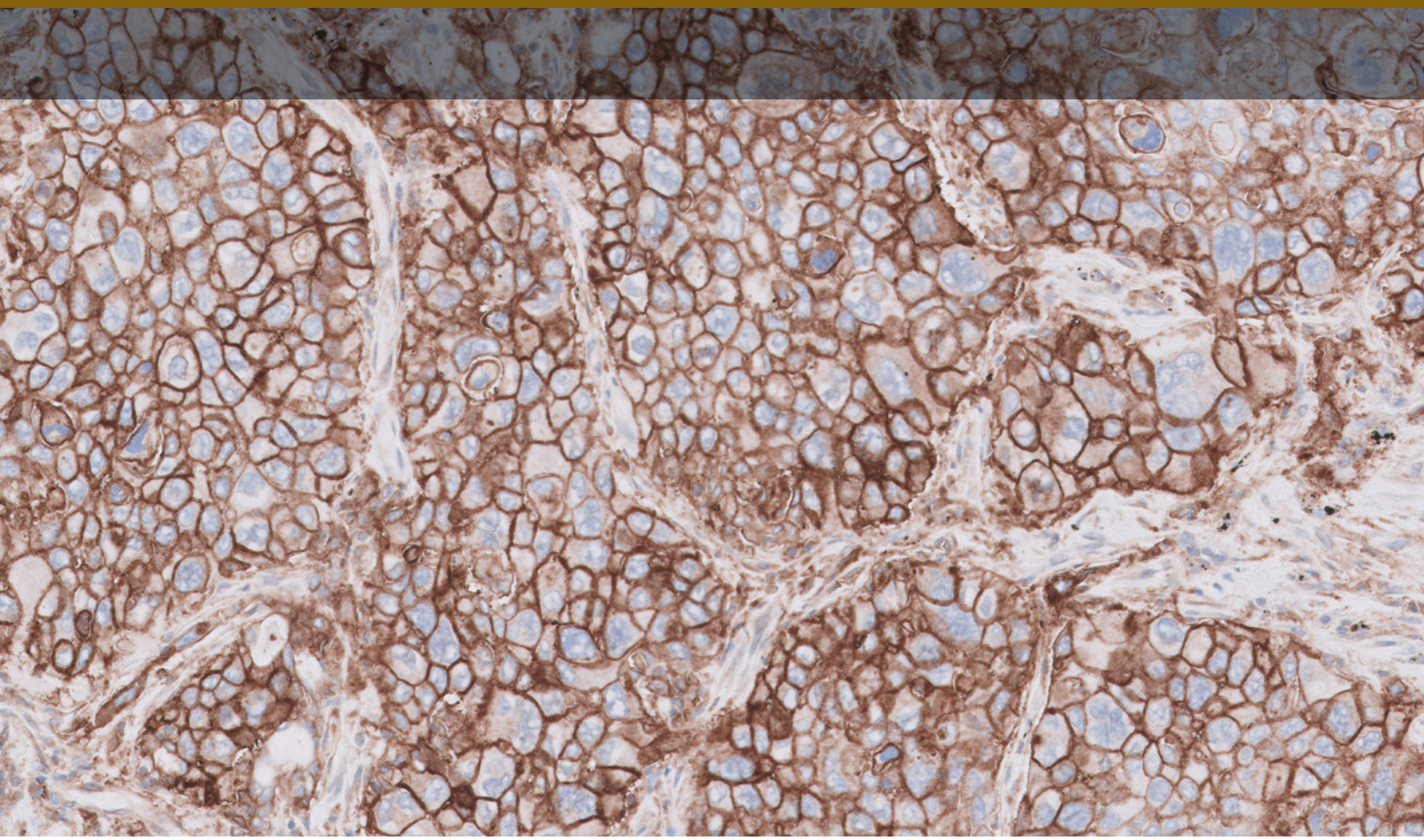
Agilent Dako

PD-L1 IHC 22C3 pharmDx Interpretation Manual – Non-small Cell Lung Cancer (NSCLC)

FDA-approved for in vitro diagnostic use



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Trusted Answers

This Interpretation Manual can be used for:
PD-L1 IHC 22C3 pharmDx, Code SK006 for use with Autostainer Link 48 and PT Link
PD-L1 IHC 22C3 pharmDx, Code GE006 for use with Dako Omnis

"PD-L1 IHC 22C3 pharmDx" in the text refers to both PD-L1 products

For countries outside of the United States, see the local KEYTRUDA product label for approved indications and expression cutoff values to guide therapy.

Table of Contents

Intended Use

PD-L1 IHC 22C3 pharmDx
Code SK006
On Autostainer Link 48

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, esophageal squamous cell carcinoma (ESCC), cervical cancer and urothelial carcinoma tissues using EnVision FLEX visualization system on Autostainer Link 48.

Non-Small Cell Lung Cancer (NSCLC)

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

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA product label for specific clinical circumstances guiding PD-L1 testing.

For descriptions of the intended use in other indications refer to the instructions for use (IFU) for PD-L1 IHC 22C3 pharmDx, Code SK006 for use with Autostainer Link 48, see references.

PD-L1 IHC 22C3 pharmDx
Code GE006
On Dako Omnis

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.

Non-Small Cell Lung Cancer (NSCLC)

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

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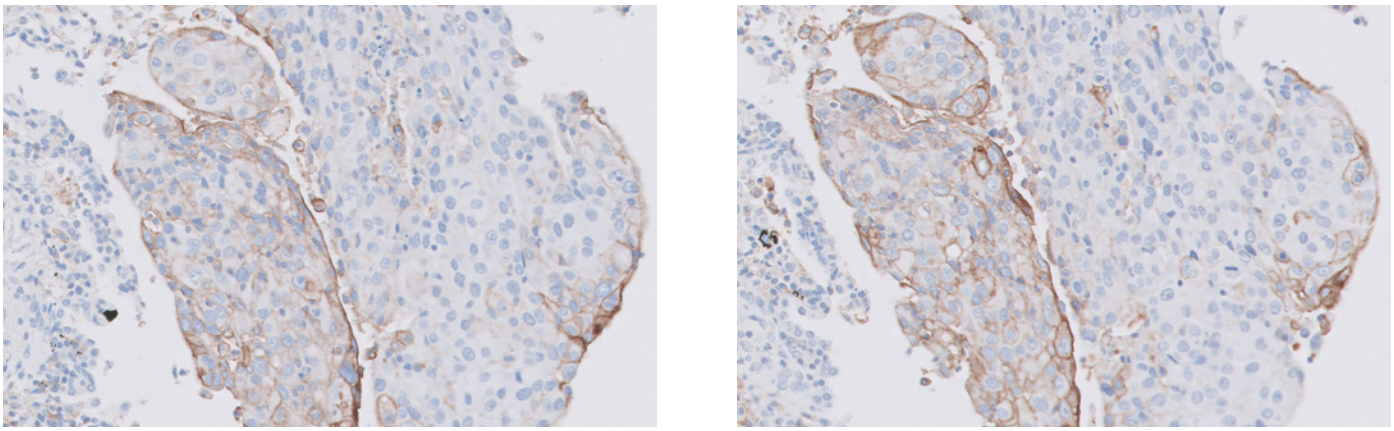


Illustration of PD-L1 IHC 22C3 pharmDx. Serial sections of NSCLC >1% TPS stained with:
Left: PD-L1 IHC 22C3 pharmDx, Code SK006
Right: PD-L1 IHC 22C3 pharmDx, Code GE006

Introduction

PD-L1 IHC 22C3 pharmDx, Code SK006 for Autostainer Link 48 is the only companion diagnostic established in the clinical trials and approved by the FDA as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab).

The assay 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 & 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 & 5. Clinical concordance has been established by a comparison study of NSCLC with PD-L1 IHC 22C3 pharmDx, Code SK006 (see GE006 IFU). Both assays are companion diagnostics approved by the FDA as an aid in identifying patients with NSCLC for treatment with KEYTRUDA (pembrolizumab). Please note that the products are not interchangeable; their reagent vials and instructions for use are specific to their respective staining platforms.

This Interpretation Manual is provided as a tool to help guide pathologists and laboratory personnel in achieving correct and reproducible results in assessing PD-L1 expression in formalin-fixed, paraffin-embedded non-small cell lung cancer (NSCLC) specimens. PD-L1 expression evaluation may be used to identify patients for anti-PD-1 immunotherapy.

The manual provides detailed scoring guidelines and technical information from the Instructions for Use (IFU) to ensure high-quality staining and diagnostic assessment. To help familiarize you with the requirements for scoring NSCLC stains with these products example cases of various PD-L1 expression levels are provided as references. These example cases and in-depth recommendations for interpretation of NSCLC specimens stained with PD-L1 IHC 22C3 pharmDx can help individual labs achieve reproducible and reliable results.

PD-L1 IHC 22C3 pharmDx is considered to be a qualitative immunohistochemical assay. 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.

NSCLC tissue specimens that are tested for PD-L1 expression are scored and divided into expression levels based on a Tumor Proportion Score (TPS):

- TPS < 1%
- TPS ≥ 1%
- TPS ≥ 50%

Note: PD-L1 expression level TPS ≥ 50% may be of interest to treating physician but does not determine eligibility for KEYTRUDA monotherapy.

For more details on staining and interpretation, please refer to the current version of the IFU provided with PD-L1 IHC 22C3 pharmDx, Code SK006 or PD-L1 IHC 22C3 pharmDx, Code GE006 or visit www.agilent.com.

Assay Interpretation

The clinical interpretation of any staining, or the absence of staining, must be complemented by the evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. These products are intended for in vitro diagnostic (IVD) use.

Reporting Results

To help understand what information should be reported to the treating physician, please refer to the Reporting Results section of this manual on page 32.

Photomicrographs

The included photomicrographs are of NSCLC unless otherwise noted.

Note: Photomicrograph magnification levels may appear different from indicated in respective annotations due to adjustment of image size.

PD-L1 Overview

The PD-1/PD-L1 Pathway Controls the Immune Response in Normal Tissue

Programmed death-ligand 1 (PD-L1) is a transmembrane protein that binds to the programmed death-1 receptor (PD-1) during immune system modulation. The PD-1 receptor is typically expressed on cytotoxic T-cells and other immune cells, while the PD-L1 ligand is typically expressed on normal cells. Normal cells use the PD-1/PD-L1 interaction as a mechanism of protection against immune recognition by inhibiting the action of T-cells (Figure 1). Inactivation of cytotoxic T-cells downregulates the immune response such that the inactive T-cell is exhausted, ceases to divide, and might eventually die by programmed cell death, or apoptosis.

The Tumor Escapes Detection by Utilizing the PD-1/PD-L1 Pathway

Many tumor cells are able to upregulate the expression of PD-L1 as a mechanism to evade the body's natural immune response. Activated T-cells recognize the PD-L1 marker on the tumor cell, similar to that of a normal cell, and PD-L1 signaling renders the T-cell inactive (Figure 2). The tumor cell escapes the immune cycle, continues to avoid detection for elimination, and is able to proliferate.

Anti-PD-1 Therapy Enables the Immune Response Against Tumors

PD-1/PD-L1 interaction between tumor cells and activated T-cells (Figure 3) is a mechanistic pathway used by immunotherapeutic agents. When the tumor cell is unable to interact with the activated T-cell, the immune system remains active, helping to prevent immunosuppression.

PD-L1 IHC 22C3 pharmDx Detects PD-L1 in NSCLC Specimens

PD-L1 upregulation in NSCLC is a biomarker for response to anti-PD-1 therapy. PD-L1 IHC 22C3 pharmDx was the only companion diagnostic used in the KEYTRUDA® (pembrolizumab) clinical trials (KEYNOTE-010, KEYNOTE-024, and KEYNOTE-042) to evaluate the relationship between PD-L1 expression and clinical efficacy. KEYTRUDA is a humanized monoclonal PD-1-blocking antibody.

The PD-1/PD-L1 Pathway

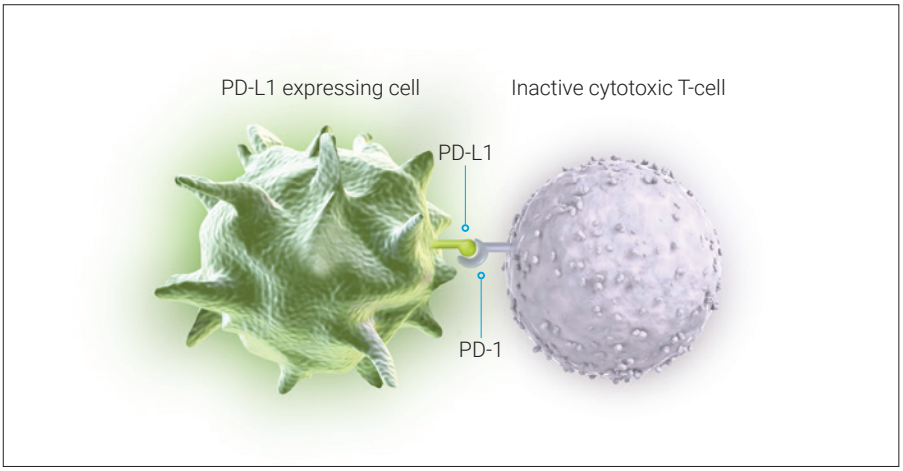


Figure 1: Inactivation of T-cells limits damage to normal tissue.

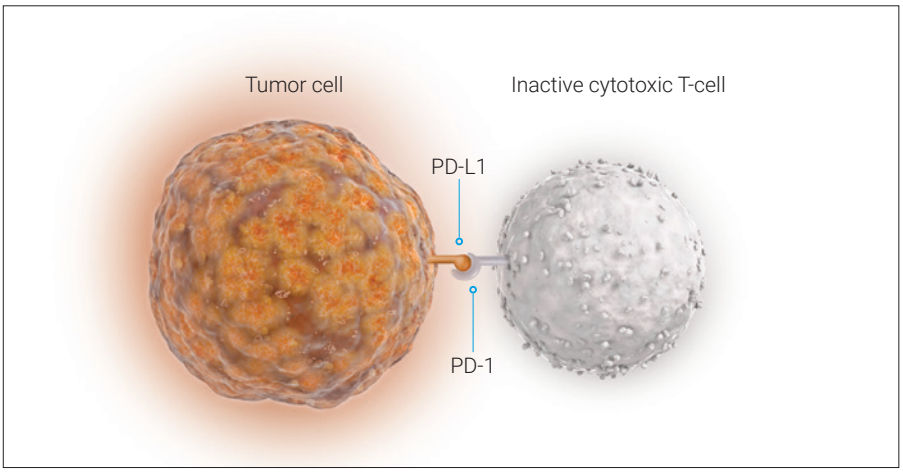


Figure 2: Inactivation of T-cells reduces tumor cell death and elimination.

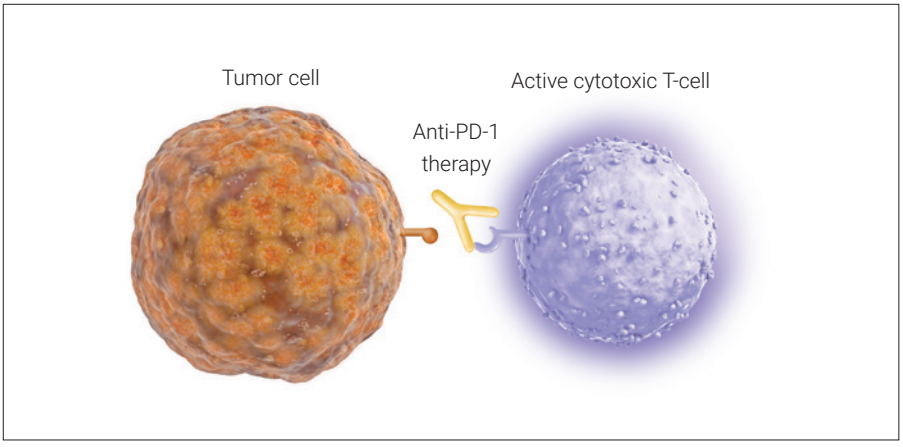
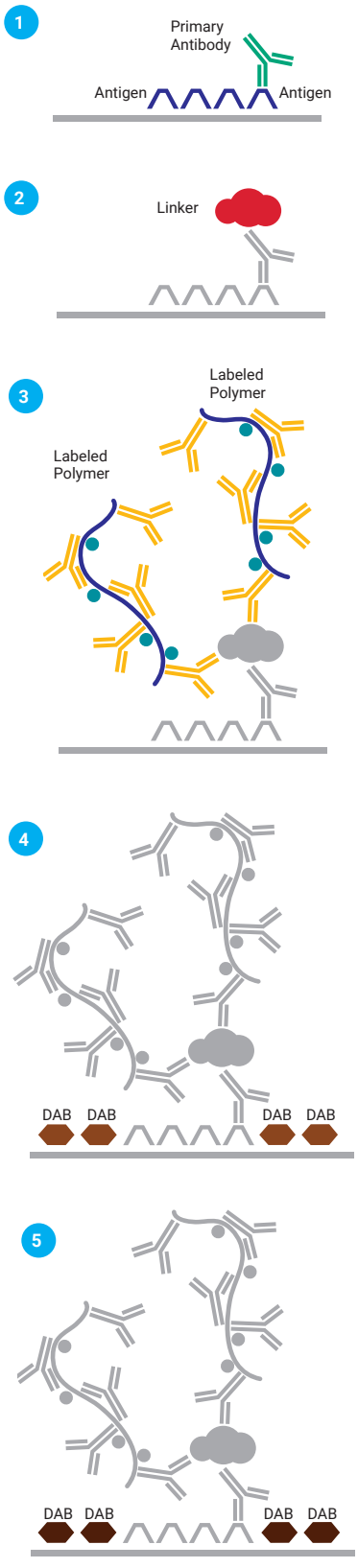


Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T-cells and tumor cell death and elimination.

PD-L1 IHC 22C3 pharmDx Overview



What is PD-L1 IHC 22C3 pharmDx?

PD-L1 IHC 22C3 pharmDx is the only companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab) in the clinical trials. PD-L1 IHC 22C3 pharmDx, Code GE006 has been reconfigured for the Dako Omnis workflow with clinical concordance established by comparison study of NSCLC between PD-L1 IHC 22C3 pharmDx, Code GE006 on Dako Omnis and PD-L1 IHC 22C3 pharmDx, Code SK006 on Autostainer Link 48. Both are qualitative immunohistochemical (IHC) assays intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue samples using EnVision FLEX visualization system.

Both assays use optimized reagents to perform an IHC staining procedure using a linker and a chromogen enhancement reagent (Figure 4).

For SK006, deparaffinization, rehydration and target retrieval are performed using a 3-in-1 procedure on the PT Link. Once on the Autostainer, specimens are incubated with peroxidase block, followed by the monoclonal antibody to PD-L1 or the Negative Control Reagent.

For GE006, deparaffinization, rehydration and target retrieval are performed on the Dako Omnis using a two step incubation of Clearify, then Low pH TRS. The specimens are then incubated with the monoclonal antibody to PD-L1 or the Negative Control Reagent (NCR) prior to the peroxidase block.

For both SK006 & GE006, after primary antibody/NCR/peroxidase blocking, the specimens are then incubated with a mouse LINKER, followed by incubation 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 the 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.

Figure 4: PD-L1 IHC 22C3 pharmDx staining procedure
1. Application of Primary Antibody
2. Application of Linker
3. Application of Visualization Reagent
4. Application of DAB+ Substrate Chromogen Solution
5. Application of DAB Enhancer.

Product Configurations

Figure 5.0: PD-L1 IHC 22C3 pharmDx components

Figure 5.1: PD-L1 IHC 22C3 pharmDx modular components

PD-L1 IHC 22C3 pharmDx, Code SK006 contains all reagents to perform 50 tests in up to 15 individual runs (Figure 5.0):

- EnVision FLEX Target Retrieval Solution, Low pH (50×)
- Peroxidase-blocking Reagent
- Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3
- Negative Control Reagent
- Mouse LINKER
- Visualization Reagent-HRP
- DAB+ Substrate Buffer
- DAB+ Chromogen
- DAB Enhancer
- PD-L1 IHC 22C3 pharmDx Control Cell Line Slides*

EnVision FLEX Wash Buffer, (20×) (Code K8007) and EnVision FLEX Hematoxylin (Code K8008) are required but not included in the kit.

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

PD-L1 IHC 22C3 pharmDx, Code GE006 is a modular assay configured for the Dako Omnis workflow. The following reagents are all required to perform 60 tests in multiple individual runs (Figure 5.1):

- PD-L1 IHC 22C3 pharmDx, Code GE006 . Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3 and Negative Control Reagent
- DAB Enhancer, Code GC806
- PD-L1 Control Cell Line Slides*, Code T1391 (optional use)

The following Dako Omnis bulk and visualization reagents are also required:

- EnVision FLEX, High pH (Dako Omnis), Code GV800 or EnVision FLEX Mini Kit, High pH (Dako Omnis), Code GV823
- 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, Code GC810
- Dako Omnis Sulfuric Acid, 0.3 M, Code GC203
- Hematoxylin (Code K8008)

Technical Considerations

The package inserts (see references) for both SK006 and GE006 contain detailed troubleshooting sections and comprehensive instructions for use (IFU). PD-L1 IHC 22C3 pharmDx reagents and protocols have been developed for optimal performance on their respective Dako instruments and are not interchangeable - refer to IFU for guidelines. Changes in instrument platform, dilution of the reagents, alteration of incubation times, temperatures, or materials may give erroneous results. All of the required steps and incubation times for staining are pre-programmed in the appropriate Dako instrument software.

Technical problems can be attributed to two areas: specimen collection and preparation prior to performing the test, and the actual performance of the test itself. Technical problems are generally related to procedural deviations and can be controlled and minimized through training and, where necessary, clarification of the product instructions.

Specimen Preparation

Specimens must be handled to preserve the tissue for immunohistochemical staining. Determine intact tumor morphology and the presence of sufficient tumor cells for evaluation. Use standard methods of tissue processing for all specimens.

In-house Control Tissue

Differences in processing and embedding in the user's laboratory may produce significant variability in results. Include positive and negative in-house control tissue in each staining run, in addition to the PD-L1 IHC 22C3 pharmDx Control Cell Line Slide*.

Select positive and negative control tissue from fresh specimens of the same tumor indication as the patient specimen. Fix, process, and embed the control tissue in the same manner. Control tissues processed differently from the patient specimen validate reagent performance only and do not verify tissue preparation.

The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate staining of tumor cells. The ideal negative control tissue gives no staining on tumor cells but contains tumor-associated macrophages/immune cells which may express PD-L1 and offer an internal positive control.

* Optional use with PD-L1 IHC 22C3 pharmDx, Code GE006.

Optional Additional In-house Control: Tonsil Tissue

For PD-L1 IHC 22C3 pharmDx, Code SK006, tonsil stained with PD-L1 should be pre-screened to exhibit strong staining in portions of the crypt epithelium and weak-to-moderate staining of the follicular macrophages in the germinal centers. PD-L1 expression of the endothelium, fibroblasts, as well as the surface epithelium should be negative. The use of tonsil tissue has not been evaluated for PD-L1 IHC 22C3 pharmDx, Code GE006.

Tissue Processing

Formalin-fixed, paraffin-embedded tissues have been validated for use. Block specimens into a thickness of 3 mm or 4 mm, fix in formalin and dehydrate and clear in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. Feasibility studies on NSCLC tissue samples were performed with fixation in 10% neutral buffered formalin for 12–72 hours. Fixation times of 3 hours or less should not be used for PD-L1 assessment. The use of PD-L1 IHC 22C3 pharmDx on decalcified tissues or tissues processed with other fixatives has not been validated and is not recommended.

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

Cut Section Storage

Store tissue sections in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C. To preserve antigenicity, stain cut sections within the time stated in the appropriate IFU.

PD-L1 IHC 22C3 pharmDx, Code SK006 for use with Autostainer Link 48 Staining Procedure

The PD-L1 IHC 22C3 pharmDx, Code SK006 reagents and protocol have been developed for optimal performance on Autostainer Link 48 with PT Link only. Further dilution of the reagents, alteration of incubation times, temperatures, or materials may give erroneous results. All of the required steps and incubation times for staining are pre-programmed in the DakoLink software.

Reagent Storage

Store all components of PD-L1 IHC 22C3 pharmDx, including Control Cell Line Slides, in the dark at 2–8 °C when not in use.

Reagent Preparation

Equilibrate all components to room temperature (20–25 °C) prior to immunostaining. Do not use after the expiration date printed on the outside of the package.

EnVision FLEX Target Retrieval Solution, Low pH

Dilute EnVision FLEX Target Retrieval Solution, Low pH (50×) 1:50 using distilled or deionized water (reagent-quality water). One 30 mL bottle of concentrate provides 1.5 L of working solution, which is sufficient to fill one PT Link tank. Discard 1× EnVision FLEX Target Retrieval Solution, Low pH after 3 uses or 5 days after dilution.

EnVision FLEX Wash Buffer

Dilute EnVision FLEX Wash Buffer (20×) 1:20 using distilled or deionized water (reagent-quality water). Store unused 1× buffer at 2–8 °C for no more than 1 month. Discard if cloudy in appearance.

DAB+ Substrate-Chromogen Solution

Add 1 drop of DAB+ Chromogen per mL of DAB+ Substrate Buffer and mix. Prepared DAB+ Substrate-Chromogen is stable for 5 days if stored in the dark at 2–8 °C. Mix the DAB+ Substrate-Chromogen Solution thoroughly prior to use. Any precipitate developing in the solution will not affect staining quality.

- *If using an entire bottle of DAB+ Substrate Buffer, add 9 drops of DAB+ Chromogen. Although the DAB+ Substrate Buffer label states 7.2 mL, this is the usable volume and does not account for the "dead volume" of DAB+ Substrate Buffer in the bottle*
- The color of the DAB+ Chromogen may vary from clear to lavender brown. This will not affect the performance of the product. Dilute per the guidelines above. Adding excess DAB+ Chromogen to the DAB+ Substrate Buffer results in deterioration of the positive signal

Controls to Assess Staining Quality

The following quality controls should be included in each staining run:

- One PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with the primary antibody
- Positive and negative in-house control tissues stained with the primary antibody
- Subsequent sections of each patient specimen stained with the Negative Control Reagent

Deparaffinization, Rehydration, and Target Retrieval

Use PT Link to perform a Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure:

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

Staining and Counterstaining

- Place the Autostainer rack with slides on the Autostainer Link 48
- Ensure slides remain wet with buffer while loading and prior to initiating the run. Dried tissue sections may display increased non-specific staining
- Select the PD-L1 IHC 22C3 pharmDx protocol. The instrument performs the staining and counterstaining procedures by applying the appropriate reagent, monitoring the incubation time, and rinsing slides between reagents
- Counterstain slides using EnVision FLEX Hematoxylin, Code K8008

Mounting

Use non-aqueous permanent mounting media.
To minimize fading, store slides in the dark at room temperature (20–25 °C).

PD-L1 IHC 22C3 pharmDx, Code GE006 for use with Dako Omnis Staining Procedure

The PD-L1 IHC 22C3 pharmDx, Code GE006 reagents and protocol have been developed for optimal performance on Dako Omnis only. Further dilution of the reagents, alteration of incubation times, temperatures, or materials may give erroneous results. The assay protocol, including all of the required steps and incubation times for staining, must be installed in the Dako Omnis software before use.

Reagent Storage

Store all components, including Control Cell Line Slides, Code T1391, according to instructions in the IFU when not in use.

Reagent Preparation

Reagents should be loaded into the instrument before starting the staining procedure, which allows sufficient time for equilibration.

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

Load all the bulk bottles and register on the Dako Omnis instrument. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module.

Controls to Assess Staining Quality

The following quality controls should be included in each staining run:

- One PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with the primary antibody (optional)
- Positive and negative in-house control tissues stained with the primary antibody
- Subsequent sections of each patient specimen stained with the Negative Control Reagent

Staining and Counterstaining

- 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.

– Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides. Load the Slide Rack in Dako Omnis. Follow the instructions on the touch screen and tap “Done” to initiate the staining procedure. The instrument performs all target retrieval, staining and counterstaining procedures.

Mounting

Use non-aqueous permanent mounting media.
To minimize fading, store slides in the dark at room temperature (20–25 °C).

Technical Checklist

Use the checklist below to ensure correct usage of PD-L1 IHC 22C3 pharmDx:

Customer Name/Institution _____

Name and Title _____

Instrument Serial Number(s) _____

Software Version _____

	Yes	No
Regular preventive maintenance is performed on the instrument according to instrument user guide?	☐	☐
Reagents used before the expiration date printed on the outside of the box?	☐	☐
All reagents and components, including Control Cell Line Slides, are stored in the dark at 2–8 °C?	☐	☐
All reagents and components, including Control Cell Line Slides (if applicable), are equilibrated according to staining procedure in IFU prior to immunostaining?	☐	☐
Appropriate positive and negative control tissue from NSCLC are identified?	☐	☐
Tissues are fixed in neutral buffered formalin?	☐	☐
Tissues are infiltrated with melted paraffin, at or below 60 °C?	☐	☐
Tissue sections of 4–5 µm are mounted on Dako FLEX IHC Microscope Slides or Fisherbrand Superfrost Plus slides?	☐	☐
Specimens are oven-dried at 58 ± 2 °C for 1 hour?	☐	☐
Specimens are stored and stained within the recommended cut section stability? (see IFU)	☐	☐
EnVision FLEX Target Retrieval Solution, Low pH is prepared properly? pH of 1× Target Retrieval Solution must be 6.1 ± 0.2.	☐	☐
EnVision FLEX Wash Buffer is prepared properly?	☐	☐
DAB+ Substrate-Chromogen Solution is prepared properly? (SK006 only)	☐	☐
Slides are counterstained with EnVision FLEX Hematoxylin?	☐	☐
The Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure is followed using PT Link (SK006 only)	☐	☐
Slides remain wet with buffer while loading and prior to initiating run on the Autostainer Link 48 (SK006 only)	☐	☐
The correct protocol is selected on the instrument?	☐	☐
Do you have all the necessary equipment to perform the PD-L1 IHC 22C3 pharmDx according to protocol? If not, specify what is missing in comments below.	☐	☐

Additional Observations or Comments:

_____

_____

Clinical Interpretation Guidelines

General Considerations

Evaluation of stained specimens should be performed by a qualified pathologist using a light microscope. Details of the PD-L1 IHC 22C3 pharmDx scoring guidelines are reviewed on page 27. Before examining the patient specimen for PD-L1 staining, it is important to examine the controls to assess staining quality.

PD-L1 expression is best assessed by requesting 3 serial tissue sections (H&E, PD-L1 stain, and NCR stain) so that if the H&E is first assessed and is acceptable.

Each PD-L1 IHC 22C3 pharmDx, Code SK006 is configured with Control Cell Line Slides that should be included in each IHC run. Guidelines on interpreting the Control Cell Line Slide are reviewed on page 20 (applicable to PD-L1 IHC 22C3 pharmDx, Code GE006 when used with Code T1391). In-house control tissue slides should also be assessed with every IHC run.

Specimen Adequacy

Confirm the Presence of at Least 100 Viable Tumor Cells

A hematoxylin and eosin (H&E) stain of the tissue specimen is evaluated first to assess tissue histology and preservation quality. PD-L1 IHC 22C3 pharmDx and the H&E staining 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.

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.

Instructions for Patient Specimens With Less Than 100 Viable Tumor Cells

Tissue from a deeper level of the block, or potentially another block, could have a sufficient number of viable tumor cells for PD-L1 IHC 22C3 pharmDx testing.

Evaluating Controls

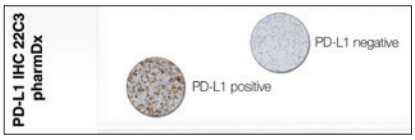


Figure 6: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

PD-L1 Control Cell Line Slide*

Examine the PD-L1 IHC Control Cell Line Slide to determine that reagents are functioning properly. Each slide contains sections of cell pellets with positive and negative PD-L1 expression (Figure 6). Assess the percentage of positive cells and the staining intensity. If any staining of the Control Cell Line Slide is not satisfactory, all results with the patient specimens should be considered invalid. Do not use the Control Cell Line Slide as an aid in interpretation of patient results.

Evaluate the overall staining intensity using the following guide:

0	Negative
1+	Weak intensity
2+	Moderate intensity
3+	Strong intensity

Positive Control Cell Pellet

The following staining is acceptable for the PD-L1 positive cell pellet (Figure 7):

- Cell membrane staining of ≥ 70% of cells
- ≥ 2+ average staining intensity
- Non-specific staining < 1+ intensity

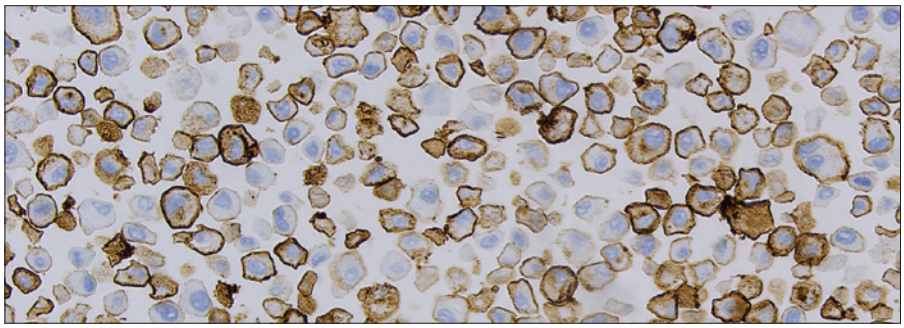


Figure 7: Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with PD-L1 Code SK006 (20× magnification).

* Optional use with PD-L1 IHC 22C3 pharmDx, Code GE006.

Negative Control Cell Pellet

For the PD-L1 negative cell pellet, the following staining is acceptable (Figure 8):

- The majority of cells should demonstrate no staining. **Note:** The presence of 10 or fewer cells with distinct cell membrane staining is acceptable
- Non-specific staining < 1+ intensity

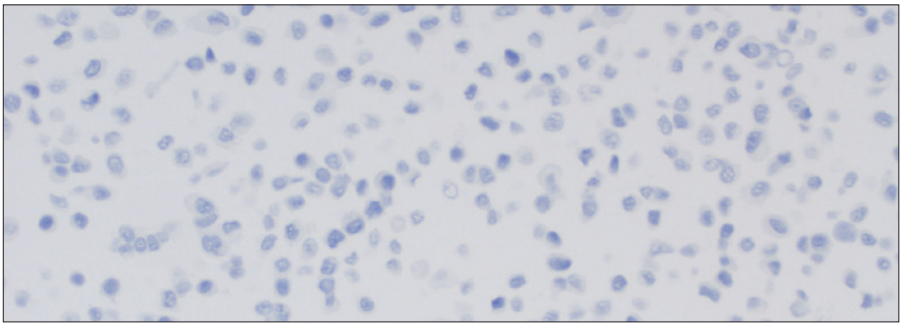


Figure 8: Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with PD-L1 Code SK006 (20× magnification).

Do not use the Control Cell Line Slide as an aid in interpretation of patient results.

Positive and Negative In-house Control Tissue (NSCLC)

Examine the positive in-house NSCLC control tissue to determine that the tissues are correctly prepared and reagents are functioning properly. The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate tumor cell membrane staining (Figure 9). If staining of positive in-house control tissue is not satisfactory, all results with the patient specimen should be considered invalid.

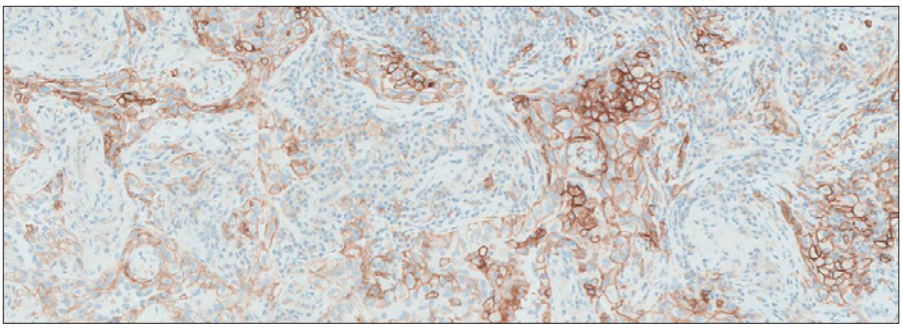


Figure 9: Ideal positive in-house control tissue stained with PD-L1 Code SK006 (10× magnification).

The ideal NSCLC negative control tissue demonstrates no staining on tumor cells but contains macrophages/immune cells that express PD-L1 and offer an internal positive control (Figure 10). Examine the negative in-house control tissue to determine the expected staining. The variety of different cell types present in most tissue sections offers internal negative control sites; this should be verified by the user.

If unwanted staining occurs in the in-house control tissues, results with the patient specimen should be considered invalid.

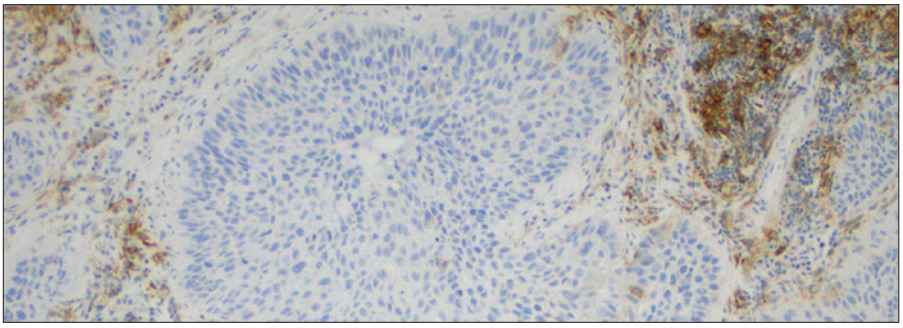


Figure 10: Ideal negative in-house control tissue demonstrating lack of staining of tumor cells stained with PD-L1 Code SK006 (10× magnification).

Optional Control Tissue

In addition to the Control Cell Line Slide and in-house control tissues, FFPE tonsil may also be used as an optional control specimen with PD-L1 IHC 22C3 pharmDx, Code SK006. Tonsil stained with PD-L1 should exhibit strong membrane staining in portions of the crypt epithelium and weak-to-moderate membrane staining of the follicular macrophages in the germinal centers (Figure 11).

PD-L1 expression of the endothelium, fibroblasts, and the surface epithelium should be absent.

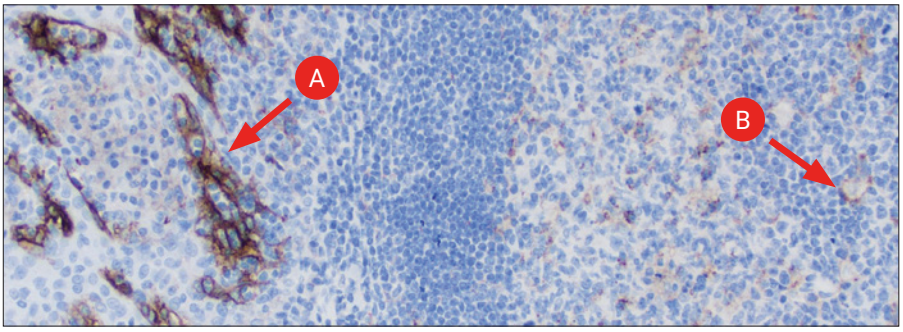


Figure 11: Tonsil stained with PD-L1 Code SK006 exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

Do not use in-house control tissue as an aid in interpretation of patient results.

Negative Control Reagent (NCR)

Examine the slides stained with the NCR to identify non-specific background staining that may interfere with PD-L1 staining interpretation, making the specimen non-evaluable. Satisfactory performance is indicated by the absence of staining (Figure 12).

Examine the patient specimens stained with the NCR to determine if there is any non-specific staining that may interfere with interpreting the PD-L1 stained slide.

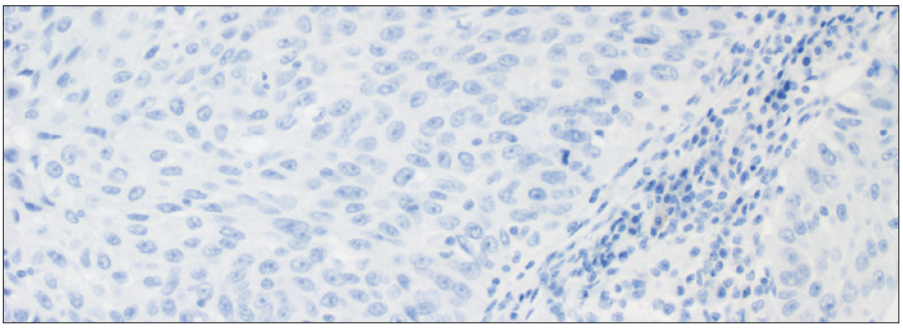


Figure 12: Ideal negative in-house control tissue stained with NCR Code SK006 (20× magnification).

NCR-stained slides indicate non-specific background staining and allow for better interpretation of patient specimens stained with the primary antibody.

Slide Evaluation Flowchart

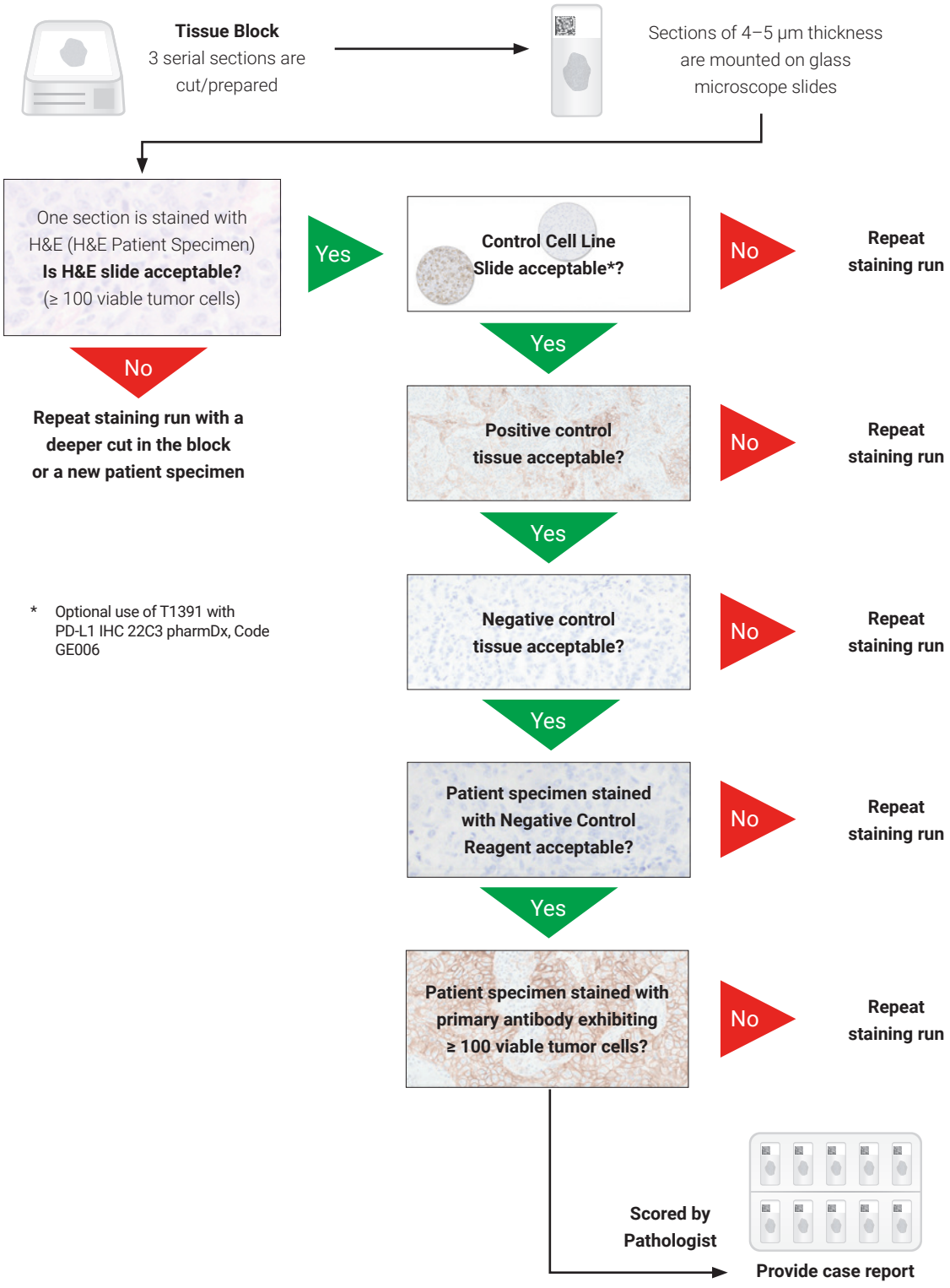


Figure 13: Recommended order of slide evaluation.

Evaluate Staining and Determine Tumor Proportion Score

Definition of Tumor Proportion Score (TPS)

The Tumor Proportion Score is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity (≥ 1+) relative to all viable tumor cells present in the sample.

TPS is defined accordingly:

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

Table 1: TPS Numerator 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 Cells	Not included	Exclude any staining of: – Normal cells adjacent to tumor cells – Stromal cells (fibroblasts) – Necrotic cells and/or cellular debris – Anthracotic pigment

Evaluation of PD-L1 Staining

Score partial or complete cell membrane staining (≥ 1+) of tumor cells that is perceived distinct from cytoplasmic staining. Cytoplasmic staining should be considered non-specific staining and is excluded in the assessment of staining intensity.

Score only viable tumor cells. Exclude any staining of immune cells, such as mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages), plasma cells, and neutrophils. Exclude any staining of normal cells adjacent to tumor cells, stromal cells (fibroblasts), necrotic cells and/or cellular debris, as well as anthracotic pigment.

Guidelines and Methods to Determine Tumor Proportion Score

- At low magnification, examine all well-preserved tumor areas. Evaluate overall areas of PD-L1 staining tumor cells, keeping in mind that partial membrane staining or ≥ 1+ membrane staining may be difficult to see at low magnification. Ensure there are at least 100 viable tumor cells in the sample
- At higher magnifications, including 10×, 20×, and 40×, observe all tumor areas with and without cell membrane staining
- At this stage of working with multiple magnifications, primary analysis involves:
  - Distinguishing tumor cells from tumor-associated immune cells
  - Determining PD-L1 staining and non-staining tumor areas
  - Determining partial and complete membrane staining (≥ 1+) of tumor cells
- Calculate the Tumor Proportion Score by evaluating the percentage of PD-L1 staining tumor cells relative to all viable tumor cells present in the specimen

  Note: Carefully consider the overall tumor area without any perceptible and convincing cell membrane staining

Make Sure to *Exclude* Immune Cells and Necrotic Tissue From Scoring

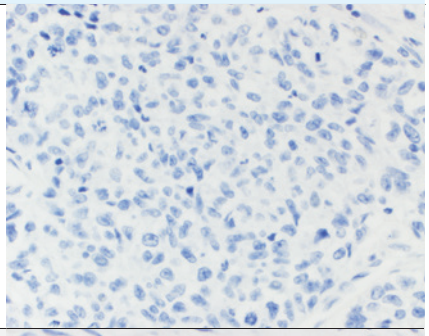
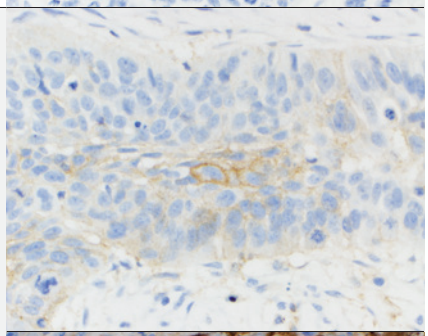
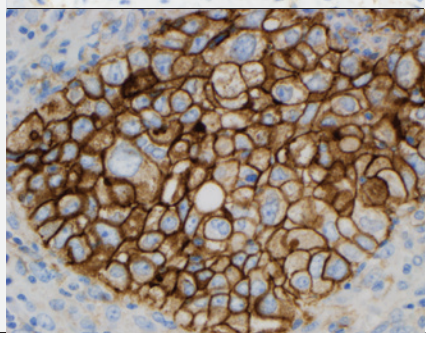
The following considerations can help distinguish tumor cells from immune cells:

- Immune cells may have smaller nuclei than tumor cells
- Macrophages may contain pigmented particles in their cytoplasm
- Macrophages may have a scattered distribution. Pulmonary macrophages are present in the alveolar space

Scoring Guidelines

The TPS determines the PD-L1 expression levels of the specimen. See the table below for scoring guideline examples.

Table 2: TPS and PD-L1 Expression Levels

TPS	Expression Level	Image (20× magnification)
< 1%	No PD-L1 Expression	
≥ 1%	PD-L1 Expression	 

Suggested Methods for Determining TPS

Agilent recommends that scoring be performed within the context of the pathologist's past experience and best judgment in interpreting IHC stains. We offer two different examples of techniques that may be used when considering various staining patterns to determine the respective Tumor Proportion Scores.

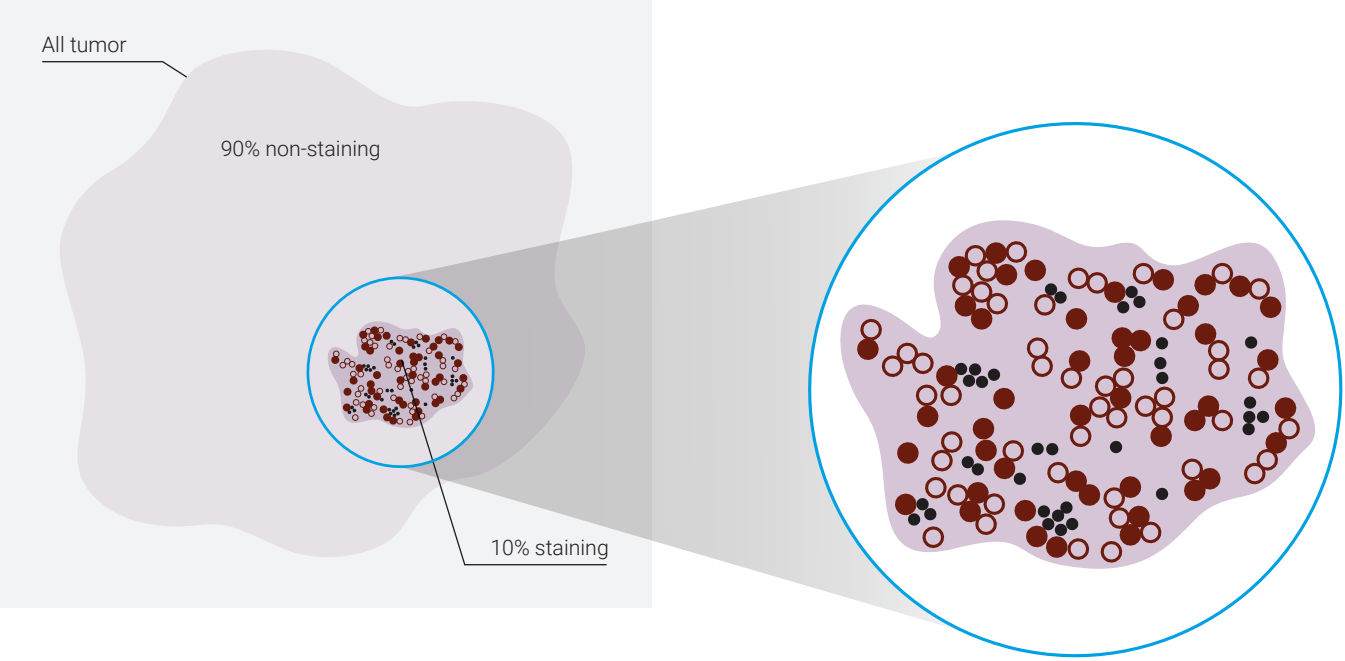
Example 1: Calculation of Tumor Proportion Score Based on a Small PD-L1 Staining Area

At lower magnification: Evaluate the tumor area for any perceptible and convincing ≥ 1+ cell membrane staining.

Assessment: *10% of area shows staining; 90% of area shows no staining*

At higher magnification: Evaluate the area of staining to estimate the percentage of PD-L1 staining tumor cells.

Assessment: *50% of tumor cells are PD-L1 staining*



Calculate Tumor Proportion Score: Determine the overall percentage of PD-L1 staining tumor cells for the entire tumor area.

Assessment: Tumor Proportion Score (TPS):
10% × 50% = 5%

Clinical Interpretation: TPS ≥ 1%, PD-L1 Expression

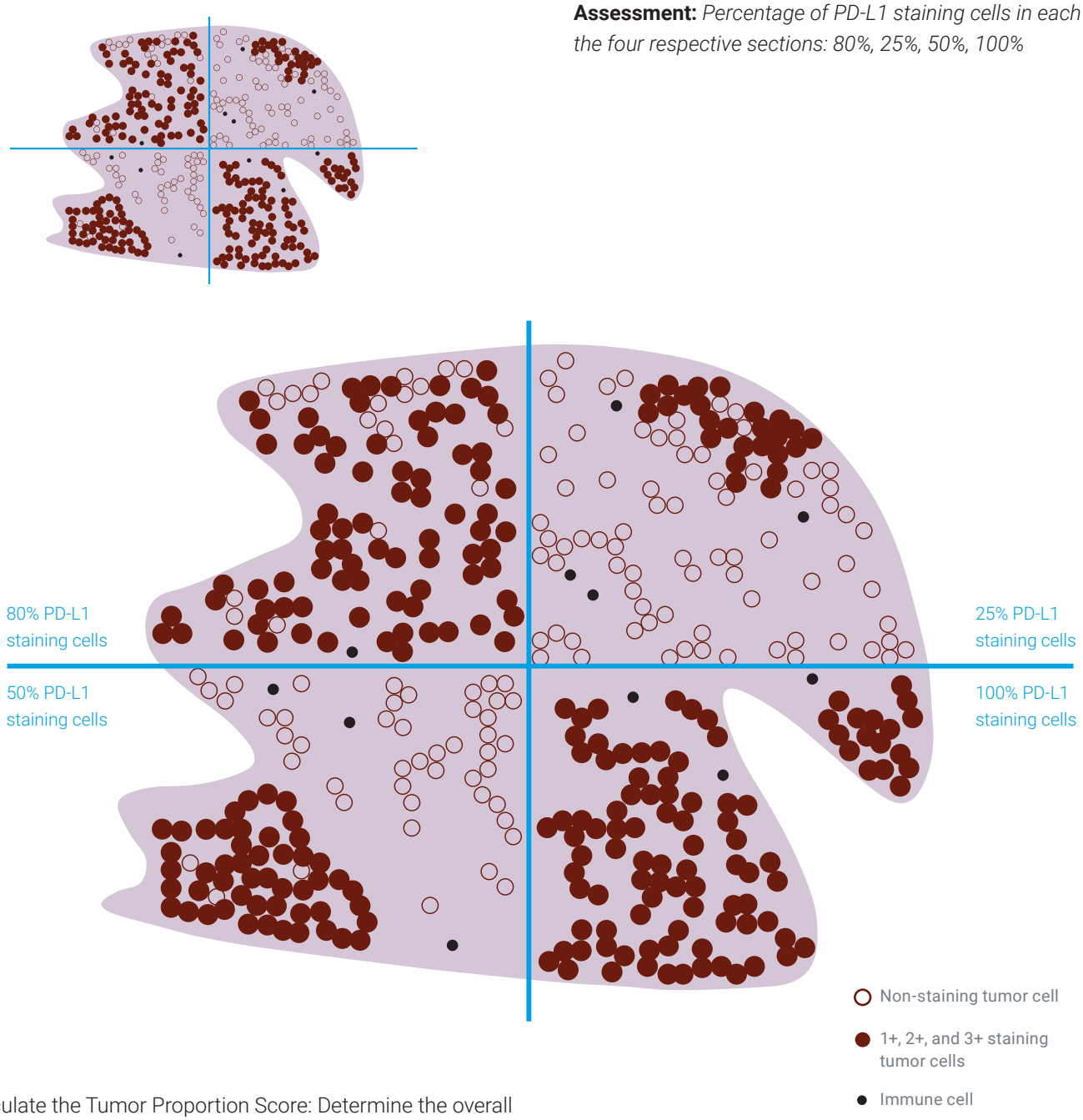
Figure 14: Example of tumor with small PD-L1 staining area.

Example 2: Calculation of Tumor Proportion Score Based on a Heterogeneous PD-L1 Staining Area

At lower magnification: Visually divide the tumor area into sections.

At higher magnification: Observe tumor areas with cell membrane staining for percentage of PD-L1 staining cells in each section.

Assessment: *Percentage of PD-L1 staining cells in each of the four respective sections: 80%, 25%, 50%, 100%*



Calculate the Tumor Proportion Score: Determine the overall percentage of PD-L1 staining tumor cells for the entire tumor area.

Assessment: Tumor Proportion Score (TPS):
(80% + 25% + 50% + 100%) / 4 ≈ 60%

Clinical Interpretation: TPS ≥ 1%, PD-L1 Expression

Figure 15: Example with heterogeneous PD-L1 staining area.

Identifying Patients With NSCLC for Treatment

PD-L1 IHC 22C3 pharmDx is the only companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab).

Clinical Validation of PD-L1 IHC 22C3 pharmDx in Previously Untreated Patients with NSCLC (First-line)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS ≥ 1%) in previously untreated patients with NSCLC is based on the KEYTRUDA KEYNOTE-042 study sponsored by Merck Sharp & Dohme Corp. Specimens from previously untreated patients with NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Only patients with TPS ≥ 1% were included in the KEYNOTE-042 study. Efficacy of KEYTRUDA treatment in patients selected by PD-L1 IHC 22C3 pharmDx, Code SK006 is presented in the Clinical Performance Evaluation section on pages 74-82.

Table 3: Patient Characteristics in Patients with NSCLC Screened for KEYNOTE-042 (N=1274)*

PD-L1 Expression Level	TPS 1–49%	TPS ≥ 50%
% of Patients	53%	47%

** Percentage calculation based on patients whose tumors expressed PD-L1 (TPS ≥ 1%). Patients whose tumors did not express PD-L1 were not enrolled*

PD-L1 IHC 22C3 pharmDx Testing Scheme

Use the following flowchart to help you understand which patients are indicated for treatment with KEYTRUDA (pembrolizumab) based on their TPS.

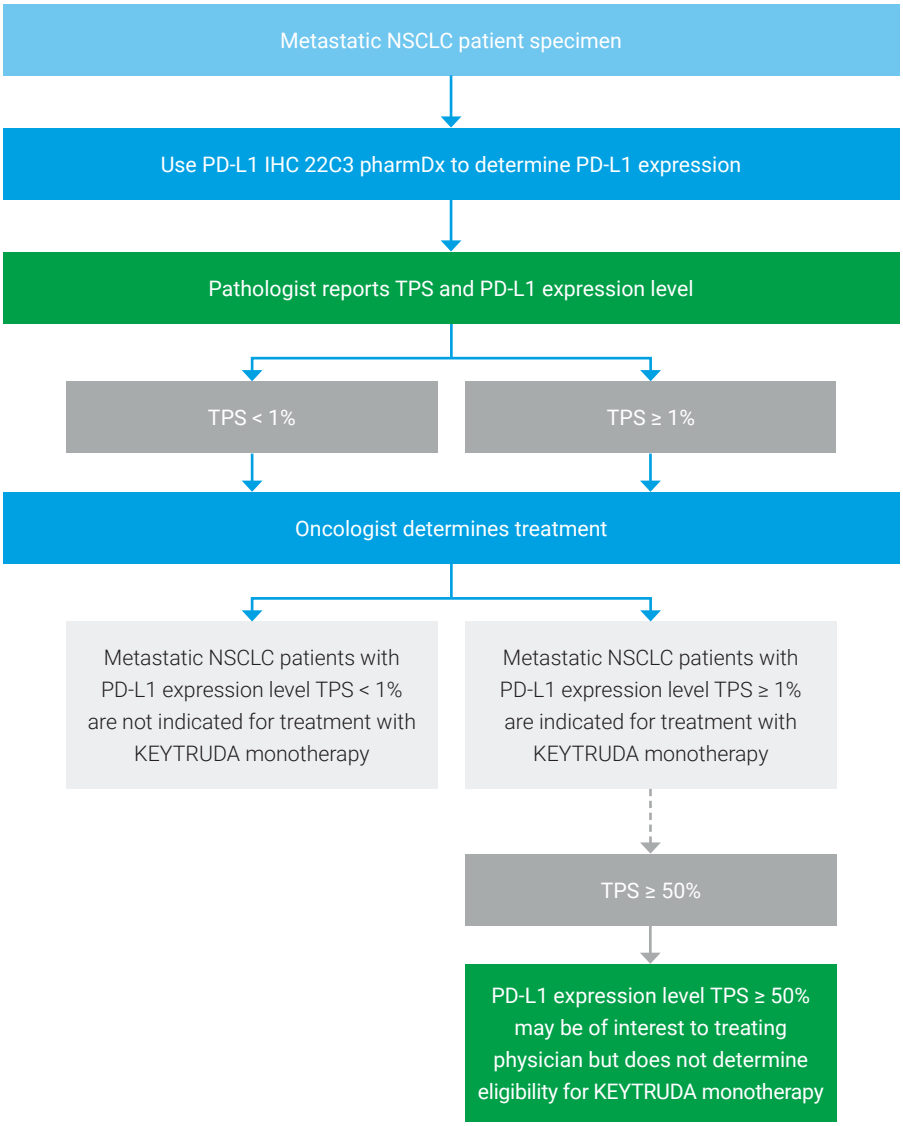


Figure 16: Testing scheme for PD-L1 IHC 22C3 pharmDx.

Reporting Results

Suggested information to include when reporting results with PD-L1 IHC 22C3 pharmDx.

PD-L1 IHC 22C3 pharmDx Summary of Sample Tested

Date of Run: _____

PD-L1 IHC 22C3 pharmDx, Code SK006, Lot: _____

or

PD-L1 IHC 22C3 pharmDx, Code GE006, Lot: _____
(Record the lot numbers for all reagents used on the instrument)

Staining Run Log ID: _____

Specimen ID: _____

Patient Identifiers: _____

Type of Service: IHC Stain With Manual Interpretation

Other: _____

PD-L1 Included in Non-small Cell Lung Cancer Comprehensive Panel: Yes: ☐ No: ☐

Type of Tissue: Squamous Cell: ☐ Non-squamous Cell: ☐

PD-L1 Testing Results

Control Cell Line Slide Results: Pass: ☐ Fail: ☐

Adequate Tumor Cells Present (≥ 100 cells): ☐

PD-L1 IHC 22C3 pharmDx Result to Treating Physician

Tumor Proportion Score (TPS): _____

TPS < 1%: ☐ TPS ≥ 1%: ☐

TPS ≥ 50%: ☐

Note: PD-L1 expression level TPS ≥ 50% may be of interest to treating physician but does not determine eligibility for KEYTRUDA® (pembrolizumab) monotherapy.

PD-L1 Staining Characteristics

Key Considerations in Scoring Stained Specimens using PD-L1 IHC 22C3 pharmDx

To successfully score stained specimens with PD-L1 IHC 22C3 pharmDx, it is critical that:

- A minimum of 100 viable tumor cells are present for evaluation
- The appropriate cells are evaluated—only viable tumor cells should be scored
- The proper cellular localization is identified—only membrane staining of tumor cells should be evaluated
- The staining is properly interpreted

The pathologist's experience and judgment are important in the evaluation of PD-L1 staining. For evaluation of the immunohistochemical staining and scoring, objectives of 10×, 20×, and 40× magnifications are appropriate.

However, below are several staining characteristic patterns that should be considered in the Tumor Proportion Score (TPS) calculation:

- Membrane staining of tumor cells at all intensities (1–3+) should be included
- Partial and/or complete membrane staining should be included
- Any perceptible and convincing membrane staining should be included
- Cytoplasmic staining **should not** be included
- Immune cells such as infiltrating lymphocytes or macrophages **should not** be included
- Granular staining must demonstrate a perceptible and convincing membrane pattern to be included

The following pages provide guidance on various staining characteristics.

Image Guide for Interpretation of PD-L1 IHC 22C3 pharmDx Staining in NSCLC

Perceptible and Convincing Membrane Staining

Scoring should include any perceptible and convincing membrane staining at any intensity (≥ 1+) and at any magnification. Review at higher magnification may be needed to confirm perceptible and convincing membrane staining.

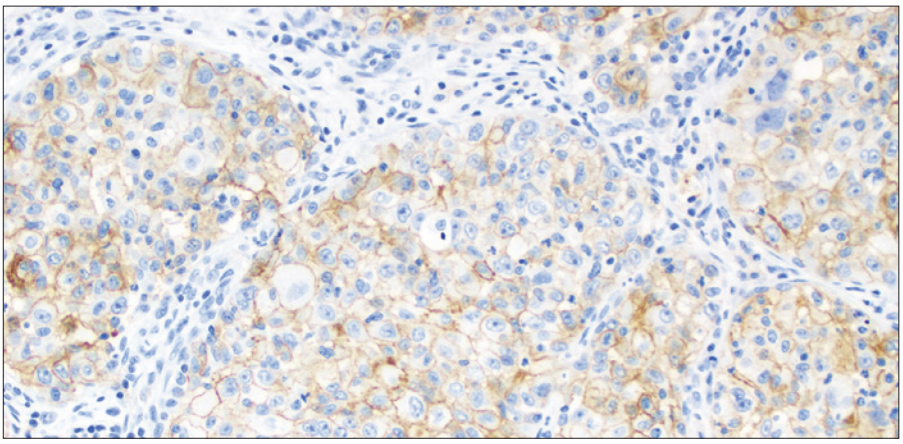


Figure 17a: NSCLC specimen stained with PD-L1 Code SK006 exhibiting weak membrane staining of tumor cells (10× magnification).

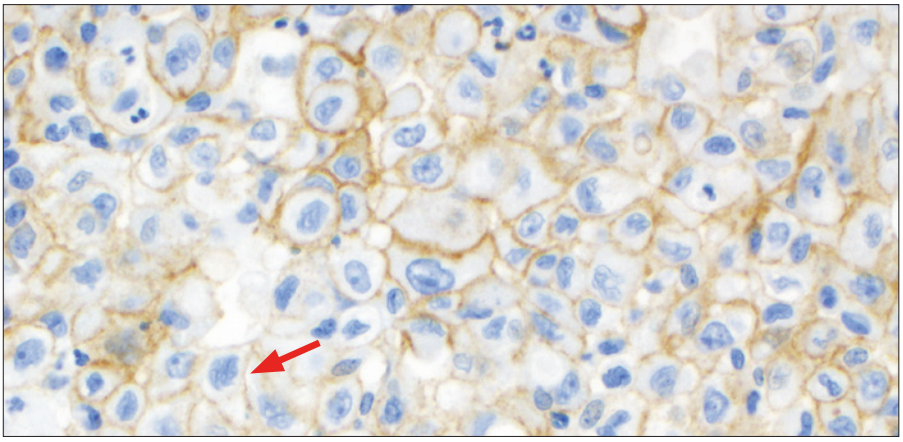


Figure 17b: NSCLC specimen stained with PD-L1 Code SK006 exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40× magnification).

Key Point

Any perceptible and convincing membrane staining of tumor cells (≥ 1+) should be included in the TPS

Weak Acceptable Membrane Staining

Scoring of tumor cells should include any perceptible and convincing membrane staining, including weak intensity of 1+.

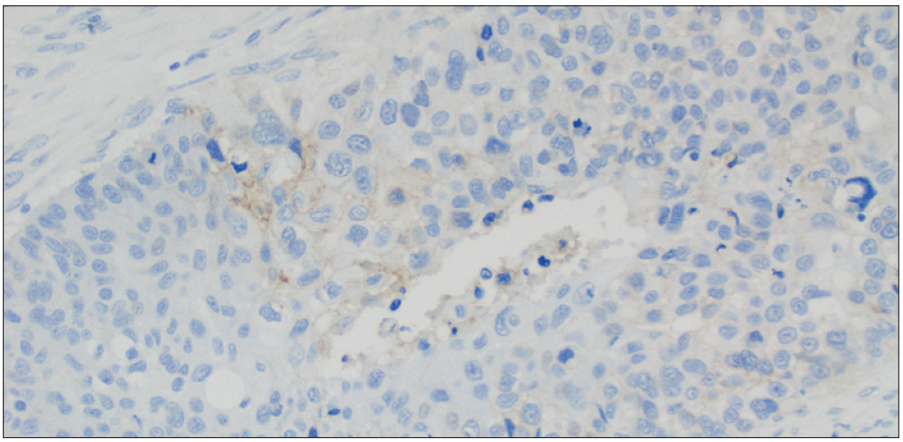


Figure 18a: NSCLC specimen stained with PD-L1 Code SK006 exhibiting weak but perceptible and convincing membrane staining of tumor cells (20× magnification).

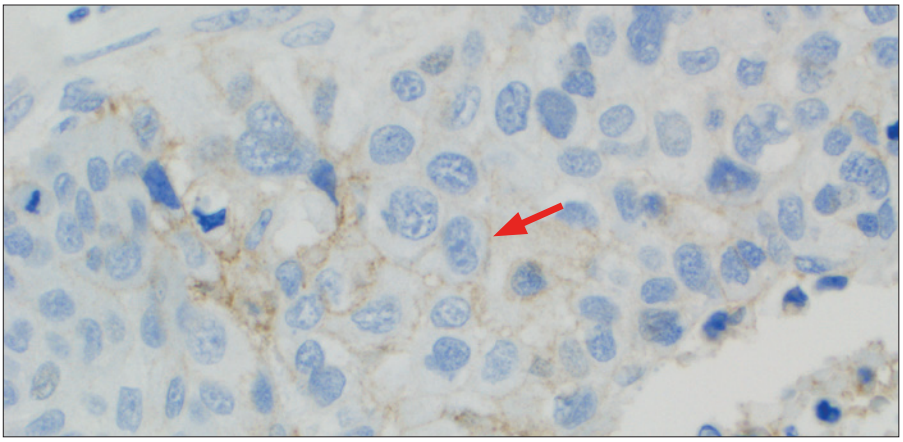


Figure 18b: NSCLC specimen stained with PD-L1 Code SK006 exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40× magnification).

Key Point

Weak but perceptible and convincing 1+ membrane staining of tumor cells should be included in the TPS

Distinguishing Tumor Cells From Immune Cells (IC)

Scoring should only include all viable tumor cells with membrane staining (≥ 1+). Tumor-associated immune cells should be excluded from scoring.

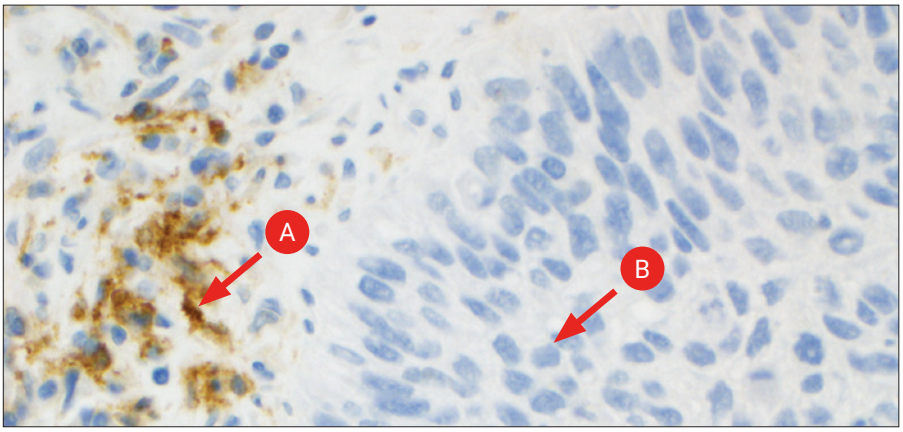


Figure 19: NSCLC specimen stained with PD-L1 Code SK006 exhibiting strong staining of the Immune Cells (A) and lack of PD-L1 staining of tumor cells (B); Immune Cell staining should be excluded from the scoring (20× magnification).

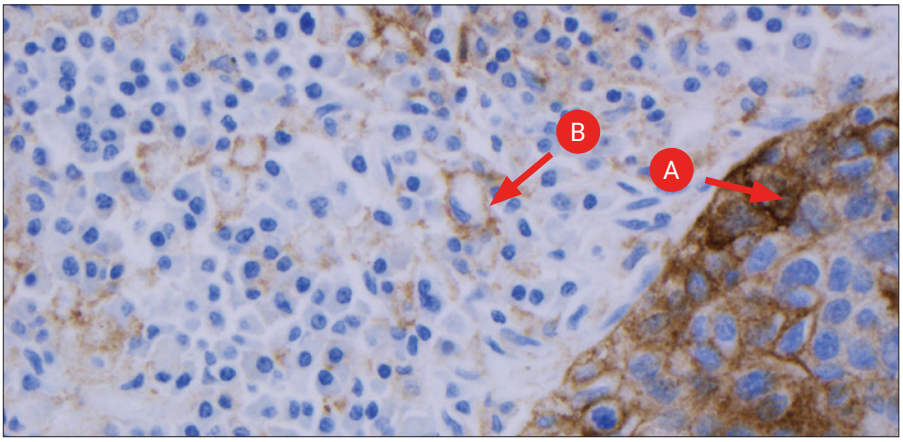


Figure 20: NSCLC specimen stained with PD-L1 Code SK006 exhibiting strong staining of tumor cells (A) and moderate staining of the Immune Cells (B); Immune Cell staining should be excluded from the scoring (20× magnification).

Key Point

Staining of Immune Cells should be excluded from the TPS

Heterogeneous Staining Intensities

Membrane staining of PD-L1 on NSCLC specimens is often heterogeneous with various staining intensities (1–3+).

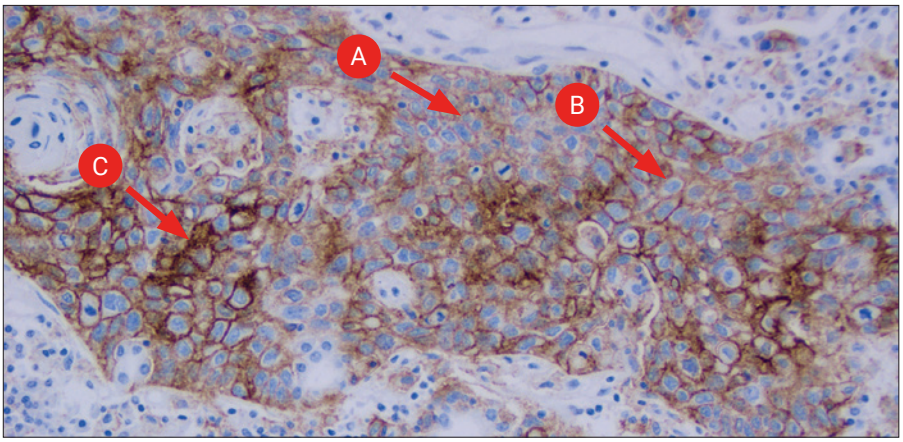


Figure 21: NSCLC specimen stained with PD-L1 Code SK006 exhibiting a heterogeneous membrane staining pattern with various staining intensities: 1+ staining (A), 2+ staining (B), and 3+ staining (C) (20× magnification).

Key Point

All membrane staining of tumor cells, at all intensities (1–3+), should be included in the TPS

Partial vs. Complete Membrane Staining

Scoring should include viable tumor cells showing partial or complete membrane staining (≥ 1+).

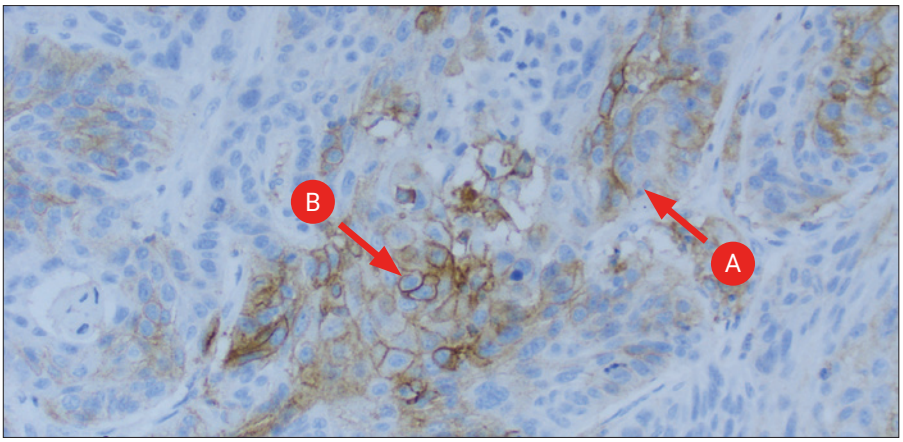


Figure 22: NSCLC specimen stained with PD-L1 Code SK006 exhibiting a heterogeneous membrane staining pattern with various staining intensities (1–3+): partial membrane staining of tumor cell (A) and complete cell membrane staining (B) (20× magnification).

Key Point

Partial and/or complete membrane staining of tumor cells (≥ 1+) should be included in the TPS

Cytoplasmic and Membrane Staining

Tumor cells can exhibit cytoplasmic and/or membrane staining. Cytoplasmic staining should be excluded from the TPS scoring assessment.

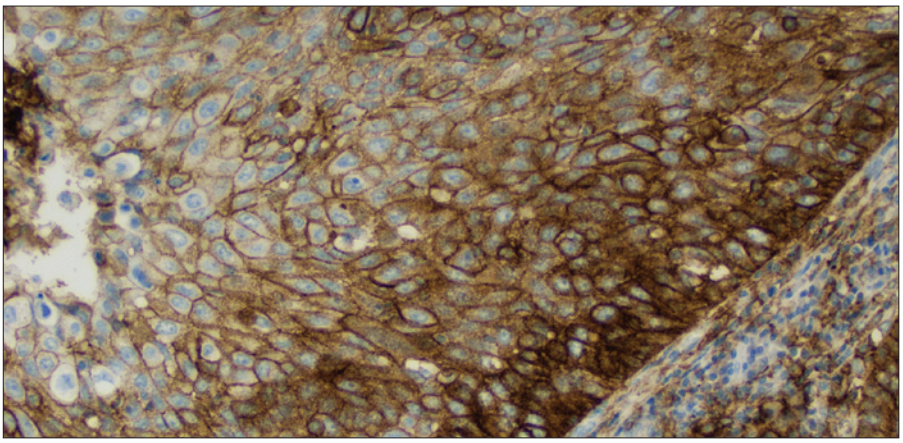


Figure 23: NSCLC specimen stained with PD-L1 Code SK006 exhibiting strong cytoplasmic and membrane staining of tumor cells (20× magnification).

Key Point

Only membrane staining of tumor cells should be included in the TPS

Granular Staining

PD-L1 membrane staining may be indistinguishable when the staining pattern appears granular. Granular staining can be difficult to interpret and easily confused with cytoplasmic staining. Only perceptible and convincing granular membrane staining should be included in the TPS scoring.

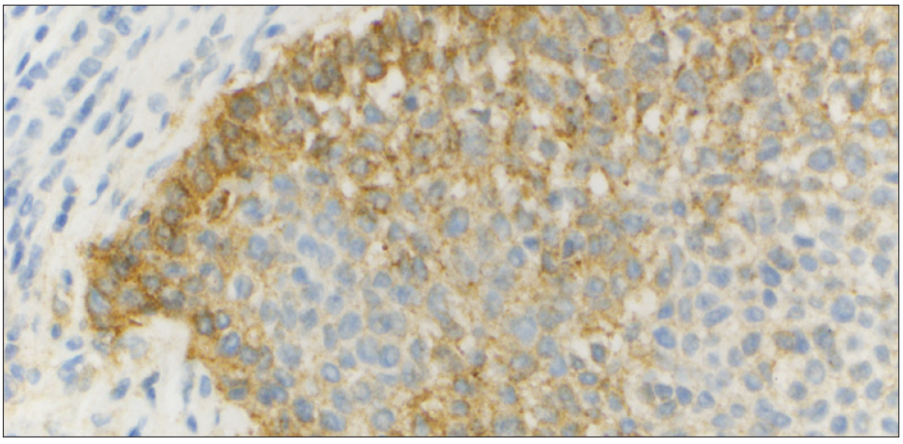


Figure 24: NSCLC specimen stained with PD-L1 Code SK006 with the majority of tumor cells exhibiting a granular pattern of perceptible and convincing membrane staining (20× magnification).

Key Point

Granular staining of tumor cells must demonstrate a perceptible and convincing membrane pattern to be included in the TPS

Patchy Staining

Staining of PD-L1 on NSCLC specimens may be patchy in appearance. A review of each portion of the specimen at high power may be needed to score accurately.

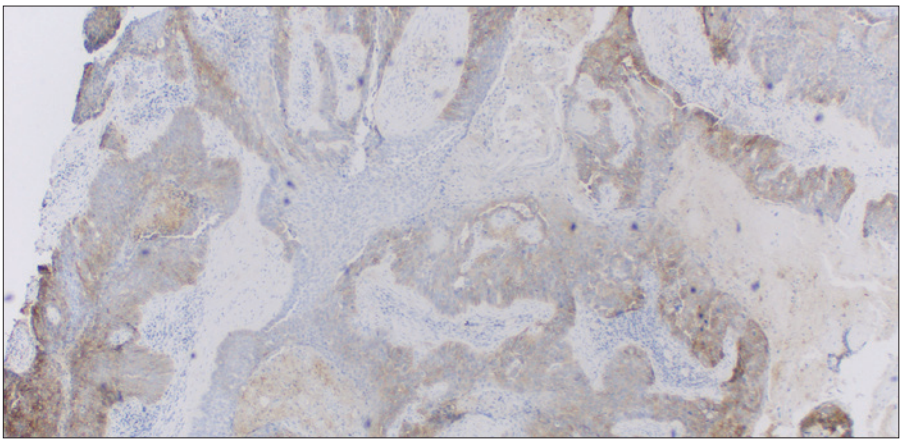


Figure 25: NSCLC specimen stained with PD-L1 Code SK006 exhibiting a patchy membrane staining pattern (10× magnification).

Key Point

Assess entire specimen to accurately determine the TPS

Anthracotic Pigment

Anthracotic pigment is an accumulation of carbon in the lungs from inhaled smoke or coal dust. It appears as granular dark spots and is often helpful to distinguish tumor cells from immune cells (IC), as anthracotic pigment is found within pulmonary macrophages and not within tumor cells.

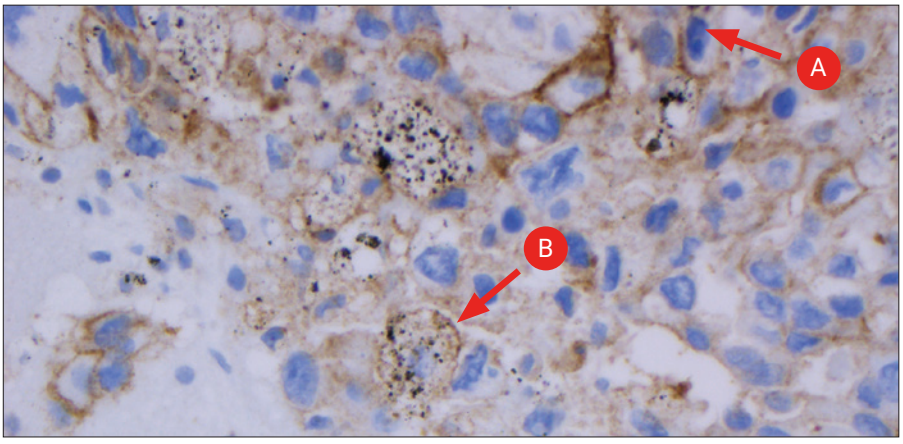


Figure 26: NSCLC specimen stained with PD-L1 Code SK006 exhibiting strong staining of tumor cells (A) and moderate staining of the IC (B); IC staining should be excluded from the scoring (20× magnification).

Key Point

Anthracotic pigment should be disregarded

PD-L1 IHC 22C3 pharmDx, Code SK006
TPS < 1% Case Examples

Case 1: TPS < 1%

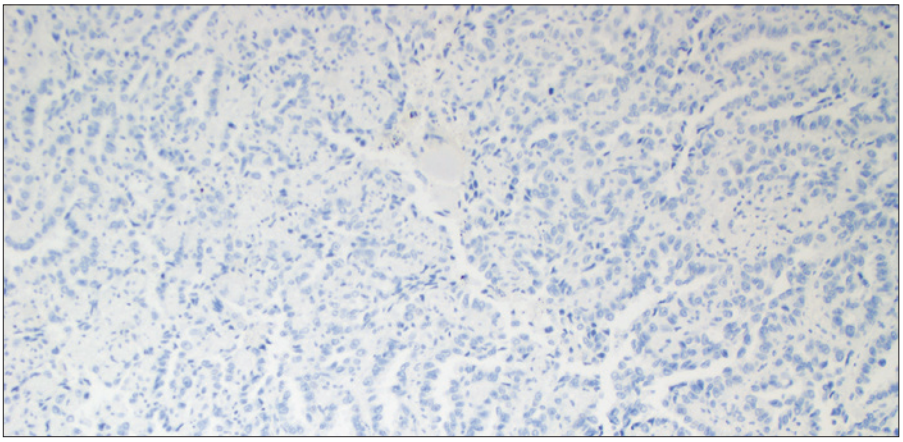


Figure 27a: 10× magnification.

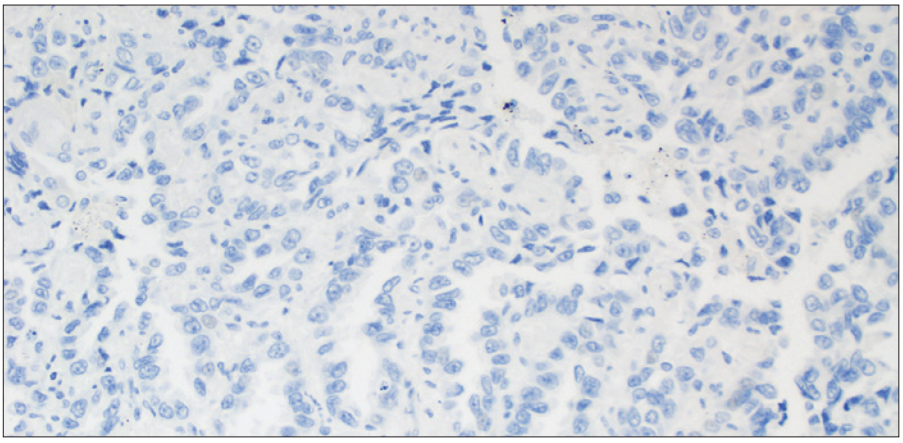


Figure 27b: 20× magnification.

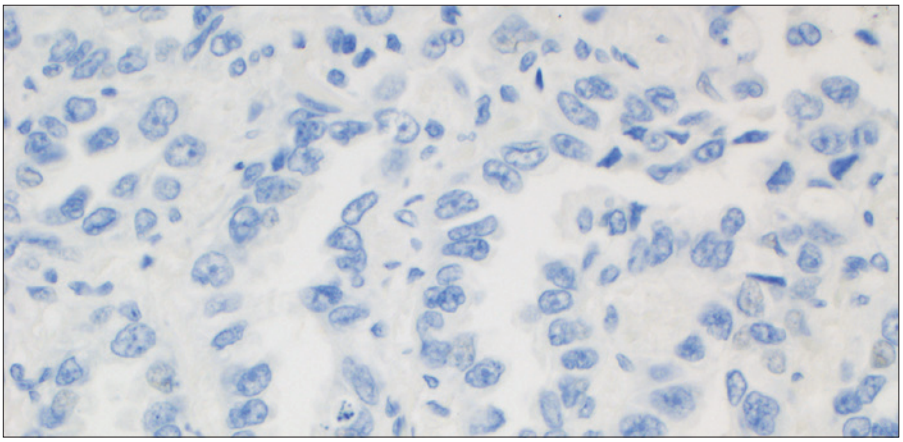


Figure 27c: 40× magnification.

Figure 27a–27c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%.

Case 2: TPS < 1%

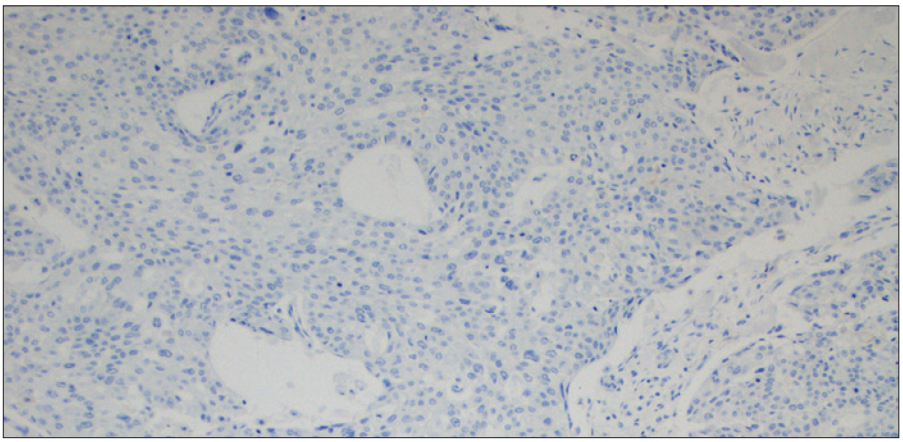


Figure 28a: 10× magnification.

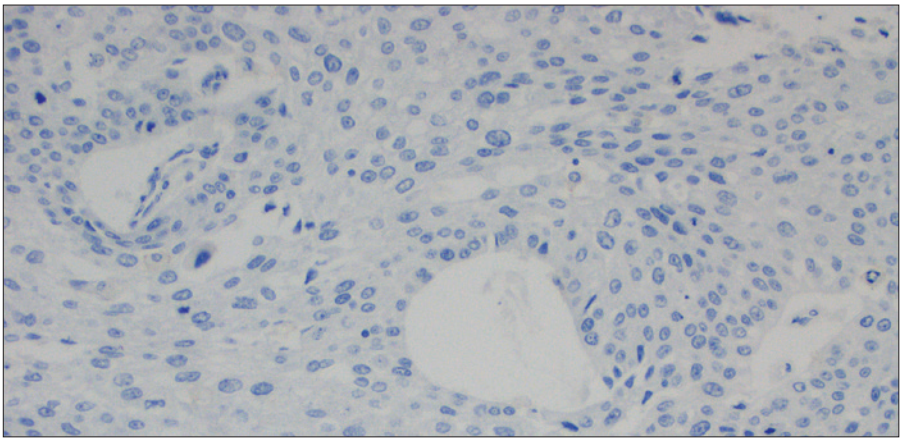


Figure 28b: 20× magnification.

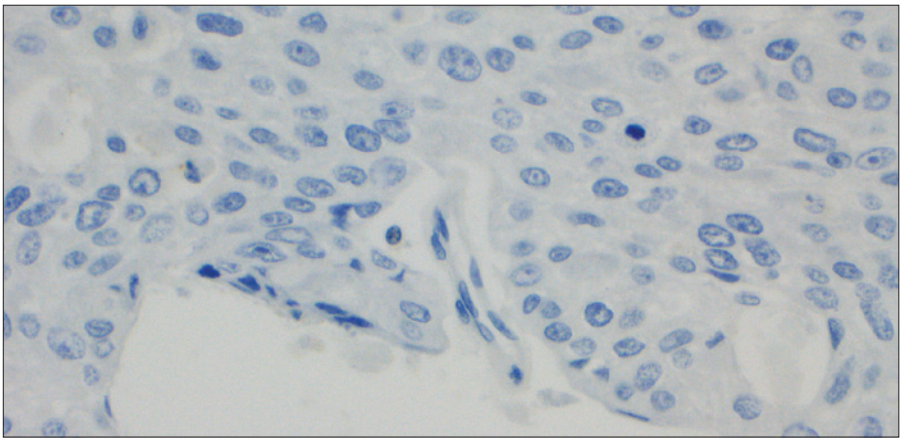


Figure 28c: 40× magnification.

Figure 28a–28c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%.

Case 3: TPS < 1%

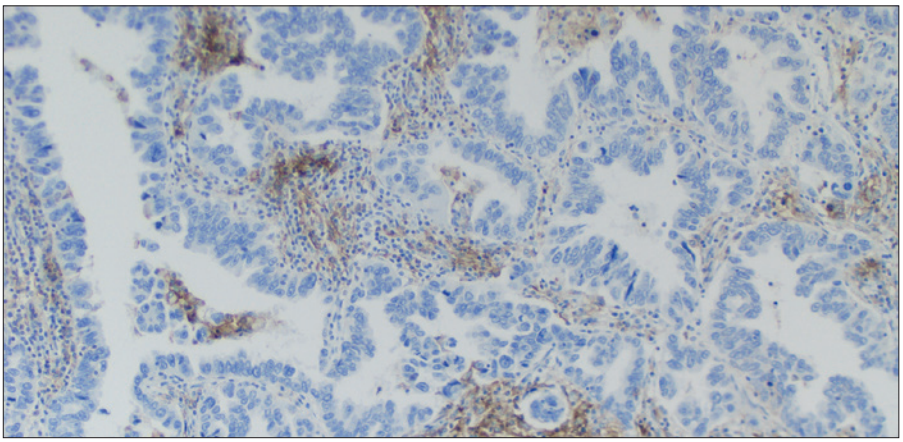


Figure 29a: 10× magnification.

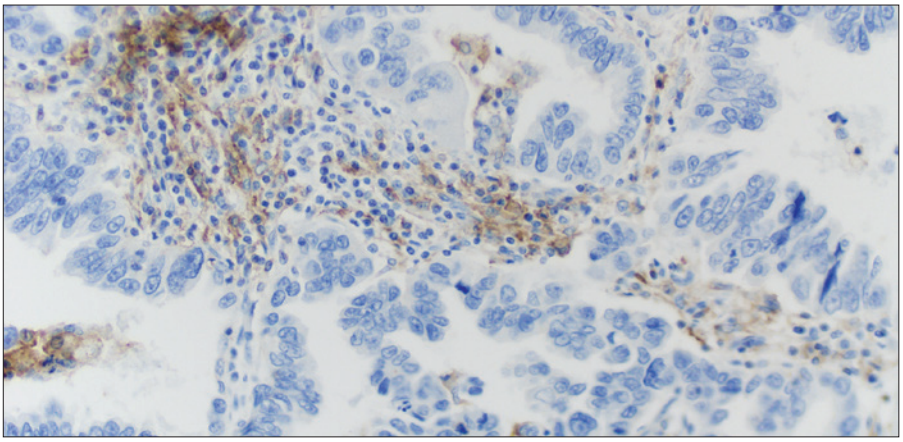


Figure 29b: 20× magnification.

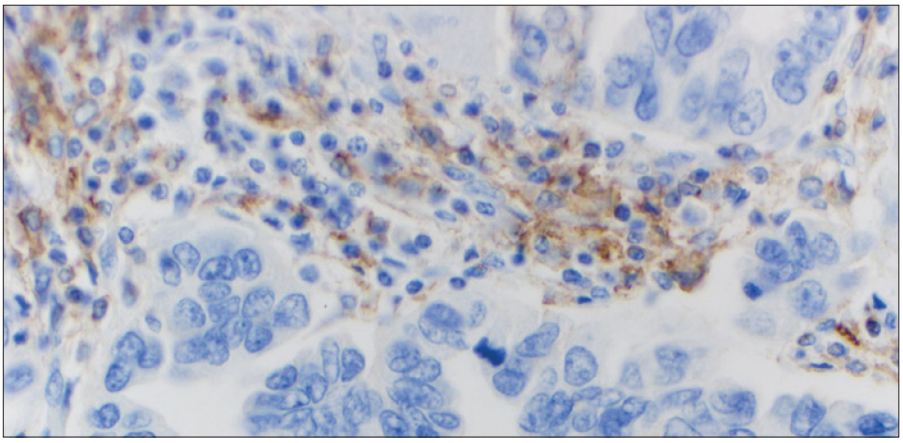


Figure 29c: 40× magnification.

Figure 29a–29c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%. Immune cells are staining, but should be excluded from scoring.

Case 4: TPS < 1%

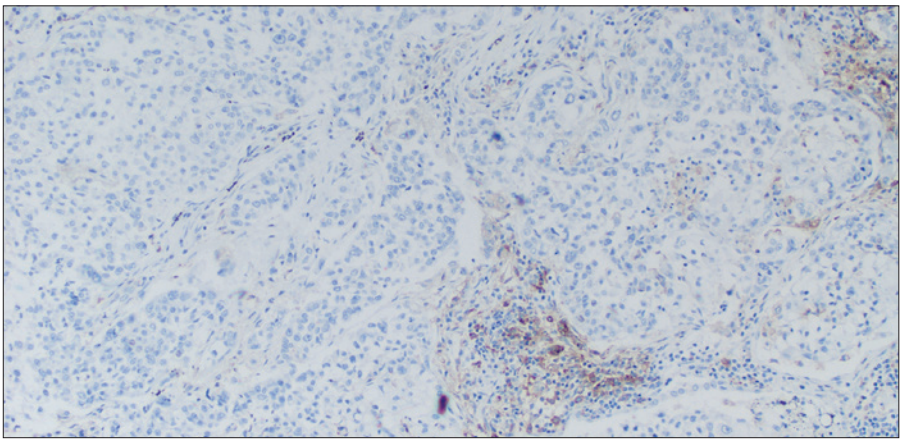


Figure 30a: 10× magnification.

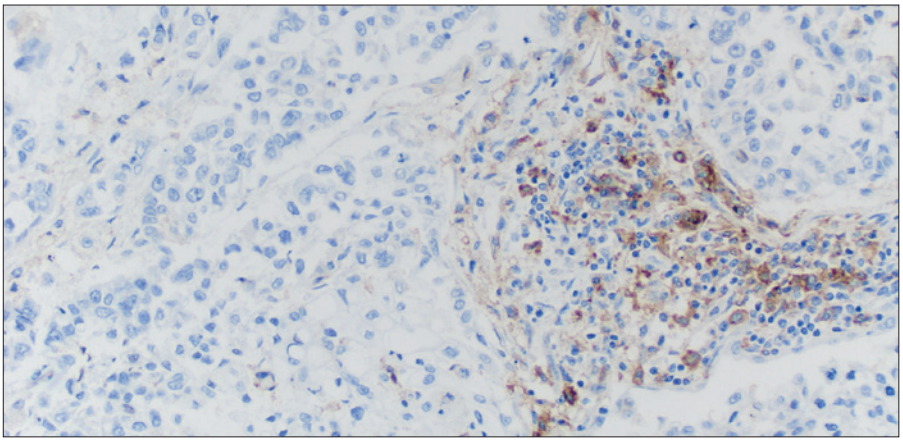


Figure 30b: 20× magnification.

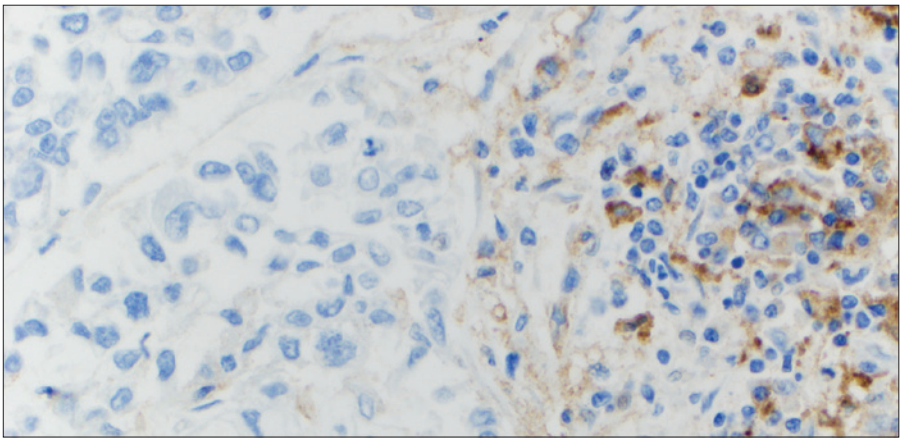


Figure 30c: 40× magnification.

Figure 30a–30c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%. Immune cells are staining, but should be excluded from scoring.

PD-L1 IHC 22C3 pharmDx, Code SK006
TPS 0–10% Case Examples

Challenging Case 1:
TPS 0–10%

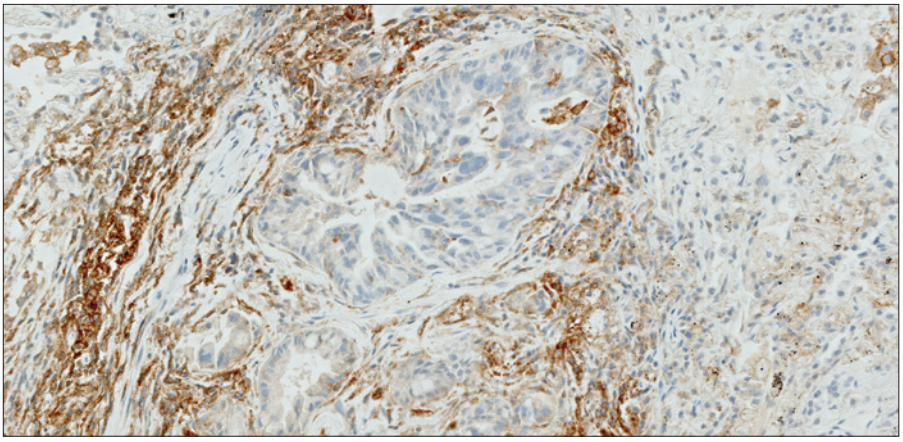


Figure 31a: 10× magnification.

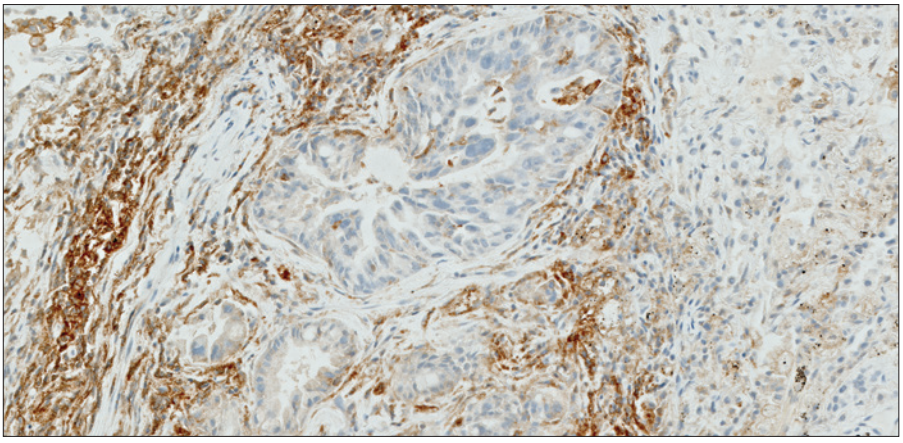


Figure 31b: 20× magnification.

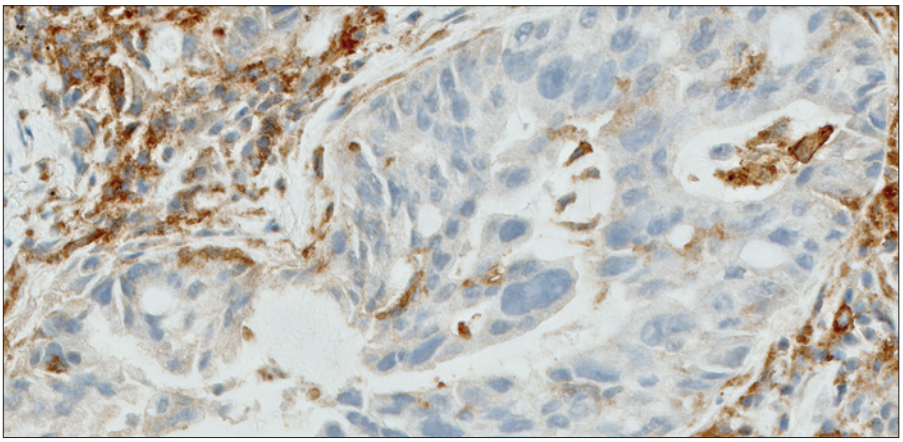


Figure 31c: 40× magnification.

Figure 31a–31c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%.

Challenging Case 2: TPS 0–10%

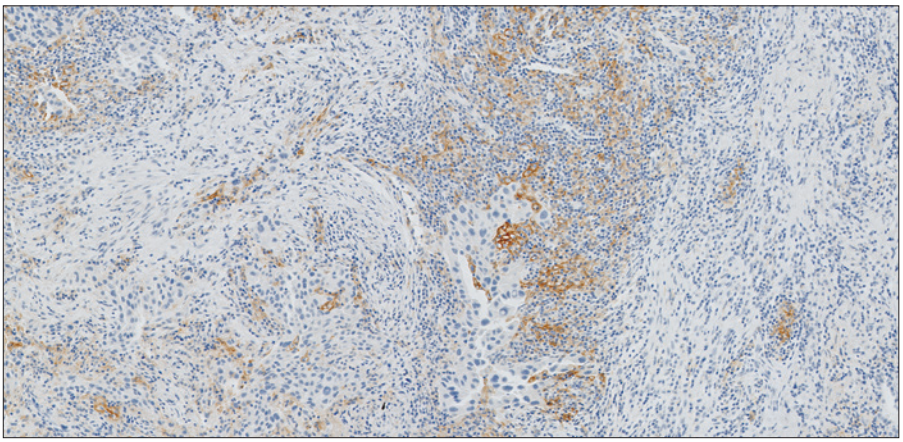


Figure 32a: 10× magnification.

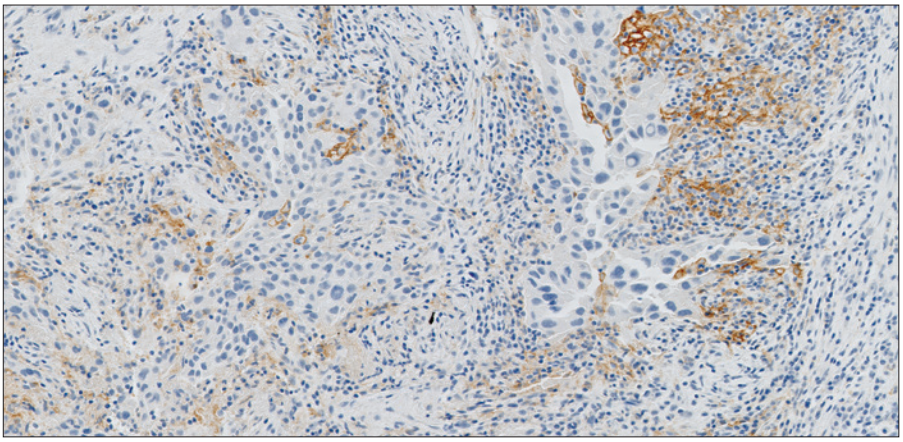


Figure 32b: 20× magnification.

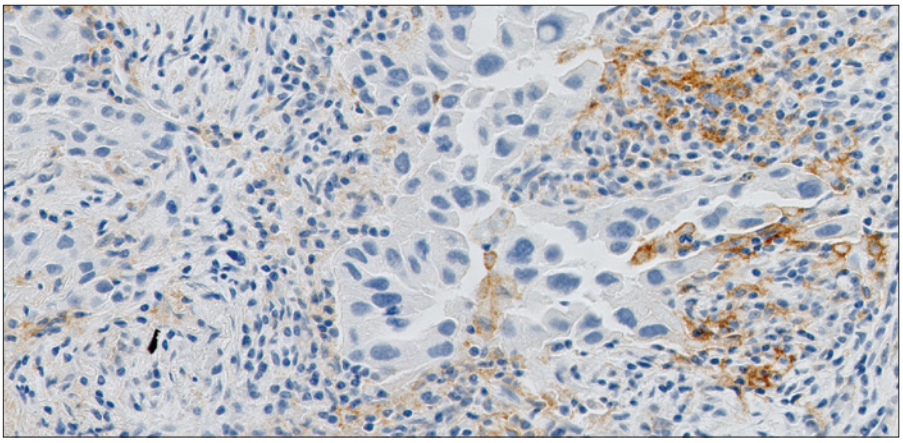


Figure 32c: 40× magnification.

Figure 32a–32c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS < 1%.

Challenging Case 3: TPS 0–10%

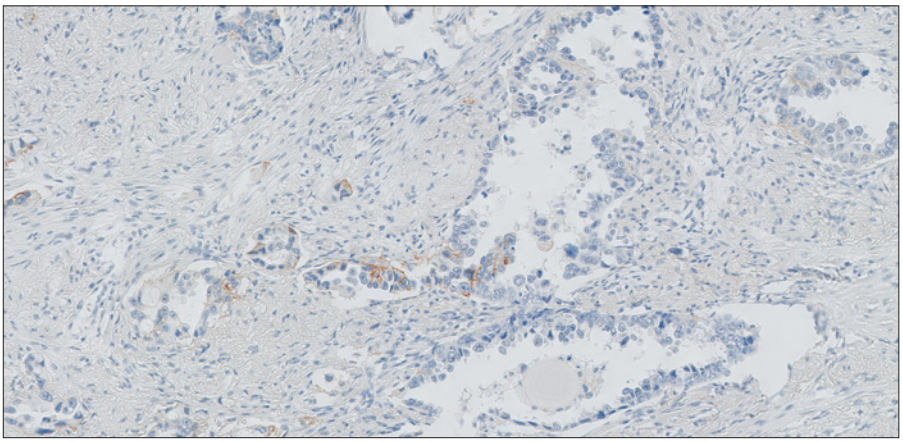


Figure 33a: 10× magnification.

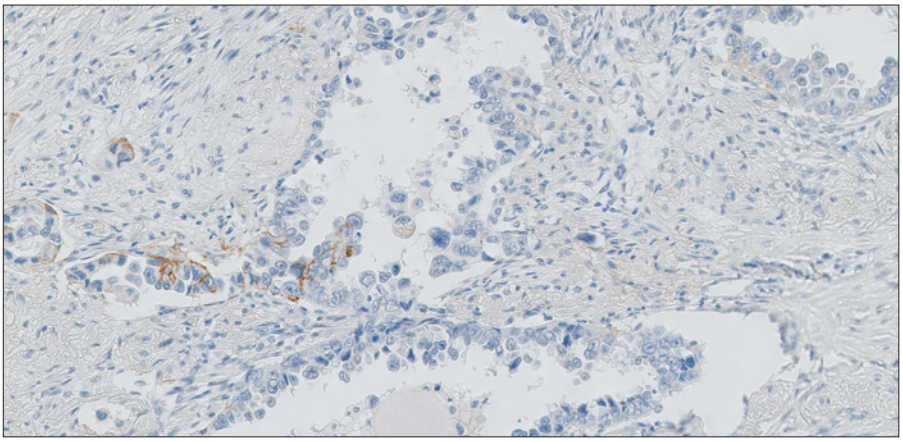


Figure 33b: 20× magnification.

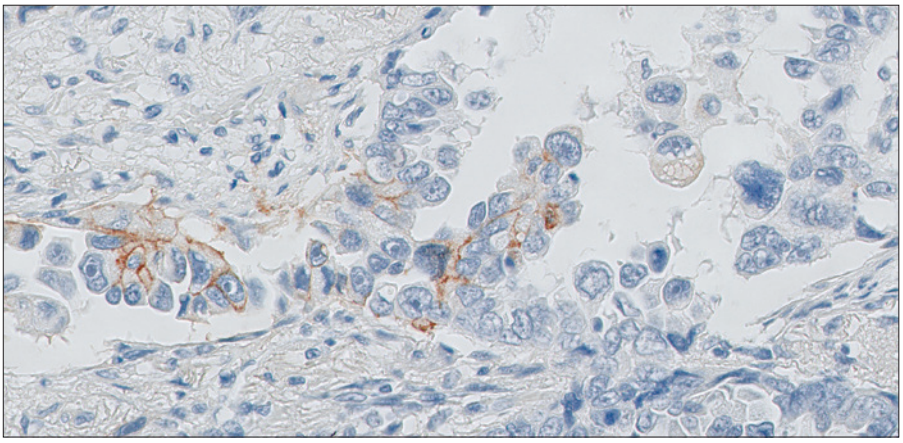


Figure 33c: 40× magnification.

Figure 33a–33c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–10%.

Challenging Case 4: TPS 0–10%

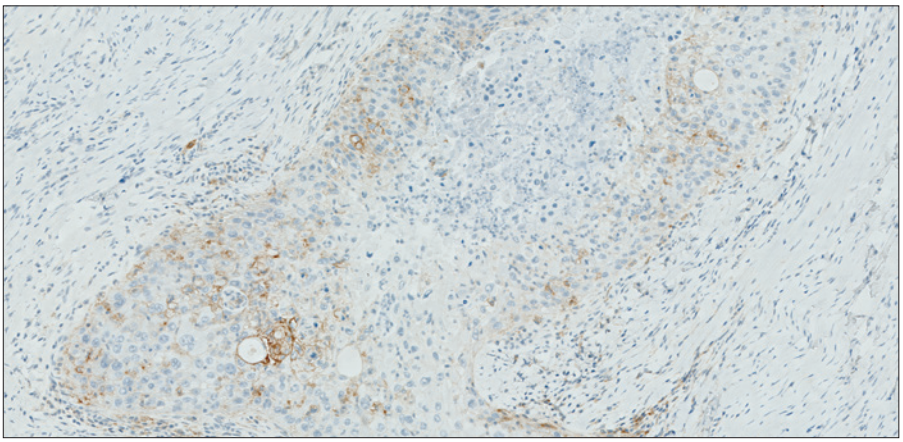


Figure 34a: 10× magnification.

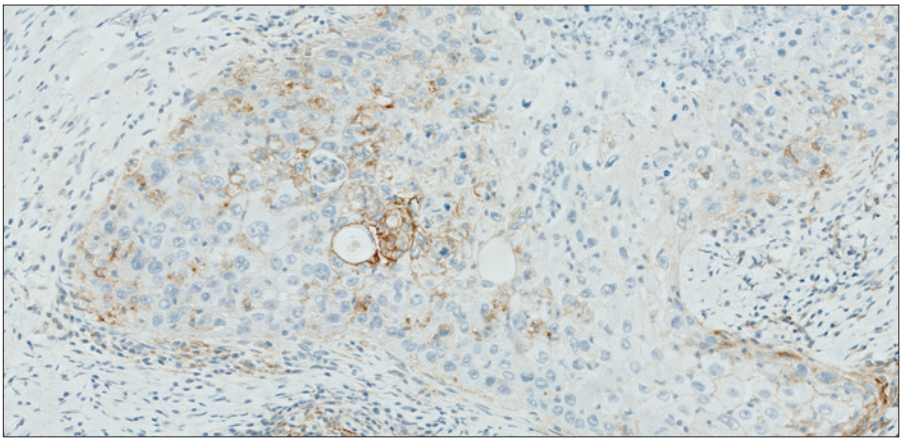


Figure 34b: 20× magnification.

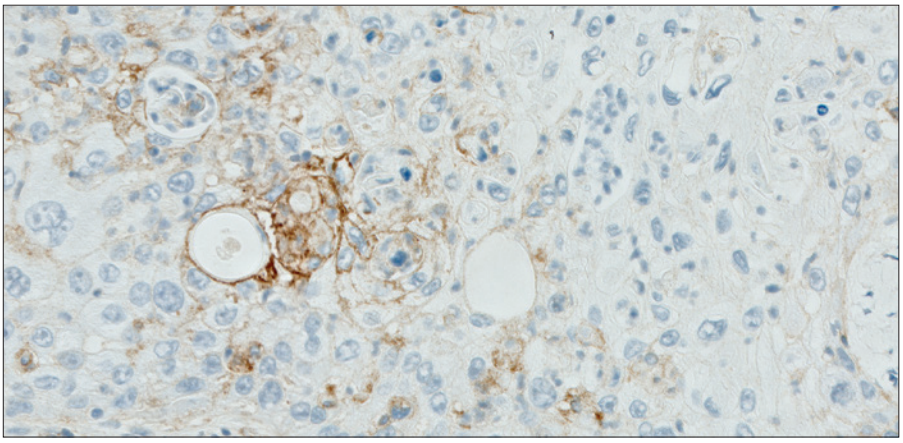


Figure 34c: 40× magnification.

Figure 34a–34c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–10%.

Challenging Case 5: TPS 0–10%

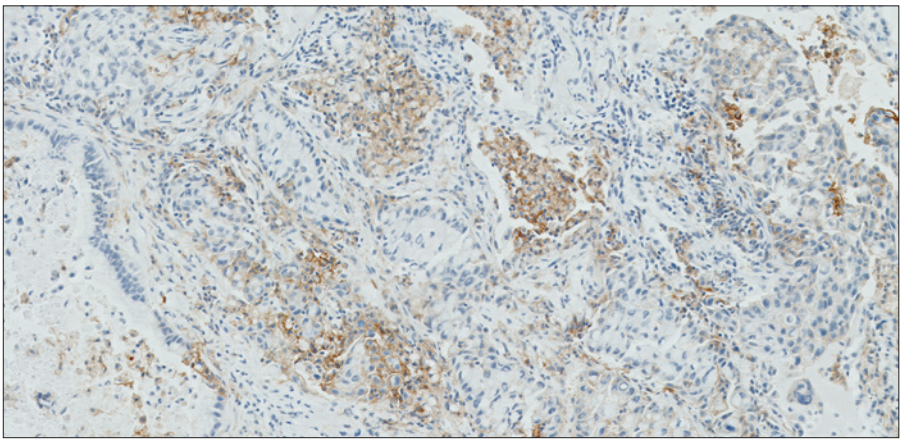


Figure 35a: 10× magnification.

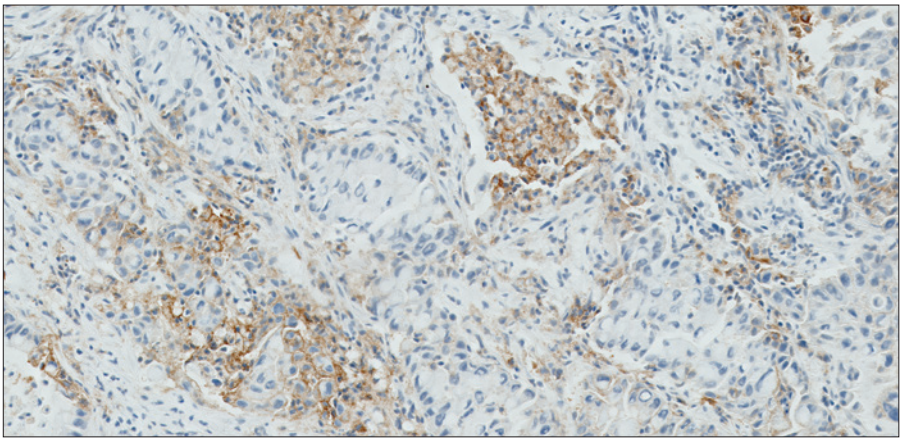


Figure 35b: 20× magnification.

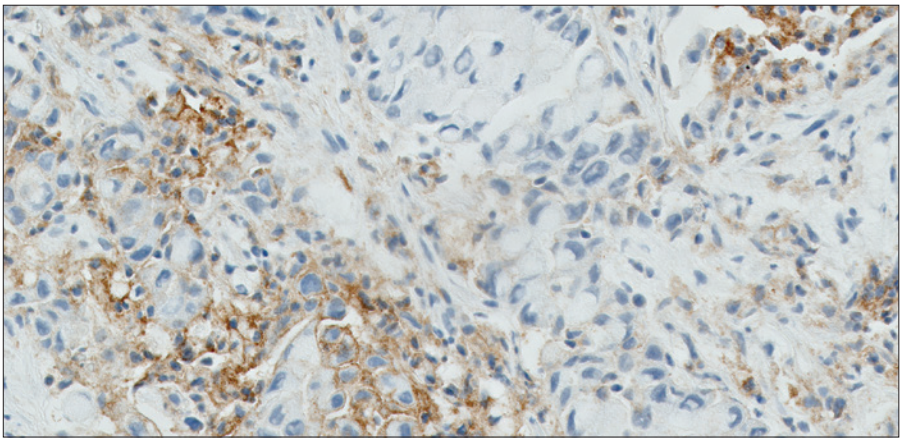


Figure 35c: 40× magnification.

Figure 35a–35c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–10%.

PD-L1 IHC 22C3 pharmDx, Code SK006 TPS 1–49% Case Examples

Case 5: TPS 1–49%

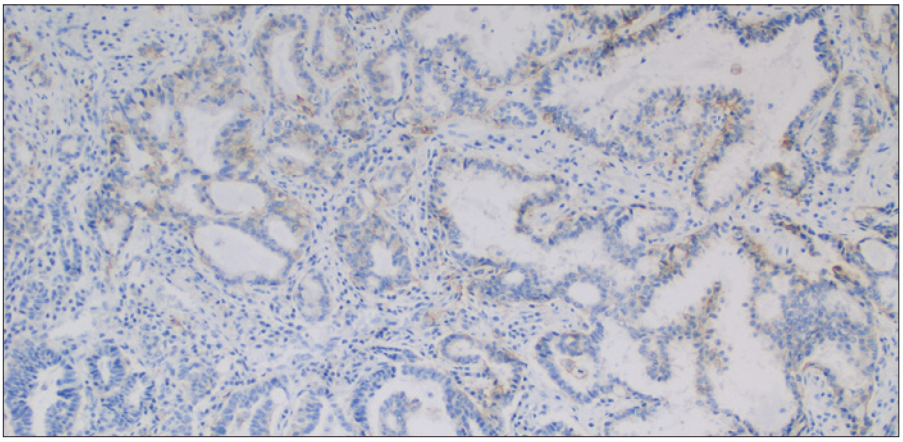


Figure 36a: 10× magnification.

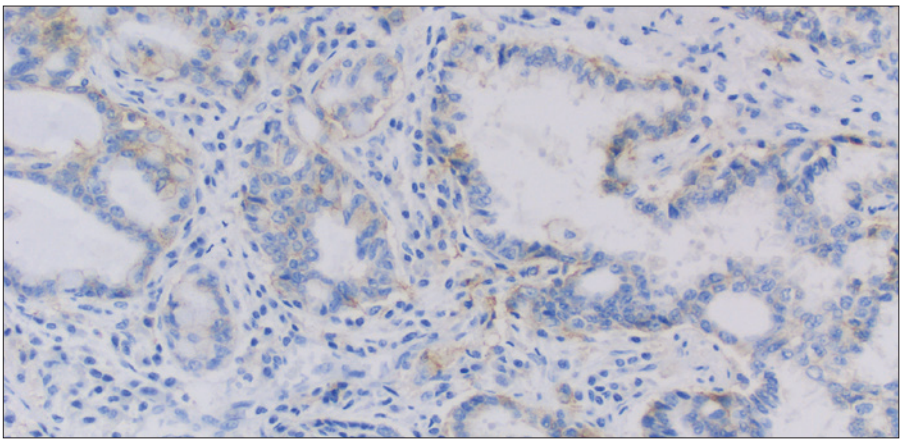


Figure 36b: 20× magnification.

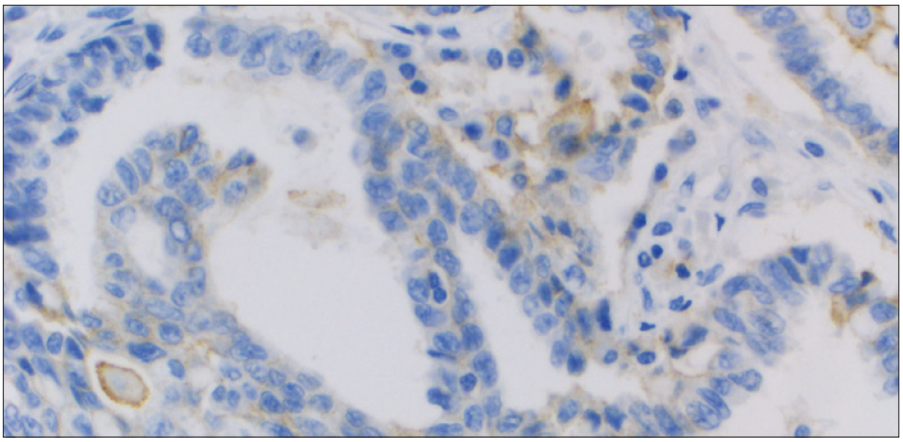


Figure 36c: 40× magnification.

Figure 36a–36c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–49%.

Case 6: TPS 1–49%

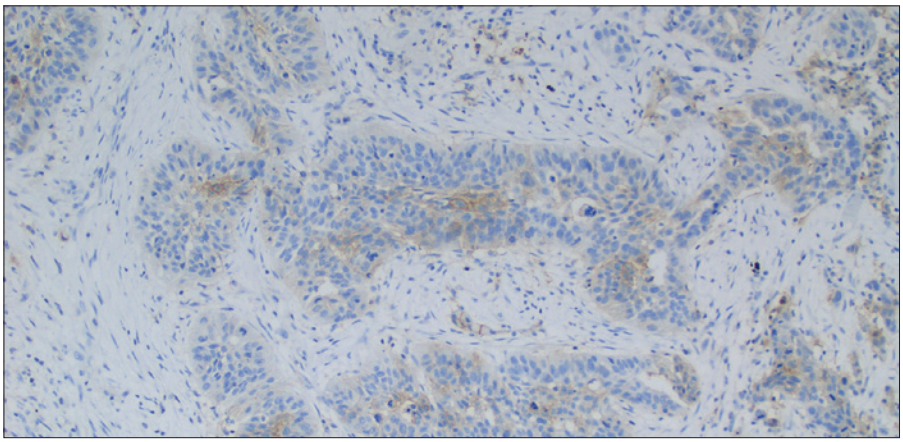


Figure 37a: 10× magnification.

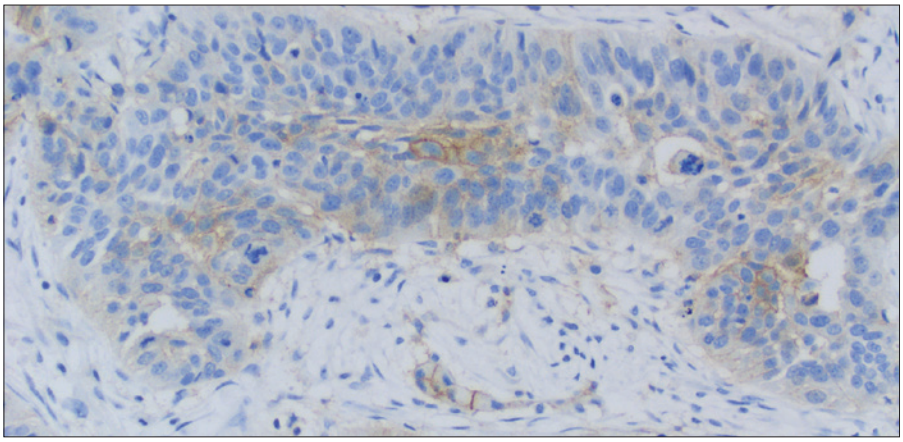


Figure 37b: 20× magnification.

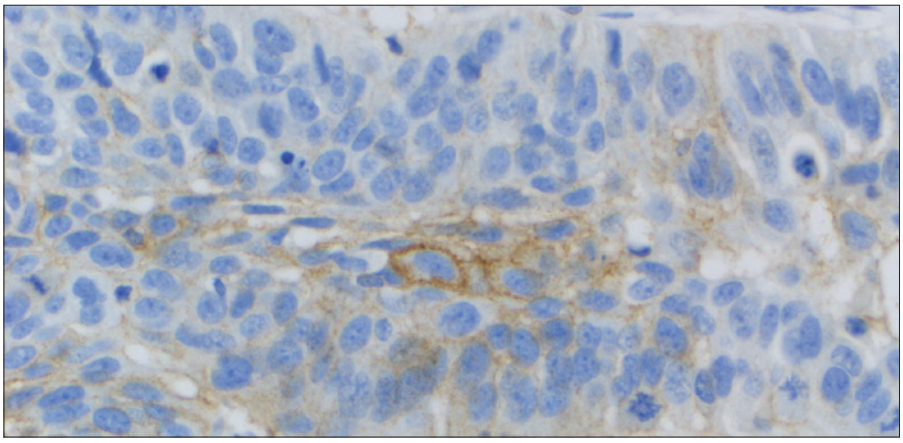


Figure 37c: 40× magnification.

Figure 37a–37c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–49%.

Case 7: TPS 1–49%

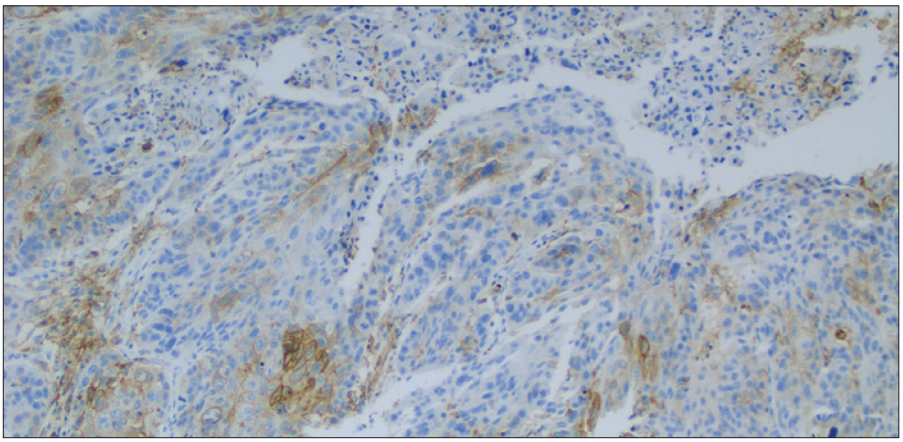


Figure 38a: 10× magnification.

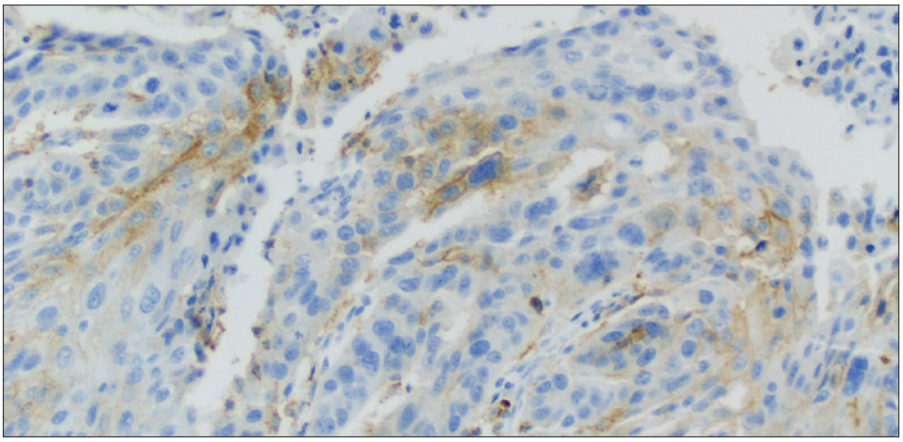


Figure 38b: 20× magnification.

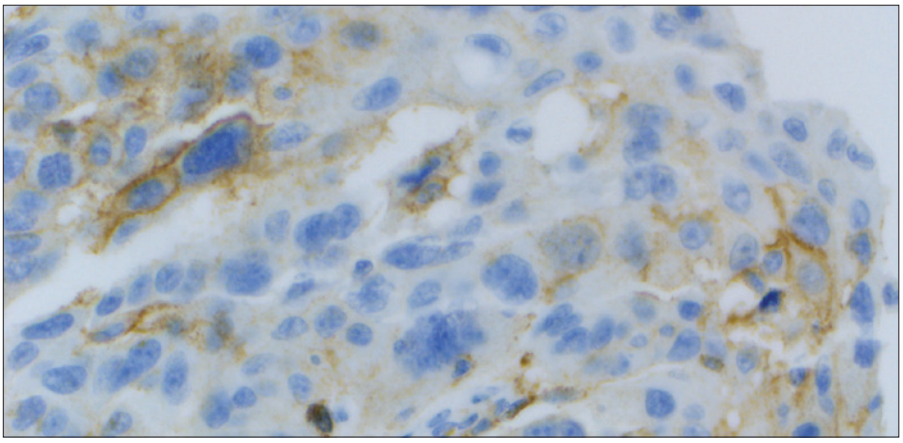


Figure 38c: 40× magnification.

Figure 38a–38c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–49%.

Case 8: TPS 1–49%

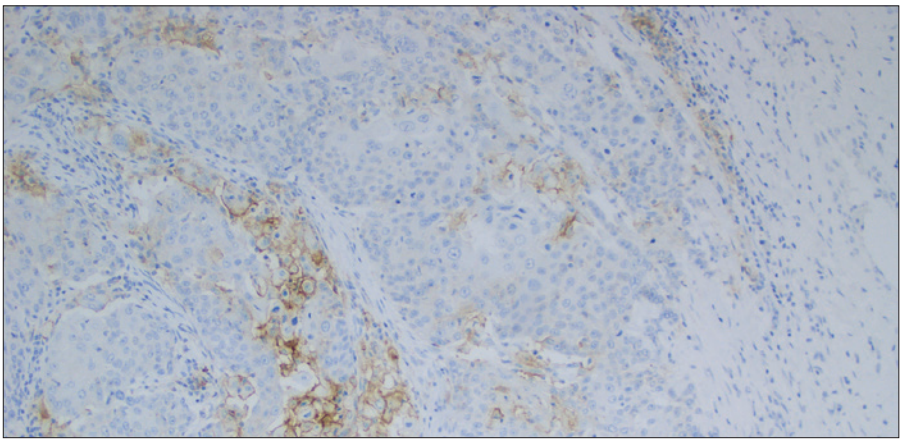


Figure 39a: 10× magnification.

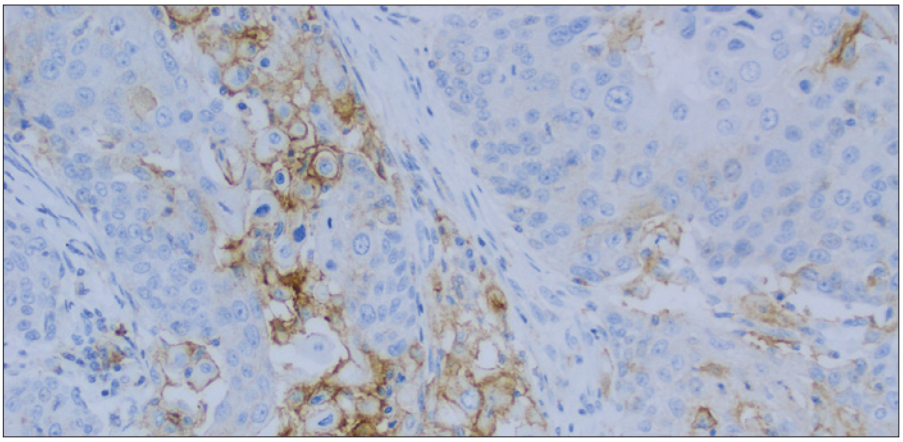


Figure 39b: 20× magnification.

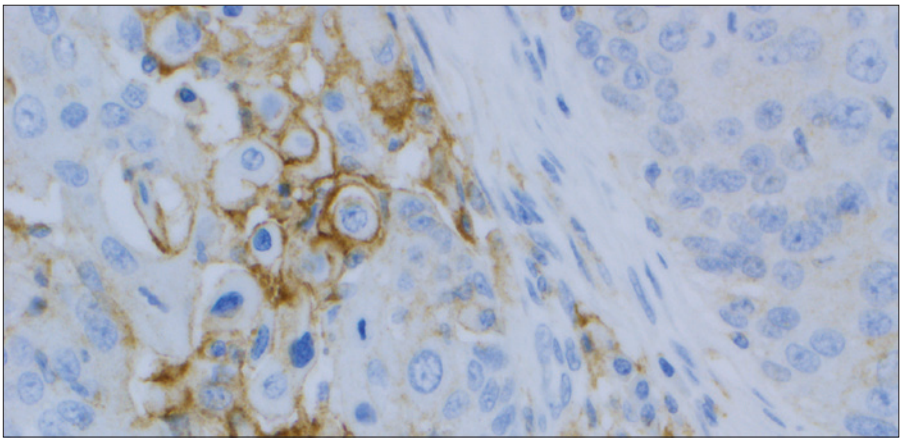


Figure 39c: 40× magnification.

Figure 39a–39c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 1–49%.

PD-L1 IHC 22C3 pharmDx, Code SK006 TPS ≥ 50% Case Examples

Case 9: TPS ≥ 50%

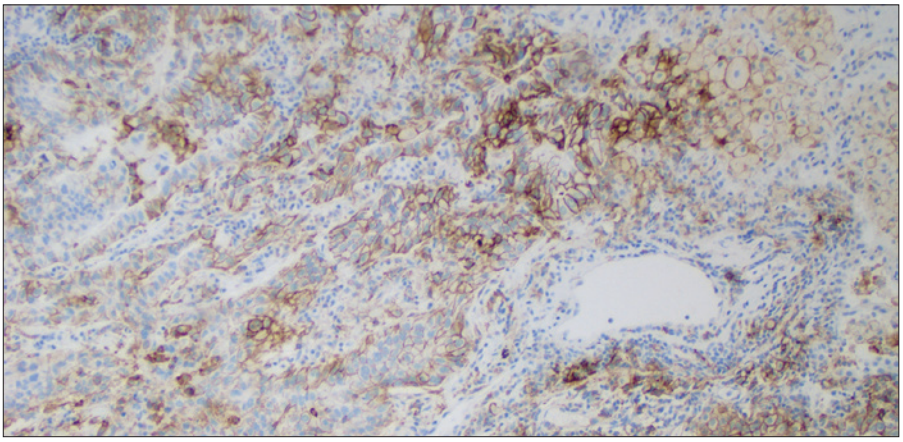


Figure 40a: 10× magnification.

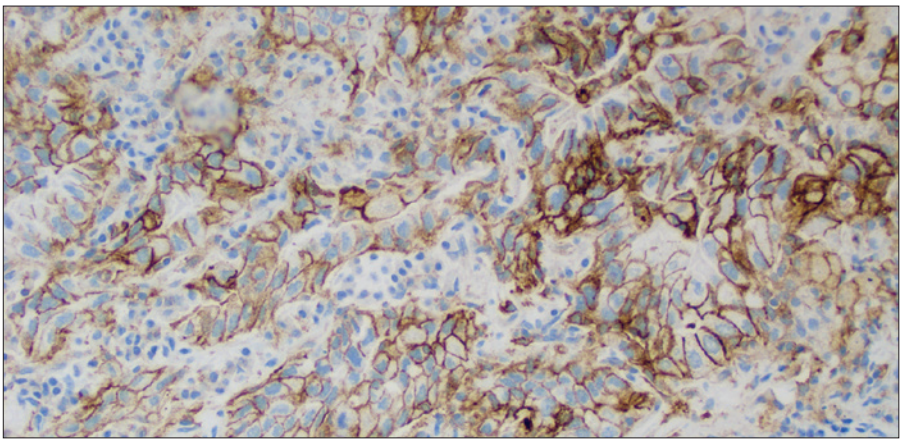


Figure 40b: 20× magnification.

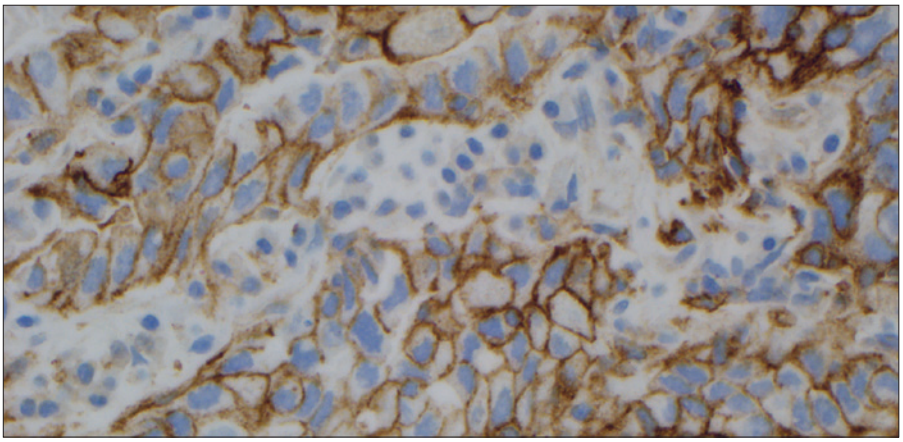


Figure 40c: 40× magnification.

Figure 40a–40c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 10: TPS ≥ 50%

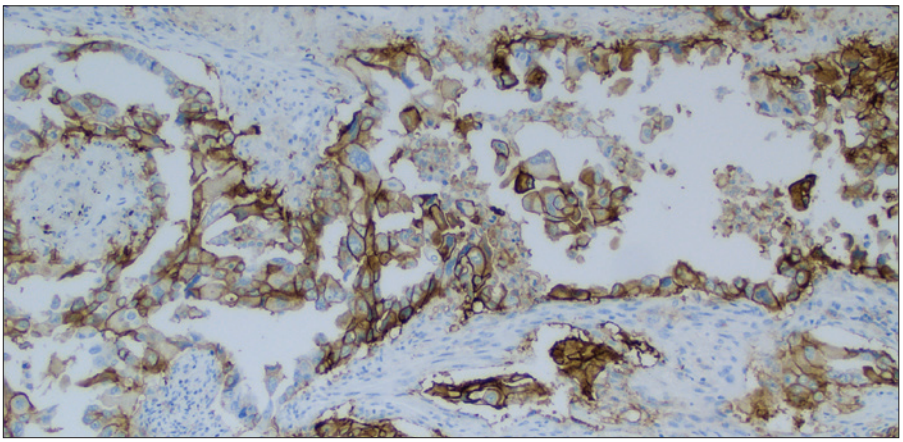


Figure 41a: 10× magnification.

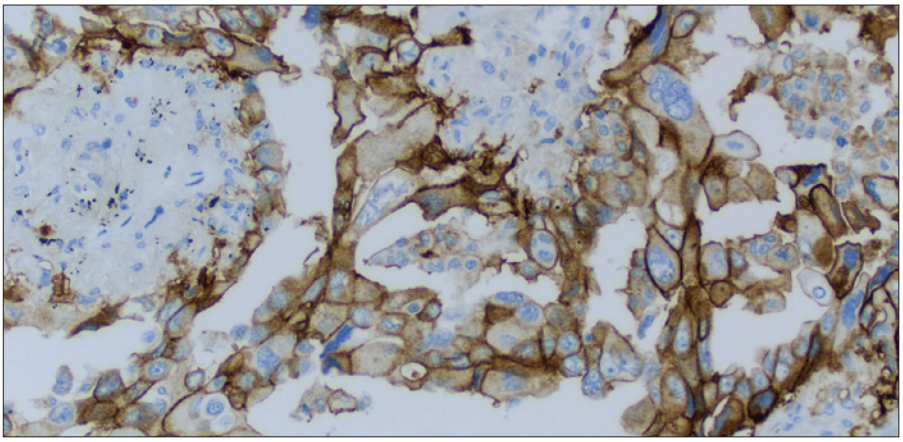


Figure 41b: 20× magnification.

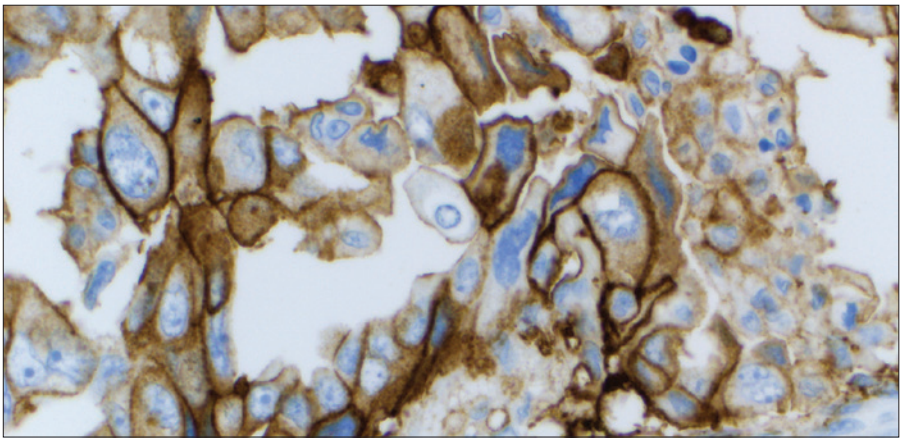


Figure 41c: 40× magnification.

Figure 41a–41c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 11: TPS ≥ 50%

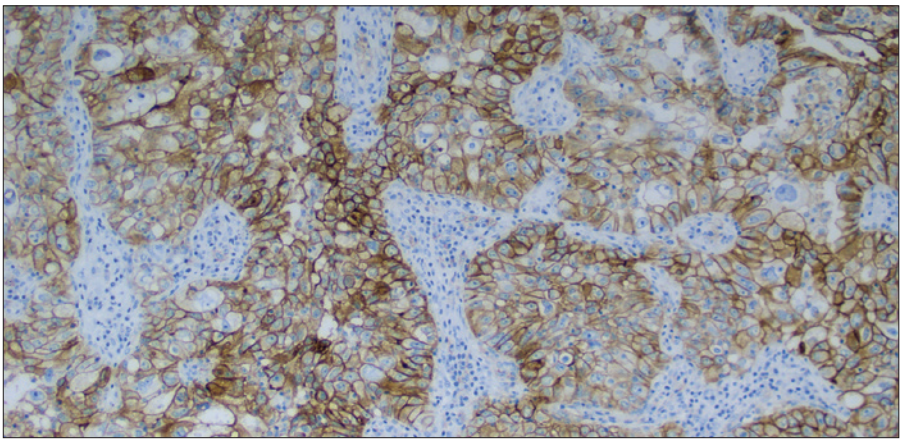


Figure 42a: 10× magnification.

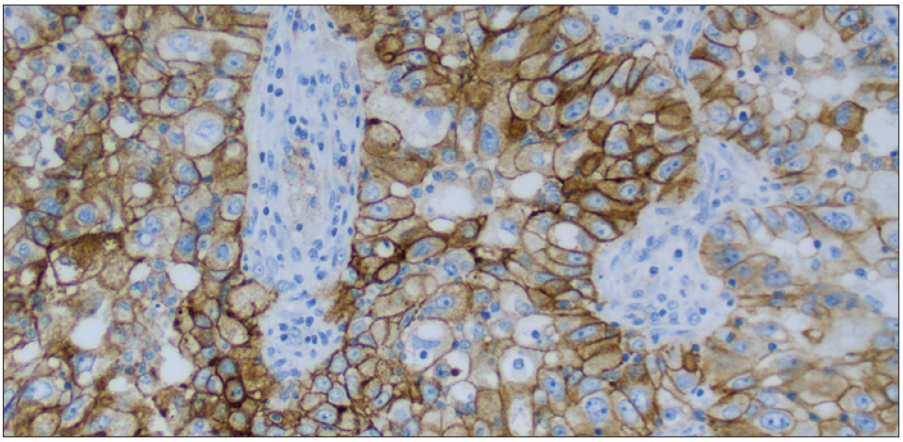


Figure 42b: 20× magnification.

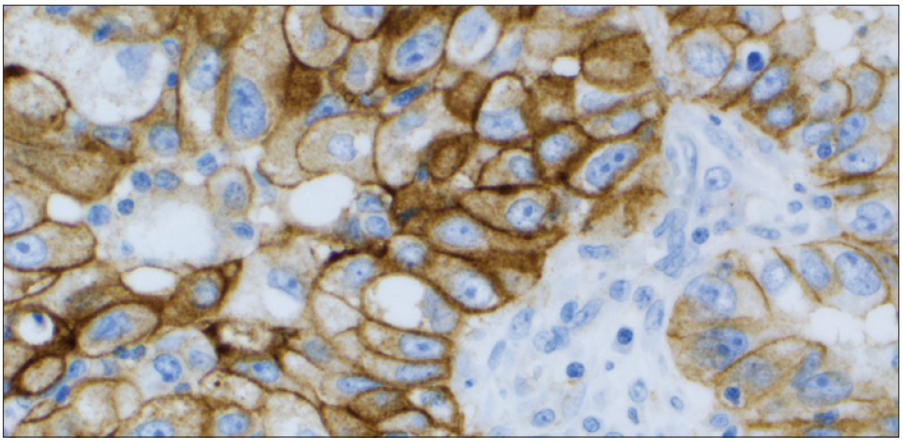


Figure 42c: 40× magnification.

Figure 42a–42c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 12: TPS ≥ 50%

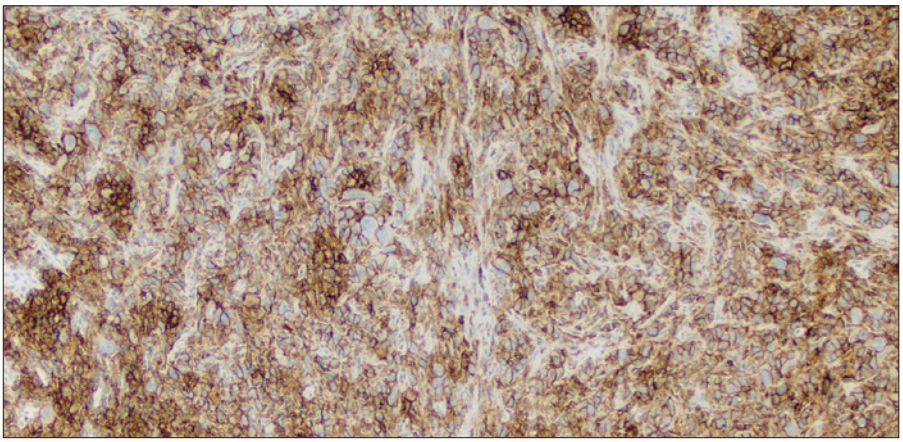


Figure 43a: 10× magnification.

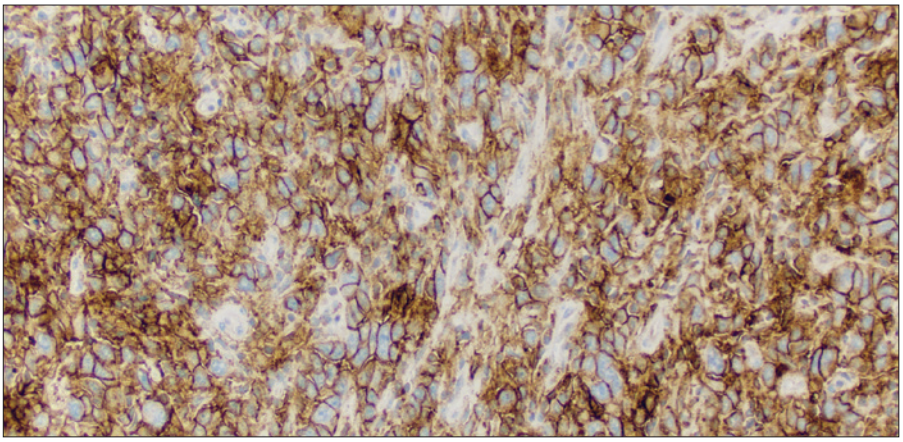


Figure 43b: 20× magnification.

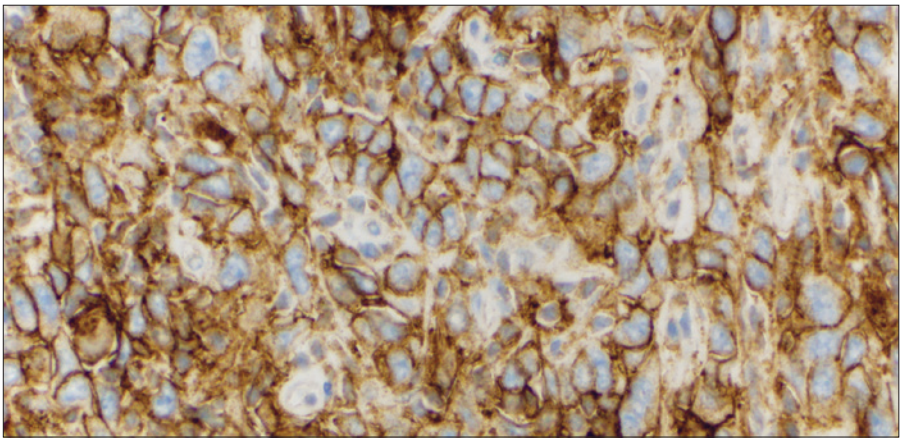


Figure 43c: 40× magnification.

Figure 43a–43c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 13: TPS ≥ 50%

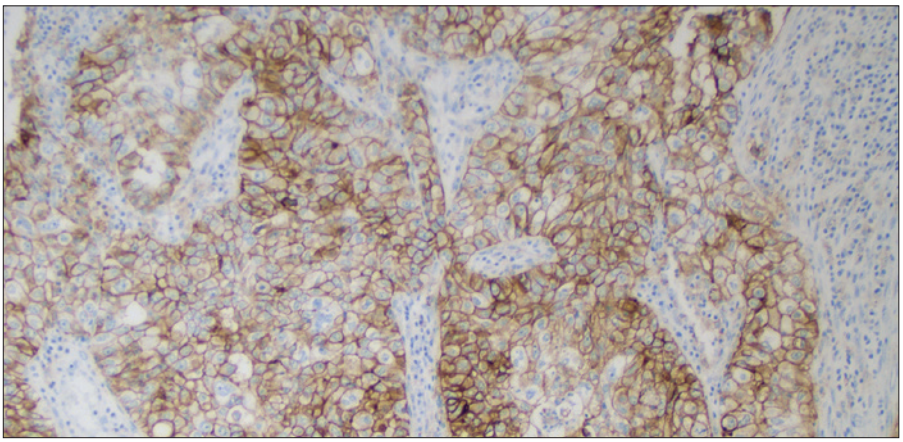


Figure 44a: 10× magnification.

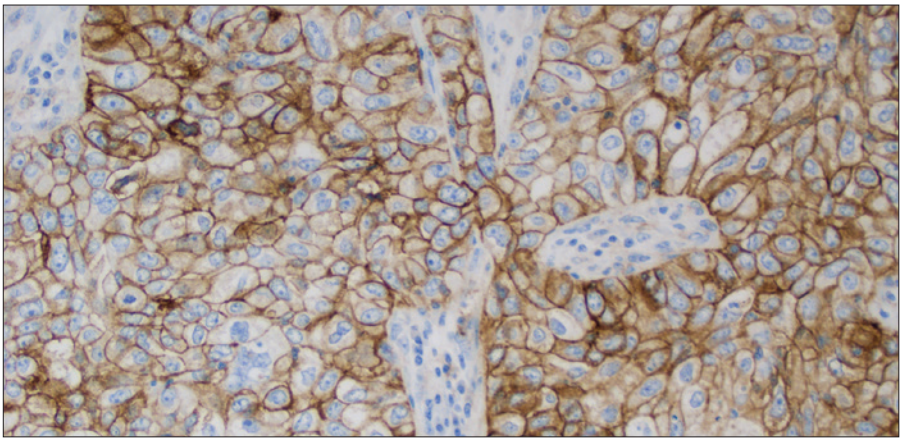


Figure 44b: 20× magnification.

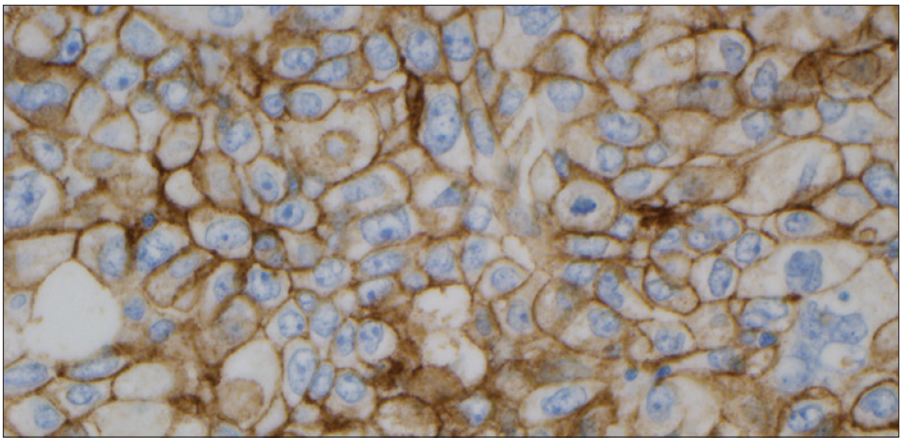


Figure 44c: 40× magnification.

Figure 44a–44c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 14: TPS ≥ 50%

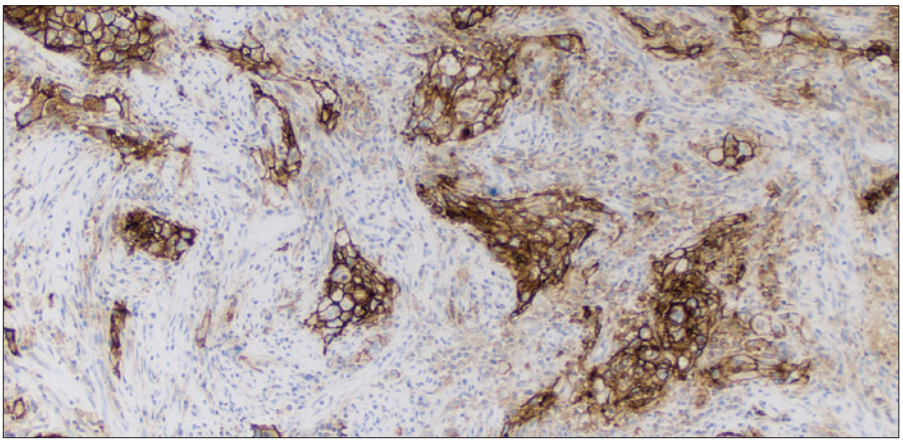


Figure 45a: 10× magnification.

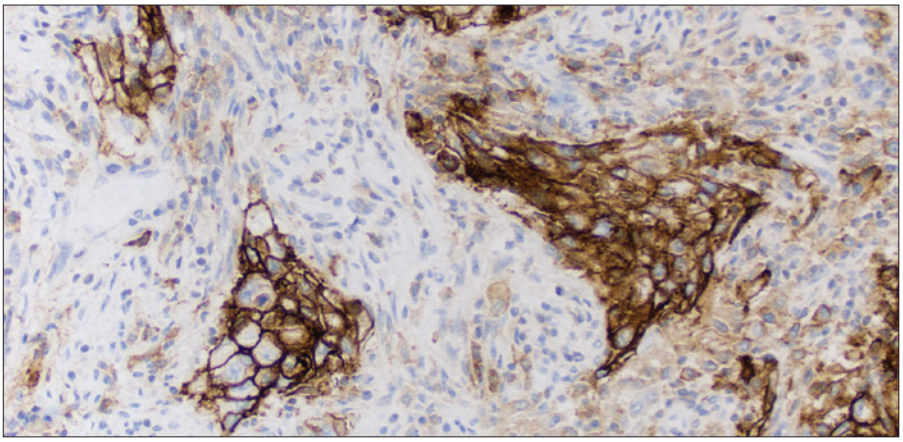


Figure 45b: 20× magnification.

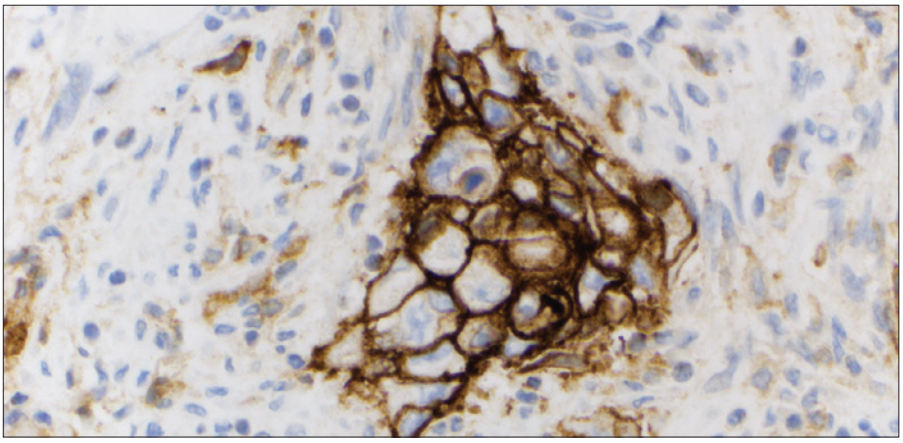


Figure 45c: 40× magnification.

Figure 45a–45c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

Case 15: TPS ≥ 50%

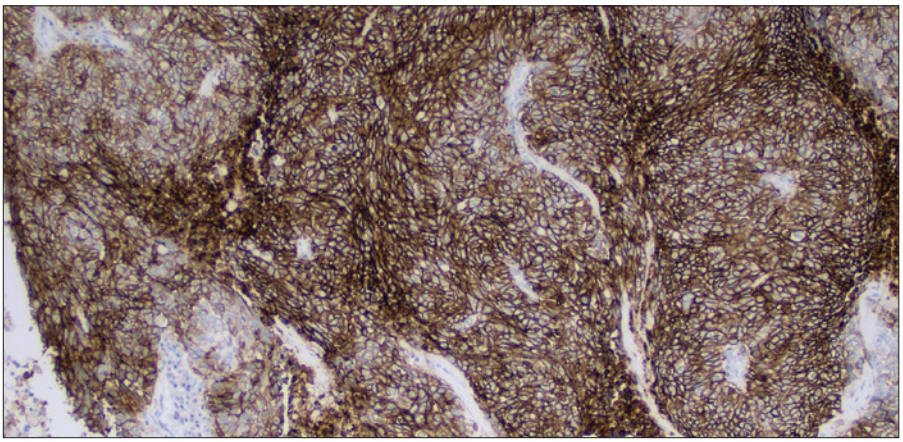


Figure 46a: 10× magnification.

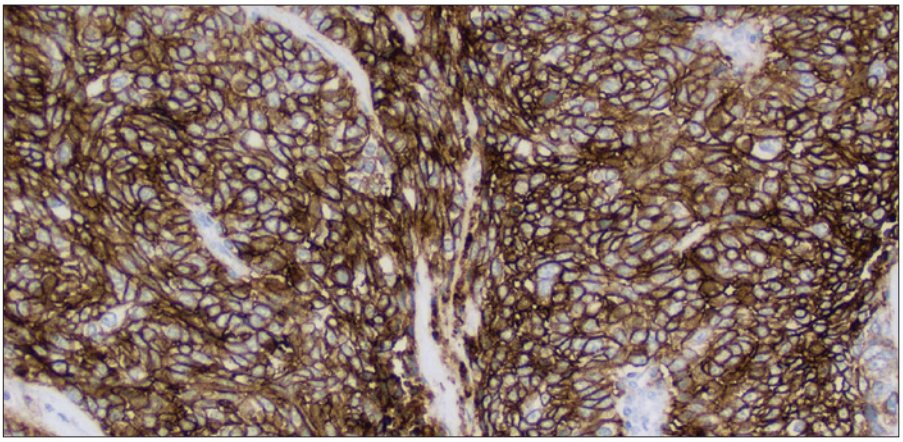


Figure 46b: 20× magnification.

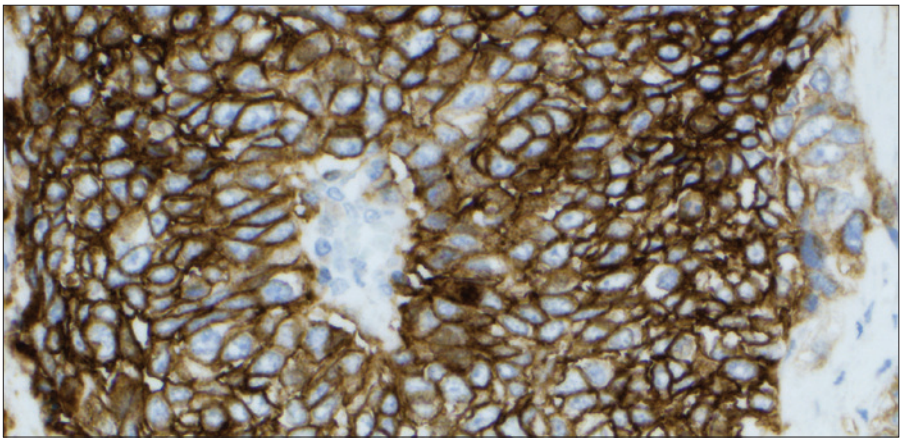


Figure 46c: 40× magnification.

Figure 46a–46c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS ≥ 50%.

PD-L1 IHC 22C3 pharmDx, Code SK006
TPS 40–60% Case Examples

Challenging Case 6:
TPS 40–60%

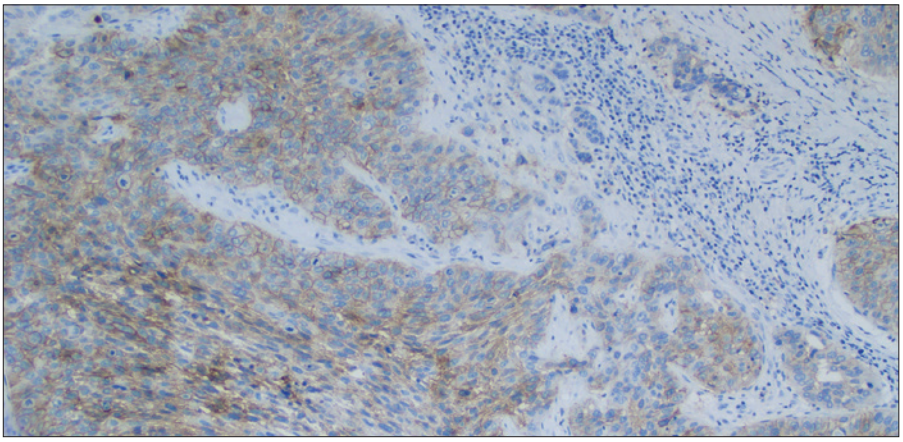


Figure 47a: 10× magnification.

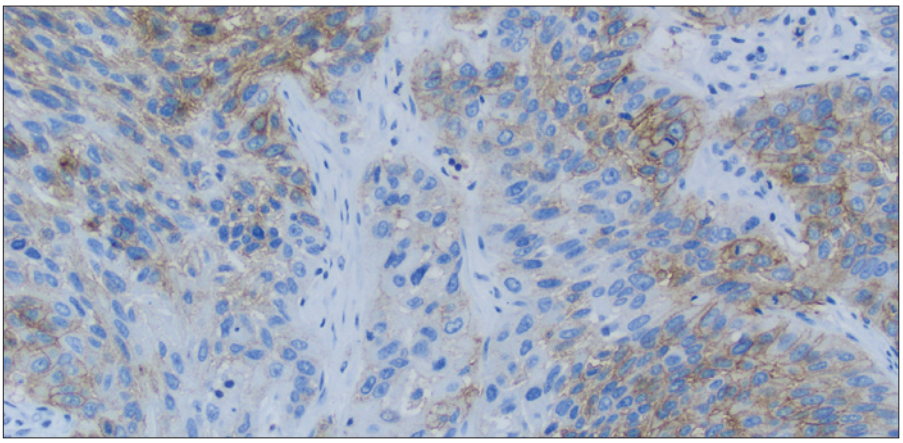


Figure 47b: 20× magnification.

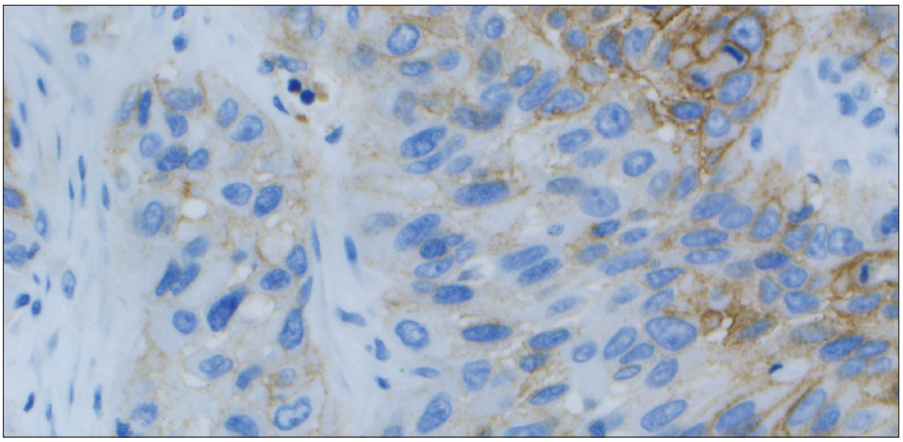


Figure 47c: 40× magnification.

Figure 47a–47c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 40%.

Challenging Case 7: TPS 40–60%

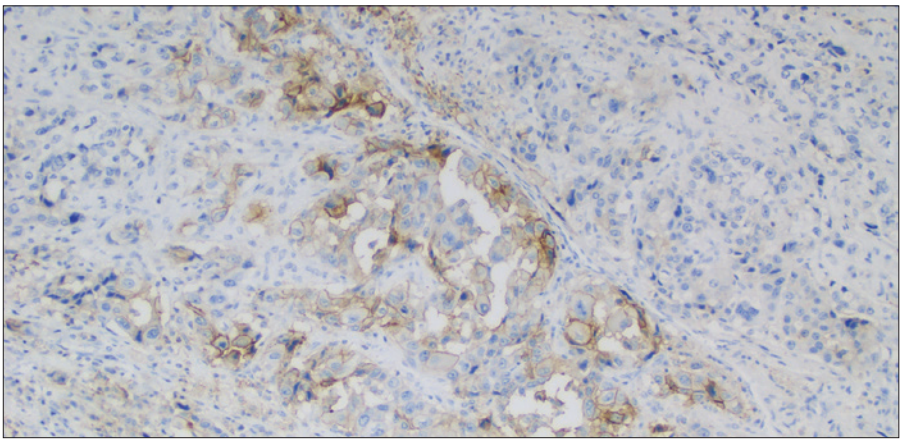


Figure 48a: 10× magnification.

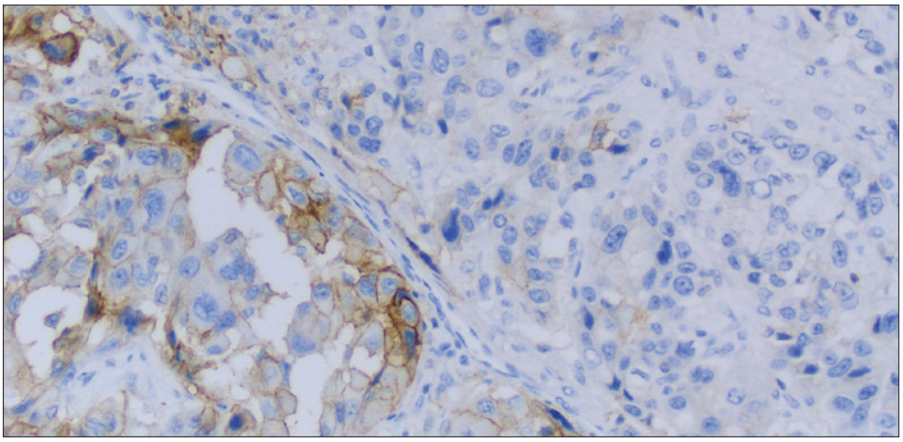


Figure 48b: 20× magnification.

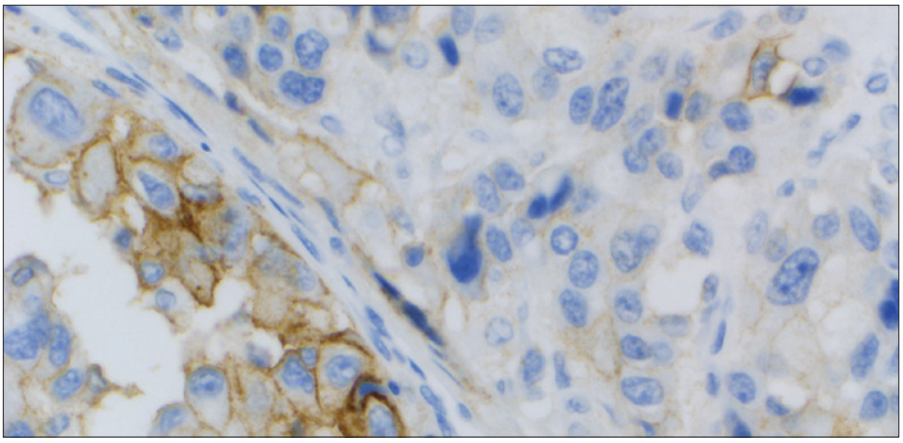


Figure 48c: 40× magnification.

Figure 48a–48c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 40%.

Challenging Case 8: TPS 40–60%

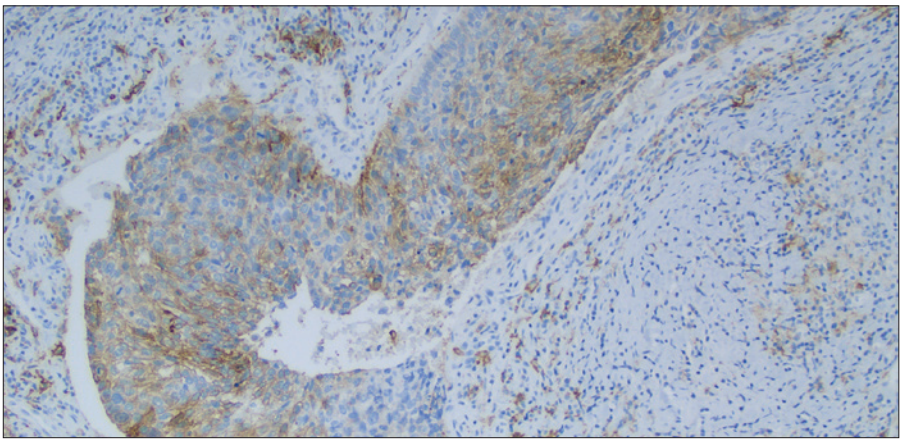


Figure 49a: 10× magnification.

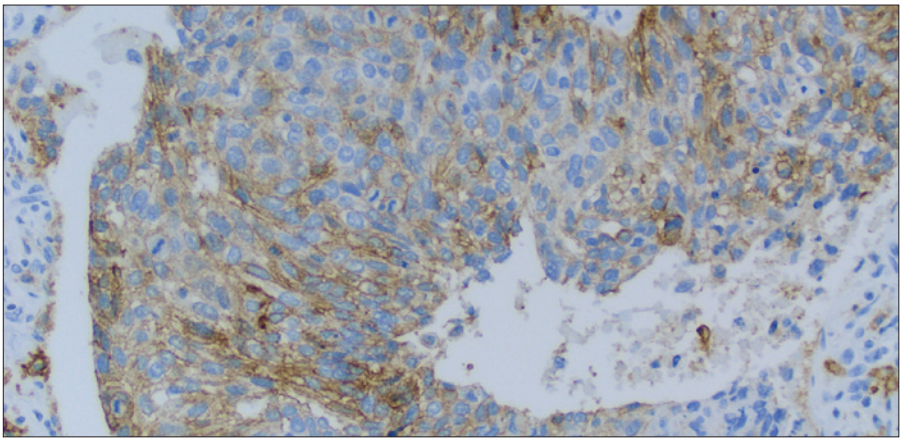


Figure 49b: 20× magnification.

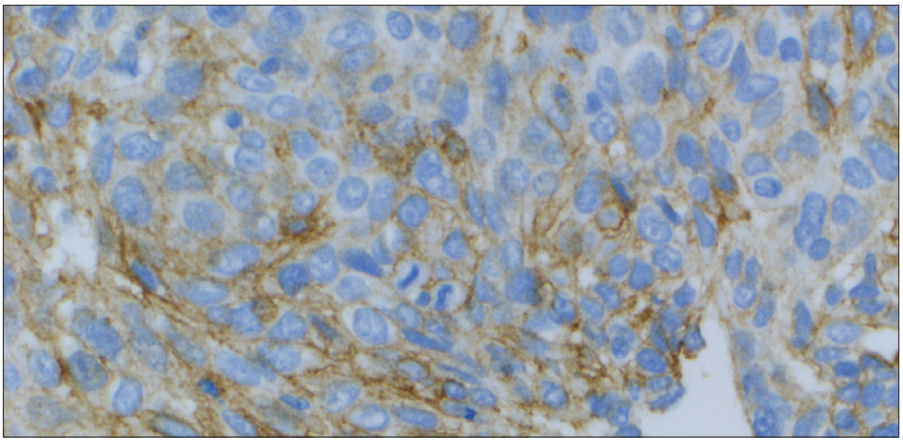


Figure 49c: 40× magnification.

Figure 49a–49c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 50%.

Challenging Case 9: TPS 40–60%

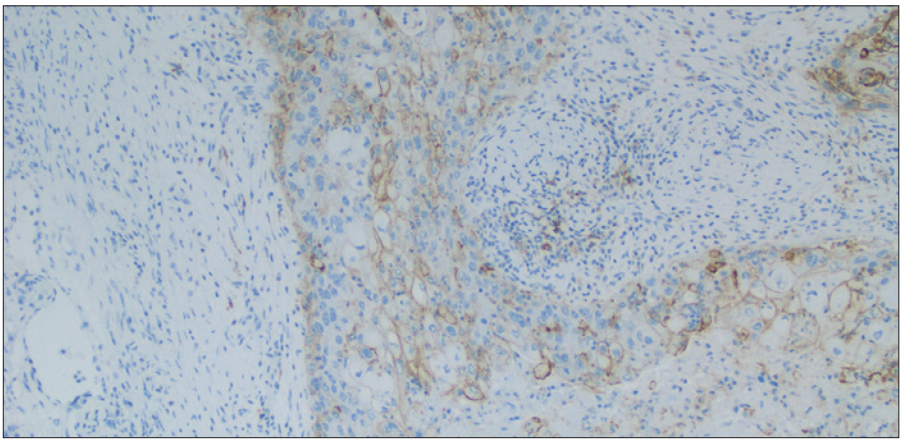


Figure 50a: 10× magnification.

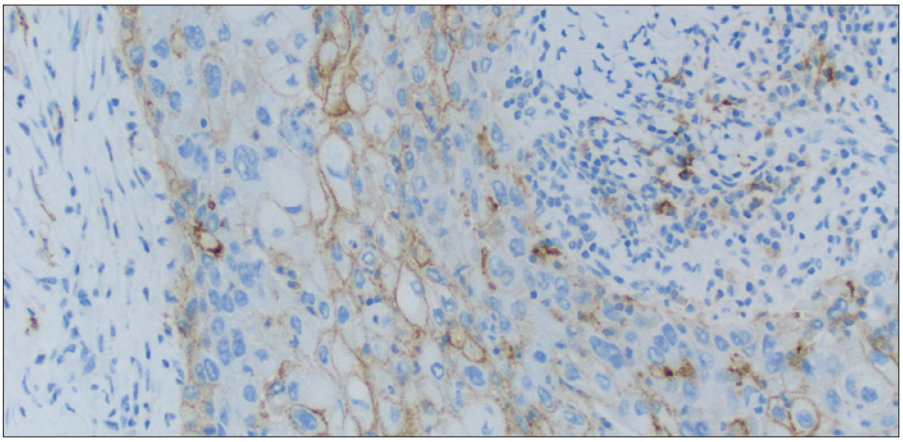


Figure 50b: 20× magnification.

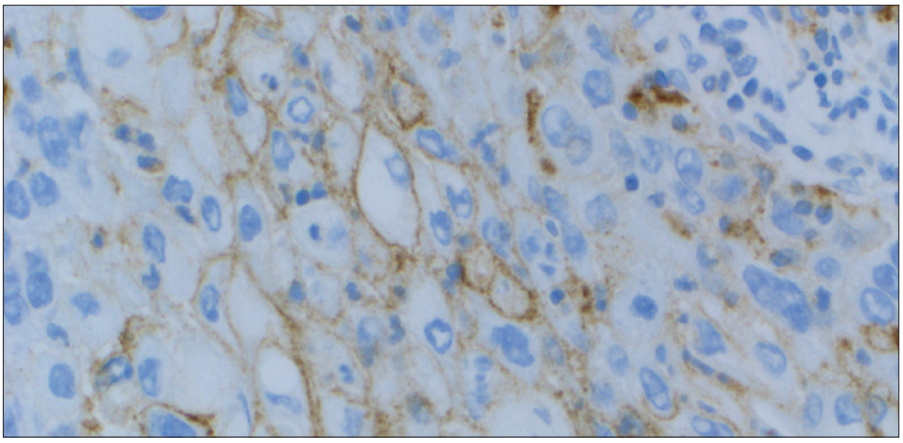


Figure 50c: 40× magnification.

Figure 50a–50c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 60%.

Challenging Case 10: TPS 40–60%

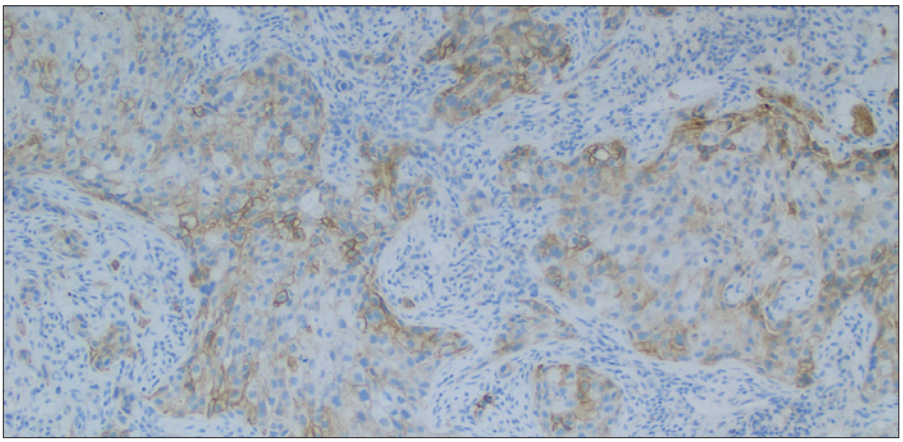


Figure 51a: 10× magnification.

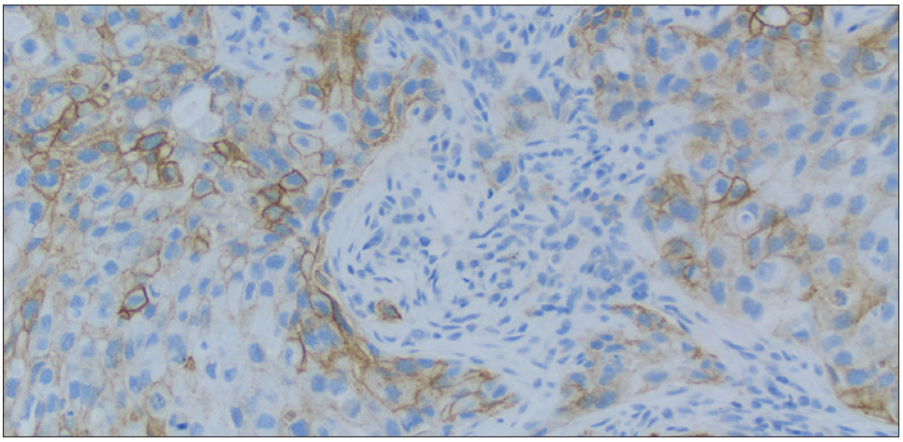


Figure 51b: 20× magnification.

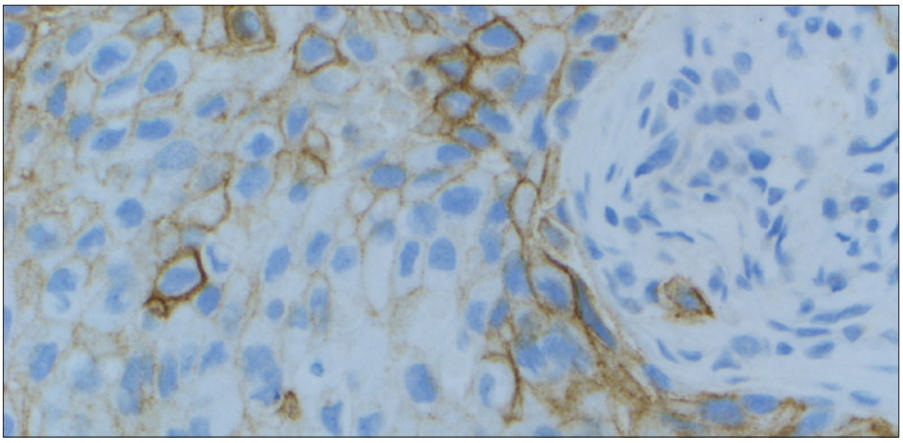


Figure 51c: 40× magnification.

Figure 51a–51c: NSCLC specimen stained with PD-L1 Code SK006 exhibiting TPS 60%.

PD-L1 IHC 22C3 pharmDx, Code GE006 TPS < 1% Case Example

Case 16: TPS < 1%

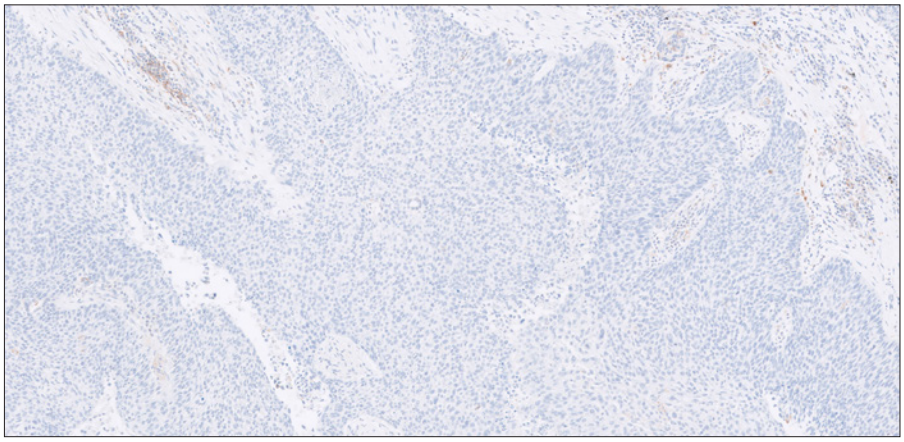


Figure 52a: 10× magnification.

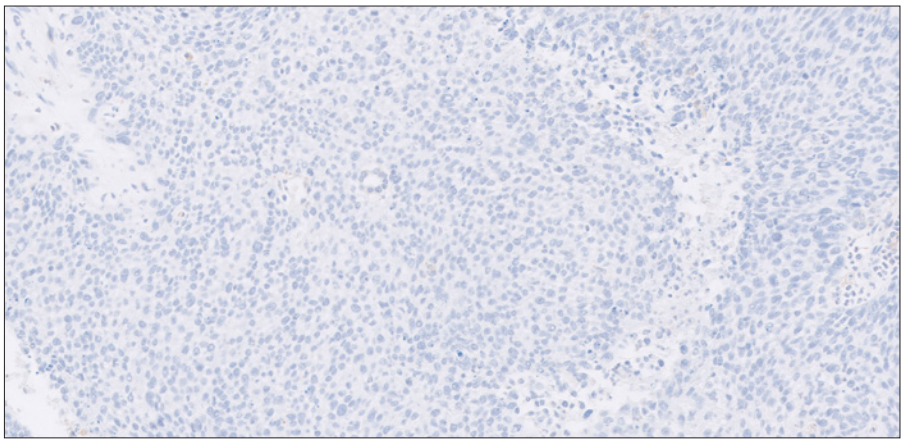


Figure 52b: 20× magnification.

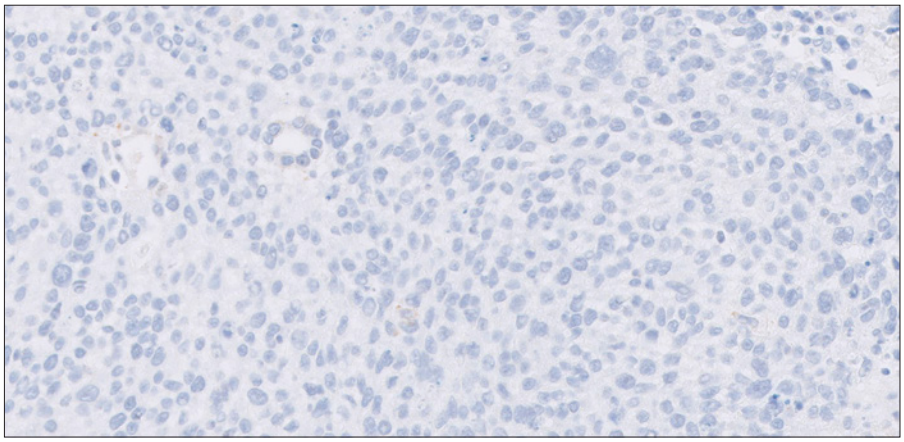


Figure 52c: 40× magnification.

Figure 52a–52: NSCLC specimen stained with PD-L1 Code GE006 exhibiting TPS < 1%.

PD-L1 IHC 22C3 pharmDx, Code GE006
TPS 1-49% Case Example

Case 17: TPS 1 - 49%

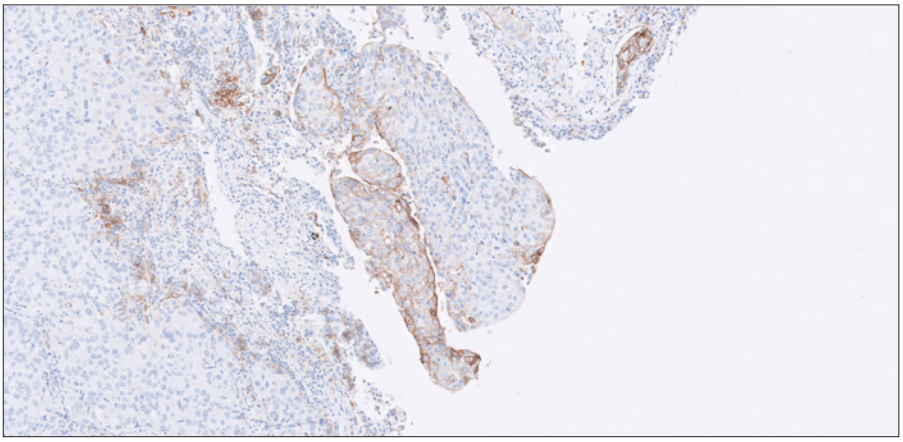


Figure 53a: 10× magnification.

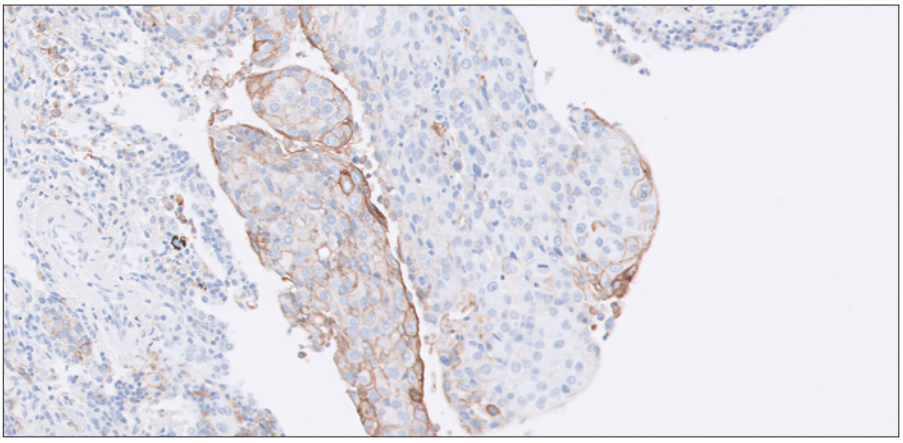


Figure 53b: 20× magnification.

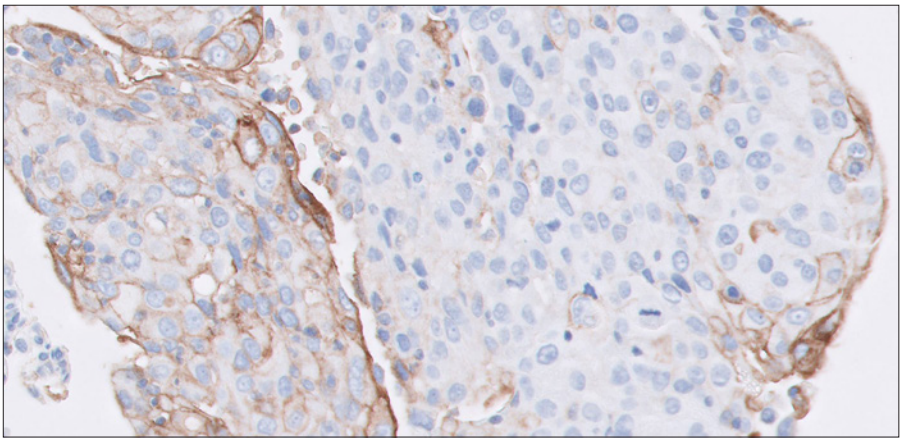


Figure 53c: 40× magnification.

Figure 53a–53c: NSCLC specimen stained with PD-L1Code GE006 exhibiting TPS 1–49%.

PD-L1 IHC 22C3 pharmDx, Code GE006 TPS ≥ 50% Case Example

Case 18: TPS ≥ 50%

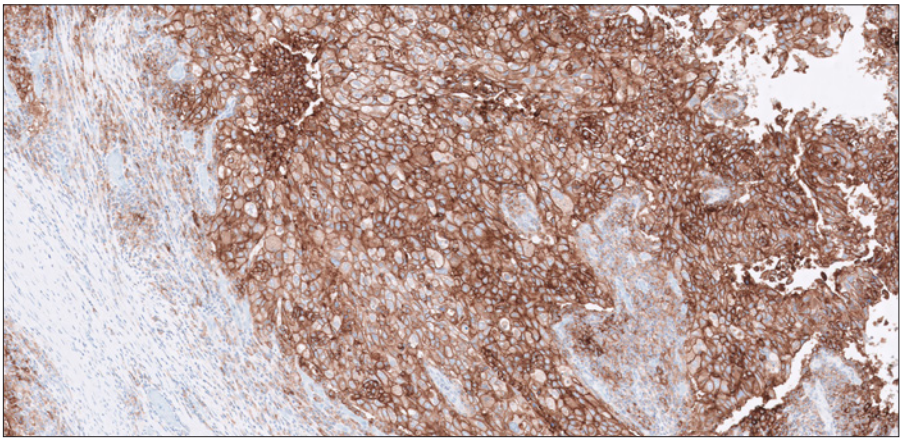


Figure 54a: 10× magnification.

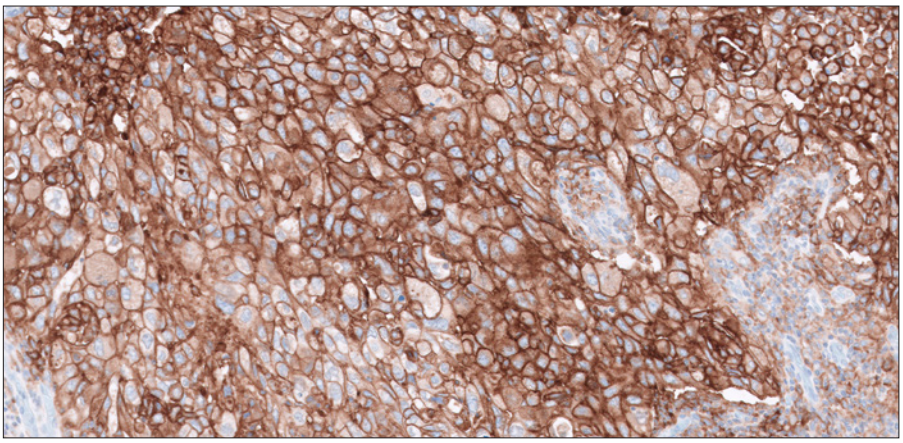


Figure 54b: 20× magnification.

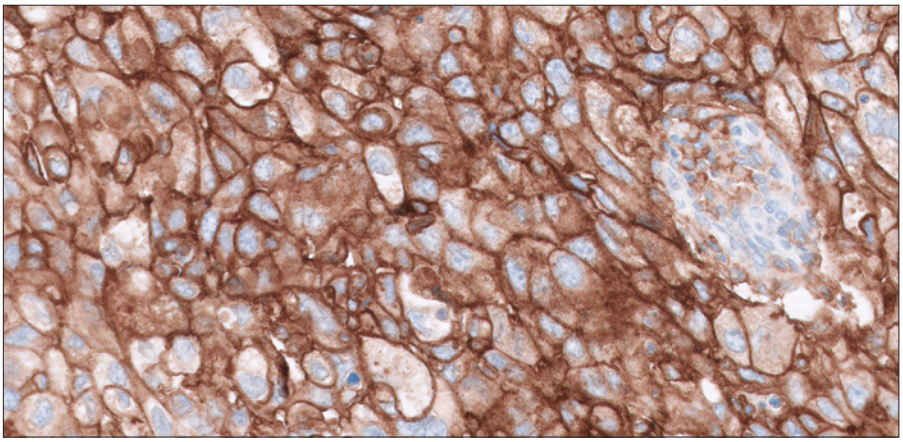


Figure 54c: 40× magnification.

Figure 54a–54c: NSCLC specimen stained with PD-L1 Code GE006 exhibiting TPS ≥ 50%.

Artifacts

The following pages provide examples of artifacts you may see when staining with PD-L1 IHC 22C3 pharmDx.

Non-specific Background Staining

Background staining is defined as diffuse, non-specific staining of a specimen. It is caused by several factors. These factors include, but are not limited to:

- Pre-analytic fixation and processing of the specimen
- Incomplete removal of paraffin from the section
- Incomplete rinsing of slides during staining
- Drying of slides; ensure slides remain wet with buffer while loading onto Autostainer Link 48 and prior to initiating run (SK006 only)
- Improper deparaffinization procedure
- Incomplete rinsing of reagents from slides

The non-specific background staining of the NCR-stained test specimen is useful in determining the level of background staining in the positive test specimen. All specimens must have ≤ 1+ non-specific background staining.

The use of fixatives other than neutral buffered formalin may be a source of background staining and is not recommended. Background staining with PD-L1 IHC 22C3 pharmDx is rare.

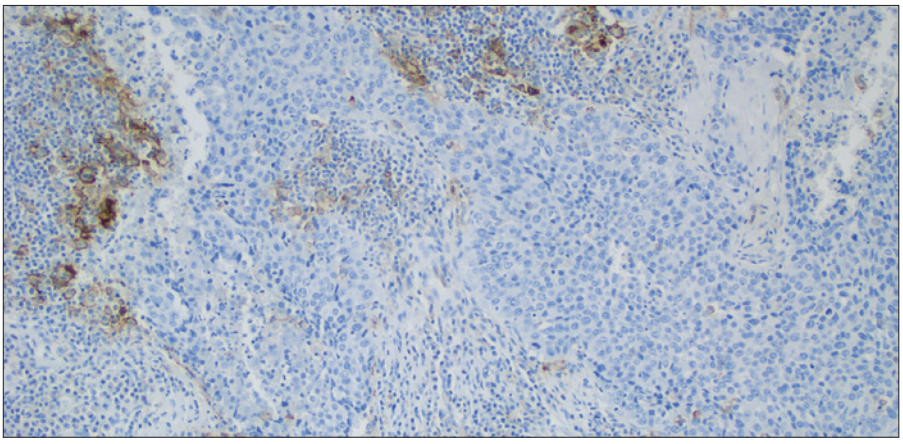


Figure 52: NSCLC specimen stained with PD-L1 Code SK006 exhibiting acceptable non-specific background staining (20× magnification).

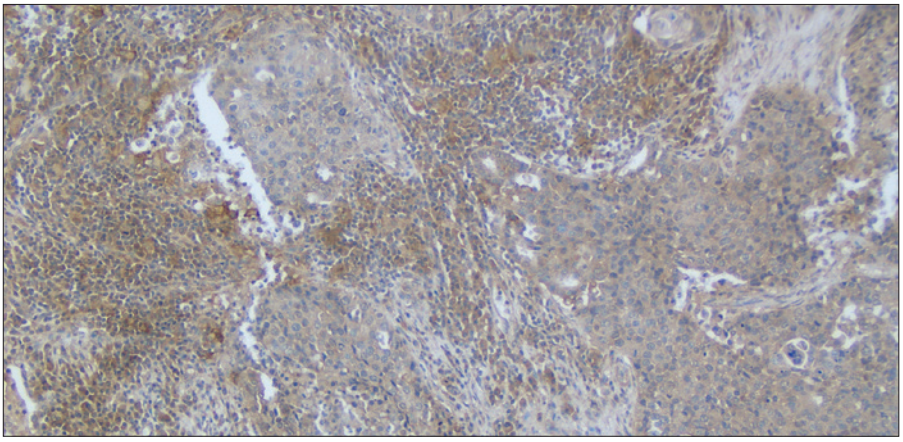


Figure 53: NSCLC specimen stained with PD-L1 Code SK006 exhibiting unacceptable non-specific background staining (> 1+) (20× magnification).

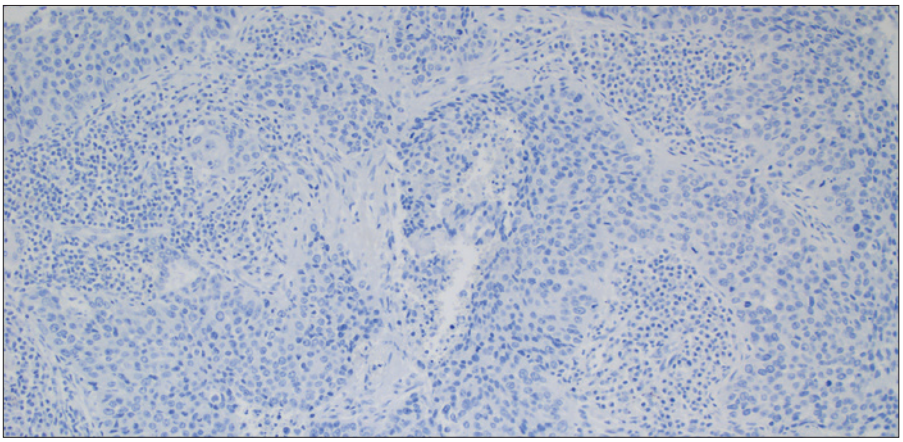


Figure 54: NSCLC specimen stained with NCR Code SK006 exhibiting acceptable non-specific background staining (20× magnification).

Key Point

All specimens must have ≤ 1+ non-specific background staining

Edge Artifact

Commonly, edge artifact is linked to the following pre-analytic factors:

- Thick tissue sections
- Drying of tissue prior to fixation or during staining procedure

Both factors can lead to accentuation of staining at the periphery of the section and minimal staining or non-staining in the central portion. Only PD-L1 staining at the edge of the tissue section is excluded from scoring.

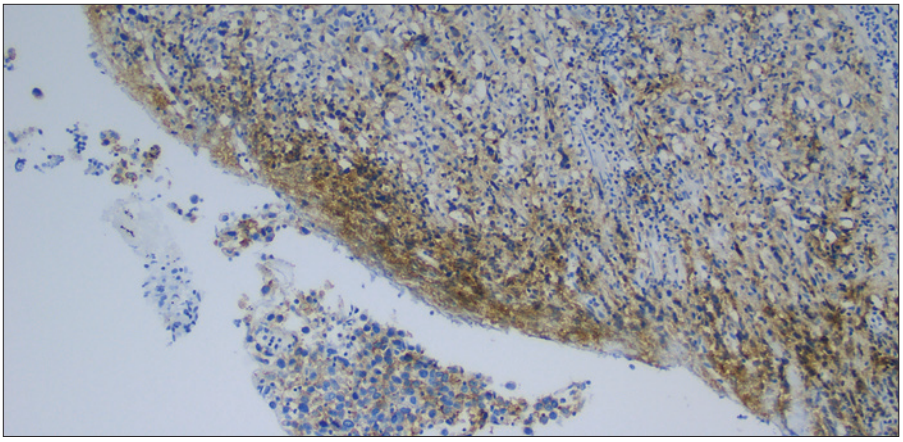


Figure 55a: NSCLC specimen stained with PD-L1 Code SK006 exhibiting edge artifact staining; edge staining should be excluded from the scoring (4× magnification).

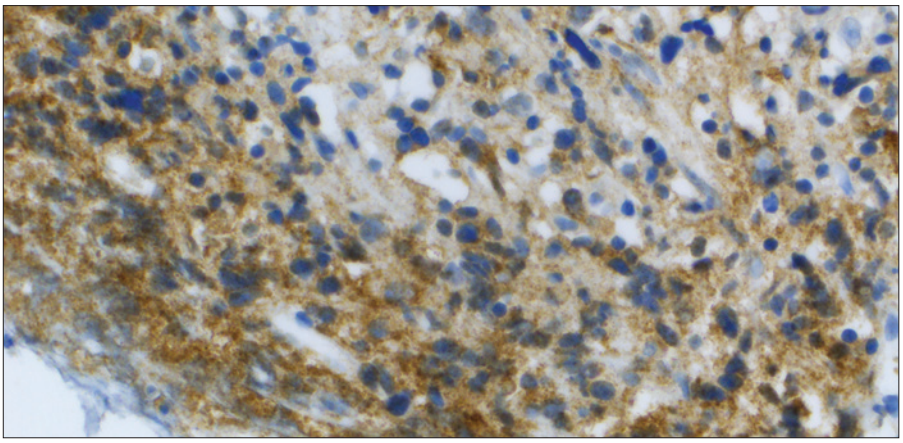


Figure 55b: NSCLC specimen stained with PD-L1 Code SK006 exhibiting edge artifact staining; edge staining should be excluded from the scoring (20× magnification).

Key Point

Scoring of the edge of a specimen should be avoided if staining is inconsistent with the rest of the specimen

Crush Artifact

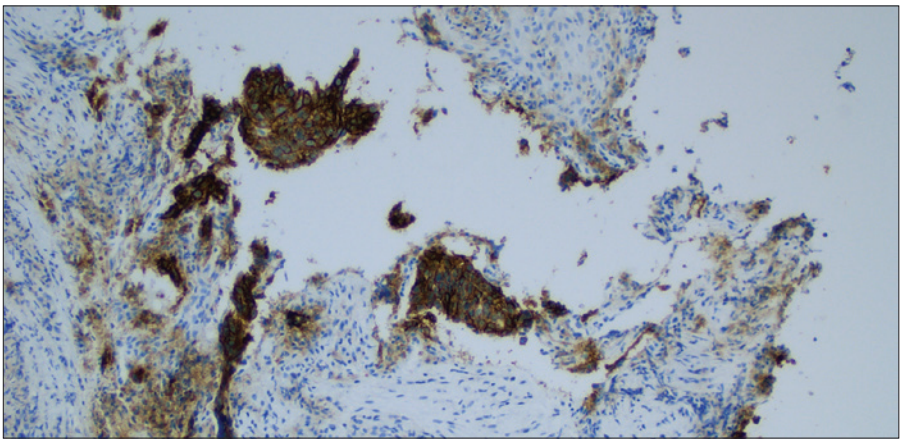


Figure 56: NSCLC specimen stained with PD-L1 Code SK006 exhibiting crush artifact (10× magnification).

Areas of the examined section exhibiting cytologically and morphologically distorted secondary crush artifact may show exaggerated staining and should be excluded from the score.

Key Point

Scoring of crush artifact should be avoided

Necrosis

Necrosis can be described as morphological changes indicative of cell death with undefined cellular detail. Necrosis is often present in NSCLC specimens and should be excluded from scoring.

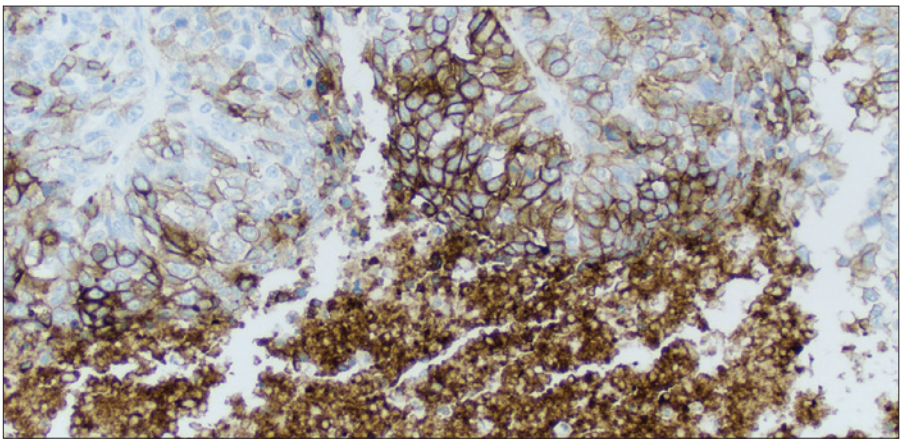


Figure 57: NSCLC specimen stained with PD-L1 Code SK006 exhibiting strong staining of necrosis and viable tumor cells; necrosis staining should be excluded from the scoring (20× magnification).

Key Point

Scoring of necrotic areas should be excluded from the TPS

Poor Fixation

Standardization of fixation is very important when using PD-L1 IHC 22C3 pharmDx. Sub-optimal fixation on tissues may give erroneous results.

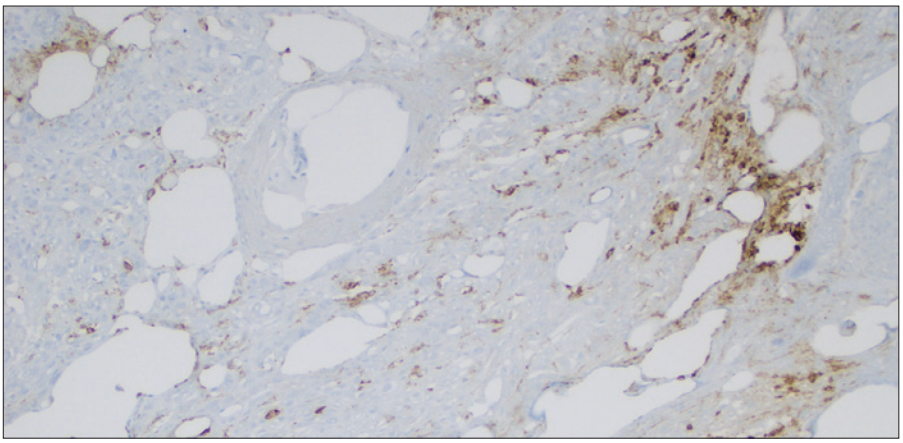


Figure 58: NSCLC specimen stained with PD-L1 Code SK006 exhibiting poor tissue fixation (10× magnification).

Key Point

Proper fixation is important for accurate diagnosis

Troubleshooting Guide

General Troubleshooting Guidelines for PD-L1 IHC 22C3 pharmDx

For assay-specific troubleshooting guidelines see the relevant product IFU. For further assistance, contact your local Agilent representative.

Problem	Probable Cause	Suggested Action
No staining of slides	Protocol error	Verify that the correct protocol was selected for programming of slides
	Lack of reaction with DAB+ Substrate-Chromogen Solution (DAB)	Verify that DAB+ Substrate-Chromogen Solution was prepared properly
	Sodium azide in wash buffer	Use only Dako Wash Buffer, see IFU
	Degradation of Control Slide	Check kit expiration date and kit storage conditions on outside of package
Weak staining of specimen slides	Inappropriate fixation method used	Ensure that only neutral buffered formalin fixative and approved fixation methods are used
	Insufficient reagent volume applied	Check size of tissue section and reagent volume applied
	Inappropriate wash buffer used	Use only Dako Wash Buffer, see IFU
Weak staining of specimen slides or of the positive cell line pellet on the Control Slide provided with the kit	Inadequate target retrieval	Verify that the 3-in-1 pre-treatment procedure was correctly performed
	Inappropriate wash buffer used	Use only Dako Wash Buffer, see IFU
Excessive background staining of slides	Paraffin incompletely removed	Verify that the 3-in-1 pre-treatment procedure was correctly performed
	Slides dried while loading onto instrument	Ensure slides remain wet with buffer while loading and prior to initiating run
	Nonspecific binding of reagents to tissue section	Check for proper fixation of the specimen and/or the presence of necrosis
	Inappropriate fixation method used	Ensure that only neutral buffered formalin fixative and recommended fixation methods are used
Tissue detached from slides	Use of incorrect microscope slides	Use Dako FLEX IHC Microscope Slides, (Code K8020), or Fisherbrand Superfrost Plus slides
	Inadequate preparation of specimens	Cut sections should be placed in a 58 ± 2 °C oven for 1 hour prior to staining
Excessively strong specific staining	Inappropriate fixation method used	Ensure that only approved fixatives and fixation methods are used
	Inappropriate wash buffer used	Only use Dako Wash Buffer, see IFU
Target Retrieval Solution is cloudy in appearance when heated	When heated the Target Retrieval Solution turns cloudy in appearance	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 (available from Agilent).

Clinical Performance Evaluation

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 PD-L1 IHC 22C3 pharmDx, 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.

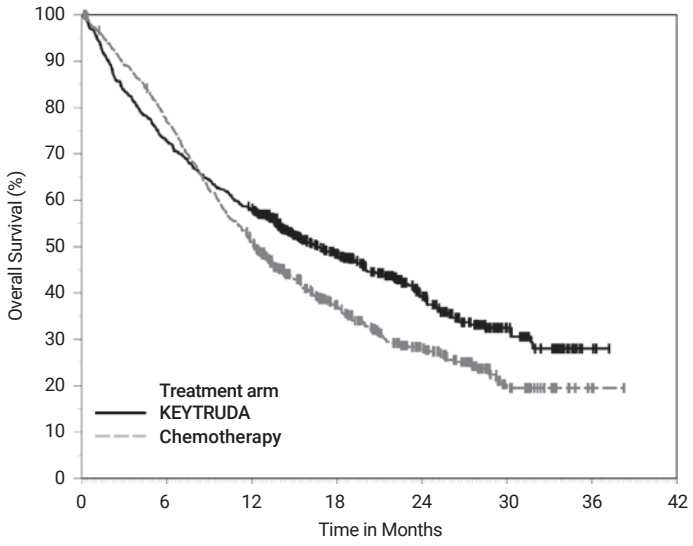
The trial demonstrated a statistically significant improvement in OS for patients randomized to KEYTRUDA as compared with chemotherapy. Table 4 and Figure 59 summarize the efficacy results in the subgroup of patients with TPS ≥ 50% and in all randomized patients with TPS ≥ 1%.

Table 4: 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).



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

Figure 59: Kaplan-Meier Curve for Overall Survival in all Randomized Patients in KEYNOTE-042 (TPS ≥ 1%)

KEYNOTE-024: Controlled Trial of First-line Treatment of Patients with NSCLC

The efficacy of KEYTRUDA was investigated in Trial 24, a randomized (1:1), open-label, multicenter, controlled trial. 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%).

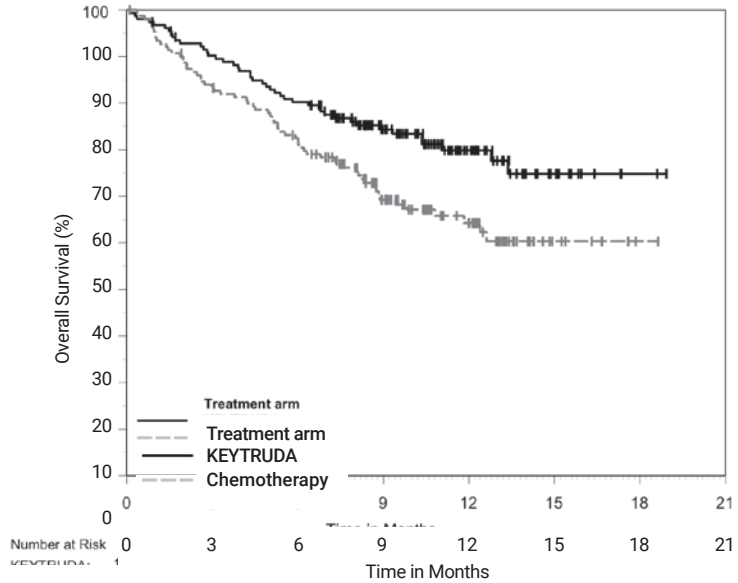
The major efficacy outcome measure was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST 1.1). Additional efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST 1.1. Table 5 summarizes key efficacy measures for the entire intent to treat (ITT) population.

Table 5: 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 60).



Number at Risk

KEYTRUDA:	154	136	121	82	39	11	2	0
Chemotherapy:	151	123	106	64	34	7	1	0

Figure 60: 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. 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 6.

Table 6: 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 7 and 8. 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 61. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 7: 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

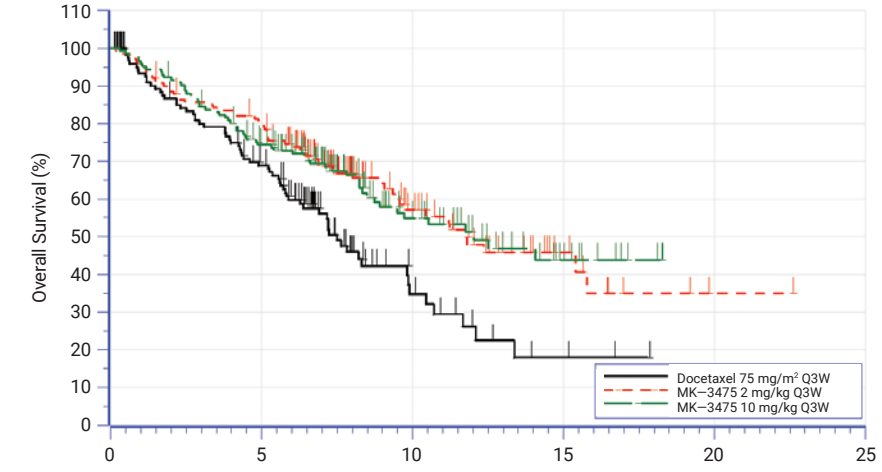
Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	344	140	346	142	343	131
OS						
Deaths (%)	172 (50%)	59 (42%)	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 8: 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



n at risk					
Docetaxel 75 mg/m² Q3W					
131	78	14	3	0	0
MK—3475 2 mg/kg Q3W					
140	109	36	10	1	0
MK—3475 10 mg/kg Q3W					
142	99	36	10	0	0

Figure 61: 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.

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Notes

Notes

Agilent Educational Resources For PD-L1

Developed with the help of expert pathologists, these education resources provide extra self directed learning opportunities to support and expand on this interpretation manual for NSCLC. Learn any time, any place, at your own pace with on-demand e-learning.

PD-L1 IHC 22C3 pharmDx NSCLC Interpretation Training Program

Online training that may help you accurately evaluate and score PD-L1 expression in patients with NSCLC.The PD-L1 IHC 22C3 pharmDx interpretation training program uses in-depth content, engaging activities, and comprehensive cases to help you confidently:

- Understand the core principles of PD-L1 pathology and learn the process for evaluating stained images for PD-L1 expression
- Recognize conditions that affect PD-L1 scoring and practice scoring images for PD-L1 expression using real cases

Online Atlas of Stains for NSCLC: PD-L1 IHC 22C3 pharmDx

The Atlas of Stains for NSCLC is a digital repository of NSCLC tissue samples stained with PD-L1 IHC 22C3 pharmDx including H&E, Negative Control Reagent (NCR), and primary antibody for each patient.

The viewer interface for the Atlas of Stains for NSCLC features:

- High-definition scans with full-screen and quadrant-view functionality. Zoom in to magnify fields of interest for detailed PD-L1 stain analysis
- Expert annotations describing areas of interest plus the ability to add your own annotations
- Provides TPS for each stain, to verify your own assessment

PD-L1 Webinar Series

Comprehensive education program for the entire laboratory led by expert speakers. Registration gives access to on-demand recordings.

For countries outside of the United States, see the local KEYTRUDA product label for approved indications and expression cutoff values to guide therapy.

This information is subject to change without notice.

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