VENTANA PD-L1 (SP142) Assay

REF 741-4860

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IVD Σ 50

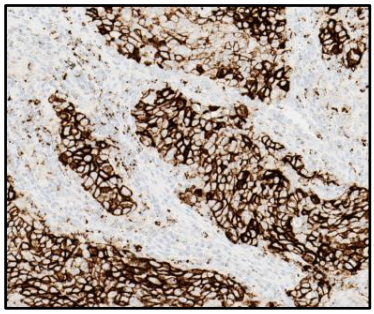

Figure 1. PD-L1 expression in non-small cell lung cancer.

INTENDED USE

VENTANA PD-L1 (SP142) Assay is intended for laboratory use in the qualitative immunohistochemical assessment of the programmed death-ligand 1 (PD-L1) protein 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.

VENTANA PD-L1 (SP142) Assay is indicated as an aid for identifying patients for treatment with the therapy listed in Table 1 for the respective indication and cutoffs in accordance with the approved therapeutic product labeling.

Table 1. VENTANA PD-L1 (SP142) Assay companion diagnostic indication.

Indication for use	Therapy	Cutoff
Non-Small Cell Lung Cancer (NSCLC)	TECENTRIQ	≥ 50% TC or ≥ 10% IC

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] Ligation of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T cells.[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 (IC) and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported across multiple cancers.[3-6]

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors.[3-6] Atezolizumab is a non-glycosylated IgG1 kappa immunoglobulin that has a calculated molecular mass of 145 kDa.

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP142) Assay utilizes a rabbit monoclonal primary antibody that binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be visualized using 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 OptiView DAB IHC Detection Kit and OptiView Amplification Kit 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 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative.

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

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

Refer to VENTANA PD-L1 (SP142) Assay Interpretation Guide for Non-Small Cell Lung Cancer (NSCLC) ≥ 50% TC or ≥ 10% IC Stepwise Scoring Algorithm (P/N 1015703) for detailed instructions for interpretation of VENTANA PD-L1 (SP142) Assay staining.

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. 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. 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. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. 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).[7,8]

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 2 months after cutting from the paraffin block of NSCLC and tonsil specimens (see the interpretation guides and the Performance Characteristics section below).

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.[9,10]
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 dialog.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. Refer to Table 2 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 instruments User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

Table 2. Recommended staining protocol and required staining procedures for VENTANA PD-L1 (SP142) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on BenchMark IHC/ISH instruments.

Staining Procedure:	**ULTRA VENTANA PDL1 (SP142) XT VENTANA PDL1 (SP142) GX VENTANA PDL1 (SP142)**
Protocol Step	**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 3 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[11] or the CLSI Approved Guideline[12]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. NSCLC tissues with known PD-L1 status, and 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 slides 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 3 (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 3. 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.

GENERAL LIMITATIONS

1. IHC is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and system-level controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. VENTANA antibodies and reagents are provided at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[13,14]
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[15]
9. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.[16]
10. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP142) Assay has been solely approved on 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. A patient specimen slide should be stained with Rabbit Monoclonal Negative Control Ig. Other negative control reagents are not suitable for this assay.
3. This assay has not been validated for use with cytology samples or decalcified bone specimens.
4. Patient tissue should be stained within 2 months of sectioning from the tissue block for NSCLC and tonsil 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.
5. It is recommended that samples be fixed between 6 and 72 hours in 10% NBF. Use of fixation times or fixative types other than those recommended can lead to false negative results. Fixatives such as AFA, PREFER fixative, and other alcohol-containing fixatives have demonstrated a loss of specific PD-L1 protein staining. Refer to the interpretation guides for further discussion.
6. 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.
7. 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 4); however, nuclear staining is not included in scoring of VENTANA PD-L1 (SP142) Assay staining.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE - GENERAL

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

General Analysis Comments

Unless otherwise noted, the two-sided 95% Confidence Interval around estimates of agreement for all studies (excluding clinical efficacy studies) were calculated using the percentile bootstrap method from 2000 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% Confidence Interval. 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% Confidence Interval.

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 4 and Table 5.

Table 4. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing FFPE normal tissues.

Tissue	# positive[a] / total cases	Tissue	# positive[a] / total cases
Adrenal gland	1/3	Muscle, cardiac	0/3
Bladder [b]	0/3	Muscle, skeletal	0/2

Tissue	# positive[a] / total cases	Tissue	# positive[a] / total cases
Breast	1/3	Myeloid	0/2
Cerebellum [c]	0/3	Nerve, peripheral	0/3
Cerebrum	0/3	Ovary	0/3
Cervix	0/2	Pancreas [d]	0/3
Colon	2/3	Parathyroid gland	0/2
Endometrium	2/3	Prostate	0/3
Esophagus	0/3	Salivary gland	2/3
Pituitary gland [d]	0/3	Skin	0/3
Intestine, small	1/3	Spleen	3/3
Kidney	2/3	Stomach	0/3
Lingual gland	0/1	Testis [c]	0/3
Liver	0/3	Thymus gland	3/3
Lung	1/25	Thyroid gland	1/3
Lymph node	3/3	Tonsil [c]	3/3
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 5. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive[a] / total cases	
	Immune cells	Tumor cells
Mesothelioma (Abdomen)	1/1	0/1
Neurofibroma (Back)	1/1	0/1
Leiomyosarcoma (Bladder)	0/1	0/1
Urothelial carcinoma (Bladder)	1/1	0/1
Osteosarcoma (Bone)	0/1	0/1
Invasive ductal carcinoma (Breast)	1/2	0/2
Intraductal carcinoma with early infiltrate (Breast)	1/1	0/1
Glioblastoma (Cerebrum)	1/1	0/1
Meningioma (Cerebrum)	0/1	0/1
Ependymoma (Cerebrum)	0/1	0/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Adenocarcinoma (Colon)	1/1	0/1
Interstitialoma (Colon)	0/1	0/1
Neuroendocrine carcinoma (Esophagus)	0/1	0/1
Adenocarcinoma (Esophagus)	1/1	0/1
Adenocarcinoma (Intestine)	1/1	0/1
Stromal sarcoma (Intestine)	1/1	0/1
Clear cell carcinoma (Kidney)	1/1	0/1
Hepatocellular carcinoma (Liver)	0/1	0/1
Hepatoblastoma (Liver)	1/1	0/1
Adenocarcinoma (Lung)	0/1	0/1
Small cell undifferentiated carcinoma (Lung)	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	0/1
Diffuse B-cell lymphoma (Lymph node) [b]	1/1	1/1
Hodgkin lymphoma (Lymph node)	1/1	1/1
Diffuse B-cell lymphoma (Mediastinum) [b]	1/1	1/1
Leiomyosarcoma (Smooth muscle)	1/1	0/1
Embryonal rhabdomyosarcoma (Striated muscle)	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
Anaplastic large cell lymphoma (Pelvic cavity) [b]	1/1	1/1
Adenocarcinoma (Prostate)	0/2	0/2
Adenocarcinoma (Rectum)	1/1	1/1
Moderate malignant interstitialoma (Rectum)	0/1	0/1
Melanoma (Rectum)	1/1	0/1
Neuroblastoma (Retroperitoneum)	1/1	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1	0/1
Basal cell carcinoma (Skin)	1/1	0/1
Squamous cell carcinoma (Skin)	1/1	0/1
Diffuse B-cell lymphoma (Spleen) [a]	1/1	1/1
Signet-ring cell carcinoma (Stomach)	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
Squamous cell carcinoma (Cervix)	2/2	0/2
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	1/1	0/1

Pathology	# positive[a] / total cases	
	Immune cells	Tumor cells
Clear cell carcinoma of endometrium (Uterus)	1/1	1/1

[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 - NSCLC

Scoring Algorithm – NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC provided in Table 6. High PD-L1 expression is defined as having PD-L1 expression on ≥ 50% TC or ≥ 10% IC. Refer to the interpretation guide (P/N 1015703) for additional instructions and representative images.

Table 6. VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC.

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

Tissue Thickness – NSCLC

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

All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 TC or IC level within the range of thickness tested. The NSCLC specimens should be cut at 4 microns 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. Sample sets consisted of 24 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.
- 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. Sample sets consisted of 24 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level tested.
- Instrument, Antibody and Detection Lot Precision – a minimum of 9 slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments. Sample sets consisted of a minimum of 18 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.
- Intra-platform Precision – 2 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay across three BenchMark ULTRA, three BenchMark XT and three BenchMark GX instruments. Sample sets consisted of 10 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level tested. 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 7 and Table 8.

Table 7. 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)	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)	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)	PPA: 99.7 (98.1-99.9) NPA: 95.2 (91.2-97.5) OPA: 97.9 (96.2-98.9)
Intra-platform precision (3 BenchMark ULTRA instruments)	PPA: 100.0 (88.6-100.0) NPA: 100.0 (88.6-100.0) OPA: 100.0 (94.0-100.0)
Intra-platform precision (3 BenchMark XT instruments)	PPA: 96.7 (83.3-99.4) NPA: 100.0 (88.6-100.0) OPA: 98.3 (91.1-99.7)
Intra-platform precision (3 BenchMark GX instruments)	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 Confidence Interval (CI)

Table 8. 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)	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)	PPA: 96.2 (91.3-98.3) [a] NPA: 98.2 (93.6-99.5) [a] OPA: 97.1 (94.1-98.6) [a]

Repeatability/Intermediate Precision Parameter	Agreement % (95% CI)
Inter-antibody and Inter-detection agreement (pairwise-comparison)	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)	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)	APA: 96.3 (93.1-98.8) ANA: 92.6 (85.9-97.8) OPA: 95.1 (91.1-98.4)
Intra-platform precision (3 BenchMark ULTRA instruments)	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 (3 BenchMark XT instruments)	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 (3 BenchMark GX instruments)	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 Confidence Interval (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) were stained with VENTANA PD-L1 (SP142) Assay on one BenchMark ULTRA (reference), one BenchMark XT 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 6). Results are summarized in Table 9.

Table 9. 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 XT	PPA: 95.2 (77.3-99.2) NPA: 100.0 (85.7-100.0) OPA: 97.7 (88.2-99.6)
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 Confidence Interval (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 6). 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 10.

Table 10. 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)

CI = Confidence Interval

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 6). Results are summarized in Table 11.

Table 11. 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 Confidence Interval (CI)

CLINICAL PERFORMANCE - NSCLC

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 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).

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression. Patient specimens were FFPE NSCLC tissue from biopsies (66.0%), resections (15.7%), or of other type (18.3%); 72.4% were from primary tumors and 27.6% from metastatic tumors.

Table 12 describes the overall staining acceptability rate for VENTANA PD-L1 (SP142) Assay among all NSCLC subjects screened for the study. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 2909 subjects, specimens for 65 subjects failed the initial staining attempt. When staining was repeated, results for the 15 of the 65 subjects remained unacceptable (14 due to unacceptable negative reagent control and 1 due to unacceptable morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial (i.e., first-pass) and final

overall staining acceptability rates: 97.8% and 99.5%, respectively. The initial and final acceptability rates for background staining and morphology were greater than 99%.

Table 12. VENTANA PD-L1 (SP142) Assay NSCLC staining performance characteristics in IMpower110.

Attribute	Acceptability rate % (n/N) (95% CI) [a]	
	Initial [b]	Final [c]
Overall staining acceptability rate	97.8 (2844/2909) (97.2-98.2)	99.5 (2894/2909) (99.2-99.7)
Morphology	99.4 (2844/2860) (99.1-99.7)	100.0 (2894/2895) (99.8-100.0)
Background	100.0 (2844/2844) (99.9-100.0)	100.0 (2894/2894) (99.9-100.0)

[a] Two-sided Wilson score method Confidence Interval (CI)

[b] Initial staining attempt [c] Final staining attempt

The IMpower110 study was conducted to evaluate the efficacy and safety of 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 atezolizumab (Arm A) or chemotherapy (Arm B). 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.

Patients were excluded if they had a history of autoimmune disease; administration of a live, attenuated vaccine within 28 days prior to randomization, administration of systemic immunostimulatory agents within 4 weeks or systemic immunosuppressive medications within 2 weeks prior to randomization, active or untreated CNS metastases. Tumor assessments were conducted every 6 weeks for the first 48 weeks following Cycle 1, Day 1 and then every 9 weeks thereafter.

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) demonstrated statistically significant improvement in OS for the patients randomized to 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) . The median survival follow-up time in patients with high PD-L1 expression was 15.7 months. In an exploratory OS analysis with longer follow up (median: 31.3 months) for these patients, the median OS for the atezolizumab arm was unchanged relative to the primary OS interim analysis (20.2 months) and was 14.7 months for the chemotherapy arm (HR of 0.76, 95% CI: 0.54, 1.09).The key results at the interim analysis are summarized in Table 13. The Kaplan-Meier curves for OS and PFS in patients with high PD-L1 expression are presented in Figures 2 and 3.

Table 13. Summary of efficacy in patients with high PD-L1 expression ≥ 50% TC or ≥ 10% IC (IMpower110)

Efficacy endpoints	Arm A (Atezolizumab)	Arm B (Chemotherapy)
Primary endpoint		
OS analysis	n = 107	n = 98
No. of deaths (%)	44 (41.1%)	57 (58.2%)
Median time to events (months)	20.2	13.1
95% CI	(16.5, NE)	(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
Secondary endpoints		
Investigator-assessed PFS (RECIST v1.1)	n = 107	n = 98
No. of events (%)	67 (62.6%)	79 (80.6%)
Median duration of PFS (months)	8.1	5.0
95% CI	(6.8, 11.0)	(4.2, 5.7)
Stratified hazard ratio [a] (95% CI)	0.63 (0.45, 0.88)	
12-month PFS (%)	36.9	21.6
Investigator-assessed ORR (RECIST 1.1)	n = 107	n = 98
No. of responders (%)	41 (38.3%)	28 (28.6%)
95% CI	(29.1, 48.2)	(19.9, 38.6)
No. of complete response (%)	1 (0.9%)	1 (1.0%)
No. of partial response (%)	40 (37.4%)	27 (27.6%)
Investigator-assessed DOR (RECIST 1.1)	n = 41	n = 28
Median in months	NE	6.7
95% CI	(11.8, NE)	(5.5, 17.3)

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

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.

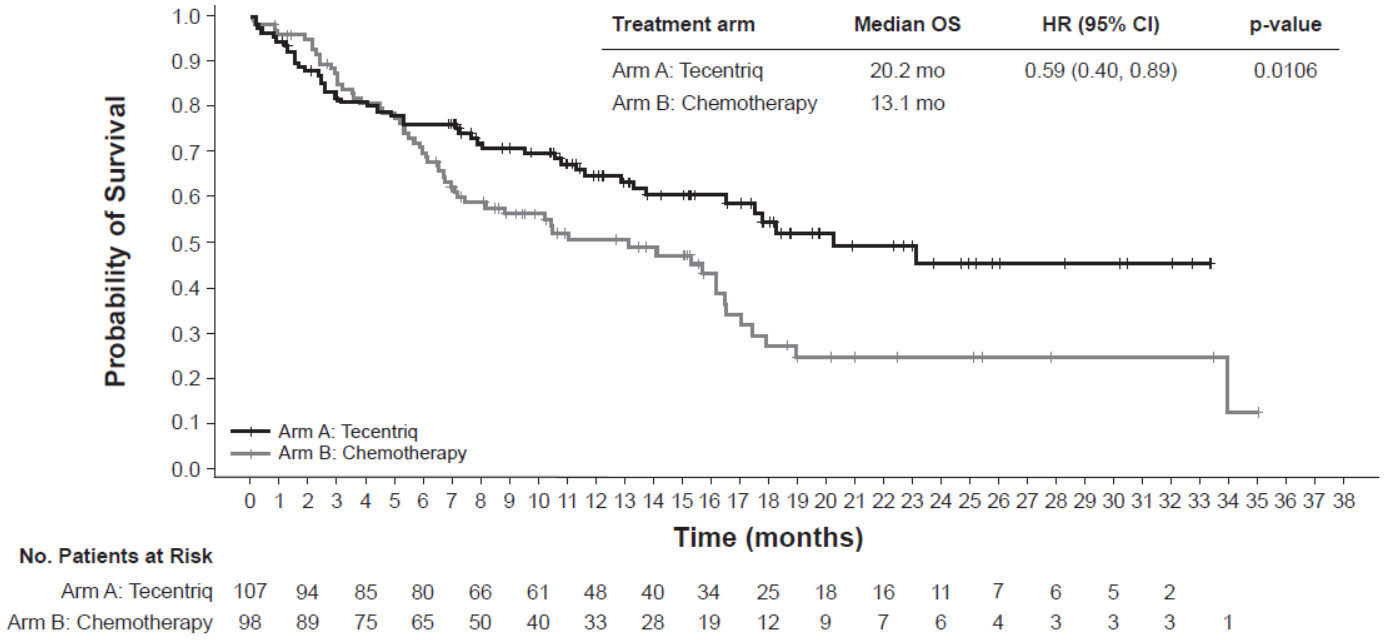

Figure 2: Kaplan-Meier curve for overall survival in patients with high PD-L1 expression ≥ 50% TC or ≥ 10% IC (IMpower110).

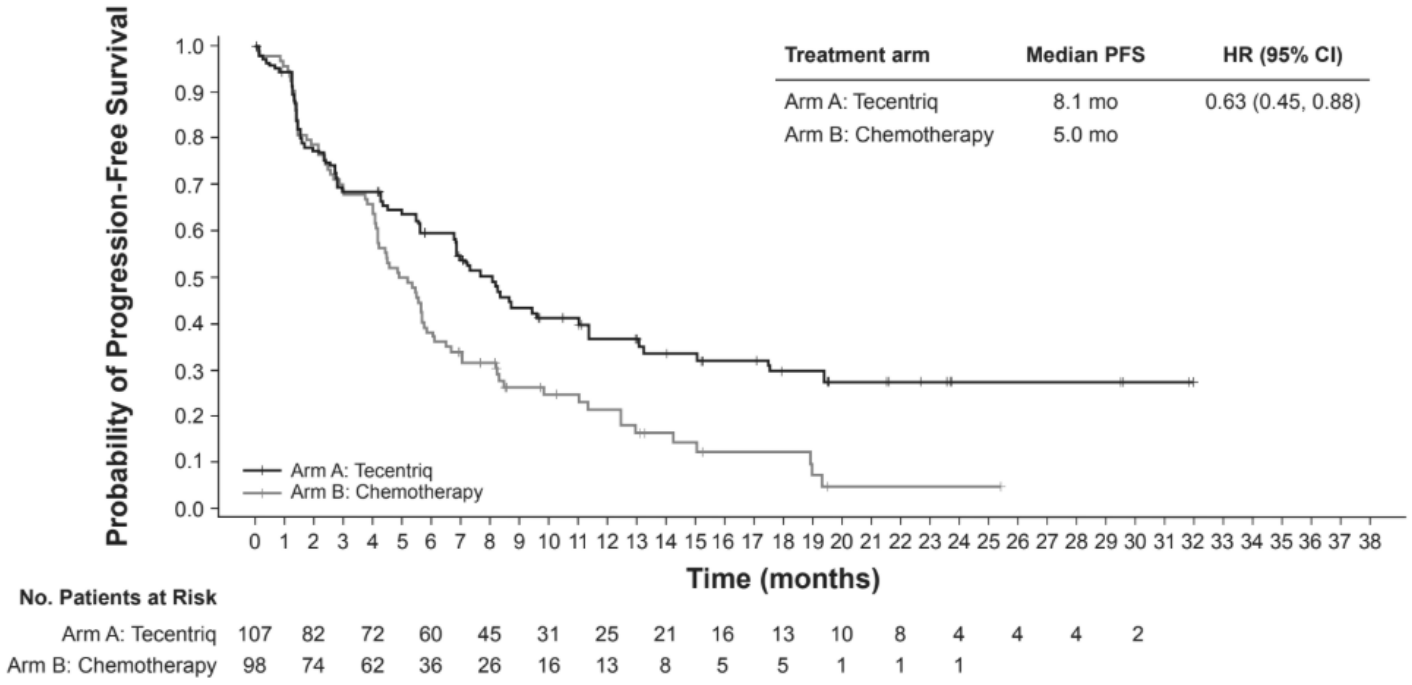

Figure 3: Kaplan-Meier curve for progression free survival in patients with high PD-L1 expression ≥ 50% TC or ≥ 10% IC (IMpower110).

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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 dialog.roche.com for definition of symbols used):

GTIN Global Trade Item Number

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