Agilent
Dako

PD-L1 IHC 22C3 pharmDx
Rx Only

SK006
50 tests for use with Autostainer Link 48

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1. Intended 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), and gastric or gastroesophageal junction (GEJ) adenocarcinoma 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, and gastric or GEJ adenocarcinoma 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	
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.

2. Summary and Explanation

2.1 KEYTRUDA (pembrolizumab)

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T-cells, inhibits T-cell proliferation and cytokine production. Up-regulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. KEYTRUDA is a humanized monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response.[1] In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.[2]

2.1.1 NSCLC (KEYTRUDA)
Merck Sharp & Dohme LLC, Rahway, NJ, USA (hereinafter "MSD") sponsored clinical study, KEYNOTE-042 (KN042), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (TPS ≥ 1%) previously untreated stage III NSCLC, who are not candidates for surgical resection or definitive chemoradiation, or metastatic NSCLC patients that may respond to KEYTRUDA treatment.[3] Refer to 'Clinical performance evaluation: NSCLC (KEYTRUDA)' Section 16.3 for KN042 study details.

MSD sponsored clinical study, KEYNOTE-024 (KN024), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (TPS ≥ 50%) previously untreated metastatic NSCLC patients that may respond to KEYTRUDA treatment.[4] Refer to 'Clinical performance evaluation: NSCLC (KEYTRUDA)' Section 16.3 for KN024 study details.

MSD sponsored clinical study, KEYNOTE-010 (KN010), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (TPS ≥ 1%) previously treated metastatic NSCLC patients that may respond to KEYTRUDA treatment.[5] Refer to 'Clinical performance evaluation: NSCLC (KEYTRUDA)' Section 16.3 for KN010 study details.

2.1.2 ESCC (KEYTRUDA)
MSD sponsored clinical study, KEYNOTE-181 (KN181), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (CPS ≥ 10) patients with recurrent locally advanced or metastatic esophageal cancer with disease progression on or after

one prior line of systemic therapy, who may respond to KEYTRUDA treatment.[6] Refer to 'Clinical performance evaluation: esophageal squamous cell carcinoma (ESCC; KEYTRUDA)' Section 16.6 for KN181 study details.

2.1.3 Cervical cancer (KEYTRUDA)
MSD sponsored clinical study, KEYNOTE-158 (KN158), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (CPS ≥ 1) cervical cancer patients, with disease progression on or after chemotherapy for recurrent or metastatic disease, that may respond to KEYTRUDA treatment.[7,8] Refer to 'Clinical performance evaluation: cervical cancer (KEYTRUDA)' Section 16.8 for KN158 Cohort E study details.

2.1.4 HNSCC (KEYTRUDA)
MSD sponsored clinical study, KEYNOTE-048 (KN048), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (CPS ≥ 1) patients with metastatic or recurrent HNSCC who had not previously received systemic therapy for metastatic disease or with recurrent disease who were considered incurable by local therapies, and who may respond to KEYTRUDA treatment.[9] Refer to 'Clinical performance evaluation: HNSCC (KEYTRUDA)' Section 16.10 for KN048 study details.

2.1.5 TNBC (KEYTRUDA)
MSD sponsored clinical study, KEYNOTE-355 (KN355), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying patients with PD-L1 expressing (CPS ≥ 10) previously untreated locally recurrent unresectable or metastatic TNBC, who may respond to KEYTRUDA treatment in combination with chemotherapy.[10] Refer to 'Clinical performance evaluation: TNBC (KEYTRUDA)' Section 16.12 for KN355 study details.

2.1.6 Gastric or GEJ adenocarcinoma (KEYTRUDA)
MSD sponsored clinical study, KEYNOTE-811 (KN811), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying patients with PD-L1 expressing (CPS ≥ 1) previously untreated, locally advanced unresectable or metastatic human epidermal growth factor receptor 2 (HER2) positive, gastric or GEJ adenocarcinoma, and who may respond to KEYTRUDA treatment. Refer to 'Clinical performance evaluation: gastric or GEJ adenocarcinoma (KEYTRUDA)', Section 16.14 for KN811 study details.

2.2 LIBTAYO (cemiplimab-rwlc)

LIBTAYO is a human monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.[11]

2.2.1 NSCLC (LIBTAYO)
Regeneron Pharmaceuticals, Inc. sponsored clinical study, EMPOWER-Lung 1, investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (TPS ≥ 50%) patients with locally advanced NSCLC who are not candidates for surgical resection or definitive chemoradiation, or with metastatic NSCLC, who may respond to LIBTAYO treatment.[12] Refer to 'Clinical performance evaluation: NSCLC (LIBTAYO)' Section 16.4 for EMPOWER-Lung 1 details.

3. Principle of Procedure

PD-L1 IHC 22C3 pharmDx contains the optimized reagents and protocol required to complete an IHC staining procedure of FFPE specimens using Autostainer Link 48. Following incubation with the primary monoclonal antibody to PD-L1 or the Negative Control Reagent (NCR), specimens are incubated with a Linker antibody specific to the host species of the primary antibody, and then are incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase (HRP) molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added chromogen results in precipitation of a visible reaction product at the site of antigen. The color of the chromogenic reaction is modified by a chromogen enhancement reagent. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope.

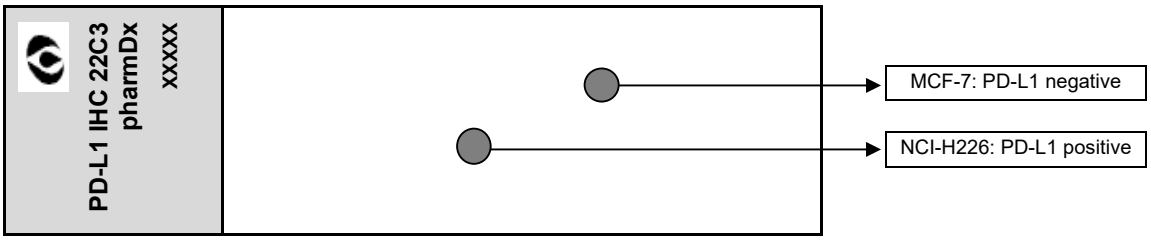
4. Materials Provided

Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3 µg/mL protein concentration) and contains the reagents necessary to perform 50 tests in up to 15 individual runs. The materials listed in this section are sufficient for 50 tests (50 slides incubated with primary antibody to PD-L1 and 50 slides incubated with the corresponding NCR; 100 slides in total). The number of tests is based on the use of 2 x 150 µL per slide of each reagent except DAB+ and Envision FLEX Target Retrieval Solution. For larger tissue sections, three drop zones (3 x 150 µL) per slide may be used. Note that this will reduce the total number of tests per kit.

The kit provides materials sufficient for a maximum of 15 individual staining runs.

Quantity	*Description*
1 x 34.5 mL	**Peroxidase-Blocking Reagent** **PEROXIDASE-BLOCKING REAGENT** Buffered solution containing hydrogen peroxide, detergent, and 0.015 mol/L sodium azide.
1 x 19.5 mL	**Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3** **MONOCLONAL MOUSE ANTI-PD-L1 CLONE 22C3**

Quantity	*Description*
	Monoclonal mouse (IgG_1) anti-PD-L1 in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.
1 x 15 mL	**Negative Control Reagent** **NEGATIVE CONTROL REAGENT** Monoclonal mouse control IgG_1 antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.
1 x 34.5 mL	**Mouse LINKER** **LINKER, ANTI-MOUSE** Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015 mol/L sodium azide.
1 x 34.5 mL	**Visualization Reagent-HRP** **VISUALIZATION REAGENT-HRP** Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution containing stabilizing protein and an antimicrobial agent.
15 x 7.2 mL	**DAB+ Substrate Buffer** **DAB+ SUBSTRATE BUFFER** Buffered solution, containing hydrogen peroxide and an antimicrobial agent.
1 x 5 mL	**DAB+ Chromogen** **DAB+ CHROMOGEN** 3,3'-diaminobenzidine tetrahydrochloride in organic solvent.
1 x 34.5 mL	**DAB Enhancer** **DAB ENHANCER** Cupric sulfate in water.
6 x 30 mL	**EnVision FLEX Target Retrieval Solution, Low pH (50x)** **EnVision FLEX TARGET RETRIEVAL SOLUTION LOW pH (50X)** Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.
15 slides	**PD-L1 IHC 22C3 pharmDx Control Slides** **CONTROL SLIDES** Each slide contains sections of two pelleted, FFPE cell lines: NCI-H226* with moderate PD-L1 protein expression and MCF-7 with negative PD-L1 protein expression.



*Dr. AF Gazdar and Dr. JD Minna at NIH are acknowledged for their contribution in developing NCI-H226 (ATCC Number: CRL-5826).[13]

Note: All components included are formulated specifically for use with this kit. In order for the test to perform as specified, no substitutions, other than EnVision FLEX Target Retrieval Solution, Low pH (50x) (Code K8005) can be made. PD-L1 IHC 22C3 pharmDx has been tailored for use with Autostainer Link 48. Please refer to the User Guides for your Autostainer Link 48 and PT Link for further information.

5. Materials Required, but Not Supplied

PT Link Pre-treatment Module (Code PT100/PT101/PT200)
Autostainer Link 48 (Code AS480)
EnVision FLEX Wash Buffer (20x) (Code K8007)
EnVision FLEX Hematoxylin (Link) (Code K8008)
Distilled or de-ionized water (reagent-grade water)*
Timer
Positive and negative tissues to use as process controls (see 'Quality Control' Section 11)
Microscope slides: FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides
Coverslips
Nonaqueous, permanent mounting medium and ancillary reagents required for mounting coverslips
Light microscope (4x–40x objective magnification)
pH meter (calibrated per manufacturer's recommendation)
Wash bottle

*Note: Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water (corresponding to Clinical Laboratory Reagent Water [CLRW] standard as specified by Clinical & Laboratory Standards Institute [CLSI][14]), or water similar in quality to be used for reagent preparation.

6. Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN_3 may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[15]
4. Primary Antibody, Negative Control Reagent, Linker, and Visualization Reagent contain material of animal origin.
5. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection, and disposed of with proper precautions.[16]
6. Incubation times, temperatures, or methods other than those specified may give erroneous results.
7. Reagents have been optimally diluted. Further dilution may result in loss of antigen staining.
8. The Visualization Reagent, liquid DAB+ Chromogen and prepared DAB+ Substrate-Chromogen solution may be affected adversely if exposed to excessive light levels. Do not store system components or perform staining in strong light, such as direct sunlight.
9. Paraffin residuals may lead to false negative results.
10. Use of reagent volumes other than recommended may result in loss of visible PD-L1 immunoreactivity.
11. Results from a small study showed a similar dynamic range of PD-L1 expression in primary and metastatic NSCLC specimen pairs. It is possible there may be differences in PD-L1 expression in primary tumors versus metastatic sites in the same patient.
12. Large tissue sections may require 3 x 150 µl of reagent.
13. As a general rule, persons under 18 years of age are not allowed to work with this product. Users must be carefully instructed in the proper work procedures, the dangerous properties of the product and the necessary safety instructions. Please refer to Safety Data Sheet (SDS) for additional information.
14. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
15. Unused solution should be disposed of according to local, State and Federal regulations.
16. Hazard information is available in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) insert contained within the product package. Safety Data Sheets are available on www.agilent.com or on request.
17. For countries outside of the United States, see the local KEYTRUDA product label for approved indications and expression cutoff values to guide therapy.

7. Storage

Store all components of PD-L1 IHC 22C3 pharmDx, including kit-supplied Control Slides, in the dark at 2–8 °C when not in use on Autostainer Link 48.

Do not use the kit after the expiration date printed on the outside of the kit box. If reagents are stored under any conditions other than those specified in this package insert, they must be validated by the user.

There are no obvious signs to indicate instability of this product, therefore, positive and negative controls should be run simultaneously with patient specimens.

8. Specimen Preparation

Tissue specimens must be handled to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens.

8.1 Paraffin-embedded specimens

FFPE tissue specimens are suitable for use. Alternative fixatives have not been validated and may give erroneous results. Fixation time for 12–72 hours in 10% neutral buffered formalin (NBF) is recommended, however, a study with limited samples showed fixation times of 4–168 hours in 10% NBF did not systematically alter PD-L1 detection. 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 formalin, dehydrated, and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. NSCLC FFPE tissue blocks which are 5 years or older may result in a loss of PD-L1 immunoreactivity.

Tissue specimens should be cut into sections of 4–5 µm. After sectioning, tissues should be mounted on FLEX IHC microscope slides (Code K8020) or Superfrost Plus slides and then placed in a 58 ± 2 °C oven for 1 hour.

8.2 Cut section storage recommendation

To preserve antigenicity, tissue sections, once mounted on slides, should be held in the dark at 2–8 °C (preferred), or at room temperature up to 25 °C. Slide storage and handling conditions should not exceed 25 °C at any point post-mounting to ensure tissue integrity and antigenicity.

8.2.1 NSCLC cut section storage recommendation

Cut sections must be stained within 6 months when stored at 2—8 ºC (preferred), or at 25 ºC.

8.2.2 ESCC cut section storage recommendation

Cut sections must be stained within 4.5 months when stored at 2–8 °C (preferred), or within 1 month when stored at 25 °C.

8.2.3 Cervical cancer cut section storage recommendation

Cut sections must be stained within 2 months when stored at 2—8 ºC (preferred), or within 1 month when stored at 25 ºC.

8.2.4 HNSCC cut section storage recommendation

Cut sections must be stained within 6 months when stored at 2–8 °C (preferred), or within 4 months when stored at 25 °C.

8.2.5 TNBC cut section storage recommendation

Cut sections must be stained within 7.5 months when stored at 2–8 °C (preferred), or within 4 months when stored at 25 °C.

8.2.6 Gastric or GEJ adenocarcinoma cut section storage recommendation

Cut sections must be stained within 5 months when stored at 2-8 ºC (preferred), or at 25 °C.

9. Reagent Preparation

The following reagents must be prepared prior to staining:

EnVision FLEX Target Retrieval Solution, Low pH (50x)
Prepare a sufficient quantity of 1x EnVision FLEX Target Retrieval Solution, Low pH (working solution) by diluting EnVision FLEX Target Retrieval Solution, Low pH (50x) 1:50 using distilled or deionized water; the pH of 1x EnVision FLEX Target Retrieval Solution, Low pH must be 6.1 ± 0.2. Do not modify the pH of 1x EnVision FLEX Target Retrieval Solution, Low pH after preparation under any circumstances. If a problem is suspected with the pH of the EnVision FLEX Target Retrieval Solution, Low pH, please refer to 'Troubleshooting' Section 17. for more information. One 30 mL bottle of EnVision FLEX Target Retrieval Solution, Low pH (50x) diluted 1:50 will provide 1.5 L of 1x reagent, sufficient to fill one PT Link tank which will treat up to 24 slides per use. Discard 1x EnVision FLEX Target Retrieval Solution, Low pH after three uses and do not use after 5 days following dilution. Note, the EnVision FLEX Target Retrieval Solution, Low pH (50x) is a red colored solution. Please refer to 'Product-specific limitations' Section 15.2 for EnVision FLEX Target Retrieval Solution, Low pH limitations in ESCC specimens.

Additional EnVision FLEX Target Retrieval Solution, Low pH (50x) if required, is available as Code K8005.

EnVision FLEX Wash Buffer (20x)
Prepare a sufficient quantity of 1x EnVision FLEX Wash Buffer by diluting EnVision FLEX Wash Buffer (20x) 1:20 using distilled or de-ionized water for the wash steps. Store unused 1x EnVision FLEX Wash Buffer at 2–8 ºC for no more than 1 month. Discard EnVision FLEX Wash Buffer if cloudy in appearance. Refer to the User Guide for your Autostainer Link 48 for further information.

EnVision FLEX Wash Buffer (20x) is available as Code K8007.

DAB+ Substrate-Chromogen Solution
This solution should be mixed thoroughly prior to use. Any precipitate developing in the solution does not affect staining quality.

To prepare DAB+ Substrate-Chromogen Solution, add one drop of liquid DAB+ Chromogen per mL of DAB+ Substrate Buffer and mix.*

Prepared DAB+ Substrate-Chromogen Solution is stable for 5 days if stored in the dark at 2—8 °C.

Important notes:

- ***If using an entire bottle of DAB+ Substrate Buffer, add nine drops of DAB+ Chromogen.** Although the label states 7.2 mL, this is the useable volume and does not account for the "dead volume" (1.8 mL) in the bottle.
- The color of the liquid DAB+ Chromogen in the bottle may vary from clear to lavender-brown. This will not affect the performance of this product. Dilute per the guidelines in this section. Addition of excess liquid DAB+ Chromogen to the DAB+ Substrate Buffer will result in deterioration of the positive signal.

10. Staining Procedure on the Autostainer Link 48 Solution

Procedural notes
The user should read these instructions carefully and become familiar with all components and instrumentation prior to use (see 'Precautions' Section 6).

All reagents should be equilibrated to room temperature (20–25 °C) prior to immunostaining. Likewise, all incubations should be performed at room temperature.

Do not allow tissue sections to dry after deparaffinization, rehydration, and target retrieval (3-in-1) procedure (specimen pretreatment) or at any time during the staining procedure. Dried tissue sections may display increased nonspecific staining (including nuclear staining).

Ensure that Autostainer slide racks are level prior to initiation of the IHC staining procedure. Level Autostainer slide racks are required for staining. Unlevel and/or warped Autostainer slide racks can result in uneven reagent distribution and improper pooling on the glass away from the specimen areas, which increases the risk for tissue drying and may lead to the appearance of nonspecific staining on the Primary Antibody and/or Negative Control Reagent (NCR)-stained slides. Perform level testing using dry untreated slides for each slide position in all Autostainer slide racks every 3 months or whenever the Autostainer Link 48 is moved or adjusted on the counter. Discard Autostainer slide racks that fail level testing in any slide position and/or have undergone ≥ 175 PT Link pretreatment cycles.

All of the required steps and incubation times for staining are preprogrammed in the DakoLink software. Please refer to the User Guides for Autostainer Link 48 and PT Link for further information on programming protocols and loading slides and reagents.

Note: The reagents and instructions supplied in this system have been designed for optimal performance when used with the recommended reagents and materials. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.

Staining protocol
Please select the PD-L1 IHC 22C3 pharmDx staining protocol from the options in the DakoLink drop down menu.

All of the required steps and incubation times for staining are preprogrammed in the Autostainer Link 48. If the appropriate PD-L1 IHC 22C3 pharmDx protocols are not on your server, please contact your local Technical Service Representative or Agilent Pathology Support to obtain the protocols.

Step 1: Deparaffinization, rehydration, and target retrieval (3-in-1) procedure (specimen pretreatment)
For details, please refer to the PT Link User Guide.
Set PT Link (Code PT100/PT101/PT200) Preheat and Cool to 65 °C. Set Heat to 97 °C for 20 minutes.

- ►Fill PT Link tanks with 1.5 L per tank of 1x EnVision FLEX Target Retrieval Solution, Low pH (working solution) to cover the tissue sections.
- ►Preheat the 1x EnVision FLEX Target Retrieval Solution, Low pH to 65 °C.
- ►Immerse Autostainer slide racks containing mounted, FFPE tissue sections into the pre-heated 1x EnVision FLEX Target Retrieval Solution, Low pH (working solution) in PT Link tank. Incubate for 20 minutes at 97 °C.
- ►When target retrieval incubation has been completed and the temperature has cooled to 65 °C, remove each Autostainer slide rack with the slides from the PT Link tank and **immediately** place the Autostainer rack with slides into a tank (e.g., PT Link Rinse Station, Code PT109) containing diluted (1x), room temperature EnVision FLEX Wash Buffer (Code K8007).
- ►Incubate slides in diluted (1x), room temperature EnVision FLEX Wash Buffer for 5 minutes.

Step 2: Staining procedure
After deparaffinization, rehydration and target retrieval (3-in-1) procedure (specimen pretreatment), the Autostainer racks with slides should be placed one rack at a time on the Autostainer Link 48. Prior to initiating the staining procedure on the Autostainer Link 48, 1x EnVision FLEX Wash Buffer should be manually applied to the slides using a wash bottle for prevention of tissue drying. 1x EnVision FLEX Wash Buffer should not be applied directly on the tissue section, but applied sufficiently to the slide so that the tissue section is amply covered. Slides should remain wet prior to the initiation of the staining procedure. The instrument will perform the staining process by applying the appropriate reagent, monitoring the incubation time and rinsing slides between reagents. The reagent times are preprogrammed in the DakoLink software. Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure.

Step 3: Counterstain
Slides should be counterstained for 5 minutes with EnVision FLEX Hematoxylin (Link) (Code K8008). The EnVision FLEX Hematoxylin (Link) incubation time is preprogrammed in the protocol.

Step 4: Mounting
Nonaqueous, permanent mounting medium is required.

Note: Some fading of stained slides may occur, depending on several factors including, but not limited to, counterstaining, mounting materials and methods, and slide storage conditions. To minimize fading, store slides in the dark at room temperature (20–25 °C).

11. Quality Control

Reagents in PD-L1 IHC 22C3 pharmDx have been quality controlled by immunohistochemistry using the target retrieval and staining procedures outlined in 'Staining Procedure on the Autostainer Link 48 Solution' Section 10. Deviations in the recommended procedures for tissue fixation, processing and embedding in the user's laboratory may produce significant variability in results. Quality controls should be included in each staining run. These quality controls are specified in Table 16 and include: a lab-supplied H&E stained patient tissue specimen; kit-supplied Control Cell Line Slide; and lab-supplied positive and negative control tissues.[17] Consult the guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry[18]; see also CLSI Quality Assurance for Immunohistochemistry, Approved Guideline[19] for additional information.

12. Assay Verification

Prior to initial use of a staining system in a diagnostic procedure, the user should verify the assay's performance by testing it on a series of lab-supplied tissues with known IHC performance characteristics representing known positive and negative tissues. Refer to the quality

control procedures outlined in 'Quality Control' Section 11. These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Troubleshooting options for potential problems, their causes and suggested corrective actions are outlined in Table 51.

13. Staining and Scoring Interpretation

13.1 NSCLC – PD-L1 expression determined by Tumor Proportion Score

All viable tumor cells on the entire tissue section must be evaluated and included in the PD-L1 scoring 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 should be performed by a pathologist using a light microscope. For evaluation of the immunohistochemical staining and scoring, an objective of 10–40x magnification is appropriate. Any perceptible membrane staining of tumor cells should be included in the scoring.

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

$$\text{TPS (\%)} = \frac{\text{\# PD-L1 staining cells (tumor cells)}}{\text{Total \# of viable tumor cells}} \times 100$$

Score partial or complete cell membrane staining (≥ 1+) that is perceived distinct from cytoplasmic staining. Cytoplasmic staining should be considered nonspecific staining and is excluded in the assessment of staining intensity. Normal cells and tumor-associated immune cells such as infiltrating lymphocytes or macrophages ***should not*** be included in the scoring for the determination of PD-L1 expression level.

Table 2 provides details about which tissue elements are included in and excluded from determining the TPS.

Table 2. TPS inclusion/exclusion criteria for NSCLC

Tissue Elements	Included in TPS Scoring for NSCLC	Excluded from TPS Scoring for NSCLC
Tumor Cells	• Convincing partial or complete cell membrane staining (at any intensity) of viable tumor cells	• Exclude any cytoplasmic staining
Immune Cells	• Not included	Exclude any staining of immune cells, such as: • Mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages) • Plasma cells • Neutrophils
Other	• Not included	Exclude any staining of: • Normal cells adjacent to tumor cells • Stromal cells (fibroblasts) • Necrotic cells and/or cellular debris • Anthracotic pigment

For each staining run, slides should be examined in the order presented in Table 16 ('Slide Evaluation' Section 14) to determine the validity of the staining run and enable assessment of the staining of the sample tissue. Examine patient specimens stained with PD-L1 and the NCR from PD-L1 IHC 22C3 pharmDx when evaluating PD-L1 expression. Specimens stained with NCR must have 0 specific staining. Nonspecific staining, including nuclear staining, should be ≤ 1+.

The specimen should be evaluated for categories of PD-L1 expression per the table below. For treatment eligibility, refer to 'Intended Use' Section 1.

Table 3. NSCLC PD-L1 expression levels

	Tumor Proportion Score		
PD-L1 Expression Levels	TPS < 1%	TPS ≥ 1%	TPS ≥ 50%

Refer to PD-L1 IHC 22C3 pharmDx NSCLC Interpretation Manual for additional guidance.

13.2 ESCC, cervical cancer, HNSCC, TNBC, gastric or GEJ adenocarcinoma – PD-L1 expression determined by Combined Positive Score

All viable tumor cells on the entire tissue section must be evaluated and included in the PD-L1 expression assessment.

PD-L1 expression 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:

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

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.

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 presented in Table 16 ('Slide Evaluation' Section 14) to determine the validity of the staining run and enable assessment of the staining of the sample tissue. Examine patient specimens stained with PD-L1 and the NCR from PD-L1 IHC 22C3 pharmDx when evaluating PD-L1 expression. Specimens stained with NCR must have 0 specific staining. Nonspecific staining, including nuclear staining, should be ≤ 1+.

Refer to 'ESCC' Section 13.2.1, 'Cervical cancer' Section 13.2.2, 'HNSCC' Section 13.2.3, 'TNBC' Section 13.2.4, and 'Gastric or GEJ adenocarcinoma' Section 13.2.5 for tumor indication-specific information.

13.2.1 ESCC

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.

Tables 4 and 5 provide details about which tissue elements are included in and excluded from the CPS numerator and denominator, respectively, in ESCC.

Table 4. CPS numerator inclusion/exclusion criteria for ESCC

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 (including 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: o Lymphocytes (including lymphocyte aggregates) o Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Nonstaining MICs • MICs associated with noninvasive neoplasia (including carcinoma in situ) • MICs associated with benign structures • MICs (including lymphoid aggregates) not directly associated with the response to the tumor • Neutrophils, eosinophils and plasma cells
Other Cells	• Not included	• Benign epithelial cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

In **MICs membrane and cytoplasmic staining are often indistinguishable due to a 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 against the tumor should be excluded.*
****Macrophages and histiocytes are considered the same cells*.

Table 5. CPS denominator inclusion/exclusion criteria for ESCC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	• All viable invasive tumor cells	• Nonviable tumor cells • Noninvasive neoplasia (including 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 ≥ 10.

Refer to PD-L1 IHC 22C3 pharmDx ESCC Interpretation Manual for additional guidance.

13.2.2 Cervical cancer
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.

Tables 6 and 7 provide details about which tissue elements are included in and excluded from the CPS numerator and denominator, respectively, in cervical cancer.

Table 6. CPS numerator inclusion/exclusion criteria for cervical cancer

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 cervical tumor cells	• Nonstaining tumor cells • Tumor cells with only cytoplasmic staining
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*** • Only MICs directly associated with the response to the tumor are scored.	• Nonstaining MICs • MICs associated with cervical intraepithelial neoplasia (CIN I-III) • MICs associated with benign cells including squamous or glandular mucosa, cervical polyps, and microglandular hyperplasia • MICs (including lymphoid aggregates) associated with ulcers, and other processes not associated with the tumor such as cervicitis • Neutrophils, eosinophils and plasma cells
Other Cells	• Not included	• CIN I-III • Benign cells including squamous or glandular mucosa, cervical polyps, and microglandular hyperplasia • 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 CPS numerator.*
*****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 7. CPS denominator inclusion/exclusion criteria for cervical cancer

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	• All viable invasive tumor cells	• Any necrotic or nonviable tumor cells
Immune Cells	• Not included	• All immune cells of any type
Other Cells	• Not included	• CIN I-III • Benign cells including squamous or glandular mucosa, cervical polyps and microglandular hyperplasia • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

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

Table 8. Cervical cancer PD-L1 expression levels

Combined Positive Score		
PD-L1 Expression Level	CPS < 1	CPS ≥ 1
PD-L1 Expression Status	No PD-L1 Expression	PD-L1 Expression

Refer to PD-L1 IHC 22C3 pharmDx cervical cancer Interpretation Manual for additional guidance.

13.2.3 HNSCC
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.

Tables 9 and 10 provide details about which tissue elements are included in and excluded from the CPS numerator and denominator, respectively, in HNSCC.

Table 9. CPS numerator inclusion/exclusion criteria for HNSCC

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 • Carcinoma in situ (CIS)

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***
	• Only MICs directly associated with the response to the tumor are scored.
		• Nonstaining MICs
		• MICs (including lymphoid aggregates) associated with ulcers or other inflammatory processes
		• MICs associated with carcinoma in situ
		• MICs associated with benign structures
		• 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 10. CPS denominator inclusion/exclusion criteria for HNSCC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	• All viable invasive tumor cells	• Any necrotic or nonviable tumor cells • Carcinoma in situ (CIS)
Immune Cells	• Not included	• All immune cells of any type
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.

Table 11. HNSCC PD-L1 expression levels

	Combined Positive Score		
PD-L1 Expression Levels	CPS < 1	CPS ≥ 1	CPS ≥ 20

Refer to PD-L1 IHC 22C3 pharmDx HNSCC Interpretation Manual for additional guidance.

13.2.4 TNBC
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.

Tables 12 and 13 provide details about which tissue elements are included in and excluded from the CPS numerator and denominator, respectively, in TNBC.

Table 12. CPS numerator inclusion/exclusion criteria for TNBC

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 • Carcinoma in situ (DCIS and LCIS)
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*** • Only MICs directly associated with the response to the tumor are scored.	• Nonstaining MICs • MICs associated with DCIS and LCIS • MICs associated with benign structures • MICs (including lymphoid aggregates) not directly associated with the response to the tumor • Neutrophils, eosinophils, and plasma cells
Other Cells	• Not included	• Benign epithelial 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 against the tumor should be excluded.
***Macrophages and histiocytes are considered the same cells.

Table 13. CPS denominator inclusion/exclusion criteria for TNBC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	• All viable invasive tumor cells	• Nonviable tumor cells • Carcinoma in situ (DCIS and LCIS)
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 ≥ 10.

Refer to PD-L1 IHC 22C3 pharmDx TNBC Interpretation Manual for additional guidance.

13.2.5 Gastric or GEJ adenocarcinoma
A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide (biopsy and resection) for the specimen to be considered adequate for PD-L1 evaluation. If patient specimens include more than one biopsy (i.e., 3-5 endoscopic biopsies) on a slide, all tissues on the slide need to be evaluated to generate a single CPS for determining the PD-L1 expression level. Each biopsy should not be reported independently A study was performed to evaluate the equivalence of tumor resection versus biopsy specimens for assessment of PD-L1 status by the PD-L1 IHC 22C3 pharmDx in gastric cancer tissues. The results demonstrated that 90% of specimens assessed using 3-5 surrogate biopsies yielded concordant PD-L1 status when compared to the resection specimen.

Tables 14 and 15 provide details about which tissue elements are included in and excluded from the CPS numerator and denominator, respectively, in gastric or GEJ adenocarcinoma.

Table 14. CPS numerator inclusion/exclusion criteria for gastric or GEJ adenocarcinoma.

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 gastric or GEJ adenocarcinoma tumor cells	• Nonstaining tumor cells • Tumor cells with only cytoplasmic staining • Adenoma, 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**: ○ Lymphocytes (including lymphocyte aggregates) ○ Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Nonstaining MICs • MICs associated with adenoma, dysplasia, and carcinoma in situ • MICs (including lymphoid aggregates) associated with ulcers, chronic gastritis, and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils, eosinophils and plasma cells
Other Cells	Not included	• Normal cells (including ganglion 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 CPS numerator.*

***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 15. CPS denominator inclusion/exclusion criteria for gastric or GEJ adenocarcinoma.

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	• All viable invasive tumor cells (PD-L1 staining or nonstaining)	• Nonviable tumor cells • Adenoma, dysplasia, and carcinoma in situ
Immune Cells	Not included	• All immune cells
Other Cells	Not included	• Normal cells (including ganglion cells) • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

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

Refer to PD-L1 IHC 22C3 pharmDx gastric or GEJ adenocarcinoma Interpretation Manual for additional guidance.

14. Slide Evaluation

Table 16. Recommended order of slide evaluation

Specimens	Rationale	Requirements
1. H&E (Lab-supplied)	A hematoxylin and eosin (H&E) stain of the tissue specimen is evaluated first to assess tissue histology and preservation quality.	The PD-L1 IHC 22C3 pharmDx and H&E stain should be performed on serial sections from the same paraffin block of the specimen. Tissue specimens should be intact, well preserved, and should confirm tumor indication.
2. Control Cell Line Slide (Kit-supplied)	The Control Cell Line Slide stained with the PD-L1 primary antibody from PD-L1 IHC 22C3 pharmDx should be examined to ascertain that all reagents are functioning properly. The Control Cell Line Slide contains the PD-L1-positive cell line pellet and PD-L1-negative cell line pellet.	One Control Cell Line Slide should be stained with the PD-L1 Primary Antibody in each staining run. *NCI-H226 (PD-L1-positive control cell line) acceptance criteria:* • Cell membrane staining of ≥ 70% of cells. • ≥ 2+ average staining intensity of cells with membrane staining. • Nonspecific staining < 1+ intensity. *MCF-7 (PD-L1-negative control cell line) acceptance criteria:* • No cells with membrane staining.* • Nonspecific staining < 1+ intensity.* *Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤ 10 total cells with distinct cell membrane staining and/or nonspecific staining with ≥ 1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable. If either of the Control Cell Lines does not meet these criteria, all results with the patient specimens should be considered invalid.
3. Positive Control Tissue Slides (Lab-supplied)	The Positive Control Tissue Slides stained with both PD-L1 primary antibody and Negative Control Reagent should be examined next. These slides verify that the fixation method and epitope retrieval process are effective. Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, NOT as an aid in formulating a specific diagnosis of patient samples.	Tissue controls should be biopsy/surgical specimens, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Control tissue must represent one of the approved tumor indications for PD-L1 IHC 22C3 pharmDx as listed in Section 1, Intended Use. Use well-preserved specimens for interpretation of staining results as necrotic or degenerated cells often demonstrate nonspecific staining. The tissues selected for use as the positive tissue controls should give weak to moderate positive staining when stained with PD-L1 to aid in detection of subtle changes in assay sensitivity. Two positive tissue control slides should be included in each staining run. **Slide stained with PD-L1**: Presence of brown cell membrane staining should be observed. Nonspecific staining, including nuclear staining, should be ≤ 1+. **Slide stained with Negative Control Reagent**: No membrane staining. Nonspecific staining, including nuclear staining, should be ≤ 1+. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid.

Specimens	Rationale	Requirements
4. Negative Control Tissue Slides (Lab-supplied)	The Negative Control Tissue Slides (known to be PD-L1 negative) stained with both PD-L1 primary antibody and Negative Control Reagent should be examined next to verify the specificity of the labeling of the target antigen by the primary antibody. Alternatively, negative portions of the Positive Control Tissue may serve as the Negative Control Tissue, but this should be verified by the user.	Tissue controls should be biopsy/surgical specimens, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Control tissue must represent one of the approved tumor indications for PD-L1 IHC 22C3 pharmDx as listed in Section 1, Intended Use. Two negative tissue control slides should be included in each staining run. **Slide stained with PD-L1**: No membrane staining in tumor cells. Nonspecific staining, including nuclear staining, should be ≤ 1+. **Slide stained with Negative Control Reagent**: No membrane staining. Nonspecific staining, including nuclear staining, should be ≤ 1+. If specific cell membrane staining occurs in the lab-supplied Negative Control Tissue Slides, results with the patient specimen should be considered invalid.
5. Tonsil Control Tissue (optional) (Lab-supplied)	Use human tonsil tissue fixed, processed and embedded in a manner similar to the patient sample(s) as an additional control material to verify sensitivity, specificity and nonspecific staining of the assay.	Strong positive staining should be detected in portions of the crypt epithelium and weak to moderate staining of the follicular macrophages in the germinal centers. Negative staining should be observed in endothelium, fibroblasts as well as surface epithelium.
6. Patient tissue slide stained using the Negative Control Reagent	Examine patient specimens stained with the Negative Control Reagent from PD-L1 IHC 22C3 pharmDx. Negative Control Reagent is used in place of the primary antibody and aids in interpretation of specific staining at the antigen site.	Absence of cell membrane staining verifies the specific labeling of the target antigen by the primary antibody. Nonspecific staining, including nuclear staining, should be ≤ 1+.
7. Patient tissue slide stained using the PD-L1 primary antibody	Examine the entire slide of the patient specimens stained with the PD-L1 primary antibody from PD-L1 IHC 22C3 pharmDx last.	Positive staining intensity should be assessed within the context of any nonspecific staining observed on the patient's Negative Control Reagent slide in the same run. Nonspecific staining, including nuclear staining, should be ≤ 1+. As with any immunohistochemical test, a negative result means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed. All viable tumor cells on the entire PD-L1 stained patient slide must be evaluated and included in the PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present for the specimen to be considered adequate for PD-L1 evaluation. Refer to 'Staining and Scoring Interpretation' Section 13 for scoring interpretation guidelines in PD-L1 expression.

15. Limitations

15.1 General limitations

1. For prescription use only.
2. Immunohistochemistry is a multi-step diagnostic process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
3. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false-negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
4. Tissue drying after specimen pretreatment may lead to appearance of > 1+ nonspecific staining, including nuclear staining, on the PD-L1 and/or NCR-stained slides.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of PD-L1 staining must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
7. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[20]
8. Reagents may demonstrate unexpected reactions in previously untested tissue types. The possibility of unexpected reactions even in tested tissue types cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Agilent Pathology Support with documented unexpected reactions.

9. False-positive results may be seen due to nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C).[17]
10. The reagents and instructions supplied in this system have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.

15.2 Product-specific limitations

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Specimens should be stained within the cut section storage recommendations (refer to 'Cut section storage recommendation' Section 8.2).
2. For optimal and reproducible results, the PD-L1 protein requires target retrieval pretreatment when tissues are routinely fixed (neutral buffered formalin) and paraffin embedded.
3. Do not substitute reagents from different lot numbers of this product, or from kits of other manufacturers. The only exception is the EnVision FLEX Target Retrieval Solution, Low pH (50x), which, if required, is available as Code K8005.
4. Stained control cell lines should be used only for validation of the staining run and should not be used to score the staining reaction in tissue sections.
5. Use of PD-L1 IHC 22C3 pharmDx on tissues with fixatives other than 10% neutral buffered formalin has not been validated.
6. Use of PD-L1 IHC 22C3 pharmDx on fine needle aspirates has not been validated.
7. Use of PD-L1 IHC 22C3 pharmDx on decalcified tissues has not been validated.
8. Clinicians should use caution when interpreting test results at the CPS ≥ 20 cutoff, because PD-L1 IHC 22C3 pharmDx failed to meet pre-specified acceptance criteria for positive percent agreement in two independent inter-site reproducibility studies and overall percent agreement in one inter-site reproducibility study conducted on HNSCC specimens at the CPS ≥ 20 cutoff. All pre-specified acceptance criteria were met in the independent inter-site reproducibility study conducted on HNSCC specimens at the CPS ≥ 1 cutoff.
9. Laboratories should pay particular attention to the pH of the 1x EnVision FLEX Target Retrieval Solution, Low pH for pretreatment of ESCC specimens as pH 5.9 may affect PD-L1 staining performance.
10. The studies carried out to assess 1x EnVision FLEX Target Retrieval Solution, Low pH use up to 3 times in esophageal cancer did not meet acceptance criteria for qualitative evaluation of PD-L1 expression status, therefore 1x EnVision FLEX Target Retrieval Solution, Low pH reuse is not recommended for ESCC specimens.
11. If PD-L1 expression is being evaluated in endoscopic biopsies of gastric or GEJ adenocarcinoma a minimum of 3-5 biopsies is recommended.

16. Performance Evaluation

16.1 Nonclinical performance evaluation: normal and neoplastic tissues

Normal tissues: Table 17 summarizes monoclonal mouse anti-PD-L1, Clone 22C3, immunoreactivity on the recommended panel of normal tissues. Cell membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. All tissues were FFPE and stained with PD-L1 IHC 22C3 pharmDx according to the instructions in this package insert. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in normal tissues.[21,23]

Table 17. Summary of PD-L1 IHC 22C3 pharmDx normal tissue reactivity

Tissue Type (# tested)	Positive Cell Membrane Staining: Tissue Elements	Positive Cytoplasmic Staining: Tissue Elements	Nonspecific Staining
Adrenal (3)	0/3	1/3 Medullary cells	0/3
Bladder (3)	0/3	0/3	0/3
Bone marrow (3)	3/3 Megakaryocytes	3/3 Megakaryocytes	0/3
Breast (3)	0/3	0/3	0/3
Cerebellum (3)	0/3	0/3	0/3
Cerebrum (3)	0/3	0/3	0/3
Cervix (3)	1/3 Epithelium	0/3	0/3
Colon (3)	2/3 Macrophages	0/3	0/3
Esophagus (3)	0/3	0/3	0/3
Kidney (3)	1/3 Tubular epithelium	0/3	0/3
Liver (3)	1/3 Macrophages 1/3 Hepatocytes	0/3	0/3
Lung (3)	3/3 Alveolar macrophages	0/3	0/3
Mesothelial cells (3)	0/3	0/3	0/3
Muscle, cardiac (3)	0/3	0/3	0/3
Muscle, skeletal (3)	0/3	0/3	0/3
Nerve, peripheral (3)	0/3	1/3 Connective tissue/vessels	0/3
Ovary (3)	0/3	0/3	0/3
Pancreas (3)	0/3	0/3	0/3
Parathyroid (3)	1/3 Glandular epithelium	0/3	0/3
Pituitary (3)	1/3 Anterior hypophysis 1/3 Posterior hypophysis	1/3 Anterior hypophysis 1/3 Posterior hypophysis	0/3
Prostate (3)	3/3 Epithelium	0/3	0/3
Salivary gland (3)	0/3	0/3	0/3
Skin (3)	0/3	0/3	0/3
Small intestine (3)	0/3	0/3	0/3
Spleen (3)	2/3 Macrophages	0/3	0/3
Stomach (3)	2/3 Lymphocytes 1/3 Gastric glands	1/3 Gastric glands	0/3

Tissue Type (# tested)	Positive Cell Membrane Staining: Tissue Elements	Positive Cytoplasmic Staining: Tissue Elements	Nonspecific Staining
Testis (3)	0/3	0/3	0/3
Thymus (3)	3/3 Medullary epithelium	0/3	0/3
Thyroid (3)	0/3	0/3	0/3
Tonsil (3)	3/3 Crypt epithelium 2/3 Germinal center (macrophages)	0/3	0/3
Uterus (3)	0/3	0/3	0/3

Neoplastic tissues: Table 18 summarizes monoclonal mouse anti-PD-L1, Clone 22C3, immunoreactivity on a panel of neoplastic tissues. Cell membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. All tissues were FFPE and stained with PD-L1 IHC 22C3 pharmDx according to the instructions in this package insert. There were no unexpected results observed in the tumor specimens tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in neoplastic tissues.[21-24]

Table 18. Summary of PD-L1 IHC 22C3 pharmDx neoplastic tissue reactivity

Tumor Type	Location	PD-L1 positive/total N=159
Adenocarcinoma	Appendix	0/1
	Breast, DCIS	0/2
	Breast, invasive ductal	0/7
	Breast, invasive ductal metastatic to lymph node	0/1
	Cervix, endocervical type	0/1
	Colon	0/5
	Colon, metastatic to liver	0/1
	Colon, mucinous	0/1
	Esophagus	0/1
	Gallbladder	1/5
	GI, metastatic to lung	0/1
	Head & neck, hard palate	0/1
	Lung	1/4
	Ovary	0/1
	Ovary, endometrioid	0/1
	Ovary, mucinous	0/1
	Ovary, serous	0/1
	Pancreas	0/2
	Pancreas, ductal	0/3
	Prostate	0/5
	Rectum	0/4
	Salivary/parotid gland	0/2
	Small intestine	0/2
	Stomach	0/6
	Stomach, mucinous	0/1
	Thyroid, follicular	0/1
	Thyroid, follicular-papillary	0/1
	Thyroid, papillary	0/3
	Uterus, clear cell	0/1
	Uterus, endometrium	0/3
Adrenocortical carcinoma	Adrenal	0/1
Astrocytoma	Cerebrum	0/3
Basal cell carcinoma	Skin	0/1
Carcinoma	Nasopharyngeal, NPC	0/1
Chondrosarcoma	Bone	0/1
Chordoma	Pelvic cavity	0/1
Embryonal carcinoma	Testis	0/1
Ependymoma	Brain	0/1
Gastrointestinal stromal tumor	Colon	0/1
	Rectum	0/1
	Small intestine	0/1
Glioblastoma	Brain	0/1
Hepatoblastoma	Liver	0/1
Hepatocellular carcinoma	Liver	0/5
Islet cell tumor	Pancreas	0/1
Leiomyosarcoma	Soft tissue, chest wall	0/1
	Bladder	0/1
Lymphoma		
Anaplastic large cell	Lymph node	0/1
Diffuse B-cell	Lymph node	0/4
Hodgkin	Lymph node	2/2
Non-Hodgkin	Lymph node	1/1

Tumor Type	Location	PD-L1 positive/total N=159
Medulloblastoma	Brain	0/1
Medullary carcinoma	Thyroid	0/1
Melanoma	Rectum	0/1
	Nasal cavity	0/1
Meningioma	Brain	0/2
Mesothelioma	Peritoneum	0/1
Neuroblastoma	Retroperitoneum	0/1
Neurofibroma	Soft tissue, lower back	0/1
Osteosarcoma	Bone	0/2
Pheochromocytoma	Adrenal	0/1
Primitive neuroectodermal tumor (PNET)	Retroperitoneum	0/1
Renal cell carcinoma		
Papillary	Kidney	0/1
Clear cell	Kidney	0/6
Rhabdomyosarcoma	Soft tissue, embryonal	0/1
	Prostate	0/1
	Retroperitoneum	0/1
Seminoma	Testis	0/2
Signet ring adenocarcinoma	Metastatic colon signet ring cell carcinoma to ovary	0/1
	Colon	0/1
Small cell carcinoma	Lung	0/1
Spermatocytoma	Testis	0/2
Squamous cell carcinoma	Metastatic esophageal squamous cell carcinoma to lymph node	0/1
	Cervix	2/5
	Esophagus	0/7
	Head & neck	0/2
	Lung	1/2
	Skin	0/2
	Uterus	0/1
Synovial sarcoma	Pelvic cavity	0/1
Thymoma	Mediastinum	1/1
Urothelial carcinoma	Bladder	0/6
	Kidney	0/1

16.2 Nonclinical performance evaluation: NSCLC

Analytical sensitivity/specificity: NSCLC
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 127 unique cases of NSCLC FFPE specimens staged I to IV using a manufactured production lot. Assessment of PD-L1 expression demonstrated staining across a range of 0–100% positive tumor cells and 0–3 staining intensity.

Precision: NSCLC
The precision of PD-L1 IHC 22C3 pharmDx was evaluated at Agilent. Average negative percent agreement (ANA), average positive percent agreement (APA), and overall percent agreement (OA) were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff. For studies which resulted in 100.0% agreement, negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with corresponding two-sided 95% Wilson score confidence intervals for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff.

Table 19. Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 1%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-operator	TPS ≥ 1%	All 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 6 analysts using 1 Autostainer Link 48 instrument.	NPA 100.0% (93.9–100.0%) PPA 100.0% (94.0–100.0%) OA 100.0% (96.9–100.0%)
Inter-instrument	TPS ≥ 1%	All 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested using 6 Autostainer Link 48 instruments.	NPA 100.0% (94.0–100.0%) PPA 100.0% (94.0–100.0%) OA 100.0% (96.9–100.0%)
Inter-lot	TPS ≥ 1%	All 24 NSCLC specimens (13 PD-L1-negative and 11 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 3 replicates and 3 reagent lots using the Autostainer Link 48 instrument.	ANA 98.3% (95.9–100.0%) APA 97.9% (94.6–100.0%) OA 98.1% (95.3–100.0%)
Inter-day	TPS ≥ 1%	All 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 6 nonconsecutive days using the Autostainer Link 48 instrument.	NPA 100.0% (94.0–100.0%) PPA 100.0% (94.0–100.0%) OA 100.0% (96.9–100.0%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Intra-run (Repeatability)	TPS ≥ 1%	All 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 6 replicates within a run using the Autostainer Link 48 instrument.	NPA 100.0% (94.0–100.0%) PPA 100.0% (93.8–100.0%) OA 100.0% (96.8–100.0%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement
ANA=Average Negative Percent Agreement; APA=Average Positive Percent Agreement; TPS=Tumor Proportion Score

Table 20. Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 50%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-operator	TPS ≥ 50%	All 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 6 analysts using 1 Autostainer Link 48 instrument.	NPA 100.0% (92.7–100.0%) PPA 100.0% (88.6–100.0%) OA 100.0% (95.4–100.0%)
Inter-instrument	TPS ≥ 50%	All 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression were tested using 6 Autostainer Link 48 instruments.	NPA 100.0% (92.9–100.0%) PPA 100.0% (88.6–100.0%) OA 100.0% (95.4–100.0%)
Inter-lot	TPS ≥ 50%	All 16 NSCLC specimens (8 PD-L1-negative and 8 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 3 replicates and 3 reagent lots using the Autostainer Link 48 instrument.	NPA 100.0% (92.6–100.0%) PPA 100.0% (92.6–100.0%) OA 100.0% (96.2–100.0%)
Inter-day	TPS ≥ 50%	All 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 6 nonconsecutive days using the Autostainer Link 48 instrument.	NPA 100.0% (92.9–100.0%) PPA 100.0% (88.6–100.0%) OA 100.0% (95.4–100.0%)
Intra-run (Repeatability)	TPS ≥ 50%	All 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 6 replicates within a run using the Autostainer Link 48 instrument.	NPA 100.0% (92.9–100.0%) PPA 100.0% (88.6–100.0%) OA 100.0% (95.4–100.0%)
Intra-day	TPS ≥ 50%	All 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 2 runs within a day, repeated over 3 days, using the Autostainer Link 48 instrument.	NPA 100.0% (88.3–100.0%) PPA 100.0% (82.4–100.0%) OA 100.0% (92.4–100.0%)

NPA=Negative Percent Agreement; PPA=Positive Percent Agreement; OA=Overall Percent Agreement; TPS=Tumor Proportion Score

External reproducibility: NSCLC
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external testing sites. Average percent agreements were calculated since no natural reference exists in reproducibility parameters such as site and observer. ANA, APA, and OA were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff.

Table 21. Reproducibility of PD-L1 IHC 22C3 pharmDx tested at three external sites (TPS ≥ 1%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	TPS ≥ 1%	All 36 NSCLC specimens (16 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 2700 pair-wise comparisons.	ANA 94.8% (90.3–98.4%) APA 95.5% (91.2–98.7%) OA 95.2% (90.8–98.6%)
Intra-site	TPS ≥ 1%	All 36 NSCLC specimens (16 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 1080 pair-wise comparisons.	ANA 96.2% (94.1–97.5%) APA 96.7% (95.0–97.9%) OA 96.5% (95.2–97.4%)
Inter-observer	TPS ≥ 1%	All 62 NSCLC specimens (28 PD-L1-negative and 34 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 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 1674 pair-wise comparisons.	ANA 85.8% (79.3–91.8%) APA 88.2% (82.2–93.3%) OA 87.1% (81.0–92.6%)
Intra-observer	TPS ≥ 1%	All 62 NSCLC specimens (28 PD-L1-negative and 34 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 nonconsecutive days.	ANA 93.7% (90.0–96.1%) APA 94.8% (91.6–96.7%) OA 94.3% (92.0–95.9%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
		Intra-observer analysis was performed for 3 sites on a total of 558 pair-wise comparisons.	

ANA=Average Negative Percent Agreement; APA=Average Positive Percent Agreement; OA=Overall Percent Agreement; TPS=Tumor Proportion Score

Table 22. Reproducibility of PD-L1 IHC 22C3 pharmDx tested at three external sites (TPS ≥ 50%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	TPS ≥ 50%	All 36 NSCLC specimens (21 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 2700 pair-wise comparisons.	ANA 90.3% (84.4–95.2%) APA 85.2% (75.6–92.9%) OA 88.3% (81.4–94.3%)
Intra-site	TPS ≥ 50%	All 36 NSCLC specimens (21 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 1080 pair-wise comparisons.	ANA 91.9% (88.8–94.8%) APA 87.6% (82.5–92.2%) OA 90.2% (86.3–93.7%)
Inter-observer	TPS ≥ 50%	All 62 NSCLC 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 1674 pair-wise comparisons.	ANA 92.6% (87.8–96.7%) APA 92.8% (88.1–96.8%) OA 92.7% (88.1–96.8%)
Intra-observer	TPS ≥ 50%	All 62 NSCLC 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 558 pair-wise comparisons.	ANA 96.4% (94.0–98.5%) APA 96.5% (94.3–98.6%) OA 96.4% (94.3–98.6%)

ANA=Average Negative Percent Agreement; APA=Average Positive Percent Agreement; OA=Overall Percent Agreement; TPS=Tumor Proportion Score

16.3 Clinical performance evaluation: NSCLC (KEYTRUDA)

KEYNOTE-042: First-line treatment of metastatic NSCLC as a single agent

The efficacy of KEYTRUDA was investigated in KEYNOTE-042 (NCT02220894), a randomized, multicenter, open-label, active-controlled trial conducted in 1274 patients with stage III NSCLC, who were not candidates for surgical resection or definitive chemoradiation, or metastatic NSCLC, whose tumors expressed PD-L1 (TPS ≥ 1%) by an immunohistochemistry assay using PD-L1 IHC 22C3 pharmDx, and who had not received prior systemic treatment for metastatic NSCLC.[3] Patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of radiation in the thoracic region within the prior 26 weeks of initiation of study were ineligible. Randomization was stratified by ECOG performance status (0 vs. 1), histology (squamous vs. nonsquamous), geographic region (East Asia vs. non-East Asia), and PD-L1 expression (TPS ≥ 50% vs. TPS 1 to 49%). Patients were randomized (1:1) to receive KEYTRUDA 200 mg intravenously every 3 weeks or investigator's choice of either of the following platinum-containing chemotherapy regimens:

- Pemetrexed 500 mg/m^2 every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for a maximum of 6 cycles followed by optional pemetrexed 500 mg/m^2 every 3 weeks for patients with nonsquamous histologies;
- Paclitaxel 200 mg/m^2 every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for a maximum of 6 cycles followed by optional pemetrexed 500 mg/m^2 every 3 weeks for patients with nonsquamous histologies.

Treatment with KEYTRUDA continued until RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ)-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 deriving clinical benefit as determined by the investigator. Treatment with KEYTRUDA could be reinitiated at the time of subsequent disease progression and administered for up to 12 months. Assessment of tumor status was performed every 9 weeks. The main efficacy outcome measure was OS. Additional efficacy outcome measures were PFS and ORR as assessed by a BICR review according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ.

The study population characteristics were: median age of 63 years (range: 25 to 90), 45% age 65 or older; 71% male; 64% White, 30% Asian, and 2% Black. Nineteen percent were Hispanic or Latino. Sixty-nine percent had ECOG performance status of 1; 39% with squamous and 61% with nonsquamous histology; 87% with M1 disease and 13% with Stage IIIA (2%) or Stage IIIB (11%) who were not candidates for surgical resection or definitive chemoradiation per investigator assessment; and 5% with treated brain metastases at baseline. Forty-seven percent of patients had TPS ≥ 50% NSCLC and 53% had TPS 1 to 49% NSCLC.

The trial demonstrated a statistically significant improvement in OS for patients randomized to KEYTRUDA as compared with chemotherapy. Table 23 and Figure 1 summarize the efficacy results in the subgroup of patients with TPS ≥ 50% and in all randomized patients with TPS ≥ 1%.

Table 23. Efficacy results of all randomized patients (TPS ≥ 1% and TPS ≥ 50%) in KEYNOTE-042

	TPS ≥ 1%		TPS ≥ 50%	
Endpoint	**KEYTRUDA 200 mg every 3 weeks n=637**	**Chemotherapy n=637**	**KEYTRUDA 200 mg every 3 weeks n=299**	**Chemotherapy n=300**
OS				
Number of events (%)	371 (58%)	438 (69%)	157 (53%)	199 (66%)
Median in months (95% CI)	16.7 (13.9, 19.7)	12.1 (11.3, 13.3)	20.0 (15.4, 24.9)	12.2 (10.4, 14.2)
Hazard ratio* (95% CI)	0.81 (0.71, 0.93)		0.69 (0.56, 0.85)	
p-Value†	0.0036		0.0006	
PFS				
Number of events (%)	507 (80%)	506 (79%)	221 (74%)	233 (78%)
Median in months (95% CI)	5.4 (4.3, 6.2)	6.5 (6.3, 7.0)	7.1 (5.9, 9.0)	6.4 (6.1, 6.9)
Hazard ratio*,‡ (95% CI)	1.07 (0.94, 1.21)		0.81 (0.67, 0.99)	
p-Value†	- ‡		NS§	
Objective Response Rate				
ORR‡ (95% CI)	27% (24, 31)	27% (23, 30)	39% (33.9, 45.3)	32% (26.8, 37.6)
Complete response rate	0.5%	0.5%	0.7%	0.3%
Partial response rate	27%	26%	39%	32%
Duration of Response				
% with duration ≥ 12 months¶	47%	16%	42%	17%
% with duration ≥ 18 months¶	26%	6%	25%	5%

* Based on the stratified Cox proportional hazard model

† Based on a stratified log-rank test; compared to a p-Value boundary of 0.0291

‡ Not evaluated for statistical significance as a result of the sequential testing procedure for the secondary endpoints

§ Not significant compared to a p-Value boundary of 0.0291

¶ Based on observed duration of response

In a pre-specified exploratory subgroup analysis for patients with TPS 1–49% NSCLC, the median OS was 13.4 months (95% CI: 10.7, 18.2) for the pembrolizumab group and 12.1 months (95% CI: 11.0, 14.0) in the chemotherapy group, with an HR of 0.92 (95% CI: 0.77, 1.11).

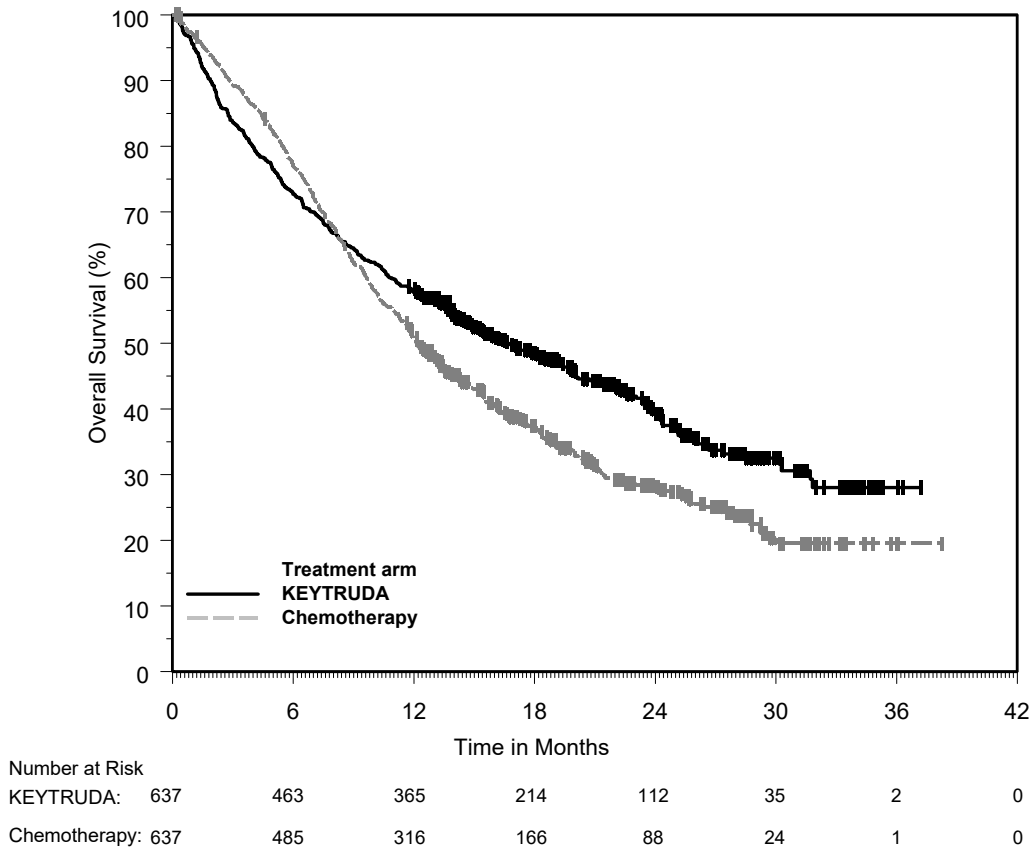


Figure 1. Kaplan-Meier curve for overall survival in all randomized patients in KEYNOTE-042 (TPS ≥ 1%)

KEYNOTE-024: Controlled trial of first-line treatment of patients with NSCLC

The efficacy of KEYTRUDA was investigated in Trial 24, a randomized (1:1), open-label, multicenter, controlled trial.[4] Key eligibility criteria were metastatic NSCLC, PD-L1 expression TPS of 50% or greater by an immunohistochemistry assay using PD-L1 IHC 22C3 pharmDx, and no prior systemic treatment for metastatic NSCLC. Patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Patients were randomized to receive KEYTRUDA 200 mg every 3 weeks (n=154) or investigator's choice platinum-containing chemotherapy (n=151; including pemetrexed + carboplatin, pemetrexed + cisplatin, gemcitabine + cisplatin, gemcitabine + carboplatin, or paclitaxel + carboplatin. Non-squamous patients could receive pemetrexed maintenance). Patients were treated with KEYTRUDA until unacceptable toxicity or disease progression, or up to 35 administrations. Subsequent disease progression could be retreated for up to 1 additional year. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving clinical benefit by the investigator. Assessment of tumor status was performed every 9 weeks. Patients on chemotherapy who experienced progression of disease were offered KEYTRUDA.

Among the 305 patients in Trial 24, baseline characteristics were: median age 65 years (54% age 65 or older); 61% male; 82% White and 15% Asian; and 35% and 65% with an ECOG performance status 0 and 1, respectively. Disease characteristics were squamous (18%) and non-squamous (82%); M1 (99%); and brain metastases (9%).

The major efficacy outcome measure was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST 1.1). Additional efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST 1.1. Table 24 summarizes key efficacy measures for the entire intent to treat (ITT) population.

Table 24. Efficacy results in Trial 24

Endpoint	KEYTRUDA 200 mg every 3 weeks n=154	Chemotherapy n=151
PFS*		
Number (%) of patients with event	73 (47%)	116 (77%)
Hazard ratio† (95% CI)	0.50 (0.37, 0.68)	---
p-Value‡	<0.001	---
Median in months (95% CI)	10.3 (6.7, NA)	6.0 (4.2, 6.2)
OS		
Number (%) of patients with event	44 (29%)	64 (42%)
Hazard ratio† (95% CI)	0.60 (0.41, 0.89)	---
p-Value‡	0.005	---
Median in months (95% CI)	Not reached (NA, NA)	Not reached (9.4, NA)
Objective Response Rate*		
ORR % (95% CI)	45% (37, 53)	28% (21, 36)
Complete response %	4%	1%
Partial response %	41%	27%

* Assessed by BICR using RECIST 1.1
† Hazard ratio (KEYTRUDA compared to chemotherapy) based on the stratified Cox proportional hazard model
‡ Based on stratified Log rank test
NA = not available

Among the 69 patients randomized to KEYTRUDA 200 mg with an objective response, response durations ranged from 1.9+ to 14.5+ months. Eighty-eight percent of these responders had a response duration of 6 months or longer (based on Kaplan-Meier estimation; Figure 2).

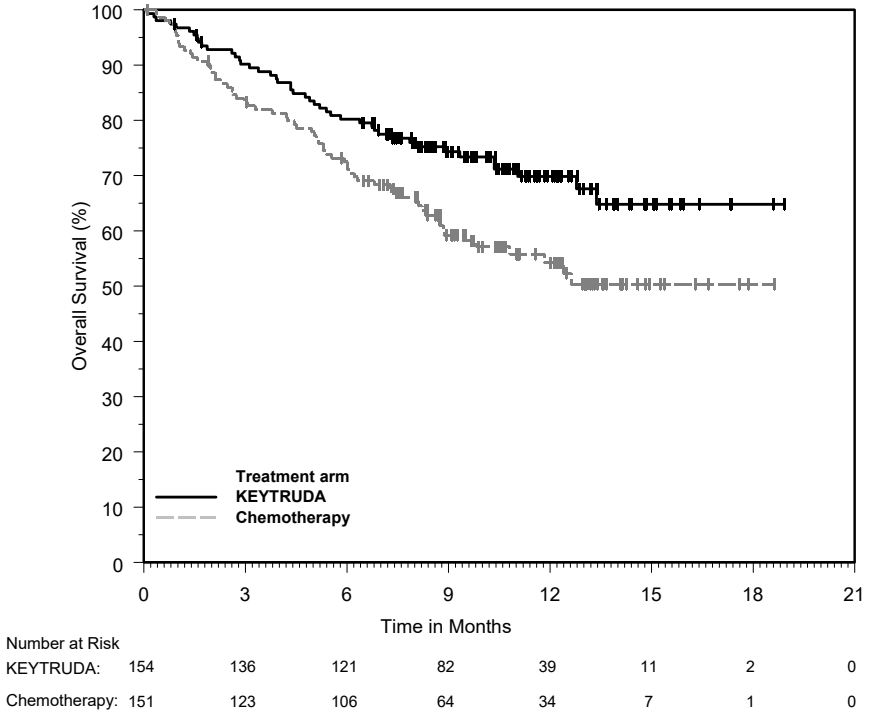


Figure 2. Kaplan-Meier curve for overall survival in Trial 24

KEYNOTE-010: Controlled trial of NSCLC patients previously treated with chemotherapy

The efficacy of KEYTRUDA was investigated in Trial 10, a randomized (1:1), open-label, multicenter, controlled trial.[5] Key eligibility criteria were advanced NSCLC that had progressed following platinum-containing chemotherapy, and if appropriate, targeted therapy for ALK or EGFR mutations, and PD-L1 expression TPS of 1% or greater by a clinical trial assay version of PD-L1 IHC 22C3 pharmDx (CTA). Forty-four and 56 percent of patients were enrolled based on testing of an archival tumor sample or a new tumor sample, respectively. Patients with autoimmune disease; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Patients were randomized (1:1:1) to receive 2 mg/kg (n=344) or 10 mg/kg (n=346) of KEYTRUDA every 3 weeks or 75 mg/m^2 of docetaxel every 3 weeks (n=343). Patients were treated with KEYTRUDA until unacceptable toxicity or disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in

performance status, or was confirmed at 4 to 6 weeks with repeat imaging. Patients without disease progression were treated for up to 24 months or 35 administrations, whichever was longer. Subsequent disease progression could be retreated for up to 1 additional year. Assessment of tumor status was performed every 9 weeks. The primary efficacy outcome measures were OS and PFS as assessed by BICR using RECIST 1.1.

Based on the CTA, a total of 1,033 NSCLC patients were randomized in the study. To evaluate the clinical utility of PD-L1 IHC 22C3 pharmDx, archived clinical study samples were retrospectively tested at a USA-based reference laboratory with PD-L1 IHC 22C3 pharmDx. Out of the 1,033 patients, tumor tissue from 529 patients was retrospectively tested with PD-L1 IHC 22C3 pharmDx. Specimens from 413 patients had PD-L1 expression TPS ≥ 1% and samples from 94 patients did not have PD-L1 expression (TPS < 1%). In these 413 patients with PD-L1 expression TPS ≥ 1%, 163 patients had PD-L1 expression TPS ≥ 50%.

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 25.

Table 25. CTA vs. PD-L1 IHC 22C3 pharmDx agreement

Agreement Rates	PD-L1 Cutoff	Negative Percent Agreement (95% Confidence Interval [CI])	Positive Percent Agreement (95% Confidence Interval [CI])
CTA vs. PD-L1 IHC 22C3 pharmDx	TPS ≥ 1%	94.5% [91.4–96.6%]	80.0% [76.9–82.8%]
	TPS ≥ 50%	98.3% [97.1–99.0%]	73.2% [67.9–77.9%]

Among randomized patients having PD-L1 expression TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, the demographic and other baseline characteristics were well balanced between the treatment arms. The median age was 63 years (44% age 65 or older). The majority of patients were white (77%) and male (58%); baseline ECOG performance status was 0 (29%) or 1 (71%). Seventy-eight percent (78%) of patients were former/current smokers. Twenty-two percent (22%) of patients had squamous histology and 69% had non-squamous histology. The baseline and demographic characteristics were similarly well balanced across pembrolizumab and docetaxel arms in the overall clinical study.

Efficacy results are summarized in Tables 26 and 27. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS ≥ 1%), which was enhanced in patients with PD-L1 expression TPS ≥ 50%, as determined by PD-L1 IHC 22C3 pharmDx. The magnitude of benefit was comparable to that in the overall clinical trial. Tables 26 and 27 summarize key efficacy measures in the overall population with PD-L1 expression TPS ≥ 1% and in the subpopulation with PD-L1 expression TPS ≥ 50% for the overall clinical study (PD-L1 expression TPS ≥ 1% based on CTA) and in the population with PD-L1 expression determined by PD-L1 IHC 22C3 pharmDx. The Kaplan-Meier curve for OS (TPS ≥ 1%), as determined by PD-L1 IHC 22C3 pharmDx, is shown in Figure 3. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 26. Response to KEYTRUDA in previously treated NSCLC patients: overall clinical trial and patients with PD-L1 expression, TPS ≥ 1%, as determined by PD-L1 IHC 22C3 pharmDx

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	344	140	346	142	343	131
OS						
Deaths (%)	172 (50%)	59 (42%)	156 (45%)	59 (42%)	193 (56%)	67 (51%)
Hazard ratio* (95% CI)	0.71 (0.58, 0.88)	0.54 (0.37, 0.78)	0.61 (0.49, 0.75)	0.57 (0.39, 0.82)	---	---
p-Value†	<0.001	<0.001	<0.001	0.00115	---	---
Median in months (95% CI)	10.4 (9.4, 11.9)	11.8 (9.6, NA)	12.7 (10.0, 17.3)	12.0 (8.7, NA)	8.5 (7.5, 9.8)	7.5 (6.3, 9.9)
PFS‡						
Events (%)	266 (77%)	97 (69%)	255 (74%)	103 (73%)	257 (75%)	94 (72%)
Hazard ratio* (95% CI)	0.88 (0.73, 1.04)	0.68 (0.50, 0.92)	0.79 (0.66, 0.94)	0.79 (0.59, 1.06)	---	---
p-Value†	0.068	0.00578	0.005	0.05767	---	---
Median in months (95% CI)	3.9 (3.1, 4.1)	4.9 (4.1, 6.2)	4.0 (2.6, 4.3)	4.0 (2.2, 4.6)	4.0 (3.1, 4.2)	3.8 (2.2, 4.2)
Overall response rate‡						
ORR %§ (95% CI)	18% (14, 23)	24% (17, 32)	18% (15, 23)	20% (14, 28)	9% (7, 13)	5% (2, 11)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

† Based on stratified Log rank test

‡ Assessed by BICR using RECIST 1.1

§ All responses were partial responses

Table 27. Response to KEYTRUDA in previously treated NSCLC patients: overall clinical trial and patients with PD-L1 high expression, TPS ≥ 50%, as determined by PD-L1 IHC 22C3 pharmDx

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	139	56	151	60	152	47
OS						
Deaths (%)	58 (42%)	18 (32%)	60 (40%)	19 (32%)	86 (57%)	25 (53%)
Hazard ratio* (95% CI)	0.54 (0.38, 0.77)	0.45 (0.24, 0.84)	0.50 (0.36, 0.70)	0.29 (0.15 0.56)	---	---
p-Value†	<0.001	0.00541	<0.001	<0.001	---	---
Median in months (95% CI)	14.9 (10.4, NA)	Not reached (9.3, NA)	17.3 (11.8, NA)	Not reached (8.3, NA)	8.2 (6.4, 10.7)	7.2 (4.4, 8.3)
PFS‡						
Events (%)	89 (64%)	33 (59%)	97 (64%)	34 (57%)	118 (78%)	33 (70%)
Hazard ratio* (95% CI)	0.58 (0.43, 0.77)	0.47 (0.28, 0.80)	0.59 (0.45, 0.78)	0.41 (0.24, 0.70)	---	---
p-Value†	<0.001	0.00221	<0.001	<0.001	---	---
Median in months (95% CI)	5.2 (4.0, 6.5)	5.9 (4.2, 9.0)	5.2 (4.1, 8.1)	4.8 (2.8, NA)	4.1 (3.6, 4.3)	3.9 (2.0, 4.3)
Overall response rate ‡						
ORR %§ (95% CI)	30% (23, 39)	37% (25, 52)	29% (22, 37)	28% (18, 41)	8% (4, 13)	4% (1, 15)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model
† Based on stratified Log rank test
‡ Assessed by BICR using RECIST 1.1
§ All responses were partial responses

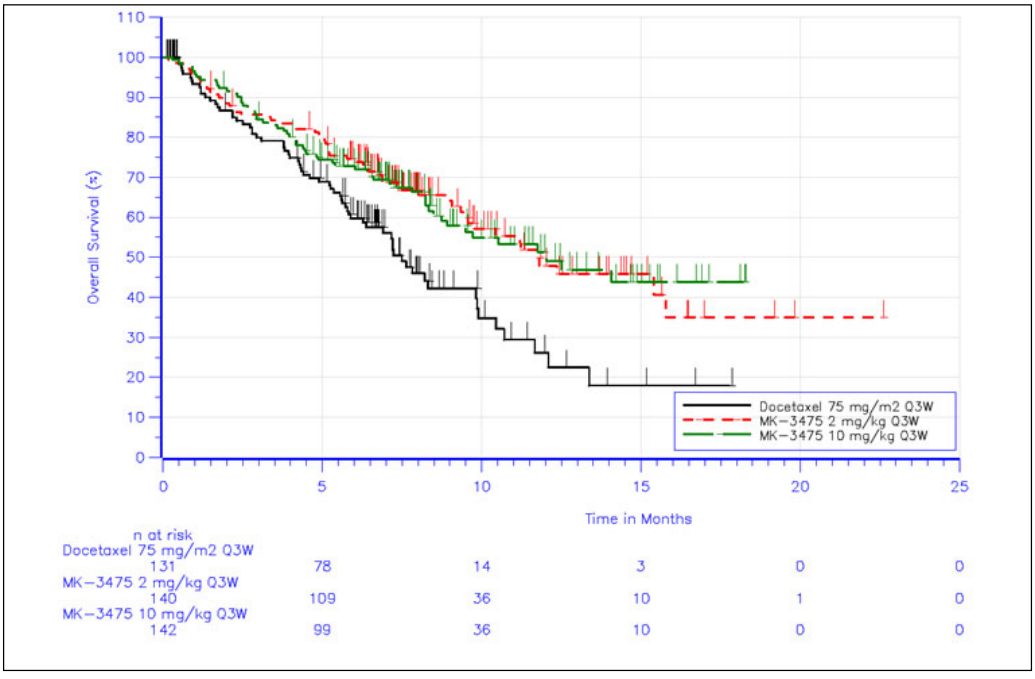


Figure 3. Kaplan-Meier curve for overall survival by treatment arm (TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, intent to treat population)

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with PD-L1 expression TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, but who may have had no PD-L1 expression (TPS < 1%) by the CTA. Patients with such test results are part of the intended use/ intent to diagnose (ITD)/ population of PD-L1 IHC 22C3 pharmDx; however, they were excluded from the clinical trial due to no PD-L1 expression upon CTA screening. To account for these missing data, a sensitivity analysis was conducted to understand the plausible range for the hazard ratio (HR) estimated based on PD-L1 IHC 22C3 pharmDx in the TPS ≥ 1% and TPS ≥ 50% subpopulations under an ITD framework to verify the consistency with the observed HR based on enrollment with the

CTA. The HR sensitivity analysis results showed that the HR estimates are robust to any assumed attenuation of the treatment effect under the ITD framework.

16.4 Clinical performance evaluation: NSCLC (LIBTAYO)

Regeneron EMPOWER-Lung 1: First-line treatment of locally advanced NSCLC in patients who are not candidates for surgical resection or definitive chemoradiation, or metastatic NSCLC

The efficacy of LIBTAYO in patients with locally advanced NSCLC who were not candidates for surgical resection or definitive chemoradiation, or with metastatic NSCLC was evaluated in a randomized, open-label, multi-center trial: EMPOWER-Lung 1 (NCT03088540).[12]

The trial was designed to enroll patients with tumor PD-L1 expression of TPS ≥ 50%. A total of 710 patients (Intent-To-Treat [ITT] population) were enrolled, and an analysis was performed on a population (n=563) who had PD-L1 expression of TPS ≥ 50% using PD-L1 IHC 22C3 pharmDx according to the approved labeling.

Patients with EGFR, ALK or ROS1 genomic tumor aberrations; a medical condition that required systemic immunosuppression; autoimmune disease that required systemic therapy within 2 years of treatment; or who had never smoked were ineligible. Patients with a history of brain metastases were eligible if they had been adequately treated and had neurologically returned to baseline for at least 2 weeks prior to randomization.

Randomization was stratified by histology (non-squamous vs squamous) and geographic region (Europe vs Asia vs Rest of world). Patients were randomized (1:1) to receive LIBTAYO 350 mg intravenously (IV) every 3 weeks for up to 108 weeks or a platinum-doublet chemotherapy regimen for 4 to 6 cycles followed by optional pemetrexed maintenance for patients with non-squamous histology who received a pemetrexed containing regimen.

Treatment with LIBTAYO continued until RECIST 1.1-defined progressive disease, unacceptable toxicity, or up to 108 weeks. Patients who experienced independent review committee (IRC)-assessed RECIST 1.1-defined progressive disease on LIBTAYO therapy were permitted to continue treatment with LIBTAYO (up to an additional 108 weeks) with the addition of 4 cycles of histology-specific chemotherapy until further progression was observed. Of the 150 patients in the population with TPS ≥ 50% randomized to receive chemotherapy who had IRC-assessed RECIST 1.1-defined disease progression, 107 (71.3%) patients crossed over to treatment with LIBTAYO. Assessment of tumor status was performed every 9 weeks. The major efficacy outcome measures were overall survival (OS) and progression-free survival (PFS). An additional efficacy outcome measure was overall response rate (ORR).

The study population characteristics of patients with PD-L1 expression of TPS ≥ 50% are included in Table 28.

Table 28: Summary of baseline patient and disease characteristics in the population with TPS ≥ 50%

	LIBTAYO N=283	Chemotherapy N=280
Patient Characteristics		
Median Age, Years (min, max)	63 (31, 79)	64 (40, 84)
Age < 65 Years, n (%)	157 (55)	147 (53)
Age ≥ 65 Years, n (%)	126 (45)	133 (48)
Gender: Male n (%)	248 (88)	231 (83)
Race: White n (%)	243 (86)	240 (86)
ECOG Performance Status n (%)		
0	77 (27)	75 (27)
1	206 (73)	205 (73)
History of brain metastasis (%)	12	12
Disease Characteristics		
Extent of Disease n (%)		
Locally Advanced	45 (16)	42 (15)
Metastatic	238 (84)	238 (85)
Histological Subtype n (%)		
Squamous	122 (43)	121 (43)
Non-squamous	161 (57)	159 (57)

In the ITT population, baseline patient and disease characteristics were consistent with those in the population with TPS ≥ 50%.

In the population with TPS ≥ 50%, the trial demonstrated statistically significant improvement in OS and PFS for patients randomized to LIBTAYO as compared with chemotherapy. Results were similar to the efficacy results for the ITT population.

Efficacy results for the population with PD-L1 expression of TPS ≥ 50% are presented in Table 29 and in Figure 4.

Table 29: Efficacy results from EMPOWER-Lung 1 in non-small cell lung cancer

	TPS ≥ 50% Population (N=563)	
Endpoints	**LIBTAYO 350 mg every 3 weeks n=283**	**Chemotherapy n=280**
Overall Survival		
Number of deaths (%)	70 (24.7)	105 (37.5)
Median in months (95% CI)[a]	NR (17.9, NE)	14.2 (11.2, 17.5)
Hazard ratio (95% CI)[b]	0.57 (0.42, 0.77)	
p-Value	0.0002	
Progression-free Survival per BICR		
Number of events (%)	147 (51.9)	197 (70.4)
Median in months (95% CI)[a]	8.2 (6.1, 8.8)	5.7 (4.5, 6.2)
Hazard ratio (95% CI)[b]	0.54 (0.43, 0.68)	
p-Value	<0.0001	
Overall Response Rate per BICR (%)[c, d]		
ORR (95% CI)	39.2 (33.5, 45.2)	20.4 (15.8, 25.6)
Complete response (CR) rate	2.1	1.1
Partial response (PR) rate	37.1	19.3
Duration of Response per BICR[c]		
Median in months (range)	16.7 (1.9+, 23.3+)	6.0 (1.3+, 14.5+)

BICR: blinded independent central review; CI: confidence interval; NE: Not evaluable; NR: Not reached
+: Ongoing response
[a] Based on Kaplan-Meier method
[b] Based on stratified proportional hazards model
[c] Not a pre-specified endpoint
[d] Clopper-Pearson exact confidence interval

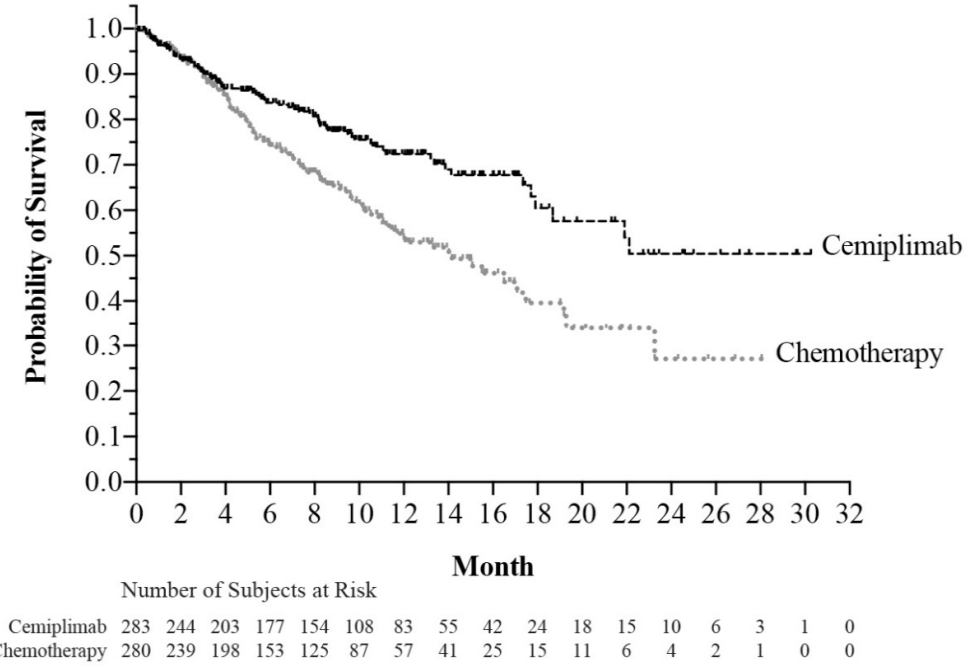


Figure 4. Kaplan-Meier curve for OS in the TPS ≥ 50% population

16.5 Nonclinical performance evaluation: esophageal cancer

The nonclinical studies were performed on FFPE esophageal cancer specimens (studies were conducted with both squamous and adenocarcinoma specimens).

Analytical sensitivity/specificity: esophageal cancer
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 FFPE esophageal cancer specimens. Assessment of PD-L1 expression demonstrated staining across a range of CPS 0–100, where 34% of the specimens had PD-L1 expression with a CPS ≥ 10. Two specimens were not evaluable due to containing fewer than 100 viable tumor cells.

Precision: esophageal cancer
The precision of PD-L1 IHC 22C3 pharmDx was evaluated at Agilent. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. For the precision studies, negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for the CPS ≥ 10 cutoff as shown in Table 30.

Table 30. Precision of PD-L1 IHC 22C3 pharmDx in esophageal cancer, tested at one site (CPS ≥ 10)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-operator, inter-instrument, inter-lot, and inter-day as combined variables)	CPS ≥ 10	All 32 esophageal cancer specimens (15 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 3 operators, using 3 Autostainer Link 48 instruments, using 3 reagent lots over 3 nonconsecutive days.	NPA 97.8% (93.3–100.0%) PPA 98.0% (94.1–100.0%) OA 97.9% (94.8–100.0%)
Intra-run precision (Repeatability)	CPS ≥ 10	All 32 esophageal cancer specimens (21 PD-L1-negative and 11 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 98.1% (95.2–100.0%) PPA 92.7% (83.6–100.0%) OA 96.2% (93.1–98.8%)
Inter-observer precision	CPS ≥ 10	All 59 esophageal cancer specimens (28 PD-L1-negative and 31 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 nonconsecutive days.	NPA 95.1% (90.5–98.8%) PPA 92.4% (87.5–96.8%) OA 93.7% (90.3–96.8%)
Intra-observer precision	CPS ≥ 10	All 60 esophageal cancer specimens (29 PD-L1-negative and 31 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 nonconsecutive days.	NPA 96.2% (93.4–98.8%) PPA 98.5% (96.5–100.0%) OA 97.3% (95.6–98.9%)

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

External reproducibility: esophageal cancer
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites using esophageal cancer specimens. NPA, PPA, and OA were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for the CPS ≥ 10 cutoff.

Table 31. Reproducibility of PD-L1 IHC 22C3 pharmDx in esophageal cancer, tested at three external sites (CPS ≥ 10)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-Site	CPS ≥ 10	All 36 esophageal cancer specimens (23 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 540 comparisons to majority call.	NPA 99.7% (99.1–100.0%) PPA 99.0% (96.9–100.0%) OA 99.4% (98.5–100.0%)
Intra-Site	CPS ≥ 10	All 36 esophageal cancer specimens (23 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Intra-site analysis was performed for 3 sites on a total of 540 comparisons to majority call.	NPA 99.7% (99.1–100.0%) PPA 99.0% (96.9–100.0%) OA 99.4% (98.5–100.0%)
Inter-observer	CPS ≥ 10	All 60 esophageal cancer specimens (31 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to majority call.	NPA 97.1% (94.3–99.3%) PPA 87.4% (81.6–92.7%) OA 92.4% (89.3–95.4%)
Intra-observer	CPS ≥ 10	All 60 esophageal cancer specimens (31 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to majority call.	NPA 97.1% (95.2–98.7%) PPA 97.0% (94.8–98.8%) OA 97.0% (95.6–98.3%)

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

External reproducibility: esophageal squamous cell carcinoma (ESCC)
The inter-observer and intra-observer reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated by three external pathologists using one pre-stained ESCC specimen set. Negative percent agreement (NPA), positive percent agreement (PPA) and overall percent agreement (OA) were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for the CPS ≥ 10 cutoff.

Table 32. Inter-Observer and Intra-Observer Reproducibility of the PD-L1 IHC 22C3 pharmDx in ESCC, tested by three external pathologists (CPS ≥ 10)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-observer	CPS ≥ 10	All 60 ESCC specimens (29 PD-L1-negative and 31 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 three sites on a total of 540 comparisons to majority call.	NPA 98.9% (96.9-100.0%) PPA 98.6% (96.1-100.0%) OA 98.7% (97.2-99.8%)
Intra-observer	CPS ≥ 10	All 60 ESCC specimens (29 PD-L1-negative and 31 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 three sites on a total of 540 comparisons to majority call.	NPA 98.5% (96.6-100.0%) PPA 99.3% (98.1-100.0%) OA 98.9% (97.6-99.8%)

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

16.6 Clinical performance evaluation: esophageal squamous cell carcinoma (ESCC; KEYTRUDA)

The efficacy of KEYTRUDA was investigated in KEYNOTE-181 (NCT02564263), a multicenter, randomized, open-label, active-controlled trial that enrolled 628 patients with recurrent locally advanced or metastatic esophageal cancer who progressed on or after one prior line of systemic treatment for advanced disease.[6] Patients with HER2/neu positive esophageal cancer were required to have received treatment with approved HER2/neu targeted therapy. All patients were required to have tumor specimens for PD-L1 testing at a central laboratory; PD-L1 status was determined using PD-L1 IHC 22C3 pharmDx. Patients with a history of noninfectious pneumonitis that required steroids or current pneumonitis, active autoimmune disease, or a medical condition that required immunosuppression were ineligible.

Patients were randomized (1:1) to receive either KEYTRUDA 200 mg every 3 weeks or investigator's choice of any of the following chemotherapy regimens, all given intravenously: paclitaxel 80-100 mg/m^2 on Days 1, 8, and 15 of every 4-week cycle, docetaxel 75 mg/m^2 every 3 weeks, or irinotecan 180 mg/m^2 every 2 weeks. Randomization was stratified by tumor histology (esophageal squamous cell carcinoma [ESCC] vs. esophageal adenocarcinoma [EAC]/Siewert type I EAC of the gastroesophageal junction [GEJ]), and geographic region (Asia vs. ex-Asia). Treatment with KEYTRUDA or chemotherapy continued until unacceptable toxicity or disease progression. Patients randomized to KEYTRUDA were permitted to continue beyond the first RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ)-defined disease progression if clinically stable until the first radiographic evidence of disease progression was confirmed at least 4 weeks later with repeat imaging. Patients treated with KEYTRUDA without disease progression could be treated for up to 24 months. Assessment of tumor status was performed every 9 weeks. The major efficacy outcome measure was OS evaluated in the following co-primary populations: patients with ESCC, patients with tumors expressing PD-L1 CPS ≥ 10, and all randomized patients. Additional efficacy outcome measures were PFS, ORR, and DoR, according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ, as assessed by BICR.

A total of 628 patients were enrolled and randomized to KEYTRUDA (n=314) or investigator's treatment of choice (n=314). Of these 628 patients, 167 (27%) had ESCC that expressed PD L1 with a CPS ≥ 10. Of these 167 patients, 85 patients were randomized to KEYTRUDA and 82 patients to investigator's treatment of choice [paclitaxel (n=50), docetaxel (n=19), or irinotecan (n=13)]. The baseline characteristics of these 167 patients were: median age of 65 years (range: 33 to 80), 51% age 65 or older; 84% male; 32% White and 68% Asian; 38% had an ECOG PS of 0 and 62% had an ECOG PS of 1. Ninety percent had M1 disease and 10% had M0 disease. Prior to enrollment, 99% of patients had received platinum-based treatment, and 84% had also received treatment with a fluoropyrimidine. Thirty-three percent of patients received prior treatment with a taxane.

The observed OS hazard ratio was 0.77 (95% CI: 0.63, 0.96) in patients with ESCC, 0.70 (95% CI: 0.52, 0.94) in patients with tumors expressing PD-L1 CPS ≥ 10, and 0.89 (95% CI: 0.75, 1.05) in all randomized patients. On further examination patients whose ESCC tumors expressed PD-L1 (CPS ≥ 10), an improvement in OS was observed among patients randomized to KEYTRUDA as compared with chemotherapy (hazard ratio of 0.64, 95% CI: 0.46, 0.90).

Ten (10) of the 167 ESCC participants with tumors expressing PD-L1 CPS ≥ 10 had specimens stained outside the stability window. These 10 participants have been excluded from the efficacy results summarized in Table 33 and the Kaplan-Meier curve for OS shown in Figure 5. The efficacy results for the population excluding the 10 participants with specimens outside the stability window are consistent with the efficacy conclusions detailed above.

Table 33. Efficacy results in patients with recurrent or metastatic ESCC (CPS ≥ 10) in KEYNOTE-181 (excluding 10 participants with specimens outside the stability window)

Endpoint	KEYTRUDA 200 mg every 3 weeks n=79	Chemotherapy n=78
OS		
Number (%) of patients with event	62 (78.5)	68 (87.2)
Median in months (95% CI)	10.3 (7.8, 13.6)	6.7 (4.8, 9.3)
Hazard ratio* (95% CI)	0.63 (0.45, 0.90)	
PFS (BCS per RECIST 1.1)		
Number (%) of patients with event	70 (88.6)	72 (92.3)
Median in months (95% CI)	3.2 (2.1, 4.3)	2.6 (2.1, 3.7)
Median follow-up (95% CI), months	3.2 (2.1, 4.4)	2.8 (2.1, 4.0)
Hazard ratio* (95% CI)	0.67 (0.48, 0.95)	
Objective Response Rate		
ORR (95% CI)	21.5 (13.1, 32.2)	7.7 (2.9, 16.0)
Number (%) of complete responses	3 (3.8)	1 (1.3)
Number (%) of partial responses	14 (17.7)	5 (6.4)
Median duration of response in months (range)	10.3 (2.8, 18.8+)	7.7 (4.3, 16.8+)

Cox proportional hazards model stratified by geographic region (Asia vs ex-Asia)
"+" indicates there is no progressive disease by the time of last disease assessment.

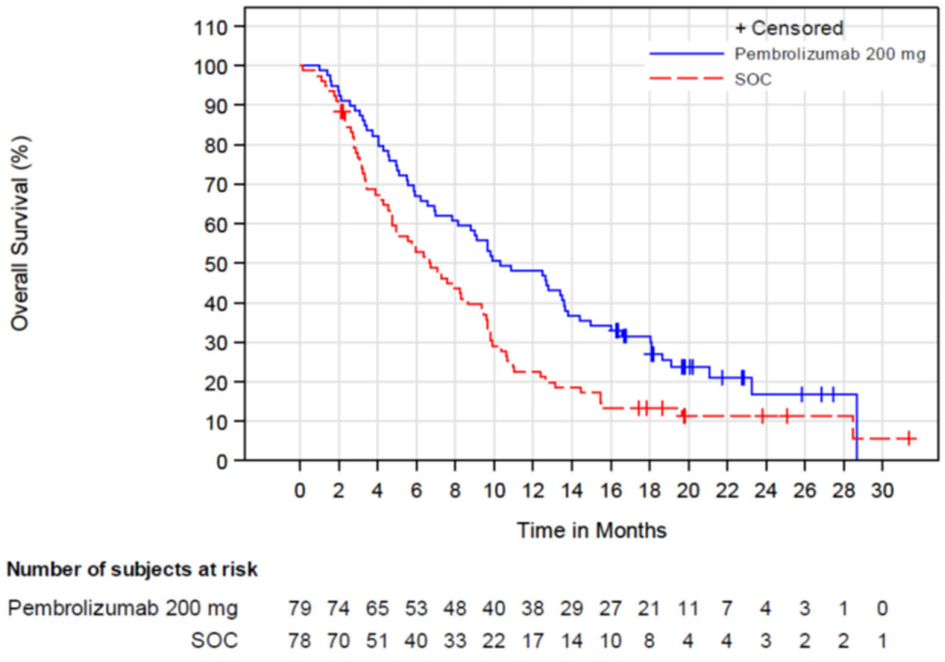


Figure 5. Kaplan-Meier curve for overall survival in KEYNOTE-181 (ESCC CPS ≥ 10) (excluding 10 participants with specimens outside the stability window)

16.7 Nonclinical performance evaluation: cervical cancer including combined squamous cancers

Nonclinical studies were performed on PD-L1 IHC 22C3 pharmDx on FFPE human cervical cancer tissue specimens. Squamous cell (SQ) cancers from vulva, anal and salivary gland were also included in these nonclinical study sample sets to supplement the SQ cervical cancer. These supplemental cancer types were also included in the MSD study KEYNOTE-158. The nonclinical studies consisted of analytical validation, stability and external reproducibility studies.

Analytical sensitivity/specificity: cervical cancer
Sensitivity of PD-L1 IHC 22C3 pharmDx was analyzed on 100 FFPE cervical cancer specimens (stage I to IV). Assessment of PD-L1 expression demonstrated staining across a range of CPS 0–100, where approximately 74% of cervical cancer specimens had PD-L1 expression, with a CPS ≥ 1.

Precision: cervical cancer Including squamous cell cancers
The precision of PD-L1 IHC 22C3 pharmDx in cervical cancers were evaluated at Agilent using squamous cell (SQ) cancers from cervical, vulva, anal and salivary gland. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. Repeatability was tested in intra-run precision. Inter- and intra-observer precision were also assessed. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were calculated with two-sided 95% Wilson score confidence intervals for the CPS ≥ 1 cutoff.

Table 34. Precision of PD-L1 IHC 22C3 pharmDx in cervical cancer including squamous cell cancers, tested at one site (CPS ≥ 1)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Squamous Cell Histological Subgroup Combined Precision (inter-operator, inter-instrument, inter-lot, inter-day as combined variables)	CPS ≥ 1	All 36 squamous cell cancer specimens (17 PD-L1 negative and 19 PD-L1-positive; 24 cervical cancer [13 PD-L1 negative and 11 PD-L1 positive]) with a range of PD-L1 expression were tested by 4 operators, using 3 Autostainer Link 48 instruments, using 3 reagent lots, over 3 nonconsecutive days.	NPA 100.0% (94.9–100.0%) PPA 98.9% (93.9–99.8%) OA 99.4% (96.5–99.9%)
Squamous Cell Histological Subgroup Intra-run precision (Repeatability)	CPS ≥ 1	All 24 squamous cell cancer specimens (12 PD-L1-negative and 12 PD-L1-positive; 18 cervical cancer [12 PD-L1 negative and 6 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 100.0% (93.8–100.0%) PPA 98.3% (91.1–99.7%) OA 99.2% (95.4–99.9%)
Squamous Cell Histological Subgroup Inter-observer precision	CPS ≥ 1	All 52 squamous cell cancer specimens (22 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 nonconsecutive days.	NPA 98.4% (95.5–99.5%) PPA 98.9% (96.8–99.6%) OA 98.7% (97.2–99.4%)
Squamous Cell Histological Subgroup Intra-observer precision	CPS ≥ 1	All 52 squamous cell cancer specimens (22 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 nonconsecutive days.	NPA 97.9% (94.8–99.2%) PPA 99.6% (97.9–99.9%) OA 98.9% (97.5–99.5%)

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

Precision: cervical cancer
The precision of PD-L1 IHC 22C3 pharmDx in cervical cancer was evaluated at Agilent. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. Repeatability was tested in intra-run precision. Inter- and intra-observer precision were also assessed. NPA, PPA, and OA were calculated with two-sided 95% Wilson Score confidence intervals for the CPS ≥ 1 cutoff.

Table 35. Precision of PD-L1 IHC 22C3 pharmDx in cervical cancer, tested at one site (CPS ≥ 1)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Cervical Cancer Combined Precision (inter-operator, inter-instrument, inter-lot, inter-day as combined variables)	CPS ≥ 1	All 24 cervical cancer specimens (13 PD-L1 negative and 11 PD-L1-positive) with a range of PD-L1 expression were tested by 4 operators, using 3 Autostainer Link 48 instruments, using 3 reagent lots, over 3 nonconsecutive days.	NPA 100.0% (92.4–100.0%) PPA 97.6% (87.4–99.6%) OA 98.9% (93.8–99.8%)
Cervical Cancer Intra-run precision (Repeatability)	CPS ≥ 1	All 18 cervical cancer specimens (12 PD-L1-negative and 6 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 100.0% (93.8–100.0%) PPA 100.0% (88.6–100.0%) OA 100.0% (95.8–100.0%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Cervical Cancer Inter-observer precision	CPS ≥ 1	All 21 cervical cancer specimens (8 PD-L1 negative and 13 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 nonconsecutive days.	NPA 100.0% (94.9–100.0%) PPA 99.1% (95.3–99.8%) OA 99.5% (97.1–99.9%)
Cervical Cancer Intra-observer precision	CPS ≥ 1	All 21 cervical cancer specimens (8 PD-L1 negative and 13 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 nonconsecutive days.	NPA 100.0% (94.9–100.0%) PPA 99.1% (95.3–99.8%) OA 99.5% (97.1–99.9%)

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

External reproducibility: cervical cancer including squamous cell cancers
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites using squamous cell cancers (SQ) from cervical, vulva, anal and salivary gland. NPA, PPA, and OA were computed with two-sided 95% Wilson score confidence intervals for the CPS ≥ 1 cutoff.

Table 36. Reproducibility of PD-L1 IHC 22C3 pharmDx in cervical cancer including squamous cell cancers, tested at three external sites (CPS ≥ 1)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 1	All 40 squamous cell cancer specimens (20 PD-L1 negative and 20 PD-L1 positive; n=25 cervical cancer) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 600 comparisons to majority call.	NPA 96.7% (94.0–98.2%) PPA 98.3% (96.2–99.3%) OA 97.5% (95.9–98.5%)
Intra-site	CPS ≥ 1	All 40 squamous cell cancer specimens (20 PD-L1 negative and 20 PD-L1 positive; n=25 cervical cancer) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Intra-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA 98.3% (96.1–99.3%) PPA 98.4% (96.2–99.3%) OA 98.3% (97.0–99.1%)
Inter-observer	CPS ≥ 1	All 49 squamous cell cancer specimens (23 PD-L1 negative and 26 PD-L1 positive; n=34 cervical cancer) 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 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 441 comparisons to majority call.	NPA 96.1% (92.6–98.0%) PPA 99.1% (96.9–99.8%) OA 97.7% (95.9–98.8%)
Intra-observer	CPS ≥ 1	All 49 squamous cell cancer specimens (23 PD-L1 negative and 26 PD-L1 positive; n=34 cervical cancer) 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 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 441 comparisons to majority call.	NPA 97.5% (94.4–98.9%) PPA 99.2% (97.0–99.8%) OA 98.4% (96.8–99.2%)

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

External reproducibility: cervical cancer
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites. NPA, PPA, and OA were computed with two-sided 95% Wilson score confidence intervals for the CPS ≥ 1 cutoff.

Table 37. Reproducibility of PD-L1 IHC 22C3 pharmDx in cervical cancer, tested at three external sites (CPS ≥ 1)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 1	All 25 cervical cancer specimens (13 PD-L1 negative and 12 PD-L1 positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 375 comparisons to majority call.	NPA 98.5% (95.6–99.5%) PPA 98.3% (95.2–99.4%) OA 98.4% (96.6–99.3%)
Intra-site	CPS ≥ 1	All 25 cervical cancer specimens (13 PD-L1 negative and 12 PD-L1 positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Intra-site analysis was performed for 3 sites on a total of 375 comparisons to majority call.	NPA 98.5% (95.6–99.5%) PPA 98.3% (95.2–99.4%) OA 98.4% (96.6–99.3%)
Inter-observer	CPS ≥ 1	All 34 cervical cancer specimens (16 PD-L1 negative and 18 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 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 306 comparisons to majority call.	NPA 97.2% (93.1–98.9%) PPA 98.8% (95.6–99.7%) OA 98.0% (95.8–99.1%)
Intra-observer	CPS ≥ 1	All 34 cervical cancer specimens (16 PD-L1 negative and 18 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 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 306 comparisons to majority call.	NPA 97.9% (94.1–99.3%) PPA 99.4% (96.6–99.9%) OA 98.7% (96.7–99.5%)

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

16.8 Clinical performance evaluation: cervical cancer (KEYTRUDA)

The efficacy of KEYTRUDA was investigated in 98 patients with recurrent or metastatic cervical cancer enrolled in a single cohort (Cohort E) in Study KEYNOTE-158 (NCT02628067), a multicenter, non-randomized, open-label, multi-cohort trial.[7,8] The trial excluded patients with autoimmune disease or a medical condition that required immunosuppression.

Patients were treated with KEYTRUDA intravenously at a dose of 200 mg every 3 weeks until unacceptable toxicity or documented disease progression. Patients with initial radiographic disease progression could receive additional doses of treatment during confirmation of progression unless disease progression was symptomatic, was rapidly progressive, required urgent intervention, or occurred with a decline in performance status. Patients without disease progression could be treated for up to 24 months. Assessment of tumor status was performed every 9 weeks for the first 12 months, and every 12 weeks thereafter. The major efficacy outcome measures were ORR according to RECIST 1.1, as assessed by blinded independent central review, and duration of response.

Among the 98 patients in Cohort E, 77 (79%) had tumors that expressed PD-L1 with a CPS ≥ 1 and received at least one line of chemotherapy in the metastatic setting. PD-L1 status was determined using PD-L1 IHC 22C3 pharmDx. The baseline characteristics of these 77 patients were: median age was 45 years (range: 27 to 75 years); 81% were White, 14% Asian, 3% Black; ECOG PS was 0 (32%) or 1 (68%); 92% had squamous cell carcinoma, 6% adenocarcinoma, and 1% adenosquamous histology; 95% had M1 disease and 5% had recurrent disease; 35% had one and 65% had two or more prior lines of therapy in the recurrent or metastatic setting.

No responses were observed in patients whose tumors did not have PD-L1 expression (CPS < 1).

Efficacy results are summarized in Table 38.

Table 38. Efficacy results in cohort E of KEYNOTE-158 (CPS ≥ 1)

Endpoint	n=77*
Objective response rate	
ORR (95% CI)	14.3% (7.4, 24.1)
Complete response rate	2.6%
Partial response rate	11.7%
Response duration	
Median in months (range)	NR (4.1, 18.6+)†
% with duration ≥ 6 months	91%

*Median follow-up time of 11.7 months (range 0.6 to 22.7 months)
†Based on patients (n=11) with a response by independent review
+Denotes ongoing
NR = not reached

16.9 Nonclinical performance evaluation: HNSCC

The nonclinical studies were performed on FFPE HNSCC specimens.

Analytical sensitivity/specificity: HNSCC
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 112 FFPE HNSCC specimens (staged I to IV) using a manufactured production lot. Assessment of PD-L1 expression demonstrated staining across a range of CPS 0–100, where 72% of the specimens had PD-L1 expression with a CPS ≥ 1 and 45% of the specimens had PD-L1 expression with a CPS ≥ 20.

Precision: HNSCC
The precision of PD-L1 IHC 22C3 pharmDx was evaluated at Agilent. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. For the precision studies, negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with corresponding two-sided 95% bootstrap confidence intervals for the CPS ≥ 1 cutoff and CPS ≥ 20 cutoff as shown in Tables 39 and 40 respectively. For studies with agreement parameters which resulted in 100.0% agreement, two-sided 95% confidence intervals were calculated using the Wilson Score method for the CPS ≥ 1 cutoff and CPS ≥ 20 cutoff.

Table 39. Precision of PD-L1 IHC 22C3 pharmDx in HNSCC, tested at one site (CPS ≥ 1)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-operator, inter-instrument, inter-lot, and inter-day as combined variables)	CPS ≥ 1	All 34 HNSCC specimens (12 PD-L1-negative and 22 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 5 operators, using 5 Autostainer Link 48 instruments, using 5 reagent lots, over 5 days.	NPA 100.0% (94.0–100.0%) PPA 99.1% (97.3–100.0%) OA 99.4% (98.2–100.0%)
Intra-run precision (Repeatability)	CPS ≥ 1	All 34 HNSCC specimens (16 PD-L1-negative and 18 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 98.8% (96.2–100.0%) PPA 97.8% (94.4–100.0%) OA 98.2% (95.9–100.0%)
Inter-observer precision	CPS ≥ 1	All 24 HNSCC specimens (11 PD-L1-negative and 13 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 nonconsecutive days with a minimum 2-week washout period in between each read.	NPA 88.9% (78.8–98.0%) PPA 99.1% (97.4–100.0%) OA 94.4% (89.8–98.6%)
Intra-observer precision	CPS ≥ 1	All 24 HNSCC specimens (11 PD-L1-negative and 13 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 nonconsecutive days with a minimum 2-week washout period in between each read.	NPA 98.8% (96.0–100.0%) PPA 95.4% (92.3–98.4%) OA 96.7% (94.0–99.1%)

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

Table 40. Precision of PD-L1 IHC 22C3 pharmDx in HNSCC, tested at one site (CPS ≥ 20)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-operator, inter-instrument, inter-lot, and inter-day as combined variables)	CPS ≥ 20	All 34 HNSCC specimens (17 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 5 operators, using 5 Autostainer Link 48 instruments, using 5 reagent lots, over 5 days.	NPA 100.0% (95.7–100.0%)* PPA 96.5% (90.6–100.0%) OA 98.2% (95.3–100.0%)
Intra-run precision (Repeatability)	CPS ≥ 20	All 34 HNSCC specimens (18 PD-L1-negative and 16 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 97.7% (92.9–100.0%) PPA 98.7% (96.2–100.0%) OA 98.2% (95.2–100.0%)
Inter-observer precision	CPS ≥ 20	All 48 HNSCC specimens (27 PD-L1-negative and 21 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 nonconsecutive days with a minimum 2-week washout period in between each read.	NPA 96.3% (91.8–100.0%) PPA 93.1% (87.3–97.9%) OA 94.9% (91.4–97.9%)
Intra-observer precision	CPS ≥ 20	All 48 HNSCC specimens (27 PD-L1-negative and 21 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 nonconsecutive days with a minimum 2-week washout period in between each read.	NPA 98.0% (95.9–99.6%) PPA 96.8% (94.4–98.9%) OA 97.5% (95.8–98.8%)

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

External reproducibility: HNSCC
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites. NPA, PPA, and OA were computed with corresponding two-sided 95% bootstrap confidence intervals for the CPS ≥ 1 cutoff and CPS ≥ 20 cutoff as shown in Tables 41 and 42, respectively.

Table 41. Reproducibility of PD-L1 IHC 22C3 pharmDx in HNSCC, tested at three external sites (CPS ≥ 1)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 1	All 38 HNSCC specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed for 3 sites on a total of 570 comparisons to majority call.	NPA 96.8% (92.6–100.0%) PPA 93.3% (86.7–98.6%) OA 95.1% (91.2–98.2%)
Intra-site	CPS ≥ 1	All 38 HNSCC specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 570 comparisons to majority call.	NPA 95.7% (91.3–99.0%) PPA 97.0% (94.5–98.9%) OA 96.3% (93.5–98.6%)
Inter-observer	CPS ≥ 1	All 62 HNSCC 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 556 comparisons to majority call.	NPA 94.0% (89.3–97.8%) PPA 97.2% (94.4–99.3%) OA 95.7% (93.0–98.0%)
Intra-observer	CPS ≥ 1	All 62 HNSCC 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 555 comparisons to majority call.	NPA 97.3% (95.4–98.9%) PPA 98.3% (96.8–99.7%) OA 97.8% (96.8–98.9%)

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

Table 42. Reproducibility of PD-L1 IHC 22C3 pharmDx in HNSCC, tested at three external sites (CPS ≥ 20)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 20	All 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 570 comparisons to majority call.	NPA 95.5% (92.0–98.4%) PPA 81.0% (71.3–90.3%) OA 90.5% (86.5–94.4%)
Intra-site	CPS ≥ 20	All 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 570 comparisons to majority call.	NPA 96.9% (94.6–98.8%) PPA 90.6% (86.3–94.9%) OA 94.9% (92.8–96.8%)
Inter-observer	CPS ≥ 20	All 62 HNSCC specimens (31 PD-L1-negative and 31 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 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 556 comparisons to majority call.	NPA 93.1% (87.2–97.8%) PPA 91.0% (85.7–95.7%) OA 92.1% (88.2–95.5%)
Intra-observer	CPS ≥ 20	All 62 HNSCC specimens (31 PD-L1-negative and 31 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 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 555 comparisons to majority call.	NPA 96.8% (94.5–98.7%) PPA 97.8% (96.0–99.3%) OA 97.3% (95.9–98.6%)

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

Note: Study results failed to meet pre-specified acceptance criteria for inter-site PPA for CPS ≥ 20 in two independent studies and inter-site OA for CPS ≥ 20 in one study.

16.10 Clinical performance evaluation: HNSCC (KEYTRUDA)

The efficacy of KEYTRUDA was investigated in KEYNOTE-048 (NCT02358031), a randomized, multicenter, open label, active controlled trial conducted in 882 patients with metastatic or recurrent HNSCC who had not previously received systemic therapy for metastatic disease or with recurrent disease who were considered incurable by local therapies.[9] Patients with active autoimmune disease that required systemic therapy within two years of treatment or a medical condition that required immunosuppression were ineligible. Randomization was stratified by tumor PD-L1 expression (TPS ≥ 50% or < 50%) according to PD-L1 IHC 22C3 pharmDx, HPV status according to p16 IHC (positive or negative), and ECOG PS (0 vs. 1). Patients were randomized 1:1:1 to one of the following treatment arms:

- KEYTRUDA 200 mg intravenously every 3 weeks

- KEYTRUDA 200 mg intravenously every 3 weeks, carboplatin AUC 5 mg/mL/min intravenously every 3 weeks or cisplatin 100 mg/m^2 intravenously every 3 weeks, and FU 1000 mg/m^2/day as a continuous intravenous infusion over 96 hours every 3 weeks (maximum of 6 cycles of platinum and FU)
- Cetuximab 400 mg/m^2 intravenously as the initial dose then 250 mg/m^2 intravenously once weekly, carboplatin AUC 5 mg/mL/min intravenously every 3 weeks or cisplatin 100 mg/m^2 intravenously every 3 weeks, and FU 1000 mg/m^2/day as a continuous intravenous infusion over 96 hours every 3 weeks (maximum of 6 cycles of platinum and FU)

Treatment with KEYTRUDA continued until RECIST v1.1-defined progression of disease as determined by the investigator, 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 6 weeks for the first year, followed by every 9 weeks through 24 months. A retrospective re-classification of patients' tumor PD-L1 status according to CPS according to PD-L1 IHC 22C3 pharmDx was conducted using the tumor specimens used for randomization.

The main efficacy outcome measures were OS and PFS as assessed by BICR according to RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ) sequentially tested in the subgroup of patients with CPS ≥ 20, the subgroup of patients with CPS ≥ 1, and the overall population.

A total of 601 patients were randomized to the KEYTRUDA as a single agent and cetuximab in combination with chemotherapy arms; 301 patients to the KEYTRUDA as a single agent arm and 300 patients to the cetuximab in combination with chemotherapy arm. The study population characteristics were: median age of 61 years (range: 22 to 94); 36% age 65 or older; 85% male; 74% White and 19% Asian, and 1.7% Black; 61% ECOG PS of 1; and 79% were former/current smokers. Twenty-two percent of patients' tumors were HPV-positive, and 96% had Stage IV disease (Stage IVA 20%, Stage IVB 6%, and Stage IVC 70%).

For the subgroup of patients randomized to KEYTRUDA as a single agent or to cetuximab in combination with chemotherapy, PD-L1 expression level for 601 patient tumor biopsy or resection tissue (159 archival and 442 newly obtained; refer to definition in Table 42) was determined using PD-L1 IHC 22C3 pharmDx. Overall, 85% (512/601) of the patients had tumors that expressed PD-L1 with CPS ≥ 1. Eighty-six percent (380/442) of patients whose tumors were newly obtained for PD-L1 testing and 83% (132/159) of patients whose archival tumors were tested expressed PD-L1 at CPS ≥ 1. Forty-three percent (255/597) of the patients had tumors that expressed PD-L1 with CPS ≥ 20; four patients had unknown PD-L1 expression status (one specimen was archival tissue and three specimens were newly obtained tissue). Forty-two percent (186/439) of patients whose tumors were newly obtained for PD-L1 testing and 44% (69/158) of patients whose archival tumors were tested expressed PD-L1 at CPS ≥ 20 (Table 43).

Table 43. Tumor PD-L1 expression by specimen type

Tumor Tissue	Number (%) with CPS < 1	Number (%) with CPS ≥ 1	Number (%) with CPS ≥ 20
Overall study n=601	89 (15)	512 (85)	255 (43)**
Archival Tissue* n=159	27 (17)	132 (83)	69 (44)**
Newly Obtained Tissue* n= 442	62 (14)	380 (86)	186 (42)**

*In the context of Clinical Trial KEYNOTE-048, newly obtained tissue biopsy was defined as the biopsy collected within 90 days of initiation of treatment with pembrolizumab. Specimens that were > 90 days were classified as archival.
**Based on patients with known PD-L1 expression; 4 patients had unknown PD-L1 expression status (one specimen was archival tissue and three specimens were newly obtained tissue).

The trial demonstrated a statistically significant improvement in OS for the subgroup of patients with PD-L1 CPS ≥ 1 randomized to KEYTRUDA as a single agent compared to those randomized to cetuximab in combination with chemotherapy. At the time of the interim analysis, there was no significant difference in OS between the KEYTRUDA single agent arm and the control arm for the overall population.

Table 44 summarizes efficacy results for KEYTRUDA as a single agent in the subgroup of patients with CPS ≥ 1 HNSCC and CPS ≥ 20 HNSCC. Figure 6 summarizes the OS results in the subgroup of patients with CPS ≥ 1 HNSCC.

Table 44. Efficacy results for KEYTRUDA as a single agent in KEYNOTE-048 (CPS ≥ 1 and CPS ≥ 20)

Endpoint	CPS ≥ 1		CPS ≥ 20	
	KEYTRUDA 200 mg every 3 weeks n=257	Cetuximab Platinum FU n=255	KEYTRUDA 200 mg every 3 weeks n=133	Cetuximab Platinum FU n=122
OS				
Number of events (%)	177 (69%)	206 (81%)	82 (62%)	95 (78%)
Median in months (95% CI)	12.3 (10.8, 14.9)	10.3 (9.0,11.5)	14.9 (11.6, 21.5)	10.7 (8.8, 12.8)
Hazard ratio* (95% CI)	0.78 (0.64, 0.96)		0.61 (0.45, 0.83)	
p-Value†	0.0171		0.0015	
PFS				
Number of events (%)	225 (88%)	231 (91%)	113 (85%)	111 (91%)
Median in months (95% CI)	3.2 (2.2, 3.4)	5.0 (4.8, 5.8)	3.4 (3.2, 3.8)	5.0 (4.8, 6.2)
Hazard ratio‡ (95% CI)	1.15(0.95, 1.38)		0.99 (0.75, 1.29)	
Objective Response Rate				
ORR‡ (95% CI)	19% (14.5, 24.4)	35% (29.1, 41.1)	23% (16.4, 31.4)	36% (27.6, 45.3)
Complete response rate	5%	3%	8%	3%
Partial response rate	14%	32%	16%	33%
Duration of Response				
Median in months (range)	20.9 (1.5+, 34.8+)	4.5 (1.2+, 28.6+)	20.9 (2.7, 34.8+)	4.2 (1.2+, 22.3+)

* Based on the stratified Cox proportional hazard model
† Based on a stratified log-rank test
‡ Response: Best objective response as confirmed complete response or partial response

In an exploratory subgroup analysis for patients with CPS 1-19 HNSCC, the median OS was 10.8 months (95% CI: 9.0, 12.6) for KEYTRUDA as a single agent and 10.1 months (95% CI: 8.7, 12.1) for cetuximab in combination with chemotherapy, with an HR of 0.90 (95% CI: 0.68, 1.18).

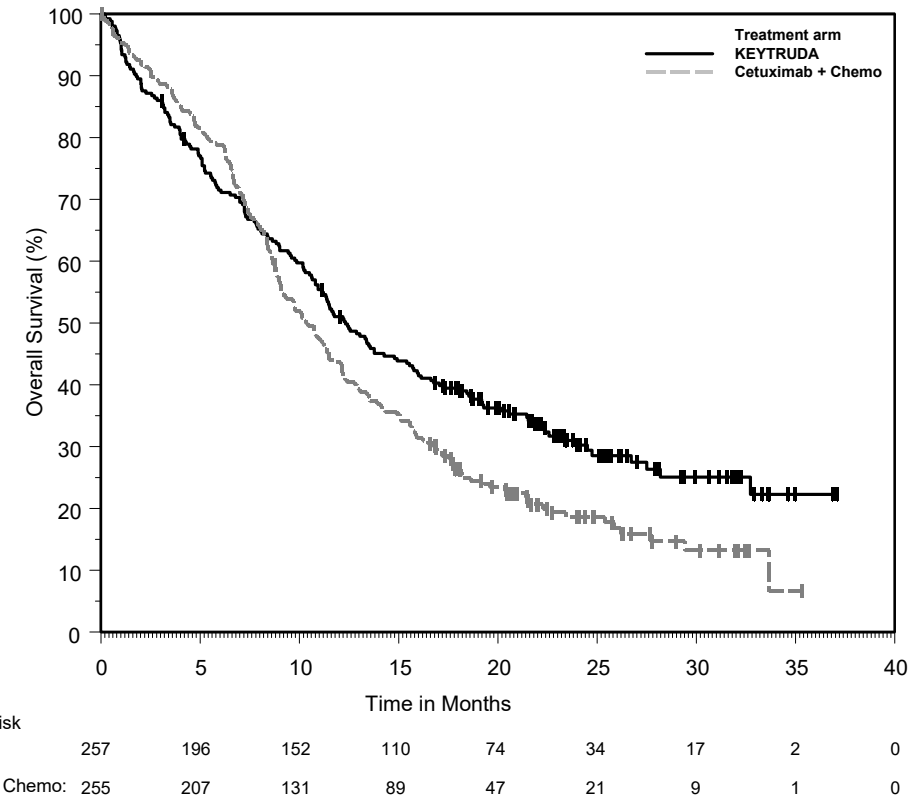


Figure 6. Kaplan-Meier curve for overall survival for KEYTRUDA as a single agent in KEYNOTE-048 (CPS ≥ 1)

16.11 Nonclinical performance evaluation: TNBC

The nonclinical studies were performed on FFPE TNBC specimens.

Analytical sensitivity/specificity: TNBC
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 FFPE TNBC specimens (staged I to IV) using a manufactured production lot. Assessment of PD-L1 expression demonstrated staining across a range of CPS 0-100, where 30% of the specimens had PD-L1 expression with a CPS ≥ 10.

Precision: TNBC
The precision of PD-L1 IHC 22C3 pharmDx was evaluated at Agilent. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. For the precision studies, negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with corresponding two-sided 95% bootstrap confidence intervals for the CPS ≥ 10 cutoff as shown in Table 45. For studies which resulted in 100.0% agreement, NPA, PPA, and OA were computed with corresponding two-sided 95% Wilson score confidence intervals for the CPS ≥ 10 cutoff.

Table 45. Precision of PD-L1 IHC 22C3 pharmDx in TNBC, tested at one site (CPS ≥ 10)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-operator, inter-instrument, inter-lot, and inter-day as combined variables)	CPS ≥ 10	All 33 TNBC specimens (21 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested by 3 operators, using 3 Autostainer Link 48 instruments, using 3 reagent lots, over 3 nonconsecutive days.	NPA 100.0% (94.3–100.0%) PPA 100.0% (90.4–100.0%) OA 100.0% (96.3–100.0%)
Intra-run precision(Repeatability)	CPS ≥ 10	All 33 TNBC specimens (16 PD-L1-negative and 17 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 100.0% (95.4–100.0%) PPA 94.0% (86.9–100.0%) OA 96.9% (93.3–100.0%)
Inter-observer precision	CPS ≥ 10	All 48 TNBC specimens (31 PD-L1-negative and 17 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 nonconsecutive days.	NPA 93.2% (87.5–97.8%) PPA 92.2% (85.6–97.4%) OA 92.8% (88.4–96.8%)
Intra-observer precision	CPS ≥ 10	All 48 TNBC specimens (31 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx,	NPA 98.5% (97.0–99.6%) PPA 94.5% (90.9–98.0%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
		were scored by 3 pathologists over 3 nonconsecutive days.	OA 97.0% (95.4–98.6%)

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

External reproducibility: TNBC
The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites. NPA, PPA, and OA were computed with corresponding two-sided 95% bootstrap confidence intervals for the CPS ≥ 10 cutoff.

Table 46. Reproducibility of PD-L1 IHC 22C3 pharmDx in TNBC, tested at three external sites (CPS ≥ 10)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 10	All 40 TNBC specimens (19 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed for 3 sites on a total of 600 comparisons to majority call.	NPA 93.0% (85.3–100.0%) PPA 92.1% (86.3–97.1%) OA 92.5% (87.8–96.7%)
Intra-site	CPS ≥ 10	All 40 TNBC specimens (19 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive 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 97.1% (94.3–99.3%) PPA 94.4% (90.0–98.1%) OA 95.7% (92.7–98.2%)
Inter-observer	CPS ≥ 10	All 60 TNBC specimens (26 PD-L1-negative and 34 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 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to majority call.	NPA 97.0% (93.6–100.0%) PPA 95.4% (91.2–98.7%) OA 96.1% (93.3–98.5%)
Intra-observer	CPS ≥ 10	All 60 TNBC specimens (26 PD-L1-negative and 34 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 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to majority call.	NPA 98.7% (96.6–100.0%) PPA 96.7% (94.6–98.7%) OA 97.6% (96.1–98.9%)

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

16.12 Clinical performance evaluation: TNBC (KEYTRUDA)

The efficacy of KEYTRUDA in combination with paclitaxel, paclitaxel protein-bound, or gemcitabine and carboplatin was investigated in KEYNOTE-355 (NCT02819518), a multicenter, double-blind, randomized, placebo-controlled trial conducted in 847 patients with locally recurrent unresectable or metastatic TNBC, regardless of tumor PD-L1 expression, who had not been previously treated with chemotherapy in the metastatic setting.[10] Patients with active autoimmune disease that required systemic therapy within 2 years of treatment or a medical condition that required immunosuppression were ineligible. Randomization was stratified by chemotherapy treatment (paclitaxel or paclitaxel protein-bound vs. gemcitabine and carboplatin), tumor PD-L1 expression (CPS ≥ 1 vs. CPS < 1) according to PD-L1 IHC 22C3 pharmDx, and prior treatment with the same class of chemotherapy in the neoadjuvant setting (yes vs. no). According to pre-specified analysis plan, the study analysis population included patient populations with tumor PD-L1 expression CPS ≥ 1 and CPS ≥ 10.

Patients were randomized (2:1) to one of the following treatment arms; all study medications were administered via intravenous infusion:

- KEYTRUDA 200 mg on Day 1 every 3 weeks in combination with paclitaxel protein-bound 100 mg/m^2 on Days 1, 8 and 15 every 28 days, paclitaxel 90 mg/m^2 on Days 1, 8, and 15 every 28 days, or gemcitabine 1000 mg/m^2 and carboplatin AUC 2 mg/mL/min on Days 1 and 8 every 21 days.
- Placebo on Day 1 every 3 weeks in combination with paclitaxel protein-bound 100 mg/m^2 on Days 1, 8 and 15 every 28 days, paclitaxel 90 mg/m^2 on Days 1, 8, and 15 every 28 days, or gemcitabine 1000 mg/m^2 and carboplatin AUC 2 mg/mL/min on Days 1 and 8 every 21 days.

Assessment of tumor status was performed at Weeks 8, 16, and 24, then every 9 weeks for the first year, and every 12 weeks thereafter. The main efficacy outcome measure was PFS as assessed by BICR according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ tested in the subgroup of patients with CPS ≥ 10. Additional efficacy outcome measures were OS as well as ORR and DoR as assessed by BICR.

The study population characteristics for patients were: median age of 53 years (range: 22 to 85), 21% age 65 or older; 100% female; 68% White, 21% Asian, and 4% Black; 60% ECOG PS of 0 and 40% ECOG PS of 1; and 68% were post-menopausal status. Seventy-five percent of patients had tumor PD-L1 expression CPS ≥ 1 and 38% had tumor PD-L1 expression CPS ≥ 10.

Table 47 and Figure 7 summarize the efficacy results for KEYNOTE-355.

Table 47. Efficacy Results in KEYNOTE-355 (CPS ≥ 10)

Endpoint	KEYTRUDA 200 mg every 3 weeks with chemotherapy n=220	Placebo every 3 weeks with chemotherapy n=103
PFS		
Number of patients with event (%)	136 (62%)	79 (77%)
Median in months (95% CI)	9.7 (7.6, 11.3)	5.6 (5.3, 7.5)
Hazard ratio* (95% CI)	0.65 (0.49, 0.86)	
p-Value†	0.0012	
ORR		
Objective confirmed response rate (95% CI)	53% (46, 60)	40% (30, 50)
Complete response rate	17%	13%
Partial response rate	36%	27%
DoR		
Median in months (95% CI)	19.3 (9.9, 29.8)	7.3 (5.3, 15.8)

* Based on stratified Cox regression model.
† One-sided p-Value based on stratified log-rank test.

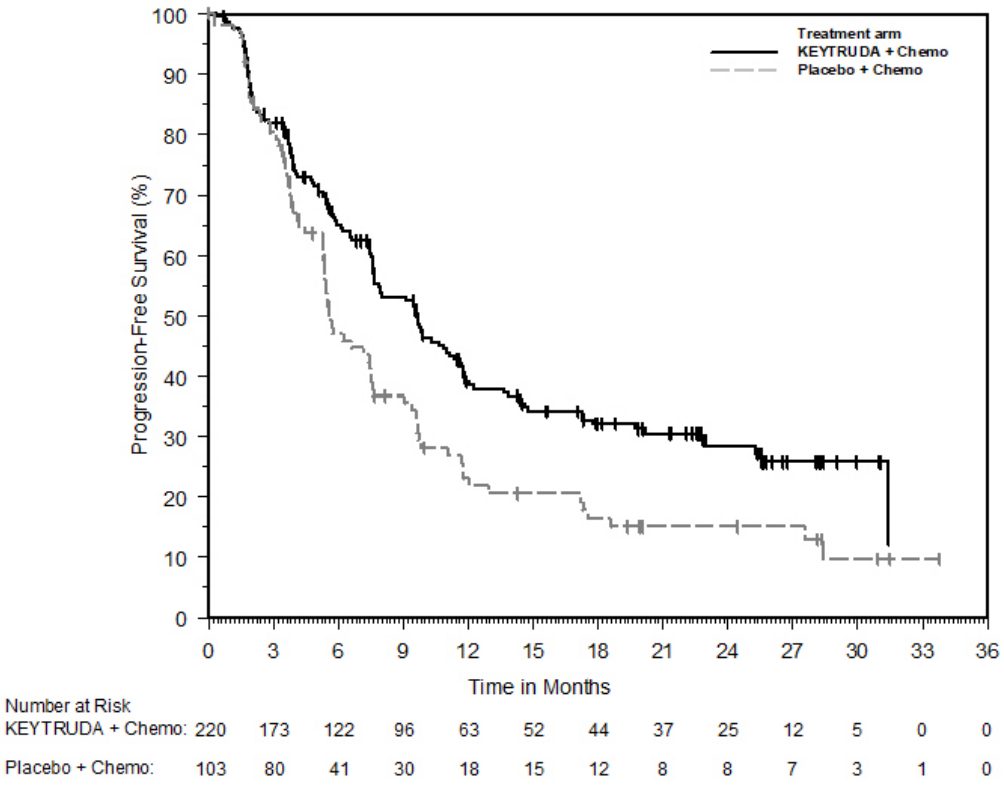


Figure 7. Kaplan-Meier curve for progression-free survival in KEYNOTE-355 (CPS ≥ 10)

16.13 Nonclinical performance evaluation: gastric or GEJ adenocarcinoma

The following histologies were tested in the non-clinical performance evaluation of gastric or GEJ adenocarcinoma: intestinal, diffuse including signet ring cell carcinoma, and mucinous types.

Analytical sensitivity/specificity: gastric or GEJ adenocarcinoma
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 100 FFPE gastric or GEJ adenocarcinoma specimens (stage I to IV) using a manufactured production lot. Assessment of PD-L1 expression demonstrated staining across a range of CPS 0-100. Sixty percent of the specimens had PD-L1 expression, with a CPS ≥ 1.

Precision: gastric or GEJ adenocarcinoma
The precision of PD-L1 IHC 22C3 pharmDx in gastric or GEJ adenocarcinoma was evaluated at Agilent. Inter-operator, inter-instrument, inter-lot, and inter-day were tested in combined precision. Repeatability was tested in intra-run precision. Intra-observer and inter-observer

precision were also assessed. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% Wilson score confidence intervals for the CPS ≥ 1 cutoff.

Table 48. Precision of PD-L1 IHC 22C3 pharmDx in gastric or GEJ adenocarcinoma, tested at one site (CPS ≥ 1).

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI):
Combined Precision (inter-operator, inter-instrument, inter-lot, inter-day)	CPS ≥ 1	All 24 gastric or GEJ adenocarcinoma specimens (12 PD-L1-positive and 12 PD-L1-negative) with a range of PD-L1 expression were tested by 4 operators, using 3 Autostainer Link 48 instruments, 3 reagent lots, over 3 nonconsecutive days.	NPA 100.0% (94.9-100.0%) PPA 95.8% (88.5-98.6%) OA 97.9% (94.1-99.3%
Intra-run precision (Repeatability)	CPS ≥ 1	All 24 gastric or GEJ adenocarcinoma specimens (13 PD-L1-negative and 11 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 96.9% (89.5-99.2%) PPA 100.0% (93.5-100.0%) OA 98.3% (94.1-99.5%)
Inter-observer precision	CPS ≥ 1	All 60 gastric or GEJ adenocarcinoma specimens (26 PD-L1-negative and 34 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 nonconsecutive days	NPA 91.5% (87.2-94.4%) PPA 96.1% (93.3-97.7%) OA 94.1% (91.8-95.8%)
Intra-observer precision	CPS ≥ 1	All 60 gastric or GEJ adenocarcinoma specimens (26 PD-L1-negative and 34 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 nonconsecutive days	NPA 96.0% (92.6-97.9%) PPA 96.8% (94.3-98.3%) OA 96.5% (94.6-97.7%)

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

External reproducibility: gastric or GEJ adenocarcinoma

The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites. NPA, PPA, and OA were computed with corresponding two-sided 95% percentile bootstrap confidence intervals (CI) for the CPS ≥ 1 cutoff. In an initial study the acceptance criteria for the CI lower bound of OA and NPA for inter-observer reproducibility and CI lower bound of NPA for intra-observer reproducibility were not met. A root cause assessment indicated that one of the three observers in the study did not pass post-study proficiency testing. A second inter- and intra-observer study was conducted with three naïve observers, and the results met the acceptance criteria. Results are shown in Table 49. Proficiency assessment is recommended to ensure correct observer scoring interpretation.

Table 49. Reproducibility of PD-L1 IHC 22C3 pharmDx in gastric or GEJ adenocarcinoma, tested at three external sites (CPS ≥ 1).

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 1	All 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 PD-L1 positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days. Inter-site analysis was performed between 3 sites on a total of 540 comparisons to majority call.	NPA 92.5% (86.2-97.5%) PPA 91.7% (84.7-97.7%) OA 92.0% (87.4-96.3%)
Intra-site	CPS ≥ 1	All 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 PD-L1 positive) with a range of PD-L1 IHC expression were tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 540 comparisons to majority call.	NPA 93.1% (89.2-96.5%) PPA 98.2% (96.4-99.6%) OA 95.7% (93.7-97.6%)
Inter-observer	CPS ≥ 1	All 68 gastric or GEJ adenocarcinoma specimens (36 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Inter-observer analysis was performed between 3 sites on a total of 612 comparisons to majority call.	NPA 96.6% (92.9-99.4%) PPA 96.5% (93.1-99.3%) OA 96.6% (94.0-98.7%)
Intra-observer	CPS ≥ 1	All 68 gastric or GEJ adenocarcinoma specimens (36 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, 1 at each of 3 study sites, on 3 nonconsecutive days. Intra-observer analysis was performed for 3 sites on a total of 612 comparisons to majority call.	NPA 97.2% (94.8-99.1%) PPA 97.2% (94.8-99.3%) OA 97.2% (95.3-98.9%)

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

16.14 Clinical performance evaluation: gastric or GEJ adenocarcinoma (KEYTRUDA)

The efficacy of KEYTRUDA in combination with trastuzumab plus fluoropyrimidine and platinum chemotherapy was investigated in KEYNOTE-811 (NCT03615326), a multicenter, randomized, double-blind, placebo-controlled trial that enrolled 698 patients with HER2-positive advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma who had not previously received systemic therapy for metastatic disease. PD-L1 status was determined using PD-L1 IHC 22C3 pharmDx. Patients with an autoimmune disease that required systemic therapy within 2 years of treatment or a medical condition that required immunosuppression were ineligible. Randomization was stratified by PD-L1 expression (CPS ≥1 or CPS <1), chemotherapy regimen (5-FU plus cisplatin [FP] or capecitabine plus oxaliplatin [CAPOX]), and geographic region (Europe/Israel/North America/Australia, Asia, or Rest of the World). Patients were randomized (1:1) to one of the following treatment arms.

- KEYTRUDA 200 mg, trastuzumab 8 mg/kg on first infusion and 6 mg/kg in subsequent cycles, followed by investigator's choice of combination chemotherapy of cisplatin 80 mg/m^2 for up to 6 cycles and 5-FU 800 mg/m^2/day for 5 days (FP) or oxaliplatin 130 mg/m^2 up to 6-8 cycles and capecitabine 1000 mg/m^2 bid for 14 days (CAPOX). KEYTRUDA was administered prior to trastuzumab and chemotherapy on Day 1 of each cycle.
- Placebo, trastuzumab 8 mg/kg on first infusion and 6 mg/kg in subsequent cycles, followed by investigator's choice of combination chemotherapy of cisplatin 80 mg/m^2 for up to 6 cycles and 5-FU 800 mg/m^2/day for 5 days (FP) or oxaliplatin 130 mg/m^2 up to 6-8 cycles and capecitabine 1000 mg/m^2 bid for 14 days (CAPOX).

All study medications, except oral capecitabine, were administered as an intravenous infusion for every 3-week cycle. Treatment with KEYTRUDA continued until RECIST v1.1-defined progression of disease as determined by BICR, unacceptable toxicity, or a maximum of 24 months. In an interim efficacy analysis, the major outcome measures assessed were ORR and DoR by BICR using RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ.

At the time of the interim analysis, ORR and DoR were assessed in the first 264 patients randomized. Among the 264 patients the population characteristics were: median age of 62 years (range: 19 to 84), 41% age 65 or older; 82% male; 63% White, 31% Asian, and 0.8% Black; 47% ECOG PS of 0 and 53% ECOG PS of 1. Ninety-seven percent of patients had metastatic disease (Stage IV) and 3% had locally advanced unresectable disease. Ninety-one percent (n=240) had tumors that were not MSI-H, 1% (n=2) had tumors that were MSI-H, and in 8% (n=22) the status was not known. Eighty-seven percent of patients received CAPOX. From the total number of patients randomized (n=698) in KEYNOTE-811, there were 354 newly obtained samples (samples obtained within 42 days of randomization), 343 archival samples (greater than 42 days of randomization), and one unknown; 620 biopsies, 77 resections, and one unknown.

A statistically significant improvement in ORR was demonstrated in patients randomized to KEYTRUDA in combination with trastuzumab and chemotherapy compared with placebo in combination with trastuzumab and chemotherapy. Efficacy results are summarized in Table 50.

Table 50. Efficacy Results for KEYNOTE-811

Endpoint	KEYTRUDA 200 mg every 3 weeks Trastuzumab Fluoropyrimidine and Platinum Chemotherapy n=133	Placebo Trastuzumab Fluoropyrimidine and Platinum Chemotherapy n=131
Objective Response Rate		
ORR* (95% CI)	74% (66, 82)	52% (43, 61)
Complete response rate	11%	3.1%
Partial response rate	63%	49%
p-Value†	<0.0001	
Duration of Response	n=99	n=68
Median in months (range)	10.6 (1.1+, 16.5+)	9.5 (1.4+, 15.4+)
% with duration ≥6 months‡	65%	53%

* Response: Best objective response as confirmed complete response or partial response
† p-Value based on stratified Miettinen and Nurminen method (compared to an alpha boundary of 0.002)
‡ Based on observed duration of response

In a pre-specified subgroup analysis of ORR based on PD-L1 status, the ORR in patients with PD-L1-positive disease (CPS ≥1) was 76% (95% CI: 67, 83) in the pembrolizumab arm (n=117) versus 51% (95% CI: 41, 60) in the control arm (n=112). In patients with tumors that were PD-L1 CPS<1, the ORR was 63% (95% CI: 35, 85) in the pembrolizumab arm (n=16) versus 58% (95% CI: 34, 80) in the control arm (n=19).

In a subsequent interim analysis of pre-specified subgroups based on PD-L1 status in the full study population (n=698), the HR for PFS and OS in patients with PD-L1 CPS<1 (N=104) was 1.03 (95% CI 0.65, 1.64) and 1.41 (95% CI: 0.90, 2.20), respectively.

17. Troubleshooting

Table 51. Troubleshooting

Problem	Probable Cause	Suggested Action
1. No staining of slides	1a. Programming error.	1a. Verify that the PD-L1 IHC 22C3 pharmDx protocol was selected for programming of slides.
	1b. Lack of reaction with DAB+ Substrate-Chromogen Solution (DAB)	1b. Verify that DAB+ Substrate-Chromogen Solution was prepared properly.
	1c. Sodium azide in wash buffer.	1c. Use only EnVision FLEX Wash Buffer (20x) (Code K8007).
	1d. Degradation of Control Slide	1d. Check kit expiration date and kit storage conditions on outside of package.
2. Weak staining of specimen slides.	2a. Inappropriate fixation method used.	2a. Ensure that only neutral buffered formalin fixative and approved fixation methods are used.
	2b. Insufficient reagent volume applied.	2b. Check size of tissue section and reagent volume applied.
	2c. Inappropriate wash buffer used.	2c. Use only EnVision FLEX Wash Buffer (20x) (Code K8007).
3. Weak staining of specimen slides or of the positive cell line on the kit-supplied Control Slide	3a. Inadequate target retrieval.	3a. Verify that the 3-in-1 pretreatment procedure was correctly performed.
	3b. Inappropriate wash buffer used.	3b. Use only EnVision FLEX Wash Buffer (20x) (Code K8007).
4. Excessive nonspecific staining of slides.	4a. Paraffin incompletely removed.	4a. Verify that the 3-in-1 pretreatment procedure was correctly performed.
	4b. Slides dried after the 3-in-1 pretreatment procedure was performed.	4b. Ensure slides remain wet with 1x Envision FLEX Wash Buffer after the 3-in-1 pretreatment procedure and after loading on the Autostainer Link 48. Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure.
	4c. Nonspecific binding of reagents to tissue section.	4c. Check for proper fixation of the specimen and/or the presence of necrosis.
	4d. Inappropriate fixation method used.	4d. Ensure that only neutral buffered formalin fixative and recommended fixation methods are used.
	4e. Warped Autostainer Link 48 slide racks used.	4e. Ensure that only level Autostainer Link 48 slide racks are used.

Problem	Probable Cause	Suggested Action
5. Tissue detached from slides.	5a. Use of incorrect microscope slides.	5a. Use FLEX IHC Microscope Slides, (Code K8020), or Superfrost Plus slides.
	5b. Inadequate preparation of specimens	5b. Cut sections should be placed in a 58 ± 2 °C oven for 1 hour prior to staining.
6. Excessively strong specific staining.	6a. Inappropriate fixation method used.	6a. Ensure that only approved fixatives and fixation methods are used.
	6b. Inappropriate wash buffer used.	6b. Use only EnVision FLEX Wash Buffer (20x) (Code K8007).
7. 1x EnVision FLEX Target Retrieval Solution, Low pH is cloudy in appearance when heated.	7. When heated the 1x EnVision FLEX Target Retrieval Solution, Low pH turns cloudy in appearance.	7. This is normal and does not influence staining.
8. 1x EnVision FLEX Target Retrieval Solution, Low pH does not meet pH specifications.	8a. pH meter is not calibrated correctly.	8a. Ensure pH meter is calibrated per manufacturer's recommendations. After re-calibration, re-test the pH of 1x EnVision FLEX Target Retrieval Solution, Low pH. Do not modify the pH of 1x EnVision FLEX Target Retrieval Solution, Low pH. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x EnVision FLEX Target Retrieval Solution, Low pH. Prepare new 1x EnVision FLEX Target Retrieval Solution, Low pH. Check the pH of the new 1x EnVision FLEX Target Retrieval Solution, Low pH.
	8b. Inferior quality water is used to dilute the EnVision FLEX Target Retrieval Solution concentrate.	8b. Ensure that distilled or deionized water is used to prepare 1x EnVision FLEX Target Retrieval Solution.
	8c. Incorrect target retrieval solution is used.	8c. Ensure that the correct EnVision FLEX Target Retrieval Solution specified in 'Materials Provided' Section 4 and 'Reagent Preparation' Section 9 is used.
9. Nonspecific > 1+ nuclear staining on PD-L1 and/or NCR slides	9a. Specimen slides dried prior to initiating the Autostainer Link 48 staining procedure.	9a. Ensure slides remain wet with 1x EnVision FLEX Wash Buffer after deparaffinization, rehydration, and target retrieval (3-in-1) procedure and after loading on the Autostainer Link 48. Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure.
	9b. Improper manual slide rinsing with 1x EnVision FLEX Wash Buffer before loading on the Autostainer Link 48.	9b. Slide racks should be placed one rack at a time on the Autostainer Link 48 and then 1x EnVision FLEX Wash Buffer should be manually applied to the slides using a wash bottle. Ensure slides remain wet prior to initiating the Autostainer Link 48 procedure.

NOTE: If the problem cannot be attributed to any of the causes in Table 51, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in the Education Guide: Immunohistochemical Staining Methods[17] (available from Agilent Technologies).

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Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code	Σ	Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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