# PD-L1 IHC 22C3 pharmDx

## Code GE006

60 tests for use with Dako Omnis

Table of Contents

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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) tissue, using EnVision FLEX visualization system on Dako Omnis.

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.

**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).* |
| | TPS ≥ 50% | |

*See the KEYTRUDA® product label for PD-L1 expression cutoff values and specific clinical circumstances guiding therapy.

### 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.[1] In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.[2]

PD-L1 IHC 22C3 pharmDx, Code SK006, is designed for use on Autostainer Link 48 and was used in the clinical trials for KEYTRUDA, for details, refer to the Instructions for Use (IFU) for Code SK006.[3] PD-L1 IHC 22C3 pharmDx, Code GE006, has been re-configured for use on Dako Omnis staining platform. PD-L1 IHC 22C3 pharmDx, Codes SK006 and GE006, use platform-specific vials, but both use the same reagents at the same concentrations. Reagents for Code GE006 are available in modular format in Dako Omnis specific vials, listed in sections 4 and 5.

**NOTE: Reagents for SK006 and GE006 are not interchangeable due to their platform-specific configuration.**

#### NCSLC

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.[4] PD-L1 expressing (TPS ≥ 50%) NSCLC patients treated with KEYTRUDA displayed improved outcomes compared to standard of care chemotherapy. 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 locally advanced or metastatic NSCLC patients that may respond to KEYTRUDA treatment where the benefit shown in KN024 was confirmed.[5] Refer to 'Clinical Performance Evaluation (NSCLC)' Section for KN024 and KN042 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.[6] PD-L1 expressing (TPS ≥ 1%) NSCLC patients treated with KEYTRUDA displayed improved outcomes compared to docetaxel. Refer to 'Clinical Performance Evaluation (NSCLC)' Section for KN010 study details.

### 3. Principle of Procedure

PD-L1 IHC 22C3 pharmDx, Code GE006, contains optimized reagents and protocol required to complete an IHC staining procedure on FFPE specimens using Dako Omnis. Following incubation with the primary monoclonal antibody to PD-L1 or the Negative Control Reagent (NCR), specimens are incubated with peroxidase block, incubated with a linker antibody specific to the host species of the primary antibody, and then 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 bright-field microscope. Please consult the Dako Omnis Basic User Guide for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

**4. Materials Provided**

The materials listed are sufficient for 60 tests: 60 slides incubated with the primary antibody to PD-L1 protein and 60 slides incubated with the Negative Control Reagent. GE006 includes 12 mL of PD-L1 Primary Antibody and 12mL of Negative Control Reagent (both approximately 3 µg/mL protein concentration). GE006 has been optimized for use with the Dako Omnis instrument. Please refer to the Dako Omnis Basic User Guide for further information.

| Quantity | Description |
|---|---|
| 1 x 12 mL | **Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3** |

**MONOCLONAL MOUSE ANTI-PD-L1 CLONE 22C3 (Dako Omnis)**

Monoclonal mouse ($IgG_1$) anti-PD-L1 in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

1 x 12 mL **Negative Control Reagent**

**NEGATIVE CONTROL REAGENT (Dako Omnis)**

Monoclonal mouse control ($IgG_1$) antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

**5. Materials Required, but Not Supplied**

Dako Omnis (Code GI100)
EnVision FLEX, High pH (Dako Omnis) (Code GV800) or EnVision FLEX Mini Kit, High pH (Dako Omnis) (Code GV823)
- EnVision FLEX DAB+ Chromogen (Dako Omnis)
- EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis)
- EnVision FLEX Substrate Buffer (Dako Omnis)
- EnVision FLEX Target Retrieval Solution, High pH (50x)*
- EnVision FLEX Visualization Reagent (Dako Omnis)

EnVision FLEX Target Retrieval Solution, Low pH (50x) (Dako Omnis) (Code GV805)
EnVision FLEX+ Mouse Linker (Dako Omnis) (Code GV821)
EnVision FLEX DAB Enhancer (Dako Omnis) (Code GC806)
Hematoxylin (Dako Omnis) (Code GC808)
Wash Buffer (20x) (Dako Omnis) (Code GC807)
Clearify™ clearing agent (GC810)
Dako Omnis Sulfuric Acid, 0.3 M (Code GC203)
Distilled or de-ionized water (reagent-grade water) **
Ethanol, absolute
Xylene, toluene, or xylene substitutes
Materials for permanent mounting
Timer
Microscope slides: Dako FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides
Bright-field microscope (4-40x objective magnification)
Positive and negative tissue to use as process controls (see Quality Control section 12)
pH meter (calibrated per manufacturer's recommendations)

***NOTE: Use EnVision FLEX Target Retrieval Solution, Low pH (50x) (Dako Omnis), Code GV805, for heat-induced epitope retrieval (HIER) with GE006. Do not use EnVision FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis) Code GV804. The color of the EnVision FLEX Target Retrieval Solution, Low pH (50x) (Dako Omnis), Code GV805, is red.**

****NOTE: Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water [corresponding to Clinical Laboratory Reagent Water (CLRW) standard as specified by CLSI], or water similar in quality to be used for reagent preparation.[7]**

**6. Optional Materials**

PD-L1 Control Slides (Code T1391)

**7. 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.[8]
4) PD-L1 Primary Antibody and Negative Control 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.[9]
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) Paraffin residuals may lead to false negative results.
9) Results from small studies showed similar dynamic ranges of PD-L1 expression in primary and metastatic NSCLC pairs. It is possible there may be differences in PD-L1 expression in primary tumors versus metastatic sites in the same patient as each tumor has unique heterogeneity.
10) 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.
11) Wear appropriate Personal Protective Equipment (PPE) to avoid contact with eyes and skin.
12) Unused solution should be disposed of according to local, State and Federal regulations.
13) Safety Data Sheets are available on www.agilent.com or on request.
14) For countries outside of the European Union, see the local KEYTRUDA product label for approved indications and expression cutoff values to guide therapy.
15) Lack of adherence to the maintenance schedule for the Dako Omnis instrument may give erroneous results. Refer to Dako Omnis Basic and Advanced User Guides for additional information and for additional instrument-related precautions.
16) PD-L1 IHC 22C3 pharmDx, Code GE006, is designed solely for Dako Omnis and should not be used on any other staining platform. Reagents designed for use on Autostainer Link 48 (PD-L1 IHC 22C3 pharmDx, Code SK006) should not be substituted for any reagents listed in Section 5.

## 8. Storage

Store all components of PD-L1 IHC 22C3 pharmDx, Code GE006, in the original container in the dark at 2-8 °C when not in use on Dako Omnis. During storage, the cap on each vial should be closed.

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

Onboard reagent stability for Code GE006 has been validated to 375 hours. After staining completion, the reagents should be removed from Dako Omnis, caps replaced securely on the vials, and stored in the dark at 2-8 °C. For onboard stability of all ancillary components including diluted working solutions of Wash Buffer and EnVision FLEX Target Retrieval Solution, refer to respective Instructions for Use. Onboard time of reagents is tracked by the Dako Omnis software; refer to the Dako Omnis Basic and Advanced User Guides for details.

**NOTE:** There are no obvious visual signs to indicate incorrect product storage or handling of this product during the product's shelf life. Positive and negative controls should be run simultaneously with patient specimens, preferably on the same slide, to monitor product performance during the product's shelf life. If a problem is suspected with the antibody during the shelf life that cannot be explained by incorrect product storage or handling, or other variations in laboratory procedures, contact Agilent Pathology Support. Refer to 'Troubleshooting' section 17 and 'Quality Control' section 12 for more information.

## 9. Specimen Preparation

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

### 9.1 Paraffin-embedded tissue

FFPE tissues are suitable for use. Alternative fixatives have not been validated and may give erroneous results. Fixation time for 12 to 72 hours in 10% neutral buffered formalin (NBF) is recommended. 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. FFPE tissue blocks which are 5 years or older may result in a loss of PD-L1 immunoreactivity.

### 9.2 Tissue sections

FFPE tissue specimens should be cut into sections of 4 to 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 calibrated oven for 1 hour.

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. Cut sections must be stained within 5 months when stored at 2 to 8 °C (preferred), or at 25 °C. Slide storage and handling conditions should not exceed 25 °C at any point post-mounting to ensure tissue integrity and antigenicity.

The tissue specimens must be mounted on the slide within the defined slide staining area. Please consult the Dako Omnis Basic User Guide for dimensions of slide staining area.

## 10. Reagent Preparation

The user should adhere to appropriate PPE requirements and become familiar with all components prior to use (see Precautions).

EnVision FLEX Target Retrieval Solution, Low pH (50x) (GV805) and Wash Buffer (20x) (GC807) must be diluted to 1x concentration according to their Instructions for Use. The color of the FLEX Target Retrieval Solution, Low pH (50x) (GV805) is red.

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. Do not modify the pH of 1x Target Retrieval Solution after preparation under any circumstance. If a problem is suspected with the Target Retrieval Solution pH, refer to the 'Troubleshooting' section for more information.

Reagents do not need to be equilibrated to room temperature before loading into the instrument. However, they should be loaded into the instrument before starting the staining procedure, which allows sufficient time for equilibration.

## 11. Staining Procedure

### 11.1 Procedural notes

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

The automated staining procedure for PD-L1 IHC 22C3 pharmDx, Code GE006, includes deparaffinization of tissue sections, target retrieval, and staining. The slides are unloaded in the wet unloading station. All protocol steps are pre-programmed into the Dako Omnis software. The "PD-L1 IHC 22C3 pharmDx" protocol is used with PD-L1 Primary Antibody: monoclonal mouse anti-PD-L1, Clone 22C3, and the "PD-L1 IHC 22C3 pharmDx Negative Control Reagent" protocol is used with the isotype-matched Negative Control Reagent. Please refer to the Dako Omnis Basic User Guide for further information on loading slides and reagents.

The reagents and instructions have been designed for optimal performance. Further dilution of the reagents or alteration of incubation temperatures may give erroneous or discordant results. Differences in tissue processing and technical procedures in the user's laboratory may invalidate the assay results.

**NOTE:** Laboratories located at high elevations should determine the best method of maintaining the required temperature (95 to 99 °C) during heat-induced epitope retrieval. Any adjustments required to address elevation concerns must be validated by the user. See the Dako Omnis Advanced User Guide for further information on creating new protocols.

### 11.2 Pre-staining procedure

1. Choose the "PD-L1 IHC 22C3 pharmDx" or "PD-L1 IHC 22C3 pharmDx Negative Control Reagent" protocol to be applied for each slide from the Dako Link Omnis Workstation software.
2. Ensure the Dako Link Omnis Workstation software is configured to print slide labels with the protocol name displayed.
3. Print slide labels and attach them to the glass slides.
4. Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides.
5. Ensure that the bulk bottles with fluids are onboard and registered by the Dako Omnis instrument. Bulk bottle fluids:
    a. Clearify clearing agent (Code GC810)
    b. EnVision FLEX Target Retrieval Solution Low pH (Code GV805) **diluted to 1x working concentration with distilled or de-ionized water**
    c. Wash buffer (Code GC807) **diluted to 1x working concentration with distilled or de-ionized water**
6. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module:
    a. Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, Code GE006
    b. Negative Control Reagent, Code GE006
    c. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis), Code GV800 or GV823
    d. EnVision FLEX Visualization Reagent (Dako Omnis), Code GV800 or GV823
    e. EnVision FLEX Substrate Buffer (Dako Omnis), Code GV800 or GV823
    f. EnVision FLEX DAB+ Chromogen (Dako Omnis), Code GV800 or GV823
    g. EnVision FLEX+ Mouse LINKER (Dako Omnis), Code GV821
    h. EnVision FLEX DAB Enhancer (Dako Omnis), Code GC806
    i. Optional: Hematoxylin (Dako Omnis), Code GC808 or equivalent
    j. Sulfuric Acid, 0.3 M, Code GC203
7. Load the Slide Rack onto Dako Omnis.
8. Follow the instructions on the touch screen and tap "Done" to initiate the staining procedure.
9. Ensure the slide unloading station is filled with distilled or de-ionized water to prevent slides from drying.

**NOTE:** The "PD-L1 IHC 22C3 pharmDx" or "PD-L1 IHC 22C3 pharmDx Negative Control Reagent" protocols on the Dako Omnis instrument can be monitored on the Dako Link Omnis Workstation

### 11.3 Counterstain

Slides should be counterstained with Dako Hematoxylin (Code GC808). The "PD-L1 IHC 22C3 pharmDx" and "PD-L1 IHC 22C3 pharmDx Negative Control Reagent" protocols on Dako Omnis include a counterstaining step that is pre-programmed for 3 minutes with Hematoxylin (Dako Omnis) (Code GC808). Slides are ready for mounting when removed from the Dako Omnis unloading station. The counterstaining step is editable when a copy of the protocol is created. If counterstains other than the recommended Dako Hematoxylin (Code GC808) are preferred and adjustments are made to the protocol, they must be validated by the user. See the Dako Omnis Advanced User Guide for further information on creating new protocols.

### 11.4 Mounting

After staining onboard Dako Omnis, the sections must be dehydrated, cleared, and mounted using non-aqueous, permanent mounting methods.

### 11.5 Stained slide storage

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 stained slides in the dark at room temperature (20-25 °C).

## 12. Quality Control

PD-L1 IHC 22C3 pharmDx, Code GE006, has been quality-controlled by immunohistochemistry using the required reagents 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. Consult the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry guidelines.[10] See also the Educational Guide, "Immunohistochemical Staining Methods", and CLSI "Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline" for additional information.[11,12]

### 12.1 System-level controls

Positive and negative control tissue (lab-supplied) should be run for each staining procedure. These quality controls are intended to ensure the validity of the staining procedure, including reagents, tissue processing and instrument performance. It is recommended that control tissue be stained on the same slide as the patient tissue. The positive control should be a tissue with positive biomarker expression fixed in the same way as the patient tissue. Internal positive elements of the patient tissue may be used as positive controls. The negative control should be a tissue with no biomarker expression. Internal negative elements of the patient tissue may be used as negative controls. If controls are not fixed in the same way as the patient tissue, the control may only be used as a staining control for reagents and instrument performance. Refer to Table 3 for more information on quality controls including H&E stained patient tissue specimen, and lab-supplied positive and negative control tissues.

### 12.2 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 and guidelines outlined in the 'Quality Control' section for additional information. 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 17.

### 12.3 Negative Control Reagent (optional)

Negative Control Reagent is used in place of the primary antibody with a section of each patient tissue to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site. Use the "PD-L1 IHC 22C3 pharmDx Negative Control Reagent" Dako Omnis protocol for slides stained with the Negative Control Reagent.

## 13. Staining and Scoring Interpretation for NSCLC

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

Slide evaluation should be performed by a pathologist using a bright-field microscope. For evaluation of the immunohistochemical staining and scoring, an objective of 10x to 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 Tumor Proportion Score (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 expression level.

Table 1 provides details about which tissue elements are included and/or excluded in determining the Tumor Proportion Score.

**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:<br>• Mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages)<br>• Plasma cells<br>• Neutrophils |
| **Other** | Not included | Exclude any staining of:<br>• Normal cells adjacent to tumor cells<br>• Stromal cells (fibroblasts)<br>• Necrotic cells and/or cellular debris<br>• Anthracotic pigment |

For each staining procedure, tissues should be examined in the order presented in Table 3 to determine the validity of the staining and enable assessment of the staining of the patient tissue. Examine patient specimens stained with PD-L1 and the Negative Control Reagent (if using) 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 (Table 2).

**Table 2. PD-L1 expression status based on Tumor Proportion Score**

| | 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 the PD-L1 IHC 22C3 pharmDx NSCLC Interpretation Manual for additional guidance.

**14. Tissue Evaluation**

**Table 3. Recommended order of tissue evaluation**

| Specimens | Rationale | Requirements |
|---|---|---|
| 1. Patient tissue stained with H&E | A hematoxylin and eosin (H&E) stain of the patient tissues 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.<br><br>Tissue specimens should be intact, well preserved, and should confirm tumor indication. |
| 2. Positive control tissue stained with PD-L1 Primary Antibody<br><br>(Lab-supplied) | The positive control tissue stained with PD-L1 Primary Antibody should be examined next. Known positive control tissue 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 tissue(s).<br><br>Use well-preserved specimens for interpretation of staining results as necrotic or degenerated cells often stain non-specifically.<br><br>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.<br><br>Positive control tissue should be included in each staining procedure. On slide tissue controls are recommended.<br><br>**Tissue sections stained with PD-L1 Primary Antibody**: Presence of brown plasma membrane staining should be observed. Non-specific staining should be ≤ 1+. |

| Specimens | Rationale | Requirements |
|---|---|---|
| | | If the positive control tissues fail to demonstrate appropriate positive staining, results with the patient tissue should be considered invalid. |
| 3. **Optional:** Positive control tissue stained with Negative Control Reagent<br><br>(Lab-supplied) | Negative Control Reagent may be used to stain the positive control tissue specimen if needed for troubleshooting purposes. | **Tissue sections stained with Negative Control Reagent:** No membrane staining. Non-specific staining should be ≤ 1+. |
| 4. Negative control tissue stained with PD-L1 Primary Antibody<br><br>(Lab-supplied) | The negative control tissue (known to be PD-L1 negative) stained with PD-L1 Primary Antibody should be examined next to verify the labeling specificity of the target antigen by the primary antibody.<br><br>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 tissue(s).<br><br>Use well-preserved specimens for interpretation of staining results as necrotic or degenerated cells often stain non-specifically.<br><br>Negative control tissue should be included in each staining procedure. On-slide tissue controls are recommended.<br><br>**Tissue sections stained with PD-L1 Primary Antibody**: No membrane staining in tumor cells. Non-specific staining should be ≤ 1+.<br><br>If specific cell membrane staining occurs in the negative control tissue, results with the patient specimen should be considered invalid. |
| 5. **Optional:** Negative control tissue stained with Negative Control Reagent<br><br>(Lab-supplied) | Negative Control Reagent may be used to stain the negative control tissue specimen if needed for troubleshooting purposes. | **Tissue sections stained with Negative Control Reagent**: No membrane staining. Non-specific staining should be ≤ 1+. |
| 6. **Optional:** Patient tissue stained with Negative Control Reagent | Examine patient tissue stained with the Negative Control Reagent from PD-L1 IHC 22C3 pharmDx, Code GE006. 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+.<br><br>If patient tissue stained with Negative Control Reagent fails to demonstrate appropriate staining, the corresponding patient tissue stained with the primary antibody is considered non-evaluable and the patient tissue must be retested. |
| 7. Patient tissue stained with PD-L1 Primary Antibody | Examine the patient specimen(s) stained with the PD-L1 Primary Antibody from PD-L1 IHC 22C3 pharmDx, Code GE006, last to assess PD-L1 protein status. 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 staining observed in the staining procedure. Negative Control Reagent is recommended for this assessment if non-specific staining is observed.<br><br>As with any immunohistochemical test, a result showing no staining means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed.<br><br>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.<br><br>Refer to Section 13 ‘Staining and Scoring Interpretation’ for details on patient tissue evaluation. |

## 15. Limitations

### 15.1 General limitations

1) 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.

2) 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.
3) Excessive or incomplete counterstaining may compromise proper interpretation of results.
4) Staining artifacts caused by non-specific DAB chromogen particles require re-test of the stained slides if the artifacts impair the interpretation of PD-L1 staining. Background staining should be evaluated by comparing tissue stained with the primary antibody to tissue stained with Negative Control Reagent.
5) The clinical interpretation of any PD-L1staining 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.[13]
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.[14] Contact Agilent Pathology 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).[11]
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. Any protocol adjustments made must be validated by the user.
10) Slides flagged in the slide log on the Dako Omnis Workstation should be investigated by qualified personnel. See Dako Omnis Basic User Guide.
11) Canceled slides indicate that a significant issue occurred during staining and should not be used. The specimen will require re-staining. Refer to the Dako Omnis User Guide for further details.

### 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) For optimal and reproducible results, the PD-L1 protein requires target retrieval pre-treatment on the Dako Omnis instrument when tissues are routinely fixed (neutral buffered formalin) and paraffin embedded.
3) Use of PD-L1 IHC 22C3 pharmDx on tissues with fixatives other than formalin has not been validated.
4) Use of PD-L1 IHC 22C3 pharmDx on fine needle aspirates has not been validated.
5) Use of PD-L1 IHC 22C3 pharmDx on decalcified tissues has not been validated.

## 16. Performance Evaluation

The normal and neoplastic tissue reactivity (specificity) data in section 16.1 are leveraged from PD-L1 IHC 22C3 pharmDx, Code SK006. The monoclonal mouse anti-PD-L1, Clone 22C3 is used in both products, GE006 and SK006. Tissue reactivity of monoclonal mouse anti-PD-L1, Clone 22C3 is not dependent on the instrument used to perform the staining procedure. For performance evaluation of sensitivity and precision on NSCLC tissues, see section 16.2

Agilent has completed a comparison study, evaluating the performance of PD-L1 IHC 22C3 pharmDx, Code GE006, on FFPE NSCLC specimens using PD-L1 IHC 22C3 pharmDx, Code SK006, as a reference. This study has demonstrated performance equivalence. The results from this study can be found in section 16.3

### 16.1 Non-clinical performance evaluation: normal and neoplastic tissues

**Normal tissues:** Table 4 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.[15, 16]

**Table 4: 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 |
| Bladder (3) | 0/3 | 0/3 | 0/3 |
| Bone marrow (3) | 3/3 Megakaryocytes | 3/3 Megakaryocytes | 0/3 |
| Breast (3) | 0/3 | 0/3 | 0/3 |
| Cerebellum (3) | 0/3 | 0/3 | 0/3 |
| Cerebrum (3) | 0/3 | 0/3 | 0/3 |
| Cervix (3) | 1/3 Epithelium | 0/3 | 0/3 |
| Colon (3) | 2/3 Macrophages | 0/3 | 0/3 |
| Esophagus (3) | 0/3 | 0/3 | 0/3 |
| Kidney (3) | 1/3 Tubular epithelium | 0/3 | 0/3 |
| Liver (3) | 1/3 Macrophages<br>1/3 Hepatocytes | 0/3 | 0/3 |
| Lung (3) | 3/3 Alveolar macrophages | 0/3 | 0/3 |

| Tissue Type (# tested) | Positive Plasma Membrane Staining: Tissue Elements | Positive Cytoplasmic Staining: Tissue Elements | Non-specific Staining |
|---|---|---|---|
| Mesothelial cells (3) | 0/3 | 0/3 | 0/3 |
| Muscle, cardiac (3) | 0/3 | 0/3 | 0/3 |
| Muscle, skeletal (3) | 0/3 | 0/3 | 0/3 |
| Nerve, peripheral (3) | 0/3 | 1/3 Connective tissue/vessels | 0/3 |
| Ovary (3) | 0/3 | 0/3 | 0/3 |
| Pancreas (3) | 0/3 | 0/3 | 0/3 |
| Parathyroid (3) | 1/3 Glandular epithelium | 0/3 | 0/3 |
| Pituitary (3) | 1/3 Anterior hypophysis<br>1/3 Posterior hypophysis | 1/3 Anterior hypophysis<br>1/3 Posterior hypophysis | 0/3 |
| Prostate (3) | 3/3 Epithelium | 0/3 | 0/3 |
| Salivary gland (3) | 0/3 | 0/3 | 0/3 |
| Skin (3) | 0/3 | 0/3 | 0/3 |
| Small intestine (3) | 0/3 | 0/3 | 0/3 |
| Spleen (3) | 2/3 Macrophages | 0/3 | 0/3 |
| Stomach (3) | 2/3 Lymphocytes<br>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<br>2/3 Germinal center (macrophages) | 0/3 | 0/3 |
| Uterus (3) | 0/3 | 0/3 | 0/3 |

**Neoplastic tissues:** Table 5 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.[15-18]

**Table 5: Summary of PD-L1 IHC 22C3 pharmDx neoplastic tissue reactivity**

| Tumor Type | Location | PD-L1 positive/total N=159 |
|---|---|---|
| Adenocarcinoma | Appendix | 0/1 |
| | Breast, DCIS | 0/2 |
| | Breast, invasive ductal | 0/7 |
| | Breast, invasive ductal metastatic to lymph node | 0/1 |
| | Cervix, endocervical type | 0/1 |
| | Colon | 0/5 |
| | Colon, metastatic to liver | 0/1 |
| | Colon, mucinous | 0/1 |
| | Esophagus | 0/1 |
| | Gallbladder | 1/5 |
| | GI, metastatic to lung | 0/1 |
| | Head & neck, hard palate | 0/1 |
| | Lung | 1/4 |
| | Ovary | 0/1 |
| | Ovary, endometrioid | 0/1 |
| | Ovary, mucinous | 0/1 |
| | Ovary, serous | 0/1 |
| | Pancreas | 0/2 |
| | Pancreas, ductal | 0/3 |
| | Prostate | 0/5 |
| | Rectum | 0/4 |
| | Salivary/parotid gland | 0/2 |
| | Small intestine | 0/2 |
| | Stomach | 0/6 |
| | Stomach, mucinous | 0/1 |
| | Thyroid, follicular | 0/1 |
| | Thyroid, follicular-papillary | 0/1 |
| | Thyroid, papillary | 0/3 |
| | Uterus, clear cell | 0/1 |
| | Uterus, endometrium | 0/3 |
| Adrenocortical carcinoma | Adrenal | 0/1 |
| Astrocytoma | Cerebrum | 0/3 |
| Basal cell carcinoma | Skin | 0/1 |
| Carcinoma | Nasopharyngeal, NPC | 0/1 |

| Tumor Type | Location | PD-L1 positive/total N=159 |
|---|---|---|
| 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: NSCLC**

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx, Code GE006, was tested on 105 unique cases of non-small cell lung cancer (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, Code GE006, was evaluated at Agilent Technologies. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff. For studies which resulted in 100.0% agreement, NPA, PPA, and OA were computed with two-sided 95% confidence intervals using the Wilson score method.

**Table 6: Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 1%)**

| Precision Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| Inter-instrument, Inter-day, Inter-lot | TPS ≥ 1% | All 32 NSCLC specimens (15 PD-L1-negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression were tested using 3 Dako Omnis instruments with 3 lots of reagents over 3 non-consecutive days. | NPA 100.0% (92.1-100.0%)<br>PPA 100.0% (93.0-100.0%)<br>OA 100.0% (96.2-100.0%) |
| Intra-rack (Repeatability) | TPS ≥ 1% | All 31 NSCLC specimens (19 PD-L1-negative and 12 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 5 replicates within a rack on the Dako Omnis instrument. | NPA 100.0% (96.1-100.0%)<br>PPA 96.7% (90.0-100.0%)<br>OA 98.7% (96.1-100.0%) |

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

**Table 7: Precision of PD-L1 IHC 22C3 pharmDx tested at one site (TPS ≥ 50%)**

| Precision Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| Inter-instrument, Inter-day, Inter-lot | TPS ≥ 50% | All 52 NSCLC specimens (24 PD-L1-negative and 28 PD-L1-positive) with a range of PD-L1 IHC expression were tested using 3 Dako Omnis instruments with 3 lots of reagents over 3 non-consecutive days. | NPA 100.0% (94.9-100.0%)<br>PPA 100.0% (95.6-100.0%)<br>OA 100.0% (97.6-100.0%) |
| Intra-rack (Repeatability) | TPS ≥ 50% | All 51 NSCLC specimens (27 PD-L1-negative and 24 PD-L1-positive) with a range of PD-L1 IHC expression were tested with 5 replicates within a rack on the Dako Omnis instrument. | NPA 98.5% (96.3-100.0%)<br>PPA 97.5% (93.3-100.0%)<br>OA 98.0% (96.1-99.6%) |

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

**External reproducibility: NSCLC**

The reproducibility of PD-L1 IHC 22C3 pharmDx, Code GE006, was evaluated at three external testing sites. Average agreements were calculated since no natural reference exists in reproducibility parameters such as site and observer. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff. For studies which resulted in 100.0% agreement, NPA, PPA, and OA were computed with two-sided 95% confidence intervals using the Wilson score method.

**Table 8: Reproducibility of PD-L1 IHC 22C3 pharmDx tested at three external sites (TPS ≥ 1%)**

| Reproducibility Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| Inter-site | TPS ≥ 1% | All 40 NSCLC specimens (20 PD-L1 negative and 20 PD-L1 positive) representing a range of PD-L1 expression were tested on 5 non-consecutive days. Inter-site analysis was performed between 3 sites on a total of 600 comparisons. | NPA 99.3% (98.3-100.0%)<br>PPA 100.0% (98.7-100.0%)<br>OA 99.7% (99.2-100.0%) |
| Intra-site | TPS ≥ 1% | All 40 NSCLC specimens (20 PD-L1 negative and 20 PD-L1 positive) representing a range of PD-L1 expression were tested on 5 non-consecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 600 comparisons. | NPA 99.3% (98.3-100.0%)<br>PPA 100.0% (98.7-100.0%)<br>OA 99.7% (99.2-100.0%) |

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

**Table 9: Reproducibility of PD-L1 IHC 22C3 pharmDx tested at three external sites (TPS ≥ 50%)**

| Reproducibility Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| Inter-site | TPS ≥ 50% | All 40 NSCLC specimens (20 PD-L1 negative and 20 PD-L1 positive) representing a range of PD-L1 expression were tested on 5 non-consecutive days. Inter-site analysis was performed between 3 sites on a total of 600 comparisons. | NPA 93.7% (87.6-98.4%)<br>PPA 91.9% (84.2-98.6%)<br>OA 92.8% (88.0-97.0%) |
| Intra-site | TPS ≥ 50% | All 40 NSCLC specimens (20 PD-L1 negative and 20 PD-L1 positive) representing a range of PD-L1 expression were tested on 5 non-consecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 600 comparisons | NPA 96.9% (94.1-99.1%)<br>PPA 97.1% (94.7-99.3%)<br>OA 97.0% (94.8-98.8%) |

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

**16.3 Performance evaluation, SK006 versus GE006 / Autostainer Link 48 versus Dako Omnis: NSCLC**

The performance equivalence of PD-L1 IHC 22C3 pharmDx, Code GE006, vs. PD-L1 IHC 22C3 pharmDx, Code SK006, was evaluated at three sites. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the TPS ≥ 1% cutoff and TPS ≥ 50% cutoff.

**Table 10: Comparison study of PD-L1 IHC 22C3 pharmDx, Code SK006 versus Code GE006, scored by three pathologists at three sites (TPS ≥ 1%).**

| Comparison Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| SK006 vs. GE006 | TPS ≥ 1% | 96 NSCLC specimens with a range of PD-L1 IHC expression were stained on both Autostainer Link 48 (Code SK006) and Dako Omnis (Code GE006). This set was scored by three different pathologists at three sites. | NPA 97.5% (94.2-100.0%)<br>PPA 98.8% (96.9-100.0%)<br>OA 98.2% (96.5-99.6%) |

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

**Table 11: Comparison study of PD-L1 IHC 22C3 pharmDx, Code SK006 versus Code GE006, scored by three pathologists at three sites (TPS ≥ 50%).**

| Comparison Study | Diagnostic Cutoff | Study Design | % Agreement (95% CI) |
|---|---|---|---|
| SK006 vs. GE006 | TPS ≥ 50% | 96 NSCLC specimens with a range of PD-L1 IHC expression were stained on both Autostainer Link 48 (Code SK006) and Dako Omnis (Code GE006). This set was scored by three different pathologists at three sites | NPA 91.6% (85.3-97.1%)<br>PPA 97.1% (94.2-99.3%)<br>OA 94.3% (91.0-97.2%) |

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

**16.4 Clinical performance evaluation**

All clinical trials were performed using PD-L1 IHC 22C3 pharmDx, Code SK006. Data in section 16.3 supports performance equivalence for GE006 as compared to SK006.

*KEYNOTE-042: Controlled trial of NSCLC patients naïve to treatment*

The safety and efficacy of pembrolizumab were investigated in KEYNOTE-042, a multicenter, controlled study for the treatment of previously untreated locally advanced or metastatic NSCLC.[5] The study design was similar to that of KEYNOTE-024 (see below), except that patients had PD-L1 expression with a TPS ≥ 1% based on PD-L1 IHC 22C3 pharmDx. Patients were randomized (1:1) to receive pembrolizumab at a dose of 200 mg every 3 weeks (n=637) or investigator's choice platinum-containing chemotherapy (n=637; including pemetrexed+carboplatin or paclitaxel+carboplatin; patients with non-squamous NSCLC could receive pemetrexed maintenance). Assessment of tumor status was performed every 9 weeks for the first 45 weeks, and every 12 weeks thereafter.

Among the 1,274 patients in KEYNOTE-042, 599 (47%) had tumors that expressed PD-L1 with TPS ≥ 50% based on PD-L1 IHC 22C3 pharmDx. The baseline characteristics of these 599 patients included: median age 63 years (45% age 65 or older); 69% male; 63% White and 32% Asian; 17% Hispanic or Latino; and ECOG performance status 0 and 1 in 31% and 69%, respectively. Disease characteristics were squamous (37%) and non-squamous (63%); stage IIIA (0.8%); stage IIIB (9%); stage IV (90%); and treated brain metastases (6%).

The primary efficacy outcome measure was OS. Secondary efficacy outcome measures were PFS and ORR (as assessed by BICR using RECIST 1.1). The trial demonstrated a statistically significant improvement in OS for patients whose tumors expressed PD-L1 TPS ≥ 1% randomized to pembrolizumab monotherapy compared to chemotherapy (HR 0.82; 95% CI 0.71, 0.93 at the final analysis) and in patients whose tumors expressed PD-L1 TPS ≥ 50% randomized to pembrolizumab monotherapy compared to chemotherapy. Table 12 summarizes key efficacy measures for the TPS ≥ 50% population at the final analysis performed at a median follow-up of 15.4 months. The Kaplan-Meier curve for OS for the TPS ≥ 50% population based on the final analysis is shown in Figure 1.

**Table 12: Efficacy results (PD-L1 TPS ≥ 50%) in KEYNOTE-042**

| Endpoint | Pembrolizumab 200 mg every 3 weeks n=299 | Chemotherapy n=300 |
|---|---|---|
| | | |
| **OS** | | |
| Number (%) of patients with event | 180 (60%) | 220 (73%) |
| Hazard ratio* (95% CI) | 0.70 (0.58, 0.86) | |
| p-Value† | 0.0003 | |
| Median in months (95% CI) | 20.0 (15.9, 24.2) | 12.2 (10.4, 14.6) |
| **PFS** | | |
| Number (%) of patients with event | 238 (80%) | 250 (83%) |
| Hazard ratio* (95% CI) | 0.84 (0.70, 1.01) | |
| Median in months (95% CI) | 6.5 (5.9, 8.5) | 6.4 (6.2, 7.2) |
| **Objective response rate** | | |

| | | |
|---|---|---|
| ORR % (95% CI) | 39% (34, 45) | 32% (27, 38) |
| Complete response % | 1% | 0.3% |
| Partial response % | 38% | 32% |
| **Response duration**‡ | | |
| Median in months (range) | 22.0 (2.1+, 36.5+) | 10.8 (1.8+, 30.4+) |
| % with duration ≥ 18 months | 57% | 34% |

* Hazard ratio (pembrolizumab compared to chemotherapy) based on the stratified Cox proportional hazard model

† Based on stratified log-rank test

‡ Based on patients with a best objective response as confirmed complete or partial response

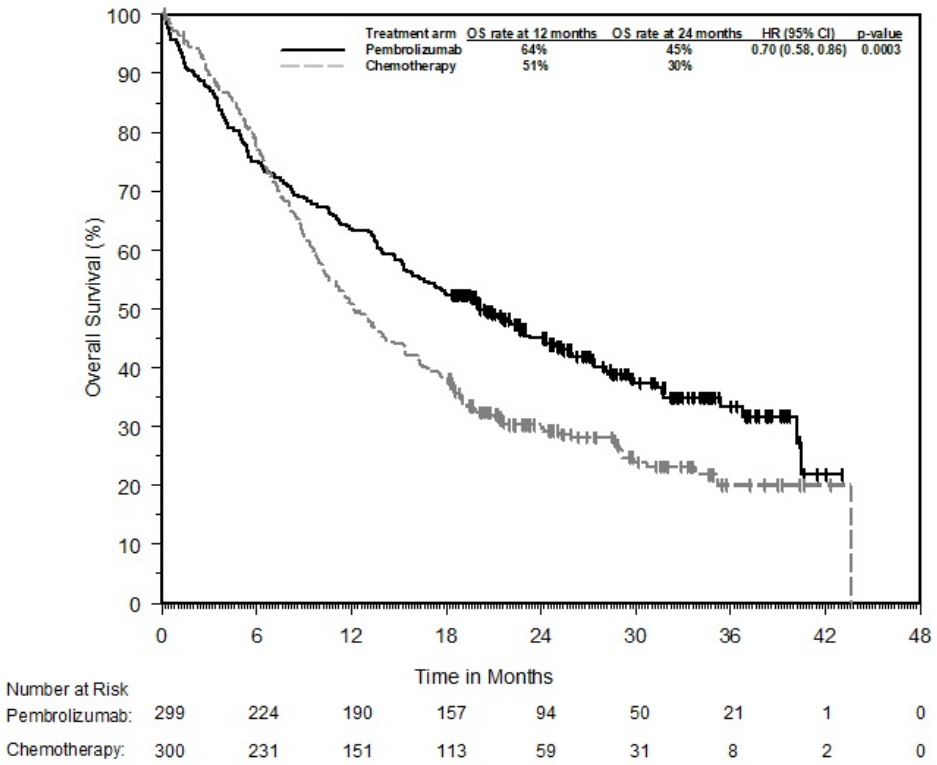


**Figure 1. Kaplan-Meier curve for overall survival by treatment arm in KEYNOTE-042 (patients with PD-L1 expression TPS ≥ 50%, intent to treat population)**

The results of a post-hoc exploratory subgroup analysis indicated a trend towards reduced survival benefit of pembrolizumab compared to chemotherapy, during both the first 4 months and throughout the entire duration of treatment, in patients who were never-smokers. However, due to the exploratory nature of this subgroup analysis, no definitive conclusions can be drawn.

*KEYNOTE-024: Controlled trial of NSCLC patients naïve to treatment*

The safety and efficacy of pembrolizumab were investigated in KEYNOTE-024, a multicenter, controlled study for the treatment of previously untreated metastatic NSCLC.[4] Patients had PD-L1 expression with a TPS ≥ 50% based on PD-L1 IHC 22C3 pharmDx. Patients were randomized (1:1) to receive pembrolizumab at a dose of 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 pembrolizumab until unacceptable toxicity or disease progression. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving clinical benefit by the investigator. Patients without disease progression could be treated for up to 24 months.The study excluded 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. Assessment of tumor status was performed every 9 weeks. Patients on chemotherapy who experienced independently-verified progression of disease were able to crossover and receive pembrolizumab.

Among the 305 patients in KEYNOTE-024, 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 primary efficacy outcome measure was PFS as assessed by blinded independent central review (BICR) using RECIST 1.1. Secondary efficacy outcome measures were OS and ORR (as assessed by BICR using RECIST 1.1). Table 13 summarizes key efficacy measures for the entire intent to treat (ITT) population. PFS and ORR results are reported from an interim analysis at a median follow up of 11 months. OS results are reported from the final analysis at a median follow up of 25 months.

**Table 1. Efficacy results in KEYNOTE-024**

| Endpoint | Pembrolizumab 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 | 73 (47%) | 96 (64%) |
| Hazard ratio* (95% CI) | 0.63 (0.47, 0.86) | |
| p-Value† | 0.002 | |
| Median in months (95% CI) | 30.0 (18.3, NA) | 14.2 (9.8, 19.0) |
| **Objective response rate** | | |
| ORR % (95% CI) | 45% (37, 53) | 28% (21, 36) |
| Complete response % | 4% | 1% |
| Partial response % | 41% | 27% |
| **Response duration‡** | | |
| Median in months (range) | Not reached (1.9+, 14.5+) | 6.3 (2.1+, 12.6+) |
| % with duration ≥ 6 months | 88%§ | 59%¶ |

* Hazard ratio (pembrolizumab compared to chemotherapy) based on the stratified Cox proportional hazard model

† Based on stratified log-rank test

‡ Based on patients with a best objective response as confirmed complete or partial response

§ Based on Kaplan-Meier estimates; includes 43 patients with responses of 6 months or longer

¶ Based on Kaplan-Meier estimates; includes 16 patients with responses of 6 months or longer

NA = not available

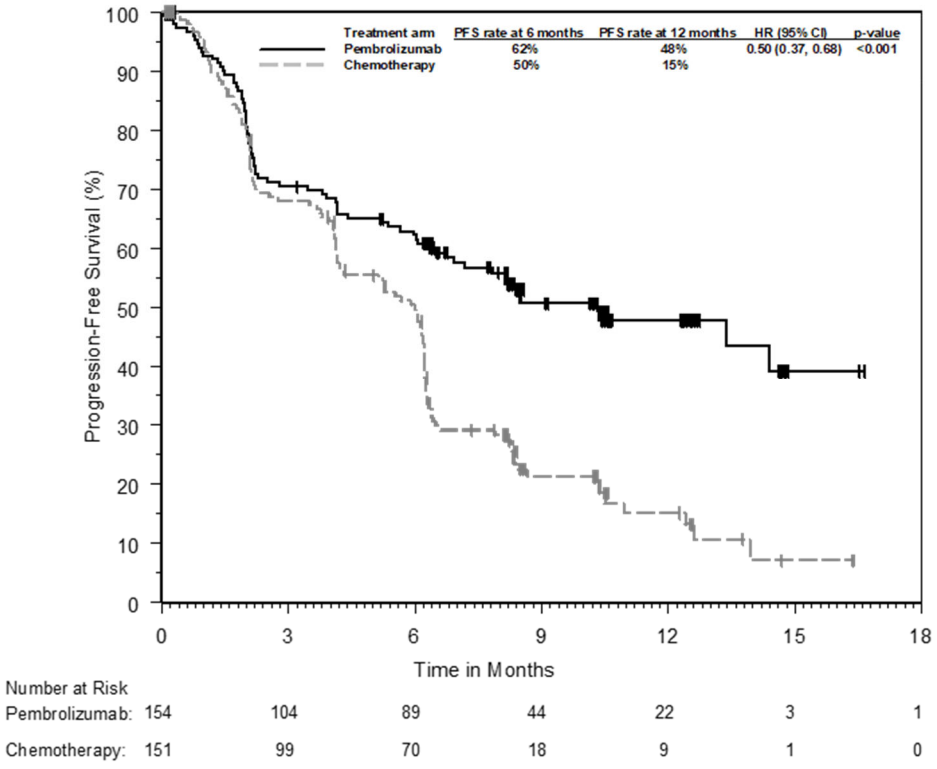


**Figure 2. : Kaplan-Meier curve for progression-free survival by treatment arm in KEYNOTE-024 (intent to treat population)**

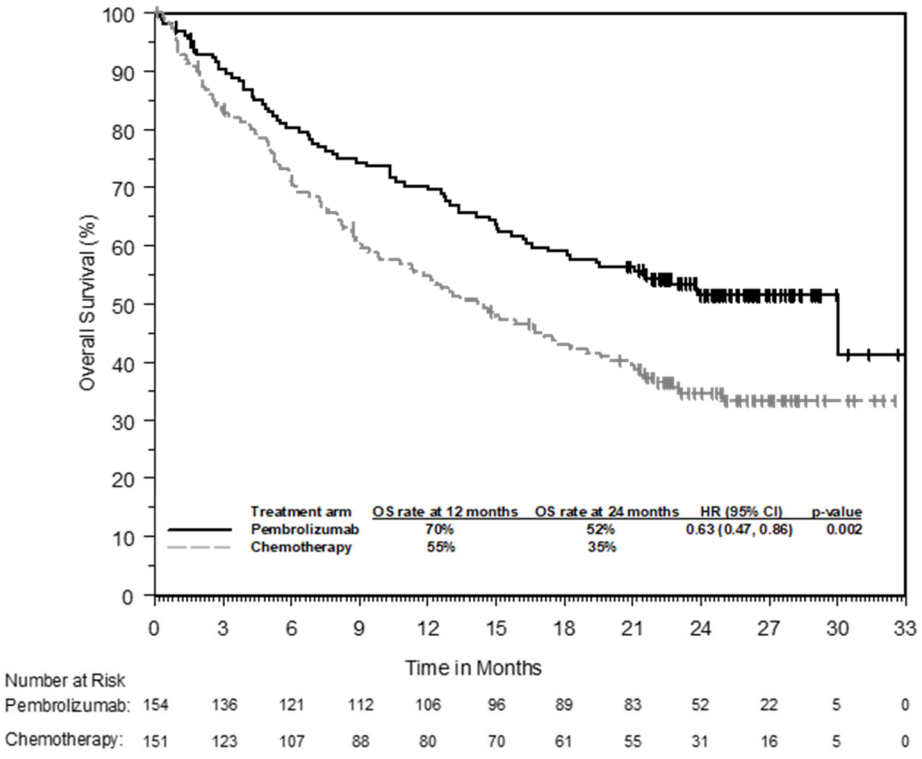


**Figure 3: Kaplan-Meier curve for overall survival by treatment arm in KEYNOTE-024 (intent to treat population)**

In a subgroup analysis, a reduced survival benefit of pembrolizumab compared to chemotherapy was observed in the small number of patients who were never-smokers; however, due to the small number of patients, no definitive conclusions can be drawn from these data.

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

The clinical benefit of PD-L1 IHC 22C3 pharmDx was investigated in KEYNOTE-010, a multicenter, open-label, randomized clinical study conducted to assess the safety and efficacy of KEYTRUDA in patients with advanced NSCLC previously treated with platinum-containing chemotherapy.[5] Patients had PD-L1 expression with a TPS ≥ 1% based on a clinical trial assay (CTA) version of PD-L1 IHC 22C3 pharmDx. Patients with EGFR activation mutation or ALK translocation also had disease progression on approved therapy for these mutations prior to receiving pembrolizumab. Patients were randomized (1:1:1) to receive pembrolizumab at a dose of 2 (n=344) or 10 mg/kg (n=346) every 3 weeks or docetaxel at a dose of 75 mg/m$^2$ every 3 weeks (n=343) until disease progression or unacceptable toxicity. The trial excluded 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. Assessment of tumor status was performed every 9 weeks. The primary efficacy outcome measures were OS and PFS as assessed by BICR using RECIST 1.1.

Based on the CTA, a total of 1,033 NSCLC patients were randomized in the study. To evaluate the clinical utility of PD-L1 IHC 22C3 pharmDx, archived clinical study samples were retrospectively tested at a USA-based reference laboratory with PD-L1 IHC 22C3 pharmDx. Out of the 1,033 patients, tumor tissue from 529 patients was retrospectively tested with PD-L1 IHC 22C3 pharmDx. Specimens from 413 patients had PD-L1 expression (≥ 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 14.

**Table 14: CTA vs. PD-L1 IHC 22C3 pharmDx agreement**

| Agreement Rates | PD-L1 Cut-off | 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 15 and 16. 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 4. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

**Table 15: Response to KEYTRUDA in previously treated NSCLC patients: overall clinical study 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 16: Response to KEYTRUDA in previously treated NSCLC patients: overall clinical study 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

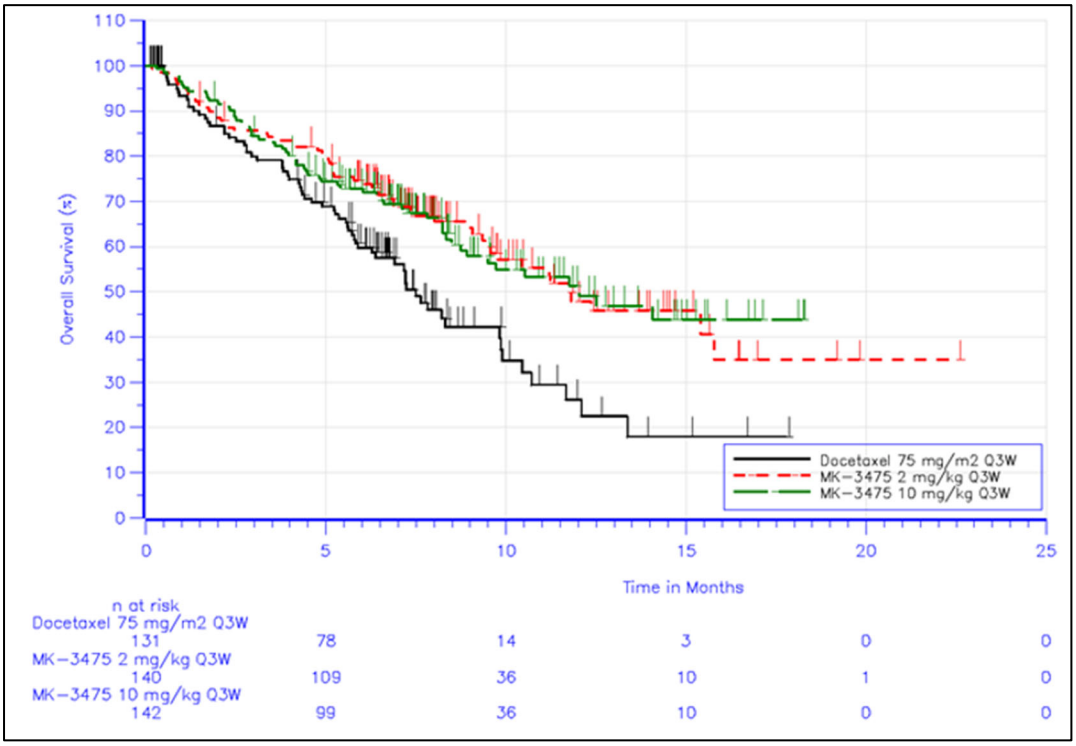


**Figure 4: Kaplan-Meier curve for overall survival by treatment arm (TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, intent to treat population)**

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.

**17. Troubleshooting**

Refer to the Troubleshooting section in the referenced Education Guide for remedial action or contact Agilent Pathology Support to report unusual staining.[8]

Dako Omnis is an automated system designed to alert the user if anything in the run has been outside of specifications. Please refer to the Dako Omnis Basic and Advanced User Guides for details on what conditions are flagged and how. Below is a troubleshooting guide for results and conditions that are not easily identified through the Dako Omnis warning and alert system.

The user should always ensure adherence to the maintenance schedule for the Dako Omnis instrument.

Always ensure to use the appropriate controls as described in the Quality Control section.

**Table 17: Troubleshooting**

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| **1. No or weak staining of slides** | 1a. Wrong storage conditions used for reagents. | 1a. Check that reagents have been stored correctly according to listed storage conditions. |
| | 1b. Reagent is used past its expiration date. | 1b. Ensure reagent is not used past its expiration date. |
| | 1c. Reagent is used past its onboard stability. | 1c. Ensure reagent is not used past its onboard stability. |
| | 1d. Inappropriate fixation method used. | 1d. Ensure that patient tissue is not fixed for too short or too long a time period, and that the correct fixative (NBF) was used. |
| | 1e. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of immunoreactivity and morphology. | 1e. Dry the tissue sections at 58 ± 2 °C for a maximum of 1 hour, using a calibrated oven with uniform heat distribution.[19] |
| | 1f. Incorrect placement of dynamic gap lids in stainer modules. | 1f. Check placement of dynamic gap lids. |
| | 1g. Damaged dynamic gap lids. | 1g. Check integrity of dynamic gap lids. |
| | 1h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 1h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 1i. Incorrect Target Retrieval Solution is used. | 1i. Ensure that correct Target Retrieval Solution specified in 'Materials Required but not Supplied' and/or 'Reagent Preparation' sections is used. |
| | 1j. 1x Target Retrieval Solution does not meet pH specifications. | 1j. Check pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check the pH of the new 1x Target Retrieval Solution. |
| **2. Excessively strong specific staining of slides** | 2a. Inappropriate fixation method used. | 2a. Ensure that only approved fixatives and fixation methods are used. |
| | 2b. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 2b. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 2c. Incorrect Target Retrieval Solution is used. | 2c. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but not Supplied' and/or 'Reagent Preparation' sections is used. |
| | 2d. 1x Target Retrieval Solution does not meet pH specifications. | 2d. Check pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check the pH of the new 1x Target Retrieval Solution. |
| **3. Excessive non-specific staining of slides** | 3a. Starch additives used in mounting sections to slides. | 3a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive. |
| | 3b. Sections dried after staining procedure. | 3b. Verify that the unloading station is filled with sufficient water. |
| | 3c. Sections dried prior to coverslipping. | 3c. Avoid stained slides drying out between unloading from Dako Omnis and coverslipping. |

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| | 3d. Inappropriate fixation method used. | 3d. Ensure that approved fixative was used. Alternative fixative may cause excessive background staining. |
| | 3e. Paraffin incompletely removed. | 3e. Check appearance of solvent couplings. Gently scrub the couplings to remove impurities. Check the integrity of the couplings on the backside of the bulk bottles after cleaning. Refer to Dako Omnis Basic User Guide for additional details. |
| | 3f. Non-specific binding of reagents to tissue. | 3f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis. |
| | 3g. Re-use of mixing strip. | 3g. Ensure that new mixing strips are used. |
| | 3h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 3h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 3i. Incorrect Target Retrieval Solution is used. | 3i. Ensure that correct Target Retrieval Solution specified in 'Materials Required but not Supplied' and/or 'Reagent Preparation' sections is used. |
| | 3j. 1x Target Retrieval Solution does not meet pH specifications. | 3j. Check pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check the pH of the new Target Retrieval Solution. |
| **4. Tissue detaches from slides** | 4a. Use of incorrect slides. | 4a. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides. |
| | 4b. Inadequate preparation of specimens | 4b. Cut sections should be placed in a 58 ± 2 °C calibrated oven for 1 hour prior to staining. |
| **5. Slide is flagged** | 5a. Reagent is used beyond its expiration date. | 5. Flagged slides should be evaluated by qualified personnel. Contact an Agilent Technologies representative if further action is needed. |
| | 5b. Reagent is stored onboard Dako Omnis beyond its validated onboard stability. | |
| | 5c. Maintenance overdue or other factors. | |
| **6. 1x Target Retrieval Solution does not meet pH specifications** | 6a. pH meter is not calibrated correctly. | 6a. Ensure pH meter is calibrated per manufacturer's recommendations. After re-calibration, re-test the pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. |
| | 6b. Inferior quality water is used to dilute the Target Retrieval Solution concentrate. | 6b. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution |
| | 6c. Incorrect Target Retrieval Solution is used. | 6c. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but not Supplied' section 5 and/or 'Reagent Preparation' section 10 is used. |

**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 contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found previously referenced the Educational Guide: Immunohistochemical Staining Methods (available from www.agilent.com), Atlas of Immunohistology and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.[11,20,21]

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**Explanation of symbols**

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


Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051
United States

Tel. +44 161 492 7050
www.agilent.com



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