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), gastric or gastroesophageal junction (GEJ) adenocarcinoma, cervical cancer, urothelial carcinoma and head and neck squamous cell carcinoma (HNSCC) tissues using EnVision FLEX visualization system on Autostainer Link48.

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 gastric or GEJ adenocarcinoma, cervical cancer, urothelial carcinoma and HNSCC 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.

Companion Diagnostic Indications

Tumor Indication	PD-L1 Expression Level	Intended Use
NSCLC	TPS ≥ 1%	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab).**
Gastric or GEJ Adenocarcinoma	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with KEYTRUDA® (pembrolizumab).
Cervical Cancer	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying cervical cancer patients for treatment with KEYTRUDA® (pembrolizumab).
Urothelial Carcinoma	CPS ≥ 10	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying urothelial carcinoma patients for treatment with KEYTRUDA® (pembrolizumab).**
HNSCC	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying HNSCC patients for treatment with KEYTRUDA® (pembrolizumab).**

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

2. Summary and Explanation

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. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth (1).

2.1 NSCLC

Merck Sharp & Dohme 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. Refer to 'Clinical Performance Evaluation (NSCLC)' section below for KN042 study details.

Merck Sharp & Dohme 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. Refer to 'Clinical Performance Evaluation (NSCLC)' section below for KN024 study details.

Merck Sharp & Dohme 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. Refer to 'Clinical Performance Evaluation (NSCLC)' section below for KN010 study details.

2.2 Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma

Merck Sharp & Dohme sponsored clinical study, KEYNOTE-059 (KN059), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (CPS ≥ 1) gastric or GEJ adenocarcinoma patients with at least two prior systemic treatments for advanced disease that may respond to KEYTRUDA treatment. Refer to 'Clinical Performance Evaluation (gastric or GEJ adenocarcinoma)' section below for KN059 study details.

2.3 Cervical Cancer

Merck Sharp & Dohme 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. Refer to 'Clinical Performance Evaluation (cervical cancer)' Section below for KN158 Cohort E study details.

2.4 Urothelial Carcinoma

Merck Sharpe & Dohme sponsored clinical study, KEYNOTE-052 (KN052), investigated the clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expressing (CPS ≥ 10) patients with advanced/unresectable or metastatic urothelial cancer who have not received prior systemic chemotherapy, and who are not eligible to receive cisplatin, who may respond to KEYTRUDA treatment. Refer to 'Clinical Performance Evaluation (UC)' section below for KN052 study details.

2.5 HNSCC

Merck Sharpe & Dohme 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. Refer to 'Clinical Performance Evaluation (HNSCC)' section below for KN048 study 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 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.

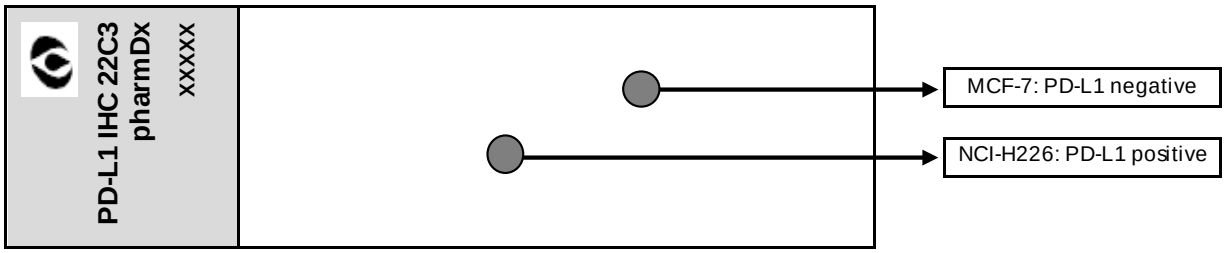
4. Materials Provided

Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and contains the reagents necessary to perform 50 tests in up to 15 individual runs. The materials listed below 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). For larger tissue sections three drop zones (3 x 150µL) per slide may be warranted. 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** 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 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.

Quantity	*Description*
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, formalin-fixed paraffin-embedded 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) (2).

Note: All reagents 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)
Hematoxylin (Code K8008)
Distilled or deionized water (reagent-quality water)
Timer
Positive and negative tissues to use as process controls (see Quality Control section)
Microscope slides: Dako FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus charged slides
Coverslips
Permanent mounting medium and ancillary reagents required for mounting coverslips
Light microscope (4x–40x objective magnification)

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 (3).
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 (4).
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 3x150 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. Safety Data Sheet available for professional users 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.

Danger

DAB+ Substrate Buffer: Contains Imidazole.

H360	May damage the unborn child.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P308 + P313	IF exposed or concerned: Get medical attention.
P405	Store locked up.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.

Danger

DAB+ Chromogen: Contains 3,3-Diaminobenzidine tetrahydrochloride.

H319	Causes serious eye irritation.
H350	May cause cancer.
H341	Suspected of causing genetic defects.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P264	Wash hands thoroughly after handling.
P308 + P313	IF exposed or concerned: Get medical attention.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical attention.
P405	Store locked up.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.

Warning

DAB Enhancer

H400	Very toxic to aquatic life.
H411	Toxic to aquatic life with long lasting effects.
P273	Avoid release to environment.
P391	Collect spillage.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.

Warning

EnVision FLEX Target Retrieval Solution, Low pH (50x)

H319	Causes serious eye irritation.
H411	Toxic to aquatic life with long lasting effects.
P280	Wear eye or face protection.
P273	Avoid release to the environment.
P264	Wash hands thoroughly after handling.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical attention.
P501	Dispose of contents and container in accordance with all local, regional, national and international regulations.

7. Storage

Store all components of PD-L1 IHC 22C3 pharmDx, including 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 and dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. NSCLC FFPE tissue blocks which are 5 years or older may result in a loss of PD-L1 immunoreactivity. Please refer to Section 15.2 (Product Specific Limitations) for gastric or GEJ adenocarcinoma specimens.

Tissue specimens should be cut into sections of 4-5 µm. After sectioning, tissues should be mounted on Dako 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 Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma Cut Section Storage Recommendation

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

8.2.3 Cervical Cancer Cut Section Storage Recommendation

Cut sections must be stained within 5 months when stored at 2-8 ºC (preferred), or within 1 month when stored at 25 ºC.

8.2.4 Urothelial Carcinoma Cut Section Storage Recommendation

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

8.2.5 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.

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 Target Retrieval Solution, Low pH by diluting Target Retrieval Solution, Low pH (50x) 1:50 using distilled or deionized water (reagent-quality water); the pH of 1x Target Retrieval Solution must be 6.1 ± 0.2. 1x Target Retrieval Solution pH below 5.9 may give erroneous results. One 30 mL bottle of 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 Target Retrieval Solution after three uses and do not use after 5 days following dilution.

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 Wash Buffer by diluting Wash Buffer (20x) 1:20 using distilled or deionized water (reagent-quality water) for the wash steps. Store unused 1x solution at 2-8 ºC for no more than one month. Discard 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 1 drop of Liquid DAB+ Chromogen per mL of DAB+ Substrate Buffer and mix. Prepared Substrate-Chromogen 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 9 drops of DAB+ chromogen.** Although the label states 7.2 mL, this is the useable volume and does not account for the "dead volume" 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 above. 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 Section 6, Precautions).

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 during the staining procedure. Dried tissue sections may display increased nonspecific staining.

All of the required steps and incubation times for staining are preprogrammed in the Dako Link 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 Dako Link 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 to obtain the protocols.

Step 1: Deparaffinization, Rehydration and Target Retrieval (3-in-1) Procedure
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 Target Retrieval Solution, Low pH, 1x working solution to cover the tissue sections.
- ►Preheat the Target Retrieval Solution to 65 °C.
- ►Immerse Autostainer racks containing mounted, FFPE tissue sections into the pre-heated Target Retrieval Solution, Low pH, (1x 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, room temperature Wash Buffer (Code K8007).
- ►Incubate slides in diluted, room temperature Wash Buffer for 5 minutes.

Step 2: Staining Procedure
After deparaffinization, rehydration and target retrieval (3-in-1) procedure, the Autostainer racks with slides are placed on Autostainer Link 48. 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 Dako Link software.

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

Step 4: Mounting
Non-aqueous, permanent mounting media 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 above. 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 6 and include: a H&E stained patient tissue specimen; lab-supplied positive and negative control tissues; and a Dako-supplied Control Cell Line Slide (5). In the USA, consult the quality control guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry; see also CLSI Quality Assurance for Immunocytochemistry, Approved Guideline (5, 6, 7) 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 the quality control section above. 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 34.

13. Staining and Scoring Interpretation

13.1 NSCLC – PD-L1 Expression Determined by Tumor Proportion Score

All viable tumor cells on the entire slide must be evaluated and included in the PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present 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 non-specific 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 positivity.

Table 1 below provides details about which tissue elements are included and/or excluded in determining the TPS.

Table 1. 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 6 (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 and ≤ 1+ non-specific staining.

The specimen should be considered to have PD-L1 expression if TPS ≥ 1% of the viable tumor cells exhibit membrane staining at any intensity. The specimen should be considered to have high PD-L1 expression if TPS ≥ 50% of the viable tumor cells exhibit membrane staining at any intensity.

Tumor Proportion Score			
PD-L1 Expression Levels	TPS < 1%	TPS ≥ 1%	TPS ≥ 50%
PD-L1 Expression Status	No PD-L1 Expression	PD-L1 Expression	High PD-L1 Expression

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

13.2 Gastric or GEJ Adenocarcinoma, Cervical Cancer, Urothelial Carcinoma, HNSCC – PD-L1 Expression Determined by Combined Positive Score

All viable tumor cells on the entire tissue section must be evaluated and included in 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:

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

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 6 (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 and ≤ 1+ nonspecific staining.

Refer to sections 13.2.1, 13.2.2, 13.2.3 and 13.2.4 for tumor indication-specific information.

13.2.1 Gastric or Gastroesophageal Junction (GEJ) Adenocarcinoma – CPS Interpretation

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 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.

The CPS denominator includes all viable invasive tumor cells (PD-L1 staining and non-staining). Dysplasia, carcinoma in situ and all other cells are excluded.

Table 2 below provides details about which tissue elements are included in and excluded from the CPS numerator in gastric or GEJ adenocarcinoma

Table 2. 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	• Non-staining 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**: o Lymphocytes (including lymphocyte aggregates) o Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Non-staining 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.*

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

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 gastric or GEJ adenocarcinoma Interpretation Manual for additional guidance.

13.2.2 Cervical Cancer – CPS Interpretation

A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for evaluation.

The CPS denominator includes all viable invasive tumor cells (PD-L1 staining and non-staining). Dysplasia, carcinoma in situ and all other cells are excluded.

Table 3 below provides details about which tissue elements are included in and excluded from the CPS numerator in cervical cancer.

Table 3. 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	• Non-staining 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**: o Lymphocytes (including lymphocyte aggregates) o Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Non-staining 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.*

For cervical cancer, NSCLC tissue with its corresponding TPS scoring algorithm may be used as a positive and/or negative control if no cervical control tissue is available.

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

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 Urothelial Carcinoma – CPS Interpretation

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.

The CPS denominator includes all viable tumor cells (PD-L1 staining and non-staining). All immune cells, normal cells, necrotic cells, ulcers, chronic cystitis, and low-grade papillary carcinoma are excluded.

Table 4 below provides details about which tissue elements are included in and excluded from the CPS numerator in urothelial carcinoma.

Table 4. CPS Numerator Inclusion/Exclusion Criteria for Urothelial Carcinoma

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	• Convincing partial or complete linear membrane staining (at any intensity) of viable urothelial carcinoma tumor cells including: • High grade papillary carcinoma • Carcinoma in situ (CIS) • Any lamina propria, muscularis, or serosal invasion • Metastatic carcinoma	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Low grade papillary carcinoma[†]
Immune Cells	• Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma:** o Lymphocytes (including lymphocyte aggregates) o Macrophages*** Only MICs directly associated with the response to the tumor are scored.	• Non-staining MICs • MICs (including lymphoid aggregates) associated with ulcers, chronic cystitis, and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils, eosinophils, and plasma cells • BCG[††]-induced granulomas
Other Cells	• Not included	• Normal cells

Tissue Elements	Included in the Numerator	Excluded from the Numerator
		• 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 are 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*
†If the tumor consists entirely of low-grade papillary carcinoma, the result should be flagged as such
††Bacillus Calmette-Guérin

The specimen should be considered to have PD-L1 expression if CPS ≥ 10.

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

13.2.4 HNSCC– CPS Interpretation

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.

The CPS denominator includes all viable invasive tumor cells (PD-L1 staining and non-staining). All immune cells, benign cells, necrotic or non-viable tumor cells, carcinoma in situ, stromal cells (including fibroblasts), and necrotic cells and/or cellular debris are excluded.

The table below provides details about which tissue elements are included in and excluded from the CPS numerator in HNSCC.

Table 5. 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	• Non-staining 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:** o Lymphocytes (including lymphocyte aggregates) o Macrophages*** • Only MICs directly associated with the response to the tumor are scored.	• Non-staining 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	• Carcinoma in situ • 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.*

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

	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.

14. Slide Evaluation

Table 6. 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 (Supplied with kit)	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.	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. • Non-specific staining < 1+ intensity.

Specimens	Rationale	Requirements
	The Control Cell Line Slide contains the PD-L1-positive cell line pellet and PD-L1-negative cell line pellet.	*MCF-7 (PD-L1-negative control cell line) acceptance criteria:* • No specific staining. • Non-specific 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 plasma membrane staining, or cytoplasmic 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.	Controls should be biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Use well-preserved specimens for interpretation of staining results as necrotic or degenerated cells often demonstrate non-specific 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 plasma membrane staining should be observed. Non-specific staining should be ≤1+. **Slide stained with Negative Control Reagent**: No membrane staining. Non-specific staining should be ≤ 1+. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid. See section 13.2.2 for additional guidance on control tissue related to cervical cancer.
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.	Controls should be biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Two negative tissue control slides should be included in each staining run. **Slide stained with PD-L1**: No membrane staining in tumor cells. Non-specific staining should be ≤ 1+. **Slide stained with Negative Control Reagent**: No membrane staining. Non-specific staining should be ≤ 1+. If specific cell membrane staining occurs in the Negative Control Tissue Slides, results with the patient specimen should be considered invalid. See section 13.2.2 for additional guidance on control tissue related to cervical cancer.
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 background 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. Non-specific 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. Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding PD-L1 IHC 22C3 pharmDx immunoreactivity.	Positive staining intensity should be assessed within the context of any non-specific background staining observed on the patient's Negative Control Reagent slide in the same run. 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.

Specimens	Rationale	Requirements
		Refer to 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. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any positive staining or its absence 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.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit non-specific staining with horseradish peroxidase (7).
7. 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 Technical Support with documented unexpected reactions.
8. False-positive results may be seen due to non-Immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C) (7).
9. 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 Section 8.2).
2. For optimal and reproducible results, the PD-L1 protein requires target retrieval pre-treatment 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 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. The clinical study in gastric or GEJ adenocarcinoma was conducted with the guidance to provide a minimum of 3 and up to 5 core needle tissue biopsies per patient. The reliability of determining patients' PD-L1 expression level based on testing fewer passes is unknown. Refer to Section 13.2 for guidance on scoring interpretation.
9. The clinical study in urothelial carcinoma was conducted with guidance to provide a minimum of 3 and up to 5 core needle tissue biopsies per patient. The reliability of determining patients' PD-L1 expression level based on testing fewer passes is unknown. Refer to Section 13.2 for guidance on scoring interpretation.
10. If PD-L1 expression is not detected in an archival* gastric or GEJ adenocarcinoma specimen, evaluate the feasibility of obtaining an additional tumor biopsy for PD-L1 testing. (See Table 20 in Clinical Section 16.5)
11. 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.

*In the context of clinical trial KN059, a newly obtained biopsy was defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1 (Cycle 1) with KEYTRUDA and with no additional anti-cancer treatment having been given after the specimen was obtained. Specimens that were >42 days were classified as archival.

16. Performance Evaluation

16.1 Non-Clinical Performance Evaluation: Normal and Neoplastic Tissues

Normal tissues: Table 7 summarizes monoclonal mouse anti-PD-L1, Clone 22C3 immunoreactivity on the recommended panel of normal tissues. Plasma 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 (8, 9).

Table 7: Summary of PD-L1 IHC 22C3 pharmDx Normal Tissue Reactivity

Tissue Type (# tested)	Positive Plasma Membrane Staining: Tissue Elements	Positive Cytoplasmic Staining: Tissue Elements	Non-specific Staining
Adrenal (3)	0/3	1/3 Medullary cells	0/3
Bone marrow (3)	3/3 Megakaryocytes	3/3 Megakaryocytes	0/3
Breast (3)	0/3	0/3	0/3
Cerebellum (3)	0/3	0/3	0/3

Tissue Type (# tested)	Positive Plasma Membrane Staining: Tissue Elements	Positive Cytoplasmic Staining: Tissue Elements	Non-specific Staining
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 (2)	0/2	0/2	0/2
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 (2)	2/2 Epithelium	0/2	0/2
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
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 8 summarizes monoclonal mouse anti-PD-L1, Clone 22C3 immunoreactivity on a panel of neoplastic tissues. Plasma 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 (8-11).

Table 8: 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

Tumor Type	Location	PD-L1 positive/total N=159
Carcinoma	Nasopharyngeal, NPC	0/1
Chondrosarcoma	Bone	0/1
Chordoma	Pelvic cavity	0/1
Embryonal carcinoma	Testis	0/1
Ependymoma	Brain	0/1
Glioblastoma	Brain	0/1
Hepatoblastoma	Liver	0/1
Hepatocellular carcinoma	Liver	0/5
Islet cell tumor	Pancreas	0/1
Interstitialoma	Colon	0/1
	Rectum	0/1
	Small intestine	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
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 cell carcinoma	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
Transitional cell carcinoma	Bladder	0/6
	Kidney	0/1

16.2 Non-Clinical Performance Evaluation: NSCLC

Analytical Sensitivity/Specificity
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 two-sided 95% confidence intervals using the bootstrap method. For studies which resulted in 100% agreement, negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using the Wilson score method for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff.

Table 9: Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 1%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-instrument	TPS ≥ 1%	Each of 24 NSCLC specimens (12	NPA 100% (94.0-100%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
		PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression was tested on each of six Autostainer Link48 instruments.	PPA 100% (94.0-100%) OA 100% (96.9-100%)
Inter-operator	TPS ≥ 1%	Each of 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression was tested using six analysts on one Autostainer Link48 instrument.	NPA 100% (93.9-100%) PPA 100% (94.0-100%) OA 100% (96.9-100%)
Inter-day	TPS ≥ 1%	Each of 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression was tested on six non-consecutive days on the Autostainer Link48 instrument.	NPA 100% (94.0-100%) PPA 100% (94.0-100%) OA 100% (96.9-100%)
Inter-lot	TPS ≥ 1%	Each of 24 NSCLC specimens (13 PD-L1-negative and 11 PD-L1-positive) with a range of PD-L1 IHC expression was tested with three replicates and each of three reagent lots on the Autostainer Link48 instrument.	ANA 98.3% (95.9-100%) APA 97.9% (94.6-100%) OA 98.1% (95.3-100%)
Intra-run (Repeatability)	TPS ≥ 1%	Each of 24 NSCLC specimens (12 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression was tested with six replicates within a run on the Autostainer Link48 instrument.	NPA 100% (94.0-100%) PPA 100% (93.8-100%) OA 100% (96.8-100%)

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 10: Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 50%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-instrument	TPS ≥ 50%	Each of 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression was tested on each of six Autostainer Link48 instruments.	NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-operator	TPS ≥ 50%	Each of 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression was tested using six analysts on one Autostainer Link48 instrument.	NPA 100% (92.7-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-day	TPS ≥ 50%	Each of 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression was tested on six non-consecutive days on the Autostainer Link48 instrument.	NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Inter-lot	TPS ≥ 50%	Each of 16 NSCLC specimens (8 PD-L1-negative and 8 PD-L1-positive) with a range of PD-L1 IHC expression was tested with three replicates and each of three reagent lots on the Autostainer Link48 instrument.	NPA 100% (92.6-100%) PPA 100% (92.6-100%) OA 100% (96.2-100%)
Intra-run (Repeatability)	TPS ≥ 50%	Each of 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression was tested with six replicates within a run on the Autostainer Link48 instrument.	NPA 100% (92.9-100%) PPA 100% (88.6-100%) OA 100% (95.4-100%)
Intra-day	TPS ≥ 50%	Each of 16 NSCLC specimens (10 PD-L1-negative and 6 PD-L1-positive) with a range of PD-L1 IHC expression was tested on two runs within a day, repeated over three days, on the Autostainer Link48 instrument.	NPA 100% (88.3-100%) PPA 100% (82.4-100%) OA 100% (92.4-100%)

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 agreements were calculated since no natural reference exists in reproducibility parameters such as site and observer. Average negative percent agreement (ANA), average positive percent agreement (APA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff.

Table 11: 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%	Each of 36 NSCLC specimens (16 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three 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%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Intra-site	TPS ≥ 1%	Each of 36 NSCLC specimens (16 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three 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%	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three 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%	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 558 pair-wise comparisons.	ANA 93.7% (90.0-96.1%) APA 94.8% (91.6-96.7%) OA 94.3% (92.0-95.9%)

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

Table 12: 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%	Each of 36 NSCLC specimens (21 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three 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%	Each of 36 NSCLC specimens (21 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three 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%	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three 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%	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three 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

KN042: 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 the PD-L1 IHC 22C3 pharmDx Kit, and who had not received 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 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 13 and Figure 1 summarize the efficacy results in the subgroup of patients with TPS ≥ 50% and in all randomized patients with TPS ≥ 1%.

Table 13: 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

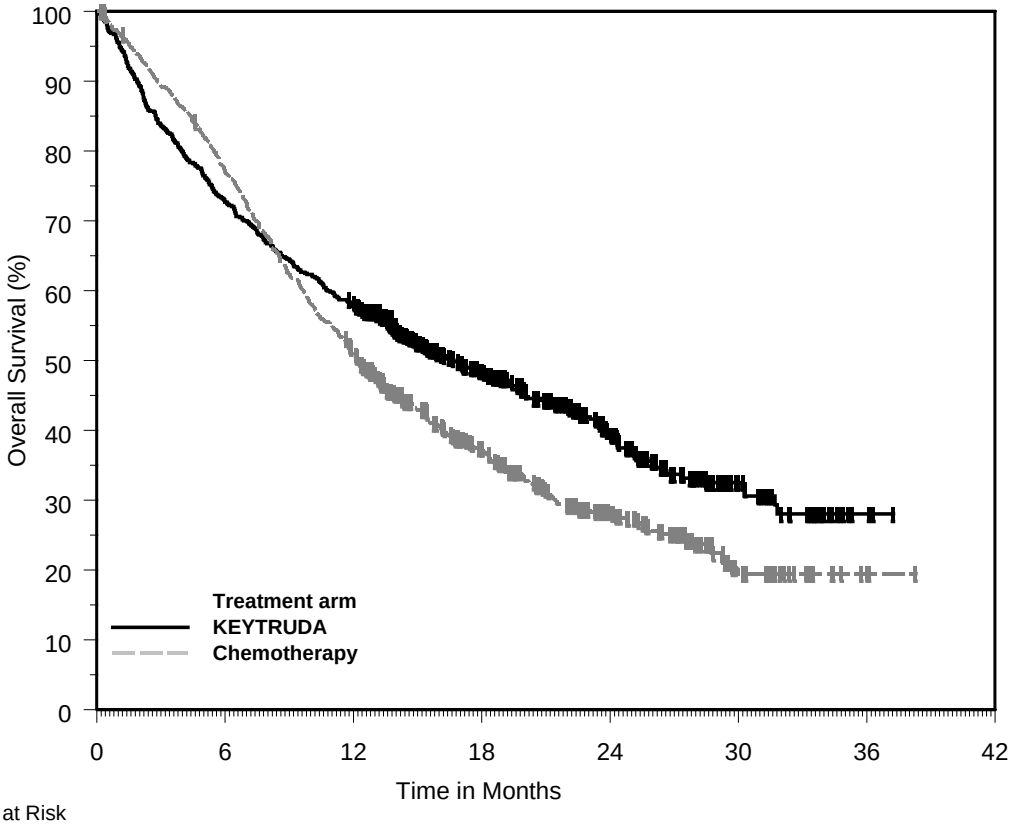
‡ Not evaluated for statistical significance as a result of the sequential testing procedure for the secondary endpoints

§ 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%)



Number at Risk								
KEYTRUDA:	637	463	365	214	112	35	2	0
Chemotherapy:	637	485	316	166	88	24	1	0

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 (12). Key eligibility criteria were metastatic NSCLC, PD-L1 expression tumor proportion score (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 14 summarizes key efficacy measures for the entire intent to treat (ITT) population.

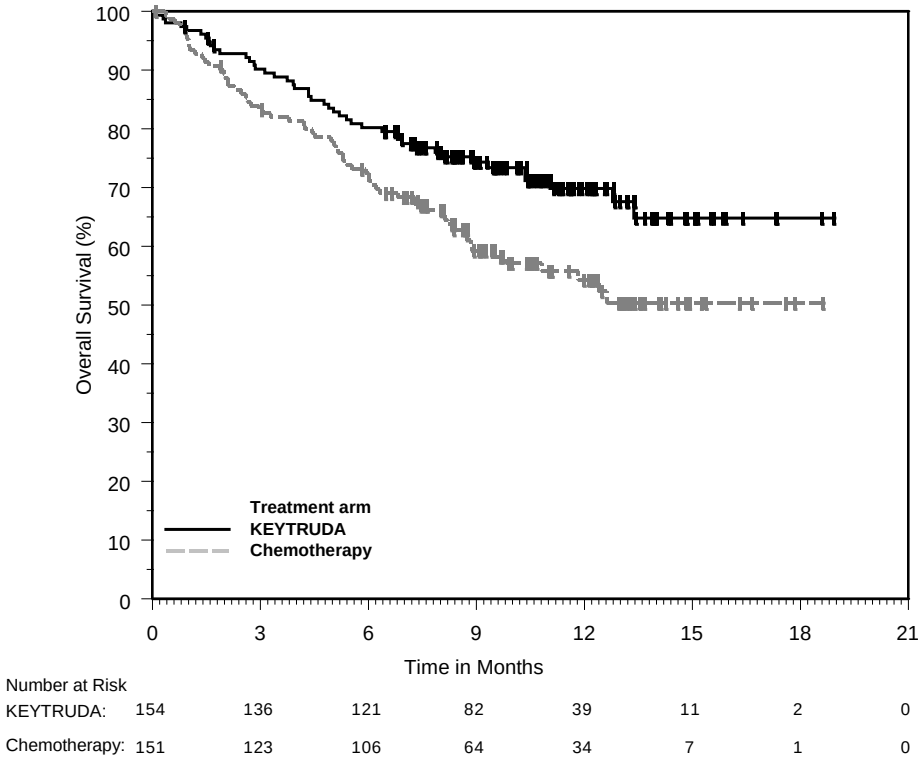
Table 14: Efficacy Results in Trial 24

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 (13). 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 tumor proportion score (TPS) of 1% or greater by a clinical trial assay (CTA) version of PD-L1 IHC 22C3 pharmDx. 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 US-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 (≥ 1% of viable tumor cells exhibiting membrane staining at any intensity) and samples from 94 patients did not have PD-L1 expression (< 1% of viable tumor cells exhibiting membrane staining at any intensity). Within these 413 patients with PD-L1 expression, specimens from 163 patients had high PD-L1 expression (≥ 50% of viable tumor cells exhibiting membrane staining at any intensity).

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 15.

Table 15: 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 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 16 and 17. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS ≥ 1%), which was enhanced in patients with high 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. The tables below summarize key efficacy measures in the overall population with PD-L1 expression (TPS ≥ 1%) and in the high PD-L1 expression (TPS ≥ 50%) subset for the overall clinical study (TPS ≥ 1% by CTA) and in the population with PD-L1 expression 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 16: 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 (63%)	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

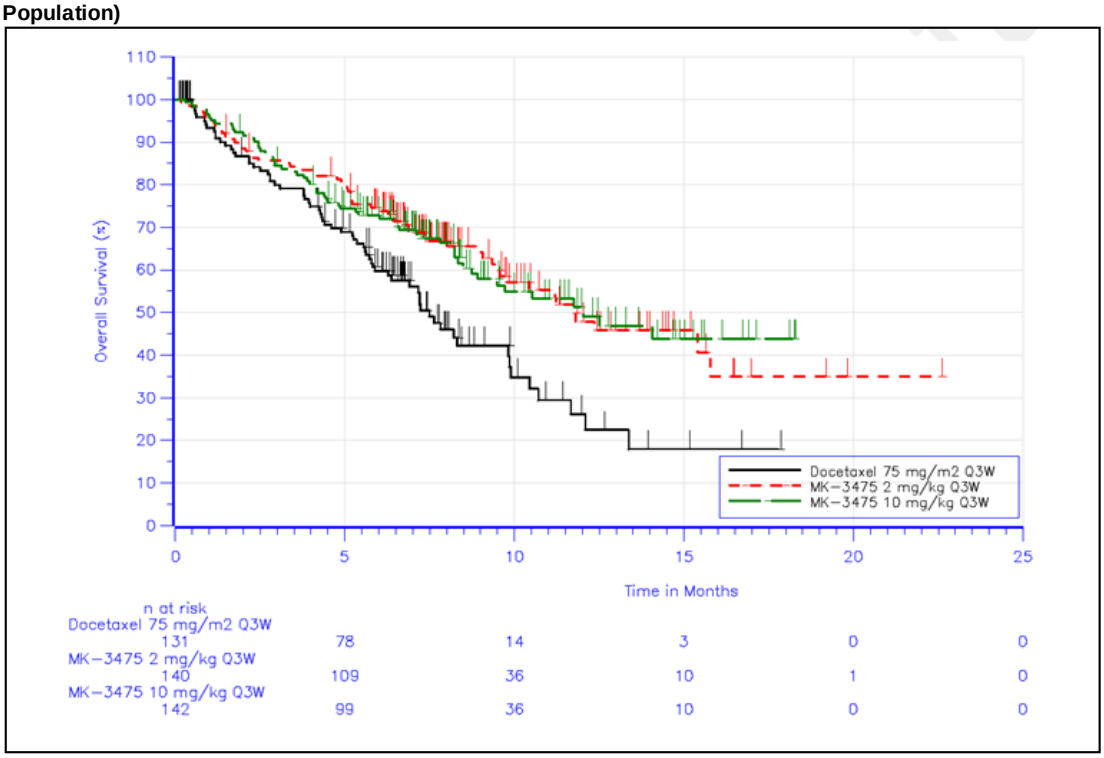
§ All responses were partial responses

Table 17: 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 Non-Clinical Performance Evaluation: Gastric or Gastroesophageal Junction (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. 60% of the specimens had PD-L1 expression, with expression defined by 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-instrument, inter-operator, inter-day, and inter-lot 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% confidence intervals using the Wilson score method for the CPS ≥ 1 cutoff.

Table 18: 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-instrument, inter-operator, inter-lot, inter-day,)	CPS ≥ 1	Each of 24 gastric or GEJ adenocarcinoma specimens (12 PD-L1-positive and 12 PD-L1-negative) with a range of PD-L1 expression was tested using three Autostainer Link48 instruments, four operators, three kit lots, over three non-consecutive days.	NPA 100% (94.9-100%) PPA 95.8% (88.5-98.6%) OA 97.9% (94.1-99.3%
Intra-run precision (Repeatability)	CPS ≥ 1	Each of 24 gastric or GEJ adenocarcinoma specimens (13 PD-L1-negative and 11 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link48 instrument.	NPA 96.9% (89.5-99.2%) PPA 100% (93.5-100%) OA 98.3% (94.1-99.5%)
Inter-observer precision	CPS ≥ 1	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 three pathologists over three non-consecutive 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	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 three pathologists over three non-consecutive 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. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals (CI) using the bootstrap method 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 19 below. Proficiency assessment is recommended to ensure correct observer scoring interpretation.

Table 19: 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	Each of 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three 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	Each of 36 gastric or GEJ adenocarcinoma specimens (16 PD-L1 negative and 20 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three 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	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three 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%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Intra-observer	CPS ≥ 1	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three 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

16.5 Clinical Performance Evaluation: Gastric or Gastroesophageal (GEJ) Adenocarcinoma

The efficacy of KEYTRUDA was investigated in KEYNOTE 059 (KN059), a multicenter, non-randomized, open-label multi-cohort trial that enrolled 259 patients with gastric or GEJ adenocarcinoma who progressed on at least two prior systemic treatments for advanced disease. Previous treatment must have included a fluoropyrimidine and platinum doublet. HER2/neu positive patients must have previously received treatment with approved HER2/neu targeted therapy. Patients with active autoimmune disease or a medical condition that required immunosuppression or with clinical evidence of ascites by physical exam were ineligible.

Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or disease progression that was symptomatic, rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at least 4 weeks later with repeat imaging. Patients without disease progression were treated for up to 24 months. Assessment of tumor status was performed every 6 to 9 weeks. The major efficacy outcome measures were ORR according to RECIST 1.1, as assessed by blinded independent central review, and duration of response.

PD-L1 expression level for 259 patient tumor biopsy or resection tissue (167 archival and 90 newly obtained (refer to definition in Table 20) was determined using PD-L1 IHC 22C3 pharmDx. PD-L1 expression level for 2 samples was not evaluable. Overall, 58% (148/257) of the patients had tumors that expressed PD-L1 with a combined positive score (CPS) ≥ 1. Seventy-three percent (66/90) of patients whose tumors were newly obtained for PD-L1 testing and 49% (82/167) of patients whose archival tumors were tested expressed PD-L1 at CPS ≥ 1 (Table 20).

Table 20: Tumor PD-L1 Expression by Specimen Type

Tumor Tissue	PD-L1 Expression (CPS ≥ 1) n (%)	No PD-L1 Expression (CPS < 1) n (%)
Overall study n=257 (%)	148 (58)	109 (42)
Archival Tissue* n=167	82 (49)	85 (51)
Newly Obtained Tissue* n= 90	66 (73)	24 (27)

*In the context of clinical trial KN059, a newly obtained biopsy was defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1 (Cycle 1) with KEYTRUDA and with no additional anti-cancer treatment having been given after the specimen was obtained. Specimens that were >42 days were classified as archival.

Of 148 patients with PD-L1 expression at CPS ≥ 1, 143 were assessed to be either microsatellite stable (MSS) tumor status or had undetermined MSI or MMR status. The baseline characteristics of these 143 patients were: median age 64 years (47% age 65 or older); 77% male; 82% White, 11% Asian; and ECOG PS of 0 (43%) and 1 (57%). Eighty-five percent had M1 disease and 7% had M0 disease. Fifty-one percent had two and 49% had three or more prior lines of therapy in the recurrent or metastatic setting.

For the 143 patients that have PD-L1 expression (CPS ≥ 1), the ORR was 13.3% (95% CI: 8.2, 20.0); 1.4% had a complete response and 11.9% had a partial response. Among the 19 responding patients, the duration of response ranged from 2.8+ to 19.4+ months, with 11 patients (58%) having responses of 6 months or longer and 5 patients (26%) having responses of 12 months or longer.

16.6 Non-Clinical Performance Evaluation: Cervical Cancer including Combined Squamous Cancers

Non-clinical 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 non-clinical study sample sets to supplement the SQ cervical cancer. These supplemental cancer types were also included in the Merck study KEYNOTE 158. The non-clinical studies comprised of analytical validation, stability and external reproducibility studies.

Analytical Sensitivity/Specificity: Cervical Cancer
Sensitivity of PD-L1 IHC 22C3 pharmDx was analyzed on 370 FFPE cervical cancer specimens (stage I to IV). Assessment of PD-L1 expression demonstrated staining across a range of CPS 0-100. Approximately 85% of cervical cancer specimens had PD-L1 expression, with expression defined by 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-instrument, inter-operator, inter-day, and inter-lot 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% confidence intervals based on the Wilson score method for the CPS ≥ 1 cutoff.

Table 21: 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	CPS ≥ 1	Each of 18 specimens of the squamous subgroup (7 PD-L1 negative and 11 PD-L1-	NPA 100% (91.4-100%) PPA 100% (94.5-100.0%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (inter-instrument, inter-operator, inter-lot, inter-day,)		positive; 6 Cervical Cancer (3 PD-L1 positive/3 PD-L1 negative)) with a range of PD-L1 expression were tested using three Autostainer Link 48 instruments, four operators, three kit lots, over three non-consecutive days.	OA 100% (96.5-100%)
Squamous Cell Histological Subgroup Intra-run precision (Repeatability)	CPS ≥ 1	Each of 8 specimens (0 PD-L1-negative and 8 PD-L1-positive; 2 Cervical Cancer (2 PD-L1 positive)) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA NA PPA 97.5% (87.1-99.6%) OA 97.5% (87.1-99.6%)
Squamous Cell Histological Subgroup Inter-observer precision	CPS ≥ 1	52 squamous specimens (22 PD-L1 negative/30 PD-L1 positive) with a range of PD-L1 IHC expression, were stained with PD-L1 IHC 22C3 pharmDx and then scored by three pathologists over three non-consecutive 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	52 squamous specimens (22 PD-L1 negative/30 PD-L1 positive) with a range of PD-L1 IHC expression, were stained with PD-L1 IHC 22C3 pharmDx and then scored by three pathologists over three non-consecutive 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-instrument, inter-operator, inter-day, and inter-lot 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% confidence intervals based on the Wilson score method for the CPS ≥ 1 cutoff.

Table 22: 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-instrument, inter-operator, inter-lot, inter-day,)	CPS ≥ 1	6 Cervical Cancer specimens (3 PD-L1 negative and 3 PD-L1-positive) with a range of PD-L1 expression were tested using three Autostainer Link 48 instruments, four operators, three kit lots, over three non-consecutive days.	NPA 100.0% (81.6-100%) PPA 100.0% (82.4-100%) OA 100.0 (90.1-100%)
Cervical Cancer Intra-run precision (Repeatability)	CPS ≥ 1	2 Cervical Cancer specimens (0 PD-L1-negative and 2 PD-L1-positive) with a range of PD-L1 IHC expression were tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA NA PPA 100.0% (72.2-100%) OA 100.0% (72.2-100.0%)
Cervical Cancer Inter-observer precision	CPS ≥ 1	21 Cervical Cancer specimens (8 PD-L1 negative/13 PD-L1 positive) with a range of PD-L1 IHC expression, were stained with PD-L1 IHC 22C3 pharmDx and then scored by three pathologists over three non-consecutive days.	NPA 100% (94.9-100%) PPA 99.1% (95.3-99.8%) OA 99.5% (97.1-99.9%)
Cervical Cancer Intra-observer precision	CPS ≥ 1	21 Cervical Cancer specimens (8 PD-L1 negative/13 PD-L1 positive) with a range of PD-L1 IHC expression, were stained with PD-L1 IHC 22C3 pharmDx and then scored by three pathologists over three non-consecutive days.	NPA 100% (94.9-100%) 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. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals based on the Wilson score method for the CPS ≥ 1 cutoff.

Table 23: 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	Each of 22 specimens of the squamous subgroup (9 PD-L1 negative and 13 PD-L1 positive; n=6 Cervical Cancer) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 329 comparisons to majority call.	NPA 94.8% (89.7-97.5%) PPA 97.4% (94.1-98.9%) OA 96.4% (93.7-97.9%)
Intra-site	CPS ≥ 1	Each of 22 specimens of the squamous subgroup (9 PD-L1 negative and 13 PD-L1 positive; n=6 Cervical Cancer) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Intra-site analysis was performed between three sites on a total of 329 comparisons to majority call.	NPA 98.5% (94.6-99.6%) PPA 97.5% (94.3-98.9%) OA 97.9% (95.7-99.0%)
Inter-observer	CPS ≥ 1	Each of 22 specimens of the squamous subgroup (9 PD-L1 negative and 13 PD-L1 positive; n=6 Cervical Cancer) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three sites on a total of 194 comparisons to majority call.	NPA 95.1% (88.0-98.1%) PPA 99.1% (95.2-99.8%) OA 97.4% (94.1-98.9%)
Intra-observer	CPS ≥ 1	Each of 22 specimens of the squamous subgroup (9 PD-L1 negative and 13 PD-L1 positive; n=6 Cervical Cancer) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 194 comparisons to majority call.	NPA 97.4% (91.1-99.3%) PPA 98.3% (93.9-99.5%) OA 97.9% (94.8-99.2%)

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 using cervical cancer. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) were computed with two-sided 95% confidence intervals based on the Wilson score method for the CPS ≥ 1 cutoff.

Table 24: 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	6 Cervical Cancer specimens (2 PD-L1 negative and 4 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 90 comparisons to majority call.	NPA 100% (88.6-100%) PPA 95.0% (86.3-98.3%) OA 96.7% (90.7-98.9%)
Intra-site	CPS ≥ 1	6 Cervical Cancer specimens (2 PD-L1 negative and 4 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Intra-site analysis was performed between three sites on a total of 90 comparisons to majority call.	NPA 100% (88.6-100%) PPA 95.0% (86.3-98.3%) OA 96.7% (90.7-98.9%)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-observer	CPS ≥ 1	6 Cervical Cancer specimens (2 PD-L1 negative and 4 PD-L1 positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three sites on a total of 54 comparisons to majority call.	NPA 100% (82.4-100%) PPA 100% (90.4-100%) OA 100% (93.4-100%)
Intra-observer	CPS ≥ 1	6 Cervical Cancer specimens (2 PD-L1 negative and 4 PD-L1 positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 54 comparisons to majority call.	NPA 100% (82.4-100%) PPA 100% (90.4-100%) OA 100% (93.4-100%)

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

16.7 Clinical Performance Evaluation: Cervical Cancer

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. 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 25.

Table 25: 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

16.8 Non-Clinical Performance Evaluation: Urothelial Carcinoma

The non-clinical studies were performed on FFPE urothelial carcinoma specimens.

Analytical Sensitivity/Specificity: Urothelial Carcinoma
Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 104 FFPE urothelial carcinoma specimens (staged III to IV) using a manufactured production lot. Assessment of PD-L1 expression demonstrated staining across a range of CPS 0-100, where 37% of the specimens had PD-L1 expression with a CPS ≥ 10. One specimen was not evaluable due to high background staining.

Precision: Urothelial Carcinoma
The precision of PD-L1 IHC 22C3 pharmDx was evaluated at Agilent. Inter-instrument, inter-operator, inter-day, and inter-lot 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 two-sided 95% confidence intervals using a percentile bootstrap method for the CPS ≥ 10 cutoff as shown in Table 26.

Table 26: Precision of PD-L1 IHC 22C3 pharmDx in urothelial carcinoma, tested at one site (CPS ≥ 10)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-Operator, Inter-	CPS ≥ 10	Each of 46 urothelial carcinoma specimens (26 PD-L1-negative and 20 PD-L1-positive) with a range of PD-L1	NPA 96.2% (92.3-100%) PPA 98.3% (95.0-100%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Instrument, Inter-Day, and Inter-Lot as combined variables)		IHC expression was tested using three operators, on three Autostainer Link 48 instruments, over three non-consecutive days, using three reagent lots.	OA 97.1% (94.2-99.3%)
Intra-run precision* (Repeatability)	CPS ≥ 10	Each of 32 urothelial carcinoma specimens (17 PD-L1-negative and 15 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA 100% (95.7-100%)* PPA 96.0% (92.0-100.0%) OA 98.1% (96.2-100.0%)
Inter-observer precision	CPS ≥ 10	60 urothelial carcinoma specimens (28 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 three pathologists over three non-consecutive days.	NPA 95.2% (90.3-99.2%) PPA 94.1% (89.9-97.6%) OA 94.6% (91.4-97.4%)
Intra-observer precision	CPS ≥ 10	60 urothelial carcinoma specimens (28 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 three pathologists over three non-consecutive days.	NPA 96.8% (94.3-99.2%) PPA 96.5% (94.1-98.6%) OA 96.7% (94.8-98.3%)

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

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson score method was used to compute confidence intervals for intra-run precision NPA agreement. Note that the Wilson score method has limitations as it assumes independence of data. Since one specimen contributes more than one comparison to majority call, the data are not independent.

External Reproducibility: Urothelial Carcinoma

The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites using urothelial carcinoma. Negative percent agreement (NPA), positive percent agreement (PPA) and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using a percentile bootstrap method.

Table 27: Reproducibility of the PD-L1 IHC 22C3 pharmDx in urothelial carcinoma, tested at three external sites (CPS ≥ 10)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 10	Each of 36 urothelial carcinoma specimens (20 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 539 comparisons to majority call.	NPA 94.0% (87.7-99.3%) PPA 84.6% (77.1-91.7%) OA 89.8% (85.0-94.1%)
Intra-site	CPS ≥ 10	Each of 36 urothelial carcinoma specimens (20 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 539 comparisons to majority call.	NPA 96.2% (92.9-98.8%) PPA 95.0% (92.4-97.4%) OA 95.7% (93.5-97.6%)
Inter-observer	CPS ≥ 10	Scoring of 60 urothelial carcinoma 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three sites on a total of 540 comparisons to majority call.	NPA 97.3% (94.3-99.6%) PPA 90.7% (86.4-94.6%) OA 93.9% (91.3-96.3%)
Intra-observer	CPS ≥ 10	Scoring of 60 urothelial carcinoma 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 540 comparisons to majority call.	NPA 95.7% (93.8-97.8%) PPA 96.1% (93.6-98.3%) OA 95.9% (94.3-97.4%)

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

16.9 Clinical Performance Evaluation: Urothelial Carcinoma

The efficacy of KEYTRUDA was investigated in Study KEYNOTE 052 (NCT02335424), a multicenter, open-label, single-arm trial in 370 patients with locally advanced or metastatic urothelial carcinoma who were not eligible for cisplatin-containing chemotherapy. The trial excluded patients with autoimmune disease or a medical condition that required immunosuppression.

Patients received KEYTRUDA 200 mg every 3 weeks until unacceptable toxicity or 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. Tumor response assessments were performed at 9 weeks after the first dose, then every 6 weeks for the first year, and then every 12 weeks thereafter. The major efficacy outcome measures were ORR according to RECIST 1.1 as assessed by independent radiology review and duration of response.

PD-L1 status was determined using PD-L1 IHC 22C3 pharmDx. Data from the first 100 patients enrolled, the training set, were used to determine the CPS ≥ 10 cutoff. Data from the remaining 270 patients, the validation set, were used to clinically validate the CPS ≥ 10 cutoff.

Among the 370 patients, 30% (n = 110) had tumors that expressed PD-L1 with CPS ≥ 10 and PD-L1 status was unknown for 9 patients. Baseline characteristics of these patients were: median age 73 years, 68% male, and 88% White. Eighty-two percent had M1 disease, and 18% had M0 disease. Eighty-one percent had a primary tumor in the lower tract, and 18% of patients had a primary tumor in the upper tract. Seventy-six percent of patients had visceral metastases, including 11% with liver metastases. Reasons for cisplatin ineligibility included: 45% with baseline creatinine clearance of <60 mL/min, 37% with ECOG performance status of 2, 10% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 8% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

Among the 270 patients in the validation set, 30% (n = 80) had tumors that expressed PD-L1 with CPS ≥ 10. Baseline characteristics of these patients were: median age 72 years, 68% male, and 86% White. Seventy-one percent had M1 disease, and 26% had M0 disease. Seventy-nine percent had a primary tumor in the lower tract, and 20% of patients had a primary tumor in the upper tract. Seventy-eight percent of patients had visceral metastases, including 8% with liver metastases. Reasons for cisplatin ineligibility included: 41% with baseline creatinine clearance of <60 mL/min, 43% with ECOG performance status of 2, 11% with ECOG 2 and baseline creatinine clearance of <60 mL/min, and 5% with other reasons (Class III heart failure, Grade 2 or greater peripheral neuropathy, and Grade 2 or greater hearing loss). Ninety percent of patients were treatment naïve, and 10% received prior adjuvant or neoadjuvant platinum-based chemotherapy.

Efficacy results are summarized in Table 28.

Table 28: Efficacy Results in KN052

Endpoint	CPS < 10 in Validation Set (N=185)	CPS ≥ 10 in Validation Set (N=80)
Objective Response Rate*		
ORR (95% CI)	22% (16, 28)	51% (40, 63)
Complete response rate	2%	16%
Partial response rate	20%	35%
Duration of Response		
Median in months (range)	9.7 (1.4+ - 11.0+)	NR (1.4+ - 11.1+)

+ Denotes ongoing
NR = not reached; *excludes patients with unknown PD-L1 status

KEYNOTE-361 (NCT02853305) is an ongoing, multicenter, randomized study in previously untreated patients with metastatic urothelial carcinoma who are eligible for platinum-containing chemotherapy. The study compares KEYTRUDA with or without platinum-based chemotherapy (i.e., cisplatin or carboplatin with gemcitabine) to platinum-based chemotherapy alone. The trial also enrolled a third arm of monotherapy with KEYTRUDA to compare to platinum-based chemotherapy alone. The independent Data Monitoring Committee (iDMC) for the study conducted a review of early data and found that in patients classified as having PD-L1 expression of CPS < 10, those treated with KEYTRUDA monotherapy had decreased survival compared to those who received platinum-based chemotherapy. The iDMC recommended to stop further accrual of patients with PD-L1 expression of CPS < 10 in the monotherapy arm, however, no other changes were recommended, including any change of therapy for patients who had already been randomized to and were receiving treatment in the monotherapy arm.

16.10 Non-Clinical Performance Evaluation: HNSCC

The non-clinical 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-instrument, inter-operator, inter-day, and inter-lot 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 two-sided 95% confidence intervals using a percentile bootstrap method for the CPS ≥ 1 and CPS ≥ 20 cutoffs as shown in Tables 29 and 30 respectively.

Table 29: 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-Day, and Inter-Lot as combined variables)	CPS ≥ 1	Each of 34 HNSCC specimens (12 PD-L1-negative and 22 PD-L1-positive) with a range of PD-L1 IHC expression was tested using five operators, on five Autostainer Link 48 instruments, over five days, using five reagent lots.	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	Each of 34 HNSCC specimens (16 PD-L1-negative and 18 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on 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	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 three pathologists over three non-consecutive days with a minimum two-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	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	NPA 98.8% (96.0-100.0%) PPA 95.4% (92.3-98.4%) OA 96.7% (94.0-99.1%)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
		by three pathologists over three non-consecutive days with a minimum two-week washout period in between each read.	

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

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson score method was used to compute confidence intervals for combined precision NPA agreement. Note that the Wilson score method has limitations as it assumes independence of data. Since one specimen contributes more than one comparison to majority call, the data are not independent.

Table 30: 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-Day, and Inter-Lot as combined variables)	CPS ≥ 20	Each of 34 HNSCC specimens (17 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression was tested using five operators, on five Autostainer Link 48 instruments, over five days, using five reagent lots.	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	Each of 34 HNSCC specimens (18 PD-L1-negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on 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	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 three pathologists over three non-consecutive days with a minimum two-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	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 three pathologists over three non-consecutive days with a minimum two-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

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson score method was used to compute confidence intervals for combined precision NPA agreement. Note that the Wilson score method has limitations as it assumes independence of data. Since one specimen contributes more than one comparison to majority call, the data are not independent.

External Reproducibility: HNSCC

The reproducibility of PD-L1 IHC 22C3 pharmDx was evaluated at three external sites. Negative percent agreement (NPA), positive percent agreement (PPA) and overall percent agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method.

Table 31: 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	Each of 38 HNSCC specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 570 comparisons to majority call.	NPA 96.8% (92.6-100.00%) PPA 93.3% (86.7-98.6%) OA 95.1% (91.2-98.2%)
Intra-site	CPS ≥ 1	Each of 38 HNSCC specimens (19 PD-L1-negative and 19 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three 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	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three 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	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three 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 32: 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	Each of 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three 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	Each of 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three 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	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Inter-observer analysis was performed between three 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	Scoring of 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, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three 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.

16.11 Clinical Performance Evaluation: HNSCC

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. 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 the PD-L1 IHC 22C3 pharmDx Kit, 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 the PD-L1 IHC 22C3 pharmDx Kit 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 33) 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 33).

Table 33: 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 34 summarizes efficacy results for KEYTRUDA as a single agent in the subgroup of patients with CPS ≥ 1 HNSCC and CPS ≥ 20 HNSCC. Figure 4 summarizes the OS results in the subgroup of patients with CPS ≥ 1 HNSCC.

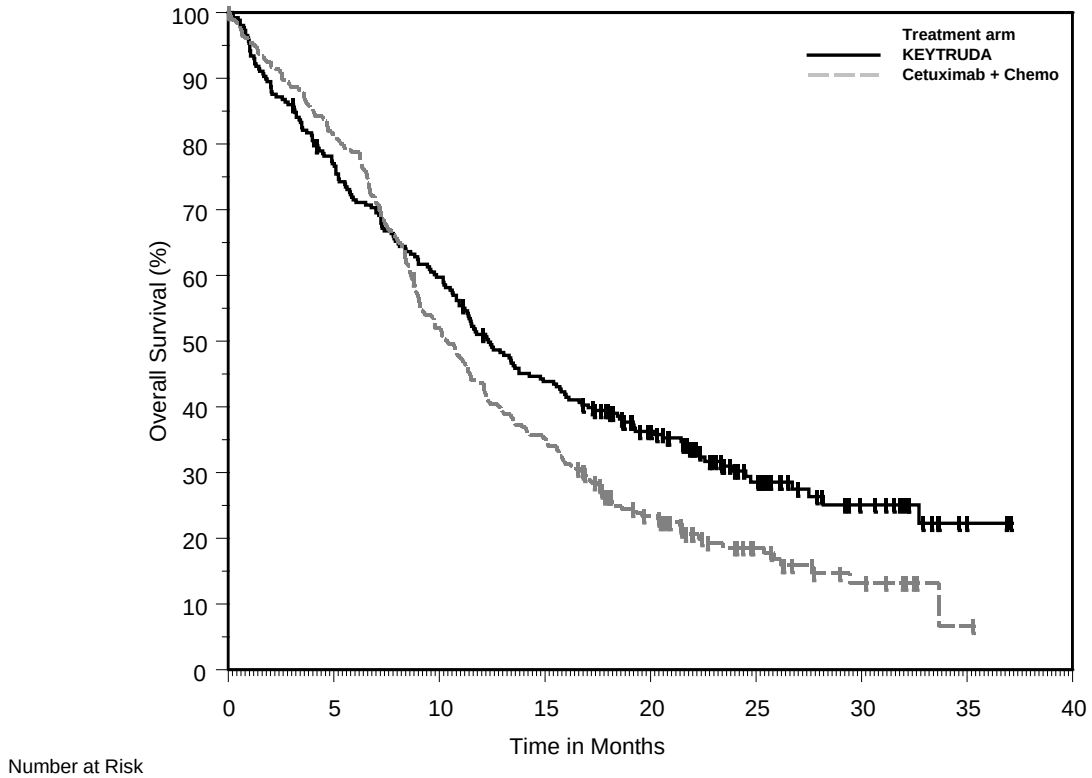
Table 34: Efficacy Results for KEYTRUDA as a Single Agent in KEYNOTE-048 (CPS ≥ 1 and CPS ≥ 20)

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 4: Kaplan-Meier Curve for Overall Survival for KEYTRUDA as a Single Agent in KEYNOTE-048 (CPS ≥ 1)



17. Troubleshooting

Table 35: Troubleshooting

Problem	Probable Cause	Suggested Action
1. No staining of slides	1a. Programming error.	1a. Verify that the PD-L1 IHC 22C3 pharmDx program 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 Dako Wash Buffer (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 Dako Wash Buffer, Code K8007.
3. Weak staining of specimen slides or of the positive cell line on the Control Slide provided with the kit	3a. Inadequate target retrieval.	3a. Verify that the 3-in-1 pre-treatment procedure was correctly performed.
	3b. Inappropriate wash buffer used.	3b. Use only Dako Wash Buffer, Code K8007
4. Excessive background staining of slides.	4a. Paraffin incompletely removed.	4a. Verify that the 3-in-1 pre-treatment procedure was correctly performed.
	4b. Slides dried while loading onto the Autostainer Link 48.	4b. Ensure slides remain wet with buffer while loading and prior to initiating run.
	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.
5. Tissue detached from slides.	5a. Use of incorrect microscope slides.	5a. Use Dako 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 Dako Wash Buffer, Code K8007.
7. Target Retrieval Solution is cloudy in appearance when heated.	7. When heated the Target Retrieval Solution turns cloudy in appearance.	7. This is normal and does not influence staining.

NOTE: If the problem cannot be attributed to any of the above causes, or if the suggested corrective action fails to resolve the problem, please call Agilent Technical Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Dako Education Guide: Immunohistochemical Staining Methods (5) (available from Agilent).

18. References

1. Keytruda® package insert.
2. R. Phelps, et al., *Journal of Cellular Biochemistry Supplement* (1996) 24:32-91.
3. Department of Health, Education and Welfare, National Institutes for Occupational Safety and Health, Rockville, MD. "Procedures for the decontamination of plumbing systems containing copper and/or lead azides." DHHS (NIOSH) Publ. No. 78-127, Current 13. August 16, 1976.
4. Clinical and Laboratory Standards Institute (formerly NCCLS). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline – Third Edition. CLSI document M29-A3 [ISBN 1-56238-567-4]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 – 1898 USA, 2000.
5. Taylor CR and Rudbeck L. Education Guide: Immunohistochemical Staining Methods. Sixth Edition. Dako, Carpinteria, California; 2013.
6. Clinical and Laboratory Standards Institute (formerly NCCCLS). Quality assurance for Immunocytochemistry; Approved guideline. CLSI document MM4-A (1-56238-396-5) CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 1999.
7. Omata M, Liew C-T, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B s surface antigen: a possible source of error in immunohistochemistry. Am J Clin Path 1980; 73:626.
8. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. International Immunol 2007(19); 7:813.
9. Brown JA, Dorfman DM, Ma F-R, Sullivan EL, Munoz O, Wood CR, et al. Blockade of Programmed Death-1 Ligands on Dendritic Cells Enhances T Cell Activation and Cytokine Production. J Immunol 2003;170:1257.
10. Cooper WA, Tran T, Vilain RE, Madore J, Seliger CI, Kohonen-Cornish M, et al. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 2015; 89:181.
11. Chen B, Chapuy B, Ouyang J et al. PD-L1 Expression Is Characteristic of a Subset of Aggressive B-cell Lymphomas and Virus-Associated Malignancies. Clin Cancer Res 2013; 19:3462-3473.
12. Reck, M, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer (KEYNOTE-024). The New England Journal of Medicine (NJEM.org) 2016 Oct 09; 1-11.
13. Herbst RS, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. The Lancet online; 2015 Dec 19.
14. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. New Eng J Med 2015; 372:2018-28.
15. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le D, Burtness B, McRee A, Lin C-C, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang Y. Pembrolizumab for patients with PD-L1–positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol. 2016;17(6):717-726.
16. Schellens JHM, Marabelle A, Zeigenfuss S, et al. Pembrolizumab for previously treated advanced cervical squamous cell cancer: preliminary results from the phase 2 KEYNOTE-158 study. J Clin Oncol. 2017;35(Suppl 15):5514.
17. Chung HC, Schellens JHM, Delord J-P, et al. Pembrolizumab treatment of advanced cervical cancer: updated results from the phase 2 KEYNOTE-158 study. J Clin Oncol. 2018;36(Suppl 15):5522.
18. Balar AV, Castellano D, O'Donnell PH, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): A multicentre, single-arm, phase 2 study. Lancet Oncol 2017;18(11):1483-1492.
19. Burtness B, et al. KEYNOTE-048: Phase 3 study of first-line pembrolizumab (P) for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC). ESMO 2018 Congress. 22 October 2018.

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

Dako North America, Inc.
6392 Via Real
Carpinteria, California 93013 USA

Tel 805 566 6655
Fax 805 566 6688
Technical Support 800 424 0021
Customer Service 800 235 5763

EC REP Dako Denmark A/S
Produktionsvej 42
DK-2600 Glostrup Denmark

Tel +45 4485 9500
Fax +45 4485 9595
www.agilent.com

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