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:	PD-L1 IHC 22C3 pharmDx
Device Procode:	PLS
Applicant's Name and Address:	Dako North America, Inc. 6392 Via Real Carpinteria, CA 93013
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P150013/S011
Date of FDA Notice of Approval:	August 16, 2018

The original PMA (P150013) for PD-L1 IHC 22C3 pharmDx intended to detect PD-L1 protein in non-small cell lung cancer (NSCLC) was approved on October 2, 2015. In supplement S001 the indication of use for NSCLC was expanded with analytical and clinical validation of an additional cut off. Subsequently, two more indications were approved for gastric and gastroesophageal junction adenocarcinomas (S006) on September 22, 2017 and cervical cancer (S009) on June 12, 2018. The SSED for these indications are available on the CDRH website and is incorporated by reference here. The current supplement was submitted to expand the indication for the PD-L1 IHC 22C3 pharmDx to include urothelial carcinoma (referred to as UC throughout this SSED).

II. INDICATIONS FOR USE

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and gastric or gastroesophageal junction (GEJ) adenocarcinoma, cervical cancer and urothelial carcinoma tissues using EnVision FLEX visualization system on Autostainer Link 48.

Non-Small Cell Lung Cancer (NSCLC)
PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The specimen should be considered to have PD-L1 expression if TPS $\geq$1% and high PD-L1 expression if TPS $\geq$50%.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA® product label for expression cutoff values guiding therapy in specific clinical circumstances.

Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma
PD-L1 protein expression in gastric or GEJ adenocarcinoma is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS $\geq$ 1.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with KEYTRUDA® (pembrolizumab).

Cervical Cancer
PD-L1 protein expression in cervical cancer is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS $\geq$ 1.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying cervical cancer patients for treatment with KEYTRUDA® (pembrolizumab).

Urothelial Carcinoma
PD-L1 protein expression in urothelial carcinoma is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS $\geq$ 10.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying urothelial carcinoma patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA® product label for specific clinical circumstances guiding PD-L1 testing.

III. CONTRAINDICATIONS

There are no known contraindications for the use of this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the PD-L1 IHC 22C3 pharmDx product labeling.

V. DEVICE DESCRIPTION

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical staining procedure for formalin-fixed and paraffin-embedded (FFPE) specimens using the Dako Autostainer Link 48 automated staining and the

EnVision FLEX visualization system. The principle component of the kit is the mouse monoclonal anti PD- L1 clone 22C3 antibody that binds to PD-L1 protein expressed on FFPE tissue. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and reagents shown in Table 1 necessary to perform 50 tests in up to 15 individual runs. Wash buffer and hematoxylin are required for the assay but not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods.

Table 1. Overview of PD-L1 IHC 22C3 pharmDx Components

Reagent	Description
Peroxidase Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015mol/L sodium azide.
Monoclonal Mouse anti–PD-L1, Clone 22C3	Monoclonal mouse anti-PD-L1 antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.
Negative Control Reagent	Monoclonal mouse control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015mol/L sodium azide.
Visualization Reagent-HRP	Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution containing stabilizing protein and an antimicrobial agent.
DAB+ Buffered Substrate	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride in an organic solvent.
DAB Enhancer	Cupric sulfate in water.
Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.
Cell Line Control Slides	Each slide contains sections of two pelleted, formalin-fixed paraffin-embedded cell lines: NCI-H226 with moderate PD-L1 protein expression and MCF-7 with negative PD-L1 protein expression.

Device Instrumentation and Software

PD-L1 IHC 22C3 pharmDx assay is performed on the Dako Autostainer Link 48 automated staining system using the DakoLink software 4.0.3 or later. The Autostainer system is designed to mimic the staining steps performed manually by a lab technician. The PD-L1 IHC 22C3 pharmDx protocol is assay specific. The DakoLink software has

been designed to recognize and group PD-L1 IHC 22C3 pharmDx reagents, requiring that all system reagents are used together. Deparaffinization, rehydration and target retrieval (3-in-1) procedures are performed in the PT Link Pre-treatment module (PT100/200 modules).

Specimen Preparation

Urothelial Carcinoma specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens.

Formalin-fixed, paraffin-embedded tissues are suitable for use. Alternative fixatives have not been validated and may give erroneous results. Fixation time for 12-72 hours in 10% neutral buffered formalin (NBF) is recommended. Fixation times of ≤3 hours may result in variable PD-L1 detection. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in formalin and dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C.

Tissue specimens should be cut into sections of 4-5 µm, mounted on charged microscope slides, and then placed in a 58 ± °C oven for 1 hour. To preserve antigenicity, tissue sections, once mounted on slides, should be held in the dark at 2-8 °C (preferred), or at room temperature up to 25°C, and stained within 1 months of sectioning. Slide storage and handling conditions should not exceed 25°C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls and Calibrators

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

1) Control cell line slides provided as part of the kit should be used to verify the staining procedure. One Control Slide should be stained with the primary antibody to PD-L1 in each staining run. Each slide contains sections of 2 pelleted, FFPE cell lines: one with moderate PD-L1 protein expression and one that is negative for PD-L1 expression. The evaluation of the Control Slide cell lines supplied in the kit indicates the validity of the staining run. The Control Slides should not be used as an aid in interpretation of patient results.

2) Run controls are to be provided by the end-user laboratory. Positive and negative run controls should be fresh biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). The positive control tissue should include weak staining for PD-L1 to detect subtle changes in assay sensitivity. Negative control tissue

is required to detect unintended antibody cross reactivity to tissue and is expected to be negative for PD-L1 expression.

3) The Kit includes a Negative Control Reagent that is used in parallel with the PD-L1 Clone 22C3 primary antibody on patient tissue. The matched negative control aids the reader in differentiating a true signal from tissue-specific background staining that occurs from reaction with detection chemistry and not the anti PD-L1 primary antibody.

Additional information about the use of controls is available in the product labeling.

Principle of Operation

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an IHC staining procedure on FFPE specimens using the Autostainer Link 48. Following deparaffinization of the tissue sections, rehydration and target retrieval, the slides are incubated with the primary monoclonal antibody to PD-L1 (Clone 22C3) or the Negative Control Reagent. The slides are then incubated with an anti-mouse Linker antibody, which is specific to the host species of the primary antibody. Following this, the slides are incubated with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added DAB+ Chromogen results in precipitation of a visible reaction product at the antigen sites. The color of the chromogenic reaction is modified by a chromogen enhancement reagent, DAB Enhancer. The specimen may then be counterstained with hematoxylin and cover-slipped.

Staining Procedure:

The PD-L1 IHC 22C3 pharmDx is designed to be run on the Autostainer Link 48 with DakoLink software.

The staining protocol on the Autostainer Link 48 is as follows:

Peroxidase-Blocking Reagent (2 drop zones x150µL): 5 minutes (± 1 minute)
Rinse in buffer
Monoclonal Mouse anti-PD-L1 (or Negative Control Reagent) (2 drop zones x150µL): 30 minutes (± 1 minute)
Rinse in buffer
Linker, anti-Mouse Ig (2 drop zones x150µL): 30 minutes (± 1 minute)
Rinse in buffer
Visualization Reagent (2 drop zones x150µL): 30 minutes (± 1 minute)
Rinse in buffer: 5 minutes
DAB+ solution (2 drop zones x150µL): 2 x 5 minutes (± 1 minute)
Rinse in buffer
DAB+ Enhancer (2 drop zones x150µL): 5 minutes (± 1 minute)
Rinse in buffer
Hematoxylin (2x150µL): 5 minutes (± 1 minute)

Rinse in deionized water
Rinse in buffer: 5 minutes
Rinse in deionized water
Remove slides from autostainer and place in bath of reagent water

Interpretation of PD-L1 Staining

The labeling instructs that all viable tumor cells on the entire tissue must be evaluated and included in PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation.

The labeling instructs that slide evaluation must be performed by a pathologist using a light microscope. For evaluation of the immunohistochemical staining, an objective of 10-20x magnification is appropriate. For determination of PD-L1 expression, an objective of 20x magnification is required.

Assessment of PD-L1 expression in urothelial carcinoma includes:

- Any partial or complete linear membrane staining (at any intensity) of tumor cells that is perceived distinct from cytoplasmic staining
- Any membrane and/or cytoplasmic staining (at any intensity) of tumor associated lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma.

Tumor PD-L1 expression in urothelial carcinoma specimens is determined by Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, macrophages, lymphocytes) divided by the total number of all viable tumor cells, multiplied by 100. Distinction of viable tumor cells, lymphocytes, and macrophages is essential for accurate denominator estimation. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100. CPS is defined as follows:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# viable tumor cells}} \times 100$$

The table below provides details about which tissue elements are included in and excluded from the CPS numerator in urothelial carcinoma.

Table 2. Tissue Elements in Determining the Combined Positive Score

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable urothelial carcinoma tumor cells including: • High grade papillary carcinoma • Carcinoma in situ (CIS) • Any lamina propria, muscularis, or serosal invasion • Metastatic carcinoma	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Low grade papillary carcinoma†
Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma.** • Lymphocytes (including lymphocyte aggregates) • Macrophages*** Only MICs directly associated with the response to the tumor are scored.	• Non-staining MICs • MICs (including lymphoid aggregates) associated with ulcers, chronic cystitis, and other processes not associated with the tumor and • MICs associated with normal structures • Neutrophils, eosinophils and plasma cells • BCG††-induced granulomas
Other Cells	Not included	• Normal cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

In **MICs membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs is included in the score.*
******Adjacent MICs*** *are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response to the tumor should be excluded.*
****Macrophages and histiocytes are considered the same cells.*
†If the tumor consists entirely of low grade papillary carcinoma, the result should be flagged as such
††bacillus Calmette- Guérin

For each staining run, slides should be examined in the order recommended in the product labeling. The labeling instructs users to examine patient specimens stained with PD-L1 and the Negative Control Reagent from PD-L1 IHC 22C3 pharmDx when evaluating PD-L1 expression. Specimens stained with NCR must have 0 specific staining and ≤ 1+ nonspecific staining.

The specimen should be considered to have PD-L1 expression if CPS ≥ 10.

	Combined Positive Score	
PD-L1 Expression Levels	**CPS < 10**	**CPS ≥ 10**
PD-L1 Expression Status	No PD-L1 Expression	PD-L1 Expression

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay available for use as an aid in identifying patients with urothelial carcinoma for treatment with KEYTRUDA (pembrolizumab).

VII. MARKETING HISTORY

PD-L1 IHC 22C3 pharmDx has been marketed in the United States since the approval of P150013 in October 2, 2015. PD-L1 IHC 22C3 pharmDx has also been marketed in Albania, Argentina, Austria, Algeria, Australia, Bahrain, Belgium, Bosnia and Herzegovina, Brazil, Bulgaria, Canada, Chile, Colombia, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, Ireland, Iraq, India, Indonesia, Israel, Italy, Japan, Jordan, South Korea, Kazakhstan, Kosovo, Kuwait, Lebanon, Latvia, Lithuania, Lichtenstein, Luxembourg, Macedonia, Malta, Malaysia, Montenegro, Morocco, Mexico, Netherlands, New Zealand, Norway, Oman, Panama, Peru, Philippines, Poland, Portugal, Qatar, Romania, Russia, Saudi Arabia, Singapore, Slovakia, Slovenia, Serbia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, Ukraine, United Arab Emirates, the United Kingdom, Vietnam, and Uruguay.

This device has not been withdrawn from marketing for any reason related to safety and effectiveness."

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 subsequently improper assignment of treatment with KEYTRUDA®. Patients with a false negative assay result may not be considered for treatment with KEYTRUDA (pembrolizumab). Patients with a false positive assay result may receive treatment with KEYTRUDA (pembrolizumab) for which there is no expectation of benefit and exposure to potential toxicity. There is also a risk of delayed results, which may lead to delay in treatment.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Preclinical studies were performed using the PD-L1 IHC 22C3 pharmDx kit to establish analytical performance of the device. The scoring algorithm used in these studies included a clinical score (i.e., PD-L1 positive or negative) and/or analytical score (CPS 0-100). Binary outcomes were assessed for all studies with the scoring algorithm developed for clinical interpretation of the PD-L1 22C3 IHC Assay. Continuous scores

were reported for some studies to ensure assay performance in borderline cases. Antibody characterization studies for clone 22C3, including specificity and tour of body/ tour of tumor, control cell line validation, kit stability and preanalytical variables were submitted and reviewed in the original PMA (P150013) for this device. Study designs and results are available in the Summary of Safety and Effectiveness Data: https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013B.pdf. Results from studies performed to support the urothelial cancer indication are summarized in the sections below.

1. Analytical Specificity

Assessment of analytical specificity of the monoclonal mouse anti -human PD-L1 clone 22C3 antibody was reviewed under P150013 and included Western blot and immunoreactivity in human tissues, both normal and tumor.

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 104 unique urothelial cancer FFPE tissue specimens using one lot of the device. The specimens were chosen at random and represented the full range of PD-L1 expression and staining intensity (i.e., CPS range 0-100). One specimen was not evaluable due to high background staining. The percentage of PD-L1 positive cases with CPS expression level ≥10 (n = 38) was 36.5%.

3. Repeatability

The objective of this study was to demonstrate that PD-L1 IHC 22C3 pharmDx would produce consistent staining in normal day-to-day testing of UC specimens with multiple lots of test kit.

Precision was assessed in 3 separate studies: intra-run, combined precision (inter-instrument/operator/day/lot) and reader precision. Intra-day/run and combined precision studies were performed with 46 UC specimens spanning the range of PD-L1 expression, and at least 25% of these represented specimens around the CPS ≥ 10 cut off. Near cut-off specimens was defined as specimens with CPS score >1 and <20. The intra-run study was performed with 32 UC specimens, and Reader precision studies included 60 unique UC specimens with 19 around the CPS ≥ 1 cut off. Study specimens along with control slides were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Specimens were determined to be positive if PD-L1 expression was at CPS ≥10, or negative if PD-L1 expression was at CPS <10. Statistical analysis using pair-wise analysis was used to calculate average negative agreement (NPA), positive agreement (PPA), and overall agreement (OA) were computed with two-sided 95%

confidence intervals using the bootstrap method for the CPS ≥ 10 cutoff as shown in Table 3. The results met the pre-specified acceptance criteria (i.e., 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%).

Table 3. Summary of Repeatability

Precision Endpoint	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-Operator, Inter-Instrument, Inter-Day, and Inter-Lot as combined variables)	CPS ≥ 10	Each of 46 urothelial carcinoma specimens (26 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression was tested using three operators, on three Autostainer Link 48 instruments, over three non-consecutive days, using three reagent lots.	NPA 96.2% (92.3-100%) PPA 98.3% (95.0-100%) OA 97.1% (94.2-99.3%)
Intra-run* (Repeatability)	CPS ≥ 10	Each of 32 urothelial carcinoma specimens (17 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA 100% (95.7-100%) PPA 96.0% (92-100%) OA 98.1% (96.2-100%)
Inter-observer precision	CPS ≥ 10	60 urothelial carcinoma specimens (28 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days with a 2 week washout between reads.	NPA 95.2% (90.3-99.2%) PPA 94.1% (89.9-97.6%) OA 94.6% (91.4-97.4%)
Intra-observer precision	CPS ≥ 10	60 urothelial carcinoma specimens (28 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days.	NPA 96.8% (94.3-99.2%) PPA 96.5% (94.1-98.6%) OA 96.7% (94.8-98.3%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson Score method was used to compute confidence intervals for Intra-run precision NPA agreement. Note that the Wilson Score method has limitations as it assumes independence of data. Since one specimen contributes more than one comparison to majority call, , the data are not independent.

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4. External Reproducibility

Reproducibility studies for UC were designed to evaluate the performance of PD-L1 IHC 22C3 pharmDx for PD-L1 detection across laboratories on the Dako Autostainer Link 48.

Reproducibility was assessed with 36 specimens (16 positive and 20 negative including 14 around cut off) being tested across 3 sites over 5 non consecutive days. The study included specimens that were pre-qualified at Dako to represent full PD-L1 expression range and a minimum of 25% of the specimens were around cut off. The specimen set was randomized and blinded prior to testing at 3 external reproducibility sites and assessed for performance with regard to site-to-site and day-to-day reproducibility, or inter and intra-site reproducibility.

Reproducibility studies also included assessment of observer-to-observer variability with specimens that spanned the expression range of PD-L1. These specimens were stained at the Dako facility and shipped to the 3 external reproducibility sites for assessment of PD-L1 expression by pathologists for both inter-observer and intra-observer reproducibility. Sixty (60) pre-stained specimens (with a minimum of 25% near the CPS ≥ 10 cut off) were assessed 3 times by 3 readers with a two week washout between reads. All IHC tests were interpreted by certified clinical pathologists to determine the positive/negative results based on the CPS ≥10 expression levels at three external sites. NPA, PPA and OA and 95% CI was calculated by pairwise comparison to the majority call as reference. The results of the reproducibility studies are included in Table 4. The results met the pre-specified acceptance criteria (i.e., 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%) except for the interlaboratory study. It was determined that the results were due to one pathologist scoring high negative specimens at the cut-off as positive. Because the cut-off specimens were over-represented and the overall performance and historical performance passed the acceptance criteria, the results were determined to be acceptable.

Table 4. Three Site Reproducibility in UC

Reproducibility Endpoint	**Cutoff**	**Study Design**	**% Agreement (95% CI)**
Inter-site	CPS ≥ 10	Each of 36 urothelial carcinoma specimens (20 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 539 comparisons to majority call.	NPA 94.0% (87.7-99.3%) PPA 84.6% (77.1-91.7%) OA 89.8% (85.0-94.1%)
Intra-site	CPS ≥ 10	Each of 36 urothelial carcinoma specimens (20 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 539 comparisons to majority call.	NPA 96.2% (92.9-98.8%) PPA 95.0% (92.4-97.4%) OA 95.7% (93.5-97.6%)

Reproducibility Endpoint	Cutoff	Study Design	% Agreement (95% CI)
Inter-observer	CPS ≥ 10	Scoring of 60 urothelial carcinoma specimens (29 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Four wild card specimens (4 at each site) were also scored but not included in final data analysis. Inter-observer analysis was performed between three sites on a total of 540 comparisons to majority call.	NPA 97.3% (94.3-99.6%) PPA 90.7% (86.4-94.6%) OA 93.9% (91.3-96.3%)
Intra-observer	CPS ≥ 10	Scoring of 60 urothelial carcinoma specimens (29 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 540 comparisons to majority call.	NPA 95.7% (93.8-97.8%) PPA 96.1% (93.6-98.3%) OA 95.9% (94.3-97.4%)
NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Agreement; CPS=Combined Positive Score			

5. **Robustness Studies:**
Robustness of the staining performance of PD-L1 IHC 22C3 pharmDx in UC was evaluated by testing the performance of the assay when varying the following conditions as described below. On one lot of reagents was assessed.

- Tissue sections cut at three thicknesses:
    - 3 µm
    - 4 µm
    - 5 µm
- Tissue sections mounted on two different types of microscope slides:
    - Fisherbrand™ Superfrost™ Plus
    - Dako FLEX IHC Microscope Slides (Dako code K8020)
- Target Retrieval Time at three incubation times
    - 18 minutes
    - 20 minutes-standard
    - 22 minutes
- Target Retrieval Temperature at three incubation temperatures
    - 95°C
    - 97°C -standard
    - 99°C
- Target Retrieval Solution pH at three pH levels
    - pH 5.9
    - pH 6.1-standard
    - pH 6.3

Tissue thickness and slide type studies included at least 29 UC specimens spanning the range of PD-L1 expression and included a minimum of 20% of specimens around the cut off. Target retrieval studies were performed with a minimum of 26 UC specimens and included a minimum of 20% specimens around the CPS ≥10 cut off. Staining performance was evaluated for both CPS score and intensity of staining. NPA, PPA and OA for pairwise comparison against the reference condition and the 95% CI calculated with Bootstrap method. Acceptance criteria for the study specified that lower bound of the 95% CI would meet or exceed 85% for each condition tested. The study passed acceptance criteria and no significant difference in results was observed for any of the recommended experimental conditions above.

6. Impact of Intra-Case Heterogeneity

The objective of these studies was to investigate whether tumor heterogeneity affects PD-L1 IHC staining results with PD-L1 IHC 22C3 pharmDx.

a. Multiple FFPE Blocks from the Same Subjects (Variability in PD-L1 expression between anatomic sites within patients)

Multiple blocks (at least 2) of 18 UC subjects obtained from the same tumor were evaluated to demonstrate within-patient concordance for PD-L1 status. In 17 of 18 sets of UC intra-subject specimens, (7 were <10%, 10 were ≥10%) the diagnostic outcomes were concordant across sister tissue blocks. One case showed discordant results at the CPS ≥10 expression level. Four of the evaluable 18 cases were near cut-off pairs (1-20%). Results may not be representative of all urothelial cancer specimens, as tumor heterogeneity is unique for each specimen.

b. Intra Block Heterogeneity

Heterogeneity within one urothelial cancer FFPE blocks was assessed. The 1st, 32nd, and 65th cut sections from 36 unique FFPE blocks were stained with PD-L1 IHC 22C3 pharmDx and assessed for PD-L1 expression. Of the 36 specimens, 75% were selected to span the dynamic range of PD-L1 expression and at least 25% of specimens were around the cut off. Equal numbers of positive and negative specimens were enrolled into the study. At the CPS ≥ 10 cut-off, 35 of 36 blocks pairs demonstrated agreement in PD-L1 expression (98.1% overall agreement) among the three cut sections. High overall agreement in PD-L1 expression within individual urothelial cancer blocks was observed to at least 200μm. Results may not be representative of all urothelial cancer specimens, as tumor heterogeneity is unique for each specimen.

7. Stability testing

a. PD-L1 IHC 22C3 pharmDx Stability

The Real-time reagent stability testing was previously performed on PD-L1 IHC 22C3 pharmDx for NSCLC and reviewed in P150013.

b. FFPE Cut Section Stability

A real-time stability study was designed to evaluate the shelf life of cut tissue sections of UC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 3 UC specimens. Based on these studies, stability dating for cut slides in UC is 1 month. A post market study was agreed to in order to support accurate determination of specimen stability when stored at 2-8 °C or 25 °C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

A. Study Design

The clinical performance of PD-L1 IHC 22C3 pharmDx was evaluated in the study Keynote-052 (KN052/ NCT02335424) which is an ongoing, phase 2 single-arm, multi-center, open-label trial of pembrolizumab in the treatment of subjects with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy. Patients with prior platinum based therapy in the neoadjuvant or adjuvant setting with recurrence > 12 months from completion of therapy were permitted to enroll. All subjects were required to provide tissue specimens for PD-L1 testing and assessment of FFPE tumor sample sections for PD-L1 expression was performed centrally.

1. Clinical Inclusion and Exclusion Criteria (abbreviated list)

   Enrollment in the KN052 study was limited to patients who met the following inclusion criteria:

   - Male/female at least 18 years of age.
   - Presence of histologically or cytologically-confirmed diagnosis of advanced/unresectable (inoperable) or metastatic urothelial cancer of the renal pelvis, ureter, bladder, or urethra. Both transitional cell and mixed transitional/non-transitional cell histologies are allowed. Subjects with non-urothelial cancer of the urinary tract are not allowed.
   - Considered cisplatin-ineligible to receive cisplatin-based combination therapy
   - Have received no prior systemic chemotherapy for advanced/unresectable (inoperable) or metastatic urothelial cancer
   - Have provided tissue for biomarker analysis from a newly obtained core or excisional biopsy of a tumor lesion not previously irradiated (mandatory). Adequacy of the biopsy specimen for PD-L1 biomarker analysis must be confirmed by the central laboratory.

- Have measurable disease based on RECIST 1.1 as determined by central review. Tumor lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
- Have a performance status of 0, 1 or 2 on the ECOG Performance Scale

Patients were not permitted to enroll in the KN052 study if they met any of the following exclusion criteria:

- Has disease that is suitable for local therapy administered with curative intent.
- Current or prior receipt of study therapy or prior participation in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
- A prior anti-cancer monoclonal antibody (mAb) for direct anti-neoplastic
- Any known additional malignancy that requires active treatment
- Treatment within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
- Active autoimmune disease that required systemic treatment in the prior 2 years
- HIV positive patients.
- Any prior therapy with an anti-PD-L1, anti-PD-L2 agent, or any agent directed to another co-inhibitory T-cell receptor.

2. Follow-up Schedule
   Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or disease progression. Patients with initial radiographic disease progression could receive additional doses of treatment during confirmation of progression unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, or occurred with a decline in performance status. Patients without disease progression could be treated for up to 24 months. Tumor response assessments were performed at 9 weeks after the first dose, then every 6 weeks for the first year, and then every 12 weeks thereafter. The major efficacy outcome measures were objective response rate (ORR) according to RECIST 1.1 as assessed by independent radiology review and duration of response.

3. Clinical Endpoints
   The primary endpoint of the study was objective response rate (ORR) per RECIST v1.1 as assessed by ICR, defined as the percentage of patients having a complete or partial response during the trial in all subject, PD-L1 positive subjects ad PD-L1 strongly positive subjects (CPS cut off determined from the training set).
   Secondary endpoints included duration of response (DoR), progression-free survival at 6 and 12 months and OS at 6 and 12 months.

B. Accountability of PMA Cohort

At the time of database lock, December 2018, 370 patients were treated after tumor PD-L1 expression status was determined. All treated subjects had a tumor tissue sample. Primary efficacy analysis was based on data from subjects whose tumors expressed PD-L1 at CPS ≥10. Of the 370 enrolled subjects in KN052, 110 subjects had tumors with PD-L1 CPS ≥ 10 expression and 251 subjects had tumors with PD-L1 CPS < 10 expression. Nine subjects had unknown PD-L1 status. Of the 270 enrolled subjects in the KN052 validation set, 80 subjects had tumors with PD-L1 CPS ≥ 10 expression and 185 subjects had tumors with PD-L1 CPS < 10 expression. Five subjects had unknown PD-L1 status. A description of the specimen characteristics is shown in Table 5. Refer to Table 6 for the PD-L1 distribution.

Table 5. Accountability of PMA Cohort in KN052

	Number of Study subjects n (%)
Enrolled	370
Training Set	100
Validation set	270
Quantifiable PD-L1 expression	361
Unevaluable PD-L1 expression	9
Site of Collection:	
Primary Site	194 (71.4%)
Metastatic Site	176(28.6%)
Specimen type	
Biopsy	250
Resection	111
Unknown	9

Table 6: Subject PD-L1 Distribution

PD-L1 Status	Overall study (N=370)	Validation set (N=270)
PD-L1 CPS ≥ 10	110	80
PD-L1 CPS < 10	251	185
Unknown	9	5
Total	370	270

C. Study Population Demographics and Baseline Parameters

In the KN052 trial, the median age was 74 years, 77% were male, and 89% were white. Eighty-seven percent (87%) had M1 disease, and 13% had M0 disease. Eighty-one percent (81%) had a primary tumor in the lower tract, and 19% of patients had a primary tumor in the upper tract. Eighty-five percent (85%) of patients had visceral metastases, including 21% with liver metastases. Reasons for cisplatin ineligibility included: 50% with baseline creatinine clearance of <60 mL/min, 32% with ECOG performance status of 2, 9% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 9% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent (90%) of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

Among the 270 patients in the validation set, 30% (n = 80) had tumors that expressed PD-L1 with CPS ≥ 10. Baseline characteristics of these patients were evaluated for imbalance between the validation set and the all-enrolled set: median age 72 years, 68% male, and 86% white. Seventy-one percent (71%) had M1 disease, and 26% had M0 disease. Seventy-nine percent (79%) had a primary tumor in the lower tract, and 20% of patients had a primary tumor in the upper tract. Seventy-eight percent (78%) of patients had visceral metastases, including 8% with liver metastases. Reasons for cisplatin ineligibility included: 41% with baseline creatinine clearance of <60 mL/min, 43% with ECOG performance status of 2, 11% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 5% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent (90%) of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

D. Safety and Effectiveness Results

1. Safety Results

   FFPE UC sections are routinely removed as part of the practice of medicine for the diagnosis of urothelial cancer by pathologists. Removal of these tissues, therefore, presents no additional safety hazard to the patient being tested. Safety for use of the device for patient management is related to effectiveness (see effectiveness results, below). As compared to the overall study population, no meaningful differences in adverse events from treatment with KEYTRUDA® was observed based on PD-L1 expression level.

2. Effectiveness Results

Clinical performance of PD-L1 IHC 22C3 pharmDx was assessed in the KN052 trial. Of 370 subjects treated in the trial, 370 had tumor specimens tested with the PD-L1 IHC 22C3 pharmDx assay. PD-L1 status was not evaluable for 9 subjects. PD-L1 status was quantifiable for all 361 (97.5%) tumor specimens. The validation set was comprised of 270 subjects of which 80 (30%) had PD-L1 expression of CPS ≥10 and 185 (68.5) subjects with CPS <10 and 5 (1.5%) subject's tumors were not evaluable for PD-L1 expression.

Efficacy in overall population and PD-L1 sub groups

The confirmed objective response rate (ORR) per RECIST 1.1 by blinded independent central review (BICR) assessments is 30% (95% CI: 25, 36) in the overall validation population (n=270), including 17 patients (6.3%) who achieved complete responses. The data from the population of evaluable results for the test validation set was separated by the clinical threshold for PDL1 expression and are summarized in Table 7.

Table 7: Efficacy Results in KEYNOTE-052

	All Validation Subjects n=270	**CPS < 10 in Validation Set (N=185)**	**CPS ≥ 10 in Validation Set (N=80)**
Objective Response Rate			
ORR (95% CI)	30% (25, 36)	22% (16, 28)	51% (40, 63)
Complete response rate	6.3%	2%	16%
Partial response rate	24%	20%	35%
Duration of Response*			
Median in months (range)	NR (1.4+, 17.8+)	9.7 (1.4+ - 11.0+)	NR (1.4+ - 11.1+)

+ Denotes ongoing; NR = not reached

In the validation set of 270 subjects, the ORR among patients with CPS ≥10 and PD-L1 CPS <10 tumors were 51.3% (95% CI: 40, 62.6) and 21.6% (95% CI: 16, 28.3), respectively. A total of 13 patients among the 80 PD-L1 CPS ≥ 10 patients achieved complete responses. Among the PD-L1 CPS < 10 sub-group, 4 of the 185 patients had a complete response.

Median duration of response in PD-L1 CPS ≥ 10 sub-group was not reached (N.R) months (1.4+, 11.1+) while in PD-L1 CPS <10 subgroup was 9.7 months (1.4 +, 11.0+). The difference in ORR for PD-L1 positive and PD-L1 negative subgroups was 27.0% (16.4, 37.4) suggesting a statistically significant difference in response rates for the two sub groups.

3. 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. The pivotal clinical study included 3 investigators. 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.

F. Other Clinical Studies

KEYNOTE-361 (NCT02853305) is an ongoing, multicenter, randomized study in previously untreated patients with metastatic urothelial carcinoma who are eligible for platinum-containing chemotherapy. The study compares KEYTRUDA with or without platinum-based chemotherapy (i.e., cisplatin or carboplatin with gemcitabine) to platinum-based chemotherapy alone. The trial also enrolled a third arm of monotherapy with KEYTRUDA to compare to platinum-based chemotherapy alone. The independent Data Monitoring Committee (iDMC) for the study conducted a review of early data and found that in patients classified as having PD-L1 expression of CPS <10, those treated with KEYTRUDA monotherapy had decreased survival compared to those who received platinum-based chemotherapy. The iDMC recommended to stop further accrual of patients with PD-L1 expression of CPS< 10 in the monotherapy arm, however, no other changes were recommended, including any change of therapy for patients who had already been randomized to and were receiving treatment in the monotherapy arm.

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

In accordance with the provisions of section 515(c) (2) 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

Clinical benefit of PD-L1 IHC 22C3 pharmDx is based upon the results of the KEYNOTE-052 study which was conducted to evaluate the safety and the efficacy of single agent KEYTRUDA (pembrolizumab) in patients with urothelial carcinoma. In

this single-arm study, the PD-L1 IHC 22C3 pharmDx was used to determine PD-L1 expression status of patient tumors. A total of 80 patients (30%) had PD-L1 expression CPS ≥10 umors, 185 patients (68.5%) has PD-L1 expression CPS <10, and 5 patients (1.5%) had unknown PD-L1 status. The data from the trial shows that the ORR in PD-L1 positive patients was 51% (95% CI: 40, 63) with 16% having a complete response and 35% had a partial response. Among the 41 responding patients, the duration of response ranged from 1.4+ to 11.1+ months, with 19 patients (79%) having responses of 6 months or longer. Therefore, the PD-L1 IHC 22C3 pharmDx supported the identification of patients who will benefit from the pembrolizumab when used in accordance with the instructions for use. The safe and effective use of the test supported accelerated approval of KEYTRUDA in this population.

The performance of PD-L1 IHC 22C3 pharmDx was also supported by the analytical validation studies.

B. Safety Conclusions

Safety for use of the device for patient management is related to effectiveness (see effectiveness conclusions, above). In general, risks of the PD-L1 IHC 22C3 pharmDx are associated with failure of the device to perform as expected or failure to correctly interpret test results (see Benefit-Risk Determination, below). The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for urothelial cancer diagnosis.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the clinical study which demonstrated improved ORR and DoR in patients who were PD-L1 positive as determined by the device. The risks of the test are associated with false negative or false positive results which may lead to patients having no benefit from the treatment. The safety and efficacy of KEYTRUDA in PD-L1 positive patients was determined to have clinical benefit when compared to the risks. The analytical validation conducted supports the test as a reliable method for detecting PD-L1 expression.

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

In conclusion, given the available information detailed above, the data supports that, for the urothelial carcinoma patients who are being considered for treatment with KEYTRUDA® (pembrolizumab), the probable benefits of PD-L1 IHC 22C3 pharmDx use 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 and product labeling. The provided studies support use of PD-L1 IHC 22C3 pharmDx as an aid in identifying patients with urothelial cancer for treatment with KEYTRUDA® (pembrolizumab)

XIII. CDRH DECISION

CDRH issued an approval order on August 16, 2018. The final conditions of approval cited in the approval order are described below:

Provide data to establish cut-slide stability for formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma (UC) specimens to support the proposed 6 months claim. This data is necessary to assure the expected performance of your device with cut-slides obtained from FFPE Urothelial Carcinoma specimens for a time frame that may be applicable to clinical laboratories.

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, Warnings, and Precautions in the device labeling.

Post-approval Requirements and Restrictions: See approval order.