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/S016

Date of FDA Notice of Approval: July 30, 2019

The original PMA (P150013) for PD-L1 IHC 22C3 pharmDx was approved on October 2, 2015 for the detection of PD-L1 protein in non-small cell lung cancer (NSCLC) tissue. Subsequently, four additional indications were approved. These are gastric and gastroesophageal junction adenocarcinomas (S006) on September 22, 2017, cervical cancer (S009) on June 12, 2018, urothelial cancer (S011) on August 16, 2018, and head and neck squamous cell carcinoma (S014) on June 10, 2019. 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 Esophageal Squamous Cell Cancer (referred to as ESCC throughout this SSED).

II. INDICATIONS FOR USE

For in vitro diagnostic 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), gastric or gastroesophageal junction (GEJ) adenocarcinoma, esophageal squamous cell carcinoma (ESCC), cervical cancer, urothelial carcinoma and head and neck squamous cell carcinoma

(HNSCC) tissues using EnVision FLEX visualization system on Autostainer Link 48.

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.

PD-L1 protein expression in gastric or GEJ adenocarcinoma, ESCC, cervical cancer, urothelial carcinoma and HNSCC 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.

Companion Diagnostic Indications

Tumor Indication	PD-L1 Expression Level	Intended Use
NSCLC	TPS ≥ 1%	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). **
Gastric or GEJ Adenocarcinoma	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with KEYTRUDA® (pembrolizumab).
ESCC	CPS ≥ 10	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying esophageal squamous cell cancer patients for treatment with KEYTRUDA® (pembrolizumab).
Cervical Cancer	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying cervical cancer patients for treatment with KEYTRUDA® (pembrolizumab).
Urothelial Carcinoma	CPS ≥ 10	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying urothelial carcinoma patients for treatment with KEYTRUDA® (pembrolizumab). **
HNSCC	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying HNSCC 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 kit contains the mouse monoclonal anti PD-L1 clone 22C3 antibody and reagents required to complete an immunohistochemical staining procedure for formalin-fixed and paraffin-embedded (FFPE) specimens using the Dako Autostainer Link 48 with DakoLink software and the EnVision FLEX visualization system. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and reagents 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. Cover-slipping is required but can be performed by either manual or automated methods. An overview of the kit components is shown in Table 1.

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
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

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. 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

ESCC specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens. Only formalin-fixed, paraffin-embedded tissues (FFPE) 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 4-5µm, 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. Information about the use of controls is available in the product labeling. The following controls should 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.

Principle of Operation

PD-L1 IHC 22C3 pharmDx contains 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. If patient specimens include more than one biopsy (i.e., 3-5 biopsies) on a slide, all tissues on the slide need to be evaluated to generate a single CPS for determining the PD- L1 expression level. Each biopsy should not be reported independently.

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

Assessment of PD-L1 expression in ESCC 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 ESCC 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:

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

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 $\leq$ 1+ nonspecific staining.

ESCC specimens are evaluated for PD-L1 expression at CPS $\geq$10 cut off.

Table 2 below provides details about which tissue elements are included in and excluded from the CPS numerator in ESCC.

Table 2. CPS Numerator Inclusion/Exclusion Criteria for ESCC

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable tumor cells including:	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining
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 associated with non-invasive neoplasia • MICs associated with benign structures • MICs (including lymphoid aggregates) not directly associated with the response to the tumor Neutrophils, eosinophils and plasma cells
Other Cells	Not included	• Carcinoma in situ • Benign 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.

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 ESCC for treatment with KEYTRUDA (pembrolizumab).

VII. MARKETING HISTORY

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

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 to establish analytical performance of the device was performed with esophageal cancer (EC) specimens including the adeno and squamous subtypes. 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 ESCC 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 provided and reviewed under P150013 and included Western blot and immunoreactivity in human tissues, both normal and tumor. Refer to the SSED associated with the original PMA for study design and results.

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 unique EC FFPE tissue specimens (including 51 unique ESCC specimens) using one lot of the device. Assessment of PD-L1 expression demonstrated staining across a range of CPS scores from 0 to 100, where 34% of EC specimens and 45% of ESCC had a PD-L1 expression of CPS ≥ 10. Two specimens were not evaluable due to containing fewer than 100 viable tumor cells.

3. Precision

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 ESCC 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 ESCC 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 for CPS≥10.

The intra-run and combined precision studies were performed with 32 EC specimens that included 12 unique ESCC specimens. Study specimens along with control slides were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Inter-/Intra- Observer precision were tested separately with 60 specimens (including 23 unique ESCC specimens). 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. Pre-specified acceptance criteria are 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%. All results met the criteria except for the PPA for the intra-run (repeatability) which had a lower bound 87.5%. It was determined that this was due to the higher than expected number of specimens (45.5%) around the cut-off.

Table 3. Summary of Precision (one site) in EC (including ESCC) for CPS≥ 10

Precision Endpoint	Study Design	% Agreement (95% CI)
Combined Precision (Inter- Operator, Inter- Instrument, Inter-Day, and Inter-Lot as combined variables)	Each of 32 esophageal cancer specimens (15 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression was tested using three operators, on 3 Autostainer Link 48 instruments, over 3 non-consecutive days, using three reagent lots.	NPA 97.8% (93.3-100%) PPA 98.0% (94.1-100%) OA 97.9% (94.8-100%)
Intra-run* (Repeatability)	Each of 32 esophageal cancer specimens (21 PD-L1-negative and 11 PD-L1-positive) with a range of PD-L1 IHC expression was tested with 5 replicates within a run on the Autostainer Link 48	NPA 98.1% (95.2-100%) PPA 92.7% (83.6-100%) OA 96.2% (93.1-98.8%)
Inter-observer precision	59 esophageal cancer specimens (28 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists over 3 non-consecutive days.	NPA 95.1% (90.5-98.8%) PPA 92.4% (87.5-96.8%) OA 93.7% (90.3-96.8%)
Intra-observer precision	60 esophageal cancer 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, were scored by 3 pathologists over 3 non-consecutive days.	NPA 96.2% (93.4-98.8%) PPA 98.5% (96.5-100%) OA 97.3% (95.6-98.9%)

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

4. External Reproducibility

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

Reproducibility was assessed in a two-part study: Study Part A assessing Inter- and Intra-Laboratory endpoints and Study Part B assessing Inter- and Intra-Observer endpoints.

Part A included a set of thirty-six (13 positive and 23 negative including 13 around cut off; 19 unique ESCC specimens were included) stained at three laboratories over 5 non-consecutive days using 1 reagent lot. One observer at each laboratory evaluated the set of specimens for each day.

Inter-reader reproducibility was assessed in the Part B with 3 independent readers evaluating PD-L1 status for pre-stained tissue sections from 64 specimens (29 positive and 31 negative and 22 around cut off including 31 unique ESCC specimens). Four (4) of the 64 specimens were designated as "wildcard" specimens to mitigate recall bias. Each pathologist performed 3 independent reads of the specimen set that included a 14 day washout.

Reproducibility was evaluated using positive/negative diagnostic determinations based on a cutoff of CPS ≥ 10 (where CPS <10 was negative for PD-L1 expression and CPS ≥ 10 was positive for PD-L1 expression). Evaluation of PD-L1 expression was performed according to the IFU of the device and scoring guidelines. NPA, PPA and OA and 95% CI were calculated by pairwise comparison to the majority call as reference. The results of the reproducibility studies are included in Table 4. All studies except the inter-reader reproducibility met the pre-specified acceptance criteria (i.e., 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%) for the CPS ≥ 10 cut off and met all study end points. In order to obtain a more precise estimate of inter-observer reproducibility for this study in the target specimens (ESCC), a post approval study to evaluate reader reproducibility in 60 ESCC was required.

Table 4. Summary of Reproducibility in EC (including ESCC) at Three Sites (CPS ≥ 10)

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 36 esophageal cancer specimens (23 PD-L1-negative and 13 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 540 comparisons to majority call.	NPA 99.7% (99.1-100%) PPA 99.0% (96.9-100%) OA 99.4% (98.5-100%)
Intra-site	Each of 36 esophageal cancer specimens (23 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Intra-site analysis was performed for three sites on a total of 540 comparisons to majority call.	NPA 99.7% (99.1-100%) PPA 99.0% (96.9-100%) OA 99.4% (98.5-100%)

Inter-observer*	60 esophageal cancer specimens (31 PD-L1-negative and 29 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. Inter-observer analysis was performed between three sites on a total of 540 comparisons to majority call.	NPA 97.1% (94.3-99.3%) PPA 87.4% (81.6-92.7%) OA 92.4% (89.3-95.4%)
Intra-observer	60 esophageal cancer specimens (31 PD-L1-negative and 29 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored 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 97.1% (95.2-98.7%) PPA 97.0% (94.8-98.8%) OA 97.0% (95.6-98.3%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement;
OA=Overall Percent Agreement; CPS=Combined Positive Score
* The inter-observer study failed to meet the acceptance criteria lower-bound 95% CI >85%. A supplemental study was conducted, and the data supported the use of the device.

5. Robustness Studies:

Robustness of the staining performance of PD-L1 IHC 22C3pharmDx in esophageal specimens was evaluated by testing the performance of the assay when varying the assay conditions as described below. Robustness studies used one PD-L1 IHC 22C3 pharmDx lot and 32 esophageal cancer specimens (including 12 ESCC specimens). On one lot of reagents was assessed.

- Tissue sections cut at three thicknesses:
  - 3 µm
  - 4 µm
  - 5 µm
- 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
- Target Retrieval Solution after first use and third use

Staining performance was evaluated for CPS≥10 cutoff and intensity of staining. All studies were performed three to four times with same specimens and conditions. Each repeat study was scored having blinded the prior results and randomized the slides.

Tissue thickness included at least 32 EC (including 12 ESCC) 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 31 EC (including 12 ESCC) specimens and included a minimum of 20% specimens around the cut off. 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. Excepting TRS- pH and TRS re-use all robustness studies passed acceptance criteria and the details for two failures are noted below.

The TRS-pH study results did not meet acceptance criteria as 95% CI lower-bound calculations for ANA/APA/OA using a bootstrap percentile method yielded the following results: 83% for ANA, 76.7% for APA and 80.6.6% for OA and did not meet the prespecified acceptance criteria. A repeat of the TRS re-use study also failed to meet pre-specified acceptance criteria.

The TRS re-use robustness study results did not meet acceptance criteria as 95% CI lower-bound calculations for ANA/APA/OA using a bootstrap percentile method yielded the following results: 76.3% for ANA, 66.7% for APA and 72.6% for OA and did not meet the prespecified acceptance criteria.

Results from the robustness studies informed the following product specific limitations were included in the label:

Product-Specific Limitations

- Laboratories should pay particular attention to pH of target retrieval solution for esophageal cancer specimens as studies for robustness failed at ph5.9 in two independent studies.

- The studies carried out to assess TRS use up to three times in esophageal cancer did not meet acceptance criteria for qualitative evaluation of PD-L1 expression status, therefore TRS reuse is not recommended for ESCC specimens.

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. Primary vs. Metastatic Tumor Tissues

A study compared PD-L1 IHC 22C3 pharmDx performance in the context of primary esophageal versus metastatic tumor tissues. Only lymph nodes were available to use as metastatic tumor specimens. Performance of PD-L1 IHC 22C3 pharmDx was evaluated in FFPE matched primary (esophageal cancer) and metastatic (lymph node) tissues. There were no acceptance criteria for this study. In 19 of 20 paired primary and metastatic tissues, there were 12 concordant negative pairs, 1 concordant positive pair, and 6 discordances for PD-L1 status when using the CPS 10 cut-off. All 6 discordant cases were positive for the metastatic tumor but negative for primary. As the sample of 19 lymph node specimens is not representative of the diversity and population size of esophageal metastatic tumors, these results may not be extrapolated to all metastatic locations.

b. Intra Block Heterogeneity

A study investigated the effect of esophageal cancer tumor intra-block heterogeneity on PD-L1 IHC 22C3 pharmDx performance. Fifty-three (53) individual FFPE esophageal cancer tissue blocks across a minimum of 200µm depth were divided in three portions: anterior (sections 1-10), middle (sections 25-35) and posterior (sections 50-70). No acceptance criteria were set for this study. Of the fifty-three (53) cases assessed, 47 cases were concordant and 6 cases were discordant at front and back sections.

c. Intra-Case Heterogeneity

A study investigated the effect of esophageal cancer tumor intra-case heterogeneity on PD-L1 IHC 22C3 pharmDx performance. Esophageal cancer tissue sister blocks were obtained from the same tumor of 22 subjects. There were no acceptance criteria for this study. In 18 of 22 sister blocks, at the CPS≥ 10 cutoff assessment, 14 pairs were concordant, and 4 pairs were discordant. Six (6) of the evaluable 18 cases were near cut off pairs and were concordant. Results may not be representative of all ESCC 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. Based on data provided in P150013 the stability of the device is established and approved at 9 months for storage at 2-8 ^{0}C.

b. FFPE Cut Section Stability

A real-time stability study was designed to evaluate the shelf life of cut tissue sections of ESCC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. There were four (4) ESCC specimens out of a total of seven (7) EC specimens that were included in the cut section stability study of esophageal cancer. Based on these studies, stability dating for cut slides in ESCC is 4.5 months (135 days) for storage at 2-8 °C and 1 months (30 days) for storage at 25 °C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of PD-L1 IHC 22C3 pharmDx in ESCC was evaluated in the study Keynote-181 (KN181/ NCT02564263). The study design and outcomes are summarized below. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

KEYNOTE-181 is an ongoing, randomized (1:1), multi-center, open-label, Phase 3 study of pembrolizumab vs standard of care (SOC) in participants with advanced/metastatic esophageal adenocarcinoma (EAC) or ESCC, or advanced/metastatic Siewert type I adenocarcinoma of the Esophagogatsric Junction (EGJ). Participants were required to have been previously treated with 1 line of chemotherapy (2L). Participants were stratified by tumor histology and geographic region (Asia vs ex-Asia). Participants receiving SOC were not allowed to cross-over to the pembrolizumab arm during the trial.

In KEYNOTE-181, of 900 total participants were assessed for eligibility in the study, 272 could not meet the eligibility criteria and were excluded from the study, 628 were randomized to either the pembrolizumab monotherapy (314 participants) or the standard treatment group (314 participants). All treated subjects had a tumor tissue sample tested with the PD-L1 IHC 22C3 pharmDx device at the CPS $\geq$ 10 cut off in a central laboratory prior to randomization.

1. Clinical Inclusion and Exclusion Criteria (abbreviated list):
   Participants were required to have histologically or cytologically confirmed advanced/metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the EGJ, and experienced documented objective radiographic or clinical disease progression on 1 previous line of standard therapy. Measurable disease based on RECIST 1.1, as determined by local site investigator/radiology assessment, ECOG PS of 0 or 1, and demonstrated adequate organ function were also required as defined in the protocol. Patients with active autoimmune disease requiring systemic treatment within the 2 years before the first dose of study treatment (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs), with a diagnosis of immunodeficiency or known history of HIV, with known active central nervous system metastases and/or carcinomatous meningitis, or with hepatitis B or C were excluded.

2. Follow up Schedule:
   Patients were randomized (1:1) to receive either KEYTRUDA 200 mg every 3weeks or investigator's choice of any of the following chemotherapy regimens, all given intravenously: paclitaxel 80-100 mg/m2 on Days 1, 8, and 15 of every 4-week cycle, docetaxel 75 mg/m2 every 3weeks, or irinotecan 180 mg/m2 every 2 weeks. Treatment with KEYTRUDA or chemotherapy continued until unacceptable toxicity or disease progression. Patients randomized to KEYTRUDA were permitted to continue beyond the first RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ)-defined disease progression if clinically stable until the first radiographic evidence of disease progression was confirmed at least 4 weeks later with repeat imaging, or for up to 24 months without disease progression. Assessment of tumor status was performed every 9 weeks.

3. Clinical Endpoints

   Primary Efficacy Endpoint:
   The primary endpoint was overall survival (OS), defined as the time from randomization to death due to any cause. The primary objectives were to compare pembrolizumab and SOC for OS in participants with ESCC, in participants with tumors expressing PD-L1 CPS ≥10, and in all participants.

   Secondary Efficacy Endpoints:
   The key secondary efficacy endpoints were:
   - Progression free survival (PFS) in all participants, defined as the time from randomization to the first documented disease progression per modified RECIST 1.1 based on central imaging vendor review, or death due to any cause.
   - Overall response rate (ORR) in all participants, defined as the proportion of the participants who have a complete response (CR) or partial response (PR) per RECIST 1.1 based on central imaging vendor review.
   - Additional secondary efficacy endpoints included PFS and ORR in the other two populations (participants with tumors expressing PD-L1 CPS ≥10 and participants with ESCC); Duration of response (DOR) in all 3 populations (PD-L1 CPS ≥10, ESCC, and all); and safety and tolerability of pembrolizumab in all subjects compared to investigator's choice of paclitaxel, docetaxel, or irinotecan.

4. Assay Cut-off Selection
   Determination of PD-L1 CPS ≥10 as the biomarker for KEYNOTE-181 was made strictly outside of KEYNOTE-181, by using data from KEYNOTE-180 before conducting any efficacy analysis of KEYNOTE-181. PD-L1 expression levels were measured in tumor tissue samples by immunohistochemistry using the Dako PD-L1 IHC 22C3 pharmDx. KEYNOTE; 180 served as a training set for evaluating PD-L1 cut points that enriched for pembrolizumab responders relative to nonresponders in esophageal cancer. An interim analysis from KEYNOTE-180 informed selection of PD-L1 expression as a biomarker with an optimal cutoff of CPS ≥10. This decision was based on the data indicating

positive predictive value, sensitivity, and prevalence evident with the CPS ≥10 cut-off.

B. Accountability of PMA Cohort

As of the data cut-off date, October 2018, in KEYNOTE-181, of 900 total participants in the study, 272 could not meet the eligibility criteria and were excluded from the study, 628 were randomized to either the pembrolizumab monotherapy (314 participants) or the standard treatment group (314 participants). All treated subjects had a tumor tissue sample and were tested with the PD-L1 IHC 22C3 pharmDx device in a central laboratory prior to randomization and PD-L1 status was evaluated at the CPS≥10 cut off. Clinical validation of the PD-L1 IHC 22C3 device was based on outcomes from 628 patients randomized to pembrolizumab monotherapy or standard of care arm. Additional information about the distribution of PD-L1 positive and negative specimens in the trial are summarized in Table 5.A summary of information of the distribution of PD-L1 results for ESCC specimens alone are shown in Table 6 and for each study arm in CPS≥10 Table 7 and Table 8.

Table 5. Accountability of PMA Cohort in KN181

	Number of Study Subjects n (%)	
Enrolled	**628**	
PD-L1 Cut off	**CPS ≥ 10**	**CPS<10**
Quantifiable PD-L1 expression	222	397
Unevaluable PD-L1 expression	9	
ESCC	157	216
EACC	51	165
Invalid for PD-L1 expression in ESCC (tested outside the cut-slide stability window)	10	18
Invalid for PD-L1 expression in EACC (tested outside the cut-slide stability window)	4	16
Site of Collection		
Primary Site	183	342
Metastatic Site	39	55
Specimen type		
Biopsy	160	306
Resection	62	91

Table 6: Tumor PD-L1 by Specimen Type in ESCC Excluding 28 Participants with Specimens Outside the Stability Window

Tumor Tissue	(N)	Number (%) with CPS ≥ 10	Number (%) with CPS < 10
Overall study*	367	157 (42.8)	210 (57.2)
Archival Tissue**	290	113 (39.0)	177 (61.0)
Newly Obtained Tissue**	77	44 (57.1)	33 (42.9)

* Based on participants with known PD-L1 expression in the ESCC population; 6 patients had unknown PD-L1expression status (3 specimens were archival tissue and 3 specimens were newly obtained tissue).
** In the context of clinical trial KEYNOTE-181, a newly-obtained tissue sample is defined as tissue that was collected between the last line of therapy and the first dose of study treatment. Archival tissue samples are those that were collected prior to the last line of therapy.

Table 7: SubjectPD-L1 Distribution (CPS ≥ 10 cut off)

PD-L1 Status	Pembrolizumab arm	SOC arm
PD-L1 CPS < 10	107	115
PD-L1 CPS ≥ 10	201	196
Unknown	6	3
Total	314	314

Table 8: Subject PD-L1 Distribution ESCC (CPS ≥ 10 cut off)

PD-L1 Status	Pembrolizumab arm	SOC arm
PD-L1 CPS <10	99	111
PD-L1 CPS ≥ 10	79	78
Unknown	4	2
Total	182	191

C. Study Population Demographics and Baseline Parameters

Among the 628 participants in the ITT population the baseline characteristics were: median age of 63 years (range: 23 to 84), 43% age 65 or older; 87% male; 56% White, 40% Asian; 39% had an ECOG performance status (PS) of 0 and 61% had an ECOG PS of 1; 64% had squamous cell and 36% had adenocarcinoma histology; and 2% had a history of brain metastases. Ninety-two percent had M1 disease and 8% had M0 disease. For all participants (ITT population), demographic and baseline characteristics were generally well-balanced across intervention arm. The prevalence of participants whose tumors express PD-L1 CPS ≥ 10 was 35.4% (n=222).

D. Safety and Effectiveness Results

1. Safety Results:
   Patients with ESCC whose specimens have PD-L1 expression with CPS <10 are not eligible for Keytruda. Safety of the device for this cut-off was demonstrated in the analytical validation studies described above. There are no safety issues related to the use of this score. Safety with respect to treatment with KEYTRUDA at the different PDL1 expression levels is addressed in the review of the associated therapeutic application and summarized 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 KN181 trial. The observed OS hazard ratios of 0.77 (95% CI: 0.63, 0.96) in patients with ESCC, 0.70 (95% CI: 0.52, 0.94) in patients with tumors expressing PD-L1 CPS ≥10, and 0.89 (95% CI: 0.75, 1.05) in all randomized patients. In an exploratory analysis conducted in patients whose ESCC tumors expressed PD-L1 (CPS ≥10), an improvement in OS was observed among patients randomized to KEYTRUDA as compared with chemotherapy.

   Of 628 subjects treated in the trial, 373 had ESCC tumor specimens tested with the PD-L1 IHC 22C3 pharmDx assay. PD-L1 status was not evaluable for 6 subjects due to inadequate tissue. PD-L1 status was quantifiable for all 367 (98.3%) tumor specimens. In total of 373 subjects, 167 (42.8%) had PD-L1 expression at CPS≥10 and 228(57.2%) had PD-L1 expression at CPS<10. Further, it was observed that 10 specimens out of 167 that expressed PD L1 with a CPS ≥10 were tested outside cut-section stability window and were excluded from the final efficacy analysis. Of these 157 patients, 79 patients were randomized to KEYTRUDA and 78 patients to investigator's treatment of choice [paclitaxel (n=50), docetaxel (n=19), or irinotecan (n=13)].

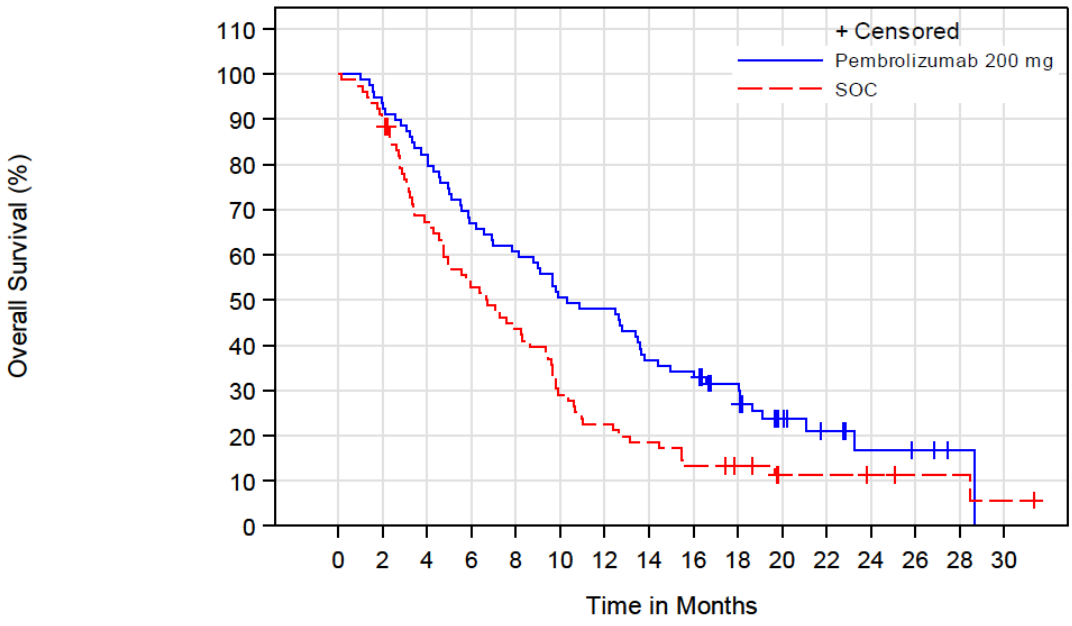
   Table 9 below summarize the key efficacy measures for KEYNOTE-181 for patients with ESCC CPS ≥10. Figure 1 shows the Kaplan Meier survival curves for patients with ESCC who received pembrolizumab separates from with ESCC who received SOC and shows the most treatment benefit.

Table 9. Efficacy Results in Patients with ESCC Whose Tumors Expressed PD-L1 CPS ≥10, KENOTE-181 (Excluding 10 Participants with Specimens Outside the Stability Window)

	Pembrolizumab (N=79)	SOC (N=78)
Primary Endpoint: OS		
Number of events, n (%)	62(78.5)	68 (87.2)
Median (95% CI), month	10.3 (7.8, 13.6)	6.7 (4.8, 9.3)
Median follow-up (95% CI), months	10.3 (7.8, 13.6)	6.9 (4.8, 9.6)
Hazard Ratio [1] (95% CI)	0.63 (0.45, 0.90)	
Secondary Endpoint: PFS (BICR per RECIST 1.1)		
Number of events, n (%)	70 (88.6)	72 (92.3)
Median (95% CI), month	3.2 (2.1, 4.3)	2.6 (2.1, 3.7)
Median follow-up (95% CI), months	3.2 (2.1, 4.4)	2.8 (2.1, 4.0)
Hazard Ratio [1] (95% CI)	0.67 (0.48, 0.95)	
Secondary Endpoints: ORR and DOR (BICR per RECIST 1.1)		
Response, n (%)		
CR	*3 (3.8)*	*1 (1.3)*
PR	*14 (17.7)*	*5 (6.4)*
SD	*26 (32.9)*	*30 (38.5)*
PD	*31 (39.2)*	*28 (35.9)*
ORR, n (%)	17 (22)	6 (8)
95% CI for ORR, %	(13, 32)	(3, 16)
Median DOR in responders (range), months[2]	10.3 (2.8, 18.8+)	7.7 (4.3, 16.8+)

[1] Stratified Cox proportional hazards model by geographic region (Asia vs ex-Asia).
[2] + means duration of response was censored at the time point and not observed.

Figure 1. Kaplan-Meier Estimates of Overall Survival ITT Population, Participants with Squamous Cell Carcinoma and PD-L1 CPS≥10 (Excluding 10 Participants with Specimens Outside the Stability Window)



Number of subjects at risk

Pembrolizumab 200 mg	79	74	65	53	48	40	38	29	27	21	11	7	4	3	1	0
SOC	78	70	51	40	33	22	17	14	10	8	4	4	3	2	2	1

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.

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-181 study, which was conducted to evaluate the safety and efficacy of pembrolizumab in patients with recurrent locally advanced or metastatic ESCC with disease progression on or after one prior line of systemic therapy. In this study, PD-L1 IHC 22C3 pharmDx was used to determine PD-L1 expression status of patient tumors. The trial demonstrated a statistically significant improvement in OS for patients whose tumors express PD-L1 with CPS ≥10 randomized to KEYTRUDA monotherapy as compared with chemotherapy.

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 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 ESCC diagnosis.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the clinical study which demonstrated improved overall survival and duration of response to treatment with KEYTRUDA as a single agent 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 as a single agent 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 ESCC 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 selecting patients with ESCC with PD-L1 CPS ≥10 who may be considered for treatment with KEYTRUDA® (pembrolizumab).

XIII. CDRH DECISION

CDRH issued an approval order on July 30, 2019. The final conditions of approval cited in the approval order are described below.

Provide updated labeling that includes the complete data from an External Reproducibility study (part B- Inter-Observer) which includes a minimum of 60 esophageal squamous cell carcinoma specimens (30 positive and 30 negative including 20% of specimens around the cut-off specimens).

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