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 28-8 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: P150025/S013

Date of FDA Notice of Approval: May 15, 2020

The original PMA (P150025) for PD-L1 IHC 28-8 pharmDx intended for use in detecting PD-L1 protein in non-squamous non-small cell lung cancer (nsNSCLC) was approved on October 9, 2015. A second PMA (P150027) for the same device was approved for use in detecting PD-L1 protein in melanoma on January 23, 2016. The Agency combined PMA submissions (P150025 and P150027) for PD-L1 IHC 28-8 pharmDx to be referenced as P150025. A supplemental PMA (P150025/S003) for PD-L1 IHC 28-8 pharmDx indicated for squamous cell carcinoma of the head and neck (SCCHN) and urothelial carcinoma (UC) was approved on September 15, 2017. The SSED to support the non-squamous NSCLC, SCCHN, and UC indications is available in the CDRH website and are incorporated by reference here.

The melanoma indication was withdrawn from the PD-L1 IHC 28-8 pharmDx label based on the removal of the PD-L1 subgroup data for study CA209067, in conjunction with the March 07, 2019 conversion of accelerated approval into regular approval of OPDIVO® alone or in combination with YERVOY® for the unresectable or metastatic melanoma indication (BLA 125554/S-042). The current supplement was submitted to expand the indication for the PD-L1 IHC 28-8 pharmDx to include detection of PD-L1 protein in NSCLC (non-squamous NSCLC and squamous NSCLC) patients for treatment with OPDIVO® (nivolumab) in combination with YERVOY® (ipilimumab).

II. INDICATIONS FOR USE

For in vitro diagnostic use.

PD-L1 IHC 28-8 pharmDx is a qualitative immunohistochemical assay using Monoclonal Rabbit Anti-PD-L1, Clone 28-8 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head and neck (SCCHN), and urothelial carcinoma (UC) tissues using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression is defined as the percentage of evaluable tumor cells exhibiting partial or complete membrane staining at any intensity.

Companion Diagnostic Indication

Tumor Indication	PD-L1 Expression Clinical Cut Off	Intended Use
NSCLC	≥1% tumor cell expression	PD-L1 IHC 28-8 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with OPDIVO® (nivolumab) in combination with YERVOY® (ipilimumab).

PD-L1 expression (≥1% or ≥5% or ≥10% tumor cell expression), as detected by PD-L1 IHC 28-8 pharmDx in non-squamous NSCLC may be associated with enhanced survival from OPDIVO®.

PD-L1 expression (≥1% tumor cell expression), as detected by PD-L1 IHC 28-8 pharmDx in SCCHN may be associated with enhanced survival from OPDIVO®.

PD-L1 expression (≥1% tumor cell expression), as detected by PD-L1 IHC 28-8 pharmDx in UC may be associated with enhanced response rate from OPDIVO®.

See the OPDIVO® and YERYOV® product labels 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 28-8 pharmDx labeling.

V. DEVICE DESCRIPTION

PD-L1 IHC 28-8 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 system and the EnVision FLEX visualization system. The principle component of the kit is the rabbit monoclonal anti

PD-L1 clone 28-8 antibody that binds to PD-L1 protein expressed on FFPE tissue. The kit includes reagents required for pre-treatment of tissue, secondary antibodies, amplification and detection reagents that are all manufactured by Dako. PD-L1 IHC 28-8 pharmDx includes reagents sufficient to perform 50 tests in up to 15 individual runs and instructions for use (IFU). A total of 28 vials containing reagents including the EnVision FLEX visualization system, and 15 cell line control slides are provided in each kit. See Table 1 below.

Table 1: Overview of PD-L1 IHC 28-8 pharmDx Components

Component	Component Description	Quantity x Volume
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015 mol/L sodium azide.	1 x 34.5 mL
Primary Antibody: Monoclonal Rabbit anti-PD-L1, Clone 28-8	Monoclonal rabbit anti-PD-L1 in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.	1 x 19.5 mL
Negative Control Reagent (NCR)	Monoclonal rabbit control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.	1 x 15 mL
Linker, anti-Rabbit	Mouse secondary antibody against rabbit immunoglobulins in a buffered solution containing stabilizing protein and 0.015 mol/L sodium azide.	1 x 34.5 mL
Visualization Reagent-HRP	Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution	1 x 34.5 mL
DAB+ Substrate Buffer	Buffered solution containing hydrogen peroxide and an antimicrobial agent.	15 x 7.2 mL
DAB+ Chromogen	3,3′-diaminobenzidine tetrahydrochloride in an organic solvent.	1 x 5 mL
DAB Enhancer	Cupric sulfate in water.	1 x 34.5 mL
EnVision FLEX Target Retrieval Solution, Low pH, 50x	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent (EnVision FLEX Target Retrieval Solution, Low pH, 50X).	6 x 30 mL
PD-L1 IHC 28-8 pharmDx Control Slides	Each slide contains sections of two pelleted, formalin-fixed, paraffin-embedded cell lines: NCI-H226 with positive PD-L1 protein expression (originating from human lung squamous cell carcinoma with positive PD-L1 protein expression) and MCF-7 with negative PD-L1 protein expression (originating from human breast adenocarcinoma with negative PD-L1 protein expression).	15 slides

Device Instrumentation and Software

PD-L1 IHC 28-8 pharmDx assay is performed on the Dako Autostainer Link 48 automated staining system using the Dako Link software (version 4.0.3, 4.1.0 and 4.2.). The Autostainer system is designed to mimic the staining steps performed manually by a lab

technician. The PD-L1 IHC 28-8 pharmDx protocol is assay specific. The Dako Link software has been designed to recognize and group PD-L1 IHC 28-8 pharmDx reagents, requiring that all system reagents are used together. Deparaffinization, rehydration, and target retrieval (3-in-1) procedures are performed in the Dako PT Link Pre-treatment module.

Specimen Preparation

Only FFPE tissues are suitable for use. Recommended handling and processing conditions are as follows: < 30 minutes ischemia time prior to immersion in fixative, and 24-48 hours fixation time in 10% neutral buffered formalin. Alternative fixative methods have not been validated and may give erroneous results. The use of PD-L1 IHC 28-8 pharmDx on decalcified tissues has not been validated and is not recommended. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in 10% neutral buffered 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. After sectioning, tissues should be mounted on Fisherbrand Superfrost Plus charged slides or Dako FLEX IHC Microscope Slides and then placed in a 58 ± 2 °C oven for 1 hour. To preserve antigenicity, tissue sections, once mounted on slides, should be stored in the dark at 2-8 °C, or room temperature up to 25 °C, and stained within 4 months of sectioning **except** for squamous NSCLC. Squamous NSCLC tissue sections stored at room temperature up to 25 °C should be stained within 2 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

Run controls are included in each staining run to establish the validity of the test results. 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) Positive run controls of the same tumor indication as the patient specimen are to be provided by the end-user laboratory. It is recommended that tissue that stains a weak to moderate intensity for PD-L1 is used to monitor for all aspects of pre-analytical variables such as fixation, processing and sectioning. Negative control tissue of the same tumor indication as the patient specimen is required to detect unintended antibody cross reactivity to tissue and is expected to be negative for PD-L1 expression. Tissue features located in patient tissue or positive control tissue that is not expected to show PD-L1 expression may be used as negative control for staining.

3) The kit includes a Negative Control Reagent that is used in parallel with the PD-L1 Clone 28-8 primary antibody on patient tissue. The matched negative control reagent aids the reader in differentiating of specific staining at the antigen site 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 28-8 pharmDx contains optimized reagents and protocol required to complete an IHC staining procedure of FFPE specimens using Autostainer Link 48 and PT Link Pre-treatment Module.

Patient FFPE tissue specimens are subject to deparaffinization, rehydration, and target retrieval in the Target Retrieval Solution Low pH (3-in-1) for 20 minutes at 97 °C in the PT Link instrument to expose the PD-L1 antigen if present on the tissue. The slides are then loaded onto the Autostainer Link 48 automated stainer and incubated with the primary monoclonal antibody to PD-L1 (Clone 28-8) or the Negative Control Reagent (NCR). This step enables binding of the primary antibody to tumor tissue section when PD-L1 antigen is expressed. The slides are then incubated with an anti- Rabbit linker antibody, specific to Fc region 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 specimens are then counterstained with hematoxylin and coverslipped and observed under a microscope to visually determine if the PD-L1 protein is expressed in patient tumor tissue.

Staining Protocol:
The staining protocol for PD-L1 IHC 28-8 pharmDx on the Dako Autostainer Link 48 is as follows:

Rinse in buffer
Peroxidase-Blocking Reagent (2 drop zones x 150 µL): 5 minutes
Rinse in buffer
Monoclonal Rabbit anti-PD-L1 (or Negative Control Reagent) (2 drop zones x 150 µL): 30 minutes
Rinse in buffer
Linker Reagent (2 drop zones x 150 µL): 30 minutes
Rinse in buffer
Visualization Reagent-HRP (2 drop zones x 150 µL): 30 minutes
Rinse in buffer: 5 minutes
DAB+ solution (2 drop zones x 150 µL): 2 x 5 minutes
Rinse in buffer

DAB Enhancer (2 drop zones x 150 µL): 5 minutes
Rinse in buffer
Hematoxylin (2 drop zones x 150 µL): 7 minutes
Rinse in deionized water
Rinse in buffer: 5 minutes
Rinse in deionized water

Interpretation of PD-L1 Staining in NSCLC

Interpretation of PD-L1 IHC 28-8 pharmDx stained slides should be performed by a pathologist using a light microscope. For evaluation of the PD-L1 immunohistochemical staining and scoring, 4x objective magnification can be used for initial assessment of the entire specimen, followed by the 10-20x objectives for scoring (40x can be utilized for confirmation if needed). PD-L1 staining is indicated with a brown (3,3'-diaminobenzidine, DAB) reaction product. All viable tumor cells on the entire PD-L1 stained NSCLC patient slide must be evaluated and included in the PD-L1 scoring assessment. A minimum of 100 viable tumor cells should be present in the PD-L1 stained NSCLC patient slide to determine the percentage of stained cells.

Cytoplasmic staining, if present, is not considered for scoring purposes. Additionally, non-malignant cells including stromal cells, immune cells (e.g., infiltrating lymphocytes or macrophages), necrotic cells and cellular debris, if stained with PD-L1, should be excluded from the scoring for the determination of PD-L1 positivity.

The specimen is considered PD-L1 positive if ≥ 1% of tumor cells exhibit circumferential and/or partial linear plasma membrane PD-L1 staining of tumor cells at any intensity. The specimen is considered PD-L1 negative if <1% of cancer cells exhibit circumferential and/or partial linear plasma membrane PD-L1 staining of tumor cells at any intensity.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or approved alternatives for the testing of PD-L1 in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) human specimens to assess PD-L1 expression levels for treatment with OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

VII. MARKETING HISTORY

PD-L1 IHC 28-8 pharmDx, as approved under P150025 in the US, is currently marketed in Argentina, Algeria, Australia, Austria, Bahrain, Belgium, Brazil, Bulgaria, Canada, Chile, Colombia, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Egypt, Estonia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, India, Ireland, Israel, Italy, Japan, Jordan, South Korea, Kuwait, Latvia, Lebanon, Lithuania, Luxembourg, Macau, Macedonia, Malaysia, Malta, Mexico, Morocco, Netherlands, New Zealand, Norway, Oman, Panama, Peru, Philippines, Poland, Portugal, Qatar, Romania, Russia, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, UAE, United States, United Kingdom, Ukraine, Uruguay, 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 interpretation of the benefit/risks for patients who are considering treatment with OPDIVO in combination with YERVOY®.

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 28-8 pharmDx kit to establish analytical performance of 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 scoring algorithm used in these studies included a clinical score (i.e., PD-L1 positive or negative), a PD-L1 expression score (i.e., percent positive cell staining), and/or an analytical quality score (0-3 scale for staining signal intensity). Clinical scores were recorded for all studies with the scoring algorithm developed for clinical interpretation of the PD-L1 28-8 IHC pharmDx. Analytical scores were assessed for some studies to evaluate assay performance across the range of staining including borderline cases. The analytical score is not part of the interpretation of PD-L1 staining status in the device labeling.

1. **Analytical Specificity**
   See Summary of Safety and Effectiveness Data for P150025.

2. **Analytical Sensitivity**
   Analytical sensitivity of PD-L1 IHC 28-8 pharmDx was tested on a set of 186 unique cases of NSCLC FFPE specimens staged I to IV using one lot of PD-L1 IHC 28-8 pharmDx device. Of those 186 specimens, 112 specimens were non-squamous NSCLC and 74 were squamous NSCLC. Assessment of PD-L1 expression demonstrated staining across a range of 0-100% positive tumor cells and 0 - 3 staining intensity. The number of positive cases at the ≥1% PD-L1 expression level was 135 (73%).

3. **Repeatability**
   The objective of this study was to demonstrate that PD-L1 IHC 28-8 pharmDx would produce consistent staining in normal day-to-day testing.

Inter-instrument, Inter-operator, Inter-day and Intra-lot studies were performed with 90 NSCLC specimens that were pre-qualified at Dako to represent a PD-L1 expression range and a minimum of 25% of these specimens were around the cut off (≥1%). Of those 90 specimens, 49 specimens were non-squamous NSCLC and 41 were squamous NSCLC. The specimen distribution were as follows: Inter-instrument: 46 negative and 44 positive, Inter-operator: 49 negative, 41 positive, Inter-day: 50 negative and 40 positive, Intra-run: 44 negative and 46 positive. Inter-lot study was performed with 36 NSCLC specimens (14 negative and 22 positive) that were pre-qualified at Dako to represent a PD-L1 expression range and a minimum of 25% of these specimens were around the cut off (≥1%). Of those 36 specimens, 20 specimens were non-squamous NSCLC and 16 were squamous NSCLC. All NSCLC specimen 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 ≥1 % or negative if PD-L1 expression was <1 %. Comparisons were made using majority diagnostic outcome (majority call) across all observations for a given specimen as reference. Statistical analysis was conducted on the dichotomized data. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) with two-sided 95% percentile bootstrap confidence intervals were calculated for each of the study endpoints.

Summaries and results of the repeatability studies are presented Table 2 below.

Table 2: Repeatability of PD-L1 IHC 28-8 pharmDx Tested at One Site, PD-L1 expression ≥1%

Precision Study	Study Design	% Agreement (95% CI)
Intra-run	Each of 90 NSCLC specimens with a range of PD-L1 IHC expression was tested with 5 replicates within a run on the Autostainer Link 48 instrument. A total of 447 comparisons to majority call were performed.	NPA 98.6 (96.4-100) PPA 98.7 (97.0-100) OA 98.7 (97.3-99.8)
Inter-instrument	Each of 90 NSCLC specimens with a range of PD-L1 IHC expression was tested on each of three Autostainer Link 48 instruments, two replicates per specimen. A total of 539 comparisons to majority call were performed.	NPA 97.8 (94.9-100) PPA 98.5 (96.2-100) OA 98.1 (96.3-99.6)
Inter-operator/ analyst	Each of 90 NSCLC specimens with a range of PD-L1 IHC expression was tested by three operators on one Autostainer Link 48, two replicates per specimen. A total of 539 comparisons to majority call were performed.	NPA 99.0 (97.6-100) PPA 100 (98.5-100) OA 99.4 (98.7-100)

Inter-day	Each of 90 NSCLC specimens with a range of PD-L1 IHC expression was tested with one replicate over five non-consecutive days on the Autostainer Link 48 instrument. A total of 445 comparisons to majority call were performed.	NPA 98.4 (96.0-100) PPA 99.0 (97.5-100) OA 98.7 (97.1-99.8)
Inter-lot	Each of 36 NSCLC specimens with a range of PD-L1 IHC expression was tested with each of three assay lots on the Autostainer Link 48 instrument, two replicate per specimen. A total of 360 comparisons to majority call were performed.	NPA 100 (97.3-100) PPA 99.1 (97.3-100) OA 99.4 (98.3-100)

4. External Reproducibility

The objective of this study was to evaluate the performance of PD-L1 IHC 28-8 pharmDx across three external laboratories using the Dako Autostainer Link 48.

Part A of the study included 80 NSCLC specimens to assess for inter-site and intra-site assay reproducibility. Of those, 40 specimens were non-squamous NSCLC (16 negative, 24 positive) and 40 (16 negative, 24 positive) specimens were squamous NSCLC. The specimens were pre-qualified at Dako to represent full PD-L1 expression range and a minimum of 20% of the specimens were around the ≥1% PD-L1 cut off. Five specimen sets of unstained sections were blinded and randomized prior to processing at 3 external reproducibility sites and assessed for performance with regard to inter-site and intra-site reproducibility.

Part B of the study included assessment of inter-observer and intra-observer reproducibility with 130 NSCLC specimens that spanned the full expression range of PD-L1. Of those, 65 specimens were non- squamous NSCLC (25 negative, 40 positive) and 65 were squamous NSCLC (25 negative, 40 positive) . These specimens were stained, then blinded and randomized 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.

All IHC tests were interpreted by qualified pathologists to determine the positive/negative results based on the ≥1% expression level, in a blinded and randomized fashion, at three external sites with a minimum of 14 days washout period between evaluations.

Positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement (OA) with two-sided 95% percentile bootstrap confidence intervals were calculated for each of the study endpoints. The pre-specified acceptance criteria was the lower bound of the 95% CI must meet or exceed 85% for PPA, NPA, and OA for inter-site, intra-site, inter-observer and intra-observer.

The results of the reproducibility study for the combined non-squamous NSCLC and squamous NSCLC analysis are summarized in Table 3.

Table 3: Reproducibility of PD-L1 IHC 28-8 pharmDx tested at three external sites, PD-L1 Expression ≥1%

	Sudy Design	% Agreement (95% CI)
Inter-site	Each of 80 NSCLC specimens 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 1198 comparisons to majority call.	NPA 98.5 (97.1-99.6) PPA 99.2 (97.6-100) OA 98.9 (97.8-99.7)
Intra-site	Each of 80 NSCLC specimens with a range of PD-L1 IHC expression was tested on five non-consecutive days. Intra-site analysis was performed for three sites on a total of 1198 comparisons to majority call.	NPA 98.1 (96.6-99.4) PPA 99.6 (98.9-100) OA 99.0 (98.1-99.7)
Inter-observer (one observer at each of three sites)	Scoring of 130 NSCLC specimens with a range of PD-L1 IHC expression was performed by three pathologists, one at each of three study sites, on three n0on-consecutive days. Inter-observer analysis was performed between three sites on a total of 1170 comparisons to majority call.	NPA 95.8 (92.9-98.2) PPA 99.2 (97.8-100) OA 97.9 (96.5-99.0)
Intra-observer	Scoring of 130 NSCLC specimens with a range of PD-L1 IHC expression 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 1170 comparisons to majority call.	NPA 97.7 (96.2-99.1) PPA 99.6 (99.0-100) OA 98.9 (98.2-99.5)

5. Robustness

Robustness of the staining performance of PD-L1 IHC 28-8 pharmDx was evaluated by testing the performance of the assay on NSCLC specimens under various laboratory conditions.

Conditions tested included:

a) Tissue section thickness (3, 4 and 5 µm)
b) Target Retrieval Solution temperature (97 °C, 95 °C, and 99 °C)
c) Target Retrieval time (20, 18 and 22 minutes)
d) Target Retrieval Solution pH (pH 6.1, 5.9 and 6.3)
e) Target Retrieval Solution re-use (up to 3 re-uses)

Eighty eight (88) NSCLC specimens were used for robustness evaluation for tests a), b), c) and d). Of those 88 specimens, 44 were non-squamous NSCLC and 44 were squamous NSCLC. One hundred (100) specimens were used for robustness evaluation for test e). Of those 100 specimens, 50 were non-squamous NSCLC and 50 were squamous NSCLC. Specimens spanning the range of PD-L1 expression and specimens around the cut off were included. All NSCLC specimens were stained, then were blinded and randomized prior to evaluation of PD-L1 expression status. Staining performance was evaluated for percent (%) tumor staining. No significant difference in

results was observed for any of the recommended experimental conditions above. Pre-specified acceptance criteria of 85% at the lower bound of a 2-sided 95% confidence interval for PPA, NPA and OPA were met in these studies.

6. Impact of Ischemia, Fixation and Fixatives

See Summary of Safety and Effectiveness Data for P150025.

7. 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 28-8 pharmDx.

a. Primary vs. Metastatic Tumor Tissues

Twenty (20) matched primary versus metastatic blocks were obtained and evaluated by PD-L1 IHC 28-8 pharmDx. Of those, 10 were non-squamous NSCLC and 10 were squamous NSCLC. In 18 of 20 matched NSCLC pairs, the diagnostic outcome was identical for primary and metastatic specimens at the ≥1% expression level. Two primary/metastatic pairs showed discordant results at the ≥1% expression.

b. Multiple FFPE Blocks from the Same Cases

Multiple blocks (minimum of 2) from each of 30 NSCLC specimens obtained from the same tumor were evaluated. Of these, 16 were non-squamous NSCLC and 14 were squamous NSCLC. In 28 of 30 sets of NSCLC intra-subject specimens the diagnostic outcomes were identical for all specimens at the ≥1% expression level. Two cases, each with five blocks, showed discordant results at the ≥1% expression.

8. Stability Testing

a. PD-L1 IHC 28-8 pharmDx Stability

The follow stability studies were conducted using NSCLC samples and 3 lots of the reagent:

- Shelf life/stability of the PD-L1 IHC 28-8 pharmDx reagents was assessed in a real-time stability testing study.
- Simulated transport stability was tested by exposing the kits to temperatures between -20-0 °C, 37 °C for 16-24 hours and 20-25 °C for 11 days or at 30 °C for 4 days.
- Kit stability during typical use in the laboratory was tested in an In-use/on-board cycling tolerance test. This test included a minimum of 15 temperature cycles from 2-8 °C to RT with 6-8 hours at RT in each cycle.
- Stability of reconstituted working solutions was tested for both DAB (one week) and target retrieval solution (3 reuses over five days).

The results support the stability claims listed below.

Finished Good Shelf Life:12 months at 2-8 °C

In-Use/On-Board Stability Testing: Fifteen 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. NSCLC FFPE Cut Section Stability

Real-time stability studies were conducted to evaluate the shelf life of cut tissue sections of non-squamous and squamous NSCLC FFPE blocks using PD-L1 IHC 28-8 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 4 non-squamous NSCLC and 8 squamous NSCLC specimens. Based on results of these studies, the cut section stability of non- squamous NSCLC is 4 months at 2-8 °C or 25 °C and squamous NSCLC is 4 months at 2-8 °C or 2 months at 25 °C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The clinical performance of PD-L1 IHC 28-8 pharmDx was evaluated in study CA209227 (CHECKMATE 227), a randomized, open-label trial in patients with metastatic or recurrent NSCLC to establish a reasonable assurance of safety and effectiveness of the PD-L1 IHC 28-8 pharmDx device for identifying patients with NSCLC for treatment with OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) in the US and 31 other countries. Data from this clinical study were the basis for the PMA approval decision. This clinical study was also used to support approval of OPDIVO in combination with YERVOY for the first-line treatment of patients with metastatic NSCLC with no EGFR or ALK genomic tumor aberrations (sBLA125554/s080 and sBLA125377/s109). A summary of the clinical study is presented below.

A. Study Design

Patients were treated between August 5, 2015 and January 6, 2017. The database for this Panel Track Supplement reflected data collected through May 15, 2019 and included 793 patients.

The study was an international, multicenter, randomized, open-label multi-part study (Parts 1a, 1b, and 2) which enrolled patients with metastatic or recurrent previously untreated PD-L1 positive (Part 1a) or negative (Part 1b). The study included patients

(18 years of age or older) with histologically confirmed Stage IV or recurrent NSCLC (per the 7th International Association for the Study of Lung Cancer [ASLC] classification), ECOG performance status 0 or 1, and no prior anticancer therapy (including EGFR and ALK inhibitors). Primary efficacy results were based on Part 1a of the study which was limited to the indicated population of patients with PD-L1 tumor expression ≥1%. Randomization was stratified by tumor histology (non-squamous versus squamous). The evaluation of efficacy relied on the comparison between:

- OPDIVO 3 mg/kg administered intravenously over 30 minutes every 2 weeks in combination with YERVOY 1 mg/kg administered intravenously over 30 minutes every 6 weeks; or
- Platinum-based doublet chemotherapy

Patients tumor samples were tested for PD-L1 expression using the PD-L1 IHC 28-8 pharmDx device. Study treatment continued until disease progression, unacceptable toxicity, or for up to 24 months.

Patient tumor PD-L1 status was determined by IHC staining of PD-L1 protein in the submitted tumor sample using the validated PD-L1 IHC 28-8 pharmDx device. Pre-study (baseline) tumor tissue specimens were systematically collected prior to initiation of treatment and the tumor tissue sample could be fresh or archival if obtained within 6 months prior to enrollment, with no systemic therapy (eg, adjuvant or neoadjuvant chemotherapy) given after the sample was obtained. Tissue was to be a core needle biopsy, excisional or incisional biopsy. PD-L1 testing was performed at a central lab. Interpretation and scoring of the PD-L1 stained slides were performed according to the scoring method described in the section titled "Interpretation of PD-L1 Staining in NSCLC".

The clinical validation for this PMA supplement focuses only on subjects with PD-L1 ≥1% tumors (Part 1a) of the study.

1. Clinical Inclusion and Exclusion Criteria

   Enrollment in the CHECKMATE 227 study was limited to patients who met the following inclusion criteria:

   a. ECOG Performance Status of ≤1
   b. Subjects with histologically confirmed Stage IV or recurrent NSCLC (per the 7th International Association for the Study of Lung Cancer classification squamous or non-squamous histology, with no prior systemic anticancer therapy (including EGFR and ALK inhibitors) given as primary therapy for advanced or metastatic disease.
   c. Measurable disease by CT or MRI per RECIST 1.1 criteria.

   Patients were not permitted to enroll in the CHECKMATE 227 study if they met any of the following exclusion criteria:

   a. Patients with known EGFR mutations or ALK translocations sensitive to available targeted inhibitor therapy

b. Patients with untreated brain metastases or carcinomatous meningitis
c. Active autoimmune disease or medical conditions requiring systemic immunosuppression

2. Follow-up Schedule
Tumor assessments were performed every 6 weeks from the first dose of study treatment for the first 12 months, then every 12 weeks until disease progression or study treatment was discontinued. The minimum follow-up for overall survival (OS) was 29.3 months.

3. Clinical Endpoints
With regards to safety, the incidence of serious adverse events and their potential relation to the investigational device were recorded and monitored during the CHECKMATE 227 study, and are presented later in this section. Potential safety issues regarding sampling and false positive and false negative test results are discussed in Section VIII.

With regards to effectiveness, the clinical performance of the PD-L1 IHC 28-8 pharmDx device in identifying NSCLC patients with PD-L1 expression ≥1% for treatment with OPDIVO in combination with YERVOY as compared to platinum-based doublet chemotherapy was assessed.

With regard to success/failure criteria, OS was the primary efficacy outcome. The type-1 error (alpha) for assessing the co-primary efficacy endpoint of OS in PD-L1 ≥1% was set at a 2-sided alpha of 0.0249. Hazard ratio of OS and the corresponding 2-sided confidence intervals (CI) (97.5% and 95%) were estimated using a stratified Cox proportional hazard model, with treatment arm as a single covariate. Kaplan-Meier (KM) product-limit methodology was used to estimate OS curves, OS medians with 95% CIs, and OS rates at 6, 12, 18, and 24 months with 95% CIs.

B. Accountability of PMA Cohort

At the time of database lock, 793 patients were enrolled in the PMA study. Accountability of the PMA cohort in provided in Table 6 below.

Table 6 : Accountability of PMA Cohort: PD-L1 ≥1%

	OPDIVO + YERVOY	Platinum doublet chemotherapy	Total
Number of subjects enrolled and randomized	396	397	793
Site of Tumor			
Primary	238 (60.10%)	242 (60.96%)	480 (60.53%)
Metastatic	142 (35.86%)	144 (36.27%)	286 (36.07%)
Not Reported	16 (4.04%)	11 (2.77%)	27 (3.41%)
Specimen Type			
Newly Obtained*	130 (32.83%)	145 (36.62%)	275 (34.68%)
Archival*	251 (63.38%)	239 (60.20%)	490 (61.80%)
Not Reported	15 (3.79%)	13 (3.27%)	28 (3.53%)
Sample Procedure			
Biopsy	298 (75.25%)	300 (75.57%)	598 (75.41%)
Resection	38 (9.60%)	35 (8.82%)	73 (9.21%)
Not Reported	60 (15.15%)	62 (15.62%)	122 (15.38%)

* A newly obtained biopsy was defined as a specimen obtained within 30 days of randomization into the study. Specimens that were collected >30 days were classified as archival.

C. Study Population Demographics and Baseline Parameters

The demographics characteristics of the intended use population is provided in Table 7 below.

Table 7: Key Baseline Characteristics in Subjects with PD-L1 ≥1%

	OPDIVO + YERVOY Arm B (N = 396)	Chemotherapy Arm C (N = 397)
Age (years)		
Median	64.0	64.0
< 65 (n, %)	199 (50.3)	207 (52.1)
≥ 65 and < 75 (n, %)	157 (39.6)	149 (37.5)
≥ 65 (n, %)	197 (49.7)	190 (47.9)

	OPDIVO + YERVOY Arm B (N = 396)	Chemotherapy Arm C (N = 397)
≥ 75 (n, %)	40 (10.1)	41 (10.3)
Male (n, %)	255 (64.4)	260 (65.5)
Race (n, %)		
White	299 (75.5)	305 (76.8)
Black	4 (1.0)	5 (1.3)
Asian	84 (21.2)	82 (20.7)
Other	5 (1.3)	3 (0.8)
Cell Type (n, %)		
SQ Carcinoma	117 (29.5)	116 (29.2)
NSQ Carcinoma		
Adenocarcinoma	267 (67.4)	269 (67.8)
Large Cell	6 (1.5)	4 (1.0)
Other	6 (1.5)	8 (2.0)
Metastasis Site		
Liver	71 (17.9)	85 (21.4)
Brain	41 (10.4)	40 (10.1)
ECOG PS (n, %)		
0	135 (34.1)	134 (33.8)
1	260 (65.7)	259 (65.2)
≥ 2	1 (0.3)	3 (0.8)
Not Reported	0	1 (0.3)
Smoking Status (n, %)		
Never smoker	56 (14.1)	51 (12.8)
Smoker*	334 (84.3)	340 (85.6)
Unknown	6 (1.5)	6 (1.5)
PD-L1 Level (n, %)		
≥ 50%	205 (51.8)	192 (48.4)
1% – 49%	191 (48.2)	205 (51.6)
≥ 1%	396 (100.0)	397 (100.0)

*Includes former and current smokers; ECOG - Eastern Cooperative Oncology Group, NSQ - non-squamous, PD - L1 - programmed cell death ligand 1, PS - performance status, SQ - squamous

D. Safety and Effectiveness Results

1. Safety Results

   The analysis of safety was based on 576 patients. In the trial, there were no device related adverse events.

Adverse effects that occurred in the PMA clinical study:

Safety with respect to treatment with the combination of nivolumab with ipilimumab was evaluated in the clinical study. The most frequent (≥2%) serious adverse reactions were pneumonia, diarrhea/colitis, pneumonitis, hepatitis, pleural effusion, and adrenal insufficiency. Fatal adverse reactions occurred in 1.7% of patients; these included events of pneumonitis (4 patients), myocarditis, acute kidney injury, shock, hyperglycemia, multi-system organ failure, and renal failure. The most common (≥20%) adverse reactions were fatigue, rash, decreased appetite, musculoskeletal pain, diarrhea/colitis, dyspnea, cough, hepatitis, nausea, and pruritus

2. Effectiveness Results

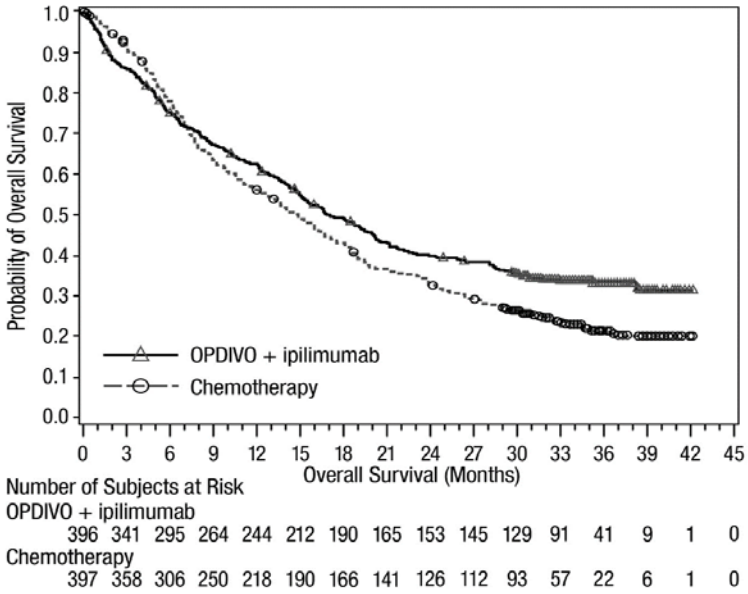
The analysis of effectiveness was based on the 793 evaluable patients. Key effectiveness outcomes (OS) are presented in Table 8 and Figure 1 below.

Table 8: Efficacy Results (PD-L1 ≥1%) – Overall Survival, CHECKMATE-227 Part 1a

	OPDIVO and YERVOY (n=396)	**Platinum-Doublet Chemotherapy (n=397)**
Events (%)	258 (65%)	298 (75%)
Median (months)[a] (95% CI)	17.1 (15, 20.1)	14.9 (12.7, 16.7)
Hazard ratio (95% CI)[b]	0.79 (0.67, 0.94)	
Stratified log-rank p-value	0.0066	

[a]Kaplan-Meier estimate; [b] Based on a stratified Cox proportional hazard model.

Figure1: Overall Survival (PD-L1 ≥1%) - CHECKMATE-227



Blinded independent central review (BICR)-assessed progression free survical (PFS) showed a hazard ratio (HR) of 0.82 (95% CI: 0.69, 0.97), with a median PFS of 5.1 months (95% CI 4.1, 6.3) in the OPDIVO plus YERVOY arm and 5.6 months (95% CI: 4.6, 5.8) in the platinum-based chemotherapy arm. The BICR-assessed confirmed overall response rate (ORR) was 36% (95% CI: 31, 41) in the OPDIVO plus YERVOY arm and 30% (95% CI: 26, 35) in the platinum-doublet chemotherapy arm. Median duration of response (DoR) observed in the OPDIVO plus YERVOY arm was 23.2 months and 6.2 months in the platinum-doublet chemotherapy arm.

3. Subgroup Analyses
   There were no applicable subgroup analyses performed.

4. 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 five investigators of which none were full-time or part-time employees of the sponsor and none had disclosable financial interests/arrangements as defined in 21 CFR 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)(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. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Effectiveness of the PD-L1 IHC 28-8 pharmDx device is based on the clinical performance and benefit to patients with metastatic NSCLC as assessed in the CHECKMATE- 227 study which was conducted to evaluate the safety and the efficacy of OPDIVO in combination with YERVOY as a first line treatment. In this study, PD-L1 IHC 28-8 pharmDx device was used to determine patient PD-L1 status.

The study demonstrated a statistically significant benefit in OS for patients with PD-L1 tumor expression ≥1% who were treated with OPDIVO in combination with YERVOY compared to chemotherapy alone. The data supports the performance of this device in identifying NSCLC patients who will benefit from the therapeutics when used in accordance with the instructions for use.

The performance of the PD-L1 IHC 28-8 pharmDx device was also supported by the analytical validation studies.

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.

The PD-L1 IHC 28-8 pharmDx is an in vitro diagnostic device, which tests tumor FFPE specimens collected from patients with NSCLC. The risks of the device are based on data collected in the clinical study. In general, risks of the PD-L1 IHC 28-8 pharmDx assay 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 NSCLC diagnosis.

C. Benefit-Risk Determination

The probable benefits of the device are also based on data collected in a clinical study conducted to support PMA approval as described above. Overall, baseline demographics and disease characteristics for subjects in Parts 1a and 1b of the pivotal clinical trial CA209227 were representative of a first-line metastatic NSCLC population and were balanced across the treatment groups. Part 1a of the study met its pre-defined co-primary endpoint of overall survival (OS) for the combination of OPDIVO + YERVOY (Arm B) compared with chemotherapy (Arm C), demonstrating a statistically significant improvement in OS in subjects with PD-L1 expressing (≥1%) tumors (Part 1a): HR = 0.79 (97.72% CI: 0.65, 0.96); stratified log-rank test p-value = 0.0066. The median OS (95% CI) was 17.08 (14.95, 20.07) months with OPDIVO + YERVOY and 14.88 (12.71, 16.72) with chemotherapy. The OS benefit was durable, with 2-year OS rates for OPDIVO + YERVOY vs chemotherapy of 40.0% vs 32.8%. Efficacy results with OPDIVO + YERVOY vs chemotherapy were consistent across most subgroups of sufficient size, including histology. These results for the co-primary endpoint of OS were supported by other efficacy measures including ORR and DoR. Responses to OPDIVO + YERVOY were deeper and more durable than with chemotherapy; the complete response (CR) rate was 5.8% vs 1.8% in the OPDIVO + YERVOY arm (Arm B) compared with the chemotherapy arm (Arm C) and the median DoR was 23.2 vs 6.2 months, respectively.

The probable risks of the device are also based on data collected in a clinical study conducted to support PMA approval as described above. The safety profiles of both OPDIVO and YERVOY are well-documented in the FDA-approved drug labels, both of which were updated in March 2020. The safety profile of OPDIVO + YERVOY in CA209227 was acceptable and manageable using the adverse event (AE) management algorithms previously established for OPDIVO, YERVOY, and the combination. The safety of OPDIVO + YERVOY vs chemotherapy was similar in subjects with PD-L1 expression (≥1%, Part 1a) and without PD-L1 expression (<1%, Part 1b). No consistent differences were observed in the frequencies of select AEs by PD-L1 expression subgroup.

1. Patient Perspectives

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

In conclusion, given the available information above, the data support that for the use of the PD-L1 IHC 28-8 pharmDx to identify NSCLC patients for first line treatment with OPDIVO in combination with YERVOY, 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. The provided studies support use of PD-L1 IHC 28-8 pharmDx in NSCLC patients who may be considered for treatment with OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

XIII. CDRH DECISION

CDRH issued an approval order on May 15, 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).

XIV. 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.