemotherapy n=280	LIBTAYO n=164	Chemotherapy n=160
Overall Survival				
Number of deaths (%)	70 (24.7)	105 (37.5)	38 (23.2)	56 (35.0)
Median in months (95% CI) [d]	NR (17.9, NE)	14.2 (11.2, 17.5)	22.1 (17.7, NE)	15.5 (11.4, NE)
Hazard ratio (95% CI) [e]	0.57 (0.42, 0.77)		0.52 (0.34, 0.80)	
p-Value	0.0002		0.0022	
Progression-free Survival per BICR				
Number of events (%)	147 (51.9)	197 (70.4)	76 (46.3)	110 (68.8)
Median in months (95% CI) [d]	8.2 (6.1, 8.8)	5.7 (4.5, 6.2)	9.8 (8.1, 14.5)	5.4 (4.2, 6.2)
Hazard ratio (95% CI) [e]	0.54 (0.43, 0.68)		0.43 (0.32, 0.59)	
p-Value	<0.0001		<0.0001	

BICR = blinded independent central review; CI = confidence interval; NE = Not evaluable; NR = Not reached; LIBTAYO = cemiplimab

[a] 22C3+ refers to subset of randomized patients with PD-L1 expression of TPS ≥ 50% tumor cell (TC) for PD-L1 IHC 22C3 pharmDx according to the approved labeling

[b] From Study 1624

[c] 22C3+, SP263+ refers to subjects with ≥ 50%TC for VENTANA PD-L1 (SP263) Assay and TPS ≥ 50% tumor cell (TC) for PD-L1 IHC 22C3

[d] Based on Kaplan-Meier method

[e] Based on stratified proportional hazards model

Clinical Performance of LIBTAYO (cemiplimab) in Study 16113 Part 2

The clinical performance of VENTANA PD-L1 (SP263) Assay was evaluated in Part 2 of Study 16113 (NCT03409614), a randomized, global, Phase III study to investigate the efficacy and safety of LIBTAYO (cemiplimab) in combination with platinum-based doublet chemotherapy as first-line treatment of patients with advanced squamous or non-squamous NSCLC.

A total of 466 patients (Full Analysis Set [FAS]) were enrolled. Patients were randomized (2:1) to receive either cemiplimab 350 mg plus platinum-based doublet chemotherapy or placebo plus platinum-based doublet chemotherapy intravenously every 3 weeks for 4 cycles depending on patient tolerability, and disease assessment. Randomization was stratified by histology (non-squamous versus squamous) and level of PD-L1 expression in TC (<1%, 1% to 49%, ≥50%). Overall 57.1% of patients presented with non-squamous histology (patients with squamous histology were capped per protocol at 50%), and the majority (85.2%) of patients had metastatic (stage IV) disease at screening versus locally advanced (14.8% [stage IIIB: 10.7% or IIIC: 4.1%]) disease.

Tumor specimens from 465 of the 466 enrolled patients were tested with VENTANA PD-L1 (SP263) Assay to determine their PD-L1 expression level. The percentage of these patients who had tumors with PD-L1 expression on ≥ 1% of tumor cells (TC) as determined by VENTANA PD-L1 (SP263) Assay was 70.1%. The final staining acceptability rate among patients in the intended use population of the VENTANA PD-L1 (SP263) Assay was 100.0%.

The primary efficacy endpoint measure of 16113 Part 2 was overall survival (OS). OS was defined as the time from randomization to the date of death due to any cause. The primary efficacy objective was to compare the OS of patients who received cemiplimab and platinum-based doublet chemotherapy with patients who received placebo and platinum-based doublet chemotherapy among the FAS. A key secondary efficacy endpoint was progression free survival (PFS) among the FAS. PFS was defined as the time from randomization to the date of the first documented tumor progression or death due to any cause, whichever occurred earlier. OS and PFS were also evaluated in patients with PD-L1 expression ≥ 1% TC as defined by VENTANA PD-L1 (SP263) Assay.

At the time of the final analysis (clinical data cutoff date: 14-Jun-2021), clinically meaningful and statistically significant improvement in OS was observed in the cemiplimab/chemotherapy arm vs the placebo/chemotherapy arm. In the FAS (N=466), the median OS was significantly greater in the cemiplimab/chemo arm (21.9 months [95% CI: 15.5 to NE]) compared with the placebo/chemo arm (13.0 months [95% CI: 11.9 to 16.1] (HR=0.706 [95% CI: 0.534 to 0.933], p=0.0140).

The OS benefit was further corroborated by significant improvement in PFS. In the FAS (N=466), median PFS was significantly greater in the cemiplimab/chemo arm (8.2 months [95% CI: 6.4, 9.3] compared with the placebo/chemo arm (5.0 months [95% CI: 4.3, 6.2]) (HR=0.556 [95% CI: 0.442 to 0.699], p<0.0001).

Clinically meaningful improvement in OS and PFS was also observed in patients with PD-L1 ≥1% TC (n=327), providing support for use of LIBTAYO in combination with platinum-based doublet chemotherapy in the 1L setting for NSCLC patients with PD-L1 ≥ 1% TC expression identified by the VENTANA PD-L1 (SP263) Assay. Efficacy results for patients with PD-L1 expression ≥ 1% TC or <1% TC are presented in Table 26.

Table 26. Clinical Efficacy of LIBTAYO in patients with PD-L1 expression ≥ 1% TC or <1% TC as Determined by the VENTANA PD-L1 (SP263) Assay

Endpoints	LIBTAYO + chemotherapy	Placebo + chemotherapy
Overall Survival		
PD-L1 ≥ 1%	(N=217)	(N=110)
Event (%)	78/217 (35.9%)	55/ 110 (50.0%)
Median (95% CI), (months) [a]	21.9 (17.3, NE)	12.6 (10.3, 16.4)
HR (95% CI) [b]	0.552 (0.390, 0.781)	
PD-L1 <1%	(N=95)	(N=44)

Endpoints	LIBTAYO + chemotherapy	Placebo + chemotherapy
Event (%)	54/95 (56.8%)	27/44 (61.4%)
Median (95% CI), (months) [a]	12.8 (9.6, 16.5)	14.2 (9.1, 18.0)
HR (95% CI) [c]	1.006 (0.633, 1.600)	
Progression-free Survival		
PD-L1 ≥ 1%	(N=217)	(N=110)
Event (%)	134/217 (61.8%)	86/110 (78.2%)
Median (95% CI), (months) [a]	8.5 (6.7, 10.7)	5.5 (4.3, 6.2)
HR (95% CI) [b]	0.475 (0.361, 0.626)	
PD-L1 <1%	(N=95)	(N=44)
Event (%)	70/95 (73.7%)	36/44 (81.8%)
Median (95% CI), (months) [a]	6.2 (4.4, 8.3)	4.4 (4.2, 6.2)
HR (95% CI) [c]	0.764 (0.509, 1.146)	

CI = confidence interval, HR = hazard ratio, NE = Not estimable, LIBTAYO = cemiplimab

[a] Based on Kaplan-Meier method

[b] Based on stratified hazards model, stratified by histology (squamous, non-squamous)

[c] Based on unstratified hazards model

Clinical Performance of TECENTRIQ (atezolizumab) in IMpower010 Study

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 (BSC) 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 BSC, following recovery from surgery. Randomization was stratified by sex, stage of disease, histology, and PD-L1 expression. Among randomized patients, 12% of patients had stage IB, 47% had stage II and 41% had stage IIIA disease.

Tumor specimens from 1169 of the 1280 enrolled patients (including 985 of the 1005 randomized patients) were tested with VENTANA PD-L1 (SP263) Assay to determine their PD-L1 expression level. The percentage of patients who had tumors with PD-L1 expression on ≥ 1% or ≥ 50% of tumor cells (TC) as determined by VENTANA PD-L1 (SP263) Assay was 55% and 26%, respectively. 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. The primary efficacy objective was to evaluate DFS in the PD-L1 ≥ 1% TC (by SP263) stage II - IIIA patient population. Key secondary efficacy objectives were to evaluate DFS in the PD-L1 ≥ 50% TC (by SP263) stage II - IIIA patient population and overall survival (OS) in the ITT population.

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 improvement in DFS in the TECENTRIQ arm compared with the BSC arm in the PD-L1 ≥ 1% TC stage II - IIIA patient population (n = 476) (stratified HR: 0.66, 95% CI: 0.50, 0.88, p-value 0.004). The median follow-up time was approximately 32 months. In the secondary objective analysis of patients with PD-L1 TC ≥ 50% stage II - IIIA (n = 229), a clinically meaningful improvement in DFS was shown with an unstratified HR of 0.43 (95% CI: 0.27, 0.68).

A clinically meaningful improvement in DFS was also observed in patients with PD-L1 ≥ 50% TC stage II - IIIA without EGFR mutations or ALK rearrangements (n = 209).

Efficacy results for the PD-L1 ≥ 50% TC stage II - IIIA patient population (with and without EGFR mutations or ALK rearrangements) are presented in Table 27.

Table 27. Efficacy results from IMpower010 in patients with stage II - IIIA NSCLC with PD-L1 (SP263) expression ≥ 50% TC.

	≥ 50% TC		≥ 50% TC without EGFR mutations or ALK rearrangements	
	Arm A (TECENTRIQ) n = 115	Arm B (BSC) n = 114	Arm A (TECENTRIQ) n = 106	Arm B (BSC) n = 103
DFS events (%)	28 (24.3%)	52 (45.6%)	24 (22.6%)	45 (43.7%)
Median DFS, months (95% CI) [a]	NE (42.3, NE)	35.7 (29.7, NE)	NE (NE, NE)	37.3 (30.1, NE)
Hazard ratio (95% CI) [b]	0.43 (0.27, 0.68)		0.43 (0.26, 0.71)	
p-value	0.0002		0.0002	

DFS = Disease-free survival; CI = confidence interval; NE = Not estimable

BSC = Best supportive care

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

[b] Unstratified

TROUBLESHOOTING

Troubleshooting guidance is provided in Table 28. 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 28. 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 navifyportal.roche.com.
		Ensure that only recommended fixatives and fixation times are used.

Problem	Probable Cause	Suggested Action
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that the recommended staining procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

REFERENCES

1. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26:677-704.
2. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 2007; 56(5):739-745.
3. Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T-cell responses. Immunity 2007; 27(1):111-122.
4. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 1999; 5(12):1365-1369.
5. Massard C, Gordon MS, Sharma S, et al. Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer. J Clin Oncol 2016; 34(26):3119-3125.
6. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
7. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
8. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
9. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small cell lung cancer. The N Engl J Med 2016; 375(19):1823-1833.
10. Herbs RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomized controlled trial. Lancet 2016; 387:1540-1550.
11. Ratcliffe MJ, Sharpe A, Midha A, et al. Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cut-offs in non-small cell lung cancer. Clin Cancer Res 2017; 23(14):3585-3591.
12. Meijuan Li (2016) Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study, Statistics in Biopharmaceutical Research, 8:3, 355-363, DOI: 10.1080/19466315.2016.1202859.
13. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced non-squamous non–small cell lung cancer. N Engl J Med 2015; 373(17):1627-1639.
14. Li M. Statistical consideration and challenges in bridging study of personalized medicine. J Biopharm Stat 2015; 25(3):397-407.

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.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN — Global Trade Item Number

Rx only — For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
E	Updated the Warnings and Precautions, Staining Procedure, Analytical Performance in Non-Small Cell Lung Cancer, and Symbols sections. Added the BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606

www.roche.com

CE 0123