VENTANA®

Roche

VENTANA PD-L1 (SP142) Assay

REF 741-4860

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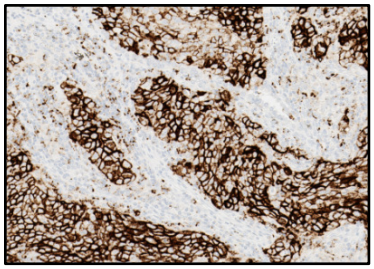

Figure 1. PD-L1 expression in non-small cell lung cancer.

INTENDED USE

VENTANA PD-L1 (SP142) Assay is intended for laboratory use in the qualitative immunohistochemical detection of programmed death-ligand 1 (PD-L1) by light microscopy in tumor cells and tumor-infiltrating immune cells in formalin-fixed, paraffin-embedded (FFPE) tissues indicated below stained with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark IHC/ISH instrument.

Determination of PD-L1 status is indication-specific and evaluation is based on either the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity.

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

Table 1. Companion Diagnostic Indications for Use.

Tumor Type	PD-L1 Expression Cut Off	Clinical Application
Urothelial Carcinoma	≥ 5% IC	PD-L1 expression in tumor-infiltrating immune cells (IC) as detected by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma is indicated as an aid for identifying patients for treatment with TECENTRIQ (atezolizumab).
Triple-Negative Breast Carcinoma (TNBC)	≥ 1% IC	PD-L1 expression in tumor-infiltrating immune cells (IC) as detected by VENTANA PD-L1 (SP142) Assay in TNBC is indicated as an aid for identifying patients for treatment with TECENTRIQ (atezolizumab).
Non-small Cell Lung Cancer (NSCLC)	≥ 50% TC or ≥10% IC	PD-L1 expression in tumor cell (TC) membrane or in tumor-infiltrating immune cells (IC) as detected by VENTANA PD-L1 (SP142) Assay in NSCLC is indicated as an aid for identifying patients for treatment with TECENTRIQ (atezolizumab).

Table 2. Additional Indications for Use.

Tumor Type	PD-L1 Expression Cut Off	Clinical Application
Non-small Cell Lung Cancer (NSCLC)	≥ 50% TC or ≥10% IC	PD-L1 expression in tumor cell (TC) membrane or in tumor-infiltrating immune cells (IC) as detected by VENTANA PD-L1 (SP142) Assay in NSCLC may be associated with enhanced patient benefit with TECENTRIQ (atezolizumab).
	≥ 1% TC or ≥1% IC	PD-L1 expression in tumor cell (TC) membrane or in tumor-infiltrating immune cells (IC) as detected by VENTANA PD-L1 (SP142) Assay in NSCLC may be associated with enhanced patient benefit with TECENTRIQ (atezolizumab).

Test results of 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.

SUMMARY AND EXPLANATION

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

PD-L1 is a transmembrane protein that downregulates 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.[1] 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.[1,2] B7.1 is a molecule expressed on antigen presenting cells and activated T-cells.[1,2] PD-L1 binding to B7.1 on T cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.[2] PD-L1 expression has been observed in immune cells and malignant cells and aberrant expression of PD-L1 on tumor cells (TC) has been reported to impede anti-tumor immunity, resulting in immune evasion.[1,3] 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 tumor cells or tumor-infiltrating immune cells and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported across multiple cancers.[3-10]

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors.[3-10]

PRINCIPLE OF THE PROCEDURE

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

MATERIAL PROVIDED

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

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

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

Specific antibody concentration is approximately 7 µg/mL.

VENTANA PD-L1 (SP142) Assay contains a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate interpretation guide for detailed instructions for interpretation of VENTANA PD-L1 (SP142) Assay staining in specific indications:

- VENTANA PD-L1 (SP142) Assay Interpretation Guide for Urothelial Carcinoma (P/N 1015704)
- VENTANA PD-L1 (SP142) Assay Interpretation Guide for NSCLC ≥ 50% TC or ≥ 10% IC Stepwise Scoring Algorithm (P/N 1015703)
- VENTANA PD-L1 (SP142) Assay Interpretation Guide for NSCLC ≥ 1% TC or ≥ 1% IC Stepwise Scoring Algorithm (P/N 1015654)
- VENTANA PD L1 (SP142) Assay Interpretation Guide for TNBC (P/N 1018231)

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. Benign human tonsil tissues for use as control tissue
2. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
3. Microscope slides, positively charged
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. General purpose laboratory equipment
15. 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. Tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours is recommended. Fixation times of less than 6 hours may result in a loss of staining for PD-L1. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[11,12]

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

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time and may be compromised 3 months after cutting from the paraffin block for urothelial carcinoma specimens, and 2 months for NSCLC, TNBC, and tonsil specimens (see the interpretation guides and the Performance Characteristics section below).

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. 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.
5. 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.[13,14]
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. 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.
9. Consult local and/or state authorities with regard to recommended method of disposal.
10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
11. 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.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. An assay-specific staining procedure must be used with VENTANA PD-L1 (SP142) Assay. Refer to Table 3 and Table 4 for the recommended staining protocol and required staining procedures. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 741-4860.

Table 3. Use the following staining procedures to perform VENTANA PD-L1 (SP142) Assay on a BenchMark IHC/ISH instrument.

Instrument	Staining Procedure
BenchMark GX	GX VENTANA PDL1 (SP142)
BenchMark ULTRA or BenchMark ULTRA PLUS	ULTRA VENTANA PDL1 (SP142)

Table 4. Recommended staining protocol for VENTANA PD-L1 (SP142) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark IHC/ISH instrument.

Procedure Type	Parameter Input
Baking	Optional
Antibody (Primary)	VENTANA PD-L1 (SP142) Selected or Negative Control Selected
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

QUALITY CONTROL PROCEDURES

Rabbit Monoclonal Negative Control Ig

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

Tonsil Tissue Control

A tissue control must be included with each staining run. Qualified benign human tonsil tissue is to be used as the control. Control tissue should be fixed as soon as possible and processed in a manner identical to patient tissues. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Tonsil tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. The positive and negative staining tissue components are used to confirm that the assay functioned properly.

Appropriate staining of tonsil tissue components is described in Table 5 and in the interpretation guides.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known IHC performance characteristics representing PD-L1 positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[15] or the CLSI Approved Guideline[16]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Urothelial carcinoma, NSCLC, and TNBC tissues with known PD-L1 status, and benign human tonsil samples, are suitable for assay verification.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the VENTANA PD-L1 (SP142) Assay antibody. The stained slide(s) are interpreted by a qualified pathologist using light microscopy. A qualified pathologist experienced in IHC procedures must evaluate tissue controls and qualify the stained product before interpreting results.

Tonsil Tissue Control Interpretation

The stained tonsil tissue control should be examined for appropriate staining. The presence of PD-L1 staining within the macrophages and lymphocytes in germinal centers and reticulated crypt epithelium of tonsil serve as positive tissue elements. Absence of staining in superficial squamous epithelium and negative immune cells in interfollicular regions of tonsil serve as negative tissue elements. Acceptability criteria are listed in Table 5. (Refer to the interpretation guides for further discussion).

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

Table 5. Tonsil tissue control evaluation criteria.

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

Negative Reagent Control

Non-specific staining, if present, will have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results; as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Examples of background staining for this assay can be found in the interpretation guides.

Patient Tissue

Tumor cells (TC) are scored as the percentage of tumor cells with the presence of discernible PD-L1 membrane staining of any intensity. Tumor-infiltrating immune cells (IC) are scored as the proportion of tumor area, including associated intratumoral and contiguous peritumoral stroma, occupied by PD-L1 staining IC of any intensity. Patient tissue must be evaluated according to the indication-specific VENTANA PD-L1 (SP142) Assay scoring algorithm provided in the Performance Characteristics section for that indication. Refer to the indication-specific interpretation guide for additional instructions and representative images.

SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP142) Assay has been solely approved on BenchMark IHC/ISH instruments with the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit and is not approved with any other detection or instruments.
2. This assay has not been validated for use with cytology samples or decalcified bone specimens.
3. Patient tissue should be stained within 2 months of sectioning from the tissue block for NSCLC, TNBC, and tonsil tissues and within 3 months for urothelial carcinoma tissues. Loss of staining performance has been observed with VENTANA PD-L1 (SP142) Assay staining of tissue sections that have been stored at room temperature for longer than these times.
4. Artifacts such as DAB spots, Blank spots, DAB dots, and/or Speckling may require repeat staining if they interfere with the interpretation of VENTANA PD-L1 (SP142) Assay. Always compare the PD-L1 stained slide to the negative reagent control to ensure that background is acceptable. Refer to the interpretation guides for further discussion.
5. Occasional DAB dots have been observed in benign human tonsil control, cerebellum and testicular tissues and focal nuclear staining has been observed in normal pancreatic (acinar cells) and hypophyseal tissue (Table 6); however, nuclear staining is not included in scoring of VENTANA PD-L1 (SP142) Assay staining.
6. All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE - GENERAL

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

General Analysis Comments

Unless otherwise noted, the two-sided 95% confidence interval (CI) around estimates of agreement for all studies (excluding clinical efficacy studies) were calculated using the percentile bootstrap method from 2,000 bootstrap samples. If the point estimate of Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), or Overall

Percent Agreement (OPA) is 0% or 100%, then Wilson score method was used to calculate 95% CI. If the point estimate of Average Positive Agreement (APA) and Average Negative Agreement (ANA) is 0% or 100% for pairwise comparison, then transformation Wilson score method was used to calculate 95% CI.

Sensitivity and Specificity

Arrays containing a variety of normal and neoplastic tissues were stained with VENTANA PD-L1 (SP142) Assay and evaluated for the presence of immune cell staining (any immune cell staining, of any intensity) as described in Table 6 and Table 7.

Table 6. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay was determined by testing FFPE normal tissues.

Tissue	# Positive[a] / Total Cases	Tissue	# Positive[a] / Total Cases
Cerebrum	0/3	Thymus	3/3
Cerebellum[c]	0/3	Bone marrow	0/2
Adrenal gland	1/3	Lung	1/25
Ovary	0/3	Heart	0/3
Pancreas[d]	0/3	Esophagus	0/3
Parathyroid gland	0/2	Stomach	0/3
Pituitary gland[d]	0/3	Small intestine	1/3
Testis[c]	0/3	Colon	2/3
Thyroid	1/3	Liver	0/3
Breast	1/66	Salivary gland	2/4
Spleen	3/3	Kidney	2/3
Tonsil[c]	3/3	Bladder[b]	3/36
Lymph node	3/3	Prostate	0/3
Endometrium	2/3	Cervix	0/2
Skeletal muscle	0/2	Skin	0/3
Nerve	0/3	Mesothelium	0/3

[a] Immune cell staining of any intensity

[b] Focal immune cell staining

[c] Focal DAB dots were observed in 1/3 cerebellum,1/3 testis tissues and normal tonsil control

[d] Nuclear staining was observed in 1/3 pancreas and 1/3 pituitary gland tissues

Table 7. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# Positive[a] / Total Cases	
	Immune Cells	Tumor Cells
Glioblastoma (Cerebrum)	1/1	0/1
Meningioma (Cerebrum)	0/1	0/1
Ependymoma (Cerebrum)	0/1	0/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Serous adenocarcinoma (Ovary)	1/1	0/1
Adenocarcinoma (Ovary)	1/1	0/1
Neuroendocrine neoplasm (Pancreas)	0/1	0/1
Adenocarcinoma (Pancreas)	1/1	0/1
Seminoma (Testis)	1/1	0/1
Embryonal carcinoma (Testis)	0/1	0/1
Medullary carcinoma (Thyroid)	0/1	0/1
Papillary carcinoma (Thyroid)	0/1	1/1
Microinvasive ductal carcinoma (Breast)	1/1	0/1
Invasive ductal carcinoma (Breast)	1/2	0/2
B-Cell Lymphoma; NOS (Spleen)[a]	1/1	1/1
Small cell carcinoma (Lung)	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	0/1
Adenocarcinoma (Lung)	0/1	0/1
Neuroendocrine carcinoma (Esophagus)	0/1	0/1
Adenocarcinoma (Esophagus)	1/1	0/1
Signet-ring cell carcinoma (Stomach)	1/1	0/1
Adenocarcinoma (Small intestine)	1/1	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	1/1	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1	0/1
Adenocarcinoma (Colon)	1/1	0/1
Adenocarcinoma (Rectum)	1/1	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1	0/1
Melanoma (Rectum)	1/1	0/1
Hepatocellular carcinoma (Liver)	0/1	0/1
Hepatoblastoma (Liver)	1/1	0/1
Clear cell carcinoma (Kidney)	1/1	0/1
Adenocarcinoma (Prostate)	0/2	0/2
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	1/1	0/1
Clear cell carcinoma (Uterus)	1/1	1/1
Squamous cell carcinoma (Cervix)	2/2	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1	0/1
Basal cell carcinoma (Skin)	1/1	0/1
Squamous cell carcinoma (Skin)	1/1	0/1
Neurofibroma (Lumbar)	1/1	0/1

Pathology	# Positive[a] / Total Cases	
	Immune Cells	Tumor Cells
Neuroblastoma (Retroperitoneum)	1/1	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1	0/1
Mesothelioma (Peritoneum)	1/1	0/1
B-Cell Lymphoma (Lymph node)[b]	1/1	1/1
Hodgkin lymphoma (Lymph node)	1/1	1/1
B-cell lymphoma; NOS (Mediastinum)[b]	1/1	1/1
Anaplastic large cell lymphoma (Lymph node)[b]	1/1	1/1
Urothelial carcinoma (Bladder)	1/1	0/1
Leiomyosarcoma (Bladder)	0/1	0/1
Osteosarcoma (Bone)	0/1	0/1
Leiomyosarcoma (Smooth muscle)	1/1	0/1

[a] Immune cell or tumor cell staining of any intensity

[b] Tumor cell and immune cell staining could not be differentiated

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE – UROTHELIAL CARCINOMA

Scoring Algorithm – Urothelial Carcinoma

Urothelial carcinoma tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma provided in Table 8. Refer to the interpretation guide (P/N 1015704) for additional instructions and representative images.

Table 8. VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma.

Immune Cell (IC) Staining Assessment[a]	PD-L1 Expression
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 5% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 5% IC

[a] PD-L1 staining in tumor cells should not be included in the scoring determination of urothelial carcinoma patient tissue.

Tissue Thickness – Urothelial Carcinoma

Tissue thickness was evaluated using 5 unique urothelial carcinoma specimens (3 PD-L1 ≥ 5% IC and 2 PD-L1 < 5% IC). Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 expression within the range of thickness tested. The specimens should be cut at 4 µm for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision – Urothelial Carcinoma

Studies for VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens were completed to demonstrate:

- Intra-day repeatability – 5 replicate slides each from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within one day.
- Inter-day precision – 10 slides from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days.
- Inter-instrument and inter-lot precision – 27 slides each from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments.
- Intra-platform precision – 2 replicate slides each from 10 unique urothelial carcinoma specimens (3 PD-L1 ≥ 5% IC and 7 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay across three BenchMark ULTRA and three BenchMark GX instruments. Agreement rates were calculated relative to the specimen mode for one platform.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma (Table 8). Results are summarized in Table 9.

Table 9. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)[a]
Intra-day repeatability (within a single day)	PPA: 98.2 (90.4-99.7) NPA: 100.0 (94.4-100.0) OPA: 99.2 (95.4-99.9)
Inter-day precision (5 non-consecutive days)	PPA: 91.8 (85.2-95.6) NPA: 100.0 (97.1-100.0) OPA: 96.3 (93.0-98.0)
Inter-instrument and Inter-lot precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	PPA: 99.4 (97.8-99.8) NPA: 99.7 (98.3-99.9) OPA: 99.5 (98.6-99.8)
Intra-platform precision (three BenchMark ULTRA instruments)	PPA: 83.3 (60.8-94.2) NPA: 100.0 (91.2-100.0) OPA: 94.8 (85.9-98.2)
Intra-platform precision (three BenchMark GX instruments)	PPA: 94.4 (74.2-99.0) NPA: 100.0 (91.4-100.0) OPA: 98.3 (91.0-99.7)

[a] Two-sided Wilson score method CI

Inter-Platform Concordance – Urothelial Carcinoma

Single slides each from 44 unique urothelial carcinoma specimens (22 PD-L1 ≥ 5% IC and 22 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay on one BenchMark ULTRA (reference) and one BenchMark GX instrument. All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma (Table 8). Results are summarized in Table 10.

Table 10. Inter-platform concordance of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Inter-platform Concordance	Agreement % (95% CI)[a]
BenchMark ULTRA: BenchMark GX	PPA: 95.2 (77.3-99.2) NPA: 100.0 (85.1-100.0) OPA: 97.7 (87.9-99.6)

[a] Two-sided Wilson score method CI

Reader Precision – Urothelial Carcinoma

To assess inter- and intra-reader precision, three pathologists evaluated 60 unique urothelial carcinoma specimens (30 PD-L1 ≥ 5% IC and 30 PD-L1 < 5% IC) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma (Table 8). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and between each pathologist's reads are summarized in Table 11.

Table 11. Inter- and intra-reader precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 92.7 (85.9-97.6) ANA: 93.9 (88.1-98.1) OPA: 93.3 (87.8-97.8)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.4 (87.3-97.7) ANA: 94.2 (88.9-98.1) OPA: 93.9 (88.8-97.8)

Inter-Laboratory Reproducibility Study – Urothelial Carcinoma

An inter-laboratory reproducibility Study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in urothelial carcinoma tissue specimens. Twenty-eight unique urothelial carcinoma specimens (14 PD-L1 ≥ 5% IC and 14 PD-L1 < 5% IC) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). Results are summarized in Table 12.

Table 12. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Inter-laboratory Reproducibility	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 98.3 (96.6-99.2)[a] NPA: 87.4 (83.8-90.2)[a] OPA: 92.8 (90.9-94.4)[a]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 90.7 (81.2-96.3) ANA: 88.3 (78.5-94.9) OPA: 89.6 (82.5-95.5)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 89.3 (78.1-96.0) ANA: 86.6 (75.1-94.6) OPA: 88.1 (84.6-90.8)[a]

[a] Two-sided Wilson score method CI

CLINICAL PERFORMANCE – UROTHELIAL CARCINOMA

The performance of VENTANA PD-L1 (SP142) Assay was investigated in IMvigor210 (NCT02951767), a phase II, multicenter, international, two-cohort, single-arm trial designed to evaluate the efficacy of TECENTRIQ (atezolizumab) in patients with locally advanced or metastatic urothelial carcinoma. Cohort 1 included previously untreated patients with locally advanced or metastatic urothelial carcinoma who were ineligible or unfit for cisplatin-based chemotherapy. In Cohort 1, 119 patients were treated with TECENTRIQ (atezolizumab) 1200 mg by intravenous infusion every 3 weeks until unacceptable toxicity or disease progression. Tumor specimens from all patients screened for IMvigor210 were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used to define subgroups for pre-specified analyses. The primary efficacy outcome measurement was confirmed objective response rate (ORR) as assessed by independent review facility (IRF) using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

The primary efficacy endpoint of IMvigor210 Cohort 1 was confirmed ORR as assessed by an IRF using RECIST v1.1. The primary analysis was performed when all patients had at least 24 weeks of follow-up. Median duration of treatment was 15.0 weeks and median duration of survival follow-up was 8.5 months in all comers. Clinically relevant IRF-assessed ORRs per RECIST v1.1 were shown; however, when compared to a pre-specified historical control response rate of 10%, statistical significance was not reached for the primary endpoint. The confirmed ORRs per IRF-RECIST v1.1 were 21.9% (95% CI: 9.3, 40.0) in patients with PD-L1 expression ≥ 5%, 18.8% (95% CI: 10.9, 29.0) in patients with PD-L1 expression ≥ 1%, and 19.3% (95% CI: 12.7, 27.6) in all comers. The median duration of response (DOR) was not reached in any PD-L1 expression subgroup or in all comers. Although, ORR did not reach statistical significance, results from IMvigor210 Cohort 1 showed clinically meaningful responses of TECENTRIQ (atezolizumab) monotherapy independent of PD-L1 expression levels, as detected by the VENTANA PD-L1 (SP142) Assay.

The performance of VENTANA PD-L1 (SP142) Assay is being investigated in IMvigor130 (NCT02807636), an ongoing multicenter, randomized study designed to evaluate the efficacy of TECENTRIQ (atezolizumab) in previously untreated patients with metastatic urothelial carcinoma who are eligible for platinum-containing chemotherapy. The study contains three arms: TECENTRIQ (atezolizumab) monotherapy, TECENTRIQ (atezolizumab) with platinum-based chemotherapy (i.e., cisplatin or carboplatin with gemcitabine), and platinum-based chemotherapy alone (comparator). Both cisplatin-eligible and cisplatin-ineligible patients are included in the study. The co-primary efficacy endpoints for the intent-to-treat (ITT) population are investigator-assessed RECIST v1.1 progression free survival (PFS) and overall survival (OS).

Tumor specimens were evaluated prospectively using the VENTANA PD-L1 (SP142) Assay at a central laboratory. The independent Data Monitoring Committee (iDMC) for the study conducted a review of early data and found that patients classified as having PD-L1 expression of < 5% when treated with TECENTRIQ (atezolizumab) monotherapy had decreased survival compared to those who received platinum-based chemotherapy.

In an exploratory analysis of OS (CCOD Jun 2020) by PD-L1 status in patients who were cisplatin-ineligible by Galsky criteria, improved OS was observed in patients with higher PD-L1 tumor status for the atezolizumab monotherapy (Atezo Mono) arm compared with the Placebo + Chemo arm (Table 13). Among cisplatin-ineligible patients with PD-L1 expression ≥ 5%, the unstratified HR for the comparison of Atezo Mono vs. Placebo +

Chemo was 0.60 (95% CI: 0.36, 1.01). The Kaplan-Meier estimated median OS was 18.63 months (95% CI: 14.0, NE) in the Atezo Mono arm (n=50) and 9.95 months (95% CI: 7.36, 18.1) in the Placebo + Chemo arm (n=43). In the subgroup of cisplatin-ineligible patients with PD-L1 expression < 5%, the unstratified HR for the comparison of Atezo Mono vs. Placebo + Chemo was 1.15 (95% CI: 0.87, 1.52). The Kaplan-Meier estimated median OS was 11.2 months (95% CI: 6.87, 14.65) in the Atezo Mono arm (n=140) and 11.17 months (95% CI: 9.89, 14.26) in the Placebo + Chemo arm (n=140).

Table 13. Summary of exploratory analyses: overall survival by PD-L1 status among cisplatin-ineligible patients (per Galsky criteria).

Overall Survival (OS)	Atezo Mono	Placebo + Chemo
PD-L1 ≥ 5%	(N=50)	(N=43)
No. of deaths (%)	28 (56.0)	30 (69.8)
Median time to events, months (95% CI)[a,b]	18.63 (14.0, NE)	9.95 (7.36, 18.1)
Unstratified hazard ratio (95% CI)[c,b]	0.60 (0.36, 1.01)	
p-value[d]	0.0509	
PD-L1 < 5%	(N=140)	(N=140)
No. of deaths (%)	105 (75.0)	96 (68.6)
Median time to events, months (95% CI)[a,b]	11.2 (6.87, 14.65)	11.17 (9.89, 14.26)
Unstratified hazard ratio (95% CI)[c,b]	1.15 (0.87, 1.52)	
p-value[d]	0.3229	

[a] Summaries of duration (median and percentiles) are Kaplan-Meier estimates.

[b] 95% CIs for the median are computed using the method of Brookmeyer and Crowley.

[c] Hazard ratios were estimated by Cox regression.

[d] Results shown for informational purposes only.

CI = confidence interval; NE = not estimable

CCOD=14-Jun-2020

The iDMC recommended closure of the monotherapy arm to further accrual of patients with low PD-L1 expression; however, no other changes were recommended for the study, including any change of therapy for patients who had already been randomized to, and were receiving treatment in, the monotherapy arm.

The IMvigor130 study is currently ongoing, and the results for the efficacy of TECENTRIQ (atezolizumab) compared to chemotherapy in the PD-L1 ≥ 5% IC population are expected to be available at the final study readout. The current companion diagnostic designation is due to the mentioned iDMC finding noting reduced survival of patients with PD-L1 < 5% IC expression treated with TECENTRIQ (atezolizumab). Results at final IMvigor130 readout will be evaluated as to whether increased TECENTRIQ (atezolizumab) efficacy is observed in the PD-L1 ≥ 5% IC population, and the rationale for a companion diagnostic will be updated accordingly.

ANALYTICAL PERFORMANCE – NSCLC

Scoring Algorithm – NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC provided in Table 14 and Table 15. The specimen should be considered to have PD-L1 expression if the specimen exhibits ≥ 1% TC or ≥ 1% IC and high PD-L1 expression if the specimen exhibits ≥ 50% TC or ≥ 10% IC. Refer to the interpretation guide (P/N 1015703 and 1015654) for additional instructions and representative images.

Table 14. VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC ≥ 50% TC or ≥ 10% IC).

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

Table 15. VENTANA PD-L1 (SP142) scoring algorithm for NSCLC ≥ 1% TC or ≥ 1% IC.

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

Tissue Thickness - NSCLC

Tissue thickness was evaluated using NSCLC specimens. Duplicate sections at 3, 4, 5, 6, and 7 µm were stained with VENTANA PD-L1 (SP142) Assay and evaluated for PD-L1 TC and IC expression. A total of 42 NSCLC specimens with a range of PD-L1 expression for each IC and TC level were evaluated.

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

Repeatability and Intermediate Precision - NSCLC

Studies for VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens were completed to demonstrate:

- Intra-day repeatability – 5 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument in a single day and evaluated for PD-L1 TC and IC expression. A total of 66 NSCLC specimens with a range of PD-L1 expression for each TC and IC level were evaluated.
- Inter-day precision – 10 slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days. A total of 66 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level were evaluated.
- Instrument, antibody and detection lot precision – a minimum of 9 slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments. A total of 92 NSCLC specimens with a range of PD-L1 expression for each TC and IC were evaluated.
- Intra-platform precision – 2 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay across three BenchMark ULTRA and three BenchMark GX instruments. A total of 38 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level were evaluated. Agreement rates were calculated relative to the specimen mode for each platform.

All slides were blinded and randomized and then evaluated for PD-L1 TC or IC expression level. Results are summarized in Table 16, Table 17, Table 18 and Table 19.

Table 16. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 50% TC).

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)[a]
Intra-day repeatability (within a single day), 24 NSCLC specimens	PPA: 100.0 (94.4-100.0) NPA: 100.0 (93.5-100.0) OPA: 100.0 (96.9-100.0)
Inter-day precision (5 non-consecutive days), 24 NSCLC specimens	PPA: 100.0 (97.1-100.0) NPA: 100.0 (96.5-100.0) OPA: 100.0 (98.4-100.0)
Inter-instrument and Inter-lot precision (compared to case-level mode, across instruments and lots), 18 NSCLC specimens	PPA: 99.7 (98.1-99.9) NPA: 95.2 (91.2-97.5) OPA: 97.9 (96.2-98.9)
Intra-platform precision (three BenchMark ULTRA instruments), 10 NSCLC specimens	PPA: 100.0 (88.6-100.0) NPA: 100.0 (88.6-100.0) OPA: 100.0 (94.0-100.0)
Intra-platform precision (three BenchMark GX instruments), 10 NSCLC specimens	PPA: 100.0 (88.6-100.0) NPA: 100.0 (88.6-100.0) OPA: 100.0 (94.0-100.0)

[a] Two-sided Wilson score method CI

Table 17. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 10% IC).

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)
Intra-day repeatability (within a single day), 24 NSCLC specimens	PPA: 98.3 (91.1-99.7)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 99.2 (95.4-99.9)[a]
Inter-day precision (5 non-consecutive days), 24 NSCLC specimens	PPA: 96.2 (91.3-98.3)[a] NPA: 98.2 (93.6-99.5)[a] OPA: 97.1 (94.1-98.6)[a]
Inter-antibody and Inter-detection agreement (pairwise-comparison), 28 NSCLC specimens	APA: 95.1 (91.1-98.1) ANA: 90.2 (82.3-96.2) OPA: 93.4 (88.7-97.5)
Inter-instrument and Inter-detection lots agreement (pairwise-comparison), 28 NSCLC specimens	APA: 96.3 (93.2-98.8) ANA: 92.7 (86.0-97.7) OPA: 95.1 (91.2-98.4)
Inter-instrument and Inter-antibody agreement (pairwise-comparison), 28 NSCLC specimens	APA: 96.3 (93.1-98.8) ANA: 92.6 (85.9-97.8) OPA: 95.1 (91.1-98.4)
Intra-platform precision (three BenchMark ULTRA instruments, 8 NSCLC specimens	PPA: 100.0 (94.0-100.0)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 100.0 (96.9-100.0)[a]
Intra-platform precision (three BenchMark GX instruments), 8 NSCLC specimens	PPA: 100.0 (94.0-100.0)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 100.0 (96.9-100.0)[a]

[a] Two -sided Wilson score method CI

Table 18. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 1% TC).

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)[a]
Intra-day repeatability (within a single day), 10 NSCLC specimens	PPA: 100.0 (86.7-100.0) NPA: 100.0 (86.7-100.0) OPA: 100.0 (92.9-100.0)
Inter-day precision (5 non-consecutive days), 10 NSCLC specimens	PPA: 100.0 (92.9- 100.0) NPA: 100.0 (92.9- 100.0) OPA: 100.0 (96.3- 100.0)
Inter-instrument and Inter-lot precision (compared to case-level mode, across instruments and lots), 18 NSCLC specimens	PPA: 99.2 (97.0-99.8) NPA: 100.0 (98.4-100.0) OPA: 99.6 (98.5-99.9)
Intra-platform precision (three BenchMark ULTRA instruments), 12 NSCLC specimens	PPA: 100.0 (86.2-100.0) NPA: 95.8 (86.0-98.8) OPA: 97.2 (90.4-99.2)
Intra-platform precision (three BenchMark GX instruments), 12 NSCLC specimens	PPA: 100.0 (86.2-100.0) NPA: 100.0 (92.6-100.0) OPA: 100.0 (94.0-100.0)

[a] Two-sided Wilson score method CI

Table 19. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 1% IC).

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)
Intra-day repeatability (within a single day), 8 NSCLC specimens	PPA: 100.0 (91.2-100.0)[a] NPA: 100.0 (91.2-100.0)[a] OPA: 100.0 (95.4-100.0)[a]
Inter-day precision (5 non-consecutive days), 8 NSCLC specimens	PPA: 100.0 (96.3-100.0)[a] NPA: 97.0 (91.6-99.0)[a] OPA: 98.5 (95.7-99.5)[a]
Inter-antibody and Inter-detection agreement (pairwise-comparison), 28 NSCLC specimens	APA: 98.4 (95.7-100.0) ANA: 98.3 (95.6-100.0) OPA: 98.4 (95.9-100.0)
Inter-instrument and Inter-detection agreement (pairwise-comparison),28 NSCLC specimens	APA: 98.4 (95.8- 100.0) ANA: 98.3 (95.4-100.0) OPA: 98.4 (95.8-100.0)
Inter-instrument and Inter-antibody agreement (pairwise-comparison), 28 NSCLC specimens	APA: 98.4 (95.9-100.0) ANA: 98.3 (95.5-100.0) OPA: 98.4 (95.8- 100.0)
Intra-platform precision (three BenchMark ULTRA instruments), 8 NSCLC specimens	PPA: 100.0 (94.0-100.0)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 100.0 (96.9-100.0)[a]
Intra-platform precision (three BenchMark GX instruments), 8 NSCLC specimens	PPA: 100.0 (94.0-100.0)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 100.0 (96.9-100.0)[a]

[a] Two-sided Wilson score method CI

Inter-Platform Concordance – NSCLC

Single slides each from 44 NSCLC specimens for PD-L1 expression ≥ 50% TC or ≥ 10% IC (21 PD-L1 ≥ 50% TC or ≥ 10% IC, and 23 PD-L1 < 50% TC and < 10% IC) and 44 NSCLC specimens for PD-L1 expression ≥1% TC or ≥1% IC (23 PD-L1 ≥ 1% TC or ≥ 1% IC, and 21 PD-L1 < 1% TC and < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on one BenchMark ULTRA (reference) and one BenchMark GX instrument.

All slides were blinded and randomized and then evaluated with the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 14 for NSCLC ≥ 50% TC or ≥ 10% IC and Table 15 for NSCLC ≥ 1% TC or ≥ 1% IC). Results are summarized in Table 20 and Table 21.

Table 20. Inter-platform concordance of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 50% TC or ≥ 10% IC).

Inter-platform Concordance	Agreement % (95% CI)[a]
BenchMark ULTRA: BenchMark GX	PPA: 95.2 (77.3-99.2) NPA: 100.0 (85.1-100.0) OPA: 97.7 (87.9-99.6)

[a] Two-sided Wilson score method CI

Table 21. Inter-platform concordance of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 1% TC or ≥ 1% IC).

Inter-platform Concordance	Agreement % (95% CI)[a]
BenchMark ULTRA: BenchMark GX	PPA: 100.0 (85.7-100.0) NPA: 100.0 (84.5-100.0) OPA: 100.0 (92.0-100.0)

[a] Two-sided Wilson score method CI

Reader Precision Study – NSCLC

To assess inter- and intra-reader precision, three pathologists evaluated 80 unique NSCLC cases, with a range of PD-L1 expression, that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 14 for NSCLC ≥ 50% TC or ≥ 10% IC and Table 15 for NSCLC ≥ 1% TC or ≥ 1% IC). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and between each pathologist's reads are summarized in Table 22 and Table 23.

Table 22. Reader precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 50% TC or ≥ 10% IC).

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 88.8 (82.0-94.1) ANA: 89.0 (82.2-94.4) OPA: 88.9 (82.8-94.1)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.7 (89.9-96.6) ANA: 93.6 (89.8-96.7) OPA: 93.6 (90.3-96.6)

Table 23. Reader precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 1% TC or ≥ 1% IC).

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 93.7 (88.8- 97.3) ANA: 90.7 (83.3- 96.2) OPA: 92.5 (87.5-96.7)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 95.4 (92.0-98.1) ANA: 93.4 (88.3-97.2) OPA: 94.6 (90.8- 97.5)

Inter-Laboratory Reproducibility Study – NSCLC

An inter-laboratory reproducibility Study for VENTANA PD-L1 (SP142) Assay staining was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in NSCLC tissue specimens. Twenty-eight unique NSCLC specimens with a range of PD-L1 expression were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers) using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 14 for NSCLC ≥ 50% TC or ≥ 10% IC and Table 15 for NSCLC ≥ 1% TC or ≥ 1% IC). Results are summarized in Table 24 and Table 25.

Table 24. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 50% TC or ≥ 10% IC).

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

[a] Two-sided Wilson score method CI

Table 25. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 1% TC or ≥ 1% IC).

Inter-laboratory Reproducibility	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 97.1 (94.9- 98.3)[a] NPA: 89.9 (86.6- 92.5)[a] OPA: 93.5 (91.6-95.0)[a]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 88.4 (78.9- 94.1) ANA: 86.6 (79.6-92.2) OPA: 87.6 (86.8- 88.3)[a]
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 89.2 (80.8- 94.3) ANA: 87.5 (81.3- 92.5) OPA: 88.4 (84.9- 91.2)[a]

[a] Two-sided Wilson score method CI

Table 26. Summary of efficacy in patients with high PD-L1 expression ≥ 50% TC or ≥ 10% IC (IMpower110)

Overall Survival (OS)	Atezolizumab (N=107)	Chemotherapy (N=98)
No. of deaths (%)	44 (41.1%)	57 (58.2%)
Median time to events, months (95% CI)	20.2 (16.5, NE)	13.1 (7.4, 16.5)
Stratified hazard ratio[a] (95% CI)	0.59 (0.40, 0.89)	
p-value[a]	0.0106	
12-month OS (%)	64.9	50.6

[a] Stratified by sex and ECOG performance status (0 vs. 1)

CI = confidence interval; NE=not estimable

CCOD = 10-Sept-2018

CLINICAL PERFORMANCE – NSCLC ≥ 50% TC OR ≥ 10% IC

The performance of VENTANA PD-L1 (SP142) Assay was investigated in IMpower110 (NCT02409342), a phase III, multicenter, international, randomized, open-label trial in 572 patients with stage IV NSCLC, including those with EGFR or ALK genomic tumor aberrations, who had received no prior chemotherapy for metastatic disease. The study was designed to evaluate the safety and efficacy of TECENTRIQ (atezolizumab) relative to chemotherapy consisting of a platinum agent (cisplatin or carboplatin per investigator discretion) in combination with either pemetrexed (non-squamous disease) or gemcitabine (squamous disease).

The IMpower110 study was conducted to evaluate the efficacy and safety of TECENTRIQ (atezolizumab) in chemotherapy-naïve patients with metastatic NSCLC. Patients had PD-L1 expression ≥ 1% TC (PD-L1 stained ≥ 1% of tumor cells) or ≥ 1% IC (PD-L1 stained tumor-infiltrating immune cells covering ≥ 1% of the tumor area) based on the VENTANA PD-L1 (SP142) Assay.

A total of 572 patients were randomized in a 1:1 ratio to receive TECENTRIQ (atezolizumab) (Arm A) or chemotherapy (Arm B). TECENTRIQ (atezolizumab) was administered as a fixed dose of 1200 mg by IV infusion every 3 weeks until loss of clinical benefit as assessed by the investigator or unacceptable toxicity. Randomization was stratified by sex, ECOG performance status, histology, and PD-L1 tumor expression on TC and IC.

The demographics and baseline disease characteristics in patients with PD-L1 expression ≥ 1% TC or ≥ 1% IC who do not have EGFR mutations or ALK rearrangements (n = 554) were well balanced between the treatment arms. The median age was 64.5 years (range: 30 to 87), and 70% of patients were male. The majority of patients were white (84%) and Asian (14%). Most patients were current or previous smokers (87%) and baseline ECOG performance status in patients was 0 (36%) or 1 (64%). Overall, 69% of patients had non-squamous disease and 31% of patients had squamous disease. The demographics and baseline disease characteristics in patients with high PD-L1 expression (PD-L1 ≥ 50% TC or ≥ 10% IC) who do not have EGFR mutations or ALK rearrangements (n = 205) were generally representative of the broader study population and were balanced between the treatment arms.

The primary endpoint was overall survival (OS). At the time of the interim OS analysis, patients with high PD-L1 expression excluding those with EGFR mutations or ALK rearrangements (n = 205) showed statistically significant improvement in OS for the patients randomized to TECENTRIQ (atezolizumab) (Arm A) as compared with chemotherapy (Arm B) (HR of 0.59, 95% CI: 0.40, 0.89; median OS of 20.2 months vs 13.1 months) with a two-sided p-value of 0.0106. The median survival follow-up time in patients with high PD-L1 expression was 15.7 months. The key results at the interim analysis are summarized in Table 26.

The performance of VENTANA PD-L1 (SP142) Assay was investigated in OAK (NCT02008227), a Phase III, multi-center, international, randomized, open-label trial designed to evaluate the efficacy and safety of TECENTRIQ (atezolizumab) treatment in patients with locally advanced or metastatic NSCLC who progressed during or following a platinum-containing regimen.

The OAK study enrolled 1225 patients with the primary analysis population consisting of the first 850 randomized patients; eligible patients were stratified by PD-L1 expression status in IC, by the number of prior chemotherapy regimens, and by histology. Patients were randomized (1:1) to receive either TECENTRIQ (atezolizumab) administered intravenously at 1200 mg every 3 weeks until unacceptable toxicity or either radiographic or clinical progression or docetaxel administered intravenously at 75 mg/m^2 every 3 weeks until loss of clinical benefit as assessed by the investigator. Tumor specimens were evaluated prospectively for PD-L1 expression on TC and IC using VENTANA PD-L1 (SP142) Assay and the results were used to define the PD-L1 expression subgroups for pre-specified analyses described below.

The major efficacy outcome measure of the OAK study was overall survival (OS) in the primary analysis population (first 850 randomized patients). The results of the OAK study, with a median follow up of 21 months, are presented in Table 27.

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

Table 27. Efficacy results in the primary analysis population from the OAK study (NCT02008227).

Overall Survival (OS)	Atezolizumab (N = 425)	Docetaxel (N = 425)
Deaths (%)	271 (64)	298 (70)
Median, months (95% CI)	13.8 (11.8, 15.7)	9.6 (8.6, 11.2)
Hazard ratio[a] (95% CI)	0.73 (0.62, 0.87)	
p-value[b]	0.0003	
12-month OS (%)	218 (55)	151 (41)
18-month OS (%)	157 (40)	98 (27)

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

[b] Based on the stratified log-rank test

CI = Confidence Interval

CLINICAL PERFORMANCE – NSCLC ≥ 1% TC OR ≥ 1% IC

The performance of VENTANA PD-L1 (SP142) Assay and efficacy of TECENTRIQ (atezolizumab) were investigated in IMpower150 (NCT02366143), a multicenter, international, randomized, open-label trial in chemotherapy-naïve patients with metastatic non-squamous NSCLC. The objectives of IMpower150 were to determine whether the addition of TECENTRIQ (atezolizumab) to platinum-based chemotherapy [carboplatin plus paclitaxel (CP)] with or without bevacizumab in chemotherapy-naïve patients with Stage IV non-squamous NSCLC results in statistically significant and clinically meaningful improvements in PFS and OS.

A total of 1202 patients were enrolled and were stratified by sex, presence of liver metastases, and PD-L1 expression status on TC and IC. Patients were randomized (1:1:1) to one of the following three treatment arms.

- TECENTRIQ (atezolizumab) 1200 mg with paclitaxel and carboplatin every 3 weeks for four or six cycles, followed by TECENTRIQ (atezolizumab) 1200mg every 3 weeks until loss of clinical benefit (Arm A)
- TECENTRIQ (atezolizumab) 1200 mg with bevacizumab, paclitaxel, and carboplatin every 3 weeks for four or six cycles, followed by TECENTRIQ (atezolizumab) 1200mg until loss of clinical benefit and bevacizumab every 3 weeks until disease progression or unacceptable toxicity (Arm B)
- Bevacizumab with paclitaxel and carboplatin every 3 weeks for four or six cycles, followed by bevacizumab every 3 weeks until disease progression or unacceptable toxicity (Arm C)

The demographics and baseline disease characteristics of the study population were well balanced between the treatment arms. The median age was 63 years (range: 31 to 90), and 60% of patients were male. The majority of patients were white (82%). Approximately 10% of patients had known EGFR mutation, 4% had known ALK rearrangements, 14% had liver metastasis at baseline, and most patients were current or previous smokers (80%). Baseline ECOG performance status was 0 (43%) or 1 (57%). 51% of patients' tumors had PD-L1 expression of ≥ 1% TC or ≥ 1% IC, and 49% of patients' tumors had PD-L1 expression of < 1% TC and < 1% IC.

At the time of the final analysis for PFS, patients had a median follow up time of 15.3 months. The intent-to-treat (ITT) population, including patients with EGFR mutations or ALK rearrangements who should have been previously treated with tyrosine kinase inhibitors, demonstrated clinically meaningful PFS improvement in Arm B as compared to Arm C (HR of 0.61, 95% CI: 0.52, 0.72; median PFS 8.3 vs. 6.8 months).

At the time of the interim OS analysis, patients had a median follow-up of 19.7 months. The key results from this analysis as well as from the updated PFS analysis in the ITT population are summarized in Table 28. Clinically meaningful improvements in OS were observed in the ITT population, with a median OS of 19.8 months in Arm B (n = 400) but 14.9 months in the Arm C (n = 400; stratified HR = 0.76; 95% CI: 0.63, 0.93).

Figure 2 summarizes the results of OS for Arm B compared to Arm C in the ITT and PD-L1 subgroups. PFS results for Arm B compared to Arm C are also presented in Figure 3.

The data indicate that all chemotherapy-naïve patients with locally advanced or metastatic non-squamous NSCLC, regardless of PD-L1 expression, may benefit from treatment with TECENTRIQ (atezolizumab) in combination with chemotherapy with bevacizumab and therefore are eligible for treatment. They also suggest that the benefits of TECENTRIQ (atezolizumab) in combination with chemotherapy with bevacizumab in this population may be greater in patients with tumor PD-L1 expression in ≥ 1% TC or ≥ 1% IC as determined by the VENTANA PD-L1 (SP142) Assay.

The OS benefit observed for Arm B compared to Arm C in the ITT population was maintained at the next analysis (CCOD Sep 2019) (Table 29) but were not formally tested per the pre-specified analytical hierarchy. The differences in median OS for Arm B compared to Arm C in the ITT population at the Sep 2019 CCOD was 4.8 months, with stratified HR of 0.80 (95% CI: 0.68, 0.95).

The OS benefit observed for Arm B compared to Arm C at the next analysis (CCOD Sep 2019), was greater in the ≥ 1% TC or ≥ 1% IC PD-L1 subgroup than the <1% TC or <1% IC PD-L1 subgroup (Table 29). In the former subgroup, median survival had increased by 8.0 months in Arm B compared to Arm C, with an unstratified HR of 0.692 (95% CI: 0.547, 0.876). In contrast in the <1% TC or <1% IC PD-L1 subgroup, median survival had increased by only 2.6 months, with an unstratified HR of 0.95 (95% CI: 0.76, 1.19).

TECENTRIQ (atezolizumab) in combination with chemotherapy with or without bevacizumab continued to be well-tolerated and safety findings were consistent with the known risks of each study treatment. No new safety signals were detected.

Collectively, these data demonstrate that PD-L1 expression in TC or IC as detected by VENTANA PD-L1 (SP142) Assay in NSCLC may be associated with enhanced patient benefit with TECENTRIQ (atezolizumab) , in combination with chemotherapy with bevacizumab, at the PD-L1 expression ≥ 1% TC or ≥ 1% IC cutoff.

Table 28. Efficacy results for the intent-to-treat population in IMpower150.

Efficacy Endpoint	Arm A (Atezolizumab + Paclitaxel + Carboplatin)	Arm B (Atezolizumab + Bevacizumab + Paclitaxel + Carboplatin)	Arm C (Bevacizumab + Paclitaxel + Carboplatin)
Secondary Endpoints[a]			
Investigator-assessed PFS (RECIST v1.1)[g]	n = 402	n = 400	n = 400
No. of events (%)	330 (82.1)	291 (72.8)	355 (88.8)
Median duration of PFS (months)	6.7	8.4	6.8
95% CI	(5.7, 6.9)	(8.0, 9.9)	(6.0, 7.0)
Stratified hazard ratio[e,f] (95% CI) p-value[b,c]	0.91 (0.78, 1.06) 0.2194	0.59 (0.50, 0.69) < 0.0001	---
12-month PFS (%)	24	38	20
OS interim analysis[g]	n = 402	n = 400	n = 400
No. of deaths (%) Median time to events (months) 95% CI	206 (51.2) 19.5 (16.3, 21.3)	192 (48.0) 19.8 (17.4, 24.2)	230 (57.5) 14.9 (13.4, 17.1)
Stratified hazard ratio[e,f] (95% CI) p-value[b,c]	0.85 (0.71, 1.03) 0.0983	0.76 (0.63, 0.93) 0.006	---
6-month OS (%)	84	85	81
12-month OS (%)	66	68	61
Investigator-assessed Overall Best Response[d,g] (RECIST v1.1)	n = 401	n = 397	n = 393
No. of responders (%)	163 (40.6)	224 (56.4)	158 (40.2)
95% CI	(35.8, 45.6)	(51.4, 61.4)	(35.3, 45.2)
No. of complete response (%)	8 (2.0)	11 (2.8)	3 (0.8)
No. of partial response (%)	155 (38.7)	213 (53.7)	155 (39.4)
Investigator-assessed DOR[g] (RECIST v1.1)	n = 163	n = 224	n = 158
Median in months	8.3	11.5	6.0
95% CI	(7.1, 11.8)	(8.9, 15.7)	(5.5, 6.9)

[a] Primary efficacy endpoints were PFS and OS and they were analyzed in the ITT-wild-type (WT) population, i.e. excluding patients with EGFR mutations or ALK rearrangements.

[b] Based on the stratified log-rank test

[c] For informational purposes; in the ITT population, comparisons between Arm B and Arm C as well as between Arm A and Arm C were not formally tested yet as per the pre-specified analysis hierarchy

[d] Overall best response for complete response and partial response

[e] Stratified by sex, presence of liver metastases and PD-L1 tumor expression on TC and IC

[f] The Arm C is the comparison group for all hazard ratios

[g] Updated PFS analysis and interim OS analysis at clinical cut-off 22 January 2018

PFS = progression-free survival; RECIST = Response Evaluation Criteria in Solid Tumors v1.1.

CI = confidence interval; DOR = duration of response; OS = overall survival.

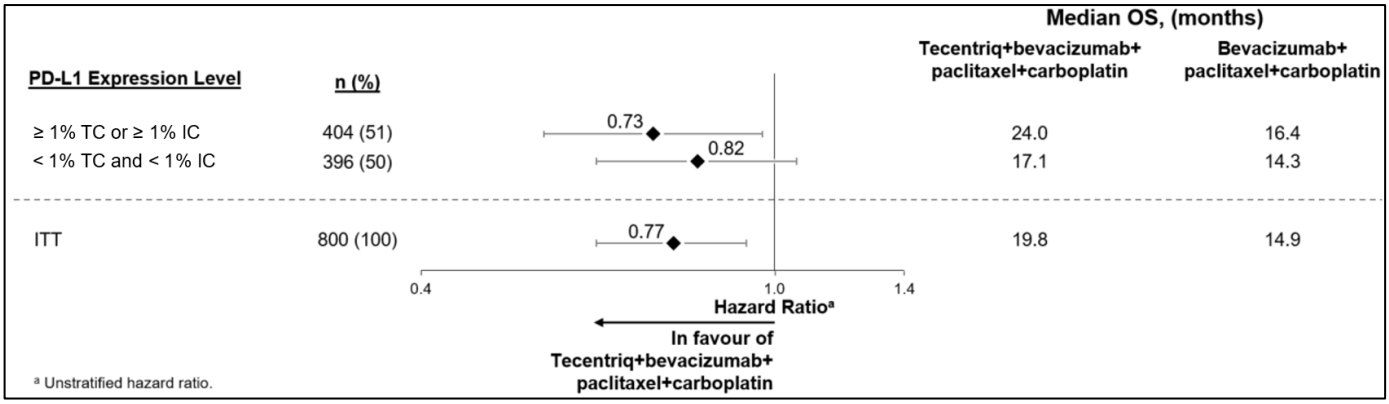

Figure 2. Forest plot of overall survival in IMpower150, Arm B vs C. (CCOD Jan 2018)

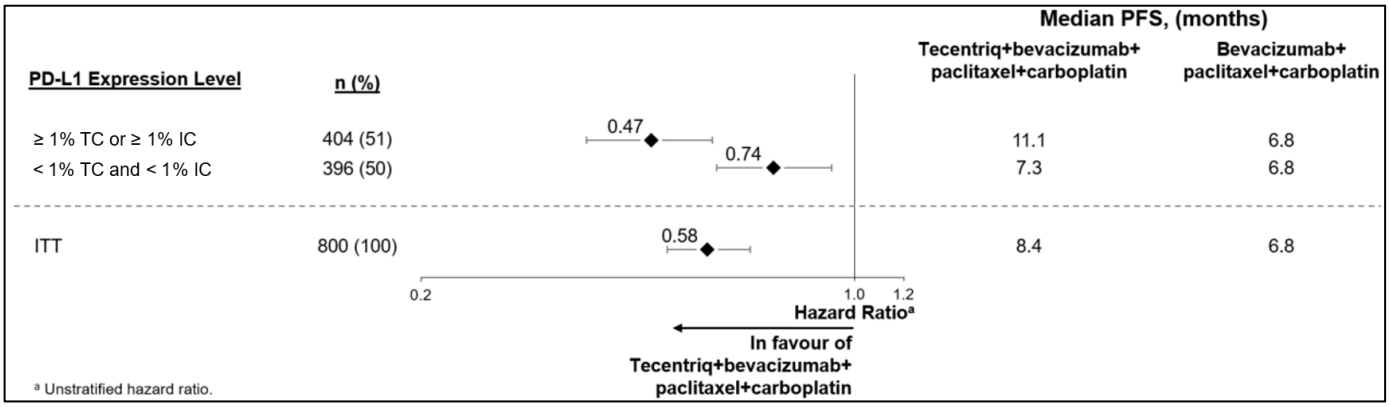

Figure 3. Forest plot of progression free survival in IMpower150, Arm B vs C. (CCOD Jan 2018)

Table 29. Overall Survival in the ITT Population and PD-L1 Subgroups (CCOD Sep 2019).

Overall Survival (OS) - CCOD Sep 2019	Arm A (Atezolizumab + Paclitaxel + Carboplatin)	Arm B (Atezolizumab + Bevacizumab + Paclitaxel + Carboplatin)	Arm C (Bevacizumab + Paclitaxel + Carboplatin)
ITT Population[a]	**n = 402**	**n = 400**	**n = 400**
Deaths, n (%)	290 (72.1)	284 (71.0)	309 (77.3)
Median duration of OS, months (95% CI)[b]	19.0 (16.3, 21.5)	19.8 (18.2, 22.5)	15.0 (13.4, 17.1)
Stratified hazard ratio (95% CI)[c,d]	0.86 (0.73, 1.01)	0.80 (0.68, 0.95)	---
Stratified log-rank p–value[f]	0.0681	0.0081	---
Unstratified hazard ratio (95% CI)[c]	0.84 (0.72, 0.99)	0.81 (0.69, 0.95)	---
Unstratified log-rank p–value[e]	0.0366	0.0084	---
6-month OS, event-free rate (%)	83.5	84.8	80.8
1-year OS, event-free rate (%)	65.7	68.0	60.1
2-year OS, event-free rate (%)	41.7	43.4	33.5
≥ 1% TC or ≥ 1% IC ITT Subgroup	**n = 213**	**n = 209**	**n = 195**
Deaths, n (%)	139 (65.3)	131 (62.7)	149 (76.4)
Median duration of OS, months[b]	24.1	24.0	16.0
Stratified hazard ratio (95% CI)[c,d]	0.729 (0.578, 0.92)	0.697 (0.55, 0.882)	---
Stratified log-rank p–value[e]	0.0074	0.0026	---
Unstratified hazard ratio (Wald 95% CI)[c]	0.72 (0.57, 0.91)	0.692 (0.547, 0.876)	---
Unstratified log-rank p–value[e]	0.0058	0.0021	---
6-month OS, event-free rate (%)	86.3	86.3	81.5
1-year OS, event-free rate (%)	70.6	71.2	55.9
2-year OS, event-free rate (%)	50.3	50.5	37.1
<1% TC or <1% IC ITT Subgroup	**n = 188**	**n = 191**	**n = 205**
Deaths, n (%)	150 (79.8)	153 (80.1)	160 (78.0)
Median duration of OS, months (95% CI)[b]	15.3 (13.1, 18.7)	17.0 (14.1, 19.8)	14.4 (13.4, 17.1)
Stratified hazard ratio (95% CI)[c,d]	1.00 (0.80, 1.25)	0.94 (0.75, 1.17)	---
Stratified log-rank p–value[e]	0.9849	0.5670	---
Unstratified hazard ratio (95% CI)[c]	1.00 (0.80, 1.26)	0.95 (0.76, 1.19)	---
Unstratified log-rank p–value[e]	0.9739	0.6628	---
6-month OS, event-free rate (%)	80.2	83.2	80.2
1-year OS, event-free rate (%)	60.4	64.6	64.1
2-year OS, event-free rate (%)	32.2	35.8	30.0

[a] The Intent-to-Treat (ITT) population includes all patients randomized to study GO29436 (whether or not they received study treatment).

[b] Time-to-event results (median, percentiles) are Kaplan-Meier estimates. The 95% CI for the median was computed using the method of Brookmeyer and Crowley.

[c] The Arm C is the comparison group for all hazard ratios

[d] Stratified by sex, presence of liver metastases and PD-L1 tumor expression on TC and IC.

[e] Results shown for informational purposes only.

CCOD = clinical data cutoff date; ITT = Intent-to-treat

ANALYTICAL PERFORMANCE – TNBC

Scoring Algorithm – TNBC

TNBC tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC provided in Table 30. Refer to the interpretation guide (P/N 1018231) for additional instructions and representative images.

Table 30. VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC.

Immune Cell (IC) Staining Assessment[a]	PD-L1 Expression
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 1% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 1% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 1% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 1% IC

[a] PD-L1 staining in tumor cells should not be included in the scoring determination of TNBC patient tissue.

Tissue Thickness – TNBC

Tissue thickness was evaluated using 10 unique TNBC specimens (5 PD-L1 ≥ 1% IC and 5 PD-L1 < 1% IC). Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 status within the range of thickness tested. The specimens should be cut at 4 µm for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision - TNBC

Studies for VENTANA PD-L1 Assay staining of TNBC specimens were completed to demonstrate:

- Intra-day repeatability – 5 replicate slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within one day.
- Inter-day precision – 10 slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days.
- Inter-instrument and inter-lot precision – 27 slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments.
- Intra-platform precision – 2 replicate slides each from 10 TNBC specimen (5 PD-L1 ≥ 1% IC and 5 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay across three BenchMark ULTRA and three BenchMark GX instruments. Agreement rates were calculated relative to the specimen mode for each platform.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC (Table 30). Results are summarized in Table 31.

Table 31. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Repeatability / Intermediate Precision Parameter	Agreement % (95% CI)
Intra-day repeatability (within a single day)	PPA: 100.0 (94.0-100.0) NPA: 95.0 (87.2-100.0) OPA: 97.5 (93.3-100.0)
Inter-day precision (5 non-consecutive days)	PPA: 100.0 (96.9-100.0) NPA: 96.7 (92.7-100.0) OPA: 98.3 (96.3-100.0)
Inter-instrument and Inter-lot precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	PPA: 98.3 (96.0-100.0) NPA: 99.2 (97.2-100.0) OPA: 98.6 (97.1-99.8)
Intra-platform precision (three BenchMark ULTRA instruments)	PPA: 100.0 (88.6-100.0) NPA: 96.7 (88.9-100.0) OPA: 98.3 (95.0-100.0)
Intra-platform precision (three BenchMark GX instruments)	PPA: 93.3 (83.3-100.0) NPA: 96.7 (88.9-100.0) OPA: 95.0 (90.0-100.0)

Inter-Platform Concordance – TNBC

Single slides each from 44 unique TNBC specimens (22 PD-L1 ≥ 1% IC and 22 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on one BenchMark ULTRA (reference) and one BenchMark GX instrument. All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC (Table 30). Results are summarized in Table 32.

Table 32. Inter-platform concordance of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Inter-platform Concordance	Agreement % (95% CI)[a]
BenchMark ULTRA: BenchMark GX	PPA: 100.0 (86.2-100.0) NPA: 85.0 (64.0-94.8) OPA: 93.2 (81.8-97.7)

[a] Two-sided Wilson score method CI

Reader Precision – TNBC

To assess inter- and intra-reader precision, three pathologists evaluated 60 unique TNBC specimens (30 PD-L1 ≥ 1% IC and 30 PD-L1 < 1% IC) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC (Table 30). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and between each pathologist's reads are summarized Table 33.

Table 33. Inter- and intra-reader precision of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 91.1 (84.2-96.6) ANA: 91.1 (84.1-96.7) OPA: 91.1 (85.6-96.7)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.8 (89.5-97.1) ANA: 93.9 (89.2-97.3) OPA: 93.9 (89.9-97.2)

Inter-Laboratory Reproducibility Study – TNBC

An inter-laboratory reproducibility study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in TNBC specimens. Twenty-eight unique TNBC specimens (14 PD-L1 ≥ 1% IC and 14 PD-L1 < 1% IC) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The final staining acceptability rate for the VENTANA PD-L1 (SP142) Assay was 98.6% in this study. Results are summarized in Table 34.

Table 34. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Inter-laboratory Reproducibility	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 93.2 (90.4-95.2)[a] NPA: 96.6 (94.4-98.0)[a] OPA: 94.8 (93.1-96.1)[a]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 91.5 (84.0-96.6) ANA: 91.3 (83.6-96.4) OPA: 91.4 (90.7-92.0)[a]
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 93.6 (88.2-97.0) ANA: 93.3 (87.8-96.7) OPA: 93.4 (90.6-95.4)[a]

[a] Two-sided Wilson score method CI

CLINICAL PERFORMANCE – TNBC

The performance of VENTANA PD-L1 (SP142) Assay was investigated in IMpassion130 (NCT02425891), a phase III, double-blind, two-arm, multicenter, international, randomized, placebo-controlled trial conducted to evaluate the efficacy and safety of TECENTRIQ (atezolizumab) in combination with nab-paclitaxel in patients with unresectable locally advanced or metastatic TNBC that had not received prior chemotherapy for metastatic disease.

IMpassion130 enrolled 902 patients; eligible patients were stratified by presence of liver metastases, prior taxane treatment, and by PD-L1 expression status in IC (IC < 1% of tumor area vs ≥ 1% of the tumor area) assessed by the VENTANA PD-L1 (SP142) Assay. Patients were randomized to receive TECENTRIQ (atezolizumab) (840 mg) or placebo by intravenous infusions on days 1 and 15 of every 28-day cycle, plus nab-paclitaxel (100 mg/m^2) administered via intravenous infusion on days 1, 8 and 15 of every 28-day cycle. Patients received treatment until radiographic disease progression per RECIST v1.1, or unacceptable toxicity.

The demographic and baseline disease characteristics of the study population were well balanced between the treatment arms. Most patients were women (99.6%), 67.5% were white and 17.8% Asian. The median age was 55 years (range: 20-86). Baseline ECOG performance status was 0 (58.4%) or 1 (41.3%). Overall, 41% of enrolled patients had PD-L1 expression ≥ 1% IC, 27% had liver metastases and 7% asymptomatic brain metastases at baseline. Approximately half the patients had received a taxane (51%) or anthracycline (54%) in the (neo)adjuvant setting. Patient demographics and baseline tumor disease in patients with PD-L1 expression ≥ 1% IC were generally representative of the broader study population.

The co-primary efficacy endpoints included investigator-assessed progression free survival (PFS) in the ITT population and in patients with PD-L1 expression ≥ 1% IC per RECIST v1.1 as well as overall survival (OS) in the ITT population and in patients with PD-L1 expression ≥ 1% IC. Secondary efficacy endpoints included objective response rate (ORR) and duration of response (DOR) per RECIST v1.1.

PFS, ORR and DOR results of IMpassion130 for patients with PD-L1 expression ≥ 1% IC at the time of the final analysis for PFS with a median survival follow up of 13 months are summarized in Table 35. Patients with PD-L1 expression < 1% IC did not show improved PFS when TECENTRIQ (atezolizumab) was added to nab-paclitaxel (HR of 0.94, 95% CI 0.78, 1.13).

An updated OS analysis was performed with a median follow up of 18 months, OS results are presented in Table 35. Patients with PD-L1 expression < 1% IC did not show improved OS when TECENTRIQ (atezolizumab) was added to nab-paclitaxel (HR of 0.97, 95% CI 0.78, 1.20). At the time of the updated OS analysis, an exploratory PFS analysis was performed as presented in Table 35.

Table 35. Efficacy results from IMpassion130 in patients with PD-L1 expression ≥ 1% IC.

Key efficacy endpoints	Atezolizumab + nab-paclitaxel	Placebo + nab-paclitaxel
Primary efficacy endpoints	n = 185	n = 184
Investigator-assessed PFS (RECIST v1.1)[c]		
No. of events (%)	138 (74.6)	157 (85.3)
Median duration of PFS (months)	7.5	5.0
95% CI	(6.7, 9.2)	(3.8, 5.6)
Stratified hazard ratio[e] (95% CI)	0.62 (0.49, 0.78)	
p-value[a]	< 0.0001	
12-month PFS (%)	29.1	16.4
Investigator-assessed PFS (RECIST v1.1) – Updated exploratory analysis[d]		
No. of events (%)	149 (80.5%)	163 (88.6%)
Median duration of PFS (months)	7.5	5.3
95% CI	(6.7, 9.2)	(3.8, 5.6)
Stratified hazard ratio[e] (95% CI)	0.63 (0.50-0.80)	
p-value[a]	< 0.0001	
12-month PFS (%)	30.3	17.3
OS[a,b,d]		
No. of deaths (%)	94 (50.8)	110 (59.8)
Median time to events (months)	25.0	18.0
95% CI	(19.55, 30.65)	(13.63, 20.07)
Stratified hazard ratio[e] (95% CI)	0.71 (0.54, 0.93)	
Secondary and exploratory endpoints		
Investigator-assessed ORR (RECIST v1.1)[c]	n = 185	n = 183
No. of responders (%)	109 (58.9)	78 (42.6)
95% CI	(51.5, 66.1)	(35.4, 50.1)
No. of complete response (%)	19 (10.3)	2 (1.1%)
No. of partial response (%)	90 (48.6)	76 (41.5)
No. of stable disease	38 (20.5)	49 (26.8)
Investigator-assessed DOR[c]	n = 109	n = 78
Median in months	8.5	5.5

Key efficacy endpoints	Atezolizumab + nab-paclitaxel	Placebo + nab-paclitaxel
95% CI	(7.3, 9.7)	(3.7, 7.1)

[a] Based on the stratified log-rank test.

[b] OS comparisons between treatment arms in patients with PD-L1 expression ≥ 1% were not formally tested, as per the pre-specified analysis hierarchy.

[c] Per final analysis for PFS, ORR, DOR and first interim analysis for OS at clinical cut off 17th April 2018

[d] Per second interim analysis for OS and exploratory PFS analysis at clinical cut off January 2nd 2019

[e] Stratified by presence of liver metastases, and by prior taxane treatment.

PFS = progression-free survival; RECIST = Response Evaluation Criteria in Solid Tumors v1.1.; CI = confidence interval; ORR = objective response rate; DOR = duration of response; OS = overall survival, NE = not estimable

PERFORMANCE OF VENTANA PD-L1 (SP142) ASSAY ON THE BENCHMARK ULTRA PLUS INSTRUMENT

Concordance Between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments for Urothelial Carcinoma, NSCLC, and TNBC

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. There were 135 unique Urothelial Carcinoma, NSCLC, and TNBC specimens (45 specimens per indication) which represented the staining range of the VENTANA PD-L1 (SP142) Assay, with approximately equal distribution between PD-L1-positive and PD-L1-negative specimens for each indication. Tissue slides from all specimens were stained with H&E as well as VENTANA PD-L1 (SP142) Assay on a BenchMark ULTRA instrument using the recommended staining protocol. Unstained tissue slides from all specimens were randomized and equally distributed (45 specimens per site such that each site received a representative sample of study specimens) for staining on a BenchMark ULTRA PLUS instrument using the recommended VENTANA PD-L1 (SP142) staining protocol. Two pathologists per site, blinded to specimen status, evaluated the slides stained on the corresponding BenchMark ULTRA PLUS instrument and determined the PD-L1 status. After a two week washout period, corresponding specimen slides previously stained at Roche on the BenchMark ULTRA instrument were distributed to the appropriate sites for clinical evaluation. Additionally, one pathologist reviewed all study slides and was included as a third pathologist for each of the sites. The results were analyzed by Roche. The OPA, PPA and NPA rates were 91.6% (359/392), 90.3% (176/195), and 92.9% (183/197), respectively. The results are summarized in Table 36.

Table 36. Pooled Agreement of PD-L1 status for Urothelial Carcinoma, NSCLC, and TNBC Specimens Stained with VENTANA PD-L1 (SP142) Assay on the BenchMark ULTRA PLUS versus BenchMark ULTRA instrument.

BenchMark ULTRA PLUS PD-L1 Status	BenchMark ULTRA PD-L1 Status		Total
	Positive	Negative	
Positive	176	14	190
Negative	19	183	202
Total	195	197	392
	n/N	% (95% CI)	
PPA	176/195	90.3 (85.2, 94.7)	
NPA	183/197	92.9 (89.0, 96.2)	
OPA	359/392	91.6 (88.4, 94.6)	

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates selected with stratification by PD-L1 clinical qualification status (positive, negative, borderline) and indication.

BenchMark ULTRA PLUS Instrument Inter-laboratory Reproducibility Study with Urothelial Carcinoma, NSCLC, and TNBC

An inter-laboratory reproducibility (ILR) study was conducted to evaluate the reproducibility of VENTANA PD-L1 (SP142) Assay to determine PD-L1 status in Urothelial Carcinoma, NSCLC, and TNBC tissue stained on the BenchMark ULTRA PLUS instrument in combination with the OptiView DAB IHC Detection Kit and OptiView Amplification Kit.

Forty-two unique Urothelial Carcinoma, NSCLC, and TNBC specimens (14 specimens per indication), which represented the staining range of the VENTANA PD-L1 (SP142) Assay, were used with an approximate equal distribution between PD-L1-positive and PD-L1-negative specimens for each indication.

Multiple tissue sections were cut from each specimen and provided to 3 external study sites. All 42 specimens were stained on a BenchMark ULTRA PLUS instrument on each of 5 non-consecutive days over a minimum of 20 days at each site. The specimens were randomized and evaluated by a total of 6 pathologists (2 readers/site).

Results are summarized in Table 37 and Table 38. The data was analyzed for PPA, NPA and OPA in Table 37 and APA, ANA, and OPA in Table 38 across all observations. For each specimen, all evaluable observations (positive vs negative) were compared against the modal result for each specimen. These comparisons were pooled across sites and days, and then results were aggregated across specimens.

Table 37. ILR: Agreement Rates with Modal Status on the BenchMark ULTRA PLUS instrument for Urothelial Carcinoma, NSCLC, and TNBC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	571/629	90.8	(85.8, 95.7)
	NPA	595/630	94.4	(90.7, 98.8)
	OPA	1166/1259	92.6	(89.8, 95.3)
Within-Site (3 sites)	PPA	586/629	93.2	(89.4, 96.6)
	NPA	610/630	96.8	(95.3, 98.5)
	OPA	1196/1259	95.0	(93.2, 96.6)
Within-Reader	PPA	589/614	95.9	(94.3, 97.4)
	NPA	628/645	97.4	(96.1, 98.7)
	OPA	1217/1259	96.7	(95.5, 97.7)

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates stratified by both indications (Urothelial Carcinoma, NSCLC, TNBC) and screening bins (positive, negative, borderline).

Table 38. ILR: Pairwise Agreement Rates on the BenchMark ULTRA PLUS instrument for Urothelial Carcinoma, NSCLC, and TNBC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site (3 sites)	APA	10640/12108	87.9	(82.5, 92.4)
	ANA	11584/13052	88.8	(85.4, 92.2)
	OPA	11112/12580	88.3	(84.1, 92.2)
Between-Reader	APA	550/605	90.9	(86.9, 94.1)
	ANA	598/653	91.6	(89.1, 93.9)
	OPA	574/629	91.3	(88.2, 94.0)
Between-Day (5 non-consecutive days)	APA	2280/2420	94.2	(92.0, 96.0)
	ANA	2472/2612	94.6	(92.9, 96.1)
	OPA	2376/2516	94.4	(92.6, 96.0)

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates stratified by both indications (Urothelial Carcinoma, NSCLC, TNBC) and screening bins (positive, negative, borderline).

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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.

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

REVISION HISTORY

Rev	Updates
D	Corrections to the Performance of VENTANA PD-L1 (SP142) Assay On The BenchMark ULTRA PLUS Instrument section. Removal of BenchMark XT Instrument. Updated to current template.

INTELLECTUAL PROPERTY

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