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/S006
Date of FDA Notice of Approval:	September 22, 2017

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. The SSED for this indication is 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 gastric and gastroesophageal junction adenocarcinomas (referred to as GC 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 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[1]. 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).

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 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.

1 Combined Positive Score (CPS) retains the same meaning with or without a percent (%) sign.

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

Gastric cancer 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 6 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. 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 aremust 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 (ie. 3-5 endoscopic 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.

Assessment of PD-L1 expression in gastric cancer 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 lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the tumor nests and/or

adjacent supporting stroma. MICs must be directly associated with the response against the tumor.

Tumor PD-L1 expression in gastric cancer 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:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells}}{\text{Total \# tumor cells}} \times 100$$

The specimen should be considered to have PD-L1 expression if CPS ≥ 1.

Combined Positive Score		
PD-L1 Expression Levels	**CPS < 1**	**CPS ≥ 1**
PD-L1 Expression Status	No PD-L1 Expression	PD-L1 Expression

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.

Table 2. Tissue Elements in Determining the Combined Positive Score

Tissue Elements	**Included in Numerator**	**Excluded from the Numerator**
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable gastric carcinoma tumor cells	• Non-Staining tumor cells • Tumor cells with only cytoplasmic staining • Adenoma, dysplasia and carcinoma in situ
Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of tumor-associated MICs within tumor nests and adjacent supporting stroma**: o Lymphocytes (lymphocyte aggregates) o Macrophages*** Only MICs directly associated with the response against the tumor.	• Non-staining MICs • MICs associated with adenoma, dysplasia, and carcinoma in situ • MICs(including lymphoid aggregates) associated with ulceration, chronic gastritis, and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils and Eosinophils and Plasma Cells

Other Cells	• Not included	• Normal cells • Stromal cells (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 CPS numerator.*
***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.*

Specimens stained with NCR must have 0 specific staining and < 1+ grade nonspecific staining.

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 gastric cancer or gastroesophageal junction adenocarcinoma for treatment with KEYTRUDA (pembrolizumab).

VII. MARKETING HISTORY

The PD-L1 IHC 22C3 pharmDx has been marketed in the United States since the approval of P150013 in October 2, 2015. It has also been marketed in Australia, Austria, Belgium, Brazil, Canada, Chile, Columbia, Denmark, Egypt, Jordan Finland, France, Germany, Hong Kong, Hungary, Iceland, Ireland,, Israel, Italy, Japan, Korea, Kuwait, Lebanon, Macau, Malaysia, Netherlands, New Zealand, Norway, Oman, Panama, Peru, Philippines, Poland, Qatar, Saudi Arabia, Serbia, Singapore, Slovakia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, and United Arab Emirates. The PD-L1 IHC 22C3 has not been withdrawn for reasons relating 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. 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. These studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, verify

precision and robustness of the assay, and establish assay stability. The study results detailed below establish sensitivity, specificity, precision and reproducibility of the device.

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. Analytical scores were reported for some studies to ensure assay performance in borderline cases. Antibody characterization studies for clone 22C3 (specificity and tour of body/ tour of tumor), control cell line validation, kit stability and pre analytical variables was leveraged from data submitted under P150013. Results from studies performed to support the gastric 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 study design and results are described in the SSED associated with P150013.

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 unique gastric 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). The number of PD-L1 positive cases with CPS expression level ≥1 was 60 (60%).

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 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 24 GC specimens spanning the range of PD-L1 expression, and at least 25% of these represented specimens around the CPS ≥ 1 cut off. Reader precision studies included 60 unique GC 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 ≥1, or negative if PD-L1 expression was at CPS <1. Statistical analysis for negative percent agreement, positive percent agreement and overall agreement (NPA, PPA and OA) was conducted on the dichotomized data and 95% confidence intervals (CI) calculated by pairwise bootstrap method. Study design and results are summarized in the Table 3 below:

Table 3. Summary of Repeatability

Precision	Study Design	% Agreement (95% CI):
Combined Precision (inter-instrument, inter-operator, inter-lot, inter-day,)	Each of 24 gastric or GEJ adenocarcinoma specimens (12 PD-L1-positive and 12 PD-L1-negative) with a range of PD-L1 expression were tested using three Autostainer Link 48 instruments, four operators, three kit lots, over three non-consecutive days.	NPA 100% (94.9-100%) PPA 95.8% (91.4, 100 %) OA 97.9% (92.5-100%)
Intra-run precision (Repeatability)	Each of 24 gastric or GEJ adenocarcinoma specimens (12 PD-L1-negative and 12 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 96.9% (92.4-100%) PPA 100% (93.5-100%) OA 98.3% (92.5-100%)
Inter-observer precision	Scoring of 60 gastric or GEJ adenocarcinoma specimens (30 PD-L1-negative and 30 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists over three non-consecutive days.	NPA 96.0% (81.4-93.3%) PPA 96.8% (86.7-95.0%) OA 96.5% (84.6-94.3%)
Intra-observer precision	Scoring of 60 gastric or GEJ adenocarcinoma specimens (30 PD-L1-negative and 30 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists over three non-consecutive days.	NPA 91.5% (87.9-95.1%) PPA 96.1% (91.2-96.5%) OA 94.1% (90.0-95.9%)

4. External Reproducibility

Reproducibility studies for GC 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 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 ≥ 1 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 ≥1 expression levels at three external sites. One specimen in the reader reproducibility was not evaluable, and was excluded from final evaluation.
The results of the reproducibility studies are included in Table 4.

Table 4. Three Site Reproducibility in GC

Reproducibility Study	**Study Design**	**% Agreement (95% CI)**
Inter-site	Each of 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 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 pair-wise comparisons.	NPA 92.5% (86.2-97.5%) PPA 91.7% (84.7-97.7%) OA 92.0% (87.4-96.3%)
Intra-site	Each of 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 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 540 pair-wise comparisons.	NPA 93.1% (89.2-96.5%) PPA 98.2% (96.4-99.6%) OA 95.7% (93.7-97.6%)
Inter-observer	Scoring of 59* gastric or GEJ adenocarcinoma specimens (23 PD-L1-negative and 36 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	NPA 76.3% (70.1-81.6%) PPA 93.8% (90.7-96.0%) OA 87.0% (83.9-89.6%)

	a total of 531 pair-wise comparisons.	
Intra-observer	Scoring of 59* gastric or GEJ adenocarcinoma specimens (23 PD-L1-negative and 36 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 531 pair-wise comparisons.	NPA 88.5% (83.1-92.4%) PPA 95.4% (92.7-97.2%) OA 93.0% (90.5-94.9%)

* 59 of 60 specimens were included due to missing data for one specimen

The acceptance criteria for the lower bound of OA and NPA for inter observer reproducibility were not met. A root cause assessment indicated that one of the three readers in the study did not pass proficiency testing. Therefore, a second study was conducted with 68 GC specimens including specimens around the cut off to assess inter-reader performance with three naïve readers and . the results met the acceptance criteria. Results are shown in the Table 5 below. The labeling notes that proficiency assessment is recommended to ensure correct observer scoring interpretation.

Table 5. Reader Reproducibility

Inter-observer	68 gastric or GEJ adenocarcinoma specimens (36 PD-L1- negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was scored 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 612 pair-wise comparisons.	NPA 96.6% (92.9 99.4%) PPA 96.5% (93.1, 99.3%) OA 96.6% (94.0, 98.7%)
Intra-observer	68 gastric or GEJ adenocarcinoma specimens (36 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx,	NPA 97.2% (94.8, 99.1%) PPA 97.2% (94.8, 99.3%) OA 97.2% (95.3, 98.9%)

	was 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 612 pair-wise comparisons.	

5. Robustness Studies:

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

a) Tissue section thickness (4 and 5 μM)
b) Microscope slide type (Fisher Brand SuperFrost Plus, Dako Flex IHC microscope slides)
c) Target retrieval solution temperature (97^0C, 95^0C, and 99^0C)
d) Target retrieval time (20, 18 and 22 minutes)
e) Target retrieval solution pH (pH 6.1, 5.9 and 6.3)
f) Target retrieval solution reuse (3 re-uses)

Tissue thickness and slide type studies included 24 GC specimens spanning the range of PD-L1 expression and included specimens around the cut off (12 positive and 12 negative and included 11-12 specimens around the cut off). Target retrieval studies were performed on PT100 and PT200 modules with 36 GC specimens (17 negative and 19 positive including 20 around cut off). Staining performance was evaluated for both CPS score and intensity of staining. A change in the PD-L1 status or drop in intensity by 0.5 on a scale of 0-3 was considered a test condition failure. No significant difference in results was observed for any of the recommended experimental conditions above.

6. Impact of Intra-Case Heterogeneity

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

a. Primary vs. Metastatic Tumor Tissues

Matched primary versus metastatic blocks were obtained from 19 subjects and evaluated by PD-L1 IHC 22C3pharmDx. Three gastric matched primary and metastatic specimen pairs were PD-L1 negative (CPS <1%), 14 pairs were PD-L1 positive (CPS ≥1%), and 2 pairs had a discordant PD-L1 status. The overall agreement (OA) in PD-L1 status between gastric matched primary and metastatic specimens is 89.5%.

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

Multiple blocks (at least 2) of 21 GC subjects obtained from the same tumor were evaluated. In 19 of 21 sets of GC intra-subject specimens the diagnostic outcomes

were concordant across sister tissue blocks. Two cases showed discordant results at the CPS ≥1 expression level. Results may not be representative of all gastric cancer specimens, as tumor heterogeneity is unique for each specimen.

c. Intra Block Heterogeneity

Heterogeneity within one gastric cancer FFPE blocks was assessed. The 1st, 32nd, and 65th cut sections from 34 unique FFPE blocks were stained with PD-L1 IHC 22C3 pharmDx and assessed for PD-L1 expression. At the CPS ≥ 1 cut-off, 31 of 34 blocks pairs demonstrated agreement in PD-L1 expression (94.1% overall agreement) among the three cut sections. High overall agreement in PD-L1 expression within individual gastric cancer blocks was observed to at least 200µm. Results may not be representative of all gastric cancer specimens, as tumor heterogeneity is unique for each specimen.

A bridging study assessing the equivalence of testing biopsy specimens and tumor resection specimens was not provided in analytical validation. Since core needle biopsies are a common tissue type tested in the clinic for gastric and gastroesophageal junction cancers, the sponsor has committed to conduct a post-approval study comparing the number of core needle biopsies that when analyzed by the PD-L1 IHC 22C3 pharmDx test establish diagnostic agreement with resected tumor specimens. The result from these studies should be included in the labeling.

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 GC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 11 GC specimens. Based on these studies, stability dating for cut slides in GC is 6 months.

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 Keynote-059 (KN059; NCT02335411) study. This is an ongoing non-randomized, multi-site, open-label, Phase 2 trial evaluating the safety and efficacy of pembrolizumab in subjects with gastric or GEJ adenocarcinoma in approximately 315 subjects divided into 3 cohorts. The evaluation of safety and efficacy of PD-L1 IHC 22C3 pharmDx was based on cohort 1 of the KN-059 trial that enrolled subjects who had progressed on at least 2 prior systemic treatments for advanced disease. Cohort1in KN059 enrolled 259 subjects from March 2, 2015 to May 26, 2016 from 52 clinical sites in the North America, Europe, Australia, Asia, and South America. A total of 259 patients received at least one dose of study drug.

Per protocol, the first 42 subjects were enrolled irrespective of PD-L1 status to evaluate futility of treatment in PD-L1 negative subjects. During the waiting period for futility analysis, the study enrolled 32 PD-L1 positive subjects. Enrollment of all comers resumed following interim analysis when futility criteria were not met. Tissue for prospective assessment of PD-L1 expression was submitted prior to treatment and was considered either archival or newly obtained tissue (in the context of clinical trial KN059, a newly obtained biopsy was defined as a specimen obtained up to 6 weeks (≤42 days) prior to initiation of treatment on Day 1 (Cycle 1) with KEYTRUDA and with no additional anti-cancer treatment having been given after the specimen was obtained. Specimens that were >42 days were classified as archival). Assessment of FFPE tumor sample sections for PD-L1 expression was performed using the PD-L1 IHC 22C3 pharmDx at a central lab in UK].

1. Clinical Inclusion and Exclusion Criteria

   Enrollment in the KN059 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 recurrent or metastatic gastric or gastroesophageal junction adenocarcinoma that is considered incurable by local therapies.
   - Evidence of disease progression on at least 2 prior chemotherapy regimens. Previous therapy regimens must have included a fluoropyrimidine and platinum doublet (as part of either a line of therapy or adjuvant therapy).
   - Patient is HER2/neu negative, or, if HER2 positive, has previously received therapy with trastuzumab
   - Presence of adequate tissue for retrospective assessment of PD-L1 status of was required.
   - Presence of measurable disease based on RECIST 1.1 as determined by central imaging vendor.
   - Presence of ECOG (Eastern Cooperative Oncology Group) performance status of 0 or 1.
   - Life expectancy of at least 3 months.

   Patients were not permitted to enroll in the KN059 study if they met any of the following exclusion criteria:

- Weight loss > 10 % over 2 months prior to first dose of study therapy.
- Evidence of ascites by physical exam.
- 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.
- Presence of active autoimmune disease that has required systemic treatment in past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) was not considered a form of systemic treatment.
- Known history of, or any evidence of active, non-infectious pneumonitis.
- Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

2. Follow-up Schedule

Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or disease progression that was symptomatic, rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at least 4 weeks later with repeat imaging. Patients without disease progression were treated for up to 24 months when tolerating the study drug. Assessment of tumor status was performed every 6 to 9 weeks.

3. Clinical Endpoints

The primary efficacy endpoint for the study was confirmed ORR defined as the proportion of subjects who had a complete response (CR) plus those who had a partial response (PR) based on RECIST 1.1, as determined by central radiology assessment, in all comers and in PD-L1 positive sub group. Secondary efficacy endpoint included duration of response (DoR) disease control rate (defined as the numbers of subjects with complete responses, partial responses, and stable disease ≥ 2 months combined).

B. Accountability of PMA Cohort

At the time of database lock, January 16, 2017, 259 patients were treated after tumor PD-L1 expression status was prospectively determined. All treated subjects had a tumor tissue sample collected at baseline. Primary efficacy analysis was based on data from subjects whose tumors expressed PD-L1 at CPA ≥1. Of these 259 patients, 148 were PD-L1 positive. Microsatellite instability high (MSI-H) cases (5) that were PD-L1 positive were later excluded from the analyzed set to yield a total of 143 PD-L1 positive subjects. . PD-L1 status was not evaluable for 2 subjects due to insufficient tissue. A description of the specimen characteristics is shown in Table 6.

Table 6. Accountability of PMA Cohort in KN059

	Number of Study subjects n (%)
Enrolled	259
Tumor sample collected at baseline	259
Specimens with insufficient tissue	2
Total evaluable PD-L1 expression	257
PD-L1 positive CPS ≥ 1	148 (57%)
PD-L1 negative CPS <1	109 (43%)
Site of Collection: (N=259)	
Primary Site	185 (71.4%)
Metastatic Site	74 (28.6%)
Specimen type (N=257)	
Newly Obtained* (≤42 days old)	90 (35%)
Archival (>42 days old)	167 (65%)
Sample Procedure (N=259)	
Biopsy	207
Resection	48
Unknown	4

* *specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1 (Cycle 1) and with no additional anti-cancer treatment having been given after the specimen was obtained.*

Specimen Age and PD-L1 status

The number of PD-L1 positive specimens was higher (73.3%) in subjects who had newly obtained tissues tested with the PD-L1 IHC 22C3 pharmDx when compared to PD-L1 positive specimens in subjects who had archival tissue tested (49.9%). The distribution of PD-L1 status based on age of specimen is shown in Table 7.

Table 7: Tumor PD-L1 Status for KN059 Study Subjects

	Total (%)	PD-L1 + n (%)	PD-L1 - n (%)
Total evaluable specimens	257	148 (57)	109 (43)
Newly Obtained (≤42 days)	90 (35.0)	66 (73.3)	24 (26.7)
Archival (>42 days)	167 (65.0)	82 (49.9)	85 (50.9)
Archival 43-900 days	121 (72.45)	64 (52.9%)	57(47.1)
Archival >900 days	46(27.5)	18 (39.1%)	28(60.9)

C. Study Population Demographics and Baseline Parameters

For the 259 subjects enrolled in KN059, the median age was 62 years (range: 24 to 89 years), and 42.9% were ≥65 years of age. Subjects were predominantly White (77.2%) or Asian (15.8%), with 48% recruited from US sites and 39% from rest of world (ROW) sites, i.e., a total of 87% from non-Asia sites. More than half of the subjects had tumors that were PD-L1 positive (148 subjects, 57.1%). Two hundred and forty three subjects (93.8%) had metastases and 16 subjects (6.2%) had locally advanced disease. A total of 151 subjects (58.3%) had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 at baseline. Approximately half of the subjects had previously received 2 lines of treatment (referred to as 3L subjects) (134 subjects, 51.7%), whereas the other half had received 3 or more lines of prior therapy (referred to as 4L+ subjects) (125 subjects, 48.3%). As per protocol requirements, all subjects had been treated with 5-FU and platinum doublet. In addition, many subjects were also pretreated with taxanes (83.8%), capecitabine (47.5%), irinotecan (44.8%), anti-VEGF/VEGFR antibodies (41.3%), anthracyclines (26.6%), and anti-HER2/neu antibodies (23.6%).

D. Safety and Effectiveness Results

1. Safety Results

   As an in vitro diagnostic test, the PD-L1 IHC 22C3 pharmDx Assay involves testing on FFPE GC sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of gastric cancer by pathologists. Removal of these tissues, therefore, presents no additional safety hazard to the patient being tested. Safety for use of the device 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 KN059 trial. Of 259 subjects treated in the trial, 259 had tumor specimens tested with the PD-L1 IHC 22C3 pharmDx assay. PD-L1 status was not evaluable for 2 subjects due to inadequate tissue. PD-L1 status was quantifiable for all 257 (99.2%) tumor

specimens. A total of 109 subjects (43%) had PD-L1 at CPS <1 and 148 (57%) had PD-L1 expression at CPS≥1.

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 11.6% (95% CI: 8.0, 16.1) in the overall population, including 6 patients (2%) who achieved complete responses. The ORR among patients with PD-L1 positive and PD-L1 negative tumors was 15.5% (95% CI: 10.1, 22.4) and 6.4% (95% CI: 2.6, 12.8), respectively. Median duration of response in PD-L1 positive tumors was 14.2 months (2.8+, 19.4+) while in PD-L1 negative subgroup was 6.9 months (2.4, 10.3+). The difference in ORR for PD-L1 positive and PD-L1 negative subgroups was 9.1% (95% CI of 1.3- 16.8) suggesting a statistically significant difference in response rates for the two sub groups.

Efficacy in PD-L1 positive population adjusted for MSI status
In retrospective assessment of MSI status for the study subjects, 5 subjects whose tumors expressed PD-L1 were also identified to be MSI-H and excluded from final efficacy analysis as efficacy based on MSI status was established in a separate study. After adjusting for the removal of the MSI subjects, the 143 evaluable PD-L1 positive patients, the ORR was 13.3% (95% CI: 8.2, 20.0); 1.4% had a complete response and 11.9% had a partial response. Among the 19 responding patients, the duration of response ranged from 2.8+ to 19.4+ months, with 11 patients (58%) having responses of 6 months or longer and 5 patients (26%) having responses of 12 months or longer.

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-059 study which conducted to evaluate the safety and the efficacy of single agent KEYTRUDA (pembrolizumab) in patients with gastric or GEJ adenocarcinoma with disease progression on or after two or more prior lines of therapy. In this single-arm study, the PD-L1 IHC 22C3 pharmDx was used to determine PD-L1 patient status. A total of 148 patients (57%) had PD-L1 positive tumors, 109 patients (42%) has PD-L1 negative tumors, and 2 patients (1%) had unknown PD-L1 status. After exclusion of 5 subjects for reasons unrelated to the test, the data from the trial shows that the ORR in PD-L1 positive patients was 13.3% (95% CI: 8.2, 20.0) with 1.4% having a complete response and 11.9% had a partial response. Among the 19 responding patients, the duration of response ranged from 2.8+ to 19.4+ months, with 11 patients (58%) having responses of 6 months or longer and 5 patients (26%) having responses of 12 months or longer. Therefore, the PD-L1 IHC 22C3 pharmDx supported the identification of patients who will benefit from the therapeutic 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

The PD-L1 IHC 22C3 pharmDx is an *in vitro* diagnostic device, which tests tumor FFPE specimens collected from patients with gastric cancer. The risks of the device are based on data collected in the clinical study. 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. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for gastric 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 support selecting PDL1 expression in gastric and gastroesophageal junction adenocarcinoma patients for treatment with pembrolizumab, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use and product labeling. The provided studies support use of PD-L1 IHC 22C3 pharmDx as an aid in identifying patients with gastric cancer or gastroesophageal junction adenocarcinoma for treatment with KEYTRUDA® (pembrolizumab).

XIII. CDRH DECISION

CDRH issued an approval order on September 22, 2017. The final conditions of approval cited in the approval order are described below:

A bridging study assessing the equivalence of testing biopsy specimens and tumor resection specimens was not provided in analytical validation. Since core needle biopsies are a common tissue type tested in the clinic for gastric and gastroesophageal junction cancers, the applicant will conduct a study to compare the number of core needle biopsies that when analyzed by PD-L1 IHC 22C3 pharmDx test establish diagnostic agreement with resected tumor specimens. The result from these studies should be included in the labeling.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Warnings, and Precautions in the device labeling.

Post-approval Requirements and Restrictions: See approval order.