SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic immunohistochemistry (IHC) for detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) human tissue sections
Device Trade Name:	VENTANA PD-L1 (SP263) Assay
Device Procode:	PLS
Applicant's Name and Address:	Ventana Medical Systems, Inc. 1910 E Innovation Park Drive Tucson, AZ 85755
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P160046

Date of FDA Notice of Approval: May 1, 2017

II. INDICATIONS FOR USE

VENTANA PD-L1 (SP263) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP263 intended for use in the assessment of the PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma tissue stained with OptiView DAB IHC Detection Kit on a VENTANA BenchMark ULTRA instrument.

PD-L1 status is determined by the percentage of tumor cells with any membrane staining above background or by the percentage of tumor-associated immune cells with staining (IC+) at any intensity above background. The percent of tumor area occupied by any tumor-associated immune cells (Immune Cells Present, ICP) is used to determine IC+, which is the percent area of ICP exhibiting PD-L1 positive immune cell staining. PD-L1 status is considered High if any of the following are met:

- $\geq$ 25% of tumor cells exhibit membrane staining; or,
- ICP > 1% and IC+ $\geq$ 25%; or,
- ICP = 1% and IC+ = 100%.

PD-L1 High status as determined by VENTANA PD-L1 (SP263) Assay was associated with increased objective response rate (ORR) in a single arm study of IMFINZI™ (durvalumab).

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VENTANA PD-L1 (SP263) Assay product labeling.

V. DEVICE DESCRIPTION

VENTANA PD-L1 (SP263) Assay contains optimized reagents required to complete an immunohistochemical staining procedure for FFPE specimens on the BenchMark ULTRA automated staining instrument visualized using the OptiView DAB IHC Detection Kit. VENTANA PD-L1 (SP263) Assay includes a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant and contains sufficient reagent for 50 tests. The antibody and detection reagents are provided as ready-to-use dispensers, as seen below in

Table 1.

Table 1. Overview of the VENTANA PD-L1 (SP142) Assay Components

Device Components	Packaged form	Description
VENTANA anti-PD-L1 (SP263) Rabbit Monoclonal Primary Antibody	Dispenser: 50 test	One 5 mL dispenser of VENTANA PD-L1 (SP263) contains approx. 8.05 µg of a rabbit monoclonal antibody. The antibody is diluted in 0.05 M Tris-HCI with 1% carrier protein, and 0.10% ProClin 300, a preservative. Total protein concentration of the reagent is approx. 10 mg/mL. Specific antibody concentration is approx. 1.61 µg/mL.
OptiView DAB IHC Detection Kit	Set of 6 dispensers packaged in a kit: 250 tests	**OptiView Peroxidase Inhibitor** contains 3.0% hydrogen peroxide solution.
		OptiView HQ Universal Linker contains a cocktail of HQ-labeled (HQ is a proprietary hapten covalently attached to the goat antibodies) antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (<50 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		OptiView HRP Multimer contains a mouse monoclonal anti-HQ-labeled HRP tertiary antibody (<40 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		OptiView H2O2 contains 0.04% hydrogen peroxide in a phosphate buffer solution.

Table 1. Overview of the VENTANA PD-L1 (SP142) Assay Components		
Device Components	**Packaged form**	**Description**
		OptiView DAB contains 0.2% 3, 3'-diaminobenzidine tetrahydrochloride (DAB) in a proprietary stabilizer solution with a proprietary preservative.
		OptiView Copper contains copper sulfate (5.0 g/L) in an acetate buffer with a proprietary preservative.
BenchMark ULTRA (IHC/ISH) automated staining instrument and VSS system software	Instrument installed with the VSS host system software	A PC that runs on Microsoft Windows controls and monitors the BenchMark ULTRA instrument via the host operating software.
		The BenchMark ULTRA software has been developed per FDA's guidance on the development of Medical Device Software.
Rabbit Monoclonal Negative Control Ig	1 dispenser packaged as 250 test kit	Intended for laboratory use as a control for nonspecific binding of rabbit immunoglobulin (Ig) in sections of FFPE tissue. One 25 mL dispenser contains approximately 250 µg of a rabbit monoclonal antibody. The antibody is diluted in 0.08 M PBS with 3% carrier protein and 0.05% ProClin 300, a preservative.

Device Instrumentation and Software

The VENTANA PD-L1 (SP263) Assay is performed on the BenchMark ULTRA automated staining instrument using the VSS Software. The VENTANA PD-L1 (SP263) Assay protocol is assay specific. The software has been designed to recognize and group VENTANA PD-L1 (SP263) Assay, requiring that all system reagents are used together.

Specimen Preparation

Routinely processed FFPE tissues are suitable for use with this VENTANA PD-L1 (SP263) Assay. The recommended tissue fixation protocol is 10% neutral buffered formalin (NBF) for a period of at least 6 hours and for a maximum of 72 hours. Acceptable fixatives for use with VENTANA PD-L1 (SP263) are Zinc Formalin and Z-5 fixatives when used with at least 6 hours of fixation time. The amount used should be 15 to 20 times the volume of tissue. Fixation can be performed at room temperature (15°C - 25°C). Other fixatives, including 95% alcohol, AFA and PREFER, are not acceptable for use with the VENTANA PD-L1 (SP263) Assay.

The package insert instructs that sections approximately 4-5 µm thick should be cut and mounted on positively-charged slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and is compromised within 6 months after cutting from the paraffin block for urothelial carcinoma specimens (UC) and 9 months for placenta specimens. See the Package Insert for additional details.

Quality Control Procedures

Run controls are included in each staining run to establish the validity of the test results. The device labeling, instructs that the following controls are to be run with the assay.

A. **Placenta Tissue Control**

A tissue control must be included with each staining run. Qualified normal human term placental 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. Placental 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.

Placental tissue shows moderate to strong uniform staining of the membrane and weak to strong uniform staining of the cytoplasm of trophoblast-lineage cells. Placental stromal tissue and vasculature can be used for assessment of any background staining.

B. **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.

Principles of Operation

Use of the VENTANA PD-L1 (SP263) Assay is fully automated on the BenchMark ULTRA automated slide stainer, from deparaffinization through counterstaining. Patient FFPE tissue specimens are cut to approximately 4-5 µm thick and mounted on positively charged glass slides. These slides are loaded into the Benchmark ULTRA instrument. This system first removes the paraffin wax from the tissue, and then subjects the tissue to heated antigen retrieval (cell conditioning). Antigen retrieval is the process by which the ability of antibodies to bind to the epitopes is restored to formalin-fixed tissues. Endogenous peroxidases that could potentially react with the horseradish peroxidase conjugates (HRP)

are blocked with OptiView Inhibitor (3% H2O2). After the endogenous peroxidase block, the VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody is dispensed during the antibody incubation step and allowed to bind to its antigen. The slides are then incubated with the reagents in the OptiView DAB IHC Detection Kit, which is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit primary antibodies which produces a visible dark brown precipitate (3,3'-Diaminobenzidine) via a horseradish peroxidase (HRP) enzymatic reaction at the antigen site. Tissues are then counterstained blue using Hematoxylin II and Bluing Reagent to create brown/blue contrast to aid the pathologist when reviewing the slides using bright field microscopy.

Interpretation of PD-L1 Staining

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the VENTANA PD-L1 (SP263) Assay. The labeling states that 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.

A. **Placenta Tissue Control**

A tissue control must be included with each staining run. Qualified normal human term placental 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. Placental 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.

Placental tissue shows moderate to strong uniform staining of the membrane and weak to strong uniform staining of the cytoplasm of trophoblast-lineage cells. Placental stromal tissue and vasculature can be used for assessment of any background staining.

B. **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. Please refer to the VENTANA PD-L1 (SP263) Assay Interpretation Guide, available in the FDA PMA database for Urothelial Cancer for additional details.

C. **Patient Tissue**

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP263) Assay scoring algorithm provided in Table 2. Refer to the VENTANA PD-L1 (SP263) Assay Package Insert and VENTANA PD-L1 (SP263) Assay Interpretation Guide for Urothelial Cancer for additional details. Background staining is also evaluated and is deemed acceptable if the background does not interfere with interpretation of PD-L1 status.

Table 2. VENTANA PD-L1 (SP263) Assay Scoring Algorithm for Urothelial Carcinoma

PD-L1 Interpretation	**Staining Description**
PD-L1 status is determined by the percentage of tumor cells with any membrane staining above background or by the percentage of tumor-associated immune cells with staining (IC+) at any intensity above background. The percent of tumor area occupied by any tumor-associated immune cells (Immune Cells Present, ICP) is used to determine IC+, which is the percent area of ICP exhibiting PD-L1 positive immune cell staining is also evaluated.	
High	PD-L1 Status is considered High if any of the following are met: • ≥ 25% of tumor cells exhibit membrane staining; or, • ICP > 1% and IC+ ≥ 25%; or, • ICP = 1% and IC+ = 100%.
Low/negative	PD-L1 Status is considered Low/negative if: • none of the criteria for PD-L1 High Status are met.

The VENTANA PD-L1 (SP263) Assay stained urothelial carcinoma tissue will be evaluated for tumor cell (TC) and immune cell (IC) staining. The cellular staining pattern for VENTANA PD-L1 (SP263) is membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membrane, diffuse cytoplasmic, and/or punctate staining.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved assay available for detection of PD-L1 in FFPE urothelial cancer tissues to estimate the likelihood of response for patients treated with IMFINZI™ (durvalumab).

VII. MARKETING HISTORY

The VENTANA PD-L1 (SP263) Assay has not been marketed in the United States or any foreign country for use as an *in vitro* diagnostic kit.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PD-L1 test results, and an inaccurate estimate of a patient's benefit from durvalumab and subsequently improper interpretation of the benefit/risks for patients with urothelial carcinoma who are considering treatment with durvalumab.

IX. SUMMARY OF NONCLINICAL STUDIES

Preclinical studies were performed using the VENTANA PD-L1 (SP263) Assay to establish analytical performance of the device. This assay was run using a VENTANA BenchMark ULTRA instrument using the VSS software version 12.2. These studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, verify precision and robustness of the assay, and establish assay stability. The study results detailed below establish sensitivity, specificity, precision and reproducibility of the device.

A. Laboratory Studies

1. Analytical Specificity

The antibody used in the VENTANA PD-L1 (SP263) Assay is Rabbit Anti-Human PD- L1/CD274 Monoclonal Antibody (Clone SP263). The molecular weight of the antibody's target (PD-L1) is 32 kDa, and the SP263 clone specifically targets a 19 amino acid sequence at the cytoplasmic tail of PD-L1. The following studies were conducted with PD-L1 (SP263) antibody to establish antibody specificity.

a. Western Blot Studies

Western blot analysis was performed on whole cell lysates from 4 cell lines with varying expression levels of PD-L1. The 4 cell lines were H820 (Lung), MDA-231 (Breast), H1975 (Lung) and Calu-3 (Lung). The cell lines were chosen for this study based on IHC staining for PD-L1 (SP263). Independent confirmation of the relative expression levels was based on assessment of mRNA expression levels for PD-L1. No unexpected staining or background was observed in any of the whole cell lysates. A second Western Blot analysis was performed to demonstrate that the PD-L1(SP263) antibody detected recombinant PD-L1 and did not detect recombinant PD-L2.

b. Blast Results For SP263 Epitope

PD-L1 is a member of the B7 family of ligands. PD-L1 (SP263) targets 7 amino acids in the cytoplasmic tail of PD-L1. NCBI BLASTp was used to search for sequence similarity using the SP263 epitope and no significant similarity was found to any other B7 family members.

c. Peptide Inhibition Studies

The specificity of primary antibody binding to PD-L1 was assessed by pre-incubating the antibody with four different concentrations of the specific peptide containing the antibody binding epitope, or a non-specific peptide. The antibody was diluted 1:1 with either the peptide solution under investigation or buffer with no peptide. One lot of antibody was used for this study. In the presence of high molar concentrations of this peptide, the PD-L1 SP263 antibody was completely inhibited from binding to tissue expressing PD-L1 protein as determined by the absence of IHC staining. The non- specific peptide had no effect on PD-L1 staining.

d. Immunoreactivity in Human Tissues:

One lot of VENTANA PD-L1 (SP263) Assay and Rabbit Monoclonal Negative Control Ig were used to stain slides of a commercially available multi-tissue array of normal and neoplastic tissue and evaluated by a PD-L1 (SP263) trained qualified reader for presence of positive staining, staining intensity, and background in tumor cells, tumor infiltrating immune cell, and normal cells.

A total of 93 normal tissues and 54 neoplastic tissues were analyzed in this study. The normal formalin-fixed, paraffin-embedded (FFPE) tissues included 25 common types of normal human organs which represent three cases per organ type from three unique individuals. Results for normal tissues are shown in Table 3 and results for neoplastic tissues are shown in Table 4.

Table 3. VENTANA PD-L1 (SP263) Assay staining of FFPE normal tissues.

Tissue	# positive/ total cases	Tissue	# positive/ total cases
Adrenal gland	0/3*	Mesothelium	0/3†
Bladder	0/3	Myeloid (bone marrow)	0/4*,†
Breast	0/3	Nerve (sparse)	0/3
Cerebellum	0/3	Ovary	0/3

Tissue	# positive/ total cases	Tissue	# positive/ total cases
Cerebrum	0/3	Pancreas	0/3*
Cervix	0/3	Parathyroid gland	0/4
Colon	0/3†	Prostate	0/3
Endometrium	0/3	Salivary gland	0/3†
Esophagus	1/3*,†	Skeletal muscle	0/3
Heart	0/3	Skin	0/4§
Hypophysis	0/3*,†	Spleen	0/3†
Intestine, small	0/3†	Stomach	0/3*,†
Kidney	0/3†	Testis	0/3
Larynx	0/3†	Thymus gland	0/3†
Liver	0/3	Thyroid	0/3*,†
Lung	0/3†	Tonsil	3/3†
Lymph node	0/3†		

Additional staining observed: * Cytoplasmic staining, † Immune cell staining, § Melanocyte staining. Percent of IC present above background cannot be evaluated in this study because there is no tumor area for which to score tumor infiltrating immune cells.

Table 4. VENTANA PD-L1 (SP263) Assay staining of FFPE neoplastic tissues for any tumor cell or immune cell staining.

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
Cerebrum	Glioblastoma	0/1	1/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	1/1
Cerebrum	Oligodendroglioma	0/1	0/1
Ovary	Serous adenocarcinoma	0/1	1/1
Ovary	Adenocarcinoma	0/1	0/1
Pancreas	Islet cell carcinoma	1/1	0/1

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
Pancreas	Adenocarcinoma	0/1	1/1
Testis	Seminoma	0/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	1/1	0/1
Breast	Intraductal carcinoma	0/1	1/1
Breast	Invasive ductal carcinoma	0/2	0/2
Spleen	Diffuse B-cell lymphoma	0/1	1/1
Lung	Small cell undifferentiated carcinoma	1/1	1/1
Lung	Squamous cell carcinoma	1/1	1/1
Lung	Adenocarcinoma	0/1	0/1
Esophagus	Neuroendocrine carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	0/1	0/1
Stomach	Signet-ring cell carcinoma	0/1	0/1
Intestine	Adenocarcinoma	0/1	0/1
Intestine	Stromal sarcoma	0/1	0/1
Colon	Adenocarcinoma	0/1	1/1
Colon	Interstitialoma	0/1	0/1
Rectum	Adenocarcinoma	0/1	0/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	0/1	0/1

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
Kidney	Clear cell carcinoma	0/1	0/1
Prostate	Adenocarcinoma	0/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	0/1	0/1
Uterus	Clear cell carcinoma of endometrium	0/1	0/1
Uterine cervix	Squamous cell carcinoma	0/2	2/2
Striated muscle	Embryonal rhabdomyosarcoma	0/1	0/1
Rectum	Malignant melanoma	0/1	0/1
Skin	Basal cell carcinoma	0/1	0/1
Skin	Squamous cell carcinoma	0/1	0/1
Back	Neurofibroma	0/1	1/1
Retroperitoneal	Neuroblastoma	0/1	0/1
Abdominal cavity	Malignant mesothelioma	0/1	0/1
Mediastinum	Diffuse B-cell lymphoma	1/1	1/1
Lymph node	Hodgkin’s lymphoma	1/1	1/1
Lymph node	Diffuse B-cell lymphoma	1/1	1/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1	1/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bone	Osteosarcoma	0/1	1/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Smooth muscle	Moderate malignant leiomyosarcoma	0/1	0/1

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
Bladder	Transitional cell carcinoma (bladder)	1/1	1/1

2. Analytical Sensitivity

Analytical sensitivity was tested on total of 211 commercially sourced unique cases of urothelial carcinoma FFPE specimens that were tested with a single lot of antibody. A total of 169 of the 211 tissues were classified as PD-L1 high using the PD-L1(SP263) scoring criteria for urothelial carcinoma.

3. Repeatability

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

a. Intra-day Repeatability − Five replicate slides each from 13 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay on a single BenchMark ULTRA instrument within one day.

b. Inter-day Precision − Two replicate slides each from 13 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay on a single Benchmark ULTRA instrument across 5 non-consecutive days spanning at least a 20 day period.

c. Inter-instrument and Inter-lot Precision − 27 replicate slides each from 22 unique urothelial carcinoma specimens were stained with VENTANA PD-L1 (SP263) Assay using three lots of VENTANA PD-L1 (SP263) antibody and three lots of OptiView DAB IHC Detection Kit, on three BenchMark ULTRA instruments.

The 13 unique cases used for the Intra-day Repeatability and Inter-day Precision Studies had a case distribution of 9 PD-L1 High and 4 PD-L1 Low with 6 borderline cases for either the tumor cell or immune cell component of the scoring criteria. For intra-day repeatability, each case had 5 antibody slides, the mode of which determined the PD-L1 status for that case. For inter-day precision, each case had 10 antibody slides, the mode of which determined the PD-L1 status for that case.

The 22 unique cases used for the Inter-instrument and Inter-lot Precision Study had an overlapping case distribution of 13 PD-L1 High and 9 PD-L1 Low. Each case contributed one observation for each of the 9 combinations of antibody lot and detection kit lot per instrument (27 observations in total per case). A

reference PD-L1 status was established as the mode of all slides for each case and stratification level.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP263) Assay scoring algorithm. Results are summarized in Table 5.

Table 5. Repeatability and Intermediate Precision of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

Repeatability/Intermediate precision parameter	Positive Agreement %* (95% CI)	Negative Agreement %* (95% CI)	Overall agreement % (95% CI)
Intra-day Repeatability (within a single day) N=65	100.0% (45/45) (92.1 – 100%)	95.0% (19/20) (76.4 – 99.1.0%)	98.5% (64/65) (91.8 – 99.7%)
Inter-day Precision (5 non-consecutive days) N = 130	100.0% (90/90) (95.9 – 100%)	100.0% (40/40) (91.2 – 100%)	100.0% (130/130) (97.1 – 100%)
Inter-instrument and Inter-lot Precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots) N=594	98.3% (345/351) (96.3 – 99.2%)	99.6% (242/243) (97.7 – 99.9%)	98.8% (587/594) (97.6 – 99.4%)

*Confidence intervals were calculated using the Wilson Score method.

4. External Reproducibility

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP263) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 expression in urothelial carcinoma tissue specimens. Twenty-six urothelial carcinoma specimens (18 PD-L1 High and 8 PD-L1 Low, with 4 borderline specimens for either the tumor or immune cell component of the scoring criteria) were stained at 3 external laboratories on each of 5 non-consecutive days over 20-days. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The final staining acceptability rate for the VENTANA PD-L1 (S263) Assay was 99.7% in this study.

Results are summarized in Table 6.

Table 6. Inter-laboratory Reproducibility of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

Inter-laboratory Reproducibility	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Overall agreement (across sites, days and readers) n =778 observations	95.2% (512/538) (93.0-96.7%)*	85.8% (206/240) (80.9-89.7%)*	92.3% (718/778) (90.2-94.0%)*
Inter-site agreement (average of site-to-site pairwise comparisons) n =7760 pairs	91.1% (84.2-95.8%)**	79.1% (61.9-89.1%)**	87.5% (86.8-88.2%)**
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site) n =389	90.1% (492/546) (82.3-95.7%)**	76.7% (178/232) (58.2-88.4%)**	86.1% (335/389) (82.3-89.2%)**
* Confidence intervals were calculated using the Wilson Score method. ** Percentile Bootstrap confidence intervals were constructed from the 2.5th and 97.5th percentiles using 2,000 replicates when sampling at the case level.			

The point estimate and lower bounds of the 95% confidence interval for NPA were below 80% for the Inter-site agreement and Inter-reader agreement analyses. The lower than expected estimates may be in part due to the few (n=8) PD-L1 Low/negative samples that were included in this study which can result in a less robust estimate of NPA. As a result, Ventana will be performing an Inter-laboratory reproducibility study with sufficient PD-L1 Low/negative samples to provide a robust estimate of NPA. Ventana will update the VENTANA PD-L1(SP263) Assay package insert when those results become available. Assay users should refer to the current package insert for any updates to these analytical studies.

5. Reader Precision

To assess Inter- and Intra-reader Precision, three pathologists evaluated fifty unique urothelial carcinoma specimens (22 PD-L1 high and 28 PD-L1 low) that were stained with VENTANA PD-L1 (SP263) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status. Readers scored all specimens twice, with a minimum of two weeks between reads. Intra-reader agreement rate between the first and second read was calculated for each reader

separately and then averaged across three readers. Inter-reader agreement rates were based on the weighted average across all reader permutations for the first set of reads (i.e., Reader 1 vs. Reader 2, Reader 1 vs. Reader 3 and Reader 2 vs. Reader 3). Agreement rates are measured as positive percent agreement, negative percent agreement, and overall percent agreement and are summarized in Table 7.

Table 7. Inter- and Intra-reader Precision of VENTANA PD-L1 (SP263) Assay staining of urothelial carcinoma specimens.

Reader Precision	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Inter-reader Precision (average of all three readers'comparisons) N=143 pairs	92.8% (128/138) (85.5-98.3%)	93.2% (138/148) (86.5-98.5%)	93.0% (133/143) (87.1-98.6%)
Intra-reader Precision (average of all three readers' agreement rates between first and second reads) N=145 pairs	92.1% (128/139) (85.4-96.7%)	92.7% (140/151) (87.1-96.8%)	92.4% (134/145) (87.2-96.6%)

6. Impact of Tissue Specimen Preparation and Treatment Studies

a. Ischemia Study (Time to Fixation)

The objective of this study was to evaluate the effects of ischemic time on PD-L1 antigenicity as detected by staining with VENTANA PD-L1 (SP263) Assay. This study examined the effects of delay to fixation (Ischemia) for mouse xenografts at zero hour, 0.5 hours, 1 hour, 2 hours, 6 hours and 24 hours post excision, under refrigeration at 2-8°C. All samples were fixed in 10% NBF for 24 hours after being delayed for fixation at their various ischemia time points. The Assay demonstrated no significant change in staining intensity from hour zero to up to 24 hours using a xenograft tissue model.

b. Fixation Study

The objective of this study was to evaluate the effects of fixative type and fixation time on PD-L1 antigenicity as detected by staining with VENTANA PD-L1 (SP263) Assay. Two tonsil tissue cases were fixed for 1, 6, 12, 24, and 72 hours in six fixatives: 10% NBF, Zinc formalin, 95%

alcohol, AFA, Z-5 and PREFER for a total of 12 tissues. The data was then compared to the reference standard of 10%NBF for 24 hours.

Tissues fixed for one hour in 10% NBF, Zinc formalin and Z-5 were unacceptable when stained with PD-L1 (SP263). All other time points (6, 12, 24 and 72 hour) in 10% NBF, Zinc formalin and Z-5 were acceptable with the VENTANA PD-L1 (SP263) Assay. The VENTANA PD-L1 (SP263) Assay demonstrated poor performance on the following fixatives at all time points: PREFER, AFA, and 95% alcohol. PREFER, AFA, and 95% alcohol should not be used as a fixative for anti-PD-L1.

7. Impact of Tissue Thickness Studies

The objective of this study was to evaluate the staining performance of the VENTANA anti-PD-L1 (SP263) Assay on seven urothelial carcinoma tissues sectioned at various thicknesses (2 to 7 microns). At each thickness, two slides from each case were stained with VENTANA PD-L1 (SP263) CDx Assay and one slide was stained with Rabbit Monoclonal Negative Control lg to serve as a negative reagent control slide. This study demonstrated 100% concordance with PD-L1 status for all seven cases at all thickness of 3-7 microns. Ventana recommends that specimens be cut at 4-5 microns for the assay.

8. Impact of Cut Slide Stability

The objective of this study is to determine a time point at which the degradation of PD-L1 antigenicity occurs in formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma tissue sectioned on positively charged microscope slides after storage in two temperature conditions, 4°C and 30°C.

Four tissues were used for this study. Slides sectioned and stained at the day 0 time point served as the baseline comparator for the remainder of the time points tested. Tissue was sectioned from each of four cases onto glass slides and separated into two different storage conditions for the duration of the study. One box was stored at refrigerated temperature condition (4°C) and one at the incubator temperature condition (30°C) to simulate room temperature. Slides were stained at each pre-defined designated time point (monthly intervals) and staining results for each time point were compared to the Day Zero baseline slides.

PD-L1 antigen stability was determined to be 6 months for slides stored at 30°C and five months for slides stored at 4°C.

9. PD-L1 (SP263) Status in Matched Primary vs. Metastatic Urothelial Cancers.

Patients were enrolled into the clinical trial on the basis of PD-L1 status testing either primary or metastatic tumor tissue. Therefore, a study to compare the concordance of PD-L1 status between primary urothelial carcinoma and matched metastatic tumors in tissues was conducted. The concordance of PD-L1 status between 49 commercially sourced patient matched Primary and Metastatic Tumor urothelial carcinoma samples demonstrated an overall concordance of 63.3% (31 /49) for PD-L1 status with 60.0% (9/15) positive percent agreement and 64.7% (22/34) negative percent agreement. Results are shown in Table 8.

Table 8. Comparison of Primary Vs Metastatic tumor urothelial carcinoma specimens staining with VENTANA PD-L1 (SP263) Assay

	Primary Tumor		
Metastatic Tumor	**Positive**	**Negative**	**Total**
Positive	9	12	21
Negative	6	22	28
Total	15	34	49
Positive Percent Agreement (PPA) n/N (%) (95% CI)	9 / 15 (60.0%) (35.7 – 80.2)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	22 / 34 (64.7%) (47.9 – 78.5)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	31 /49 (63.3) (49.3 – 75.3%)		

10. Impact of Tissue Block and Intra-Case Heterogeneity

a) Intra-Block Heterogeneity

The intent of this study was to characterize the intra-block heterogeneity in urothelial carcinoma tissue blocks by evaluating the PD-L1 status for tumor cells and tumor-associated immune cells of multiple slide cuts from the same tissue block when stained with VENTANA PD-L1 (SP263) Assay. Five unique formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma tissues, which included three transurethral resections of the bladder (TURB) and two resection specimens, were sectioned to exhaustion. Duplicate slides from approximately every 5th section for the first 30 sections and approximately every 10th section thereafter were stained with VENTANA PD-L1 (SP263) Assay. Three out of the five cases tested maintained the same PD-L1 status throughout the block. Both cases with inconsistent PD-

L1 status were TURB specimens that were borderline positive based on either immune cell staining or tumor cell staining.

b) Intra-case heterogeneity

The intent of this study was to characterize UC case heterogeneity when multiple blocks from the same patient case were stained with the VENTANA PD-L1 (SP263) Assay. Forty three cases with two blocks per case were evaluated in this study. A total of thirty-four of the forty-three (79%) cases maintained the same PD-L1 status between blocks.

11. Stability Testing

The objective of this study is to assess the stability (shelf-life and in-use) and shipping category on 3 lots of the VENTANA PD=L1 (SP263) Assay. Dispensers from each of the 3 antibody lots were subjected to different stress conditions and then placed at intended storage (2-8°C) for the duration of the study. The stress conditions were as follows:

a. Intended storage (2-8°C) for the duration of the study.
b. Product held at 30±5°C for 192±5 hours, and then places at 2-8°C for the duration of the study.
c. Product held at 15±5°C for 192±5 hours, and then places at 2-8°C for the duration of the study.
d. Product held at -20±5°C for 192±5 hours, and then places at 2-8°C for the duration of the study.

Four urothelial carcinoma, 4 squamous carcinoma head and neck and 4 non-small cell lung carcinoma tissues were used for this study. Tissues are tested in triplicate at each time point. All lots passed for all conditions and support an 11 month stability claim.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Study Design

The clinical performance of the VENTANA PD-L1(SP263) Assay was evaluated in a single arm, multi-center, open label study of IMFINZI™ (durvalumab) on patients with locally advanced or metastatic urothelial carcinoma. Patients had progressed while on or after a platinum-based therapy, including those who progressed within 12 months of receiving therapy in a neo-adjuvant or adjuvant setting. All patients received IMFINZI at 10mg/kg every 2 weeks for up to 12 months until unacceptable toxicity or confirmed disease progression.

Tumor specimens were evaluated prospectively using VENTANA PD-L1 (SP263) Assay at a central laboratory and the results were used to define subgroups based on PD-L1 expression. The first 20 patients were enrolled regardless of PD-L1 expression. The next 43 patients that were enrolled required tumor expression of PD-L1 ≥ 5% positive staining. The remaining 128 patients were enrolled regardless of PD-L1 status.

1. Clinical Inclusion and Exclusion Criteria

   Enrollment was limited to patients who met the following inclusion criteria:
   - Adult (>18 years)
   - Patients must have histologically or cytologically confirmed inoperable or metastatic transitional cell carcinoma of the urothelium (including transitional cell and mixed transitional cell/nontransitional cell histologies) carcinoma of the urothelium (including the urinary bladder, ureter, urethra, and renal pelvis).
   - Subjects must have received and have progressed or are refractory to at least 1 but not more than 2 prior lines of systemic therapy for inoperable or metastatic disease, including a standard platinum-based regimen. Interval progression between 2 lines of therapy defines separate lines of therapy. Prior definitive chemoradiation for locally advanced disease, adjuvant treatment, or neoadjuvant treatment will be considered a prior line of therapy, provided that progression has occurred < 12 months from therapy [for chemoradiation and adjuvant treatment] or < 12 months from surgery [for neoadjuvant treatment]
   - Subjects must have consented to provide an archived tumor specimen from within 6 months prior to study entry or provide a pretreatment fresh biopsy.
   - Eastern Cooperative Oncology Group (ECOG) status of 0 or 1

   Patients were not permitted to enroll if they met any of the following exclusion criteria:

   - History of primary immunodeficiency
   - Receipt of any immunotherapy, or investigational anticancer therapy within 4 weeks prior to the first dose of IMFINZI and no mAb therapy (for investigational use or immunotherapy) within 6 weeks prior to the first dose of durvalumab.

   Prior treatment with immunotherapy agents including, but not limited to, tumor necrosis factor receptor superfamily agonists or checkpoint inhibitors or natural killer

(NK) cell inhibitors including agents targeting KIR, PD-1, PD-L1, CTLA-4, OX40, CD27, CD137 (4-1BB), CD357 (GITR), and CD40. Prior treatment with Bacillus Calmette-Guerin therapy was permitted.

2. Follow-up Schedule

   Follow up for Efficacy:
   Tumor assessments were performed at Weeks 6, 12 and 16, then every 8 weeks for the first year and every 12 weeks thereafter.

   Follow up for Safety:
   Patients were closely monitored for safety and tolerability throughout the study at each patient contact. Safety assessments consisted of monitoring and recording adverse events, including serious adverse events and non-serious adverse events of special interest, measurement of protocol-specified safety laboratory assessments, measurement of protocol-specified vital signs, and other protocol-specified tests that are deemed critical to the safety evaluation of the study. An adverse event is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. Patients were followed for safety for 30 days following their last dose of study treatment (in both the initial treatment period and the re-treatment period) or until they receive another anti-cancer therapy, whichever comes first.

3. Clinical Endpoints

   The primary efficacy outcome measures were confirmed Objective Response Rate (ORR) according to RECIST v1.1 as assessed by Blinded Independent Central Review (BICR) and duration of response (DoR).

B. Accountability of PMA Cohort

A total of 451 subjects were screened for enrollment in study 1. Eighty seven (of 451) subjects were excluded prior to submitting a tissue sample for testing with the VENTANA PD-L1 (SP263) Assay because they either did not meet inclusion/exclusion criteria (n=68), consent was withdrawn (n=12), no tissue was available for testing (n=5) or subject enrolled in another study (n=2). This resulted in 364 patients with sufficient tissue available to be considered in the intent to diagnose population.

Three hundred sixty three (363) of 364 subjects were tested with VENTANA (SP263) assay and 173 subjects were excluded from the clinical trial because they did not meet inclusion criteria based on PD-L1 testing (n=89), they did not meet other inclusion/exclusion criteria (n=74), or withdrawal of consent (n=10).

One hundred and ninety one patients were treated with IMFINZI. Nine patients were excluded from efficacy analysis of IMFINZI because they were not 2nd-line post-platinum patients. The remaining 182 subjects were included in the primary efficacy

analysis for IMFINZI. Of the 182 enrolled patients who met the inclusion criteria, 128 were enrolled without regard to PD-L1 status and after the PD-L1 scoring algorithm were finalized. The analyses with respect to drug efficacy by PD-L1 status are limited to these 128 patients.

C. Study Population Demographics and Baseline Parameters

The median age of the patients was 66 years, 72% were male, 64% patients were Caucasian. Thirty-four percent had non-bladder urothelial carcinoma and 66% of patients had visceral metastases, including 34% with metastases to the liver, which portends a worse prognosis. Nineteen percent of patients had disease progression following prior platinum-containing neoadjuvant or adjuvant chemotherapy. Thirty-five percent of patients had received ≥ 2 prior systemic regimens in the metastatic setting. Seventy (70) percent of patients received prior cisplatin and 30% had prior carboplatin. One patient was previously treated with oxaliplatin.

D. Safety and Effectiveness Results

1. Safety Results

   The VENTANA PD-L1 (SP263) Assay involves testing on FFPE urothelial carcinoma specimens. These tissues are routinely removed as part of the practice of medicine for the diagnosis of urothelial carcinoma by pathologists. Removal of these tissues, therefore, presents no additional safety hazard to the patient being tested. For adverse effects related to IMFINZI refer to the drug label.

2. Effectiveness Results

   Tissues from 363 patients were submitted for testing with VENTANA PD-L1(SP263) Assay. PD-L1 status was obtained for 332 (91.5%) and 31 (8.5%) were not evaluable. PD-L1 status was not evaluable for 31 patients due to insufficient tumor cells present in sample (n=25), inappropriate tissue being submitted (n=3), tissue folding (n=1), dye trapping (n=1) or tissue washing off the slide (n=1). There were no instances where a sample was non-evaluable for PD-L1 status due to assay failure.

   The analysis of effectiveness of VENTANA PD-L1(SP263) Assay was based on the 128 patients that were enrolled into study 1 without regard to PD-L1 status and after the PD-L1 scoring algorithm was finalized. The primary efficacy outcome measures were confirmed Objective Response Rate (ORR) according to RECIST v1.1 as assessed by Blinded Independent Central Review (BICR) and duration of response (DoR). Table 9 summarizes the results in Study 1. The median follow-up time was 5.6 months.

Table 9. Efficacy Results for Study 1

	Final PD-L1 Assay[1] N = 128		
	PD-L1 High N = 58	**PD-L1 Low/Negative N = 56**	**PD-L1 NE N = 14**
Objective Response Rate by BICR n (%) (95% CI)	11 (19.0%) (9.9, 31.4)	2 (3.6%) (0.4, 12.3)	3 (21.4%) (4.7, 50.8)
Complete Response	2 (3.4%)	0	1 (7.1%)
Partial Response	9 (15.5%)	2 (3.6%)	2 (14.3%)
Median Duration of Response months (range)	4.24 (0.9+, 4.2)	NR (1.9+, 4.2+)	NR (2.3+, 2.6+)

[1]Enrolled regardless of PD-L1 status; BICR = Blinded Independent Central Review; NE = Not Evaluable; NR = Not Reached, + denotes a censored value

The VENTANA PD-L1 (SP263) Assay is intended to be used on either primary tumor tissue or metastases. Concordance of the VENTANA PD-L1 (SP263) Assay PD-L1 comparing matched primary and metastatic urothelial cancers found 18/49 pairs (36.6%) pairs gave discordant results. As an exploratory analysis, efficacy data from Study 1 was stratified by source of biopsy (tumor vs. metastatic). Similar associations were observed between PD-L1 status and drug efficacy in patients whose PD-L1 status was determined by primary tissue compared to those patients whose status was determined by metastatic tissue.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical performance of VENTANA PD-L1 (SP263) Assay investigated in Study 1, was evaluated in a non-randomized, multi-center, open label study of IMFINZI (durvalumab) on patients with locally advanced or metastatic urothelial carcinoma. Patients had progressed while on or after a platinum-based therapy, including those who progressed within 12 months of receiving therapy in a neo-adjuvant or adjuvant setting. All patients received IMFINZI (durvalumab) at 10mg/kg every 2 weeks for up to 12 months until unacceptable toxicity or confirmed disease progression. High PD-L1 status as determined by VENTANA PD-L1 (SP263) Assay was associated with increased objective response rate (ORR) in this trial (see Table 9, above).

The performance of the VENTANA PD-L1 (SP263) Assay was also supported by the analytical validation studies.

B. Safety Conclusions

The VENTANA PD-L1 (SP263) Assay is an in vitro diagnostic device, which tests tumor FFPE specimens collected from patients with urothelial carcinoma. The risks of the device are based on data collected in the clinical study.

Failure of the device to perform as expected may lead to a failure to correctly interpret test results. The process of testing an FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for diagnosis.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in a clinical study conducted to support PMA approval as described above. High PD-L1 status, as determined by VENTANA PD-L1(SP263) Assay, is associated with increased ORR for patients with locally advanced or metastatic urothelial carcinoma that have progressed while on or after a platinum-based therapy who are treated with IMFINZI (durvalumab). This assay may help physicians determine the best treatment regimen and tailor therapies accordingly. The main risk of the VENTANA PD-L1 (SP263) Assay is in getting a false result and the potential to adversely affect patient management. However, since this test result does not dictate the choice of therapy and patients that are classified as either PD-L1 High or PD-L1 Low/Negative may

receive a benefit from IMFINZI (see Table 9), the risk caused by a false result is relatively low.

1. Patient Perspectives

   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for indications for use stated above.the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

XIII. CDRH DECISION

CDRH issued an approval order on May 1, 2017.

The final conditions of approval cited in the approval order are described below.

1. You have agreed to submit results from the ongoing, confirmatory studies of durvalumab in urothelial bladder cancer to provide an estimate of the association between PD-L1 status determined by the VENTANA PD-L1(SP263) Assay and the clinical response to durvalumab. The additional postmarket results are to provide assurance that the results are generalizable to the post-approval target population. You have also agreed to provide the updated labeling that includes results of these ongoing confirmatory studies.

2. You have agreed to submit additional testing and analyses of samples from the intended use specimen type, using an appropriate study design, to robustly characterize assay precision and reproducibility.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See labeling including warnings and precautions.

Post-approval Requirements and Restrictions: See approval order.