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 (SP142) 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: P160002

Date of FDA Notice of Approval: May 18, 2016

II. INDICATIONS FOR USE

VENTANA PD-L1 (SP142) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP142 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 and OptiView Amplifcation Kit on a VENTANA BenchMark ULTRA instrument. PD-L1 status is determined by the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity.

PD-L1 expression in ≥ 5% IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is associated with increased objective response rate (ORR) in a non-randomized study of TECENTRIQ™ (atezolizumab).

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VENTANA PD-L1 (SP142) Assay labeling.

V. DEVICE DESCRIPTION

Device Kit Components

The VENTANA PD-L1 (SP142) 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 and the OptiView Amplification Kits. The VENTANA PD-L1 (SP142) 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 PD-L1 (SP142) Assay	Dispenser: 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.
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 H_2O_2 contains 0.04% hydrogen peroxide in a phosphate buffer solution.
		OptiView DAB contains 0.2% 3, 3'-diaminobenzidine tetrahydrochloride (DAB) in a proprietary stabilizer solution with a proprietary preservative.

Table 1– Overview of the VENTANA PD-L1 (SP142) Assay Components		
Device Components	**Packaged form**	**Description**
		OptiView Copper contains copper sulfate (5.0 g/L) in an acetate buffer with a proprietary preservative.
OptiView Amplification Kit	3 dispensers packaged in a kit of 50 tests or 250 tests	**OptiView Amplification** contains 0.003% HQ conjugated tyramide complex in a sodium borate solution.
		OptiView H_2O_2 contains 0.04% H_2O_2 in a sodium phosphate buffer
		OptiView Multimer contains a mouse monoclonal anti-HQ-labeled HRP tertiary antibody (<40 µg/mL) in a buffer containing protein with ProClin 300, a 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 (SP142) Assay is performed on the BenchMark ULTRA automated staining instrument using the VSS software version 12.2. The VENTANA PD-L1 (SP142) Assay protocol is assay specific. The software has been designed to recognize and group the VENTANA PD-L1 (SP142) Assay, requiring that all system reagents are used together.

Specimen Preparation

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. Ventana recommends tissue fixation in 10% neutral buffered formalin (NBF)

for at least 6 hours and for a maximum of 72 hours. 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. Fixation can be performed at room temperature (15-25°C).

Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative, or other alcohol-containing fixatives have demonstrated a loss of specific staining for PD-L1 at all fixation times tested (1−72 hours); they are not recommended for use with this assay.

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised within 3 months after cutting from the paraffin block for urothelial carcinoma specimens and 2 months for tonsil specimens.

Quality Control Procedures

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

A. 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. The Rabbit Monoclonal Negative Control Ig is required, but is not provided in the assay kit.

B. 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.

Principles of Operation

The VENTANA PD-L1 (SP142) Assay is fully automated for use on the BenchMark ULTRA automated slide stainer from deparaffinization through counterstaining. Patient FFPE tissue specimens are cut to approximately 4 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% H_2O_2). After the endogenous peroxidase block, the VENTANA PD-L1 (SP142) Rabbit Monoclonal Primary Antibody is dispensed during the antibody incubation step and allowed to bind to its antigen for 16 minutes. The slides are then incubated with the reagents in the OptiView DAB IHC Detection Kit and OptiView Amplification Kit. The OptiView DAB IHC Detection Kit 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. The OptiView Amplification Kit is used in combination with the OptiView DAB IHC Detection Kit reagents to achieve preferred staining of target cells. The OptiView Amplification Kit includes an HQ hapten conjugate (OptiView Amplifier), corresponding substrate (OptiView Amplification H2O2), and mouse anti-HQ monoclonal antibody containing HRP (OptiView Amplification Multimer). 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 (SP142) Assay antibody. A qualified pathologist experienced in IHC procedures must evaluate tissue controls with a light microscope and qualify the stained product before interpreting results.

A. 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. Specimen acceptability criteria are listed in Table 2.

Table 2 – Tonsil tissue control evaluation criteria.

Acceptable	Unacceptable
Positive tissue elements: Moderate to strong PD-L1 (SP142) 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 (SP142) positive cells.

Negative tissue elements: PD-L1 (SP142) negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Weak to no PD-L1 (SP142) staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.

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.

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. The package insert notes that 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.

C. Patient Tissue

The package insert instructs that patient tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm provided in Table 3.

Table 3 – Scoring algorithm for the VENTANA PD-L1 (SP142) Assay.

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

The VENTANA PD-L1 (SP142) Assay stained urothelial carcinoma tissue will only be evaluated for immune cell (IC) staining. IC are scored as the proportion of tumor area that is occupied by PD-L1 staining immune cells of any intensity. Any IC staining irrespective of type of cells or localization is included. Tumor area is defined as area occupied by viable tumor cells, and their associated intra- and contiguous peritumoral stroma.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay available for detection of PD-L1 in FFPE urothelial carcinoma tissues.

VII. MARKETING HISTORY

The VENTANA PD-L1 (SP142) Assay has not been marketed in the United States or any foreign country.

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 atezolizumab and subsequently improper interpretation of the benefit/risks for patients with urothelial bladder cancer who are considering treatment with atezolizumab.

For the specific adverse events that occurred in the clinical study, please see Section X below.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Preclinical studies were performed using the VENTANA PD-L1 (SP142) Assay to establish analytical performance of 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.

1. Analytical Specificity

The antibody used in the VENTANA PD-L1 (SP142) Assay is Rabbit Anti-Human PD-L1/CD274 Monoclonal Antibody (Clone SP142). The molecular weight of the antibody's target is 32 kDa, and the SP142 clone targets amino acids 284-290 at the cytoplasmic tail of PD-L1. The following studies were conducted with PD-L1 (SP142) 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 (SP142). The relative levels of PD-L1 protein observed in these four cell lines on the Western Blot correlate with PD-L1 protein expression levels determined by IHC staining and also with mRNA expression levels for PD-L1. No

unexpected staining or background was observed in any of the whole cell lysates.

b. BLAST Results for SP142 Epitope.
PD-L1 is a member of the B7 family of ligands. PD-L1 (SP142) targets 7 amino acids in the cytoplasmic tail of PD-L1. NCBI BLASTp was used to search for sequence similarity using the SP142 epitope and no significant similarity was found to any other B7 family members.

c. Specificity of PD-L1 SP(142) on Cell Lines Expressing PD-L1 or PD-L2
A series of HEK293 transfected cells lines was generated using empty vector, vector containing the PD-L1 gene, and a vector containing the PD-L2 gene. IHC staining demonstrated that PD-L1 (SP142) stains PD-L1 transfected HEK293 cells but not PD-L2 transfected HEK293 cells.

d. Peptide inhibition studies
One lot of primary antibody was incubated with either: no peptide; an unrelated non-specific peptide; or four different concentrations of the specific peptide containing the antibody binding epitope. Each of these solutions represented half the optimal concentration of the primary antibody, as the antibody was diluted 1:1 with either a peptide solution or buffer alone. In the presence of high molar concentrations of this peptide, anti-PD-L1 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.

e. Immunoreactivity in Human Tissues
One lot of VENTANA PD-L1 (SP142) antibody 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 (SP142) trained pathologist for presence of positive staining, staining intensity, and background in tumor cells, tumor infiltrating immune cell, and normal cells.

There were 93 normal tissues and 54 neoplastic tissues analyzed in this study. The normal 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 4 and results for neoplastic tissues are shown in Table 5.

Table 4. VENTANA PD-L1 (SP142) Assay staining on normal tissues.

Tissue type	# positive*/ total cases	Tissue type	# positive*/ total cases
Adrenal gland	1/3	Muscle, cardiac	0/3
Bladder	3/36	Muscle, skeletal	0/2
Breast	1/3	Myeloid	0/2
Cerebellum	0/3**	Nerve, peripheral	0/3
Cerebrum	0/3	Ovary	0/3
Cervix	2/2	Pancreas	0/3***
Colon	2/3	Parathyroid	0/2
Endometrium	1/3	Prostate	0/3
Esophagus	0/3	Salivary gland	2/3
Hypophysis	0/3***	Skin	0/3
Intestine, small	1/3	Spleen	3/3
Kidney	2/3	Stomach	0/3
Lingual gland	0/1	Testis	0/3**
Liver	0/3	Thymus gland	3/3
Lung	1/3	Thyroid gland	1/3
Lymph node	3/3	Tonsil	3/3
Mesothelium	0/3		

*Immune cell staining

**Focal DAB dots were observed in 1/3 cerebellum and1/3 testis tissues

***Nuclear staining was observed in 1/3 pancreas and 1/3 hypophysis tissues

Table 5. VENTANA PD-L1 (SP142) Assay Staining on Neoplastic Tissues.

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
Abdomen	Malignant mesothelioma	1/1	0/1
Back	Neurofibroma	1/1	0/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bladder	Transitional cell carcinoma	1/1	0/1
Bone	Osteosarcoma	0/1	0/1
Breast	Invasive ductal carcinoma	1/1	0/1
Breast	Intraductal carcinoma with early infiltrate	1/1	0/1
Cerebrum	Glioblastoma	0/1	0/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	0/1
Cerebrum	Oligodendroglioma	0/1	0/1
Colon	Adenocarcinoma	1/1	0/1
Colon	Interstitialoma	0/1	0/1
Esophagus	Squamous cell carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	1/1	0/1
Intestine	Adenocarcinoma	1/1	0/1
Intestine	Stromal sarcoma	1/1	0/1
Kidney	Clear cell carcinoma	1/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	1/1	0/1
Lung	Adenocarcinoma	0/1	0/1
Lung	Small cell	1/1	0/1

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
	undifferentiated carcinoma		
Lung	Squamous cell carcinoma	1/1	0/1
Lymph node	Diffuse B-cell lymphoma	1/1*	1/1*
Lymph node	Hodgkin's lymphoma	1/1	0/1
Mediastinum	Diffuse B-cell lymphoma	1/1*	1/1*
Muscle, smooth	Moderate malignant leiomyosarcoma	1/1	0/1
Muscle, striated	Embryonal rhabdomyosarcoma	0/1	0/1
Ovary	Serous adenocarcinoma	1/1	0/1
Ovary	Adenocarcinoma	1/1	0/1
Pancreas	Islet cell tumor	0/1	0/1
Pancreas	Adenocarcinoma	1/1	0/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1*	1/1*
Prostate	Adenocarcinoma	0/2	0/2
Rectum	Adenocarcinoma	1/1	1/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Rectum	Malignant melanoma	1/1	0/1
Retroperitoneum	Neuroblastoma	1/1	0/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Skin	Basal cell carcinoma	1/1	0/1
Skin	Squamous cell carcinoma	1/1	0/1
Spleen	Diffuse B-cell	1/1*	1/1*

Origin	Pathology	# positive/total cases	
		Immune cells	Tumor cells
	lymphoma		
Stomach	Signet-ring cell carcinoma	1/1	0/1
Testis	Seminoma	1/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	0/1	1/1
Uterine cervix	Squamous cell carcinoma	2/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	1/1	0/1
Uterus	Clear cell carcinoma of endometrium	1/1	1/1
* Tumor cell and immune cell staining could not be differentiated			

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 (SP142) was tested on 3750 urothelial carcinoma specimens, of which 3695 (98.5%) were primary tumor tissue and 55 (1.5%) were metastases. There were 466/3750 (12.4%) tissues that had discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma.

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 10 unique urothelial carcinoma specimens (4 PD-L1 ≥ 5% and 6 PD-L1 < 5%) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within one day.
b. Inter-day Precision – Two replicate slides each from 10 unique urothelial carcinoma specimens (4 PD-L1 ≥ 5% and 6 PD-L1 < 5%) were stained with VENTANA PD-L1 (SP142) Assay on a single Benchmark ULTRA instrument across 5 non-consecutive days.

c. Inter-instrument and Inter-lot Precision - Twenty seven replicate slides each from 18 unique urothelial carcinoma specimens (9 PD-L1 ≥ 5% and 9 PD-L1 < 5%) 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.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm. Results are summarized in Table 6. The overall agreement (OA) rates in each study (measured as overall percent agreement between replicate slides) were greater than 90%.

Table 6. Repeatability and Intermediate Precision of VENTANA PD-L1 (SP142) 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)	100.0% (83.9-100.0%)	100.0% (88.6-100.0%)	100.0% (92.9-100.0%)
Inter-day Precision (5 non-consecutive days)	90.0% (76.9-96.0%)	100.0% (94.0-100.0%)	96.0% (90.2-98.4%)
Inter-instrument and Inter-lot Precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	99.6% (97.7-99.9%)	99.6% (97.7-99.9%)	99.6% (98.5-99.9%)
* Positive Percent Agreement** Negative Percent Agreement			

Lot to lot variability was assessed in the Inter-instrument and Inter-lot precision study with 3 lots of antibody. The study met the acceptance criteria of >85% OA at the lower bounds of the 95% confidence interval.

4. External Reproducibility

An inter-laboratory reproducibility study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in urothelial carcinoma tissue specimens. Twenty-eight unique urothelial carcinoma specimens (14 PD-L1 ≥ 5% and 14 PD-L1 < 5%) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The sample set consisted of a total of 420 case slides (140 slides per site) generated from 28 unique urothelial

carcinoma specimens. The final staining acceptability rate for the VENTANA PD-L1 (SP142) Assay was 99.9% in this study. Results are summarized in Table 7.

Table 7. Inter laboratory Reproducibility of VENTANA PD-L1 (SP142) Assay.

Inter-laboratory Reproducibility	**Positive agreement % (95% CI)**	**Negative agreement % (95% CI)**	Overall agreement % (95% CI)
Overall agreement (across sites, days and readers)	98.3% (96.6-99.2%)*	87.4% (83.8-90.2%)**	92.8% (90.9-94.4%)
Inter-site agreement (average of site-to-site pairwise comparisons)	90.7% (81.2-96.3%)†	88.3% (78.5-94.9%)††	89.6% (82.5-95.5%)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	89.3% (78.1-96.0%)†	86.6% (75.1-94.6%)††	88.1% (84.6-90.8%)
n = 419 evaluable case slides * Positive Percent Agreement ** Negative Percent Agreement † Average Positive Agreement †† Average Negative Agreement			

5. Reader Precision

To assess inter- and intra-reader precision, three pathologists evaluated sixty unique urothelial carcinoma specimens (30 PD-L1 ≥ 5% and 30 PD-L1 < 5%) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status. Readers scored all specimens twice, according to the scoring criteria in Table 3, with a minimum of two weeks between reads. The agreement rates between the readers compared to a consensus score across two reads and between each pathologist's reads (measured as positive percent agreement, negative percent agreement, and overall percent agreement) are summarized in Table 8.

Table 8. Inter- and Intra-reader Precision of VENTANA PD-L1 (SP142) Assay

Reader Precision	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Inter-reader Precision (average of all three readers' comparisons)	92.6% (84.7-96.6%)	98.9% (92.7-99.8%)	95.8% (91.3-98.0%)
Intra-reader Precision (average of all three readers' agreement rates between first and second reads)	89.3% (77.6-95.3%)	97.8% (86.0-99.7%)	93.6% (86.8-97.0%)

6. Impact of Tissue Specimen Preparation and Treatment Studies

The objective of this study was to assess the effects of fixative type, fixation time, and delay to fixation time on PD-L1 antigenicity in formalin-fixed, paraffin-embedded tissues xenograft tissues. Slides containing CaSKi, Karpas 299, and NCI-H820 xenograft tissues with different fixative type fixation time, and delay to fixation time were stained in duplicate with VENTANA PD-L1 (SP142) assay with BenchMark Ultra instrument in order to identify limitations. Xenograft Array blocks and Xenograft Uniblocks were utilized for this study. For fixative and fixation time, each case was fixed in 10% NBF, Zinc Formalin, 95% Ethanol, AFA, Z-5, and Prefer fixatives for 1, 6, 12, 24, and 72 hours. For delay to fixation, each case had fixation time delayed for 0, 0.5, 1, 2, 6, and 24 hours prior to fixation in 10% NBF. The VENTANA anti-PD-L1 (SP142) Rabbit Monoclonal Primary Antibody demonstrated loss of stain intensity in tissues that are under fixed (less than 6 hours). Prefer fixative showed diminished staining and should not be used as a fixative for anti-PD-L1. There was no degradation of PD-L1 antigenicity in response to delay in fixation with VENTANA anti-PD-L1 (SP142) Rabbit Monoclonal Primary Antibody. All time points in delay to fixation scored the same as immediate fixation for each of the three Xenograft cell lines.

7. Impact of Tissue Thickness

The objective of this study was to evaluate the staining performance of the VENTANA anti-PD-L1 (SP142) Assay on urothelial cancer tissues sectioned at various thicknesses (2 to 7 microns) when assessing around a 5% diagnostic cut-off. Duplicate slides from five urothelial cancer cases (2 PD-L1 < 5%, 2 PD-L1 > 5%, 1 PD-L1 = 5%) were cut at thicknesses of 2, 3, 4, 5, 6 and 7 microns and stained on one BenchMark ULTRA with one lot of antibody and read by one pathologist. The acceptance criteria were that the antibody demonstrate the same PD-L1 status across 90% of urothelial cancer tissue sections. The VENTANA anti-PD-L1 (SP142) Assay met acceptance criteria for tissues sectioned at thicknesses of 2-7 μm.

8. Impact of Cut Slide Stability

The objective of this study was to determine the time point at which degradation of PD-L1 antigenicity in formalin-fixed, paraffin-embedded tissue sections stored under two different storage conditions, 4°C and 30°C, impacts the staining performance of the VENTANA PD-L1 (SP142) Assay on urothelial carcinoma (UC). Cut slide stability was evaluated on UC tissues from five cases with IC staining that spanned a range of PD-L1 expression. 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 the five 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 three months for slides stored at 30°C and four months for slides stored at 4°C.

9. PD-L1 (SP142) Staining in Matched Primary vs. Metastatic Urothelial Cancers

The intent of this study was to characterize the correlation between PD-L1 protein expression in tumor infiltrating immune cells in matched primary and metastatic urothelial cancer tissues using the VENTANA anti-PD-L1 (SP142) Assay since patients were enrolled into the clinical trial on the basis of PD-L1 testing of either primary or metastatic tumor tissue. Sections (4μm) of paraffin embedded tissue from 50 urothelial cancer primary cases and 50 matched metastatic cases were used in the study. Slides were stained in duplicate with VENTANA PD-L1 (SP142) Assay and scored by a single pathologist according to the scoring criteria in Table 3. Forty seven matched pairs of tissues were evaluable and 13/47 (27.7%) give discordant results at the 5% cutoff (Table 9). Nine of those discordant resulted in the metastatic tumor being considered positive and the primary tumor was considered negative. Four of the discordant results resulted in the metastatic tumor being considered negative while the primary tumor was considered negative.

Table 9: Comparison of PD-L1(SP142) positivity of primary tumors and paired metastases.

		PD-L1 (SP142) positivity of Primary Tumor	
		<5% IC N (%)	≥ 5% IC N (%)
PD-L1 (SP142) Positivity of Metastatic Tumor	<5% IC	26 (55.3%)	4 (8.5%)
	≥ 5% IC	9 (19.1%)	8 (17.0%)
Tissues were scored by a single pathologist according to criteria in Table 3.			

10. Impact of Tissue Block and Intra-Case Heterogeneity

The objective of this protocol was to identify and analyze existing UC heterogeneity data for both block (throughout a block) and case (multiple blocks from the same case). Eight blocks were included in the block heterogeneity study based on the scoring criteria in Table 3. For case heterogeneity, 22 cases with multiple blocks were used. For block heterogeneity, 0/8 (0%) showed heterogeneity around the 5% cutoff. For case heterogeneity, 2/22 (9.1%) showed heterogeneity around the 5% cutoff.

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 (SP142) 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 placed at 2-8°C for the duration of the study.
c. Product held at 15±5°C for 192±5 hours, and then placed at 2-8°C for the duration of the study.
d. Product held at -20±5°C for 192±5 hours, then placed at 2-8°C for the duration of the study.

Four urothelial carcinoma and 4 non-small cell lung carcinoma tissues were used for this study. Tissues are tested in triplicate at each time point. Testing was performed at 0, 3, 6 and 9 months. All lots passed for all conditions and support a 7 month stability claim.

These data also demonstrate that the VENTANA PD-L1 (SP142) Assay antibody is stable for up to eight days at 30^0 C. Assay performance beyond these limits has not been established.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Study Design

Study 1, was a multicenter, open-label, two-cohort trial designed to evaluate the efficacy of TECENTRIQ in patients with locally advanced or metastatic urothelial carcinoma. The efficacy of PD-L1(SP142) Assay was evaluated in Cohort 2 of study 1. This was a non-randomized study of TECENTRIQ on 310 patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following a platinum-containing chemotherapy regimen in the metastatic setting, or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen. Tumor specimens from these patients were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used to define subgroups based on PD-L1 expression for pre-specified analyses. If multiple biopsies were submitted for a patient, the result with the higher estimate of PD-L1 staining was used to define the subgroups. Regardless of PD-L1 subgroup status, all patients received an intravenous infusion of 1200 mg of TECENTRIQ every 3 weeks until unacceptable toxicity or symptomatic disease progression. Treatment beyond radiographic disease progression was permitted for patients experiencing ongoing clinical benefit if disease-related symptoms and tumor growth at critical anatomical sites were controlled.

1. Clinical Inclusion and Exclusion Criteria

   Enrollment in the cohort 2 of Study 1 was limited to patients who met the following inclusion criteria:

   - Adult (>18 years)
   - Histologically or cytologically documented locally advanced (TNM stage T4b, any N; or any T, N 2-3) or metastatic (M1, Stage IV) transitional cell carcinoma of the urothelium (including renal pelvis, ureters, urinary bladder, urethra)
   - Patients have had disease progression during or following a platinum-containing chemotherapy regimen or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen
   - ECOG performance status of 0 or 1
   - Life expectancy $\geq$ 12 weeks
   - Measurable disease, as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1
   - Appropriate Tissue Specimen for PD-L1 testing
     - Specimen had to be an archival or fresh representative urothelial tumor specimen with sufficient viable tumor content.
     - Acceptable samples included core needle biopsies for deep tumor tissue (at least 3 cores) or excisional, incisional, punch, or forceps biopsies for cutaneous, subcutaneous, or mucosal lesions.
     - Specimen had to be a formalin-fixed, paraffin embedded (FFPE) tissue block (preferred) or consist of at least 15 unstained slides,

with an associated pathology report. If fewer than 15 slides were available at baseline (but no fewer than 10), the specimen may still have been eligible, upon discussion with the Medical Monitor.

Patients were not permitted to enroll in the cohort 2 of study 1 if they met any of the following exclusion criteria:

- a history of autoimmune disease
- active or corticosteroid-dependent brain metastases
- administration of a live, attenuated vaccine within 28 days prior to enrollment
- administration of systemic immunostimulatory agents or systemic immunosuppressive medications

2. Follow-up Schedule

Patients were followed for efficacy:
Tumor response assessments were conducted every 9 weeks for the first 54 weeks and every 12 weeks thereafter. Major efficacy outcome measures included confirmed objective response rate (ORR) as assessed by independent review facility (IRF) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1) and duration of response (DOR).

Patients were followed 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

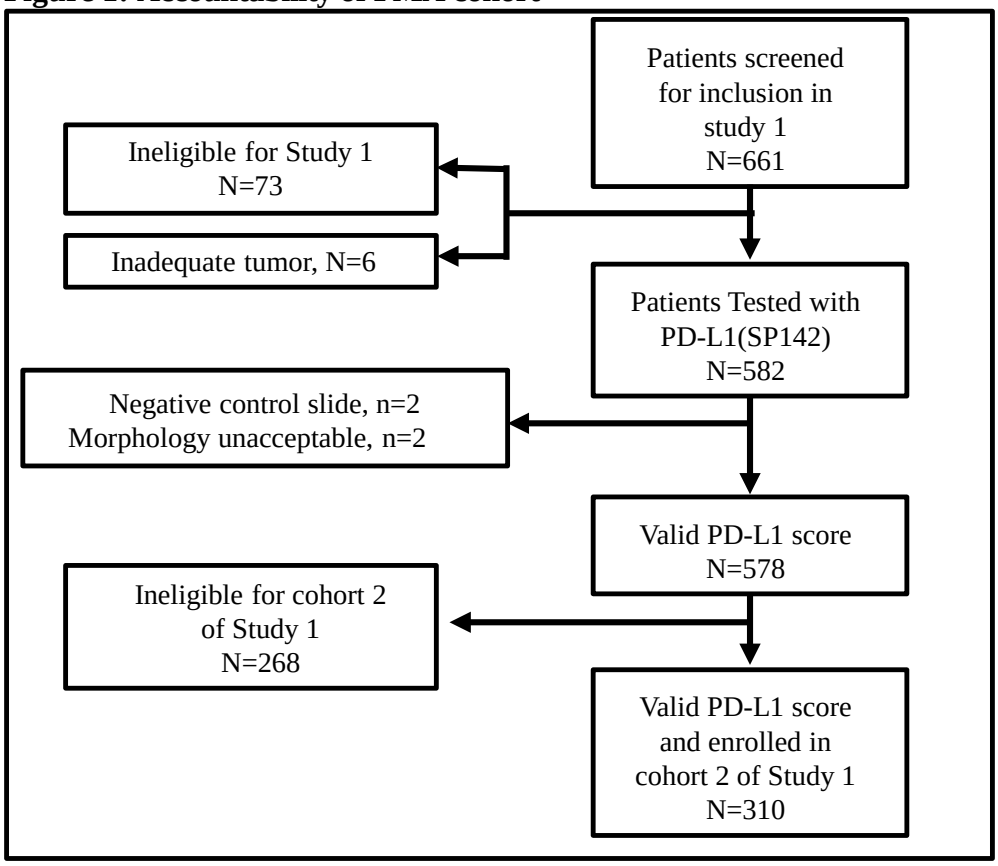
With regards to effectiveness, the primary efficacy endpoint of this study was ORR, defined as the proportion of patients whose confirmed best overall response is either a PR or CR based upon two distinct methods: (1) IRF-assessed per RECIST v1.1 and (2) investigator-assessed per modified RECIST (immune-related criteria). For the first method, OR is defined as a confirmed CR or PR as determined by the IRF per RECIST v 1.1. RECIST v1.1 criteria.

With regards to safety, safety was assessed for adverse events, including protocol-defined events of special interest, changes in laboratory test results, changes in vital signs, and exposure to TECENTRIQ.

B. Accountability of PMA Cohort

A total of 661 patients were evaluated for inclusion in Study 1 (Figure 1). Seventy three patients did not meet inclusion/exclusion criteria for Study 1, and 6 patients did not have adequate tumor tissue for evaluation per the pathologist's review of the H&E slide and were excluded. The remaining 582 were selected to be tested with the VENTANA PD-L1 (SP142) Assay. Tissues from 2 patients had unacceptable staining on the negative control reagent slide, and 2 had unacceptable morphology. Five hundred and sevety eight patients had a valid PD-L1 score. A total of 310 of the 578 were eligible to be enrolled in cohort 2 of study 1.

Figure 1: Accountability of PMA cohort



Patients could submit more than one biopsy for this study. A total of 650 specimens from 582 patients of the intent to diagnose (ITD) population of Study 1 were screened with the VENTANA PD-L1 (SP142) Assay. Patient specimens were FFPE urothelial carcinoma tissue from biopsies (21.2%), resections (46.6%), transurethral resection of

bladder tumor (TURBT, 30.5%), or of unknown type (1.6%); 74.9% were from primary tumors and 25.1% from metastatic tumors.

C. Study Population Demographics and Baseline Parameters (Cohort 2 of Study 1)

There were 310 patients in cohort 2 of study 1. Of the 310 patients, 32% were characterized as having ≥ 5% PD-L1 IC expression while 68% were characterized as having < 5% PD-L1 IC expression. The median age of the cohort was 66 years, 78% were male, 91% patients were Caucasian. Twenty-six percent had non-bladder urothelial carcinoma and 78% of patients had visceral metastases, including, but not limited to metastases to the lungs, liver and bone. Sixty-two percent of patients had an ECOG score of 1 and 34% of patients had a baseline creatinine clearance of < 60 mL/min. Nineteen percent of patients had disease progression following prior platinum-containing neoadjuvant or adjuvant chemotherapy. Forty-one percent of patients had received ≥ 2 prior systemic regimens in the metastatic setting. Seventy-three percent of patients received prior cisplatin, 26% had prior carboplatin, and 1% were treated with other platinum-based regimens.

D. Safety and Effectiveness Results

1. Safety Results

   As an in vitro diagnostic test, the VENTANA PD-L1 (SP142) 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.

2. Effectiveness Results

   Six hundred and fifty patient specimens from cohort 1 were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD L1 expression. VENTANA PD-L1 (SP142) Assay demonstrated initial (i.e., first-pass) and final overall staining acceptability rates; 96.2% and 98.0%, respectively. Final morphology and background acceptability rates were greater than 99%.

   Major efficacy outcome measures included confirmed objective response rate (ORR) as assessed by independent review facility (IRF) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1) and duration of response (DOR). Confirmed ORR in all patients and the two PD-L1 subgroups are summarized in Table 10. The median follow-up time for this cohort was 14.4 months. In 59 patients with disease progression following neoadjuvant or adjuvant therapy, the ORR was 22.0% (95% CI: 12.3%, 34.7%). PD-L1 expression in ≥ 5% IC is associated with increased ORR.

Table 10. Summary of Efficacy from Cohort 2 of Study 1

	All Patients (N = 310)	PD-L1 Expression Subgroups	
		< 5%[1] (N = 210)	≥ 5%[1] (N = 100)
Number of IRF-assessed Confirmed Responders	46	20	26
ORR % (95% CI)	14.80% (11.1, 19.3)	9.50% (5.9, 14.3)	26.00% (17.7, 35.7)
Complete Response (CR) (%)	5.50%	2.40%	12.00%
Partial Response (PR) (%)	9.40%	7.10%	14.00%
Median DOR, months (range)	NR (2.1+, 13.8+)	12.7 (2.1+, 12.7)	NR (4.2, 13.8+)

The median follow-up time for this cohort was 14.4 months.
NR= Not reached
+ Denotes a censored value
[1] PD-L1 expression in tumor-infiltrating immune cells (IC)

Median DOR in all patients was not reached at the time of data cutoff for the ORR analysis. In the 46 responding patients, response durations ranged from 2.1+ to 13.8+ months. Of these patients, 87% had ongoing responses of ≥ 6 months. Among 43 responders with ≥ 12 months of follow-up, 86% (37/43) had ongoing responses (range: 12.4, 17.2 months). Response durations in the PD-L1 subgroups were similar to that in all patients.

The VENTANA anti-PD-L1 (SP142) Assay is intended to be used on either primary tumor tissue or metastases. Concordance of the VENTANA anti-PD-L1 (SP142) Assay PD-L1 comparing matched primary and metastatic urothelial cancers found 13/47 (27.7%) pairs gave discordant results at the 5% cutoff. As an exploratory analysis, efficacy data from cohort 2 of study 1 was stratified by source of biopsy (tumor vs. metastatic). For Cohort 2 of study 1, PD-L1 subgroups were defined based on primary tumor for 233/311 (74.9%) patients and based on metastatic tumor for 78/311 (25.1%) of patients. For patients in which primary tissue was used for PD-L1 status, 78/233 (33.4%) of patients were considered to have PD-L1 expression

≥ 5%. For patients in which metastatic tissue was used for PD-L1 status, 22/78 (28.2%) were considered to have PD-L1 expression ≥ 5%. 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.

Cohort 2 of Study 1 was a non-randomized trial in which all patients received TECENTRIQ. Therefore, is not possible to distinguish if the associations between PD-L1 expression and ORR are predictive and/or prognostic.

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 because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical performance of VENTANA PD-L1 (SP142) Assay was investigated in a non-randomized, a multicenter, open-label, trial designed to evaluate the efficacy of TECENTRIQ in patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following a platinum-containing chemotherapy regimen in the metastatic setting or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen. PD-L1 expression in ≥ 5% IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is associated with increased objective response rate (ORR) from TECENTRIQ in this trial.

The performance of the VENTANA PD-L1 (SP142) Assay was also supported by the analytical validation studies.

B. Safety Conclusions

The VENTANA PD-L1 (SP142) 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 on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for diagnosis.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in a clinical study conducted to support PMA approval as described above. The results from the VENTANA PD-L1 (SP142) assay are associated with ORR in patients treated with TECENTRIQ (atezolizumab) and may help physicians determine the best treatment regimen and tailor therapies accordingly. The main risk of the VENTANA PD-L1 (SP142) 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 with either PD-L1 $\geq$ 5% or PD-L1 <5% may receive a benefit from TECENTRIQ, the risks caused by a false result is relatively low.

In conclusion, given the available information above, the data support that, for patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following a platinum-containing chemotherapy regimen in the metastatic setting or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen, the probable benefits outweigh the probable risks.

Patient Perspective Information

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

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. The provided studies support the use of the VENTANA PL-L1 (SP142) assay in urothelial carcinoma patients who are being considered for treatment with atezolizumab.

XIII. CDRH DECISION

CDRH issued an approval order on May 18, 2016. The final conditions of approval cited in the approval order are described below.

Abbreviated study design and limited numbers of samples from the intended use specimen type were used in repeatability and precision studies for the PD-L1 (SP142) Assay in urothelial carcinoma. Additional testing of samples and analyses with

appropriate study design is required for a more robust characterization of these analytical validation studies of your device. The results from these studies will be included in the labeling.

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 Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.