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/S020

Date of FDA Notice of Approval: November 13, 2020

The original PD-L1 IHC 22C3 pharmDx PMA (P150013) was approved on October 2, 2015 for the qualitative detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on the 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 indication for use of the device as approved in P150013 is to aid in identifying patients with NSCLC whose tumors are considered PD-L1 positive if TPS $\geq$ 50% and for whom safety and efficacy of KEYTRUDA® (pembrolizumab) have been established. The SSED to support the 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 triple negative breast cancer (TNBC) for treatment with KEYTRUDA® (pembrolizumab). PD-L1 protein expression is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS $\geq$ 10.

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, head and neck squamous cell carcinoma (HNSCC), and triple-negative breast cancer (TNBC) 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, HNSCC and TNBC 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 ESCC 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). **
TNBC	CPS ≥ 10	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying TNBC 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.

IV. WARNINGS AND PRECAUTIONS

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

V. DEVICE DESCRIPTION

Device Kit Components

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical (IHC) staining procedure for formalin-fixed and paraffin-embedded (FFPE) specimens using the EnVision FLEX visualization system and the Dako Autostainer Link 48 with DakoLink software version 4.2. The principal component of the kit is the mouse monoclonal anti PD-L1 clone 22C3 antibody that binds to PD-L1 protein expressed on FFPE tissue. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and reagents shown in Table 1 necessary to perform 50 tests in up to 15 individual runs. Wash buffer and hematoxylin are required for the assay but not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods. Device kit components are shown in Table 1 below.

Table 1: Device Kit Components

Reagent	Description	Qty x Vol
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015 mol/L sodium azide.	1 x 34.5 mL
Monoclonal Mouse anti-PD-L1, Clone 22C3	Monoclonal mouse anti-PD-L1 antibody in a buffered solution, containing stabilizing protein(3 µg/mL protein concentration) , and 0.015 mol/L sodium azide.	1 x 19.5 mL
Negative Control Reagent	Monoclonal mouse control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.	1 x 15 mL
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015 mol/L sodium azide.	1x 34.5 mL
Visualization Reagent-Horseradish Peroxidase (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 34.5 mL
DAB+ Buffered Substrate	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.	15 x 7.2 mL

Reagent	Description	Qty x Vol
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride (DAB) in an organic solvent.	1 x 5 mL
DAB Enhancer	Cupric sulfate in water.	1 x 34.5 mL
Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.	6 x 30 mL
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 (positive control), and MCF-7 with negative PD-L1 protein expression (negative control).	15 slides

PD-L1 IHC 22C3 pharmDx comes with individually labeled reagent components that are recognized together. The kit components can only be used on the specified instrument with the PD-L1 IHC 22C3 pharmDx protocol. The DakoLink software has been designed to recognize and group PD-L1 IHC 22C3 pharmDx reagents, mandating that the reagents are run together.

Device Instrument 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; deparaffinization performed seperately in PT100).

Specimen Preparation

TNBC specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens. FFPE tissues are suitable for use. Fixation time for 12-72 hours in 10% neutral buffered formalin (NBF) is recommended. Alternative fixatives have not been validated and may give erroneous results. 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 10% NBF. Specimens are then processed through a tissue processor where they are dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. After specimen processing, tissue specimens should be cut into sections of 4-5 µm using a tissue microtome, mounted on charged microscope slides. Slides are then placed in a 58 ± 2°C oven for 1 hour as an initial step for deparafinization.

To preserve antigenicity, tissue sections, once mounted on slides, should be stored in the dark at 2-8 °C (preferred), or at room temperature up to 25°C. Tissue sections should be stained within 7.5 months of sectioning when stored at 2-8°C, or within 4 months when

stored at 25 °C. Slide storage and handling conditions should not exceed 25°C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls

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

1) Control cell line slides provided as part of the kit should be used to verify the staining procedure. One Control Slide should be stained with the primary antibody to PD-L1 in each staining run. Each slide contains sections of two pelleted, FFPE cell lines: one with moderate PD-L1 protein expression and one that is negative for PD-L1 protein expression. The evaluation of the Control Slide 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 to moderate positive 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 may occur 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 Procedure

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 HRP 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 is then counterstained with hematoxylin and cover-slipped.

Staining Protocol

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

Interpretation of stained slides should be performed by a pathologist using a light microscope with an objective of 20x magnification. All viable tumor cells on the entire slide must be evaluated and included in the PD-L1scoring assessment together with tumor associated PD-L1 positive lymphocytes and macrophages. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for evaluation. For specimens with less than 100 viable tumor cells, tissue from a deeper level of the block or potentially another block, could present sufficient tumor cells for PD-L1 evaluation.

PD-L1 expression in TNBC is determined by 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. 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:

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

By definition, PD-L1 staining cells are:

- Tumor cells with convincing partial or complete linear membrane staining (at any intensity) that is perceived distinct from cytoplasmic staining and
- Lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma with convincing membrane and/or cytoplasmic staining (at any intensity). MICs must be directly associated with the response against the tumor.

For each staining run, slides should be examined in the order recommended in the labeling to determine the validity of the staining run and enable assessment of the staining of the sample tissue. Patient specimens stained with PD-L1 and the negative control reagent (NCR) from PD-L1 IHC 22C3 pharmDx should be examined when evaluating PD-L1 expression. Specimens stained with NCR must have 0 specific staining and ≤ 1+ nonspecific staining.

The tables below provide details about which tissue elements are included in and excluded from the CPS numerator and denominator in TNBC.

Table 2. CPS Numerator Inclusion/Exclusion Criteria for TNBC

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor cells	• Convincing partial or complete linear membrane staining (at any intensity) of viable invasive tumor cells	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Ductal carcinoma in situ (DCIS) • Lobular carcinoma in situ (LCIS)

Immune cells	• Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma**:
	  • Lymphocytes (lymphocyte aggregates)
	  • Macrophages***
	• Only MICs directly associated with the response to the tumor are scored.
		• Non-staining MICs
		• MICs (including lymphoid aggregates) not directly associated with the response to the tumor
		• MICs associated with benign structures
		• Neutrophils, eosinophils, and plasma cells

Other Cells	• Not Included	• Benign epithelial 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.

Table 3. CPS Denominator Inclusion/Exclusion Criteria for TNBC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable invasive tumor cells	• Non-viable tumor cells • DCIS and LCIS
Immune Cells	Not included	• All immune cells
Other Cells	Not included	• Benign Cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

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

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 TNBC 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.

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.

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

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Preclinical studies were performed using PD-L1 IHC 22C3 pharmDx to establish analytical performance of the device. 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. There have been no changes to the device design including reagent formulation or kit configuration since approval of the original PMA (P150013). Results from studies performed to support the TNBC indication at CPS ≥ 10 are summarized in the sections below.

1. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 unique TNBC FFPE tissue specimens. The specimens were chosen at random and represented the full range of PD-L1 expression (i.e., CPS range 0-100). Seventy percent of the specimens were negative based on the CPS ≥ 10 cutoff (CPS < 10) and 30.0% were positive (CPS ≥ 10).

2. Analytical Specificity

a. Western Blot

See Summary of Safety and Effectiveness Data for P150013.

b. Immunoreactivity in Human Tissues

See Summary of Safety and Effectiveness Data for P150013.

3. Robustness

Robustness of the staining performance of PD-L1 IHC 22C3 pharmDx in TNBC was evaluated by testing the performance of the assay when varying the following conditions as described below:

- Target retrieval time at three incubation times
  - 18 minutes
  - 20 minutes-standard
  - 22 minutes
- Target Retrieval Solution 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

Studies were performed with 36 TNBC specimens. Specimens were blinded and randomized prior to evaluation at a predetermined cutoff. Negative percent agreement

(NPA), positive percent agreement (PPA), and overall percent agreement (OA) and 95% confidence interval (CI) were calculated for CPS≥10 cutoff by pairwise comparison to the standard condition. No significant difference in results was observed for the experimental conditions above when compared to the standard conditions.

4. Precision

Precision was assessed in three separate studies: Intra-run repeatability and combined precision (interoperator/instrument/day/lot). Intra-run and combined precision studies were evaluated with 33 TNBC specimens. Inter- and Intra-observer precision study was performed using 24 positive and 24 negative specimens. Study specimens spanned the range of PD-L1 expression, and at least 15% of these represented specimens around the CPS ≥ 10 cut off. Near cut-off specimens were defined as specimens with CPS scores of 5 - 15.

Study specimens were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Specimens were assessed for qualitative PD-L1 status as PD-L1 positive or PD-L1 negative based on the CPS ≥ 10 cutoff. NPA, PPA, and OA with two-sided 95% CIs using the bootstrap method or Wilson score method as applicable. The results are shown in Table 4.

Table 4: Summary of Precision Study Results

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision* (Inter-Operator, Inter-Instrument, Inter-Day, and Inter-Lot as combined variables)	CPS ≥ 10	33 TNBC specimens (21 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 3 operators, using 3 Autostainer Link 48 instruments, over 3 non-consecutive days, using 3 reagent lots.	NPA 100.0% (94.3-100.0%)* PPA 100.0% (90.4-100.0%)* OA 100.0% (96.3-100.0%)*
Intra-run precision* (Repeatability)	CPS ≥ 10	33 TNBC specimens (16 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 5 replicates within a run using the Autostainer Link 48 instrument.	NPA 100.0% (95.4-100.0%)* PPA 94.0% (86.9-100.0%) OA 96.9% (93.3-100.0%)
Inter-observer precision	CPS ≥ 10	17 positive and 31 negative specimens. One replicate from each of the 48 unique test specimens was stained with PD-L1 IHC 22C3 pharmDx. All tissues were scored by three certified pathologists three	NPA 93.2% (87.5-97.8%) PPA 92.2% (85.6-97.4%) OA 92.8% (88.4-96.8%)

		times, with at least a two-week washout between reads.	
Intra-observer precision	CPS ≥ 10	17 positive and 31 negative specimens. One replicate from each of the 48 unique test specimens was stained with PD-L1 IHC 22C3 pharmDx. All tissues were scored by three certified pathologists three times, with at least a two-week washout between reads.	NPA 98.5% (97.0-99.6%) PPA 94.5% (90.9-98.0%) OA 97.0% (95.4-98.6%)

* The percentile bootstrap method cannot compute confidence bounds if 100% agreement is observed. Therefore, the Wilson score method was used to compute confidence intervals when the agreement (PPA/NPA/OA) was 100.0% .

5. External Reproducibility

The external reproducibility study was designed to evaluate the reproducibility of PD-L1 IHC 22C3 pharmDx in determining PD-L1 expression in TNBC tissue specimens on the Dako Autostainer Link 48. Reproducibility was assessed in a two-part study: Study Part A assessing Inter- and Intra-Site endpoints and Study Part B assessing Inter- and Intra-Observer endpoints.

In Part A, reproducibility at the CPS ≥ 10 cutoff was assessed with 40 specimens 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. The specimen set was randomized and blinded prior to testing at three external sites and assessed for performance with regard to site-to-site and day-to-day reproducibility, or inter- and intra-site reproducibility.

Part B included assessment of observer-to-observer variability with specimens that spanned the PD-L1 expression range. These specimens were stained at Dako North America and shipped to the three external sites for assessment of PD-L1 expression by pathologists for both inter-observer and intra-observer reproducibility. Sixty pre-stained specimens were assessed three times by three readers with a minimum of 2 weeks between reads.

All IHC tests were interpreted by certified pathologists to determine the positive/negative results based on the CPS ≥ 10 expression level at three external sites. NPA, PPA, and OA with corresponding 95% CI were calculated by using comparisons to the majority call. The results of the reproducibility study for the CPS ≥ 10 cutoff are included in Table 5.

Table 5. Three Sites Reproducibility in TNBC (CPS ≥ 10)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 10	40 TNBC specimens (19 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 non-consecutive days. Inter-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA 93.0% (85.3-100.0%) PPA 92.1% (86.3-97.1%) OA 92.5% (87.8-96.7%)
Intra-site	CPS ≥ 10	40 TNBC specimens (19 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 non-consecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA 97.1% (94.3-99.3%) PPA 94.4% (90.0-98.1%) OA 95.7% (92.7-98.2%)
Inter-observer	CPS ≥ 10	60 TNBC specimens (26 PD-L1-negative and 34 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists, 1 at each of 3 study sites, on 3 non-consecutive days. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to majority call.	NPA 97.0% (93.6-100.0%) PPA 95.4% (91.2-98.7%) OA 96.1% (93.3-98.5%)
Intra-observer	CPS ≥ 10	60 TNBC specimens (26 PD-L1-negative and 34 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists, 1 at each of 3 study sites, on 3 non-consecutive days. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to majority call.	NPA 98.7% (96.6-100.0%) PPA 96.7% (94.6-98.7%) OA 97.6% (96.1-98.9%)

6. Stability Studies

a. PD-L1 IHC 22C3 pharmDx Stability

Product Shelf Life: Real time shelf life stability testing with three reagent lots of PD-L1 IHC 22C3 pharmDx was previously performed for NSCLC and presented in P150013. The protocol included transport simulation, working stability and in-use/on-board stability testing. The shelf life for PD-L1 IHC 22C3 pharmDx remains unchanged and is 9 months when stored at 2-8 ºC.

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. FFPE Cut Section Stability
A stability study was conducted to evaluate the stability of the antigen in cut tissue sections of TNBC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 8 TNBC specimens. Based on these studies, stability dating for cut slides in TNBC is 7.5 months for cut sections stored at 2-8 °C and 4 months for cut sections stored at 25 °C.

7. Impact of Tumor 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 22C3 pharmDx. When specimens were determined to be positive or negative based on the CPS ≥ 10 cutoff, there were 13 concordant negative pairs, 4 concordant positive pairs, and 4 pairs had a discordant PD-L1 status. Results may not be representative of all TNBC specimens, as tumor heterogeneity is unique for each specimen.

b. Intra-Case Heterogeneity
Two sister FFPE blocks from 19 TNBC subjects obtained from the same tumor were evaluated to demonstrate within-patient concordance for PD-L1 status. In 17 of 19 cases, the diagnostic outcomes based on a predetermined cutoff were concordant across intra-case (sister) blocks. Two cases showed discordant results across sister blocks. Results may not be representative of all TNBC specimens, as tumor heterogeneity is unique for each specimen.

c. Intra-Block Heterogeneity
Thirty-four individual FFPE TNBC tissue blocks were divided in 3 portions across a minimum of 200 μm: anterior, middle, and posterior. Of the 34 cases assessed, 32 cases were concordant and 2 cases were discordant when comparing the diagnostic status of front and back sections based on the CPS ≥ 10 cutoff. Results may not be representative of all TNBC specimens, as tumor heterogeneity is unique for each specimen.

B. Animal Studies

None

C. Additional Studies

1. Impact on Ischemia/Fixation
See SSED for P150013.

2. Control Cell Line Validation
See SSED for P150013.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of PD-L1 IHC 22C3 pharmDx as a companion diagnostic for PD-L1 detection (CPS ≥ 10) in TNBC patients to determine eligibility for treatment with pembrolizumab (KEYTRUDA®) was demonstrated in study KEYNOTE-355. This study was conducted in the US and geographical regions including, Asia, Europe, Australia and rest of the world. A summary of the clinical study is presented below.

A. Study Design

KEYNOTE-355 part 2, a randomized, double-blind, phase III study evaluated the efficacy of KEYTRUDA in combination with paclitaxel, paclitaxel protein bound, or gemcitabine and carboplatin in 847 patients with locally recurrent unresectable or metastatic TNBC, regardless of tumor PD-L1 expression, who had not been previously treated with chemotherapy. Randomization was stratified by chemotherapy treatment (paclitaxel or paclitaxel protein-bound vs. gemcitabine and carboplatin), tumor PD-L1 expression (CPS ≥ 1 vs. CPS < 1) according to the PD-L1 IHC 22C3 pharmDx kit, and prior treatment with the same class of chemotherapy in the neoadjuvant setting (yes vs. no). According to KN 355 protocol amendment 5, the study analysis population included both patient population with PD-L1 CPS ≥1 and PD-L1 CPS ≥10.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the KEYNOTE-355 study was limited to to male and female subjects with locally recurrent inoperable or metastatic TNBC, which had not been previously treated with chemotherapy, and who were at least 18 years of age.

A summary of the additional inclusion criteria are as follows:

- Have centrally confirmed TNBC.

Note: Subjects initially diagnosed with hormone receptor–positive and/or HER2-positive breast cancer must have central confirmation of TNBC in a tumor biopsy obtained from a local recurrence or distant metastasis site.

- Have measurable disease based on RECIST 1.1 as determined by local radiology review.
- Have provided recently or newly obtained core or excisional biopsy from a locally recurrent inoperable or metastatic tumor lesion for central

determination of TNBC status and PD-L1 expression, unless contraindicated due to site inaccessibility and/or subject safety concerns.

2. Follow-up Schedule

Treatment with KEYTRUDA continued until RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ)-defined progression of disease as determined by the investigator, unacceptable toxicity, or up to 24 months in patients without disease progression. Assessment of tumor status was performed at Weeks 8, 16, and 24, and then every 9 weeks for the first year, and every 12 weeks thereafter.

3. Clinical Endpoints

Primary endpoint:

1. Progression-free survival (PFS), defined as the time from randomization to the first documented disease progression as measured by Blinded independent central review (BICR) per RECIST 1.1 or death due to any cause, whichever occurred first.

Secondary efficacy endpoints::

1. Objective response rate (ORR), defined as the proportion of participants in the analysis population who had a response (complete response [CR] or partial response [PR]) as measured by BICR per RECIST 1.1.
2. Duration of response (DOR), defined as the time from the first documented evidence of CR or PR until disease progression as measured by BICR per RECIST 1.1 or death due to any cause, whichever occurred first.

B. Accountability of PMA Cohort

Participants with locally recurrent inoperable or metastatic TNBC in KEYNOTE-355 comprise the primary participant population for this application. A total of 847 participants were randomly assigned in a 2:1 ratio to pembrolizumab + chemotherapy (566 participants) or placebo + chemotherapy (281 participants). All 847 participants had a tumor tissue sample tested with PD-L1 IHC 22C3 pharmDx. Most participants (75.1%, n=636) had a tumor tissue PD-L1 expression score of CPS ≥1, and 38.1% of participants (n=323) had a tumor tissue PD-L1 expression score of CPS ≥10. The patient/sample accountability is shown in Table 6 below.

Table 6: Accountability of PMA Cohort

	Number of Study Subjects
Enrolled	847
Tumor sample collected at baseline	847
Specimens with insufficient tissue	0
Total evaluable PD-L1 expression	847
PD-L1 positive CPS ≥ 10	323

PD-L1 negative CPS <10	524
Site of Tumor: (N=847)	
Primary Site	456
Metastatic Site	391
Specimen type (Derived based on dates*, N=847)	
Newly Obtained	561
Archival	286
Sample Procedure (N= 847)	
Biopsy	616
Resection	231
*Specimen type derived based on sample collection date. If the sample was obtained at or after the diagnosis of locally recurrent inoperable or metastatic breast cancer, or if the participant was diagnosed with de novo metastasis, then the sample is categorized as 'Newly Obtained'; otherwise the sample is categorized as 'Archival'. Of note, three subjects had missing data for 'date become metastasis or locally recurrent inoperable', and their samples were categorized as 'Newly re collected within 60 days of randomization.	

C. Study Population Demographics and Baseline Parameters

The baseline characteristics for study subjects with PD-L1 CPS ≥10 are shown in the table 7 below.

Table 7: Subject Characteristics

Subjects with PD-L1 (CPS ≥10)

	Pembrolizumab + Chemotherapy		**Placebo + Chemotherapy**		**Total**	
	n	(%)	n	(%)	n	(%)
Subjects in population	220		103		323	
Gender						
Female	220	(100.0)	103	(100.0)	323	(100.0)
Age (Years)						
< 65	178	(80.9)	79	(76.7)	257	(79.6)
>= 65	42	(19.1)	24	(23.3)	66	(20.4)
Mean	52.5		53.3		52.7	
SD	12.5		13.2		12.7	
Median	52.0		55.0		53.0	
Range	25 to 83		22 to 77		22 to 83	
Race						
American Indian Or Alaska Native	2	(0.9)	0	(0.0)	2	(0.6)
Asian	44	(20.0)	20	(19.4)	64	(19.8)

Black Or African American	9	(4.1)	6	(5.8)	15	(4.6)
Multiple	6	(2.7)	3	(2.9)	9	(2.8)
White	153	(69.5)	70	(68.0)	223	(69.0)
Missing	6	(2.7)	4	(3.9)	10	(3.1)
Ethnicity						
Hispanic Or Latino	37	(16.8)	21	(20.4)	58	(18.0)
Not Hispanic Or Latino	174	(79.1)	76	(73.8)	250	(77.4)
Not Reported	6	(2.7)	4	(3.9)	10	(3.1)
Unknown	2	(0.9)	2	(1.9)	4	(1.2)
Missing	1	(0.5)	0	(0.0)	1	(0.3)
Geographic Region						
Asia	38	(17.3)	18	(17.5)	56	(17.3)
Europe	108	(49.1)	52	(50.5)	160	(49.5)
Australia	5	(2.3)	2	(1.9)	7	(2.2)
North America	33	(15.0)	12	(11.7)	45	(13.9)
Rest of the World	36	(16.4)	19	(18.4)	55	(17.0)
Chemotherapy on Study						
Nab-Paclitaxel	63	(28.6)	36	(35.0)	99	(30.7)
Paclitaxel	33	(15.0)	11	(10.7)	44	(13.6)
Gemcitabine/Carboplatin	124	(56.4)	56	(54.4)	180	(55.7)
Chemotherapy on Study (Actual)						
Nab-Paclitaxel	61	(27.7)	36	(35.0)	97	(30.0)
Paclitaxel	33	(15.0)	11	(10.7)	44	(13.6)
Gemcitabine/Carboplatin	125	(56.8)	56	(54.4)	181	(56.0)
Missing	1	(0.5)	0	(0.0)	1	(0.3)
Prior Treatment with Same Class Chemotherapy in the Neoadjuvant or Adjuvant Setting						
Yes	46	(20.9)	19	(18.4)	65	(20.1)
No	174	(79.1)	84	(81.6)	258	(79.9)
Prior Treatment with Same Class Chemotherapy in the Neoadjuvant or Adjuvant Setting (Actual)						
Yes	44	(20.0)	17	(16.5)	61	(18.9)
No	175	(79.5)	86	(83.5)	261	(80.8)
Missing	1	(0.5)	0	(0.0)	1	(0.3)
Disease Status						
Metastatic, De Novo	68	(30.9)	35	(34.0)	103	(31.9)
Metastatic, Recurrence	144	(65.5)	62	(60.2)	206	(63.8)
Locally Recurrent Inoperable	7	(3.2)	6	(5.8)	13	(4.0)

Missing	1	(0.5)	0	(0.0)	1	(0.3)
ECOG						
0	134	(60.9)	62	(60.2)	196	(60.7)
1	86	(39.1)	41	(39.8)	127	(39.3)
HER2 Status						
0-1+ by IHC	153	(69.5)	80	(77.7)	233	(72.1)
2+ by IHC	67	(30.5)	23	(22.3)	90	(27.9)
History of Brain Metastasis						
Yes	5	(2.3)	6	(5.8)	11	(3.4)
No	215	(97.7)	97	(94.2)	312	(96.6)
Menopausal Status						
Pre-menopausal	74	(33.6)	34	(33.0)	108	(33.4)
Post-menopausal	146	(66.4)	69	(67.0)	215	(66.6)
Disease Free Interval						
de novo metastasis	68	(30.9)	35	(34.0)	103	(31.9)
< 12 months	49	(22.3)	17	(16.5)	66	(20.4)
>= 12 months	102	(46.4)	51	(49.5)	153	(47.4)
Unknown	1	(0.5)	0	(0.0)	1	(0.3)
Baseline Lactate Dehydrogenase (LDH)						
Normal	115	(52.3)	51	(49.5)	166	(51.4)
> ULN and < 2 x ULN	75	(34.1)	35	(34.0)	110	(34.1)
>= 2 x ULN	25	(11.4)	12	(11.7)	37	(11.5)
Missing	5	(2.3)	5	(4.9)	10	(3.1)
Sum of Target Lesion Size at Baseline (Central) (mm)						
Subjects with data	199		98		297	
Mean	69.2		72.4		70.2	
SD	47.6		51.4		48.9	
Median	57.0		59.5		59.0	
Range	11.0 to 290.0		15.0 to 271.0		11.0 to 290.0	
Sum of Target Lesion Size at Baseline (Investigator) (mm)						
Subjects with data	217		103		320	
Mean	77.3		78.1		77.6	
SD	59.9		47.8		56.2	
Median	60.0		66.0		60.0	
Range	10.0 to 352.0		10.0 to 237.1		10.0 to 352.0	

D. Safety and Effectiveness Results

1. Safety Results

The analysis of safety was based on 847 patients. In the trial, there were no device related adverse events.
The results from KEYNOTE-355 demonstrate that pembrolizumab in combination with chemotherapy has a tolerable and manageable safety profile, which is consistent with the known safety profiles of commonly used chemotherapy in locally recurrent inoperable or metastatic TNBC and pembrolizumab monotherapy. Please refer to Drugs@FDA for complete safety information on KEYTRUDA® (pembrolizumab).

2. Effectiveness Results

A summary of the efficacy results for the participants with tumors expressing PD-L1 CPS ≥10 are provided in Table 8. The trial demonstrated a statistically significant improvement in PFS in patients with PD-L1 positive tumors (CPS ≥10) randomized to KEYTRUDA in combination with chemotherapy compared to placebo in combination with chemotherapy (Table 8 and Figure 1). The trial also demonstrated a clinically meaningful improvement in ORR and DoR (Table 8).

Table 8. Summary of Efficacy Results in Participants with PD-L1 Positive Tumors (CPS≥10)

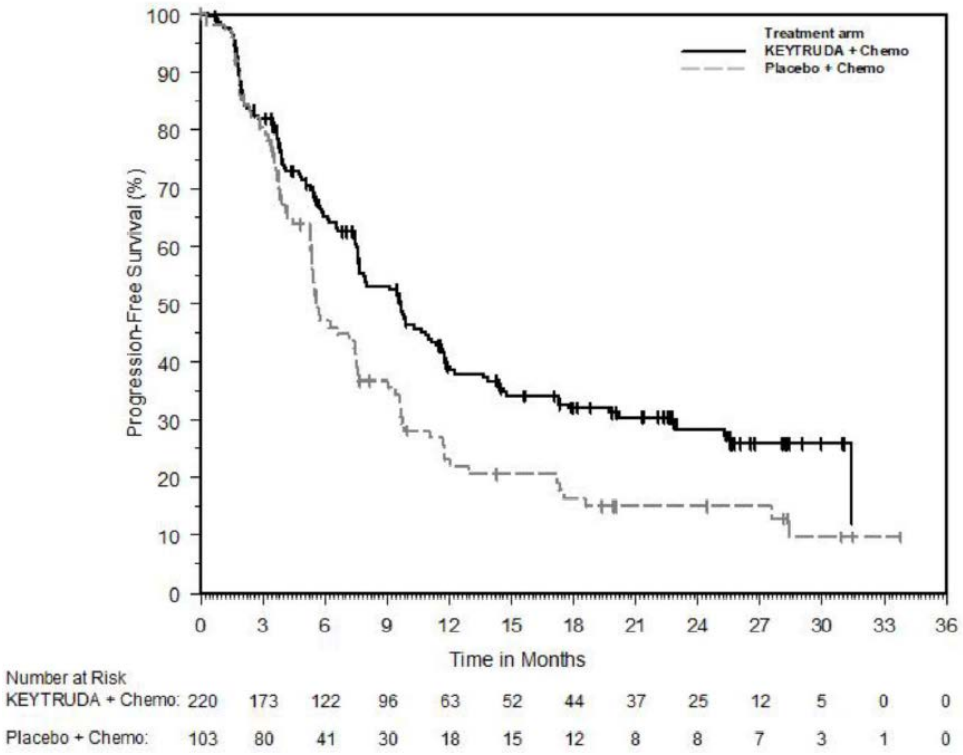
Endpoint	KEYTRUDA 200 mg every 3 weeks with chemotherapy* n=220**	Placebo every 3 weeks with chemotherapy* n=103**
PFS		
Number of patients with event (%)	136 (62%)	79 (77%)
Median in months (95% CI)	9.7 (7.6, 11.3)	5.6 (5.3, 7.5)
Hazard ratio† (95% CI)	0.65 (0.49, 0.86)	
p-Value‡	0.0012	
ORR		
Objective confirmed response rate (95% CI)	53% (46, 60)	40% (30, 50)
Complete response rate	17%	13%
Partial response rate	36%	27%
DoR		
Range (months)	19.3 (1.6+, 29.8)	7.3 (1.5, 32.5+)
% with duration ≥6 months§	83%	58%
% with duration ≥12 months§	56%	39%

* Chemotherapy: paclitaxel, paclitaxel protein-bound, or gemcitabine and carboplatin

† Based on Cox regression model with Efron's method of tie handling with treatment as a covariate stratified by chemotherapy on study (taxane vs. gemcitabine and carboplatin) and prior treatment with same class of chemotherapy in the neoadjuvant setting (yes vs. no).

‡ One-sided p-Value based on log-rank test stratified by chemotherapy on study (taxane vs. gemcitabine and carboplatin) and prior treatment with same class of chemotherapy in the neoadjuvant setting (yes vs. no).
§ From product-limit (Kaplan-Meier) method for censored data.
+ Denotes ongoing
** Since 2 patients that were PD-L1 CPS ≥ 10 did not have central confirmation of TNBC status, sensitivity analysis was performed excluding these 2 patients from the analysis population. Re-analysis of clinical effectiveness of the device excluding these 2 patients did not alter the hazard ratio.

Figure 1: Kaplan-Meier Curve for Progression-Free Survival in KEYNOTE-355 (CPS ≥10)



3. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.
4. Subgroup Analyses
   There were no subgroup analyses performed in this clinical study.
5. 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 4 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. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical performance of PD-L1 IHC 22C3 pharmDx for the detection of PD-L1 in breast tumor specimens from TNBC patients who may benefit from treatment with pembrolizumab is based on results from KEYNOTE-355. The analysis of 323 participants from KEYNOTE-355 demonstrated the effectiveness of PD-L1 IHC 22C3 pharmDx in identifying TNBC patients whose tumors express PD-L1 (CPS ≥10) for treatment with pembrolizumab in combination with chemotherapy. Treatment with pembrolizumab in combination with chemotherapy provided a statistically significant and clinically meaningful improvement in PFS compared with placebo + chemotherapy in participants with PD-L1 positive tumors (CPS ≥10). These PFS data are further supported by clinically meaningful improvements in ORR in participants receiving pembrolizumab + chemotherapy combination compared with placebo + chemotherapy. Responses were durable in participants with PD-L1 positive tumors (CPS ≥10) who responded to pembrolizumab + chemotherapy, suggesting a long-term benefit for the use of pembrolizumab in this population of participants with locally recurrent inoperable or metastatic TNBC.

Taken together with the nonclinical studies, these results demonstrate the effectiveness of the PD-L1 IHC 22C3 pharmDx as an aid in identifying TNBC patients for treatment with pembrolizumab.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies as well as data collected in a clinical study conducted to support PMA approval as described above.

Safety of the device for patient management is related to the safety and effectiveness of the therapeutic. Pembrolizumab + chemotherapy combination in KEYNOTE-355 has a tolerable and manageable safety profile that is consistent with known safety profiles for pembrolizumab monotherapy and chemotherapy. No new safety signals were identified. 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.The process of testing FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for TNBC cancer diagnosis.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the clinical study, which demonstrated pembrolizumab + chemotherapy provides a statistically significant and clinically meaningful improvement in PFS compared with placebo + chemotherapy in participants with PD-L1 positive tumors (CPS ≥10), as determined by the device.

The probable 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 combination with chemotherapy in PD-L1 positive patients was determined to have clinical benefit. The analytical validation conducted further supports the test as a reliable method for detecting PD-L1 expression in TNBC tumor samples.

Additional factors to be considered in determining probable risks and benefits for the PD-L1 IHC 22C3 pharmDx included: 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. Thus, the probable benefits are based on evaluation that the test performs consistently and provides clinically relevant results for evaluating PD-L1 status (CPS ≥ 10) in TNBC who are being considered for treatment with KEYTRUDA.

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 that, for the TNBC patients who are being considered for treatment with KEYTRUDA®, the probable benefits of PD-L1 IHC 22C3 pharmDx use outweigh the probable risks.

D. Overall Conclusions

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

XIV. CDRH DECISION

CDRH issued an approval order on November 13, 2020.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.
Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.