SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic immunohistochemistry (IHC) test for detection of PD-L1 in formalin-fixed, paraffin-embedded (FFPE) human tissue sections
Device Trade Name:	PD-L1 IHC 22C3 pharmDx
Device Procode:	PLS
Applicant's Name and Address:	Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Carpinteria, CA 95051
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P150013/S033

Date of FDA Notice of Approval: February 10, 2026

The original PD-L1 IHC 22C3 pharmDx PMA (P150013) was approved on October 2, 2015, for the qualitative detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on the Autostainer Link 48. PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. The indication for use of the device as approved in P150013 is to aid in identifying patients with NSCLC whose tumors are considered PD-L1 positive if TPS ≥ 50% and for whom safety and efficacy of KEYTRUDA® (pembrolizumab) have been established. The SSEDs to support the previously approved indications are available on the CDRH website and are incorporated by reference here.

The current supplement was submitted to expand the indication for the PD-L1 IHC 22C3 pharmDx to include patients with epithelial ovarian, fallopian tube, or primary peritoneal carcinoma (EOC) for treatment with KEYTRUDA® (pembrolizumab). PD-L1 protein expression is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS ≥ 1.

II. INDICATIONS FOR USE

For in vitro diagnostic use.

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using monoclonal mouse anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin embedded (FFPE) non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma (ESCC), cervical cancer, head and neck squamous cell carcinoma (HNSCC), triple-negative breast cancer (TNBC), gastric or gastroesophageal junction (GEJ) adenocarcinoma, and epithelial ovarian, fallopian tube, or primary peritoneal carcinoma (EOC) tissues using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

PD-L1 protein expression in ESCC, cervical cancer, HNSCC, TNBC, gastric or GEJ adenocarcinoma, and EOC is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying patients for treatment with the therapies for the indications listed in Table 1.

Table 1. PD-L1 IHC 22C3 pharmDx companion diagnostic indications, PD-L1 expression levels, and therapies

Tumor Indication	PD-L1 Expression Level	Therapy
NSCLC	TPS ≥ 1%	KEYTRUDA® (pembrolizumab)*
ESCC	CPS ≥ 10	
Cervical Cancer	CPS ≥ 1	
HNSCC	CPS ≥ 1	
TNBC	CPS ≥ 10	
Gastric or GEJ adenocarcinoma	CPS ≥ 1	
Epithelial ovarian, fallopian tube, or primary peritoneal carcinoma (EOC)	CPS ≥ 1	
NSCLC	TPS ≥ 50%	LIBTAYO® (cemiplimab)**

*See the KEYTRUDA® product label for specific clinical circumstances guiding PD-L1 testing.
**See the LIBTAYO® product label for specific clinical circumstances guiding PD-L1 testing.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

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

V. DEVICE DESCRIPTION

Device Kit Components

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical (IHC) staining procedure for formalin-fixed and paraffin-embedded (FFPE) specimens using the EnVision FLEX visualization system and the Dako Autostainer Link 48 with DakoLink software version 4.2. The principal component of the kit is the mouse monoclonal anti PD-L1 clone 22C3 antibody that binds to PD-L1 protein expressed on FFPE tissue. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and reagents shown in Table 2 necessary to perform 50 tests in up to 15 individual runs. Wash buffer and hematoxylin are required for the assay but not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration, and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods. Device kit components are shown in Table 2 below.

Table 2: Device Kit Components

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

Reagent	Description	Qty x Vol
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride (DAB) in an organic solvent.	1 x 5 mL
DAB Enhancer	Cupric sulfate in water.	1 x 34.5 mL
Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.	6 x 30 mL
Cell Line Control Slides	Each slide contains sections of two pelleted, formalin-fixed paraffin-embedded cell lines: NCI-H226 with moderate PD-L1 protein expression (positive control), and MCF-7 with negative PD-L1 protein expression (negative control).	15 slides

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

Device Instrument and Software

PD-L1 IHC 22C3 pharmDx assay is performed on the Dako Autostainer Link 48 automated staining system using the DakoLink software version 4.2. The Autostainer system is designed to mimic the staining steps performed manually by a lab technician. The PD-L1 IHC 22C3 pharmDx protocol is assay specific. The DakoLink software has been designed to recognize and group PD-L1 IHC 22C3 pharmDx reagents, requiring that all system reagents are used together. Deparaffinization, rehydration, and target retrieval (3-in-1) procedures are performed in the PT Link Pre-treatment module (PT100/200 modules; deparaffinization performed separately in PT100).

Specimen Preparation

Epithelial ovarian, fallopian tube, or primary peritoneal carcinoma specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens. FFPE tissues are suitable for use. Fixation time for 12-72 hours in 10% neutral buffered formalin (NBF) is recommended. Alternative fixatives have not been validated and may give erroneous results. Fixation times of ≤ 3 hours may result in variable PD-L1 detection. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in 10% NBF. Specimens are then processed through a tissue processor where they are dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. After specimen processing, tissue specimens should be cut into sections of 4-5 µm using a tissue microtome, mounted on charged microscope slides. Slides are then placed in a 58 ± 2°C oven for 1 hour as an initial step for deparaffinization.

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

stained within 7.5 months of sectioning when stored at 2-8°C, or within 4 months when stored at 25 °C. Slide storage and handling conditions should not exceed 25°C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls

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

1) Control cell line slides provided as part of the kit should be used to verify the staining procedure. One Control Slide should be stained with the primary antibody to PD-L1 in each staining run. Each slide contains sections of two pelleted, FFPE cell lines: one with moderate PD-L1 protein expression and one that is negative for PD-L1 protein expression. The evaluation of the Control Slide supplied in the kit indicates the validity of the staining run. The Control Slides should not be used as an aid in interpretation of patient results.

2) Run controls are to be provided by the end-user laboratory. Positive and negative run controls should be fresh biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). The positive control tissue should include weak to moderate positive staining for PD-L1 to detect subtle changes in assay sensitivity. Negative control tissue is required to detect unintended antibody cross reactivity to tissue and is expected to be negative for PD-L1 expression.

3) The kit includes a Negative Control Reagent that is used in parallel with the PD-L1 clone 22C3 primary antibody on patient tissue. The matched negative control aids the reader in differentiating a true signal from tissue-specific background staining that may occur from reaction with detection chemistry and not the anti PD-L1 primary antibody.

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

Principle of Procedure

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an IHC staining procedure on FFPE specimens using the Autostainer Link 48. Following deparaffinization of the tissue sections, rehydration and target retrieval, the slides are incubated with the primary monoclonal antibody to PD-L1 (clone 22C3) or the Negative Control Reagent. The slides are then incubated with an anti-mouse Linker antibody, which is specific to the host species of the primary antibody. Following this, the slides are incubated with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and HRP molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added DAB+ Chromogen results in precipitation of a visible reaction product at the antigen sites. The color of the chromogenic reaction is modified by a chromogen enhancement reagent, DAB Enhancer. The specimen is then counterstained with hematoxylin and cover slipped.

Staining Protocol

PD-L1 IHC 22C3 pharmDx is designed to be run on the Autostainer Link 48 with DakoLink software.

The staining protocol on the Autostainer Link 48 is as follows:

Peroxidase-Blocking Reagent (2 drop zones x150µL):	5 minutes (± 1 minute)
Rinse in buffer	
Monoclonal Mouse anti-PD-L1 (or Negative Control Reagent) (2 drop zones x150µL):	30 minutes (± 1 minute)
Rinse in buffer	
Linker, anti-Mouse Ig (2 drop zones x150µL):	30 minutes (± 1 minute)
Rinse in buffer	
Visualization Reagent (2 drop zones x150µL):	30 minutes (± 1 minute)
Rinse in buffer:	5 minutes
DAB+ solution (2 drop zones x150µL):	2 x 5 minutes (± 1 minute)
Rinse in buffer	
DAB+ Enhancer (2 drop zones x150µL):	5 minutes (± 1 minute)
Rinse in buffer	
Hematoxylin (2x150µL):	5 minutes (± 1 minute)
Rinse in deionized water	
Rinse in buffer:	5 minutes
Rinse in deionized water	

Remove slides from autostainer and place in bath of reagent water.

Slides may then be permanently mounted using non-aqueous, permanent mounting medium.

Interpretation of PD-L1 Staining

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

PD-L1 expression in epithelial ovarian, fallopian tube, or primary peritoneal carcinoma is determined by CPS, which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. Distinction of viable tumor cells, lymphocytes, and macrophages is essential for accurate denominator estimation. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100. CPS is defined as follows:

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

By definition, PD-L1 staining cells are:

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

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

Due to the histological characteristics of epithelial ovarian, fallopian tube and primary peritoneal carcinoma they are grouped together as epithelial ovarian cancer (EOC) in this document. The tables below provide details about which tissue elements are included in and excluded from the CPS numerator and denominator in EOC.

Table 3. CPS Numerator Inclusion/Exclusion Criteria for EOC

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor cells	• Convincing partial or complete linear membrane staining (at any intensity) of viable invasive tumor cells	• Nonstaining tumor cells • Tumor cells with only cytoplasmic staining • Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)

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

Other Cells	• Not Included	• Benign epithelial cells • Stromal cells (fibroblasts, endothelial cells) • Necrotic cells and/or cellular debris, cystic fluid

*In **MICs**, membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs is included in the score.
**__Adjacent MICs__ are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response to the tumor should be excluded.
***Macrophages and histiocytes are considered the same cells.

Table 4. CPS Denominator Inclusion/Exclusion Criteria for EOC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable invasive tumor cells	• Nonviable tumor cells • Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)
Immune Cells	Not included	• All immune cells
Other Cells	Not included	• Benign epithelial cells • Stromal cells (fibroblasts) • Necrotic cells and/or cellular debris

The specimen should be considered to have PD-L1 expression if CPS ≥ 1.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay available for use as an aid in identifying patients with PD-L1 expression in EOC tissues for treatment with KEYTRUDA® (pembrolizumab).

VII. MARKETING HISTORY

PD-L1 IHC 22C3 pharmDx has been marketed in the United States since approval of P150013 on October 2, 2015. PD-L1 IHC 22C3 pharmDx has also been marketed in Albania, Algeria, Argentina, Australia, Austria, Azerbaijan, Belgium, Bahrain, Bosnia-Herzegovina, Botswana, Brazil, Brunei, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Ethiopia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, India, Indonesia, Iraq, Ireland, Israel, Italy, Japan, Jordan, Kazakhstan, Kosovo, Kuwait, Latvia, Lebanon, Liechtenstein, Lithuania, Luxembourg, Macau, Macedonia, Malaysia, Malta, Mexico, Montenegro, Morocco, Netherlands, New Zealand, Norway, Oman, Panama, Paraguay, Peru, Philippines, Poland, Portugal, Puerto Rico, Qatar, Romania, Russia, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, South Africa, South Korea, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, Ukraine, United Arab Emirates, United Kingdom, Uruguay and Vietnam.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PD-L1 test results and subsequently improper assignment of treatment with KEYTRUDA®. Patients with a false negative assay result may not be considered for treatment with KEYTRUDA (pembrolizumab). Patients with a false positive assay result may receive treatment with KEYTRUDA (pembrolizumab) for which there is no expectation of benefit and exposure to potential toxicity. There is also a risk of delayed results, which may lead to delay in treatment.

For the specific adverse events that occurred in the clinical study, please see the KEYTRUDA® (pembrolizumab) FDA approved package insert which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Non-clinical studies were performed using PD-L1 IHC 22C3 pharmDx to establish analytical performance of the device. Antibody characterization studies for clone 22C3, including specificity and tour of body/ tour of tumor, control cell line validation, kit stability and preanalytical variables were submitted and reviewed in the original PMA (P150013) for this device. There have been no changes to the device design including reagent formulation or kit configuration since approval of the original PMA (P150013). Results from studies performed to support the ovarian carcinoma indication at CPS ≥ 1 are summarized in the sections below.

1. Analytical Sensitivity

Analytical sensitivity was assessed by calculating prevalence of PD-L1 positivity in participants with platinum-resistant EOC from the KEYNOTE-B96 study. A total of 643 participants were randomly assigned in a 1:1 ratio to pembrolizumab + chemotherapy (322 participants) or placebo + chemotherapy (321 participants). All 643 participants had a tumor tissue sample tested with PD-L1 IHC 22C3 pharmDx. Most participants (72.5%, n=466) had a tumor tissue PD-L1 expression score of CPS ≥1, and 27.5% of participants had a tumor tissue PD-L1 expression score of CPS < 1. While 201 of 466 (31.3%) participants with CPS ≥1 had a tumor tissue PD-L1 expression score of CPS ≥10.

2. Analytical Specificity

a. Western Blot
See Summary of Safety and Effectiveness Data for P150013.

b. Immunoreactivity in Human Tissues
See Summary of Safety and Effectiveness Data for P150013.

3. Precision

Precision of the PD-L1 IHC 22C3 pharmDx was evaluated at Agilent Technologies, Inc. to demonstrate that the assay produces consistent results in normal day-to-day testing of FFPE EOC specimens. Testing included repeatability, combined precision, and intra-/inter-reader precision:

- Repeatability was measured within a run
- Combined precision was measured between Autostainer Link 48 instruments, operators, testing days, and lots
- Precision was measured within and between readers

Intermediate Precision and Repeatability Studies (Combined Precision):
Thirty-eight EOC FFPE tissue blocks, each representing a unique study sample (19 PD-L1 positive and 19 PD-L1 negative, including 13 near cutoff samples at CPS ≥1) that encompassed PD-L1 IHC staining status range of positive and negative were used in this study. This study was performed using the following design factors on Autostainer Link 48 instruments:

- Three kit lots of PD-L1 IHC 22C3 pharmDx
- Three operators
- Three Autostainer Link 48 instruments
- Across three days
- One pathologist reader
- 3 replicates per condition
- Across all intermediate precision conditions (within-staining run)

All slides were blinded and randomized and evaluated using the PD-L1 IHC 22C3 pharmDx scoring algorithm. Each sample had 3 results and a majority PD-L1 status was assigned based on 3 results. A total of 38 unique test samples were included for assessment at the CPS ≥1 cutoff. For each sample, median CPS and range of CPS of 3 results were calculated. In addition, percent positive (CPS ≥1, "Positive" with regard to PD-L1 therapy) results were calculated. Results are presented in Table 5, below:

Table 5. Per-Specimen Agreement Results: Combined Precision: Results per Specimen (Days)

Specimen	Consensus* Result	Number of Results	Median (CPS)	Range (CPS)	Between Day (SD)	Percent Positive (n/N) **	Percent Agreement with Consensus (n/N)	95% CI***
1	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
2	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
3	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
4	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
5	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
6	Negative	3	0	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%

Specimen	Consensus* Result	Number of Results	Median (CPS)	Range (CPS)	Between Day (SD)	Percent Positive (n/N) **	Percent Agreement with Consensus (n/N)	95% CI***
7	Negative	3	0	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
8	Negative	3	0	0, 0	0.000	0% (0/3)	100% (3/3)	43.9-100%
9	Negative	3	0	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
10	Negative	3	0	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
11	Negative	3	0	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
12	Negative	3	0.5	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
13	Negative	3	0.5	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
14	Negative	3	0.5	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
15	Negative	3	0.5	0, <1	0.289	0% (0/3)	100% (3/3)	43.9-100%
16	Negative	3	0.5	<1, <1	0.000	0% (0/3)	100% (3/3)	43.9-100%
17	Negative	3	0.5	<1, <1	0.000	0% (0/3)	100% (3/3)	43.9-100%
18	Negative	3	0.5	<1, <1	0.000	0% (0/3)	100% (3/3)	43.9-100%
19	Negative	3	0.5	<1, 1	0.289	33.3% (1/3)	66.7% (2/3)	20.8-93.9%
20	Positive	3	1	1, 1	0.000	100% (3/3)	100% (3/3)	43.9-100%
21	Positive	3	4	3, 8	2.646	100% (3/3)	100% (3/3)	43.9-100%
22	Positive	3	7	3, 9	3.055	100% (3/3)	100% (3/3)	43.9-100%
23	Positive	3	8	4, 13	4.509	100% (3/3)	100% (3/3)	43.9-100%
24	Positive	3	10	8, 20	6.429	100% (3/3)	100% (3/3)	43.9-100%
25	Positive	3	12	12, 25	7.506	100% (3/3)	100% (3/3)	43.9-100%
26	Positive	3	13	10, 30	10.786	100% (3/3)	100% (3/3)	43.9-100%
27	Positive	3	15	5, 30	12.583	100% (3/3)	100% (3/3)	43.9-100%
28	Positive	3	15	7, 20	6.557	100% (3/3)	100% (3/3)	43.9-100%
29	Positive	3	20	15, 30	7.638	100% (3/3)	100% (3/3)	43.9-100%
30	Positive	3	20	20, 30	5.774	100% (3/3)	100% (3/3)	43.9-100%
31	Positive	3	25	18, 30	6.028	100% (3/3)	100% (3/3)	43.9-100%
32	Positive	3	25	18, 25	4.041	100% (3/3)	100% (3/3)	43.9-100%
33	Positive	3	25	20, 35	7.638	100% (3/3)	100% (3/3)	43.9-100%
34	Positive	3	25	20, 25	2.887	100% (3/3)	100% (3/3)	43.9-100%
35	Positive	3	30	15, 45	15.000	100% (3/3)	100% (3/3)	43.9-100%
36	Positive	3	30	20, 30	5.774	100% (3/3)	100% (3/3)	43.9-100%
37	Positive	3	30	30, 35	2.887	100% (3/3)	100% (3/3)	43.9-100%
38	Positive	3	40	30, 60	15.275	100% (3/3)	100% (3/3)	43.9-100%

SD = Standard Deviation; CI = Confidence Interval; CPS = Combined Positive Score; n = number of results; N = Total number of results

*Consensus result was determined based on the majority call across all testing sites and days
**Percent positive represents the proportion of specimens scoring positive (CPS ≥ 1) out of total number of results
***95% confidence intervals were calculated using the Wilson score method

Intra-Run Repeatability

Five replicate sections from each EOC specimen were stained with PD-L1 IHC 22C3 pharmDx within the same run on the Autostainer Link 48. A total of 38 unique test specimens were included for assessment at the CPS ≥1 cutoff. Results are presented in Table 6, below:

Table 6. Per-Specimen Agreement Results: Intra-Run Precision: Results per specimen (Replicates)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Within Run (SD)	Percent Positive (n/N) **	Percent Agreement with Consensus (n/N)	95% CI***
1	Negative	5	0	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
2	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
3	Negative	5	0	0, <1	0.22	0% (0/5)	100% (5/5)	56.6-100%
4	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
5	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
6	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
7	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
8	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
9	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
10	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
11	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
12	Negative	5	0.5	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
13	Negative	5	0.5	0, <1	0.22	0% (0/5)	100% (5/5)	56.6-100%
14	Negative	5	0.5	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
15	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
16	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
17	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
18	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
19	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
20	Positive	5	2	1, 4	1.14	100% (5/5)	100% (5/5)	56.6-100%
21	Positive	5	2	1, 4	1.10	100% (5/5)	100% (5/5)	56.6-100%
22	Positive	5	4	1, 8	2.88	100% (5/5)	100% (5/5)	56.6-100%
23	Positive	5	4	4, 5	0.45	100% (5/5)	100% (5/5)	56.6-100%
24	Positive	5	6	5, 9	1.64	100% (5/5)	100% (5/5)	56.6-100%
25	Positive	5	10	2, 30	11.65	100% (5/5)	100% (5/5)	56.6-100%
26	Positive	5	10	10, 10	0.00	100% (5/5)	100% (5/5)	56.6-100%
27	Positive	5	11	11, 11	0.00	100% (5/5)	100% (5/5)	56.6-100%
28	Positive	5	12	10, 22	5.02	100% (5/5)	100% (5/5)	56.6-100%
29	Positive	5	14	14, 18	1.79	100% (5/5)	100% (5/5)	56.6-100%
30	Positive	5	16	14, 22	3.65	100% (5/5)	100% (5/5)	56.6-100%
31	Positive	5	21	20, 31	4.72	100% (5/5)	100% (5/5)	56.6-100%

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Within Run (SD)	Percent Positive (n/N) **	Percent Agreement with Consensus (n/N)	95% CI***
32	Positive	5	22	11, 25	6.34	100% (5/5)	100% (5/5)	56.6-100%
33	Positive	5	28	22, 39	6.32	100% (5/5)	100% (5/5)	56.6-100%
34	Positive	5	30	23, 42	7.12	100% (5/5)	100% (5/5)	56.6-100%
35	Positive	5	45	45, 45	0.00	100% (5/5)	100% (5/5)	56.6-100%
36	Positive	5	60	40, 67	11.28	100% (5/5)	100% (5/5)	56.6-100%
37	Positive	5	60	50, 70	8.94	100% (5/5)	100% (5/5)	56.6-100%
38	Positive	5	85	60, 100	17.10	100% (5/5)	100% (5/5)	56.6-100%

SD = Standard Deviation; CI = Confidence Interval; CPS = Combined Positive Score; n = number of results; N = Total number of results

*Consensus was determined based on majority call across all test conditions
**Percent positive represents the proportion of specimens scoring positive (CPS ≥ 1) out of total number of results
***95% confidence intervals were calculated using the Wilson score method

Reader Precision Studies (Inter-Reader and Intra-Reader Precision)

In the observer precision studies for PD-L1 IHC 22C3 pharmDx, Within-Reader and Between-Reader components of precision for EOC tissue reads were evaluated. The study included specimens that were stained with PD-L1 IHC 22C3 pharmDx. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the PD-L1 IHC 22C3 pharmDx scoring algorithm. The study included three readers (pathologists). Readers scored all 62 specimens three times each, with a minimum of two-week wash-out period between reads. Results are presented in Table 7, below:

Table 7. Per-Specimen Agreement Results: Observer Precision: Results per Specimen (3 per reader)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Agreement (n/N) **			
					Inter-Read	Inter Observer	Total	Obs 1	Obs 2	Obs 3	All Observers
1	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
2	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
3	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
4	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
5	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
6	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
7	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
8	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
9	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
10	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
11	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
12	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
13	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Agreement (n/N) **			
					Inter-Read	Inter Observer	Total	Obs 1	Obs 2	Obs 3	All Observers
14	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
15	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
16	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
17	Negative	9	0	0, <1	0.00	0.10	0.10	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
18	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
19	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
20	Negative	7	0	0, 0	0.00	0.00	0.00	0% (0/1)	0% (0/3)	0% (0/3)	0% (0/7)
21	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
22	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
23	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
24	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
25	Negative	8	0	0, 0	0.00	0.00	0.00	0% (0/2)	0% (0/3)	0% (0/3)	0% (0/8)
26	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
27	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
28	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
29	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
30	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
31	Positive	9	1	0, 2	0.00	0.63	0.63	0% (0/3)	66.7% (2/3)	100% (3/3)	55.6% (5/9)
32	Positive	9	1	0, 2	0.24	0.24	0.33	100% (3/3)	66.7% (2/3)	100% (3/3)	88.9% (8/9)
33	Positive	9	2	1, 3	0.00	0.79	0.79	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
34	Positive	9	3	1, 6	0.01	1.92	1.92	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
35	Positive	9	3	1, 10	0.00	3.04	3.04	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
36	Positive	9	3	2, 5	0.00	0.51	0.51	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
37	Positive	9	5	3, 8	1.49	0.75	1.67	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
38	Positive	9	5	3, 10	0.00	2.01	2.01	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
39	Positive	9	6	2, 8	0.00	0.77	0.77	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
40	Positive	9	8	0, 25	0.00	11.90	11.90	100% (3/3)	0% (0/3)	100% (3/3)	66.7% (6/9)
41	Positive	9	8	2, 12	0.00	2.18	2.18	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
42***	Positive	3	8	5, 8	1.73	N/A	1.73	N/A	N/A	100% (3/3)	100% (3/3)
43	Positive	9	10	4, 10	0.00	1.25	1.25	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
44	Positive	9	15	3, 40	0.00	16.69	16.69	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
45	Positive	9	15	5, 35	0.00	6.75	6.75	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
46	Positive	9	15	6, 30	2.11	6.06	6.41	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
47	Positive	9	15	7, 35	1.18	4.12	4.28	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
48	Positive	9	15	10, 80	7.25	22.75	23.87	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
49	Positive	9	15	10, 40	3.54	5.89	6.87	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
50	Positive	9	15	10, 60	0.00	10.09	10.09	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
51	Positive	9	15	12, 20	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
52	Positive	9	20	10, 75	3.12	22.02	22.24	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
53	Positive	9	20	12, 40	0.00	8.18	8.18	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
54	Positive	9	25	10, 35	0.00	0.96	0.96	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Agreement (n/N) **			
					Inter-Read	Inter Observer	Total	Obs 1	Obs 2	Obs 3	All Observers
55	Positive	9	25	15, 60	0.11	11.90	11.90	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
56	Positive	9	30	15, 40	0.00	6.57	6.57	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
57	Positive	9	35	25, 60	5.77	4.08	7.07	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
58	Positive	9	60	25, 80	11.30	6.45	13.02	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
59	Positive	9	60	50, 90	7.70	0.00	7.70	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
60	Positive	9	65	25, 100	0.00	22.11	22.11	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
61	Positive	9	80	30, 100	14.43	15.55	21.21	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
62	Positive	9	90	60, 100	0.00	17.95	17.95	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

SD = Standard Deviation; CPS = Combined Positive Score; n = number of agreements; n = number of results; N = Total number of results

* Consensus was determined based on majority call across all test conditions
*Consensus result was determined based on the majority call across all test conditions
**Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results
*** This sample was excluded from analysis due to insufficient washout period

Precision Agreement Results Summary

The overall precision performance across all validation studies is summarized in Table 8, below:

Table 8. Precision of PD-L1 IHC 22C3 pharmDx in epithelial ovarian carcinoma, tested at one site (CPS ≥ 1)

Precision Study	Diagnostic Cutoff	Study Design	Agreement		
			Parameter	n/N	Percent Agreement (95% CI)
Combined Precision (Inter-Operator, Inter-Instrument, Inter-Day, and Inter-Lot as combined variables)	CPS ≥ 1	All 38 epithelial ovarian carcinoma specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 3 operators, using 3 Autostainer Link 48 instruments, over 3 non-consecutive days, using 3 reagent lots.	NPA	56/57	98.2% (94.7-100.0%)
			PPA	57/57	100.0% (93.7-100.0%)*
			OA	113/114	OA 99.1% (97.4-100.0%)
Intra-run precision (Repeatability)	CPS ≥ 1	All 38 epithelial ovarian carcinoma specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 5 replicates within a run using the Autostainer Link 48 instrument.	NPA	95/95	100.0% (96.1-100.0%) *
			PPA	95/95	100.0% (96.1-100.0%) *
			OA	190/190	100.0% (98.0-100.0%) *
Inter-observer precision	CPS ≥ 1	All 61 epithelial ovarian carcinoma specimens (30 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1	NPA	267/267	100.0% (98.6-100.0%) *
			PPA	274/282	97.1% (93.2-100.0%)

Precision Study	Diagnostic Cutoff	Study Design	Agreement		
			Parameter	n/N	Percent Agreement (95% CI)
		IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists over 3 non-consecutive days.	OA	541/549	98.5% (96.5-100.0%)
Intra-observer precision	CPS ≥ 1	All 62 epithelial ovarian carcinoma specimens (30 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists over 3 non-consecutive days.	NPA	272/272	100.0% (98.6-100.0%) *
			PPA	274/276	99.3% (98.2-100.0%)
			OA	546/548	99.6% (99.1-100.0%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

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

4. External Reproducibility

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

All IHC tests were interpreted by certified pathologists to determine the positive/negative results based on the CPS ≥ 1 expression level at three external sites. NPA, PPA, and OA with corresponding 95% CI were calculated by using comparisons to the majority call. The results of the reproducibility study for the CPS ≥ 1 cutoff are summarized in tables below.

In Part A, reproducibility at the CPS ≥ 1 cutoff was assessed with 40 specimens tested across 3 sites over 5 non-consecutive days. The study included specimens that were pre-qualified at Dako to represent full PD-L1 expression range. The specimen set was randomized and blinded prior to testing at three external sites and assessed for performance with regard to site-to-site and day-to-day reproducibility, or inter- and intra-site reproducibility. Each specimen block was tested at three external sites over five non-consecutive days, yielding 15 total results per specimen (5 results per site). Inter-day variability was assessed within each site, while inter-site variability was assessed across all three sites. Results are presented in Table 9, below:

Table 9. Per-Specimen Agreement Results: Inter-Site External Reproducibility: Results per Specimen (5 per Site)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Positive (n/N) **			
					Inter-Day	Inter-Site	Total	Site 1	Site 2	Site 3	All Sites
1	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
2	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
3	Negative	15	0	0, <1	0.13	0.00	0.13	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
4	Negative	15	0	0, <1	0.16	0.16	0.22	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
5	Negative	15	0	0, <1	0.13	0.00	0.13	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
6	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
7	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
8	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
9	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
10	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
11	Negative	15	0	0, <1	0.16	0.09	0.18	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
12	Negative	15	0	0, <1	0.16	0.09	0.18	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
13	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
14	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
15	Negative	15	0	0, <1	0.13	0.22	0.26	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
16	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
17	Negative	15	0	0, <1	0.16	0.16	0.22	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
18	Negative	15	0	0, <1	0.00	0.11	0.11	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
19	Negative	15	0	0, <1	0.13	0.22	0.26	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
20	Negative	15	0	0, <1	0.13	0.22	0.26	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
21	Positive	15	1	1, 9	2.18	1.40	2.59	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
22	Positive	15	2	0, 5	1.10	1.19	1.62	80% (4/5)	100% (5/5)	100% (5/5)	93.3% (14/15)
23	Positive	15	5	2, 9	1.44	1.66	2.19	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
24	Positive	15	5	3, 20	3.58	3.15	4.77	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
25	Positive	15	8	3, 30	6.65	5.22	8.46	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
26	Positive	15	10	2, 40	7.73	10.35	12.92	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
27	Positive	15	15	0, 50	9.09	15.99	18.39	100% (5/5)	80% (4/5)	100% (5/5)	93.3% (14/15)
28	Positive	15	15	8, 35	4.87	6.55	8.16	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
29	Positive	15	20	12, 80	15.77	22.94	27.84	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
30	Positive	15	25	10, 81	17.56	9.12	19.79	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
31	Positive	15	30	7, 95	17.43	28.05	33.03	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
32	Positive	15	30	10, 100	26.13	19.81	32.79	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
33	Positive	15	35	15, 80	14.87	7.05	16.45	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
34	Positive	15	35	24, 100	19.15	19.45	27.30	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
35	Positive	15	50	12, 100	26.85	10.29	28.76	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
36	Positive	15	52	35, 100	19.49	13.25	23.57	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
37	Positive	15	70	37, 95	10.59	21.06	23.58	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
38	Positive	15	75	16, 100	20.79	11.69	23.85	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
39	Positive	15	75	31, 100	15.65	18.28	24.06	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Positive (n/N) **			
					Inter-Day	Inter-Site	Total	Site 1	Site 2	Site 3	All Sites
40	Positive	15	95	81, 100	5.76	0.00	5.76	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)

SD = Standard Deviation; CPS = Combined Positive Score; n = number of positive results; N = Total number of results

* Consensus result was determined based on the majority call across all test conditions
** Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results.

Part B included assessment of reader-to-reader variability with specimens that spanned the PD-L1 expression range. These specimens were stained at Agilent (Dako North America) and shipped to the three external sites for assessment of PD-L1 expression by pathologists for both inter-reader and intra-reader reproducibility. Fifty-nine pre-stained specimens were assessed three times by three readers with a minimum of 2 weeks between reads. Fifty-nine pre-stained specimens were assessed three times by three certified pathologists with a minimum of 2 weeks between reads, yielding 9 total results per specimen (3 results per observer). Inter-read variability was assessed within each observer, while inter-observer variability was assessed across all three observers. Results are presented in Table 10, below:

Table 10. Per-Specimen Agreement Results: Inter-Reader External Reproducibility: Results per Specimen (3 per Reader)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Positive (n/N) **			
					Inter-Read	Inter-Observer	Total	Obs 1	Obs 2	Obs 3	All Observers
1	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
2	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
3	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
4	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
5	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
6	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
7	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
8	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
9	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
10	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
11	Negative	9	0	0, <1	0.17	0.00	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
12	Negative	9	0	0, <1	0.24	0.10	0.25	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
13	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
14	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
15	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
16	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
17	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
18	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
19	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
20	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)

Specimen	Consensus*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)			Percent Positive (n/N) **			
					Inter-Read	Inter-Observer	Total	Obs 1	Obs 2	Obs 3	All Observers
21	Negative	9	0	0, <1	0.17	0.00	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
22	Negative	9	0	0, <1	0.17	0.17	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
23	Negative	9	0	0, <1	0.17	0.24	0.29	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
24	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
25	Negative	9	0	0, <1	0.24	0.10	0.25	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
26	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
27	Negative	9	0	0, <1	0.17	0.00	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
28	Negative	9	0	0, 12	4.18	0.00	4.18	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	33.3% (3/9)
29	Negative	9	0.5	0, 1	0.37	0.14	0.40	0% (0/3)	0% (0/3)	66.7% (2/3)	22.2% (2/9)
30	Positive	9	3	1, 9	2.11	1.86	2.81	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
31	Positive	9	4	2, 9	0.82	2.87	2.99	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
32	Positive	9	5	1, 20	5.61	0.82	5.67	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
33	Positive	9	6	3, 15	2.98	2.46	3.87	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
34	Positive	9	7	2, 18	4.03	1.93	4.47	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
35	Positive	9	8	5, 30	5.27	7.10	8.84	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
36	Positive	9	10	3, 45	4.96	16.55	17.28	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
37	Positive	9	10	5, 75	20.11	9.22	22.12	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
38	Positive	9	12	5, 95	19.33	30.80	36.36	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
39	Positive	9	15	2, 38	4.89	15.05	15.82	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
40	Positive	9	15	2, 60	14.68	16.20	21.86	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
41	Positive	9	15	10, 70	5.11	26.91	27.39	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
42	Positive	9	15	10, 45	5.49	14.66	15.66	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
43	Positive	9	15	10, 60	5.61	20.40	21.16	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
44	Positive	9	16	1, 90	14.15	31.79	34.79	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
45	Positive	9	16	10, 60	11.30	12.60	16.93	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
46	Positive	9	20	13, 100	11.73	39.75	41.45	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
47	Positive	9	30	20, 45	7.95	0.00	7.95	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
48	Positive	9	35	9, 100	35.28	15.97	38.73	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
49	Positive	9	40	15, 100	10.32	38.41	39.77	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
50	Positive	9	40	20, 100	7.05	32.71	33.46	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
51	Positive	9	45	30, 95	13.25	19.16	23.29	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
52	Positive	9	50	19, 100	5.53	37.11	37.52	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
53	Positive	9	50	25, 85	14.18	11.71	18.39	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
54	Positive	9	50	37, 100	4.24	28.47	28.78	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
55	Positive	9	50	37, 100	1.94	32.17	32.23	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
56	Positive	9	55	40, 100	8.82	29.58	30.87	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
57	Positive	9	65	25, 100	8.50	33.10	34.17	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
58	Positive	9	80	50, 100	15.18	13.26	20.16	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
59	Positive	9	95	60, 100	8.50	16.69	18.73	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

SD = Standard Deviation; CPS = Combined Positive Score; n = number of positive results; N = Total number of results

*Consensus result was determined based on the majority call across all test conditions
**Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results

External Reproducibility Percent Agreement Results

The overall external reproducibility percent agreement results across all validation studies is summarized in Table 11, below:

Table 11. Reproducibility of PD-L1 IHC 22C3 pharmDx in epithelial ovarian carcinoma, tested at three external sites (CPS ≥ 1)

Reproducibility Study	Diagnostic Cutoff	Study Design	Agreement		
			Parameter	n/N	Percent Agreement (95% CI)
Inter-site	CPS ≥ 1	All 40 epithelial ovarian carcinoma specimens (20 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 non-consecutive days. Inter-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA	300/300	100.0% (98.7-100.0%)*
			PPA	298/300	99.3% (98.3-100.0%)
			OA	598/600	99.7% (99.2-100.0%)
Intra-site	CPS ≥ 1	All 40 epithelial ovarian carcinoma specimens (20 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 non-consecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA	300/300	100.0% (98.7-100.0%)*
			PPA	298/300	99.3% (98.3-100.0%)
			OA	598/600	99.7% (99.2-100.0%)
Inter-observer	CPS ≥ 1	All 59 epithelial ovarian carcinoma specimens (29 PD-L1-negative and 30 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists, 1 at each of 3 study sites, on 3 non-consecutive days. Inter-observer analysis was performed between 3 sites on a total of 531 comparisons to majority call.	NPA	256/261	98.1% (95.0-100.0%)
			PPA	270/270	100.0% (98.6-100.0%)*
			OA	526/531	99.1% (97.6-100.0%)

Reproducibility Study	Diagnostic Cutoff	Study Design	Agreement		
			Parameter	n/N	Percent Agreement (95% CI)
Intra-observer	CPS ≥ 1	All 59 epithelial ovarian carcinoma specimens (29 PD-L1-negative and 30 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by 3 pathologists, 1 at each of 3 study sites, on 3 non-consecutive days. Intra-observer analysis was performed for 3 sites on a total of 531 comparisons to majority call.	NPA	255/258	NPA 98.8% (96.5-100.0%)
			PPA	272/273	PPA 99.6% (98.9-100.0%)
			OA	527/531	OA 99.2% (97.9-100.0%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

*The percentile bootstrap method cannot compute confidence intervals if 100.0% agreement is observed. Therefore, the Wilson score method was used to compute confidence intervals when the agreement (PPA/NPA/OA) was observed100%.

5. Robustness

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

- Target retrieval time at three incubation times
  - 18 minutes
  - 20 minutes-standard
  - 22 minutes
- Target Retrieval Solution temperature at three incubation temperatures
  - 95 °C
  - 97 °C - standard
  - 99 °C
- Target Retrieval Solution pH at three pH levels
  - pH 5.9
  - pH 6.1- standard
  - pH 6.3

Studies were performed with 38 EOC specimens. Specimens were blinded and randomized prior to evaluation at a predetermined cutoff. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) and 95% confidence interval (CI) were calculated for CPS≥1 cutoff by pairwise comparison to the standard condition. No significant difference in results was observed for the experimental conditions above when compared to the standard conditions.

6. Stability Studies

a. PD-L1 IHC 22C3 pharmDx Stability

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

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

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

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

b. FFPE Cut Section Stability

A stability study was conducted to evaluate the stability of the antigen in cut tissue sections of EOC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 10 EOC specimens. Based on these studies, stability dating for cut slides in EOC is 3.5 months for cut sections stored at 2-8 °C and 3 months for cut sections stored at 25 °C.

7. Impact of Tumor Heterogeneity

The objective of this study was to investigate whether tumor heterogeneity affects PD-L1 IHC staining results with PD-L1 IHC 22C3 pharmDx.

a. Primary vs. Metastatic Tumor Tissues

Matched primary versus metastatic blocks were obtained from 11 subjects and evaluated by PD-L1 IHC 22C3 pharmDx and specimens were determined to be positive or negative based on the CPS $\geq$ 1 cutoff. There were 8 concordant negative pairs, 1 concordant positive pair, and 2 pairs with discordant PD-L1 status. Results may not be representative of all EOC specimens, as tumor heterogeneity is unique for each specimen.

b. Intra-Case Heterogeneity

Two sister FFPE blocks from 24 EOC subjects obtained from the same tumor were evaluated to assess concordance for PD-L1 status. In 20 of 24 cases, the diagnostic outcomes based on the CPS $\geq$ 1 cutoff were concordant across intra-case (sister) blocks. Four cases showed discordant results across sister blocks. Results may not be

representative of all EOC specimens, as tumor heterogeneity is unique for each specimen.

c. Intra-Block Heterogeneity

Thirty individual FFPE EOC tissue blocks were divided in 3 portions across a minimum of 200 µm: anterior, middle, and posterior. Of the 30 cases assessed, all 30 cases were concordant when compared to the diagnostic status of front and back sections based on the CPS ≥ 1 cutoff. Results may not be representative of all EOC specimens, as tumor heterogeneity is unique for each specimen.

B. Animal Studies

None

C. Additional Studies

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

2. Control Cell Line Validation
See SSED for P150013.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of PD-L1 IHC 22C3 pharmDx as a companion diagnostic for PD-L1 detection (CPS≥1) in EOC patients to determine eligibility for treatment with KEYTRUDA® (pembrolizumab) was demonstrated in the study KEYNOTE-B96. This study was conducted in the US and geographical regions including, Asia, Europe, Australia and the rest of the world (ROW). A summary of the clinical study is presented below.

A. Study Design

KEYNOTE-B96, a randomized, double-blind, phase III study evaluated the efficacy of KEYTRUDA in combination with paclitaxel, with or without bevacizumab, in 643 patients with platinum-resistant EOC who had received ≤ 2 prior lines of therapy. Randomization was stratified by bevacizumab use in the study (yes vs. no), region (US vs. EU vs. ROW), and PD-L1 status (CPS <1 vs. CPS 1 to <10 vs. CPS ≥10) as determined using the PD-L1 IHC 22C3 pharmDx kit. Patients were enrolled regardless of tumor PD-L1 expression status. The primary efficacy endpoint was progression-free survival (PFS) per RECIST v1.1 by investigator assessment in participants with CPS ≥1. Key secondary endpoints included overall survival (OS) in participants with CPS ≥1.

1. Clinical Inclusion and Exclusion Criteria

   Enrollment in the KEYNOTE-B96 study was limited to female subjects with platinum-resistant EOC, who had received one or two prior lines of systemic therapy, and who were at least 18 years of age.

   A summary of key additional inclusion criteria are as follows:

   - Have histologically confirmed epithelial (including high-grade serous or predominantly serous, low-grade serous, any-grade endometrioid, malignant mixed Müllerian tumors [carcinosarcoma], or clear cell) ovarian, fallopian tube, or primary peritoneal carcinoma.
   - Have received 1 or 2 prior lines of systemic therapy for EOC, including at least 1 prior platinum-based therapy.
     Note: Participants must have received at least 4 cycles but not more than 9 cycles of platinum-based therapy in first-line treatment.
   - Have radiographic evidence of disease progression within 6 months (180 days) after the last dose of platinum-based chemotherapy for EOC (i.e., platinum-resistant disease).
     Note: Participants with secondary platinum-refractory disease who were either platinum-resistant or platinum-sensitive in the first line were eligible.
   - Have been a candidate for paclitaxel chemotherapy (and bevacizumab, if used).
   - Have an ECOG performance status of 0 to 1 assessed within 3 days before randomization.
   - Provided tumor tissue sample for prospective determination of PD-L1 status.

2. Follow-up Schedule
   Treatment with KEYTRUDA continued until RECIST v1.1-defined progression of disease, unacceptable toxicity, or a maximum of 24 months. Administration of KEYTRUDA was permitted beyond RECIST-defined disease progression if the patient was clinically stable and considered to be deriving clinical benefit by the investigator. Assessment of tumor status was performed at Week 9 and then every 9 weeks for the first year, followed by every 12 weeks thereafter.

3. Clinical Endpoints

Primary endpoint:

1. Progression-free survival (PFS), defined as the time from randomization to the first documented disease progression as assessed by the investigator according to RECIST v1.1, or death due to any cause, whichever occurred first.

Secondary efficacy endpoints:

1. Overall survival (OS), defined as the time from randomization to the time of death due to any cause.

2. PFS by blinded independent central review (BICR), defined as the time from randomization to the first documented disease progression per RECIST v1.1 as assessed by BICR, or death due to any cause, whichever occurs first.
3. Change from baseline and time to deterioration (TTD) of the quality of life (QoL) and symptom scores from the Global Health Status/QoL scale (items 29 and 30) of the EORTC QLQ-C30 and the abdominal/GI symptom scale (items 31 to 36) of the EORTC QLQ-OV28, respectively.

B. Accountability of PMA Cohort

Participants with platinum-resistant EOC in KEYNOTE-B96 comprise the primary participant population for this application. A total of 643 participants were randomly assigned in a 1:1 ratio to pembrolizumab + paclitaxel, with or without bevacizumab (322 participants) or placebo + paclitaxel, with or without bevacizumab (321 participants). All 643 participants had a tumor tissue sample tested with PD-L1 IHC 22C3 pharmDx. Most participants (72.5%, n=466) had a tumor tissue PD-L1 expression score of CPS ≥1, and 27.5% of participants had a tumor tissue PD-L1 expression score of CPS < 1. While, 201 of 466 (31.3%) participants with CPS ≥1 had a tumor tissue PD-L1 expression score of CPS ≥10. The patient/sample accountability is shown in Table 12 below.

Table 12: Accountability of PMA Cohort

	Number of Study Subjects n (%)
Enrolled	643 (100%)
Tumor sample collected at baseline	643 (100%)
Specimens with insufficient tissue	0 (0.0%)
Total evaluable PD-L1 expression	643 (100%)
PD-L1 positive CPS ≥ 1	466 (72.5%)
PD-L1 positive CPS ≥ 10	201* (31.3)
PD-L1 negative CPS < 1	177 (27.5%)
Site of Tumor: (N=643)	
Primary Site	452 (70.3%)
Metastatic Site	188 (29.2%)
Unknown	3 (0.5%)
Specimen type (N=643)	
Newly Obtained**	29 (4.5%)
Archival	611 (95%)
Unknown	3 (0.5%)
Sample Procedure (N= 643)	
Biopsy	128 (19.9%)
Resection	512 (79.6%)
Unknown	3 (0.5%)

*CPS ≥10 is a subset of CPS ≥1 population (201 of 466 participants with CPS ≥1 had CPS ≥10).

**Specimen type derived based on sample collection date. If the sample was obtained within 42 days of randomization, the sample is categorized as 'Newly

Obtained'; otherwise, the sample is categorized as 'Archival'. Three subjects had unknown specimen type classification.

C. Study Population Demographics and Baseline Parameters

The baseline characteristics for study subjects with PD-L1 CPS ≥1 is shown in the table 13 below.

Table 13: Patient Characteristics With PD-L1 CPS ≥1

	Pembrolizumab + paclitaxel, with or without bevacizumab		Placebo + with paclitaxel, with or without bevacizumab		Total	
	n	(%)	n	(%)	n	(%)
Participants in population	234		232		466	
Sex						
Female	234	(100.0)	232	(100.0)	466	(100.0)
Age (Years)						
< 65	145	(62.0)	144	(62.1)	289	(62.0)
≥65	89	(38.0)	88	(37.9)	177	(38.0)
Mean	61.5		61.3		61.4	
SD	10.2		9.8		10.0	
Median	62.0		61.5		62.0	
Range	37 to 85		37 to 82		37 to 85	
Race						
Asian	51	(21.8)	40	(17.2)	91	(19.5)
Black Or African American	5	(2.1)	4	(1.7)	9	(1.9)
Multiple	5	(2.1)	11	(4.7)	16	(3.4)
American Indian Or Alaska Native, White	3	(1.3)	7	(3.0)	10	(2.1)
Black Or African American, White	1	(0.4)	3	(1.3)	4	(0.9)
White, Asian	1	(0.4)	1	(0.4)	2	(0.4)
Native Hawaiian Or Other Pacific Islander	1	(0.4)	1	(0.4)	2	(0.4)
White	155	(66.2)	158	(68.1)	313	(67.2)
Missing	17	(7.3)	18	(7.8)	35	(7.5)
Ethnicity						
Hispanic Or Latino	27	(11.5)	35	(15.1)	62	(13.3)
Not Hispanic Or Latino	195	(83.3)	186	(80.2)	381	(81.8)
Not Reported	8	(3.4)	7	(3.0)	15	(3.2)
Unknown	4	(1.7)	4	(1.7)	8	(1.7)
IIIA1	7	(3.0)	5	(2.2)	12	(2.6)
IIIA2	5	(2.1)	7	(3.0)	12	(2.6)

IIIB	16	(6.8)	14	(6.0)	30	(6.4)
IIIC	96	(41.0)	98	(42.2)	194	(41.6)
IVA	28	(12.0)	19	(8.2)	47	(10.1)
IVB	61	(26.1)	70	(30.2)	131	(28.1)
Histology Subtype						
High Grade Serous	206	(88.0)	208	(89.7)	414	(88.8)
Low Grade Serous	4	(1.7)	6	(2.6)	10	(2.1)
Carcinosarcoma	3	(1.3)	3	(1.3)	6	(1.3)
Clear Cell	14	(6.0)	13	(5.6)	27	(5.8)
Endometrioid	5	(2.1)	2	(0.9)	7	(1.5)
Others (Carcinoma)	2	(0.9)	0	(0.0)	2	(0.4)
Prior Lines of Therapy						
One line	88	(37.6)	81	(34.9)	169	(36.3)
Two lines	145	(62.0)	151	(65.1)	296	(63.5)
Three lines	1	(0.4)	0	(0.0)	1	(0.2)
Prior Anti-PD-1 or Anti-PD-L1						
Yes	5	(2.1)	7	(3.0)	12	(2.6)
No	229	(97.9)	225	(97.0)	454	(97.4)
Prior Bevacizumab/bev-biosimilar						
Yes and bevacizumab treated in study	68	(29.1)	66	(28.4)	134	(28.8)
Yes and bevacizumab not treated in study	40	(17.1)	42	(18.1)	82	(17.6)
No	126	(53.8)	124	(53.4)	250	(53.6)
Prior PARPi						
Yes	85	(36.3)	96	(41.4)	181	(38.8)

	Pembrolizumab + paclitaxel, with or without bevacizumab		Placebo + paclitaxel, with or without bevacizumab		Total	
	n	(%)	n	(%)	n	(%)
No	149	(63.7)	136	(58.6)	285	(61.2)
Prior Anti-VEGF Therapy						
Yes, both bevacizumab/bev-biosimilar and other VEGF inhibitors	0	(0.0)	2	(0.9)	2	(0.4)
Yes, bevacizumab/bev-biosimilar only	108	(46.2)	106	(45.7)	214	(45.9)
Yes, other VEGF inhibitors only	1	(0.4)	0	(0.0)	1	(0.2)
No	125	(53.4)	124	(53.4)	249	(53.4)
Platinum Free Interval						
<3 months from last platinum therapy to subsequent progression	103	(44.0)	115	(49.6)	218	(46.8)
3-6 months from last platinum therapy to subsequent progression	130	(55.6)	115	(49.6)	245	(52.6)
>6 months from last platinum therapy to subsequent progression	1	(0.4)	2	(0.9)	3	(0.6)

SD=Standard deviation.

Missing values in race are mainly because this information is not permitted to report in France and Netherlands.

Western Europe includes countries in the European Economic Area, United Kingdom, and Switzerland.

Database Cutoff Date: 05MAR2025.

D. Safety and Effectiveness Results

1. Safety Results

The analysis of safety was based on 643 patients. In the trial, there were no device-related adverse events. The results from KEYNOTE-B96 demonstrate that pembrolizumab in combination with paclitaxel, with or without bevacizumab, has a manageable safety profile consistent with the well-established safety profiles of pembrolizumab monotherapy, paclitaxel, and bevacizumab. The overall incidence and nature (severity, outcome) of adverse events of special interest (AEOSI) in the pembrolizumab in combination with paclitaxel, with or without bevacizumab group were generally consistent with pembrolizumab monotherapy. No new safety signals were identified. Please refer to Drugs@FDA for complete safety information on KEYTRUDA® (pembrolizumab).

2. Effectiveness Results

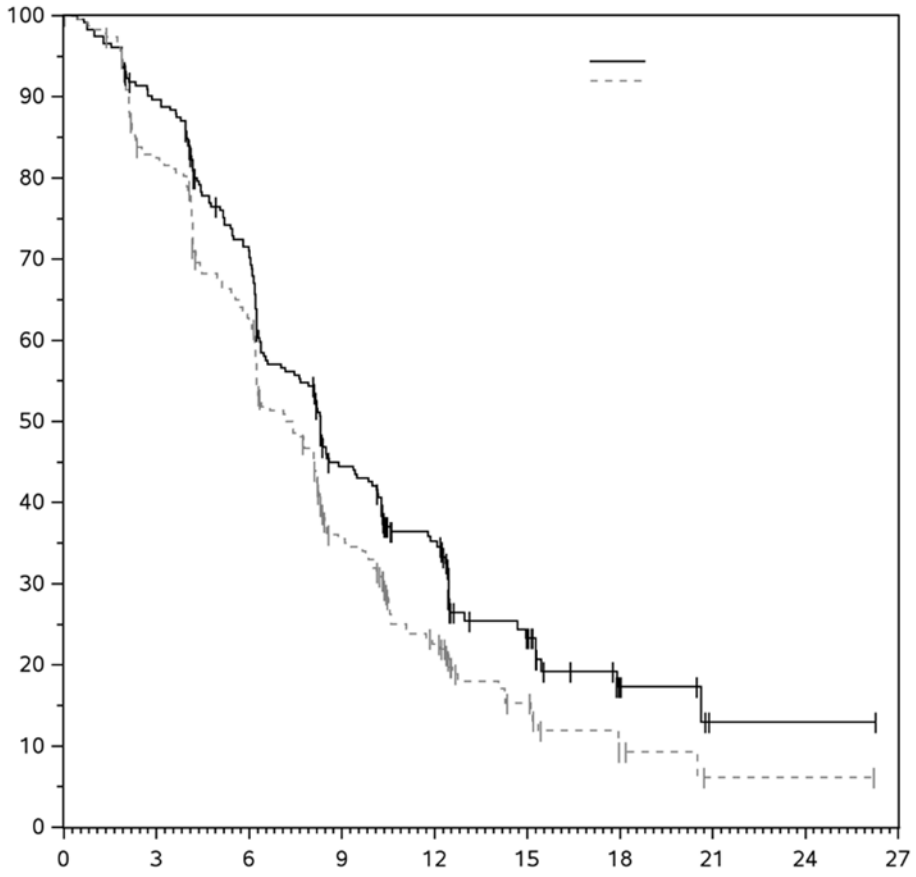
A summary of the efficacy results for the participants with tumors expressing PD-L1 CPS ≥1 is provided in Table 14. The trial demonstrated a statistically significant improvement in PFS in patients with PD-L1 positive tumors (CPS ≥1) randomized to KEYTRUDA in combination with paclitaxel, with or without bevacizumab compared to placebo in combination with paclitaxel, with or without bevacizumab at the first interim analysis (Table 14 and Figure 1). At the second interim analysis, the trial demonstrated statistically significant improvements in both PFS and OS in participants with PD-L1 positive tumors (CPS ≥1) (Table 14 and Figures 1 and 2).

Table 14. Efficacy Results in KEYNOTE-B96(CPS≥1)

Endpoint	**KEYTRUDA 400 mg every 6 weeks plus paclitaxel with or without bevacizumab n=234**	**Placebo plus paclitaxel with or without bevacizumab n=232**
PFS		
Number (%) of patients with event	162 (69)	180 (78)
Median in months (95% CI)	8.3 (7.0, 9.4)	7.2 (6.2, 8.1)
Hazard ratio (95% CI)	0.72 (0.58, 0.89)	
p-Value	0.0014*	
OS		
Number of patients with event (%)	157 (67)	175 (75)
Median in months (95% CI)	18.2 (15.3, 21.0)	14.0 (12.5, 16.1)
Hazard ratio (95% CI)	0.76 (0.61, 0.94)	
p-Value	0.0053†	

*Based on stratified log-rank test (p-Value [one-sided] is compared to an alpha boundary of 0.0116)
†Based on stratified log-rank test (p-Value [one sided] is compared to an alpha boundary of 0.0083)

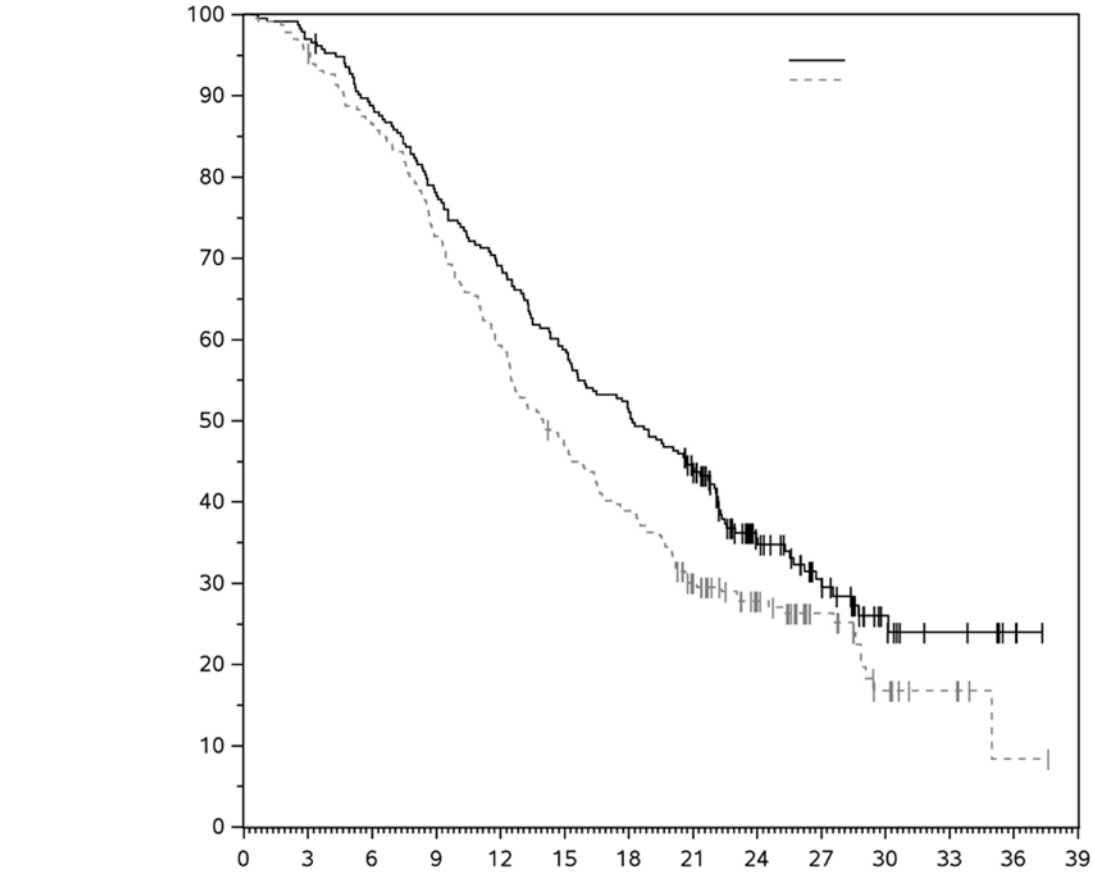
Figure 1: Kaplan-Meier Curve for Progression-Free Survival by Treatment Arm in KEYNOTE-B96 (CPS≥1)



Number at Risk										
KEYTRUDA + Chemo*	234	206	158	92	56	21	6	1	1	0
Placebo + Chemo*	232	186	138	69	36	16	5	1	1	0

*Chemotherapy (paclitaxel) with or without bevacizumab

Figure 2: Kaplan-Meier Curve for Overall Survival by Treatment Arm in KEYNOTE-B96 (CPS≥1)



*Chemotherapy (paclitaxel) with or without bevacizumab

3. Subgroup Analyses
   There was no subgroup analyses performed in this clinical study.

4. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

   Patients aged 18 years and older were eligible for enrollment into the KEYNOTE-B96 trial if they fulfilled other inclusion criteria. The youngest patient enrolled in the study was 37 years old. For the KEYNOTE-B96 study, data from adult patients aged 37 years and older are considered generalizable to the pediatric population aged 18-21 years and can be relied on to establish safety and effectiveness within the 18-21 years age group.

XI. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 2 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

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

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical performance of PD-L1 IHC 22C3 pharmDx for the detection of PD-L1 in EOC tumor specimens from patients with platinum-resistant EOC who may benefit from treatment with pembrolizumab is based on results from KEYNOTE-B96. The analysis of 466 participants from KEYNOTE-B96 demonstrated the effectiveness of PD-L1 IHC 22C3 pharmDx in identifying patients with EOC whose tumors express PD-L1 (CPS ≥1) for treatment with pembrolizumab in combination with paclitaxel with or without bevacizumab. Treatment with pembrolizumab in combination with paclitaxel with or without bevacizumab, provided a statistically significant and clinically meaningful improvement in PFS compared with placebo plus paclitaxel with or without bevacizumab in participants with PD-L1 positive tumors (CPS ≥1) at the first interim analysis (HR 0.72, 95% CI: 0.58-0.89, p=0.0014), representing a 28% reduction in the risk of disease progression or death. At the second interim analysis, pembrolizumab plus paclitaxel with or without bevacizumab demonstrated statistically significant and clinically meaningful improvements in both PFS (HR 0.75, 95% CI: 0.61-0.91, p=0.0022) and OS (HR 0.76, 95% CI: 0.61-0.94, p=0.0053) in participants with PD-L1 positive tumors (CPS ≥1).

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

B. Safety Conclusions

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

Safety of the device for patient management is related to the safety and effectiveness of the therapeutic. Pembrolizumab plus paclitaxel, with or without bevacizumab, in KEYNOTE-B96 has a manageable safety profile that is consistent with the well-established safety profiles of pembrolizumab monotherapy, paclitaxel, and bevacizumab. No new safety signals were identified. In general, risks of PD-L1 IHC 22C3 pharmDx are associated with failure of the device to perform as expected or failure to correctly interpret test results. The process of testing FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for EOC diagnosis.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with KEYTRUDA without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with KEYTRUDA. There is also a risk of delayed results, which may lead to delay of treatment with KEYTRUDA.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the clinical study, which demonstrated pembrolizumab plus paclitaxel, with or without bevacizumab, provides a statistically significant and clinically meaningful improvement in PFS and OS compared with placebo plus paclitaxel, with or without bevacizumab, in participants with PD-L1 positive tumors (CPS $\geq$1), as determined by the device.

The probable risks of the device are also based on data collected from the described studies as conducted to support PMA approval. The risks associated with the use of this device are mainly due to 1) false negatives, false positives, and failure to provide a result and 2) incorrect interpretation of test results. Erroneous device results could adversely influence clinical interpretation and consultation for patients. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in treatment of patients EOC. Patients with false positive results may be treated with KETRUDA in combination with paclitaxel, with or without bevacizumab, without clinical benefit, and may experience adverse reactions associated with the therapy. A false negative result may prevent a patient from accessing appropriate treatment. There is also a risk of delayed results /failure to provide a result, which may lead to delay of treatment with indicated therapy. These risks are partially mitigated by the analytical and clinical performance of the device and device labeling; therefore, the risk of this device is considered to be clinically acceptable for the indication listed.

Additional factors considered in determining probable risks and benefits for the PD-L1 IHC 22C3 pharmDx included lack of availability of alternative tests. Thus, the probable benefits are based on evaluation that the test performs consistently and provides clinically relevant results for evaluating PD-L1 status (CPS ≥ 1) in EOC patients who are being considered for treatment with KEYTRUDA.

1. Patient Perspectives
   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information detailed above, the data support that, for EOC patients with platinum-resistant disease who are being considered for treatment with KEYTRUDA® in combination with paclitaxel, with or without bevacizumab, the probable benefits of PD-L1 IHC 22C3 pharmDx use outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use and product labeling. The provided studies support use of PD-L1 IHC 22C3 pharmDx as an aid in identifying patients with EOC for treatment with KEYTRUDA® in combination with paclitaxel, with or without bevacizumab.

XV. CDRH DECISION

CDRH issued an approval order on February 10, 2026.

The applicant's manufacturing facility was inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820), which was in effect at the time of the inspection. As of February 2, 2026, the revised part 820, referred to as the Quality Management System Regulation (QMSR), is effective.

XVI. 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.