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: P150027

Date of FDA Notice of Approval: January 23, 2016

A PMA (P150025) for PD-L1 IHC 28-8 pharmDx indicated for non-squamous NSCLC was approved on October 9, 2015. The SSED to support the indication is available on the CDRH website and is incorporated by reference here. The current PMA was submitted to expand the indication for melanoma.

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) melanoma 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).

Positive PD-L1 status as determined by PD-L1 IHC 28-8 pharmDx in melanoma is correlated with the magnitude of the treatment effect on progression-free survival from OPDIVO®.

III. CONTRAINDICATIONS

There are no known contraindications for the use of this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in 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. 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, 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

Component	Component Description	Quantity x Volume
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 specimens must be processed appropriately to preserve the tissue for IHC staining. Handling and processing instructions recommended in the labeling are : < 30 minutes ischemia time prior to immersion in fixative, and 24-48 hours fixation time in neutral buffered formalin. Alternative fixative methods 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

Run controls are included in each staining run to establish the validity of the test results. Dako in the device labeling 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 are to be provided by the end-user laboratory. It is recommended that tissue that stains weakly for PD-L1is used to monitor for all aspects of pre-analytical variables such as fixation, processing and sectioning. Negative control tissue 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 aids the reader in differentiating a true signal from tissue-specific background staining that occurs from reaction with detection chemistry and not the anti PD-L1 primary antibody.

Additional information about the use of controls is available in the product labeling.

Principle of Operation

Patient FFPE tissue specimens are subject to deparaffinization, rehydration, and target retrieval in the Target Retrieval Solution (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. 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 cover-slipped and observed under a microscope to visually determine if the PD-L1 protein is expressed in patient melanoma tissue.

Interpretation of PD-L1 Staining

The device labeling states that interpretation of specimens 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. The entire specimen must be evaluated. All viable tumor cells on the entire PD-L1 stained 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 patient slide to determine the percentage of stained cells. Record the percentage of stained cells and if the specimen is positive or negative based on the PD-L1 expression level. Specimen is considered PD-L1 positive if $\geq$ 1% of melanoma cells exhibit circumferential and/or partial linear plasma membrane PD-L1 staining of tumor cells at any intensity. Specimen is considered PD-L1 negative if < 1% of melanoma cells exhibit circumferential and/or partial linear plasma membrane PD-L1 staining of tumor cells at any intensity.

Cytoplasmic staining, if present, is not considered for scoring purposes. Non-malignant cells and immune cells (e.g., infiltrating lymphocytes or macrophages) may also stain with PD-L1; however, these should not be included in the scoring for the determination of PD-L1 positivity.

NOTE: When interpreting melanoma patient specimens, brown melanin pigmentation may be present. Melanin should be excluded when scoring plasma membrane staining; comparison to a sequential slide stained with NCR may be useful for identifying and excluding melanin content. If highly elevated melanin precludes scoring of plasma membrane staining of tumor cells, the specimen may be excluded from interpretation and deemed to be indeterminate.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or approved alternatives for the testing of PD-L1 in FFPE melanoma human specimens.

VII. MARKETING HISTORY

PD-L1 IHC 28-8 pharmDx has been marketed in the United States following FDA approval of the device on October 9, 2015, for determining PD-L1 expression in FFPE tumor tissue from patients with advanced non-squamous NSCLC for whom OPDIVO ® treatment is considered.

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 with melanoma who are considering treatment with OPDIVO® (nivolumab) alone or OPDIVO® in combination with YERVOY®.

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

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Preclinical studies were performed using the 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 study results detailed below establish sensitivity, specificity, precision and reproducibility of the device.

The scoring algorithm used in these studies included a clinical score (i.e., PD-L1 positive or negative) and/or 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 Assay. Analytical scores were assessed for some studies to ensure assay performance in borderline cases. The analytical score is not part of the interpretation of PD-L1 staining status in the device labeling.

1. 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.The following studies were conducted with one lot of the PD-L1 28-8 antibody to establish antibody specificity

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 Clone 28-8 with 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. Anti PD-L1 clone 28-8 binding to the PD-L1 overexpressing HT29 cell lines 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 were 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 sequence. 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

1) Normal tissues:

One lot of PD-L1 IHC 28-8 pharmDx kit was used to detect PD-L1 in 30 FFPE normal 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 within acceptable range (<1 on 0-3 intensity scale) in all specimens tested. There were no unexpected results.

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

Tissue Type (number 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	0/3

Tissue Type (number tested)	Positive Plasma Membrane Staining	Positive Cytoplasmic Staining	Non-specific Staining
		neurohypophysis	
*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)

2) 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
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

Tumor Type	Location	PD-L1 positive/total (N=162)
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 cell carcinoma to lymph node	1/1
	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

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 28-8 pharmDx was tested on 104 unique cases of FFPE melanoma 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. PD-L1 expression in tumor specimens ranged from 0-100% and 0-3 staining intensity. The number of PD-L1 positive cases was 54 (52%) for the ≥1% expression level and 37 (36%) for the≥5% expression level.

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. The study was performed with 16 melanoma specimens spanning the range of PD-L1 expression, and 4 of these specimens represented specimens around cut off. The 16 melanoma specimens along with control slides were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Specimens were determined to be positive if PD-L1 expression was ≥1 % or negative if PD-L1 expression was <1 %. Statistical analysis was conducted on the dichotomized data.

Results for average negative percent agreement (ANA), average positive percent agreement (APA), 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 Wilson Score method.

For precision studies OA (95%CI) was at or above 90.0% (86.0-92.9), ANA was at or above 89.47% (83.23-93.57), and APA was at or above 90.5% (84.8-94.2).

Study design 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 Level
Inter-instrument	Each of 16 melanoma specimens with a range of PD-L1 IHC expression was tested with 3 replicates on each of 3 Autostainer Link 48 instruments. A total of 160 pair-wise comparisons were performed.	ANA 89.5% (83.2, 93.6%) APA 90.5% (84.8, 94.2%) OA 90.0% (86.0, 92.9%)
Inter-analyst	Each of 16 melanoma specimens with a range of PD-L1 IHC expression was tested	ANA 96.4% (92.9, 98.2%) APA 96.8% (93.8, 98.4%) OA 96.6% (94.5, 97.9%)

	with 3 replicates by 3 analysts on 1 Autostainer Link 48 instrument. A total of 240 pair-wise comparisons were performed.	
Inter-day	Each of 16 melanoma specimens with a range of PD-L1 IHC expression was tested with 3 replicates over 5 non-consecutive days on the Autostainer Link 48 instrument. A total of 160 pair-wise comparisons were performed.	ANA 95.5% (90.3, 97.9%) APA 96.8% (93.1, 98.6%) OA 96.3% (93.5, 97.9%)
Inter-lot	Each of 16 melanoma specimens with a range of PD-L1 IHC expression was tested with 2 replicates with each of 3 reagent lots on the Autostainer Link 48 instrument. A total of 640 pair-wise comparisons were performed.	ANA 98.6% (97.3, 99.3%) APA 98.8% (97.8, 99.4%) OA 98.7% (98.0, 99.2%)
Intra-run	Each of 16 melanoma specimens with a range of PD-L1 IHC expression was tested with 8 replicates within a run on the Autostainer Link 48 instrument. A total of 160 pair-wise comparisons were performed.	ANA 97.1% (92.6, 98.9%) APA 97.7% (94.1, 99.1%) OA 97.4% (94.9, 98.7%)

4. External Reproducibility

A reproducibility study was designed to evaluate the performance of PD-L1 IHC 28-8 pharmDx for PD-L1 detection across laboratories on the Dako Autostainer Link 48. The study included 18 melanoma specimens that were pre-qualified at Dako to represent full PD-L1 expression range and a minimum of 25% of the specimens were around cut off . This specimen set was randomized and blinded prior to testing at 3 external reproducibility sites and assessed for performance with regard to site-to-site and day-to-day reproducibility, or intra-site reproducibility.

Reproducibility studies also included assessment of observer-to-observer variability with 30 melanoma specimens that spanned the expression range of PD-L1. These specimens were stained at the Dako facility and shipped to the 3 external reproducibility sites for assessment of PD-L1 expression by pathologists for both inter-observer and intra-observer reproducibility.

All IHC tests were interpreted by certified clinical pathologists to determine the positive/negative results based on the ≥1% expression levels at three external sites. The results of the reproducibility study are included in Table 5. The acceptance criteria were met for all sources of variability that were examined.

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

Reproducibility	**Method**	**% Agreement (95% CI)**
		≥1% Expression Level
Inter-site assay (three sites)	Each of 18 melanoma specimens with a range of PD-L1 IHC expression was tested on 5 non-consecutive days. Inter-site analysis was performed between 3 sites on a total of 1350 pair-wise comparisons.	ANA 99.3% (98.8, 99.7%) APA 99.3% (98.8, 99.7%) OA 99.3% (98.7, 99.7%)
Intra-site assay	Each of 18 melanoma specimens with a range of PD-L1 IHC expression was tested on 5 non-consecutive days at each of three study sites. Intra-site analysis was performed for 3 sites on a total of 540 pair-wise comparisons.	ANA 99.3% (98.4, 99.8%) APA 99.3% (98.5, 99.8%) OA 99.3% (98.5, 99.8%)
Inter-observer (one observer at each of three sites)	Scoring of 30 melanoma specimens with a range of PD-L1 IHC expression, stained with PD-L1 IHC 28-8 pharmDx, was performed by 3 pathologists, one at each of 3 study sites, on 3 non-consecutive days. Inter-observer analysis was performed between 3 sites on a total of 810 pair-wise comparisons.	ANA 90.4% (88.1, 92.5%) APA 91.7% (89.7, 93.6%) OA 91.1% (89.1, 93.1 %)
Intra-observer (one observer at each of three sites)	Scoring of 30 melanoma specimens with a range of PD-L1 IHC expression, stained with PD-L1 IHC 28-8 pharmDx, was performed by 3 pathologists, one at each of 3 study sites, on 3 non-consecutive days. Intra-observer analysis was performed for 3 sites on a total of 270 pair-wise comparisons.	ANA 99.2% (97.9, 100%) APA 99.3% (98.2, 100%) OA 99.3% (98.1, 100%)

5. Robustness

Robustness of the staining performance of PD-L1 IHC 28-8 pharmDx was evaluated by testing the performance of the assay when varying the following conditions as described below. One lot of reagents was assessed.

a) Tissue section thickness (4 and 5 µM)
b) Microscope slide type (Fisherbrand Superfrost Plus, Dako Flex IHC microscope slides, and Dako Salinized slides)
c) Target retrieval solution temperature (97^0C, 95^0C, and 99^0C)
d) Target retrieval time (20, 18 and 22 minutes)
e) Target retrieval solution pH (pH 6.1, 5.8 and 6.4)
f) Target retrieval solution reuse (3 re-uses)

The study included 16 melanoma specimens spanning the range of PD-L1 expression and included specimens around the cut off. Staining performance was evaluated for both percent (%) tumor staining and intensity of staining. A change in the PD-L1 status or drop in intensity by 0.5 on a scale of 0-3 was considered a test condition failure. No significant difference in results was observed for any of the recommended experimental conditions above.

6. Impact of Pre-analytical Variables on Assay Performance

The objective of this study was to assess the effect of pre-analytical variables of ischemia, fixative type and fixation time on the performance of PD-L1 IHC 28-8 pharmDx in 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.

Alternate fixation methods utilizing other fixatives were not evaluated in the study and therefore performance of the PD-L1 IHC 28-8 PharmDx assay is validated for 10% neutral buffered formalin fixation.

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

Matched primary versus metastatic blocks were obtained from 20 subjects and

evaluated by PD-L1 IHC 28-8 pharmDx. In 15 of 20 matched melanoma pairs the diagnostic outcome was identical for primary and metastatic specimens. Five primary/metastatic pairs showed discordant results at the ≥1% expression level such that in 3 cases the primary tumor was negative for PD-L1 and metastatic tumor positive and in 2 cases the primary tumor was PD-L1 negative and metastatic tumor was positive.

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

Multiple blocks (2 or more) from each of 15 melanoma subjects obtained from the same tumor were evaluated. In 13 of 15 sets of melanoma intra-subject specimens the diagnostic outcomes were identical for all specimens. Two cases showed discordant results at the ≥1% expression level.

8. Stability testing

a. PD-L1 IHC 28-8 pharmDx Stability

The real-time shelf life/stability of the PD-L1 IHC 28-8 pharmDx reagents was assessed using a variety of tumor tissue: 16 NSCLC, 2 non squamous NSCLC, 3 renal cell carcinoma and 3 melanoma and 8 Control cell line blocks. Three reagent lots were evaluated and several studies were conducted. Transport simulation tested kit stability by exposing the kits to temperatures between -20-0°C, 37°C for 16-24 hrs 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 18 temperature cycles from 2-8 C to RT with 6-8hrs at RT in each cycle. Stability of reconstituted working solutions was tested for both DAB (one week) and target retrieval solution (3 reuses over one week). The results support the stability claims listed below.

Total Shelf Life
7 months at 2-8°C

Finished Goods 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. Melanoma FFPE Cut Section Stability

A real-time stability study was designed to evaluate the shelf life of cut tissue sections of melanoma FFPE blocks using PD-L1 IHC 28-8 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 3 melanoma specimens. Based on these studies, stability dating is 4 months.

B. Animal 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 Clinical Study CA209067/ NCT01844505, a Phase 3, randomized, double-blind study of nivolumab (Opdivo®; Bristol Meyer Squibb) monotherapy or nivolumab in combination with ipilimumab (Yervoy® Bristol Meyer Squibb) versus ipilimumab monotherapy in subjects with previously untreated, unresectable or metastatic melanoma. A total of 1296 subjects were enrolled at 137 sites in 21 countries (Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Finland, France, Germany, Ireland, Israel, Italy, Netherlands, New Zealand, Norway, Poland, Spain, Sweden, Switzerland, United Kingdom, and United States). The first subject was enrolled on June 11, 2013, the last patient visit date for the purposes of the database lock was on December 31, 2014. The clinical database lock for final progression free survival (PFS) analysis occurred on February 17, 2015, which provided the basis for this PMA.

Tumor tissues were collected prior to randomization from primary or metastatic sites in the metastatic or unresectable setting. PD-L1 IHC expression status was assessed as one of the stratification factors using a prototype PD-L1 IHC 28-8 pharmDx assay. Upon completion of manufacture and validation of the PD-L1 IHC 28-8 PharmDx kit, all subject tissue specimens were re-tested for all pre-specified efficacy and safety analyses summarized below. All PDL1 tests were performed in one central laboratory (LabCorp Clinical Trials, Los Angeles, CA, US) on FFPE tumor specimens by PD-L1 IHC 28-8 pharmDx manufactured at Dako North America. PD-L1 expression status was not used as a criterion for subject selection into the treatment arms.

1. Clinical Inclusion and Exclusion Criteria

Study inclusion criteria were as follows:

- Adult ($\geq$18 years) treatment naïve subjects with histologically- or cytologically-stage III or Stage IV melanoma per AJCC staging system and who had measurable disease with European Cooperative Oncology Group (ECOG) performance status $\leq$1.
- Subject should know or consent to test for BRAF V600 mutation status during screening period and provide tissue from an unresectable or metastatic site of disease to determine PD-L1 expression level for randomization.

Study exclusion criteria were as follows:

- The trial excluded patients with Ocular Melanoma
- 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 12, then every 6 weeks for the first 12 months and every 12 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. 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

Primary efficacy end points for the study were progression free survival (PFS) and overall survival (OS) on nivolumab monotherapy compared to ipilimumab monotherapy, and that of nivolumab combined with ipilimumab and ipilimumab monotherapy. Secondary end points: 1) To compare objective response rates (ORR) of nivolumab monotherapy to ipilimumab monotherapy and that of nivolumab combined with ipilimumab to ipilimumab monotherapy, 2) To evaluate differences in OS, PFS, and ORR between nivolumab combined with ipilimumab and nivolumab monotherapy, 3) to evaluate whether PD-L1 expression is a predictive biomarker for OS.

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

Of the 1296 subjects enrolled, a total of 945 subjects were randomized to the study, into one of the following treatment groups: nivolumab (n=316), nivolumab plus ipilimumab (n=314) and ipilimumab (n=315). Patients were stratified based on PD-L1 status expression as assessed by the verified PD-L1 IHC nivolumab pharmDx.

PD-L1 status was retrospectively determined with the PD-L1 IHC 28-8 pharmDx on tumor samples from 308, 304 and 303 subjects in the nivolumab, nivolumab plus ipilimumab and ipilimumab groups, respectively. In total 873 patient samples: 288 in nivolumab, 278 in nivolumab plus ipilimumab and 277 in ipilimumab arms- yielded a quantifiable PD-L1 result.

A total of 55 study subject specimens for who PD-L1 status could not be evaluated due to melanin interference were classified as "PD-L1 Indeterminate" and numbered 17 in Nivolumab arm and 19 each in nivolumab plus ipilimumab and ipilimumab arms. PD-L1 status was not determined for 47 study subjects either due to withdrawal of consent and unavailable or insufficient tissue for the IHC test: 9 in nivolumab arm, 17 in nivolumab plus ipilimumab arm and 19 in ipilimumab arm. Table 6 summarizes the disposition of subjects in the clinical study.

Table 6: Accountability of PMA cohort for PD-L1 IHC 28-8 pharmDx testing.

	Nivolumab	**Nivolumab+ Ipilimumab**	**Ipilimumab**	**Total**
Number of subjects enrolled and randomized	316	314	315	945
Tested by PD-L1 IHC pharmDx				
Number of subjects tested	308	304	303	915
Reason for not testing				
Subject withdrew/did not consent	1	1	4	6
No/insufficient tumor sample	7	9	8	24
Quantifiable PD-L1 results				
Number of Subjects with Quantifiable PD-L1 results	288	278	277	843
Reason not quantifiable:				
Non-evaluable PD-L1 result due withdrawal/not consented	3	7	7	17
Indeterminate PD-L1 Results due to melanin/background interference	17	19	19	55

C. Study Population Demographics and Baseline Parameters

Among the 945 randomized patients, the baseline study population characteristics were generally balanced across the three treatment groups. Median age was 61 years (range: 18 to 90 years); 65% of patients were male; 97% were white; the ECOG performance score was 0 (73%) or 1 (27%). The majority of the patients had AJCC Stage IV disease (93%); 58% had tumors characterized as M1c. Twenty-two percent of patients had received prior adjuvant therapy; Elevated LDH was reported at study entry in 36% of patients and history of brain metastases in 4% of patients. Thirty-two percent of patients had BRAF mutation-positive melanoma. A total of 11.9% of the tumor specimens collected for PD-L1 screening was obtained from primary site while 85.8 specimens were obtained from metastatic sites. PD-L1 status as determined by the PD-L1 IHC 28-8 prototype assay was also balanced across the three treatment groups.

D. Safety and Effectiveness Results

1. Safety Results

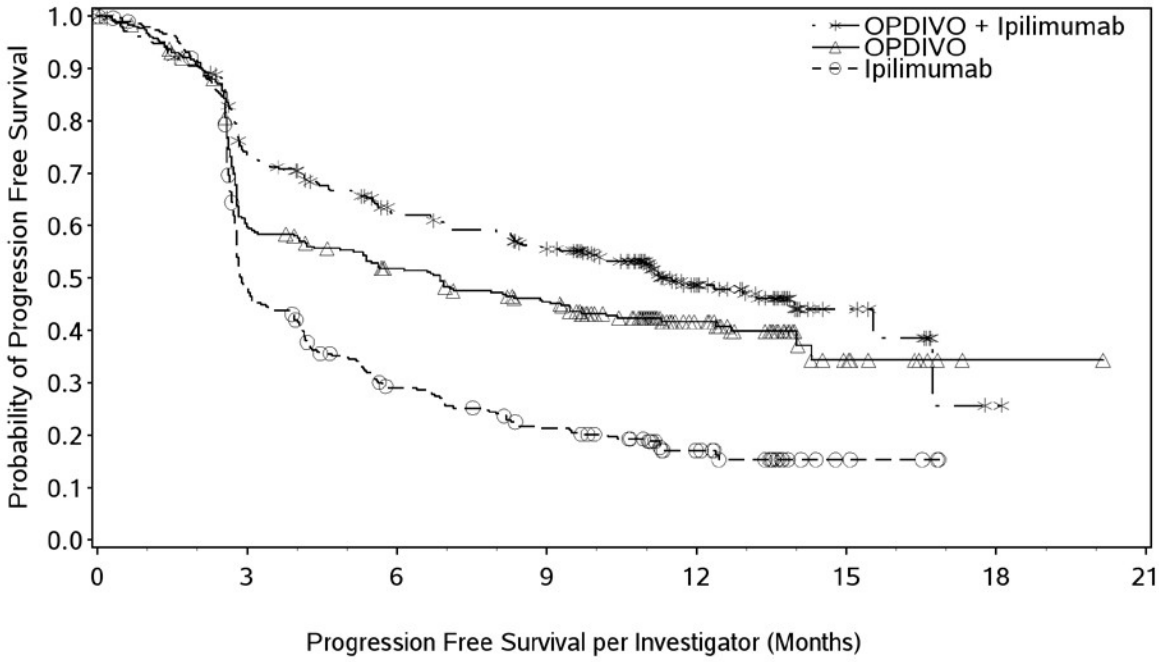
   As an in vitro diagnostic test, the PD-L1 IHC 28-8 pharmDx Assay involves testing on FFPE non-squamous melanoma sections. These tissues are routinely removed as part of the practice of medicine for the diagnosis of melanoma by pathologists. Removal of these tissues, therefore, presents no additional 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 within each treatment arm.

2. Effectiveness Results

   Drug Efficacy in all enrolled study subjects for Clinical study CA209067:
   The clinical performance of PD-L1 IHC 28-8 pharmDx was based on clinical trial CA206097 a Phase 3, randomized, double-blind study of nivolumab monotherapy or nivolumab in combination with ipilimumab versus ipilimumab monotherapy in subjects with previously untreated, unresectable or metastatic melanoma. Efficacy analysis for the study subjects irrespective of their PD-L1 status was based on 945 subjects randomized into the clinical study with 316, 314 and 315 subjects in nivolumab, nivolumab plus ipilimumab and ipilimumab arms respectively. Median PFS in the nivolumab monotherapy arm was 6.9 months when compared to 2.9

months in the ipilimumab group (HR=0.57, 99.5% CI: 0.43, 0.76; p<0.0001). The median PFS was 11.5 months in the nivolumab plus ipilimumab group, as compared with 2.9 months in the ipilimumab group (HR=0.42, 99.5% CI: 0.31, 0.57; p<0.0001). The nivolumab plus ipilimumab vs nivolumab comparison was not a primary objective of the study and thus the study was not powered to detect this difference. However, the comparison of median PFS between the nivolumab plus ipilimumab group with the nivolumab group yielded a Hazard Ratio of 0.74 (95% CI: 0.60, 0.92). The results are graphically represented in Figure 1.

Figure 1: **PFS by treatment in All Randomized Subjects**



Number of Subjects at Risk

OPDIVO + Ipilimumab							
314	219	173	151	65	11	1	0
OPDIVO							
316	177	147	124	50	9	1	0
Ipilimumab							
315	137	77	54	24	4	0	0

Efficacy Results based on pre-study PD-L1 expression status

The clinical performance of PD-L1 IHC 28-8 pharmDx was based on clinical trial CA206097, a Phase 3, randomized, double-blind study of nivolumab monotherapy or nivolumab in combination with ipilimumab versus ipilimumab monotherapy in subjects with previously untreated, unresectable or metastatic melanoma. Of the 945 study subjects, retrospective testing data for pre-treatment PD-L1 status with PD-L1 IHC 28-8 PharmDx assay was available for 843 subjects: 288 in nivolumab arm, 278 in nivolumab plus ipilimumab arm and 277 in the ipilimumab arm.

Data for 102 study subjects was not available due to missing specimens (47), or melanin interference (55). Pre specified analyses of PFS endpoint in different PD-L1 expression subgroups (<1% and ≥1%) was based on 843 randomized subjects with quantifiable PD-L1 test results. PD-L1 status (<1% and ≥1%) for study subjects was balanced across the three arms of the study and 171 (59%) patients in the nivolumab arm, 155 (56%) patients in the nivolumab plus ipilimumab arm and 164 (59%) patients in the ipilimumab arm were ≥1% PD-L1 by the PD-L1 IHC 28-8 PharmDx assay. of the distribution of subjects on the basis of PD-L1 expression in all randomized subjects in CA209067 with evaluable specimen are presented in Table 7.

Table 7. Frequency of PD-L1 Expression at Pre-Study in all Randomized Subjects

	Number of subjects, n (%)		
	Nivolumab	**Nivolumab + ipilimumab**	**Ipilimumab**
PD-L1 quantifiable subjects[a]	288	278	277
PD-L1 expression level:			
<1%	117 (40.6)	123 (44.2)	113 (30.8)
≥1%	171 (59.4)	155 (55.8)	164 (59.2)

[a] Number of quantifiable PD-L1 results only; does not include the number indeterminate PD-L1 results

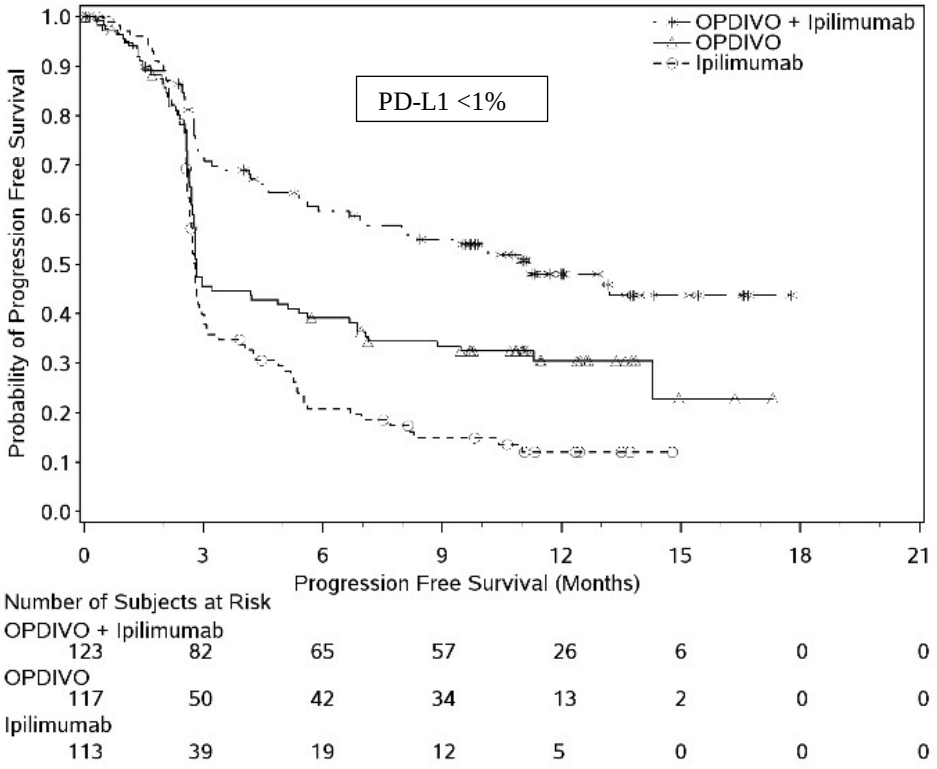
Efficacy analysis for response to nivolumab monotherapy when compared to ipilimumab monotherapy in PD-L1 subgroups (<1% and ≥1%) suggests improved median PFS in the PD-L1 positive (≥1%) subgroup as illustrated by hazard ratio (HR) of 0.65 in PD-L1 negative (<1%) vs. 0.46 in PD-L1 positive (≥1%) subjects. For subjects in PD-L1 negative (<1%) sub-group, median PFS in months was superior with nivolumab plus ipilimumab combination therapy (11.17, 95% CI: 8.11, NR) than with nivolumab monotherapy (2.83, 95% CI: 2.76, 5.13) or ipilimumab monotherapy (2.79, 95% CI: 2.66, 2.96). In PD-L1 ≥1%, subgroup, median PFS was longer in both the nivolumab monotherapy (12.39, 95% CI: 8.11, NR) and nivolumab plus ipilimumab combination therapy (12.35, 95% CI: 8.51, NR) when compared to ipilimumab monotherapy (3.91, 95% CI: 2.83, 4.17). Exploratory efficacy analysis in PD-L1 subgroups comparing outcomes with nivolumab monotherapy vs. nivolumab plus ipilimumab combination therapy, suggests increased clinical benefit for PD-L1 <1% subgroup with combination therapy (11.17 mos., 95% CI: 8.51 NR vs. 2.83 mos., 95% CI 2.76, 5.83). While in subjects with PD-L1 expression ≥1% the median PFS in nivolumab plus ipilimumab combination therapy and nivolumab monotherapy was comparable (12.39 mos.,95% CI 8.11, NR vs. 12.35 mos., 95% CI: 8.51, NR). The PFS trends are illustrated in the Table 8 and the Kaplan Meier curves shown in Figure 2.

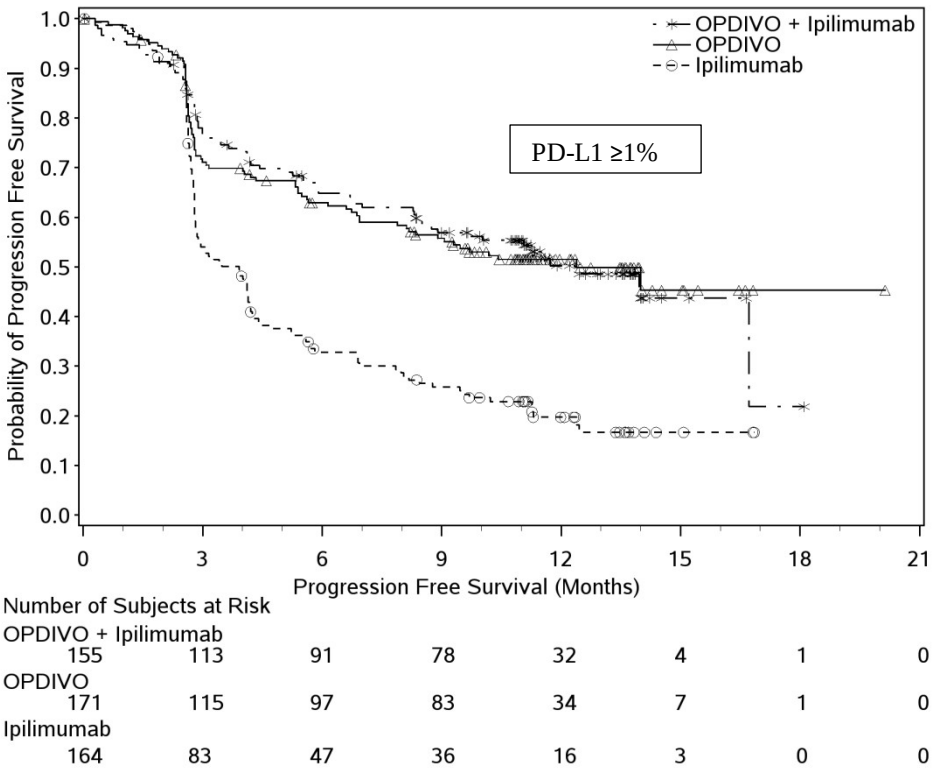
Table 8. Summary of PFS and Hazard ratios for CA209067 study by PD-L1 expression status

Median Progression Free Survival (95% CI)			
PD-L1 Expression Level	**Nivolumab monotherapy**	**Nivolumab +ipilimumab combination therapy**	**Ipilimumab monotherapy**
<1%	2.83 (2.76 , 5.13)	11.17 (6.93, NR)	2.79 (2.66, 2.96)
≥1%	12.39 (8.11, NR)	12.35 (8.51, NR)	3.91 (2.83, 4.17)
Hazard Ratios (95% CI)			
	Nivolumab vs. ipilumumab	Nivolumab + ipilimumab vs. ipilimumab	Nivolumab + ipilimumab vs. nivolumab*
<1%	0.69 (0.5, 0.93)	0.37 (0.26, 0.52)	0.58 (0.41, 0.81)
≥1%	0.45 (0.35, 0.57)	0.43 (0.32, 0.58)	0.96 (0.70, 1.33)

*Exploratory analysis

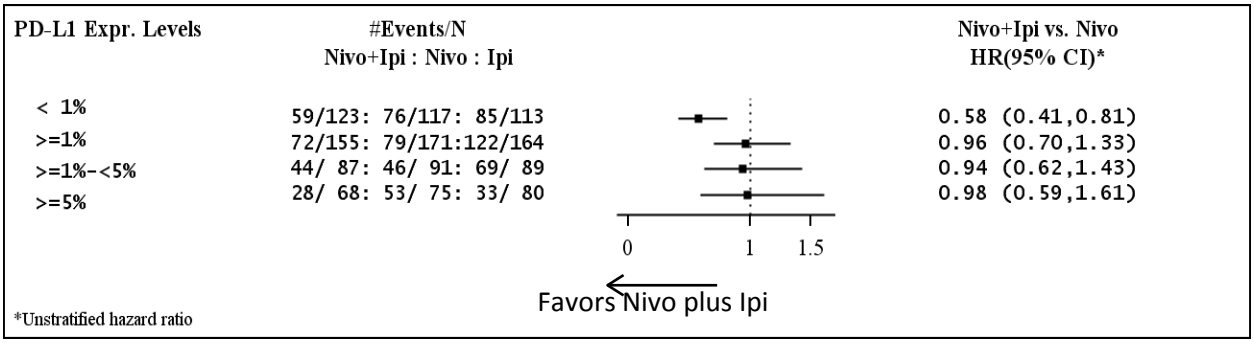
Figure 2: PFS by treatment and PD-L1 status at 1% Expression Level





The unstratified hazard ratio and the corresponding 95% CI were estimated in a Cox proportional hazards model using the randomized treatment arm as a single covariate. Hazard ratios for pre-specified PD-L1 subgroup analysis are summarized in Table 8 and the forest plots in Figure 3. Exploratory efficacy analysis comparing nivolumab monotherapy to nivolumab plus ipilimumab combination therapy suggests a greater benefit with combination therapy in the <1% PD-L1 subgroup while in the ≥1% PD-L1 subgroups, study subjects had comparable outcomes in the two treatment arms. The hazard ratios (95% CI) for for the combination versus nivolumab in <1% PD-L1 subgroup the HR was 0.58 (0.41, 0.81). The hazard ratio (95% CI) for nivolumab plus ipilimumab versus nivolumab were 0.96 (0.70, 1.33) for the ≥1% PD-L1 subgroups.

Figure 3: Forest Plots for efficacy outcomes based on PD-L1 expression levels comparing nivolumab monotherapy vs. nivolumab plus ipilimumab combination therapy.



The efficacy analysis of outcomes in the PD-L1 subgroups did not consider missing PD-L1 results for 102 specimens. In order to evaluate the potential impact of the missing specimens on the efficacy data, a sensitivity analysis with two extreme scenarios were considered: imputing missing PD-L1 results as low expression and imputing missing PD-L1 results as high expression. Imputation to PD-L1 low expression status as well as imputation to PD-L1 high expression preserved the Hazard Ratio for the Nivolumab monotherapy and combination therapy arms when compared to ipilimumab monotherapy. The imputed hazard ratios are summarized in Table 9. These results are consistent with data obtained for samples with PD-L1 results and are indicative with robustness of treatment comparisons with respect to missing PD-L1 results.

Table 9. PFS Hazard Ratios when Missing data is imputed for PD-L1 sub group analysis

Hazard Ratios (95% CI)				
	Imputation to Low PD-L1 Expression		Imputation to High PD-L1 Expression	
PD-L1 Expression Status	Nivolumab vs. Ipilumumab	Nivolumab +ipilumumab vs. ipilumumumab	Nivolumab vs. Ipilumumab	Nivolumab +ipilumumab vs. ipilumumumab
< 1%	0.72 (0.55, 0.95)	0.41 (0.31, 0.55)	0.67 (0.49, 0.92)	0.38 (0.27, 0.53)
≥ 1%	0.46 (0.34, 0.61)	0.44 (0.31, 0.58)	0.50 (0.39, 0.65)	0.45 (0.35, 0.59)

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 involved in PD-L1 IHC 28-8 pharmDx device evaluation 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.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of PD-L1 IHC 28-8 pharmDx was evaluated in a retrospective analysis of melanoma patients enrolled in CA209067, a Phase 3, randomized, double- blind study of nivolumab monotherapy or nivolumab in combination with ipilimumab versus ipilimumab monotherapy in subjects with previously untreated, unresectable or metastatic melanoma. Pre-study (baseline) tumor specimens were tested using a 1% threshold for positivity. The results demonstrated a magnitude of effect based on PD-L1 expression in the nivolumab arm. The median PFS in the nivolumab monotherapy was 2.83 (95% CI; 2.76, 5.13) in patients with with PD-L1 negative expression status <1% , and 12.39 (8.11, NR) in patients with PD-L1 positive (≥1%) expression status. When patients were treated with ipilimumab in combination with nivolumab, the PFS benefit appeared to be greatly enhanced in the PD-L1 negative patients (11.17; 6.93, NR). In contrast, the PFS benefit was similar for PD-L1 positive patients when treated with either nivolumab monotherapy (12.39; 8.11, NR) or nivolumab plus ipilimumab combination therapy (12.35; 8.51, NR). The differential treatment effect with nivolumab plus ipilimumab vs nivolumab alone was evident in subjects with <1% PD-L1, with Hazard Ratio (HR (95% CI)) of 0.58 (0.41, 0.81). HRs in subjects with PD-L1 ≥1% were 0.96 (0.70, 1.33). PD-L1 expression status in CA209067 subjects appeared to identify a subpopulation (PD-L1<1%) which derived greater benefit with the addition of ipilimumab to nivolumab as compared to nivolumab alone or ipilimumab alone (see Table 8), whereas patients with PD-L1 positive tumors (PD-L1 ≥1%) appeared to have similar PFS treatment effects in both nivolumab (Hazard Ratio: 0.43 vs. 0.45) containing arms as compared to ipilimumab alone.

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 melanoma. 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. The process of testing FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for melanoma diagnosis.

C. Benefit-Risk Conclusions

The use of PD-L1 IHC 28-8 pharmDx test to determine the PD-L1 expression level in melanoma tumor tissue from patients may help physicians determine the best treatment regimen and tailor therapies according to patients benefit/risk profile. The probable benefits of the device are based on data obtained from retrospective testing of melanoma specimens from patients enrolled into the clinical trial as described above and support PMA approval of the PD-L1 IHC 28-8 pharmDx test, and are shown to outweigh the risks. Nivolumab is an antibody to PD-L1 and prevents binding to PD-L1 protein on target cells. Changes in magnitude of treatment effect on progression free survival were observed in the nivolumab arm based on the presence or absence of PD-L1 expression (<1%, ≥1%). In the PD-L1 negative (<1%) population, it appears that a greater magnitude of treatment benefit is observed for patients receiving combination nivolumab with ipilimumab therapy than PD-L1 negative (<1%) patients receiving nivolumab alone. Treatment effects were similar for patients receiving nivolumab with ipilimumab or nivolumab monotherapy in the PD-L1 positive patients (≥ 1%). Patients receiving the combination nivolumab with ipilimumab experienced increased adverse events (grade 3 or 4) compared to nivolumab monotherapy. Frequency of treatment related adverse events of grade 3 or 4 did not meaningfully vary with PD-L1 expression status. Thus the use of this device in melanoma population may assist in determining the probable PFS benefit compared to the probable risks for individual patients' on the basis of tumor PD-L1 expression using a 1% threshold.

False positive results and false negative results may lead to improper expectation of treatment outcomes with nivolumab monotherapy or nivolumab plus ipilimumab combination therapy with the possibility of exposure to increased adverse events or forgoing greater benefit from effective therapy. However, both indications are approved for patients irrespective of PD-L1 expression. Therefore, 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 melanoma patients who may be considered for treatment with nivolumab in combination with ipilimumab.

XIII. CDRH DECISION

CDRH issued an approval order on January 23, 2016.

The final conditions of approval cited in the approval order include post-approval analytical validation studies. Because an abbreviated study design and 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 in melanoma, additional testing of samples with appropriate study design is required for robust characterization of analytical performance of the device.

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, Warnings, Precautions, Limitations, and performance evaluations in the device labeling.

Post-approval Requirements and Restrictions: See approval order.