SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic immunohistochemistry (IHC) test for detection of PD-L1 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

Date of FDA Notice of Approval: October 2, 2015

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) tissue using EnVision FLEX visualization system on Autostainer Link 48. PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. The specimen should be considered PD-L1 positive if TPS $\geq$ 50% of the viable tumor cells exhibit membrane staining at any intensity.

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

III. CONTRAINDICATIONS

There are no known contraindications for 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

Device Kit Components

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical staining procedure for Formalin-Fixed and Paraffin-Embedded (FFPE) specimens on the Dako Autostainer Link 48 automated staining system using the EnVision FLEX visualization system. These are listed in Table 1 below:

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

Reagent	Description	Qty x Vol
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015mol/L sodium azide.	1 x 34.5mL
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. Approximate protein concentration is 3 µg/mL.	1 x 19.5mL
Negative Control Reagent	Monoclonal mouse control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.	1 x 15mL
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015mol/L sodium azide.	1x 34.5mL
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.	1 x 34.5mL
DAB+ Buffered Substrate	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.	15 x 7.2mL
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride in an organic solvent.	1 x 5mL
DAB Enhancer	Cupric sulfate in water.	1 x 34.5mL
EnVision™ FLEX Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.	6 x 30mL
Cell Line Control Slides	Each slide contains sections of two pelleted, FFPE cell lines: NCI-H226 with moderate PD-L1 protein expression, and MCF-7 with negative PD-L1 protein expression.	3 x 5 slides

Each kit contains the reagents necessary to perform 50 tests in up to 15 individual runs. PD-L1 IHC 22C3 pharmDx comes with individually labeled reagent components that are recognized together. Coverslipping can be manual or automated, so capabilities for this are required, but not supplied.

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 (version 4.0.3). 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 is performed in the PT Link Pre-treatment module.

Specimen Preparation

NSCLC specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of FFPE tissue processing should be used for all specimens. Tissue specimens should be cut into sections of 4-5 µm, mounted on charged microscope slides and stored in the dark at 2-8 °C until staining, which should be performed within 6 months of sectioning.

Test Controls and Calibrators

Control cell line slides listed in the above table 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. They should not be used as an aid in interpretation of patient results. Additional information about the use of controls are avalailable in the product labeling.

Principles 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 coverslipped.

Staining protocol

The PD-L1 IHC 22C3 pharmDx is designed to be run on the Autostainer Link 48 with DakoLink software. The staining protocol on the Dako Autostainer Link 48 is as follows:

Peroxidase-Blocking Reagent (2 drop zones x150 µL): 5 minutes (± 1 minute)
Rinse in buffer
Monoclonal Mouse anti-PD-L1 (or Negative Control Reagent) (2 drop zones x150 µL): 30 minutes (± 1 minute)
Rinse in buffer
Linker, anti-Mouse Ig (2 drop zones x150 µL): 30 minutes (± 1 minute)
Rinse in buffer
Visualization Reagent (2 drop zones x150 µL): 30 minutes (± 1 minute)
Rinse in buffer: 5 minutes
DAB+ solution (2 drop zones x150 µL): 2 x 5 minutes (± 1 minute)
Rinse in buffer
DAB+ Enhancer (2 drop zones x150 µL): 5 minutes (± 1 minute)
Rinse in buffer
Hematoxylin (2 drop zones x150 µL): 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 device labeling states that interpretation of specimens should be performed by a pathologist using a light microscope. An objective of 10-40x magnification is appropriate. All viable tumor cells on the entire slide must be evaluated and included in the PD-L1scoring assessment. A minimum of 100 viable tumor cells must be present for the specimen to be considered adequate for PD-L1 evaluation. To successfully score PD-L1 IHC 22C3 pharmDx stained specimens, it is critical that the appropriate cells are evaluated, the proper cellular localization is identified and the staining intensity is properly interpreted. Any perceptible membrane staining (partial or complete) should be included in the scoring. Cytoplasmic staining should be considered non-specific staining. Tumor associated immune cells such as infiltrating lymphocytes or macrophages are not included in the scoring for the determination of PD-L1 positivity using this assay.

The specimen should be considered PD-L1 positive if TPS is ≥50% of the viable tumor cells exhibit membrane staining at any intensity (i.e., ≥1+). The specimen should be considered PD-L1 negative if TPS is ≤49% of the viable tumor cells exhibit membrane staining at any intensity (i.e., ≥1+). A PD-L1 IHC 22C3 pharmDx Interpretation Manual

for NSCLC (containing color images of representative staining patterns and known artifacts) is available to the end user to assist in the interpretation of assay results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay available for detection of PD-L1 in formalin-fixed, paraffin embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissues for the selection of patients to be treated with KEYTRUDA® (pembrolizumab).

VII. MARKETING HISTORY

The PD-L1 IHC 22C3 pharmDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PD-L1 test results, and subsequently improper patient management decisions in non-small cell lung carcinoma (NSCLC) treatment.

A false negative test result may lead to KEYTRUDA® (pembrolizumab) treatment being withheld from a patient who might have benefited. A false positive test result may lead to administration of KEYTRUDA® (pembrolizumab) treatment to a patient who may experience adverse side effects.

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

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

1. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 127 unique cases of formalin-fixed, paraffin embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue using one kit lot. The specimens were chosen at random and represented the full range of PD-L1 expression. Assessment of PD-L1 expression demonstrated staining across a range of 0-100% positive tumor cells and 0-3 staining intensity. The rate of positivity when the TPS was ≥50% was 18%.

2. Analytical Specificity

a. Western Blot

Western blot analysis was performed on tumor cell lysates, positive (NCI-H226) and negative (MCF7) for PD-L1 by IHC assay. Varying concentrations of purified PD-L1

were blotted as a positive control. The results indicated that monoclonal anti-PD-L1 clone 22C3 detected purified PD-L1 protein in western blotting and low cross-reactivity to other proteins in the cell lysates was observed.

b. Immunoreactivity in Human Tissues

Analytical specificity of PD-L1 IHC 22C3 pharmDx was tested on FFPE tissue specimens and cell lines as follows: 3 cases from each of 30 normal human tissue types (90 specimens in total) using one kit lot, 1 multi-block containing 24 different normal human tissue types (24 specimens in total) using three lots, and on 6 FFPE tumor cell lines with known PD-L1 expression using one lot. The staining of the cells lines was compared to established mRNA and flow cytometry data for PD-L1.

The testing demonstrated that PD-L1 IHC 22C3 pharmDx detects PD-L1 protein localized on the cell membrane of cell types known to express PD-L1. Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. Background staining was ≤0.5 grade in all specimens tested. PD-L1 expression patterns were consistent between the 3 lots in the multi-block study. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in normal tissues. The FFPE tumor cells lines demonstrated equivalent PD-L1 expression when compared to mRNA analysis, flow cytometry analysis and IHC analysis.

A summary of the PD-L1 IHC 22C3 pharmDx immunoreactivity on the panel of 30 normal human tissues is presented in Table 2 below.

Table 2: Summary of PD-L1 IHC 22C3 pharmDx Normal Tissue Reactivity

Tissue Type (# tested)	**Positive Plasma Membrane Staining**	**Positive Cytoplasmic Staining**	**Non-specific Staining**
Adrenal (3)	0/3	1/3 Medullary cells	0/3
Bone marrow (3)	3/3 Megakaryocytes	3/3 Megakaryocytes	0/3
Breast (3)	0/3	0/3	0/3
Cerebellum (3)	0/3	0/3	0/3
Cerebrum (3)	0/3	0/3	0/3
Cervix (3)	1/3 Epithelium	0/3	0/3
Colon (3)	2/3 Macrophages	0/3	0/3
Esophagus (3)	0/3	0/3	0/3
Kidney (3)	1/3 Tubular epithelium	0/3	0/3
Liver (3)	1/3 Macrophages 1/3 Heptatocytes	0/3	0/3
Lung (3)	3/3 Alveolar macrophages	0/3	0/3

Tissue Type (# tested)	Positive Plasma Membrane Staining	Positive Cytoplasmic Staining	Non-specific Staining
Mesothelial cells (2)	0/2	0/2	0/2
Muscle, cardiac (3)	0/3	0/3	0/3
Muscle, skeletal (3)	0/2	0/2	0/2
Nerve, peripheral (3)	0/3	1/3 connective tissue/vessels	0/3
Ovary (3)	0/3	0/3	0/3
Pancreas (3)	0/3	0/3	0/3
Parathyroid (3)	1/3 Glandular epithelium	0/3	0/3
Pituitary (3)	1/3 Anterior hypophysis 1/3 Posterior hypophysis	1/3 Anterior hypophysis 1/3 Posterior hypophysis	0/3
Prostate (2)	2/2 Epithelium	0/2	0/2
Salivary gland (3)	0/3	0/3	0/3
Skin (3)	0/3	0/3	0/3
Small intestine (3)	0/3	0/3	0/3
Spleen (3)	2/3 Macrophages	0/3	0/3
Stomach (3)	2/3 Lymphocytes 1/3 Gastric glands	1/3 Gastric glands	0/3
Testis (3)	0/3	0/3	0/3
Thymus (3)	3/3 Medullary epithelium	0/3	0/3
Thyroid (3)	0/3	0/3	0/3
Tonsil (3)	3/3 Crypt epithelium 2/3 Germinal center (macrophages)	0/3	0/3
Uterus (3)	0/3	0/3	0/3

A summary of the PD-L1 IHC 22C3 pharmDx immunoreactivity on the panel of neoplastic tissues is presented in Table 3 below. Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. There were no unexpected results observed in the tumor specimens tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in neoplastic tissues.

Table 3: Summary of PD-L1 IHC 22C3 pharmDx Neoplastic Tissue Reactivity

Tumor Type	Location	PD-L1 positive/total
Adenocarcinoma	Appendix	0/1
	Breast, DCIS	0/2

Tumor Type	Location	PD-L1 positive/total
	Breast, Invasive Ductal	0/7
	Breast, Invasive Ductal metastatic to lymph node	0/1
	Cervix, Endocervical type	0/1
	Colon	0/5
	Colon, metastatic to liver	0/1
	Colon, mucinous	0/1
	Esophagus	0/1
	Gallbladder	1/5
	GI, metastic to lung	0/1
	Head & Neck, hard palate	0/1
	Lung	1/4
	Ovary	0/1
	Ovary, endometriod	0/1
	Ovary, mucinous	0/1
	Ovary, serous	0/1
	Pancreas	0/2
	Pancreas, ductal	0/3
	Prostate	0/5
	Rectum	0/4
	Salivary/Parotid gland	0/2
	Small Intestine	0/2
	Stomach	0/6
	Stomach, mucinous	0/1
	Thyroid, Follicular	0/1
	Thyroid, Follicular-Papillary	0/1
	Thyroid, Papillary	0/3
	Uterus, Clear Cell	0/1
	Uterus, endometrium	0/3
Adrenocortical carcinoma	Adrenal	0/1
Astrocytoma	Cerebrum	0/3
Basal cell carcinoma	Skin	0/1
Carcinoma	Nasopharyngeal, NPC	0/1
Chondrosarcoma	Bone	0/1
Chordoma	Pelvic Cavity	0/1
Embryonal carcinoma	Testis	0/1
Ependymoma	Brain	0/1
Glioblastoma	Brain	0/1
Hepatoblastoma	Liver	0/1
Hepatocellular carcinoma	Liver	0/5
Islet Cell tumor	Pancreas	0/1
Interstitialoma	Colon	0/1

Tumor Type	Location	PD-L1 positive/total
	Rectum	0/1
	Small intestine	0/1
Leiomyosarcoma	Soft tissue, chest wall	0/1
	Bladder	0/1
Lymphoma		
Anaplastic Large Cell	Lymph node	0/1
Diffuse B-Cell	Lymph node	0/4
Hodgkin	Lymph node	2/2
Non-Hodgkin	Lymph node	1/1
Medulloblastoma	Brain	0/1
Medullary carcinoma	Thyroid	0/1
Melanoma	Rectum	0/1
	Nasal cavity	0/1
Meningioma	Brain	0/2
Mesothelioma	Peritoneum	0/1
Neuroblastoma	Retroperitoneum	0/1
Neurofibroma	Soft tissue, lower back	0/1
Osteosarcoma	Bone	0/2
Pheochromocytoma	Adrenal	0/1
Primitive Neuroectodermal Tumor (PNET)	Retroperitoneum	0/1
Renal Cell carcinoma		
Papillary	Kidney	0/1
Clear Cell	Kidney	0/6
Rhabdomyosarcoma	Soft tissue, embryonal	0/1
	Prostate	0/1
	Retroperitoneum	0/1
Seminoma	Testis	0/2
Signet Ring Cell carcinoma	Metastatic colon Signet Ring Cell carcinoma to ovary	0/1
	Colon	0/1
Small Cell carcinoma	Lung	0/1
Spermatocytoma	Testis	0/2
Squamous Cell carcinoma	Metastatic esophageal Sq Cell carcinoma to lymph node	0/1
	Cervix	2/5
	Esophagus	0/7
	Head & Neck	0/2
Squamous Cell carcinoma	Lung	1/2
	Skin	0/2
	Uterus	0/1
Synovial Sarcoma	Pelvic cavity	0/1

Tumor Type	Location	PD-L1 positive/total
Thymoma	Mediastinum	1/1
Transitional Cell carcinoma	Bladder	0/6
	Kidney	0/1

3. Robustness

Robustness of PD-L1 IHC 22C3 pharmDx was tested using one lot. All robustness studies were completed with 16 NSCLC specimens (8 PD-L1-negative and 8 PD-L1-positive). The robustness conditions tested were the following:

- Tissue Thickness
  - 4 µm
  - 5 µm

In addition, tissue thickness was evaluated during assay verification using 3 NSCLC cases (2 PD-L1-negative and 1 PD-L1-positvie) using tissues sectioned at 2, 3, 4, 5 and 6 µm. The slides sectioned at 2 µm did not have equivalent staining when compared to the standard 4 µm thick sections and therefore did not meet the acceptance criteria. The slides sectioned at 3-6 µm demonstrated equivalent staining. Dako recommends specimens to be sectioned at a thicknoess of 4-5 µm.

Additional parameters tested as part of the robustness studies are listed below:

- Microscope slide Type
  - Fisherbrand™ Superfrost™ Plus
  - Dako FLEX IHC Microscope Slides (Dako code K8020)
- Target Retrieval Solution: Time
  - 18 minutes
  - 20 minutes-standard
  - 22 minutes
- Target Retrieval Solution: Temperature
  - 95°C
  - 97°C -standard
  - 99°C
- Target Retrieval Solution: pH
  - pH 5.8
  - pH 5.9
  - pH 6.1-standard
  - pH 6.4
- Target Retrieval Solution: 3 Re-uses
- Target Retrieval Solution: 3 lots

No significant difference in results was observed for any of the experimental conditions listed above, with the exception of the Target Retrieval (TRS) at pH 5.8.

The 1x Target Retrieval Solution pH must be 6.1+/-0.2; a pH below 5.9 may give erroneous results.

4. Precision

Intra-run repeatability

Intra-run repeatability was tested in one run on one Autostainer Link 48 on 6 consecutive sections from 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) representing the range of PD-L1 expression. The results demonstrated 100% overall agreement between 6 sections per specimen stained within the same run.

Intra-day repeatability

Intra-day repeatability was tested in two runs performed within one day, repeated over three days, on one Autostainer Link 48 on 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) representing the range of PD-L1 expression. The results demonstrated 100% overall agreement, between two different runs per day, repeated over three days.

Inter-day reproducibility

Inter-day reproducibility was tested in 6 runs performed over 6 non-consecutive days on one Autostainer Link 48 on 16 NSCLC specimens (10 PD-L1 negative and 6 PD-L1 positive) representing the range of PD-L1 expression. The results demonstrated 100% overall agreement between 6 days.

Inter-instrument reproducibility

Inter-instrument reproducibility was tested in a total of 6 runs performed on 6 different Autostainer Link 48 instruments by one operator on 16 NSCLC specimens (10 PD-L1 negative and 6 PD-L1 positive) representing the range of PD-L1 expression. The results demonstrated 100% overall agreement between 6 instruments.

Inter-operator reproducibility

Inter-operator reproducibility was tested in a total of 6 runs performed by 6 different operators on one Autostainer Link 48 on 16 NSCLC specimens (10 PD-L1 negative and 6 PD-L1positive) representing the range of PD-L1 expression. The results demonstrated 100% overall agreement between 6 operators.

Inter-lot reproducibility

Inter-lot reproducibility was tested on 16 NSCLC specimens (8 PD-L1 negative and 8 PD-L1positive) representing the range of PD-L1 expression, using 3 lots of all PD-L1 IHC 22C3 pharmDx reagents. The results demonstrated 100% overall agreement between 3 lots.

Table 4 below shows the agreement rates for the PD-L1 IHC 22C3 pharmDx repeatability studies.

Table 4: Repeatability of PD-L1 IHC 22C3 pharmDx Tested at One Site

Repeatability Study	% Agreement (95% CI)
Intra-run	≥50% cut-off: NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Intra-day	≥50% cut-off: NPA 100% (88.3-100%) PPA 100% (82.4-100%) OA 100% (92.4-100%)
Inter-day	≥50% cut-off: NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-instrument	≥50% cut-off: NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-operator	≥50% cut-off: NPA 100% (92.7-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-lot	≥50% cut-off: NPA 100% (92.6-100%) PPA 100% (92.6-100%) OA 100% (96.2-100%)

NPA= Negative Percent Agreement;
PPA= Positive Percent Agreement;
OA=Overall Agreement.

5. External Reproducibility

Reproducibility of the PD-L1 IHC 22C3 pharmDx tested at three external sites are summarized in Table 5 below.

Table 5: Inter-laboratory Reproducibility of PD-L1 IHC 22C3 pharmDx

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 36 NSCLC specimens (21 PD-L1-negative and 15 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 2700 pair-wise comparisons.	≥50% cut-off: ANA 90.3% (84.4-95.2%) APA 85.2% (75.6-92.9%) OA 88.3% (81.4-94.3%)
Intra-site	Each of 36 NSCLC specimens (21 PD-L1-negative and 15 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 1080 pair-wise comparisons.	≥50% cut-off: ANA 91.9% (88.8-94.8%) APA 87.6% (82.5-92.2%) OA 90.2% (86.3-93.7%)
Inter-observer	Scoring of 62 NSCLC specimens (30 PD-L1-negative and 32 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 1674 pair-wise comparisons.	≥50% cut-off: ANA 92.6% (87.8-96.7%) APA 92.8% (88.1-96.8%) OA 92.7% (88.1-96.8%)
Intra-observer	Scoring of 62 NSCLC specimens (30 PD-L1-negative and 32 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 558 pair-wise comparisons.	≥50% cut-off: ANA 96.4% (94.0-98.5%) APA 96.5% (94.3-98.6%) OA 96.4% (94.3-98.6%)

ANA= Average Negative Agreement; APA= Average Positive Agreement; OA=Overall Agreement

6. Stability Studies

a. Cut Section Stability

The stability of the PD-L1 antigen in NSCLC tissue after sectioning and storage at room temperature and 2-8 °C was evaluated. Positive cases and cases near the cut-off were included in the study. At each timepoint a freshly sectioned slide was included as a reference control. The results showed equivalent staining to the reference control

for up to 6 months of storage at both room temperature and 2-8 °C. A decrease in PD-L1 signal was observed at 12 months at both storage conditions. Based on real time testing results, NSCLC tissue sections should be stained within 6 months of sectioning.

b. Block Stability

The results from a retrospective block stability study using 44 NSCLC blocks with 37 PD-L1-negative and 7 PD-L1-positive blocks demonstrated that in NSCLC there was no difference in the percentage of PD-L1-positive tumor cells and average IHC staining intensity in NSCLC tissue sections from FFPE blocks stored for up to 5 years when compared to tissue blocks tested that were less than a year old.

c. PD-L1 IHC 22C3 pharmDx Stability

Real time stability study including transport simulation, working stability and in-use/on-board stability were conducted with three lots of PD-L1 IHC 22C3 pharmDx components. Six NSCLC blocks were used, including 3 PD-L1-negative and 3 PD-L1-positive blocks. Two of the positive blocks were near the cut-off. Based on these study results, the stability dating is as follows:

Total Shelf Life :
9 months at 2-8 °C

Finished Good Shelf Life:
9 months at 2-8 °C

In-Use/On-Board Stability Testing
Eighteen cycles to room temperature

Working/Reconstituted Stability Testing
DAB Substrate-Chromogen Solution: 5 Days at 2-8 °C, protected from light.
Target Retrieval Solution: 5 days at Room Temperature in a PT-Link with up to 3 uses for 3-in-1 Pretreatment.

B. Animal Studies

None

C. Additional Studies

1. Primary vs. Metastatic Tumor

Expression data from 23 scorable pairs of primary and metastatic NSCLC tissues (8 PD-L1-negative and 15 PD-L1-positive) were analyzed. The results showed a similar dynamic range of PD-L1 expression in primary and metastatic NSCLC specimen pairs, with 87% (20/23 specimen pairs) being concordant for positive/negative status.

2. Impact on Ischemia/Fixation

A cohort of 11 FFPE multi-blocks manufactured by Dako using normal and tumor lung tissues were processed in 10% neutral buffered formalin (NBF) at the following fixation times: 3, 6, 24, 48, 72 and 168 hours. Each multi-block was stained with PD-L1 IHC 22C3 pharmDx. Fixation times of 4-168 hours in 10% NBF did not systematically alter PD-L1 detection. Fixation times of ≤3 hours may be incompatible with reproducible and robust PD-L1 detection.

Freshly excised human placenta tissues were obtained from 9 donors, macro-dissected into 24 pieces (216 FFPE blocks) and placed in specimen containers containing saline-soaked gauze at ambient temperature for indicated ischemia time before placing into fixative. Specimens were then moved to 10% NBF at ambient temperature for the indicated time and processed into FFPE blocks on an automated tissue processor. In this study, the results demonstrated that the PD-L1 IHC 22C3 pharmDx detects a similar range of PD-L1 expression in placenta tissues with ischemic time varying from 0-24 hours.

3. Intra-Case Heretogeinity

Multiple (2-5) specimen blocks per case (patient) were tested with PD-L1 IHC 22C3 pharmDx for a total of 20 individual cases (18 PD-L1-negative and 2 PD-L1-positive). The results showed that 100% (20 of 20 sets) of NSCLC intra-case specimens obtained diagnostically concordant scores.

4. Intra-Block Heretogeinity

The first and the 50^{th} consecutive tissue section cut from a NSCLC FFPE tissue block from a total of 20 blocks (15 PD-L1-negative and 5 PD-L1-positive) were tested with PD-L1 IHC 22C3 pharmDx. The results showed that 100% (20 of 20 pairs) of the section pairs obtained diagnostically concordant scores.

5. Control Cell Line Validation

The Control Cell Line (CCL) slides which are included in the PD-L1 IHC 22C3 pharmDx were evaluated for repeatability, reproducibility and robustness with cut sections from three unique CCL blocks. Replicate slides were tested to evaluate the following studies: inter-instrument, inter-operator, inter-day, intra-run, inter-lot and inter-observer. Various robustness tests were also evaluated with replicate sections from three unique CCL blocks. The acceptance criteria were as follows: PD-L1 22C3 positive cell line (NCI-H226) must exhibit plasma membrane staining of ≥80% of cells at ≥2.25 grade average staining intensity and background staining <1.0; PD-L1 22C3 negative cell line (MCF-7) must exhibit no specific staining. All tests met the pre-defined acceptance criteria.

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Study Design

The clinical performance of PD-L1 IHC 22C3 pharmDx was investigated in a retrospective analysis of patient samples from a multicenter, open-label, randomized phase 1 clinical study conducted to assess the safety and efficacy of KEYTRUDA® (pembrolizumab) in patients with advanced NSCLC. Patients were PD-L1 positive by an enrollment assay, which is different from PD-L1 IHC 22C3 pharmDx, and had progression of disease following treatment with platinum-containing chemotherapy. Patients enrolled in the trial with EGFR or ALK genomic tumor aberrations had disease progression on FDA-approved therapies for these aberrations prior to receiving KEYTRUDA® (pembrolizumab). Patients were randomized to receive 10 mg/kg of KEYTRUDA® (pembrolizumab) every 2 (n=69) or 3 (n=87) weeks until unacceptable toxicity or disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at 4 to 6 weeks with repeat imaging. Assessment of tumor status was performed every 9 weeks. The major efficacy outcome measures were overall response rate (ORR according to RECIST 1.1 as assessed by blinded independent central review) and duration of response (DoR).

Archived clinical study samples were retrospectively tested at a single U.S.-based CLIA certified laboratory with PD-L1 IHC 22C3 pharmDx.

1. Clinical Inclusion and Exclusion Criteria
   Enrollment in the study was limited to patients who met the following key inclusion criteria:

   - Patients with histologically confirmed or cytologically confirmed diagnosis of non-small cell lung cancer, and who had progression of disease following treatment with platinum-containing chemotherapy;
   - Patients who had primary, recurrent or metastatic tumor(s) amenable to biopsy and who were PD-L1 positive by the enrollment assay;
   - Patients with EGFR or ALK genomic tumor aberrations had disease progression on FDA-approved therapy for these aberrations prior to receiving KEYTRUDA® (pembrolizumab);
   - Patients ≥18 years of age with an estimated life expectancy of at least 12 weeks;
   - Patients with a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) Performance Scale and had adequate organ function.

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

   - Patients with autoimmune disease;

- Patients with a medical condition that required immunosuppression;
- Patients who had received more than 30 Gy of thoracic radiation within the prior 26 weeks.

2. Follow-up Schedule
   Tumor assessments were performed at baseline and every 9 weeks thereafter.

3. Clinical Endpoints
   With regard to safety, information about adverse events was collected from time of signed informed consent throughout the treatment period and least 30 days after the last dose of study drug or until initiation of a new anti-cancer treatment, whichever occured first. The safety analysis was performed in all patients who had received at least 1 dose of study treatment

   With regard to effectiveness, the efficacy evaluation was performed in patients with positive PD-L1 expression as determined by PD-L1 IHC 22C3 pharmDx and had progression of disease following treatment with platinum-containing chemotherapy. The major efficacy outcome measures were ORR (according to RECIST 1.1 as assessed by blinded independent central review). The secondary efficacy measure was duration of response (DoR).

B. Accountability of PMA Cohort

The PMA cohort consisted of a total of 223 previously treated NSCLC patients who were enrolled in the study based on testing with a PD-L1 IHC enrollnment assay. Among the 223 patients, tumor tissue from 220 patients was retrospectively tested with the PD-L1 IHC 22C3 pharmDx test. Specimens from 61 patients were positive for PD-L1 expression (≥50% of viable tumor cells exhibiting membrane staining at any intensity) and samples from 104 patients were negative for PD-L1 expression (<50% of viable tumor cells exhibiting membrane staining at any intensity). Fifty-eight (58) patients had unknown PD-L1 expression status by the PD-L1 IHC 22C3 pharmDx test: specifically, 44 patients had tissue sections outside the 6 month cut section stability window; 11 patients had tumor tissues that were unevaluable (because the specimen contained insufficient numbers of tumor cells [n=9] or because bone tissue was present within the specimen [n=2]); and 3 samples were not tested.

C. Study Population Demographics and Baseline Parameters

Study enrollment occurred at 44 centers in 10 countries. Thirty out of the 61 patients with positive PD-L1 expression by PD-L1 IHC 22C3 pharmDx were enrolled in the U.S. The baseline characteristics for these 61 patients included:

Table 6: Baseline Characteristics

Baseline Characteristics	n (%)
Subjects in population	61
Gender	
Male	37 (61)
Female	24 (39)
Age (Years)	
< 65	40 (66)
>=65	21 (34)
Race	
Asian	7 (11)
Black Or African American	6 (10)
White	48 (79)
Ethnicity	
Hispanic Or Latino	1 (2)
Not Hispanic Or Latino	60 (98)
Region	
Australia	1 (2)
Canada	1 (2)
EU	25 (41)
East Asia	4 (6)
US	30 (49)
ECOG	
0	21 (34)
1	39 (64)
Histology	
Squamous	13 (21)
Non-Squamous	46 (75)
Adenosquamous	1 (2)
Smoking Status	
Never	12 (20)
Former	42 (69)
Current	7 (11)
Brain Metastases	
Yes	7 (11)
No	54 (89)
Number of Unique Prior Systemic Therapies	
1	16 (26)
2	18 (30)
3 or more	27 (44)
EGFR Mutation	6 (10)
KRAS Mutation	10 (16)
ALK Gene Rearrangement	1 (0)

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 NSCLC sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of NSCLC by pathologists. Removal of these tissues, therefore, presents no additional safety hazard to the patient being tested.

In the clinical study, the rate of discontinuations of KEYTRUDA® (pembrolizumab) due to adverse effects (AE) was 13.8%. The most frequent AEs in the study population tested with this drug were fatigue (34.9%), decreased appetite (25.1%), dyspnea (22.5%), and cough 21.6%).

2. Effectiveness Results

The clinical performance of PD-L1 IHC 22C3 pharmDx was established using the efficacy data used to support KEYTRUDA® (pembrolizumab) approval. Efficacy evaluation was performed in 61 patients with positive PD-L1 expression as determined by the PD-L1 IHC 22C3 pharmDx and had progression of disease following treatment with platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations had disease progression on FDA-approved therapies for these aberrations prior to receiving KEYTRUDA® (pembrolizumab). Table 7 below summarizes the responses to KEYTRUDA® (pembrolizumab) in previously treated NSCLC patients with PD-L1 tumor proportion score ≥50%.

Table 7: Response to KEYTRUDA® (pembrolizumab) in Previously Treated NSCLC Patients with PD-L1 tumor proportion score ≥50%

Endpoint	N=61
Overall Response Rate	
ORR %, (95% CI)	41% (29, 54)*
Complete Response	0%
Partial Response	41%
Response Duration	
Median in months (range)	Not reached (2.1+, 9.2+)
% ongoing	84%†
* In patients with a PD-L1 tumor proportion score <50% (n = 104), ORR was 13% (8, 22). † Includes 11 patients with ongoing responses of ≥6 months.	

Among the 61 patients whose response to pembrolizumab is described in Table 7, 25 patients (41%) experienced a partial response. Of these 25 patients, 21 (84%) had an ongoing response at the time of the data analysis cut-off date. At that time, the duration of response was between 2.1 and 9.2 months for all 25 responders. The patients with response durations of 2.1 and 9.2 months were both ongoing at the time of data cutoff, denoted by appending a + sign to those values in the

table. Median DoR is calculated based on the number of events and their timing and had not been reached at the data cut-off date.

3. Subgroup Analyses

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with a positive PD-L1 IHC 22C3 pharmDx test result, but who may have been negative by the enrollment assay. Patients with such test results are part of the intended use population of the PD-L1 IHC 22C3 pharmDx; however, they were excluded from the clinical trial due to negative results at the time of study screening. Analysis of the data confirmed that none of the samples that were PD-L1-negative by the enrollment assay were positive for PD-L1 expression (i.e., TPS≥50%) by PD-L1 IHC 22C3 pharmDx.

Robustness analyses were also conducted to assess the potential impact of missing data arising from samples that were unavailable or unevaluable at the time of retrospective evaluation of the archived clinical study samples with PD-L1 IHC 22C3 pharmDx. A sensitivity analysis examining the distribution of ORR estimates was conducted for the 223 enrolled patients over plausible values of missing PD-L1 status by the PD-L1 IHC 22C3 pharmDx for those patients whose PD-L1 status was unavailable or unevaluable. Plausible values of missing PD-L1 status were imputed using the posterior predictive distribution model that is conditioned on patient objective response status and PD-L1 expression level as obtained using the enrollmentassay. Computation of the distribution of ORR over these imputed PD-L1 IHC 22C3 pharmDx values reveals the level of support for the potential values of the ORR statistic while accounting for uncertainty imposed by the missing data and unknown model parameters.

The posterior predictive mean from this sensitivity analysis is 37.4% and the 95% credible interval is (34.5%, 40.5%) for the TPS≥50% group, which is similar to the primary analysis findings of a 41% response rate. Examination of the 95% credible interval shows that the most plausible ORR values acknowledging the uncertainty driven by the missing data dimension lie within 34.5 to 40.5%. In the TPS<50% group, the statistics are 11.3% and (10.2%, 12.7%) for the mean and the credible interval, respectively. These imputation-based analyses show that the higher response rate in the TPS≥50% subset versus the TPS<50% subset is robust after accounting for the missing data due to unavailable or unevaluable samples.

E. Financial Disclosure

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

XI. PANEL MEETING RECOMMENDATION

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

XII. CONCLUSION.S DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of PD-L1 IHC 22C3 pharmDx was demonstrated in a retrospective analysis for NSCLC patients enrolled in the multicenter, open-label, randomized phase 1clinical study to assess the safety and efficacy of KEYTRUDA® (pembrolizumab) treatment. KEYTRUDA® (pembrolizumab) demonstrated a robust overall response rate in NSCLC patients with positive PD-L1 expression as determined by PD-L1 IHC 22C3 pharmDx. The ORR in the 61 patient cohort was 41.0% (95% CI 28.6, 54.3). Results supported the improvement in response rate in NSCLC patients who are positive for PD-L1 (i.e., TPS≥50%), as determined by PD-L1 IHC 22C3 pharmDx.

The performance of the 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 specimens collected from patients with NSCLC. 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. As PD-L1 IHC 22C3 pharmDx is intended for use to identify patients for KEYTRUDA® (pembrolizumab) therapy, if incorrect or false results are reported, then NSCLC patients may not receive the proper treatment. Patients with false positive results may undergo treatment with KEYTRUDA® (pembrolizumab) without significant clinical benefit, and may experience adverse reactions associated with KEYTRUDA® (pembrolizumab) therapy. Patients with false negative results may not be considered for treatment with KEYTRUDA® (pembrolizumab), and therefore, may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with KEYTRUDA® (pembrolizumab).

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in the clinical study, which were used to support PMA approval as described above.

Data from the open-label, randomized clinical study support the performance of PD-L1 IHC 22C3 pharmDx as an aid in selecting patients with previously treated NSCLC who may be eligible for treatment with KEYTRUDA® (pembrolizumab). There was demonstrated improvement in objective response rate in NSCLC cancer patients who are positive for PD-L1 expression as identified by PD-L1 IHC 22C3 pharmDx and who have progression of disease following treatment with platinum-containing chemotherapy (patients with EGFR or ALK genomic tumor aberrations had disease progression on FDA-approved therapies for these aberrations) prior to receiving KEYTRUDA® (pembrolizumab).

Additional factors to be considered in determining probable risks and benefits for the PD-L1 IHC 22C3 pharmDx included the analytical performance of the device, and the availability of alternative tests. The primary risks associated with the PD-L1 IHC 22C3 pharmDx are the possibility of inaccurate, or false, results that may lead to mismanagement of patient treatment. The performance of the device is supported by analytical validation studies. There is currently no FDA-approved or -cleared device for the selection of previously treated NSCLC patients with positive PD-L1 expression for treatment with KEYTRUDA® (pembrolizumab). Thus, the probable benefits are based on results demonstrating that the test performs consistently and provides clinically relevant results for evaluating PD-L1 status in NSCLC patients who are being considered for KEYTRUDA® (pembrolizumab).

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 eligible for treatment with KEYTRUDA® (pembrolizumab).

XIII. CDRH DECISION

CDRH issued an approval order on October 2, 2015.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Limitations in the device labeling. Refer to the drug label for KEYTRUDA® (pembrolizumab) for additional information related to use of the drug.

Post-approval Requirements and Restrictions: See approval order.