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
Date of FDA Notice of Approval:	October 9, 2015

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-squamous non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on Autostainer Link 48.

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

PD-L1 expression as detected by PD-L1 IHC 28-8 pharmDx in non-squamous NSCLC may be associated with enhanced survival from OPDIVO® (nivolumab).

III. CONTRAINDICATIONS

There are no known contraindications for use of this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in PD-L1 IHC 28-8 pharmDx product 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. 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, as shown in 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.	1x 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 containing stabilizing protein and an antimicrobial agent.	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 moderate PD-L1 protein expression and MCF-7 with negative PD-L1 protein expression (PD-L1 IHC 28-8 pharmDx Control Slides).	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 DakoLink software (version 4.0.3). The Autostainer system is designed to mimic the staining steps performed manually by a lab technician. The PD-L1 IHC 28-8 pharmDx protocol is assay specific. The DakoLink 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 PT Link Pre-treatment module.

Specimen Preparation

FFPE NSCLC tissues specimens must be processed appropriately to preserve the tissue for IHC staining. Recommended handling and processing conditions are: <30 minutes ischemia time prior to immersion in fixative, and 24-48 hours fixation time in neutral buffered formalin. Alternative fixatives have not been validated and may give erroneous results. Tissue specimens should be cut into sections of 4-5 µm, mounted on charged slides and stored in the dark at 2-8 °C until staining, which should be performed within 4 months of sectioning.

Test Controls and Calibrators

Control cell line slides listed in the above table should be used to verify the staining procedure. One control slide should be stained with the primary antibody to PD-L1 in each staining run. The evaluation of the Control Slide cell lines supplied in the kit indicates the validity of the staining run. The Control Slides should not be used as an aid in interpretation of patient results. Additional information about the use of controls is available in the product labeling.

Principles of Operation

Deparaffinization, rehydration, and target retrieval are performed in the Target Retrieval Solution (3-in-1) for 20 minutes at 97 °C in the PT Link instrument. 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. The slides are then incubated with an anti-mouse Linker antibody, which is specific to the host species of the primary antibody. Following this, the slides are incubated with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added DAB+ Chromogen results in precipitation of a visible reaction product at the antigen sites. The color of the chromogenic reaction is modified by a chromogen enhancement reagent, DAB Enhancer. The specimen may then be counterstained with hematoxylin and coverslipped.

Staining Protocol

The PD-L1 IHC 28-8 pharmDx is designed to be run on the Autostainer Link 48 with DakoLink software. The staining protocol on the Dako Autostainer Link 48 under PD-L1 program is as follows:

Peroxidase-Blocking Reagent (2 drop zones x 150 µL): 5 minutes (± 1 minute)
Rinse in buffer
Monoclonal Rabbit anti-PD-L1 (or Negative Control Reagent) (2 drop zones x 150 µL): 30 minutes (± 1 minute)
Rinse in buffer
Linker, anti-Rabbit Ig (2 drop zones x 150 µL): 30 minutes (± 1 minute)
Rinse in buffer
Visualization Reagent (2 drop zones x 150 µL): 30 minutes (± 1 minute)
Rinse in buffer: 5 minutes
DAB+ Chromogen (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
Remove slides from autostainer and place in bath of reagent water

Interpretation of PD-L1 Staining

The device labeling states that interpretation of specimens should be performed by a pathologist using a light microscope. An objective of 10-40x magnification is appropriate. All viable tumor cells on the entire slide must be evaluated and included in the PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present for the specimen to be considered adequate for PD-L1 evaluation. Positive PD-L1 staining is defined as complete and/or partial circumferential linear plasma membrane staining at any intensity that can be differentiated from background and cytoplasmic staining.

Positive PD-L1 staining is computed for tumor cells in the entire slide. The diagnostic status of the patient tissue slide is derived by determination of the percentage of positive tumor cells at any staining intensity present in the entire slide. Tumor associated immune cells such as infiltrating lymphocytes or macrophages are not included in the scoring for the determination of PD-L1 positivity using this assay. A PD-L1 IHC 28-8 pharmDx Interpretation Manual for non-squamous NSCLC (containing color images of representative staining patterns and known artifacts) is available to the end user to assist in the interpretation of assay results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or approved alternatives for the testing of PD-L1 in formalin-fixed, paraffin-embedded (FFPE) non-squamous non-small cell lung cancer (NSCLC) human specimens to assess improved survival to OPDIVO® (nivolumab).

VII. MARKETING HISTORY

PD-L1 IHC 28-8 pharmDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PD-L1 test results, and subsequently improper expectation of patient benefit from non-squamous NSCLC treatment with OPDIVO® (nivolumab).

A patient with a false positive result may undergo treatment with OPDIVO® (nivolumab) with inappropriate expectation of enhanced survival. A patient with a false negative result may have inappropriate expectation of not having enhanced survival from OPDIVO® (nivolumab). For specific adverse events that occurred in the clinical studies, see Section X below.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

1. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 28-8 pharmDx was tested on 112 unique cases of FFPE non-squamous NSCLC tissue using one lot of the device. The specimens were chosen at random and represented the full range of PD-L1 expression and tumor stage. 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% expression level was 81 (72%), at the ≥5% expression level was 54 (48%) and at the ≥10% expression level was 45 (40%).

2. Analytical Specificity

The primary antibody for PD-L1 IHC 28-8 pharmDx is a rabbit monoclonal anti-human PD-L1 antibody, clone 28-8.The immunogen used for the antibody generation is a purified recombinant human PD-L1 containing the extracellular domain (Phe19-Thr239) of human PD-L1.

a. Western Blot

Western blot analysis was performed on cell lysates from CHO-PD-L1, CHO-s, ES-2 (positive) and Colo205 (negative) cell lines with and without pre-absorption by purified recombinant PD-L1 protein overnight at 4 ºC. Clone 28-8 specifically reacted with the lysates expressing PD-L1 proteins and the reactivity was blocked by the pre-absorption of PD-L1 protein.

b. Specific detection of engineered PD-L1 and endogenous PD-L1in tumor cell lines
Human colorectal tumor cell line HT-29 with non-detectable PD-L1 was engineered to overexpress full-length human PD-L1. FFPE cell pellets of the parental and PD-L1 overexpressing HT-29 cell lines were stained by PD-L1 IHC 28-8 pharmDx. The level of PD-L1 detected by IHC was consistent with the PD-L1 level detected by FACS, and was completely abolished by the addition of PD-L1 antigen to the protein-rich antibody diluents.

Human ovarian clear cell carcinoma tumor cell line ES-2 and human lung adenocarcinoma tumor cell line L-2987 with high level of PD-L1 was genetically engineered to knock out PD-L1 expression. FFPE cell pellets of the parental and PD-L1 Knock-out ES-2 and L-2987 cell lines were stained by PD-L1 IHC 28-8 pharmDx. The membrane staining of PD-L1 was not detectable in the knock-out cell pellets. The membrane staining of PD-L1 in the parental cell lines was completely abolished by the addition of PD-L1 antigen to the protein-rich antibody diluents. As a control, PD-L1 antigen did not block the IHC staining of CK8 and CK18, a pair of broadly-expressed control epithelial biomarkers.

c. Specificity of PD-L1 primary antibody for PD-L1 over PD-L2
PD-L2 is an isoform of PD-L1 protein with 40% identity in amino acid sequences. Human PD-L1 and PD-L2 were each engineered into Chinese Hamster Ovary (CHO) cells. FFPE cell pellets of the PD-L1, PD-L2 overexpressing and parental CHO cell lines were stained by PD-L1 IHC 28-8 pharmDx. IHC staining with PD-L1 primary antibody showed strong positive staining in human PD-L1 transfected CHO FFPE cells, and negative staining in human PD-L2 transfected CHO cells and parental CHO cells.

d. Immunoreactivity in Human Tissues

Normal tissues:
PD-L1 IHC 28-8 pharmDx was used to detect PD-L1 in 30 FFPE human specimens (one sample of each tissue type from 3 different cases). PD-L1 IHC 28-8 pharmDx detected PD-L1 protein localized in the plasma membrane of cell types known to express the PD-L1 antigen such as immune cells and cells of epithelial origin as shown in Table 2. Cytoplasmic staining was noted in some cell types but was not considered as positive staining. Background staining was <1 grade in all specimens tested. There were no unexpected results.

Table 2: Summary of PD-L1 IHC 28-8 pharmDx Normal Tissue Reactivity

Tissue Type (# tested)	**Positive Plasma Membrane Staining**	**Positive Cytoplasmic Staining**	**Non-specific Staining**
Adrenal (3)	3/3 Medullary cells	3/3 Medullary cells	0/3
Bone marrow (3)	3/3 Megakaryocytes	3/3 Megakaryocytes	0/3
Breast (3)	0/3	0/3	0/3
Cerebellum (3)	0/3	0/3	0/3
Cerebrum (3)	0/3	0/3	0/3
Cervix (3)	1/3 Epithelium	1/3 Epithelium	0/3
Colon (3)	2/3 Macrophages	0/3	0/3
Esophagus (3)	0/3	0/3	0/3
Kidney (3)	3/3 Tubular epithelium	3/3 Tubular epithelium	0/3
Liver (3)	2/3 Immune cells	2/3 Immune cells	0/3
Lung (3)	3/3 Alveolar macrophages	0/3	0/3
Mesothelial cells (3)	0/3	0/3	0/3
*Muscle, cardiac (2)	0/2	0/2	0/2
*Muscle, skeletal (2)	0/2	0/2	0/2
Nerve, peripheral (3)	0/3	0/3	0/3
Ovary (3)	0/3	0/3	0/3
Pancreas (3)	3/3 Epithelium (mainly islet cells)	3/3 Epithelium (mainly islet cells)	0/3
Parathyroid (3)	3/3 Epithelium	0/3	0/3
Pituitary (3)	1/3 Anterior adenohypophysis	1/3 Anterior adenohypophysis 3/3 Posterior neurohypophysis	0/3
*Prostate (2)	0/2	0/2	0/2
Salivary gland (3)	0/3	0/3	0/3
Skin (3)	0/3	1/3 Epithelium	0/3
*Small intestine (2)	0/2	0/2	0/2
Spleen (3)	1/3 Macrophages 3/3 Littoral cell	0/3	0/3
Stomach (3)	0/3	0/3	0/3
Testis (3)	0/3	1/3 Leydig cells	0/3
Thymus (3)	3/3 Medullary epithelium	0/3	0/3
Thyroid (3)	0/3	0/3	0/3
Tonsil (3)	3/3 Crypt epithelium 3/3 Germinal center (immune cells)	0/3	0/3
Uterus (3)	0/3	0/3	0/3

* One of the three tissues missing or with poor quality (Not evaluable)

Neoplastic tissues:

A summary of the PD-L1 IHC 28-8 pharmDx immunoreactivity on the panel of neoplastic tissues is presented in Table 3 below. Multi-tumor tissue microarrays were tested with PD-L1 IHC 28-8 pharmDx. Expression ranged from <1% to 95% positive tumor cells, with the majority of specimens in the <1% to 10% expression range. Positive staining was observed primarily in carcinomas and tissues of lymphoid origin, which is consistent with tissue types known to express PD-L1 antigen.

Table 3: Summary of PD-L1 IHC 28-8 pharmDx Neoplastic Tissue Reactivity

Tumor Type	Location	PD-L1 positive/total (N=162)
Adenocarcinoma	Appendix	1/1
	Breast, DCIS	0/2
	Breast, invasive ductal	3/7
	Breast, invasive ductal metastatic to lymph node	1/1
	Bronchoalveolar carcinoma, lung	0/1
	Cervix, endocervical type	0/1
	Colon	2/5
	Colon, metastatic to liver	1/1
	Colon, mucinous	0/1
	Esophagus	1/1
	Gallbladder	2/4
	GI, metastatic to lung	0/1
	Head & neck, hard palate	0/1
	Lung	2/5
	Ovary	0/1
	Ovary, endometrioid	0/1
	Ovary, mucinous	0/1
	Ovary, serous	0/1
	Pancreas	½
	Pancreas, ductal	0/3
	Prostate	2/4
	Rectum	2/4
	Salivary/parotid gland	0/2
	Small Intestine	0/2
	Stomach	1/6
	Stomach, mucinous	0/1
	Thyroid, follicular	0/1
	Thyroid, follicular-papillary	0/1
	Thyroid, papillary	0/3
	Uterus, clear cell	1/1
	Uterus, endometrium	1/3
Adrenocortical carcinoma	Adrenal	0/1
Astrocytoma	Cerebrum	0/3
Basal cell carcinoma	Skin	0/1
Carcinoma	Nasopharyngeal, NPC	0/1

Tumor Type	Location	PD-L1 positive/total (N=162)
Chordoma	Pelvic cavity	0/1
Embryonal carcinoma	Testis	0/1
Ependymoma	Brain	0/1
Glioblastoma	Brain	0/1
Hepatoblastoma	Liver	0/1
Hepatocellular carcinoma	Liver	1/5
Islet cell tumor	Pancreas	0/1
Interstitialoma	Colon	0/1
	Rectum	0/1
	Small intestine	0/1
Large cell carcinoma	Lung	1/1
Liposarcoma	Abdominal cavity, mucinous	0/1
Lymphoma		
Anaplastic large cell	Lymph node	1/1
Diffuse B-cell	Lymph node	2/4
Hodgkin	Lymph node	2/2
Non-Hodgkin	Lymph node	1/1
Medullablastoma	Brain	0/1
Medullary carcinoma	Thyroid	0/1
Melanoma	Rectum	0/1
	Nasal cavity	0/1
Meningioma	Brain	0/2
Mesothelioma	Peritoneum	0/1
Neuroblastoma	Retroperitoneum	0/1
Neurofibroma	Soft tissue, lower back	0/1
Primitive neuroectodermal	Retroperitoneum	0/1
Renal cell carcinoma		
Papillary	Kidney	0/1
Clear cell	Kidney	0/6
Sarcoma		
Chondrosarcoma	Bone	0/1
Clear cell	Abdominal wall	0/1
Osteosarcoma	Bone	0/2
Leiomyosarcoma	Soft tissue, chest wall	0/1
	Bladder	0/1
Liposarcoma	Abdominal cavity, mucinous	0/1
Rhabdomyosarcoma	Soft tissue, embryonal	0/1
	Prostate	0/1
	Retroperitoneum	0/1
Synovial sarcoma	Pelvic cavity	0/1
Seminoma	Testis	0/2
Signet ring cell carcinoma	Metastatic colon signet ring cell carcinoma to ovary	0/1
	Colon	0/1
Small cell carcinoma	Lung	1/2
Spermatocytoma	Testis	0/2
Squamous cell carcinoma	Metastatic esophageal squamous	1/1

Tumor Type	Location	PD-L1 positive/total (N=162)
	cell carcinoma to lymph node	
	Cervix	2/4
	Esophagus	4/7
	Head & neck	0/2
	Lung	1/3
	Skin	1/2
	Uterus	1/1
Thymoma	Mediastinum	1/1
Transitional cell carcinoma	Bladder	3/6
	Kidney	0/1

3. Precision

The objective of this study was to demonstrate that PD-L1 IHC 28-8 pharmDx would produce consistent staining (repeatability) in normal day-to-day testing. The performance of the assay was evaluated on the determination of PD-L1 results based on ≥1%, ≥5% and ≥10% PD-L1 tumor expression levels. Specimens included in each repeatability test represented the full PD-L1 expression range and a minimum of 25% of the specimens were within 10% PD-L1 expression.

Results for Negative Percent Agreement (NPA), Positive Percent Agreement (PPA) and Overall Percent Agreement (OA) were calculated for all non-redundant pair-wise comparisons across all the conditions in a test. The respective 95% confidence intervals were calculated based on the percentile bootstrap method. The pre-specified acceptance criteria were 1) The lower bound of the 95% CI computed on % agreement must meet or exceed 85% for average negative agreement (ANA), average positive agreement (APA), and overall agreement (OA) (Bootstrap score), or NPA, PPA, and OA (Wilson score), 2) Non-specific background staining on specimens tested must be <1 grade and 3) Specimens stained with the NCR must exhibit 0 specific staining and <1 background staining.

The acceptance criteria were met in all studies.

Summaries and results of the repeatability tests are presented Table 4.

Table 4: Repeatability of PD-L1 IHC 28-8 pharmDx tested at one site

Repeatability	Method*		% Agreement (95% CI)		
			≥1% Expression	≥5% Expression	≥10% Expression
Inter-instrument	Each of 10 non-squamous NSCLC specimens with a range of PD-L1 expression was tested with 3 replicates on each of 3 Autostainer Link 48 instruments. A total of 60 independent pair-wise comparisons were performed.	NPA	100 (82.4, 100)	100 (86.2, 100)	100 (91.6, 100)
		PPA	100 (91.6, 100)	100 (90.4, 100)	100 (82.4, 100)
		OA	100 (94.0, 100)	100 (94.0, 100)	100 (94.0, 100)
Inter-analyst	Each of 12 non-squamous NSCLC specimens with a range of PD-L1 expression was tested with 3 replicates by 3 analysts on one Autostainer Link 48 instrument. A total of 72 independent pair-wise comparisons were performed.	NPA	100 (86.2, 100)	100 (91.6, 100)	100 (93.4, 100)
		PPA	100 (92.6, 100)	100 (88.6, 100)	100 (82.4, 100)
		OA	100 (94.9, 100)	100 (94.9, 100)	100 (94.9, 100)
Inter-day	Each of 10 non-squamous NSCLC specimens with a range of PD-L1 expression was tested with 3 replicates over 5 non-consecutive days on the Autostainer Link 48 instrument. A total of 80 independent pair-wise comparisons were performed.	NPA	100 (86.2, 100)	100 (89.3, 100)	98.2 (90.6, 99.7)
		PPA	100 (93.6, 100)	100 (92.6, 100)	100 (86.2, 100)
		OA	100 (95.4, 100)	100 (95.4, 100)	98.8 (93.3, 99.8)
Inter-lot	Each of 20 non-squamous NSCLC specimens with a range of PD-L1 expression was tested with 2 replicates with each of 5 reagent lots on the Autostainer Link 48 instrument. A total of 160 independent pair-wise comparisons were performed.	NPA	100 (94.3, 100)	100 (95.4, 100)	100 (96.4, 100)
		PPA	100 (96.2, 100)	100 (95.4, 100)	100 (93.6, 100)
		OA	100 (97.7, 100)	100 (97.7, 100)	100 (97.7, 100)
Intra-run	Each of 10 non-squamous NSCLC specimens with a range of PD-L1 expression was tested with 8 replicates within a run on the Autostainer Link 48 instrument. A total of 70 independent pair-wise comparisons were performed.	NPA	100 (84.5, 100)	100 (87.9 100)	100 (92.7, 100)
		PPA	100 (92.7, 100)	100 (91.6, 100)	100 (84.5, 100)
		OA	100 (94.8, 100)	100 (94.8, 100)	100 (94.8, 100)

* The number of comparisons are not equal to the number of test results because the Wilson Score interval was calculated using the number of pairs that were deemed to be independent.

4. External Reproducibility

Reproducibility study was designed to evaluate the performance of PD-L1 IHC 28-8 pharmDx for PD-L1 detection with regard to day-to-day, site-to-site, observer-to-observer reproducibility on the Dako Autostainer Link 48. All IHC tests were interpreted by certified clinical pathologists to determine the positive/negative results based on the ≥1% and ≥5% expression levels at three external sites.

Both squamous and non-squamous NSCLC specimens were included. Specimens for the reproducibility study were pre-qualified at Dako to represent full PD-L1 expression range and a minimum of 25% of the specimens were within 10% PD-L1 expression. The performance analysis and the acceptance criteria were the same as described in repeatability study. Results of the reproducibility study are presented in Table 5.

Table 5: Reproducibility of PD-L1 IHC 28-8 pharmDx tested at three external sites

Reproducibility	Method	Agreement		
		Parameter	≥1% Expression % (95% CI)	≥5% Expression % (95% CI)
Inter-site assay (three sites)	Each of 10 non-squamous NSCLC with a range of PD-L1 expression was tested on 5 non-consecutive days. Inter-site analysis was performed between 3 sites on a total of 140 independent pair-wise comparisons.	NPA	100 (93.6, 100)	91.4 (82.5, 96.0)
		PPA	98.8 (93.6, 99.8)	97.1 (90.2, 99.2)
		OPA	9.3 (96.1, 99.9)	94.3 (89.1, 97.1)
Intra-site assay	Each of 10 non-squamous NSCLC with a range of PD-L1 expression was tested on 5 non-consecutive days at each of 3 study sites. Intra-site analysis was performed for three sites on a total of 120 independent pair-wise comparisons.	NPA	100 (92.6, 100)	96.4 (87.9, 99.0)
		PPA	98.6 (92.5, 99.8)	95.3 (87.1, 98.4)
		OPA	99.2 (95.4, 99.9)	95.8 (90.6, 98.2)
Inter-observer (one observer at each of three sites)	Scoring of 15 non-squamous NSCLC specimens with a range of PD-L1 expression was performed by 3 pathologists, 1 at each of 3 study sites, on three non-consecutive days. Inter-observer analysis was performed between 3 sites on a total of 120 independent pair-wise comparisons.	NPA	96.9 (89.3, 99.1)	100 (94.3, 100)
		PPA	100 (93.6, 100)	89.3 (78.5, 95.0)
		OPA	98.3 (94.1, 99.5)	95.0 (89.5, 97.7)
Intra-observer (one observer at each of three sites)	Scoring of 15 non-squamous NSCLC specimens with a range of PD-L1 expression was performed 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 90 independent pair-wise comparisons.	NPA	95.8 (86.0, 98.8)	100 (93.1, 100)
		PPA	100 (91.6, 100)	100 (90.8, 100)
		OPA	97.8 (92.3, 99.4)	100 (95.9, 100)

The acceptance criteria were met for all sources of variability that were examined.

5. Robustness

Robustness of the staining performance of PD-L1 IHC 28-8 pharmDx was tested when varying the following conditions on one lot of material: tissue section thickness, microscope slide type, target retrieval solution temperature, target retrieval time, target retrieval solution pH, target retrieval solution reuse, and target retrieval solution lot-to-lot.

Non-squamous NSCLC specimens were included in the study. Specimens represented a dynamic expression range.

The following conditions were tested with 8 non-squamous NSCLC specimens (4 PD-L1-negative and 4 PD-L1-positive):

- Tissue Thickness
  - 4 µm
  - 5 µm

- Microscope slide Type
  - Fisherbrand™ Superfrost™ Plus
  - Dako FLEX IHC Microscope Slides (Dako code K8020)

In addition, tissue thickness was evaluated during assay verification using 3 NSCLC cases sectioned at 2, 3, 4, 5 and 6µm. The slides sectioned at 2 µm did not have equivalent staining when compared to the standard 4 µm thick sections and therefore did not meet the acceptance criteria; however, the slides sectioned 3-6 µm demonstrated equivalent staining. Dako recommends specimens to be sectioned 4-5 µm.

The following robustness studies were completed with 3 non-squamous NSCLC specimens (1 PD-L1-negative and 2 PD-L1-positive).

- Target Retrieval Solution: Time
  - 18 minutes
  - 20 minutes - standard
  - 22 minutes
- Target Retrieval Solution: Temperature
  - 95 °C
  - 97 °C - standard
  - 99 °C
- Target Retrieval Solution: pH
  - pH 5.7
  - pH 5.9
  - pH 6.1 - standard
  - pH 6.3
  - pH 6.5
- Target Retrieval Solution Re-use - 3 lots
- Target Retrieval Solution - 3 lots

No significant difference in results was observed for any of the recommended experimental conditions above.

6. Impact of Pre-analytical Variables on Assay Performance

The objective of this study was to assess the effect of pre-analytical variables on the performance of PD-L1 IHC 28-8 pharmDx in the detecting PD-L1 protein.

A freshly-excised human tonsil was divided into pieces of similar size and these specimens were placed in containers with saline-soaked gauze at ambient temperature for ischemia time ranging from 0 to 12 hours before fixation. Specimens were then transferred to 10% neutral buffered formalin (10% NBF) for fixation time ranging from 6 to 72 hours and processed into FFPE blocks. Sections from each specimen in this study were tested in duplicate and the mean of the two scores was used for data analysis.

No significant differences were observed in PD-L1 staining in freshly-excised tonsil tissues under Dako recommended pre-analytical processing conditions: <30 minutes ischemia time, 24-48 hours fixation in 10% NBF.

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

Ten (10) non-squamous NSCLC matched primary versus metastatic blocks from the same individual were evaluated by PD-L1 IHC 28-8 pharmDx. In 7 of 10 matched non-squamous NSCLC pairs the diagnostic outcome based on the PD-L1 expression was identical for primary and metastatic specimens for all expression levels. One primary/metastatic pair showed discordant results at the ≥5% expression level. One primary/metastatic pair showed discordant results at the ≥5% and ≥10% expression levels. One primary/metastatic pair showed discordant results at all three expression levels.

b. Multiple FFPE Blocks from the Same Cases

Multiple blocks from 16 non-squamous NSCLC cases with at least 2 blocks were evaluated. In 14 of 16 sets of non-squamous NSCLC intra-case specimens the diagnostic outcomes based on PD-L1 expression were identical for all specimens. One case with five specimens showed discordant results at the ≥1% expression level. One case with four specimens showed discordant results at the ≥5% and ≥10% expression levels.

7. Stability testing

a. PD-L1 IHC 28-8 pharmDx Stability

A real-time shelf life/stability study for three lots was conducted to determine the shelf life of the reagent components. This study included transport simulation, in-use/on-

board cycling tolerance and stability of reconstituted working solutions. The results support the stability claims listed below.

Total Shelf Life
7 months at 2-8°C

Finished Good Shelf Life
7 months at 2-8°C

In-Use/On-Board Stability Testing
Six 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. Non-squamous NSCLC FFPE Cut Section

A real-time stability study was designed to evaluate the shelf life of cut tissue sections of FFPE non-squamous NSCLC blocks using PD-L1 IHC 28-8 pharmDx when stored in the dark at 2-8 °C or 25 °C. Based on these studies, stability dating is as follows:

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Study Design

The clinical performance of PD-L1 IHC 28-8 pharmDx was investigated in a retrospective analysis of patient samples from a Phase 3, randomized, open-label study of nivolumab vs docetaxel in adult (≥ 18 years) subjects with advanced or metastatic non-squamous cell NSCLC after failure of prior platinum doublet -based chemotherapy. Patients with known epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (CD246, ALK) translocations were allowed an additional line of tyrosine kinase inhibitor (TKI) therapy before enrolling in this study. A total of 582 subjects were randomized at 112 sites in 22 countries (Argentina, Australia, Austria, Brazil, Canada, Chile, Czech Republic, France, Germany, Hong Kong, Hungary, Italy, Mexico, Norway, Peru, Poland, Romania, Russian Federation, Singapore, Spain, Switzerland, and United States). Subjects were randomized 1:1 and stratified according to the following factors: prior use of maintenance therapy vs. no use of maintenance therapy and second-line vs. third-line therapy (to account for prior TKI use).

Pre-study (baseline) tumor tissue specimens were collected prior to randomization and prior to first treatment to conduct pre-planned analyses of efficacy according to PD-L1 expression status. All PD-L1 tests were performed in one central CLIA certified laboratory on FFPE tumor specimens using PD-L1 IHC 28-8 pharmDx. PD-L1 expression status was not used as a criterion for subject randomization.

1. Clinical Inclusion and Exclusion Criteria

Study inclusion criteria were as follows:

- Adult (≥18 years) subjects with histologically- or cytologically-documented locally advanced non-squamous cell NSCLC who presented with Stage IIIB/ Stage IV or recurrent or progressive disease following multimodal therapy (radiation therapy, surgical resection, or definitive chemoradiation therapy for locally advanced disease) and who had measurable disease with European Cooperative Oncology Group (ECOG) performance status ≤1.
- Patients with disease recurrence or progression during or after one prior platinum doublet-based chemotherapy regimen for advanced or metastatic disease; received platinum-containing adjuvant, neoadjuvant, or definitive chemoradiation therapy given for locally advanced disease, and developed recurrent (local or metastatic) disease within 6 months of completing therapy; or experienced disease recurrence or progression during or after a separate EGFR or ALK tyrosine kinase inhibitor (TKI) regimen in addition to one prior platinum doublet-based chemotherapy regimen (regardless of order of administration) and had documented EGFR mutation or ALK translocations.

Study exclusion criteria were as follows:

- Patients with autoimmune disease, medical conditions requiring systemic immunosuppression, symptomatic interstitial lung disease, or untreated brain metastasis.
- Patients with treated brain metastases were eligible if neurologically returned to baseline at least 2 weeks prior to enrollment, and either off corticosteroids, or on a stable or decreasing dose of <10 mg daily prednisone equivalents.

2. Follow-up Schedule

Patients were followed for efficacy:
Radiographic tumor assessments were performed at baseline, at Week 9 and every 6 weeks thereafter until disease progression (or discontinuation of study therapy in subjects receiving nivolumab beyond progression) or other protocol-defined reasons. Survival was followed continuously while subjects were on the study drug and every 3 months via in-person or phone contact after subjects discontinued the study drug.

Patients were followed for safety:
Safety and tolerability were measured by the incidence of adverse events, serious adverse events, deaths, and laboratory abnormalities. Adverse event assessments and laboratory tests were performed at baseline, and continuously throughout the study at the beginning of each subsequent cycle. Adverse events were recorded as they were encountered during the study and until 100 days after final administration of study medication and classified according to the NCI-CTC AE v4.0.

3. Clinical Endpoints

With regard to efficacy of nivolumab vs. docetaxel, the primary endpoint was overall survival (OS). Secondary endpoints were objective response rate (ORR), progression-free survival (PFS), PD-L1 expression as a predictive biomarker for OS and ORR, and disease-related symptom improvement by 12 weeks, as measured by the Lung Symptom Cancer Scale (LCSS).

Endpoints for the safety assessments were frequency of deaths, serious adverse events (SAEs), adverse events (AEs) leading to discontinuation or dose modification, select AEs, clinical laboratory assessments (hematology, serum chemistry, and liver and thyroid function tests), and vital sign measurements. Endpoints for the assessment of immunogenicity were serum antidrug antibodies (ADA) and neutralizing ADA.

B. Accountability of PMA Cohort

At the time of database lock, a total of 792 subjects were enrolled into the study, of which 582 were randomized to a treatment group (292 nivolumab, 290 docetaxel). A total of 555 subjects were treated (287 nivolumab, 268 docetaxel). Subjects with an

available tumor biopsy specimen and ≥ 0% PD-L1 test result were classified as PD-L1 quantifiable subjects of which there were a total of 455 (231 nivolumab and 224 docetaxel). Quantifiable subjects were defined as those with an available tumor biopsy specimen and number of viable tumor cells per validated PD-L1 IHC nivolumab pharmDx is ≥100 and percentage of tumor PD-L1+ is ≥0%.

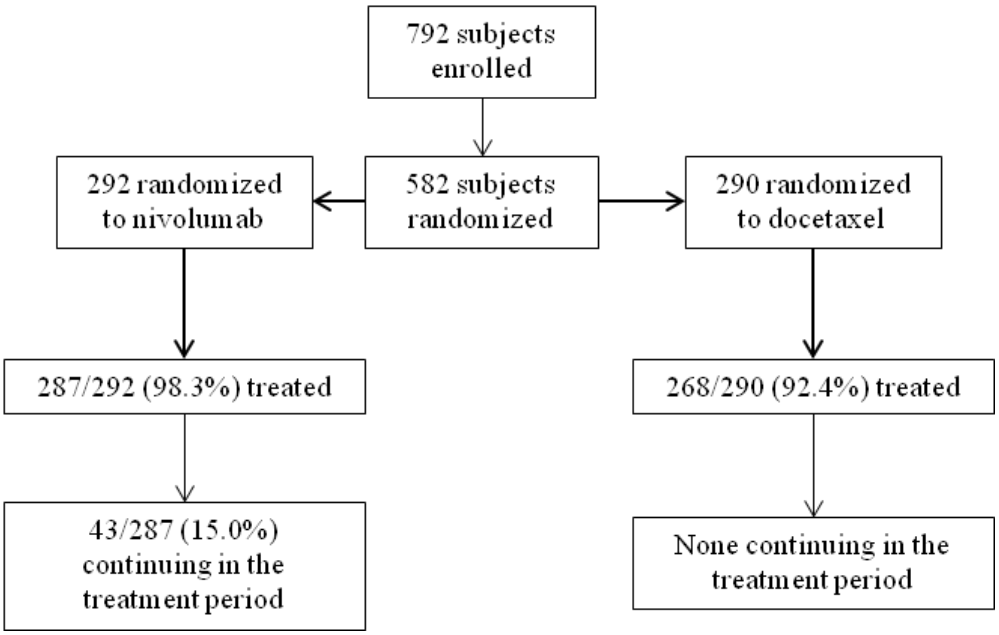
Tumor biopsy specimens without quantifiable PD-L1 expression were defined as:

Indeterminate: Subjects with an available tumor biopsy specimen but tumor cell membrane staining was hampered for reasons attributed to the biology of the tumor biopsy specimen and not because of improper sample preparation or handling.

Not evaluable: Subjects with an available tumor biopsy specimen but tumor biopsy specimen was not optimally collected or prepared (e.g., PD-L1 expression is neither quantifiable nor indeterminate). Not evaluable was to be determined from H&E process before the tumor biopsy specimen was sent for PD-L1 evaluation or from the H&E process during PD-L1 evaluation.

A flow chart of the subjects accounted for the study is presented in Figure 1.

Figure 1: Disposition of Subjects



C. Study Population Demographics and Baseline Parameters

The baseline demographic and disease characteristics were generally balanced between randomized subjects in the nivolumab and docetaxel groups. The mean age was 62 years old (range: 21 to 85) with 41% ≥65 years of age and 7% ≥75 years of age. The majority of patients were white (92%) and male (55%); and the baseline ECOG performance status was 0 (31%) or 1 (69%). Seventy-nine percent (79%) of patients were former/current smokers.

D. Safety and Effectiveness Results

1. Safety Results

   As an in vitro diagnostic test, the PD-L1 IHC 28-8 pharmDx Assay involves testing on FFPE non-squamous NSCLC sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of NSCLC by pathologists. Removal of these tissues, therefore, presents no additional safety hazard to the patient being tested. As compared to the overall study population, no meaningful differences in safety were observed based on PD-L1 expression level.

2. Effectiveness Results

   The clinical performance of PD-L1 IHC 28-8 pharmDx was established using the efficacy data used to support OPDIVO® (nivolumab) approval. Nivolumab monotherapy demonstrated superior OS compared with docetaxel in the all randomized population of subjects with advanced, previously treated non-squamous NSCLC, with a clinically meaningful and statistically significant improvement observed (HR=0.73 [95.92% CI: 0.59, 0.89]; stratified log-rank test p-value = 0.0015). The median OS was 12.2 months in the nivolumab group and 9.4 months in the docetaxel group. Based on the 413 observed deaths and O'Brien-Fleming alpha spending function, the nominal significance level for declaring OS superiority during the pre-planned interim analysis was $p<0.0408$.

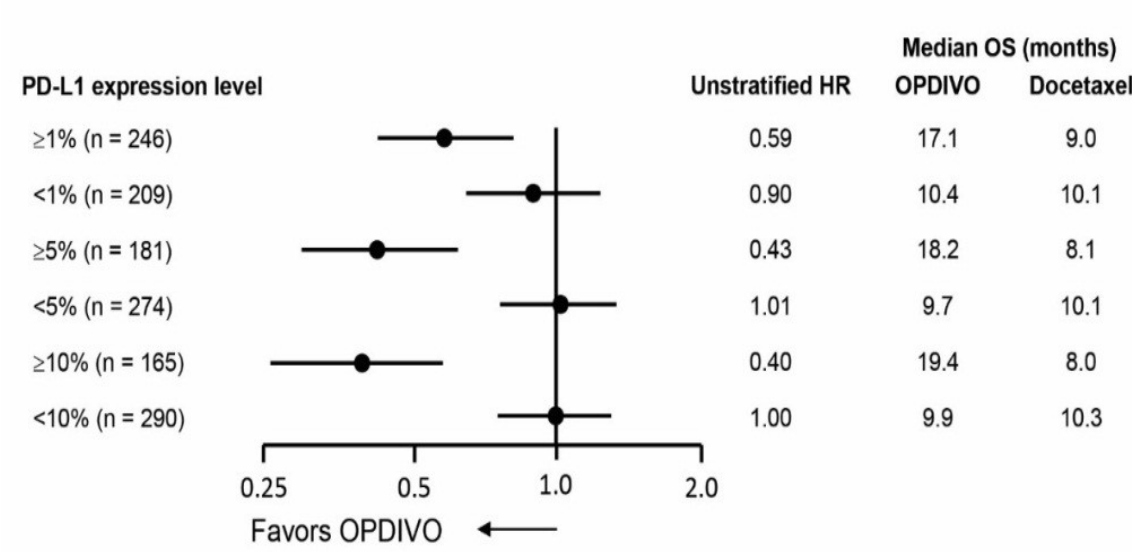
   Pre-planned analyses of efficacy endpoints of nivolumab over docetaxel were conducted in different PD-L1 expression subgroups based on 582 subjects in all randomized population including samples with non-quantifiable PD-L1 test results. Frequencies of PD-L1 expression in all randomized subjects in CA209057 are presented in Table 6.

Table 6: Frequency of PD-L1 Expression at Pre-Study in All Randomized Subjects

PD-L1 Expression Category	Nivolumab 3 mg/kg N = 292	Docetaxel N = 290	Total N = 582
PD-L1 Quantifiable at Baseline (N(%))	231 (79.1)	224 (77.2)	455 (78.2)
Baseline PD-L1 Expression ≥1%	123/231 (53.2)	123/224 (54.9)	246/455 (54.1)
Baseline PD-L1 Expression < 1%	108/231 (46.8)	101/224 (45.1)	209/455 (45.9)
Baseline PD-L1 Expression ≥5%	95/231 (41.1)	86/224 (38.4)	181/455 (39.8)
Baseline PD-L1 Expression < 5%	136/231 (58.9)	138/224 (61.6)	274/455 (60.2)
Baseline PD-L1 Expression ≥10%	86/231 (37.2)	79/224 (35.3)	165/455 (36.3)
Baseline PD-L1 Expression < 10%	145/231 (62.8)	145/224 (64.7)	290/455 (63.7)
PD-L1 Non-Quantifiable (N(%))	61 (20.9)	66 (22.8)	127 (21.8)

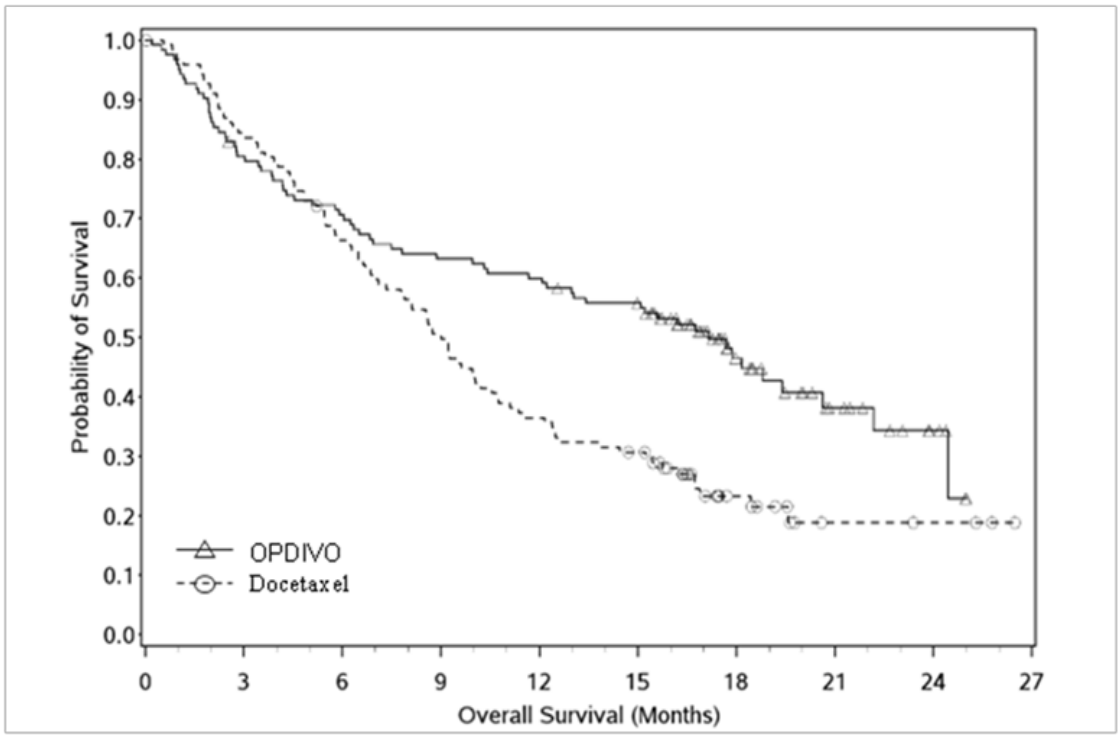
Patients with PD-L1 expression by all predefined expression levels in the nivolumab group were associated with enhanced survival compared to docetaxel, whereas survival was similar to docetaxel in patients with <1% PD-L1 expression groups as reflected in the Forest Plot of OS in Figure 2.

Figure 2: Forest Plot, OS Based on PD-L1 Expression



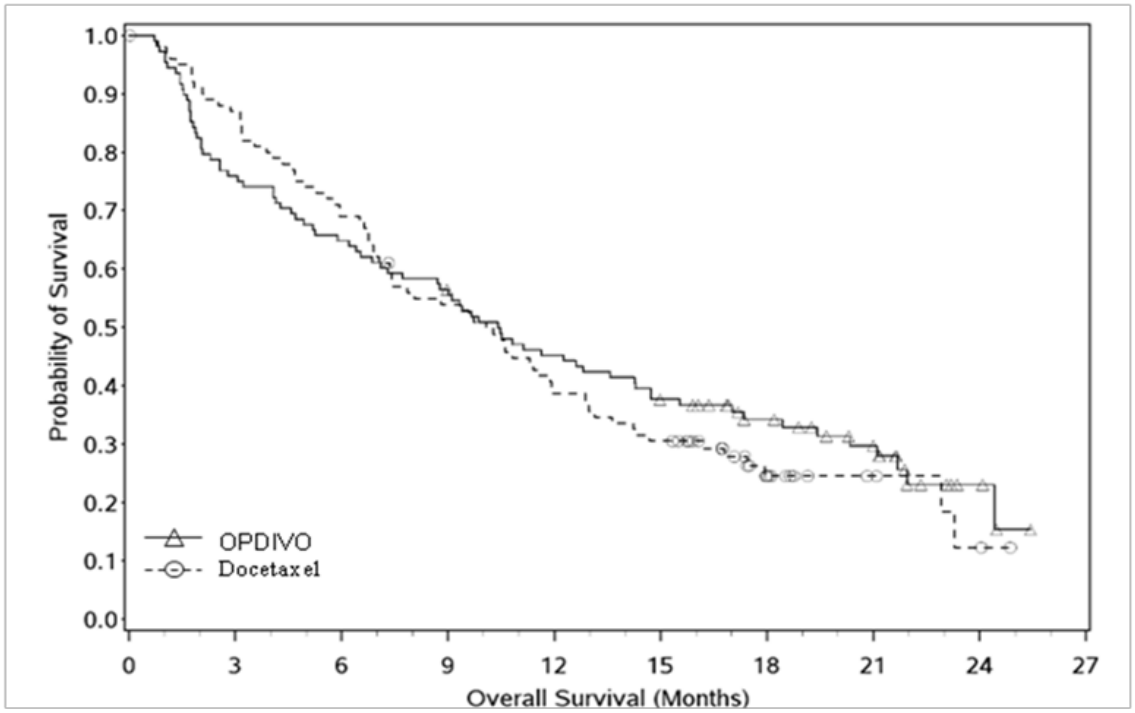
The unstratified hazard ratio and the corresponding 95% CI were estimated in a Cox proportional hazards model using the randomized arm as a single covariate. Differences in median OS were observed in nivolumab over docetaxel subgroups when analyzed by PD-L1 expression level (≥1%, ≥5%, and ≥10%). Median OS was 17.1, 18.2, and 19.4 months for nivolumab subjects compared to 9.0, 8.1, and 8.0 months for docetaxel subjects with ≥1%, ≥5%, and ≥10% expression levels, respectively. There were no differences in OS between the treatment groups in subjects with <1%, <5%, and <10% expression levels, with ranges of median OS of 9.7 to 10.4 months for nivolumab and 10.1 to 10.3 months for docetaxel. Kaplan-Meier plots for subgroups by PD-L1 expression level are shown in Figure 3 and Figure 4.

Figure 3: Overall Survival, Patients with ≥1% PD-L1 Expression



Number at Risk										
OPDIVO	123	98	86	77	73	65	27	13	5	0
Docetaxel	123	102	80	61	44	36	13	4	3	0

Figure 4: Overall Survival, Patients with <1% PD-L1 Expression



Number at Risk									
OPDIVO									
108	82	70	60	48	39	26	17	4	0
Docetaxel									
101	87	69	53	38	30	13	5	2	0

E. Financial Disclosure

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

XI. PANEL MEETING RECOMMENDATION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of PD-L1 IHC 28-8 pharmDx was demonstrated in a retrospective analysis of non-squamous NSCLC patients enrolled in a Phase 3, randomized, open-label study to assess the safety and efficacy of nivolumab vs docetaxel. PD-L1 expression status by all 3 expression levels (≥1%, ≥5%, and ≥10%) in pre-study (baseline) tumor specimens was predictive for benefit from nivolumab. Interaction p-values for PD-L1 expression subgroups by each of the pre-defined expression levels suggested a clinically important signal of a predictive association. In subjects with PD-L1 expressing tumors by predefined ≥1%, ≥5% and ≥10% expression levels, nivolumab demonstrated improved efficacy versus docetaxel across all efficacy endpoints (OS, ORR, and PFS), with increasing expression correlating to a greater likelihood of benefit. In contrast, there were no clinically relevant differences in efficacy between the treatment groups in subjects with <1%, <5% and <10% PD-L1 expression levels.

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

B. Safety Conclusions

The PD-L1 IHC 28-8 pharmDx is an *in vitro* diagnostic device, which tests tumor FFPE specimens collected from patients with non-squamous NSCLC. The risks of the device are based on data collected in the clinical study. Risks of the PD-L1 IHC 28-8 pharmDx are associated with failure of the device to perform as expected or failure to correctly interpret test results. In addition, testing on FFPE tumor specimens does not present significant safety concerns, as these samples are routinely removed for NSCLC cancer diagnosis. This test, therefore, presents no additional safety hazard to the patient being tested.

C. Benefit-Risk Conclusions

Non-squamous non-small cell lung cancer patients may potentially benefit in the intended population by use of the device as higher PD-L1 expression based on the PD-L1 IHC 28-8 pharmDx assay may be associated with enhanced survival from OPDIVO® (nivolumab). Erroneous device results could adversely influence expectation of benefit from OPDIVO® (nivolumab) treatment of non-squamous non-small cell lung cancer patients due to false negative or false positive results. Based on the data collected in the clinical study which were used to support PMA approval as described above, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use and product labeling. The provided studies support use of PD-L1 IHC 28-8 pharmDx in non-squamous NSCLC patients who may be considered for treatment with OPDIVO® (nivolumab).

XIII. CDRH DECISION

CDRH issued an approval order on October 9, 2015. The final conditions of approval cited in the approval order are described below.

Limited numbers of samples from the intended use specimen type were used in some of the analytical validation studies for the PD-L1 IHC 28-8 pharmDx device. Additional testing of samples is required to demonstrate the analytical performance characteristics of your device using the appropriate sample type, i.e., non-squamous non-small cell lung cancer (NSCLC) to cover the range of PD-L1 expression. The results from these studies should be included in the labeling.

The applicant's manufacturing facilities were 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.