SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic 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 Santa Clara, CA 95051
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P150013/S032

Date of FDA Notice of Approval: February 11, 2026

The original PMA (P150013) for PD-L1 IHC 22C3 pharmDx was approved on October 2, 2015, for the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer tissue using EnVision FLEX visualization system on the Autostainer Link 48. Subsequently, additional indications were approved: cervical cancer (S009) on June 12, 2018, head and neck squamous cell carcinoma (S014) on June 10, 2019, esophageal squamous cell carcinoma (S016) on July 30, 2019, triple-negative breast cancer (S020) on November 13, 2020, gastric or gastroesophageal junction (GEJ) adenocarcinoma (S027) on November 7, 2023, and epithelial ovarian, fallopian tube, or primary peritoneal carcinoma (S033) on February 10, 2026. The SSED for these indications are available on the CDRH website and are incorporated by reference here.

The current supplement was submitted to expand the indications for PD-L1 IHC 22C3 pharmDx to include esophageal or GEJ carcinoma, an indication which encompasses adenocarcinoma or squamous cell carcinoma of the esophagus and Siewert type 1 adenocarcinoma of the GEJ (tumors with epicenter 1 to 5 centimeters above the GEJ).

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 or

gastroesophageal junction (GEJ) carcinoma, esophageal squamous cell carcinoma (ESCC), cervical cancer, head and neck squamous cell carcinoma (HNSCC), triple negative breast cancer (TNBC), gastric or GEJ adenocarcinoma, and epithelial ovarian, fallopian tube, or primary peritoneal carcinoma 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 esophageal or GEJ carcinoma, ESCC, cervical cancer, HNSCC, TNBC, gastric or GEJ adenocarcinoma, and epithelial ovarian, fallopian tube, or primary peritoneal carcinoma 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)*
Esophageal or GEJ carcinoma	CPS ≥ 1	
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-rwlc)**

*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 for use of this test.

IV. WARNINGS AND PRECAUTIONS

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

V. DEVICE DESCRIPTION

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical staining procedure for formalin-fixed, paraffin-embedded (FFPE) specimens using the Autostainer Link 48 automated staining system and the EnVision FLEX visualization system.

The principal component of the kit is the mouse monoclonal anti PD-L1 clone 22C3 antibody that binds to PD-L1 protein expressed on FFPE tissue. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration), and reagents shown in Table 1 necessary to perform 50 tests in up to 15 individual runs. Wash buffer and hematoxylin are required for the assay but not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods.

Table 1. Overview of PD-L1 IHC 22C3 pharmDx Components

Reagent	Description
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015 mol/L sodium azide.
Monoclonal Mouse anti-PD-L1, Clone 22C3	Monoclonal mouse (IgG1) anti-PD-L1 antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.
Negative Control Reagent	Monoclonal mouse control IgG1 antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015 mol/L sodium azide.
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.
DAB+ Substrate Buffer	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride (DAB) in an organic solvent.
DAB Enhancer	Cupric sulfate in water.
EnVision FLEX Target Retrieval Solution, Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.

Reagent	Description
Control Cell Line 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).

Device Instrumentation and Software

PD-L1 IHC 22C3 pharmDx is performed on 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

Esophageal or GEJ carcinoma specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens. Only 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 and dehydrated, and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. After specimen processing, tissue specimens should be cut into sections of 4-5 um, mounted on charged microscope slides, and 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 4.5 months of sectioning when stored at 2-8 °C, or within 1 month 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, Agilent recommends the following controls to be run with the assay:

1) Control Cell Line (CCL) 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 from one of the approved indications for

PD-L1 IHC 22C3 pharmDx, 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 (NCR) that is used in parallel with the PDL1 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 Operation

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 horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added DAB+ Chromogen results in precipitation of a visible reaction product at the antigen sites. The color of the chromogenic reaction is modified by a chromogen enhancement reagent, DAB Enhancer. The specimen may then be counterstained with hematoxylin and 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 x 150µL): 5 minutes (± 1 minute)
- Rinse in buffer
- Monoclonal Mouse anti-PD-L1 (or Negative Control Reagent) (2 drop zones x 150µL): 30 minutes (± 1 minute)
- Rinse in buffer
- Linker, anti-Mouse Ig (2 drop zones x 150µL): 30 minutes (± 1 minute)
- Rinse in buffer
- Visualization Reagent (2 drop zones x 150µL): 30 minutes (± 1 minute)
- Rinse in buffer: 5 minutes
- DAB+ solution (2 drop zones x 150µL): 2 x 5 minutes (± 1 minute)
- Rinse in buffer
- DAB+ Enhancer (2 drop zones x 150µL): 5 minutes (± 1 minute)
- Rinse in buffer
- Hematoxylin (2x 150µL): 5 minutes (± 1 minute)
- Rinse in deionized water

- Rinse in buffer: 5 minutes
- Rinse in deionized water
- Remove slides from Autostainer and place in bath of reagent water

Interpretation of PD-L1 Staining

The labeling instructs that all viable tumor cells on the entire tissue section must be evaluated and included in PD-L1 expression assessment. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation.

Slide evaluation must be performed by a pathologist using a light microscope. For evaluation of the immunohistochemical staining, an objective of 10-20x magnification is appropriate. For determination of PD-L1 expression, an objective of 20x magnification is required.

PD-L1 expression in esophageal or GEJ 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:

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

By definition, PD-L1 staining cells are:

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

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

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

Table 2. CPS Numerator Inclusion/Exclusion Criteria for Esophageal or GEJ carcinoma

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 (including intramucosal adenocarcinoma component)	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Any noninvasive neoplasia including adenoma, glandular and squamous dysplasia (e.g., high grade glandular dysplasia and carcinoma in situ)
Immune cells	• Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma**, such as: ○ Lymphocytes (including lymphocyte aggregates) ○ Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Non-staining MICs • MICs associated with noninvasive neoplasia (including carcinoma in situ) • MICs associated with benign structures • MICs (including lymphoid aggregates) associated with ulcers, chronic gastritis, and other processes not directly associated with the response to the tumor • Neutrophils, eosinophils and plasma cells
Other Cells	• Not included	• Benign cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

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

Table 3. CPS Denominator Inclusion/Exclusion Criteria for Esophageal or GEJ carcinoma

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor cells	All viable invasive tumor cells	• Non-viable tumor cells • Any noninvasive neoplasia including adenoma, glandular and squamous dysplasia (e.g., high grade glandular dysplasia and carcinoma in situ)
Immune cells	Not included	• All immune cells
Other Cells	Not included	• Benign cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

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

To accurately assess PD-L1 expression status, challenging borderline cases (slides) with scores near the CPS ≥ 1 cutoff (CPS > 0 and < 10) should be reviewed by an additional pathologist and the reported results should be based on a consensus score.

This medical device product has functions subject to FDA premarket review as well as functions that are not subject to FDA premarket review. For this application, if the product has functions that are not subject to FDA premarket review, FDA assessed those functions only to the extent that they either could adversely impact the safety and effectiveness of the functions subject to FDA premarket review or they are included as a labeled positive impact that was considered in the assessment of the functions subject to FDA premarket review.

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 esophageal or GEJ carcinoma for treatment with KEYTRUDA (pembrolizumab).

VII. MARKETING HISTORY

PD-L1 IHC 22C3 pharmDx has been marketed in the United States since approval of P150013 on October 2, 2015. PD-L1 IHC 22C3 pharmDx has also been marketed in Albania, Algeria, Argentina, Australia, Austria, Bahrain, Belgium, Bosnia and Herzegovina, Brazil, Canada, Chile, Colombia, Costa Rica, Denmark, Ecuador, Egypt, Finland, France, Germany, Hong Kong, India, Indonesia, Hungary, Iceland, Ireland, Iraq, Israel, Italy, Japan, Jordan, South Korea, Kazakhstan, Kosovo, Kuwait, Lebanon, Lichtenstein, Macau, Macedonia, Malaysia, Montenegro, Morocco, Netherlands, New

Zealand, Norway, Oman, Panama, Peru, Philippines, Poland, Qatar, Russia, Saudi Arabia, Singapore, Slovakia, Serbia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, Ukraine, United Arab Emirates, Uruguay and Vietnam. Note that the labeling provided to some countries outside of the United States may differ from the PMA-approved labeling.

This device has not been withdrawn from marketing for any reason related to safety and effectiveness.

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 subsequent 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 test 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 NON-CLINICAL STUDIES

A. Laboratory Studies

Non-clinical studies were performed using PD-L1 IHC 22C3 pharmDx to establish analytical performance of the device. The scoring algorithm used in these studies resulted in a PD-L1 positive or negative output with an underlying score of CPS 0-100.

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. Study designs and results are available in the Summary of Safety and Effectiveness Data (SSED) for the original PMA, https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013B.pdf. Results from studies performed to support the esophageal or GEJ carcinoma indication are summarized in the sections below. Studies were conducted with both squamous cell carcinoma (SCC) and adenocarcinoma (AC) specimens.

1. Analytical Specificity

Assessment of analytical specificity of the monoclonal mouse anti-human PD-L1 clone 22C3 antibody was reviewed under P150013 and included Western blot and immunoreactivity in human tissues, both normal and tumor. Refer to the SSED for the original PMA P150013.

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 unique FFPE esophageal carcinoma specimens using one lot of the device. Assessment of PD-L1 expression demonstrated staining across a range of CPS scores from 0 to 100, and 65% of specimens had a PD-L1 expression of CPS ≥ 1. Two specimens were not evaluable due to lack of convincing invasive tumor.

3. Precision

The objective of this study was to demonstrate that PD-L1 IHC 22C3 pharmDx would produce consistent staining in normal day-to-day testing of esophageal or GEJ carcinoma with multiple reagent lots.

Precision was assessed in 3 separate studies: intra-run, combined precision (inter-instrument/operator/day/lot) and reader precision. Intra-run and combined precision studies were performed with esophageal or GEJ carcinoma specimens spanning the range of PD-L1 expression. Near cut-off specimens were defined as specimens with CPS score > 0 and < 10, and at least 46% of specimens tested were near cut-off specimens around the CPS ≥ 1 cutoff.

The intra-run and combined precision studies were performed with 32 esophageal or GEJ carcinoma specimens (9 SCC and 23 AC). Study specimens along with control slides were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Inter-/Intra-Observer precision were tested separately with 59 specimens (16 SCC and 43 AC). Comparisons were made using majority diagnostic outcome (majority call) across all observations for a given specimen as reference. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the CPS ≥ 1 cutoff as shown in Table 4. Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Table 4. Summary of Precision (one site) for CPS ≥ 1

Precision Endpoint	Study Design	% Agreement (95% CI)
Combined Precision (Inter-Operator, Inter-Instrument, Inter-Day, and Inter-Lot as combined variables)	Each of 32 esophageal or GEJ carcinoma specimens (15 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression was tested using three operators, on 3 Autostainer Link 48 instruments, over 3 non-consecutive days, using three reagent lots.	NPA 97.8% (93.3-100%) PPA 98.0% (94.1-100%) OA 97.9% (94.8-100%)
Intra-run (Repeatability)	Each of 32 esophageal or GEJ carcinoma specimens (16 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested with 5 replicates within a run on the Autostainer Link 48.	NPA 100% (95.4-100%) PPA 100% (95.4-100%) OA 100% (97.7-100%)
Inter-observer precision	59 esophageal or GEJ carcinoma specimens (30 PD-L1-negative and 29 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 94.3% (88.8-98.5%) PPA 97.7% (95.4-99.6%) OA 96.0% (93.1-98.5%)
Intra-observer precision	59 esophageal or GEJ 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 over 3 non-consecutive days.	NPA 98.4% (96.7-99.6%) PPA 97.1% (94.9-98.9%) OA 97.7% (96.3-98.9%)

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

To accurately assess PD-L1 expression status, challenging borderline cases (slides) with scores near the CPS ≥ 1 cutoff (CPS > 0 and < 10) should be reviewed by an additional pathologist and the reported results should be based on a consensus score.

Table 5. Combined Precision Results per Specimen (inter-instrument/operator/day/lot)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Between Day (SD)	Percent Positive Results** (n/N)	Percent Agreement with Consensus (n/N)	95% CI***
1	EAC	Negative	3	0	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
2	GEJ AC	Negative	3	0	0, 0	0.00	0% (0/3)	100% (3/3)	43.9-100%
3	ESCC	Negative	3	0	0, 0	0.00	0% (0/3)	100% (3/3)	43.9-100%
4	EAC	Negative	3	0	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
5	EAC	Negative	3	0	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
6	EAC	Negative	3	0	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
7	EAC	Negative	3	0	0, 0	0.00	0% (0/3)	100% (3/3)	43.9-100%
8	GEJ AC	Negative	3	0.5	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
9	EAC	Negative	3	0.5	0, <1	0.29	0% (0/3)	100% (3/3)	43.9-100%
10	EAC	Negative	3	0.5	0, 2	1.04	33.3% (1/3)	66.7% (2/3)	20.8-93.9%
11	GEJ AC	Negative	3	0.5	<1, <1	0.00	0% (0/3)	100% (3/3)	43.9-100%
12	EAC	Negative	3	0.5	<1, <1	0.00	0% (0/3)	100% (3/3)	43.9-100%
13	EAC	Negative	3	0.5	<1, <1	0.00	0% (0/3)	100% (3/3)	43.9-100%
14	EAC	Negative	3	0.5	<1, <1	0.00	0% (0/3)	100% (3/3)	43.9-100%
15	EAC	Negative	3	0.5	<1, <1	0.00	0% (0/3)	100% (3/3)	43.9-100%
16	GEJ AC	Positive	3	2	1, 5	2.08	100% (3/3)	100% (3/3)	43.9-100%
17	EAC	Positive	3	3	<1, 3	1.44	66.7% (2/3)	66.7% (2/3)	20.8-93.9%
18	EAC	Positive	3	3	1, 8	3.61	100% (3/3)	100% (3/3)	43.9-100%
19	EAC	Positive	3	3	1, 5	2.00	100% (3/3)	100% (3/3)	43.9-100%
20	EAC	Positive	3	4	2, 5	1.53	100% (3/3)	100% (3/3)	43.9-100%
21	ESCC	Positive	3	5	1, 7	3.06	100% (3/3)	100% (3/3)	43.9-100%
22	GEJ AC	Positive	3	6	2, 12	5.03	100% (3/3)	100% (3/3)	43.9-100%
23	ESCC	Positive	3	10	10, 20	5.77	100% (3/3)	100% (3/3)	43.9-100%
24	EAC	Positive	3	12	7, 25	9.29	100% (3/3)	100% (3/3)	43.9-100%
25	EAC	Positive	3	12	10, 15	2.52	100% (3/3)	100% (3/3)	43.9-100%
26	ESCC	Positive	3	25	25, 25	0.00	100% (3/3)	100% (3/3)	43.9-100%
27	ESCC	Positive	3	40	40, 40	0.00	100% (3/3)	100% (3/3)	43.9-100%
28	EAC	Positive	3	50	50, 100	28.87	100% (3/3)	100% (3/3)	43.9-100%
29	ESCC	Positive	3	60	50, 70	10.00	100% (3/3)	100% (3/3)	43.9-100%
30	GEJ SCC	Positive	3	90	60, 95	18.93	100% (3/3)	100% (3/3)	43.9-100%
31	ESCC	Positive	3	95	90, 100	5.00	100% (3/3)	100% (3/3)	43.9-100%
32	ESCC	Positive	3	98	60, 100	22.54	100% (3/3)	100% (3/3)	43.9-100%

EAC = Esophageal Adenocarcinoma; GEJ AC = GEJ adenocarcinoma; ESCC = Esophageal Squamous Cell Carcinoma; GEJ SCC = GEJ squamous cell carcinoma; 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 test conditions

**Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results
***95% confidence intervals were calculated using the Wilson score method

Table 6. Intra-Run Precision Results per Specimen (Replicates)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Within Run (SD)	Percent Positive** (n/N)	Percent Agreement with Consensus (n/N)	95% CI***
1	EAC	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
2	GEJ AC	Negative	5	0	0, <1	0.22	0% (0/5)	100% (5/5)	56.6-100%
3	ESCC	Negative	5	0	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
4	EAC	Negative	5	0	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
5	EAC	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
6	EAC	Negative	5	0	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
7	EAC	Negative	5	0	0, 0	0.00	0% (0/5)	100% (5/5)	56.6-100%
8	ESCC	Negative	5	0.5	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
9	EAC	Negative	5	0.5	0, <1	0.22	0% (0/5)	100% (5/5)	56.6-100%
10	EAC	Negative	5	0.5	0, <1	0.27	0% (0/5)	100% (5/5)	56.6-100%
11	ESCC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
12	EAC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
13	GEJ AC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
14	EAC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
15	EAC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
16	EAC	Negative	5	0.5	<1, <1	0.00	0% (0/5)	100% (5/5)	56.6-100%
17	EAC	Positive	5	5	1, 8	2.49	100% (5/5)	100% (5/5)	56.6-100%
18	EAC	Positive	5	5	5, 5	0.00	100% (5/5)	100% (5/5)	56.6-100%
19	EAC	Positive	5	5	5, 8	1.34	100% (5/5)	100% (5/5)	56.6-100%
20	GEJ AC	Positive	5	5	5, 5	0.00	100% (5/5)	100% (5/5)	56.6-100%
21	ESCC	Positive	5	10	5, 30	10.01	100% (5/5)	100% (5/5)	56.6-100%
22	ESCC	Positive	5	10	10, 25	8.22	100% (5/5)	100% (5/5)	56.6-100%
23	ESCC	Positive	5	25	20, 55	15.25	100% (5/5)	100% (5/5)	56.6-100%
24	EAC	Positive	5	30	25, 30	2.24	100% (5/5)	100% (5/5)	56.6-100%
25	EAC	Positive	5	30	25, 35	4.18	100% (5/5)	100% (5/5)	56.6-100%
26	EAC	Positive	5	30	25, 35	3.54	100% (5/5)	100% (5/5)	56.6-100%
27	GEJ AC	Positive	5	45	40, 50	3.54	100% (5/5)	100% (5/5)	56.6-100%
28	EAC	Positive	5	50	50, 60	5.48	100% (5/5)	100% (5/5)	56.6-100%
29	ESCC	Positive	5	60	35, 80	18.51	100% (5/5)	100% (5/5)	56.6-100%
30	GEJ SCC	Positive	5	60	60, 70	5.48	100% (5/5)	100% (5/5)	56.6-100%
31	ESCC	Positive	5	65	60, 70	5.00	100% (5/5)	100% (5/5)	56.6-100%
32	ESCC	Positive	5	75	70, 85	6.52	100% (5/5)	100% (5/5)	56.6-100%

EAC = Esophageal Adenocarcinoma; GEJ AC = GEJ adenocarcinoma; ESCC = Esophageal Squamous Cell Carcinoma; GEJ SCC = GEJ squamous cell carcinoma; 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 test conditions
**Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results
***95% confidence intervals were calculated using the Wilson score method

Table 7. Reader Precision Results per Specimen

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)**			Percent Agreement (n/N) ***			
						Inter-Read	Inter-Reader	Total	Reader 1	Reader 2	Reader 3	All Readers
1	EAC	Negative	9	0	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
2	EAC	Negative	9	0	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
3	EAC	Negative	9	0	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
4	EAC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
5	GEJ AC	Negative	9	0	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
6	EAC	Negative	9	0	0, <1	0.12	0.12	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
7	EAC	Negative	9	0	0, <1	0.00	0.10	0.10	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
8	EAC	Negative	9	0	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
9	GEJ AC	Negative	2	0.25	0, <1	N/A	0.35	0.35	N/A	0% (0/1)	0% (0/1)	0% (0/2)
10	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
11	ESCC	Negative	9	0.5	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
12	GEJ AC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
13	ESCC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
14	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
15	EAC	Negative	8	0.5	0, <1	0.00	0.15	0.15	0% (0/2)	0% (0/3)	0% (0/3)	0% (0/8)
16	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
17	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
18	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
19	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
20	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
21	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
22	GEJ AC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
23	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
24	EAC	Negative	9	0.5	0, <1	0.00	0.14	0.14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
25	EAC	Negative	9	0.5	0, <1	0.00	0.24	0.24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
26	GEJ AC	Negative	9	0.5	0, 2	0.00	0.41	0.41	0% (0/3)	33.3% (1/3)	0% (0/3)	11.1% (1/9)
27	EAC	Negative	9	0.5	0, 3	0.02	1.03	1.03	0% (0/3)	66.7% (2/3)	0% (0/3)	22.2% (2/9)
28	EAC	Negative	9	0.5	0, 4	0.33	1.34	1.38	0% (0/3)	100% (3/3)	33.3% (1/3)	44.4% (4/9)
29	EAC	Negative	9	0.5	0, 2	0.00	0.60	0.60	0% (0/3)	33.3% (1/3)	100% (3/3)	44.4% (4/9)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation (SD)**			Percent Agreement (n/N) ***			
						Inter-Read	Inter-Reader	Total	Reader 1	Reader 2	Reader 3	All Readers
30	EAC	Negative	9	0.5	0, 6	0.50	0.50	0.71	66.7% (2/3)	66.7% (2/3)	0% (0/3)	44.4% (4/9)
31	GEJ AC	Positive	9	1	0, 2	0.24	0.41	0.47	66.7% (2/3)	100% (3/3)	100% (3/3)	88.9% (8/9)
32	EAC	Positive	9	1	<1, 8	0.37	3.14	3.17	100% (3/3)	100% (3/3)	33.3% (1/3)	77.8% (7/9)
33	ESCC	Positive	9	1	1, 5	0.01	1.25	1.25	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
34	EAC	Positive	9	3	1, 5	0.00	0.61	0.61	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
35	EAC	Positive	2	3.75	<1, 7	4.60	N/A	4.60	N/A	50% (1/2)	N/A	50% (1/2)
36	EAC	Positive	9	4	2, 10	1.47	0.00	1.47	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
37	GEJ AC	Positive	9	6	2, 20	0.00	5.69	5.69	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
38	ESCC	Positive	9	6	3, 25	3.28	0.94	3.42	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
39	EAC	Positive	9	6	3, 20	0.41	2.78	2.81	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
40	EAC	Positive	9	7	0, 40	0.00	7.19	7.19	66.7% (2/3)	100% (3/3)	100% (3/3)	88.9% (8/9)
41	EAC	Positive	9	8	2, 10	1.51	2.55	2.96	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
42	EAC	Positive	9	8	4, 20	0.00	3.71	3.71	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
43	ESCC	Positive	9	10	<1, 15	2.64	0.80	2.76	100% (3/3)	66.7% (2/3)	100% (3/3)	88.9% (8/9)
44	ESCC	Positive	9	10	1, 40	7.64	9.89	12.50	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
45	EAC	Positive	9	10	2, 40	0.00	14.01	14.01	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
46	ESCC	Positive	9	10	6, 30	2.03	2.49	3.21	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
47	ESCC	Positive	9	10	6, 30	0.00	3.12	3.12	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
48	EAC	Positive	9	15	3, 20	0.00	6.84	6.84	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
49	ESCC	Positive	9	15	5, 30	3.73	8.84	9.60	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
50	EAC	Positive	9	15	8, 40	4.20	8.50	9.48	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
51	ESCC	Positive	9	20	<1, 30	0.00	0.00	0.00	100% (3/3)	66.7% (2/3)	100% (3/3)	88.9% (8/9)
52	ESCC	Positive	9	20	15, 40	1.67	10.41	10.54	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
53	EAC	Positive	9	25	5, 40	0.00	11.90	11.90	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
54	ESCC	Positive	9	40	15, 85	0.00	23.23	23.23	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
55	GEJ SCC	Positive	9	50	30, 100	0.00	18.05	18.05	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
56	ESCC	Positive	9	60	40, 80	0.00	18.26	18.26	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
57	EAC	Positive	9	60	40, 98	0.00	15.44	15.44	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
58	ESCC	Positive	9	90	70, 98	0.00	6.92	6.92	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
59	EAC	Positive	9	100	20, 100	0.00	33.00	33.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
60	ESCC	Positive	9	100	95, 100	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

EAC = Esophageal Adenocarcinoma; GEJ AC = GEJ adenocarcinoma; ESCC = Esophageal Squamous Cell Carcinoma; GEJ SCC = GEJ squamous cell carcinoma; SD = Standard Deviation; CPS = Combined Positive Score; n = number of results; N = Total number of results

*Consensus result was determined based on the majority call across all test conditions
**Standard deviations were calculated for inter-read, inter-reader, and total variability components
***95% confidence intervals were calculated using the Wilson score method

4. External Reproducibility

The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external testing sites. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method. Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Reproducibility at the CPS ≥ 1 cutoff was assessed in a two-part study: Part A assessed Inter- and Intra-Site endpoints and Part B assessed Inter- and Intra-Observer endpoints.

In Part A, Inter-site reproducibility was assessed with 40 specimens (24 SCC and 16 AC) stained at three sites over 5 non-consecutive days using 1 reagent lot. One observer at each site evaluated the set of specimens for each day.

In Part B, Inter-observer reproducibility was assessed with 3 independent observers from 3 sites, evaluating 60 specimens (33 SCC and 27 AC). The specimens were stained with PD-L1 IHC 22C3 pharmDx at Agilent and scored three times by each of three external pathologists, with a washout period of 14 to 44 days between Reads. All slides were relabeled between Reads to blind the study pathologists from specimen identity from previous evaluations. The slide sets were scored by study pathologists trained and certified on the CPS scoring algorithm.

Table 8. Summary of Reproducibility (three sites) for CPS ≥ 1

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-Site	40 esophageal or GEJ carcinoma specimens (20 PD-L1 positive and 20 PD-L1 negative) with a range of PD-L1 IHC expression were tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 600 comparisons to majority call.	NPA 100% (98.7-100%) PPA 99.0% (97.3-100%) OA 99.5% (98.7-100%)
Intra-site	40 esophageal or GEJ carcinoma specimens (20 PD-L1 positive and 20 PD-L1 negative) with a range of PD-L1 IHC expression were tested on five non-consecutive days. Intra-site analysis was performed for three sites on a total of 600 comparisons to majority call.	NPA 100% (98.7-100%) PPA 99.0% (97.3-100%) OA 99.5% (98.7-100%)

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-observer	60 esophageal or GEJ carcinoma specimens (30 PD-L1 positive and 30 PD-L1 negative) with a range of PD-L1 IHC expression were scored three times, by three pathologists, one at each of three study sites, with a washout period of 14-44 days between each read. Inter-observer analysis was performed between three sites on a total of 537* comparisons to majority call.	NPA 96.6% (93.0-99.3%) PPA 100% (98.6-100%) OA 98.3% (96.5-99.6%)
Intra-observer	60 esophageal or GEJ carcinoma specimens (30 PD-L1 positive and 30 PD-L1 negative) with a range of PD-L1 IHC expression were scored three times, by three pathologists, one at each of three study sites, with a washout period of 14-44 days between each read. Intra-observer analysis was performed for three sites on a total of 537* comparisons to majority call.	NPA 97.3% (94.4-99.3%) PPA 99.6% (98.9-100%) OA 98.5% (97.0-99.6%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement

*One specimen was scored as N/A in all three reads by one of the three observers, resulting in three less comparisons to consensus than expected.

To accurately assess PD-L1 expression status, challenging borderline cases (slides) with scores near the CPS ≥ 1 cutoff (CPS > 0 and < 10) should be reviewed by an additional pathologist and the reported results should be based on a consensus score.

Table 9. Inter-Site Reproducibility Results per Specimen (Part A)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation**			Percent Positive (n/N) ***			
						Inter-Day	Inter-Site	Total	Site 1	Site 2	Site 3	All Sites
1	EAC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
2	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
3	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
4	ESCC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
5	ESCC	Negative	15	0	0, <1	0.00	0.11	0.11	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
6	ESCC	Negative	15	0	0, <1	0.09	0.13	0.16	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
7	GEJ AC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
8	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
9	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation**			Percent Positive (n/N) ***			
						Inter-Day	Inter-Site	Total	Site 1	Site 2	Site 3	All Sites
10	ESCC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
11	EAC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
12	GEJ AC	Negative	15	0	0, <1	0.00	0.16	0.16	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
13	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
14	ESCC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
15	EAC	Negative	15	0	0, <1	0.00	0.16	0.16	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
16	ESCC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
17	EAC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
18	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
19	EAC	Negative	15	0	0, 0	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
20	EAC	Negative	15	0	0, <1	0.00	0.00	0.00	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/15)
21	ESCC	Positive	15	2	<1, 8	0.70	1.71	1.85	100% (5/5)	80% (4/5)	80% (4/5)	86.7% (13/15)
22	ESCC	Positive	15	2	1, 8	0.00	1.20	1.20	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
23	EAC	Positive	15	3	1, 8	0.48	1.82	1.88	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
24	ESCC	Positive	15	3	2, 10	0.37	2.64	2.66	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
25	ESCC	Positive	15	4	1, 35	0.97	6.99	7.06	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
26	ESCC	Positive	15	5	2, 12	0.00	3.12	3.12	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
27	ESCC	Positive	15	8	1, 40	0.00	11.50	11.50	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
28	ESCC	Positive	15	8	3, 35	0.00	13.36	13.36	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
29	ESCC	Positive	15	8	5, 50	0.00	12.95	12.95	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
30	ESCC	Positive	15	10	2, 35	0.00	11.28	11.28	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
31	ESCC	Positive	15	10	3, 45	0.00	13.32	13.32	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
32	ESCC	Positive	15	15	<1, 75	0.00	27.76	27.76	100% (5/5)	80% (4/5)	100% (5/5)	93.3% (14/15)
33	ESCC	Positive	15	15	3, 65	3.63	26.97	27.22	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
34	ESCC	Positive	15	15	5, 80	5.94	24.03	24.75	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
35	ESCC	Positive	15	15	12, 50	0.00	15.63	15.63	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
36	ESCC	Positive	15	40	14, 90	8.69	29.69	30.94	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
37	ESCC	Positive	15	65	15, 90	0.00	25.08	25.08	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
38	ESCC	Positive	15	70	23, 95	0.00	22.93	22.93	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
39	ESCC	Positive	15	90	60, 100	1.26	12.26	12.32	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)
40	GEJ AC	Positive	15	90	65, 100	0.00	11.65	11.65	100% (5/5)	100% (5/5)	100% (5/5)	100% (15/15)

EAC = Esophageal Adenocarcinoma; GEJ AC = GEJ adenocarcinoma; ESCC = Esophageal Squamous Cell Carcinoma; CPS = Combined Positive Score; n = number of results; N = Total number of results

* Consensus result was determined based on the majority call across all test conditions
**Standard deviation was calculated to assess variability in CPS values across sites and days
***Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results

Table 10. Inter-Observer Reproducibility Results per Specimen (Part B)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation**			Percent Positive (n/N) ***			
						Inter-Read	Inter-Observer	Total	Reader 1	Reader 2	Reader 3	All Readers
1	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
2	EAC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
3	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
4	GEJ AC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
5	EAC	Negative	9	0	0, <1	0.00	0.17	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
6	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
7	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
8	GEJ AC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
9	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
10	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
11	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
12	ESCC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
13	ESCC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
14	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
15	EAC	Negative	9	0	0, <1	0.12	0.12	0.17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
16	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
17	EAC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
18	EAC	Negative	9	0	0, <1	0.00	0.10	0.10	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
19	ESCC	Negative	6	0	0, 0	0.00	0.00	0.00	0% (0/3)	N/A	0% (0/3)	0% (0/6)
20	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
21	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
22	ESCC	Negative	9	0	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
23	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
24	EAC	Negative	9	0	0, 0	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
25	ESCC	Negative	9	0.5	0, <1	0.00	0.00	0.00	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/9)
26	EAC	Negative	9	0.5	0, 1	0.12	0.20	0.24	0% (0/3)	33.3% (1/3)	0% (0/3)	11.1% (1/9)
27	GEJ AC	Negative	9	0.5	0, 1	0.10	0.00	0.10	0% (0/3)	33.3% (1/3)	0% (0/3)	11.1% (1/9)
28	EAC	Negative	9	0.5	0, 1	0.00	0.10	0.10	0% (0/3)	33.3% (1/3)	0% (0/3)	11.1% (1/9)
29	ESCC	Negative	9	0.5	0, 1	0.12	0.20	0.24	33.3% (1/3)	0% (0/3)	66.7% (2/3)	33.3% (3/9)
30	EAC	Negative	9	0.5	<1, 2	0.00	0.00	0.00	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	33.3% (3/9)
31	ESCC	Positive	9	6	2, 15	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
32	ESCC	Positive	9	6	2, 13	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
33	ESCC	Positive	9	7	3, 12	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
34	ESCC	Positive	9	8	4, 20	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
35	EAC	Positive	9	10	4, 20	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
36	ESCC	Positive	9	12	5, 40	3.79	0.00	3.79	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
37	EAC	Positive	9	15	7, 35	0.00	9.61	9.61	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
38	GEJ AC	Positive	9	16	11, 45	0.00	6.81	6.81	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

Specimen	Subtype	Consensus Result*	Number of Results	Median (CPS)	Range (CPS)	Standard Deviation**			Percent Positive (n/N) ***			
						Inter-Read	Inter-Observer	Total	Reader 1	Reader 2	Reader 3	All Readers
39	EAC	Positive	9	20	13, 43	7.56	4.14	8.62	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
40	ESCC	Positive	9	25	7, 35	0.00	5.36	5.36	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
41	EAC	Positive	9	25	12, 55	2.89	0.00	2.89	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
42	GEJ AC	Positive	9	25	15, 50	0.00	12.41	12.41	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
43	EAC	Positive	9	30	18, 60	0.00	13.49	13.49	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
44	ESCC	Positive	9	35	20, 55	6.01	0.00	6.01	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
45	ESCC	Positive	9	35	20, 70	0.00	11.06	11.06	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
46	ESCC	Positive	9	40	20, 60	3.12	15.59	15.90	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
47	GEJ AC	Positive	9	40	30, 80	2.36	7.67	8.03	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
48	ESCC	Positive	9	50	25, 65	3.12	8.74	9.28	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
49	ESCC	Positive	9	55	25, 80	0.00	17.61	17.61	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
50	ESCC	Positive	9	60	33, 75	2.69	10.38	10.72	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
51	ESCC	Positive	9	60	45, 95	4.35	18.81	19.31	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
52	ESCC	Positive	9	70	60, 85	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
53	ESCC	Positive	9	80	58, 100	0.00	10.18	10.18	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
54	ESCC	Positive	9	80	60, 100	2.31	11.44	11.67	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
55	ESCC	Positive	9	90	70, 100	6.45	10.41	12.25	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
56	ESCC	Positive	9	90	75, 100	0.00	4.41	4.41	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
57	ESCC	Positive	9	90	80, 100	3.12	6.43	7.15	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
58	ESCC	Positive	9	90	80, 100	0.00	7.93	7.93	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
59	ESCC	Positive	9	90	80, 100	0.00	0.00	0.00	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)
60	ESCC	Positive	9	95	90, 100	1.18	4.25	4.41	100% (3/3)	100% (3/3)	100% (3/3)	100% (9/9)

EAC = Esophageal Adenocarcinoma; GEJ AC= GEJ adenocarcinoma; ESCC = Esophageal Squamous Cell Carcinoma; 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
**Standard deviation was calculated to assess variability in CPS values across observers/readers and reading days
*** Percent positive represents the proportion of results scoring positive (CPS ≥ 1) out of the total number of results

5. Robustness Studies

Robustness of the staining performance of PD-L1 IHC 22C3 pharmDx in esophageal or GEJ carcinoma specimens was evaluated by testing the performance of the assay when varying the assay conditions as described below. Robustness studies used one lot of PD-L1 IHC 22C3 pharmDx reagents and 32 esophageal cancer specimens.

- Tissue sections cut at three thicknesses
  - 3 µm
  - 4 µm

    - 5 µm
- Target Retrieval Time at three incubation times
    - 18 minutes
    - 20 minutes-standard
    - 22 minutes
- Target Retrieval Temperature at three incubation temperatures
    - 95°C
    - 97°C-standard
    - 99°C
- Target Retrieval Solution pH at three pH values
    - pH 5.9
    - pH 6.1-standard
    - pH 6.3
- Target Retrieval Solution Re-Use after first and third use

Staining performance was evaluated for the CPS ≥ 1 cutoff and intensity of staining.

Tissue thickness included 32 esophageal cancer specimens spanning the range of PD-L1 expression and included 50% of specimens around the cutoff. Target retrieval studies were performed with a minimum of 31 esophageal cancer specimens spanning the range of PD-L1 expression and included at least 30% of specimens around the cutoff.

NPA, PPA, and OA based on comparisons to consensus were computed with two-sided 95% confidence intervals using the bootstrap method for the CPS ≥ 1 cutoff. Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

6. Impact of Tumor Heterogeneity

The objective of the studies described below was to investigate whether tumor heterogeneity affects PD-L1 IHC staining results with PD-L1 IHC 22C3 pharmDx.

a. Primary vs. Metastatic Tumor Tissues

A study compared PD-L1 IHC 22C3 pharmDx performance in the context of primary versus metastatic tumor tissues. Only lymph nodes were available to use as metastatic tumor specimens. Performance of PD-L1 IHC 22C3 pharmDx was evaluated in FFPE matched primary (esophageal cancer) and metastatic (lymph node) tissues. There were no acceptance criteria for this study. In 19 of 20 paired primary and metastatic tissues, there were 4 concordant negative pairs, 10 concordant positive pairs, and 5 discordances for PD-L1 status when using the CPS ≥ 1 cutoff. Four of the 5 discordant cases were positive for the metastatic tumor but negative for the primary tumor. As the sample of 19 lymph node specimens is not representative of the diversity and population size of esophageal metastatic tumors, these results may not be extrapolated to all metastatic locations.

b. Intra-Block Heterogeneity

A study investigated the effect of esophageal cancer tumor intra-block heterogeneity on PD-L1 IHC 22C3 pharmDx performance. Fifty-three individual FFPE esophageal cancer tissue blocks across a minimum of 200µm depth were divided in three portions: anterior (sections 1-10), middle (sections 25-35) and posterior (sections 50-70). No acceptance criteria were set for this study. Of the 53 cases assessed, 51 cases were concordant, and 2 cases were discordant.

c. Intra-Case Heterogeneity

A study investigated the effect of esophageal cancer tumor intra-case heterogeneity on PD-L1 IHC 22C3 pharmDx performance. Esophageal cancer tissue sister blocks were obtained from the same tumor of 22 subjects. No acceptance criteria were set for this study. Eighteen of the sister blocks qualified for the study and were evaluated for the CPS ≥ 1 cutoff. All 18 pairs were concordant, and there were no discordant pairs. Results may not be representative of all esophageal cancer specimens, as tumor heterogeneity is unique for each specimen.

7. Stability Testing

a. PD-L1 IHC 22C3 pharmDx Stability

Real-time reagent stability testing was previously performed on PD-L1 IHC 22C3 pharmDx for NSCLC and reviewed in P150013. Based on data provided in P150013, the stability of the device is established and approved at 9 months for storage at 2-8 °C.

b. FFPE Cut Section Stability

Real-time cut section stability testing was previously performed on PD-L1 IHC 22C3 pharmDx for esophageal cancer and reviewed in P150013/S016. Based on data provided in P150013/S016, stability dating for cut slides in esophageal cancer is 4.5 months (135 days) for storage at 2-8°C and 1 month (30 days) for cut sections stored at 25 °C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Study Design

The clinical performance of PD-L1 IHC 22C3 pharmDx as a companion diagnostic for PD-L1 detection (CPS ≥ 1) to identify esophageal or GEJ carcinoma patients eligible for treatment with pembrolizumab (KEYTRUDA, MK3475), was

demonstrated in KEYNOTE-590. KEYNOTE-590 was a randomized, double-blind, placebo-controlled, multicenter Phase 3 study designed with pembrolizumab in combination with chemotherapy (cisplatin and 5-FU) as first line (1L) treatment in participants with locally advanced unresectable metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the GEJ.

1. Clinical Inclusion and Exclusion Criteria

   Participation in KEYNOTE-590 was limited to male and female subjects with locally advanced unresectable or metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the GEJ, who were at least 18 years of age.

   Each subject was also required to meet the following key inclusion criteria:
   - Was ≥ 18 years of age on the day of signing informed consent.
   - Had histologically or cytologically confirmed diagnosis of locally advanced unresectable or metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the GEJ.
   - Had measurable disease per RECIST 1.1 as determined by the local site investigator/radiology assessment.
   - Had an ECOG PS of 0 to 1.
   - Had provided either a newly obtained or archival tissue sample for PD-L1 by immunohistochemistry analysis. Newly obtained tissue was preferred.

   The key exclusion criteria were:
   - Had locally advanced esophageal carcinoma that was resectable or potentially curable with radiation therapy (as determined by local investigator).
   - Had previous therapy for advanced/metastatic adenocarcinoma or squamous cell cancer of the esophagus or advanced/metastatic Siewert type 1 adenocarcinoma of the GEJ.
   - Had a known additional malignancy that was progressing or required active treatment.
   - Had known active central nervous system metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may have participated provided they met specific criteria defined in the protocol.
   - Had an active autoimmune disease that had required systemic treatment in past 2 years.
   - Had a diagnosis of immunodeficiency or was receiving chronic systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment, or had a history of organ transplant, including allogeneic stem cell transplant.

- Had a history of (non-infectious) pneumonitis that required steroids or had current pneumonitis.
- Had an active infection requiring systemic therapy.
- Had received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another co-inhibitory T-cell receptor or had previously participated in a pembrolizumab (MK-3475) clinical trial.

2. Study Design

Patients were randomized (1:1) to one of the following treatment arms; all study medications were administered via intravenous infusion:

- KEYTRUDA 200 mg on Day 1 of each three-week cycle in combination with cisplatin 80 mg/m^2 IV on Day 1 of each three-week cycle for up to six cycles and FU 800 mg/m^2 IV per day on Day 1 to Day 5 of each three-week cycle, or per local standard for FU administration, for up to 24 months.
- Placebo on Day 1 of each three-week cycle in combination with cisplatin 80 mg/m^2 IV on Day 1 of each three-week cycle for up to six cycles and FU 800 mg/m^2 IV per day on Day 1 to Day 5 of each three-week cycle, or per local standard for FU administration, for up to 24 months.

Treatment with KEYTRUDA (pembrolizumab) or chemotherapy continued until unacceptable toxicity or disease progression. Patients could be treated with KEYTRUDA (pembrolizumab) for up to 24 months in the absence of disease progression.

3. Clinical Endpoints

Primary Efficacy Endpoints:

The dual primary efficacy endpoints were:

- Progression free survival (PFS) as defined in the time from randomization to the first documented disease progression per RECIST 1.1 based on investigator assessment or death due to any cause, whichever occurs first.
- Overall survival (OS), defined as the time from randomization to death due to any cause.

Secondary Efficacy Endpoints:

The secondary efficacy endpoints were:

- Overall response rate (ORR), defined as the proportion the subjects in the analysis who have complete response (CR) or partial response (PR) per RECIST 1.1 by investigator assessment.
- Duration of response (DOR), defined as the time from first documented evidence of CR or PR until disease progression per RECIST 1.1 based on investigator assessment or death due to any cause, whichever occurs first.

4. Assay Cut-Off Selection

On September 26, 2024, FDA convened an Oncologic Drugs Advisory Committee (ODAC) to discuss the risk benefit assessment of the use of immune checkpoint inhibitors (ICIs) in combination with chemotherapy for the first line treatment of

patients with unresectable or metastatic esophageal squamous cell carcinoma (ESCC) and gastric cancer (GC) at different levels of PD-L1 protein expression. FDA asked members of the ODAC if the risk-benefit assessment was favorable for the use of these agents in patients with PD-L1 expression <1. After extensive discussion and ODAC's agreement with FDA's position, a decision was made to limit use of these products to patients with PD-L1 expression ≥1. Subsequently, the esophageal carcinoma indication labeling for KEYTRUDA (pembrolizumab) was revised to PD-L1 CPS ≥ 1. Although not designated as a pre-specified scoring cutoff for KEYNOTE-590, the PD-L1 cutoff of CPS ≥ 1 has been previously utilized in earlier esophageal studies (e.g., KEYNOTE-180) and has been analytically validated.

B. Accountability of PMA Cohort

Participants with previously untreated locally advanced unresectable or metastatic carcinoma of the esophagus and gastroesophageal junction were enrolled in KEYNOTE-590. Among the 749 participants in the intent-to-treat (ITT) population, 373 and 376 participants were randomized to the pembrolizumab plus chemotherapy group and the chemotherapy group, respectively. All 749 participants in the study had a tumor tissue sample tested with PD-L1 IHC 22C3 pharmDx. Most participants, 86.4% (n=647), had a tumor tissue PD-L1 expression score of CPS ≥ 1, and 51.1% (n=383) had a tumor tissue PD-L1 expression score of CPS ≥ 10. CPS ≥ 10 was the only pre-specified subgroup; the CPS ≥1 subgroup analyses were performed post-hoc. In the CPS ≥1 subgroup, the primary diagnosis was ESCC in 73.9% (n=478) and EAC in 26.2% (n=169; including 80 participants with GEJ adenocarcinoma, Siewert Type 1), which were well balanced between treatment groups (320 and 327 participants in the pembrolizumab plus chemotherapy group and the chemotherapy group, respectively).

C. Study Population Demographics and Baseline Parameters

Among the 749 patients randomized in the study the baseline characteristics were: median age of 64 years (range: 28 to 94), 46% age 65 or older; 82% male; 37% White, 54% Asian, and 1% Black; 40% had an ECOG PS of 0 and 60% had an ECOG PS of 1. Ninety-two percent had M1 disease and 8% had M0 disease. Seventy-four percent had a tumor histology of squamous cell carcinoma, and 26% had adenocarcinoma. Eighty-six percent of patients had tumor PD-L1 expression CPS ≥ 1. The baseline characteristics in the CPS ≥ 1 population was generally consistent with all participants and well balanced, with comparable distribution between the pembrolizumab plus chemotherapy group and the chemotherapy group.

D. Safety and Effectiveness Results

1. Safety Results

   Safety of the device for the CPS ≥ 1 cutoff was demonstrated in the analytical validation studies described above. There are no safety issues related to the use of

this score. The safety profile of pembrolizumab plus chemotherapy was generally consistent with the individual safety profiles of chemotherapy (cisplatin and 5-FU) and pembrolizumab monotherapy. The safety profile of pembrolizumab plus chemotherapy is similar across PD-L1 subgroups.

Please refer to Drugs@FDA for complete safety information on KEYTRUDA (pembrolizumab).

2. Effectiveness Results

The KEYNOTE-590 trial demonstrated a clinically meaningful improvement in survival benefit for patients randomized to KEYTRUDA (pembrolizumab) in combination with chemotherapy, compared to chemotherapy. Table 11 below summarizes the key efficacy measures for patients with esophageal or GEJ carcinoma with CPS ≥ 1 and CPS ≥ 10. Figure 1 and Figure 2 show the Kaplan-Meier overall survival curves for esophageal or GEJ carcinoma patients with CPS ≥ 1 and CPS ≥ 10, respectively.

Table 11. Efficacy Results in Patients with Esophageal or GEJ carcinoma Whose Tumors Expressed PD-L1 CPS ≥ 1 and CPS ≥ 10 in KEYNOTE-590

Endpoint	KEYTRUDA 200 mg every 3 weeks Cisplatin FU n=320	Placebo Cisplatin FU n=327	KEYTRUDA 200 mg every 3 weeks Cisplatin FU n=186	Placebo Cisplatin FU n=197
	CPS ≥ 1		CPS ≥ 10	
OS				
Number (%) of events	222 (69)	271 (83)	124 (67)	165 (84)
Median in months (95% CI)	12.7 (10.5, 14.4)	9.8 (8.8, 10.8)	13.5 (11.1, 15.6)	9.4 (8.0, 10.7)
Hazard ratio* (95% CI)	0.71 (0.59, 0.84)		0.62 (0.49, 0.78)	
p-Value†			<0.0001	
PFS				
Number of events (%)	252 (79)	291 (89)	140 (75)	174 (88)
Median in months (95% CI)	6.3 (6.2, 7.1)	5.7 (4.6, 6.0)	7.5 (6.2, 8.2)	5.5 (4.3, 6.0)
Hazard ratio* (95% CI)	0.62 (0.52, 0.73)		0.51 (0.41, 0.65)	
p-Value†			<0.0001	
Objective Response Rate				
ORR, %‡ (95% CI)	45 (40, 51)	29 (24, 34)	51 (44, 59)	27 (21, 34)
Number (%) of complete responses	19 (6)	9 (2.8)	11 (6)	5 (2.5)
Number (%) of partial responses	126 (39)	85 (26)	84 (45)	48 (24)
Duration of Response				
Median in months (range)	8.6 (1.2+, 31.0+)	5.8 (1.5+, 25.0+)	10.4 (1.9+, 28.9+)	5.6 (1.5+, 25.0+)

* Based on the stratified Cox proportional hazard model

† Based on a stratified log-rank test; p-Value for CPS ≥ 1 not included (not pre-specified subgroup)

‡ Confirmed complete response or partial response

Figure 1: Kaplan-Meier Curve for Overall Survival in KEYNOTE-590 (CPS ≥1)

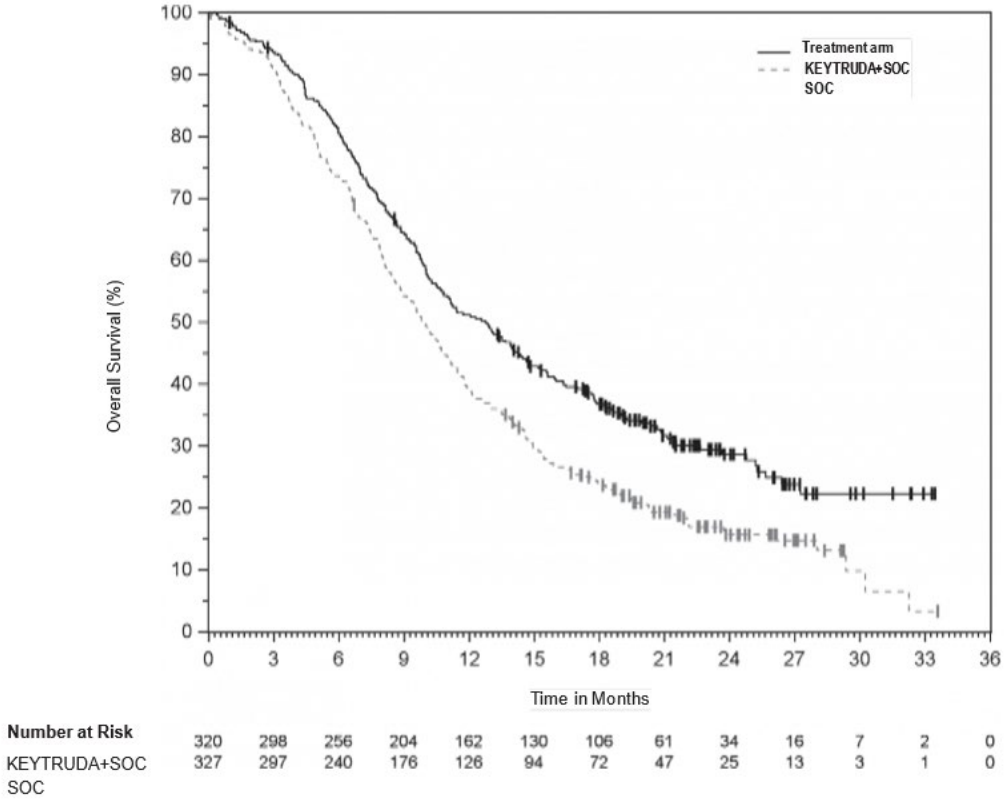
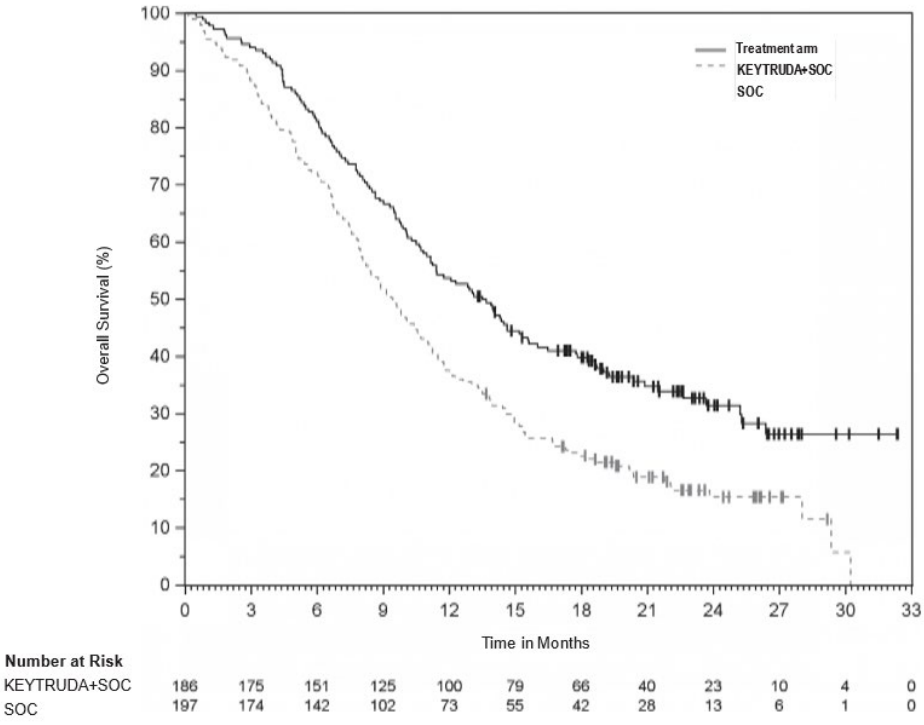


Figure 2: Kaplan-Meier Curve for Overall Survival in KEYNOTE-590 (CPS ≥10)



3. Pediatric Extrapolation
   Patients aged 18 years and older were eligible for enrollment into the KEYNOTE-590 trial if they fulfilled other inclusion criteria. The youngest patient enrolled in the study was 27 years old. For the KEYNOTE-590 study, data from adult patients aged 27 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 three 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 benefit of PD-L1 IHC 22C3 pharmDx for the esophageal or GEJ carcinoma indication is based upon the results of the KEYNOTE-590 study, which was conducted to evaluate the safety and efficacy of pembrolizumab in combination with chemotherapy (cisplatin and 5-FU) in first line (1L) treatment of esophageal or GEJ carcinoma patients. In this study, PD-L1 IHC 22C3 pharmDx was used to determine PD-L1 expression status of patient tumors. Clinically meaningful improvement in OS, PFS, and ORR were observed in patients whose tumors expressed PD-L1 CPS $\geq$ 1. Additionally, pembrolizumab plus chemotherapy provided a clinically meaningful improvement in durability of response with median DOR of 8.6 months in CPS $\geq$ 1 participants in the pembrolizumab plus chemotherapy versus 5.8 months in the chemotherapy group. Approximately, 40% (CPS $\geq$ 1) of the responders in the pembrolizumab plus

chemotherapy group had a response lasting ≥12 months, compared with 20% of responders in the chemotherapy group.

The performance of PD-L1 IHC 22C3 pharmDx was also supported by the analytical validation studies.

B. Safety Conclusions

Safety for use of the PD-L1 IHC 22C3 pharmDx device for patient management is related to effectiveness (see effectiveness conclusions above). In general, the 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 (see Benefit-Risk Determination below). The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely collected for esophageal or GEJ carcinoma diagnosis.

C. Benefit-Risk Determination

The probable benefits of this device are based on data from the KEYNOTE-590 clinical study, which demonstrated clinically meaningful improvements in overall survival (OS), progression-free survival (PFS), and overall response rate (ORR) in esophageal or gastroesophageal junction (GEJ) carcinoma patients whose tumors expressed PD-L1 combined positive score (CPS) ≥ 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 with esophageal or GEJ carcinoma. Patients with false positive results may be treated with KEYTRUDA in combination with chemotherapy 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 or failure to provide a result, which may lead to the 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.

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

In conclusion, given the available information detailed above, the data supports that, for the patients with esophageal or GEJ carcinoma who are being considered for treatment with KEYTRUDA (pembrolizumab), 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 esophageal or GEJ carcinoma with PD-L1 CPS ≥1 who may be considered for treatment with KEYTRUDA (pembrolizumab).

XV. CDRH DECISION

CDRH issued an approval order on February 11, 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.