VENTANA PD-L1 (SP142) Assay

REF 740-4859

07709374001

IVD Σ 50

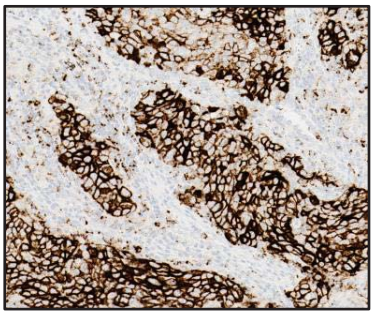


Figure 1. PD-L1 expression in non-small cell lung cancer.

INTENDED USE

VENTANA PD-L1 (SP142) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP142 intended for use in the assessment of the programmed death-ligand 1 (PD-L1) protein in tumor cells and tumor-infiltrating immune cells in the formalin-fixed, paraffin-embedded (FFPE) tissues indicated below stained with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark ULTRA instrument.

Determination of PD-L1 status is indication-specific, and evaluation is based on either the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity.

VENTANA PD-L1 (SP142) Assay is indicated as an aid for identifying patients for treatment with the therapies for the respective cutoffs listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. Companion diagnostic indications for the VENTANA PD-L1 (SP142) Assay

Indication for use	Therapy	Cutoff
Urothelial Carcinoma	TECENTRIQ	≥ 5% IC
Triple-Negative Breast Carcinoma (TNBC)	TECENTRIQ	≥ 1% IC
Non-Small Cell Lung Cancer (NSCLC)	TECENTRIQ	≥ 50% TC or ≥ 10% IC

Depending on therapeutic setting, PD-L1 expression in ≥ 50% TC or ≥ 10% IC determined by VENTANA PD-L1 (SP142) Assay in non-small cell lung cancer (NSCLC) patients may be associated with enhanced overall survival from TECENTRIQ (atezolizumab). Refer to the approved therapeutic product labeling for further information.

Test results of this product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA PD-L1 (SP142) Assay is an immunohistochemical (IHC) assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody to recognize the programmed death ligand 1 (PD-L1) protein. This assay was co-developed by Roche/Ventana Medical Systems, Inc. (Ventana) and Roche/Genentech to identify patients who are most likely to respond to treatment with TECENTRIQ®.

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors, programmed death-1 (PD-1) and B7.1. PD-1 is an inhibitory receptor expressed on T cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[1] Ligation of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T cells. B7.1 is a molecule expressed on antigen presenting cells and activated T cells. PD-L1 binding to B7.1 on T cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.[2] PD-L1 expression has been observed in immune cells and malignant cells and aberrant expression of PD-L1 on tumor cells (TC) has been reported to impede anti-tumor immunity, resulting in immune evasion.[1,3] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment. The association between PD-L1 expression in TC or tumor-infiltrating immune cells (IC) and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported across multiple cancers.[3-10]

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors. Atezolizumab is a non-glycosylated IgG1 kappa immunoglobulin that has a calculated molecular mass of 145 kDa.

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP142) Assay utilizes a rabbit monoclonal primary antibody that binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) followed by the OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test)). Refer to the appropriate OptiView DAB IHC Detection Kit and OptiView Amplification Kit package inserts for further information.

MATERIAL PROVIDED

VENTANA PD-L1 (SP142) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PD-L1 (SP142) Assay contains approximately 36 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 7 µg/mL.

VENTANA PD-L1 (SP142) Assay contains a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate interpretation guide for detailed instructions for interpretation of VENTANA PD-L1 (SP142) Assay staining in specific indications:

- VENTANA PD-L1 (SP142) Assay Interpretation Guide for Urothelial Carcinoma (P/N 1014987US)
- VENTANA PD-L1 (SP142) Assay Interpretation Guide for NSCLC (P/N 1015703EN)
- VENTANA PD-L1 (SP142) Assay Interpretation Guide for TNBC (P/N 1018231EN)

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: Principles of the Procedure, Materials and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Benign human tonsil tissues for use as control tissue
2. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
3. Microscope slides, positively charged
4. Bar code labels
5. Xylene (Histological grade)
6. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
7. Deionized or distilled water
8. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
9. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))

10. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
11. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
12. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
15. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
16. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
17. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
18. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
19. Light microscope
20. Absorbent wipes

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. Tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours is recommended. Fixation times of less than 6 hours may result in a loss of staining for PD-L1. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[11,12]

Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative and other alcohol-containing fixatives have demonstrated a loss of specific staining for PD-L1 at all fixation times tested (1-72 hours); they are not recommended for use with this assay. See the interpretation guides for further discussion of the impact of specimen preparation on PD-L1 staining intensity.

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised 3 months after cutting from the paraffin block for urothelial carcinoma specimens, 2 months for NSCLC, TNBC, and tonsil specimens (see the interpretation guides and the Performance Characteristics section below).

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any IHC assay (for example, lack of primary antibody or counterstain on the tissue). Ask your Roche representative for a copy of "Impacts of Environmental Stresses on IHC Positively Charged Slides" to better understand how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[13,14]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark ULTRA instrument Operator's Manual, and instructions for use of all necessary components.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.
13. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA PD-L1 (SP142) Assay has been developed for use on a BenchMark ULTRA instrument in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, OptiView Amplification Kit, and ancillary reagents. An assay-specific staining procedure must be used with VENTANA PD-L1 (SP142) Assay. Refer to Table 2 for the recommended staining protocol and required staining procedure. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 2. Recommended staining protocol for VENTANA PD-L1 (SP142) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark ULTRA instrument.

Staining Procedure: U OptiV DAB VENTANA PDL1 (SP142)	
Protocol Parameter	**Selection**
Deparaffinization	Selected
Cell Conditioning	CC1 Cell Conditioning 48 minutes
Pre-primary antibody peroxidase	Selected
Primary Antibody	VENTANA PD-L1 (SP142) Selected[a] or Negative Control Selected[a] 16 minutes, 36°C
OptiView HQ Linker	8 minutes (default)
OptiView Multimer	8 minutes (default)
Amplifier and Amplification	Selected
Amplifier and Amplification H2O2	8 minutes
Amplification Multimer	8 minutes
Counterstain	Hematoxylin II, 4 minutes[a]
Post Counterstain	Bluing Reagent, 4 minutes[a]

[a] User-selectable

QUALITY CONTROL PROCEDURES

Rabbit Monoclonal Negative Control Ig

A matched negative reagent control slide must be run for every specimen to aid in the interpretation of results. Rabbit Monoclonal Negative Control Ig, a negative reagent control antibody, is specifically matched for this assay and is used in place of the primary antibody to evaluate nonspecific staining. The staining procedure for the negative reagent control should equal the primary antibody incubation period. Use of a different negative control reagent, or failure to use the recommended negative control reagent, may cause false results.

Tonsil Tissue Control

A tissue control must be included with each staining run. Qualified benign human tonsil tissue is to be used as the control. Control tissue should be fixed as soon as possible and processed in a manner identical to patient tissues. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Tonsil tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. The positive and negative staining tissue components are used to confirm that the assay functioned properly.

Appropriate staining of tonsil tissue components is described in Table 3 and in the interpretation guides.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known IHC performance characteristics representing PD-L1 positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[15] or the CLSI Approved Guideline.[16] These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Urothelial carcinoma, NSCLC, and TNBC tissues with known PD-L1 status, and benign human tonsil samples, are suitable for assay verification.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the VENTANA PD-L1 (SP142) Assay antibody. The stained slide(s) are interpreted by a qualified pathologist using light microscopy. A qualified pathologist experienced in IHC procedures must evaluate tissue controls and qualify the stained product before interpreting results.

Tonsil Tissue Control Interpretation

The stained tonsil tissue control should be examined for appropriate staining. The presence of PD-L1 staining within the macrophages and lymphocytes in germinal centers and reticulated crypt epithelium of tonsil serve as positive tissue elements. Absence of staining in superficial squamous epithelium and negative immune cells in interfollicular regions of tonsil serve as negative tissue elements. Acceptability criteria are listed in Table 3. (Refer to the interpretation guides for further discussion).

If the tissue control fails to demonstrate appropriate staining, any results with the patient specimens should be considered unevaluable and repeat staining should be performed.

Table 3. Tonsil tissue control evaluation criteria.

Acceptable	Unacceptable
Positive tissue elements: Moderate to strong PD-L1 staining noted in lymphocytes and macrophages in germinal centers, with diffuse staining in reticulated crypt epithelial cells.	Excessive non-specific background staining obscuring the identification of PD-L1 positive cells.
Negative tissue elements: PD-L1 negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Weak to no PD-L1 staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.

Negative Reagent Control

Non-specific staining, if present, will have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results; as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Examples of background staining for this assay can be found in the interpretation guides.

Patient Tissue

Tumor cells (TC) are scored as the percentage of tumor cells with the presence of discernible PD-L1 membrane staining of any intensity. Tumor-infiltrating immune cells (IC) are scored as the proportion of tumor area, including associated intratumoral and contiguous peritumoral stroma, occupied by PD-L1 staining IC of any intensity. Patient tissue must be evaluated according to the indication-specific VENTANA PD-L1 (SP142) Assay scoring algorithm provided in the Performance Characteristics section for that indication. Refer to the indication-specific interpretation guide for additional instructions and representative images.

GENERAL LIMITATIONS

1. IHC is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and system-level controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[17,18]
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[19]
9. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.[20]
10. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

SPECIFIC LIMITATIONS

1. VENTANA PD-L1 (SP142) Assay has been solely approved on the BenchMark ULTRA instrument with the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit and is not approved with any other detection or instruments.
2. A patient specimen slide should be stained with Rabbit Monoclonal Negative Control Ig. Other negative control reagents are not suitable for this assay.
3. VENTANA PD-L1 (SP142) Assay antibody is stable for up to eight days at 30°C. Assay performance beyond these limits has not been established.
4. This assay has not been validated for use with cytology samples or decalcified bone specimens.
5. Patient tissue should be stained within 2 months of sectioning from the tissue block for NSCLC, TNBC, and tonsil tissues and within 3 months for urothelial carcinoma tissues. Loss of staining performance has been observed with VENTANA PD-L1 (SP142) Assay staining of tissue sections that have been stored at room temperature for longer than these times.
6. It is recommended that samples be fixed between 6 and 72 hours in 10% NBF. Use of fixation times or fixative types other than those recommended can lead to false negative results. Fixatives such as AFA, PREFER fixative, and other alcohol-containing fixatives have demonstrated a loss of specific PD-L1 protein staining. Refer to the interpretation guides for further discussion.
7. Artifacts such as DAB spots, Blank spots, DAB dots, and/or Speckling may require repeat staining if they interfere with the interpretation of VENTANA PD-L1 (SP142) Assay. Always compare the PD-L1-stained slide to the negative reagent control to ensure that background is acceptable. Refer to the interpretation guides for further discussion.
8. Occasional DAB dots have been observed in benign human tonsil control, cerebellum and testicular tissues and focal nuclear staining has been observed in

normal pancreatic (acinar cells) and hypophyseal tissue (Table 4), however nuclear staining is not included in scoring of VENTANA PD-L1 (SP142) Assay staining.

PERFORMANCE CHARACTERISTICS

Tests for staining specificity, sensitivity, impact of tissue thickness, repeatability, and intermediate precision, as well as tests for reader precision, inter-laboratory reproducibility, and clinical outcome were conducted and the results are listed in the following section.

General Analysis Comments

Unless otherwise noted, the two-sided 95% confidence interval (CI) around estimates of agreement for all studies (excluding clinical efficacy studies) were calculated using the percentile bootstrap method from 2000 bootstrap samples. If the point estimate of Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) or Overall Percent Agreement (OPA) is 0% or 100%, then Wilson score method was used to calculate 95% CI. If the point estimate of Average Positive Agreement (APA) and Average Negative Agreement (ANA) is 0% or 100% for pairwise comparison, then transformation Wilson score method was used to calculate 95% CI.

Sensitivity and Specificity

Arrays containing a variety of normal and neoplastic tissues were stained with VENTANA PD-L1 (SP142) Assay and evaluated for the presence of immune cell staining (any immune cell staining, of any intensity) as described in Table 4 and Table 5.

3750 urothelial carcinoma specimens (including 55 metastases) were evaluated with VENTANA PD-L1 (SP142) Assay. Of these, 2545 (67.9%) showed immune cell staining of any percentage; 466 (12.4%) showed ≥ 5% immune cell staining; 512 (13.7%) showed tumor cell staining of any percentage. In addition, an array of neoplastic tissues was evaluated for IC and TC staining with VENTANA PD-L1 (SP142) Assay as described in Table 5.

Table 4. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing FFPE normal tissues.

Tissue	# Positive[a]/ Total Cases	Tissue	# Positive[a]/ Total Cases
Adrenal gland	1/3	Muscle, cardiac	0/3
Bladder	3/36[b]	Muscle, skeletal	0/2
Breast	1/66	Myeloid	0/2
Cerebellum	0/3[c]	Nerve, peripheral	0/3
Cerebrum	0/3	Ovary	0/3
Cervix	0/2	Pancreas	0/3[d]
Colon	2/3	Parathyroid	0/2
Endometrium	2/3	Prostate	0/3
Esophagus	0/3	Salivary gland	2/3
Hypophysis	0/3[d]	Skin	0/3
Intestine, small	1/3	Spleen	3/3
Kidney	2/3	Stomach	0/3
Lingual gland	0/1	Testis	0/3[c]
Liver	0/3	Thymus gland	3/3
Lung	1/25	Thyroid gland	1/3
Lymph node	3/3	Tonsil	3/3[c]
Mesothelium	0/3		

[a] Immune cell staining of any intensity [b] Focal immune cell staining

[c] Focal DAB dots were observed in 1/3 cerebellum, 1/3 testis tissues and normal tonsil control

[d] Nuclear staining was observed in 1/3 pancreas and 1/3 hypophysis tissues

Table 5. Sensitivity/Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing a variety of FFPE neoplastic tissues.

Origin	Pathology	# Positive[a]/Total Cases	
		Immune cells	Tumor cells
Abdomen	Malignant mesothelioma	1/1	0/1
Back	Neurofibroma	1/1	0/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bladder	Transitional cell carcinoma	1/1	0/1
Bone	Osteosarcoma	0/1	0/1
Breast	Invasive ductal carcinoma	1/2	0/2
Breast	Intraductal carcinoma with early infiltrate	1/1	0/1
Cerebrum	Glioblastoma	1/1	0/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	0/1
Cerebrum	Oligodendroglioma	0/1	0/1
Colon	Adenocarcinoma	1/1	0/1
Colon	Interstitialoma	0/1	0/1
Esophagus	Neuroendocrine carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	1/1	0/1
Intestine	Adenocarcinoma	1/1	0/1
Intestine	Stromal sarcoma	1/1	0/1
Kidney	Clear cell carcinoma	1/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	1/1	0/1
Lung	Adenocarcinoma	0/1	0/1
Lung	Small cell undifferentiated carcinoma	1/1	1/1
Lung	Squamous cell carcinoma	1/1	0/1
Lymph node	Diffuse B-cell lymphoma	1/1[b]	1/1[b]
Lymph node	Hodgkin's lymphoma	1/1	1/1
Mediastinum	Diffuse B-cell lymphoma	1/1[b]	1/1[b]
Muscle, smooth	Moderate malignant leiomyosarcoma	1/1	0/1
Muscle, striated	Embryonal rhabdomyosarcoma	0/1	0/1
Ovary	Serous adenocarcinoma	1/1	0/1
Ovary	Adenocarcinoma	1/1	0/1
Pancreas	Islet cell tumor	0/1	0/1
Pancreas	Adenocarcinoma	1/1	0/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1[b]	1/1[b]

Origin	Pathology	# Positive[a]/Total Cases	
		Immune cells	Tumor cells
Prostate	Adenocarcinoma	0/2	0/2
Rectum	Adenocarcinoma	1/1	1/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Rectum	Malignant melanoma	1/1	0/1
Retroperitoneum	Neuroblastoma	1/1	0/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Skin	Basal cell carcinoma	1/1	0/1
Skin	Squamous cell carcinoma	1/1	0/1
Spleen	Diffuse B-cell lymphoma	1/1[b]	1/1[b]
Stomach	Signet-ring cell carcinoma	1/1	0/1
Testis	Seminoma	1/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	0/1	1/1
Uterine cervix	Squamous cell carcinoma	2/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	1/1	0/1
Uterus	Clear cell carcinoma of endometrium	1/1	1/1

[a] Immune cell or tumor cell staining of any intensity.
[b] Tumor cell and immune cell staining could not be differentiated.

PERFORMANCE CHARACTERISTICS

UROTHELIAL CARCINOMA

Scoring Algorithm – Urothelial Carcinoma

Urothelial carcinoma tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma provided in Table 6. Refer to the interpretation guide (P/N 1014987US) for additional instructions and representative images.

Table 6. VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma.

Immune Cell (IC) Staining Assessment[a]	PD-L1 Expression
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 5% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 5% IC

[a] PD-L1 staining in tumor cells should not be included in the scoring determination of urothelial carcinoma patient tissue.

Tissue Thickness – Urothelial Carcinoma

Tissue thickness was evaluated using 5 unique urothelial carcinoma specimens (3 PD-L1 ≥ 5% IC and 2 PD-L1 < 5% IC). Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 expression within the range of thickness tested. Ventana recommends that specimens be cut at 4 microns for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision – Urothelial Carcinoma

Repeatability studies for VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens were completed to demonstrate:

- Intra-day Repeatability – 5 replicate slides each from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within 1 day.
- Inter-day Precision – 10 slides each from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days.
- Inter-instrument and Inter-lot Precision – 27 slides each from 24 unique urothelial carcinoma specimens (12 PD-L1 ≥ 5% IC and 12 PD-L1 < 5% IC) were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma (Table 6). Results are summarized in Table 7.

Table 7. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Repeatability/Intermediate Precision Parameter	Agreement % (95% CI)[a]
Intra-day repeatability (within a single day)	PPA: 98.2 (90.4-99.7) NPA: 100.0 (94.4-100.0) OPA: 99.2 (95.4-99.9)
Inter-day precision (5 non-consecutive days)	PPA: 91.8 (85.2-95.6) NPA: 100.0 (97.1-100.0) OPA: 96.3 (93.0-98.0)
Inter-instrument and Inter-lot precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	PPA: 99.4 (97.8-99.8) NPA: 99.7 (98.3-99.9) OPA: 99.5 (98.6-99.8)

[a] Two-sided Wilson score method CI

Reader Precision – Urothelial Carcinoma

To assess Inter- and Intra-reader Precision, three pathologists evaluated 60 unique urothelial carcinoma specimens (30 PD-L1 ≥ 5% IC and 30 PD-L1 < 5% IC) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 expression using the VENTANA PD-L1 (SP142) Assay scoring algorithm for urothelial carcinoma (Table 6). Readers scored all specimens twice, with a minimum of 2 weeks between reads. The agreement rates between the readers between each pathologist's reads are summarized in Table 8.

Table 8. Inter- and intra-reader precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 92.7 (85.9-97.6) ANA: 93.9 (88.1-98.1) OPA: 93.3 (87.8-97.8)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.4 (87.3-97.7) ANA: 94.2 (88.9-98.1) OPA: 93.9 (88.8-97.8)

Inter-laboratory Reproducibility Study – Urothelial Carcinoma

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in urothelial carcinoma tissue specimens. Twenty-eight unique urothelial carcinoma specimens (14 PD-L1 ≥ 5% IC and 14 PD-L1 < 5% IC) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The sample set consisted of a total of 420 case slides (140 slides per site) generated from 28 unique urothelial carcinoma specimens. The final staining acceptability rate for the VENTANA PD-L1 (SP142) Assay was 99.9% in this study. Results are summarized in Table 9.

Table 9. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Inter-laboratory Reproducibility[a]	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 98.3 (96.6-99.2)[b] NPA: 87.4 (83.8-90.2)[b] OPA: 92.8 (90.9-94.4)[b]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 90.7 (81.2-96.3) ANA: 88.3 (78.5-94.9) OPA: 89.6 (82.5-95.5)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 89.3 (78.1-96.0) ANA: 86.6 (75.1-94.6) OPA: 88.1 (84.6-90.8)[b]

[a] n = 419 evaluable case slides

[b] Two-sided Wilson score method CI

Clinical Outcome Study – Urothelial Carcinoma

The performance of VENTANA PD-L1 (SP142) Assay was investigated in IMvigor210 (NCT02108652), a multicenter, open-label, two-cohort trial designed to evaluate the efficacy of TECENTRIQ in patients with locally advanced or metastatic urothelial carcinoma.

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression per the scoring algorithm specified in Table 6. Patient specimens were FFPE urothelial carcinoma tissue from biopsies (21.2%), resections (46.6%), transurethral resection of bladder tumor (TURBT, 30.5%), or of unknown type (1.6%); 74.9% were from primary tumors and 25.1% from metastatic tumors.

Table 10 describes the overall staining acceptability rate for the VENTANA PD-L1 (SP142) Assay among all urothelial carcinoma specimens screened for IMvigor210. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 650 specimens, 25 failed the initial staining attempt and staining was repeated. On the final staining attempt, 13 of the 25 samples remained unacceptable (7 due to unacceptable negative reagent control, 5 due to unacceptable morphology, and 1 for both unacceptable background and morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial and final overall staining acceptability rates; 96.2% and 98.0%, respectively. Final morphology and background acceptability rates were greater than 99%.

Table 10. VENTANA PD-L1 (SP142) Assay staining performance characteristics for urothelial carcinoma clinical study specimens of IMvigor210.

Attribute	Acceptability Rate % (n/N) (95% CI)[a]	
	Initial[b]	Final[c]
Overall staining acceptability rate	96.2 (625/650) (94.4-97.4)	98.0 (637/650) (96.6-98.8)
Morphology	98.1 (628/640) (96.8-98.9)	99.1 (637/643) (98.0-99.6)
Background	98.9 (625/632) (97.7-99.5)	99.8 (637/638) (99.1-100.0)

[a] Two-sided Wilson score method CI

[b] Initial staining attempt [c] Final staining attempt

In Cohort 2 of IMvigor210 (NCT02108652), 310 patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following a platinum-containing chemotherapy regimen or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen were treated with TECENTRIQ. This study excluded patients who had: a history of autoimmune disease, active or corticosteroid-dependent brain metastases, administration of a live, attenuated vaccine within 28 days prior to enrollment, or administration of systemic immunostimulatory agents or systemic immunosuppressive medications. Patients received an intravenous infusion of 1200 mg of TECENTRIQ every 3 weeks until unacceptable toxicity or either radiographic or clinical progression. Tumor response assessments were conducted every 9 weeks for the first 54 weeks and every 12 weeks thereafter. Major efficacy outcome measures included confirmed objective response rate (ORR) as assessed by independent review facility (IRF) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1) and duration of response (DOR).

In this cohort, the median age was 66 years, 78% were male, 91% patients were Caucasian. Twenty-six percent had non-bladder urothelial carcinoma and 78% of patients had visceral metastases. Sixty-two percent of patients had an ECOG score of 1, and 35% of patients had a baseline creatinine clearance of < 60 mL/min. Nineteen percent of patients had disease progression following prior platinum-containing neoadjuvant or adjuvant chemotherapy. Forty-one percent of patients had received ≥ 2 prior systemic regimens in the metastatic setting. Seventy-three percent of patients received prior cisplatin, 26% had prior carboplatin, and 1% were treated with other platinum-based regimens.

Tumor specimens were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory, and the results were used to define subgroups for pre-specified analyses. Of the 310 patients, 32% were classified as having PD-L1 expression of ≥ 5% IC; the remaining 68% of patients were classified as having PD-L1 expression of < 5% IC.

Confirmed ORR in all patients and the two PD-L1 subgroups are summarized in Table 11. The median follow-up time for this cohort was 14.4 months. In 59 patients with disease progression following neoadjuvant or adjuvant therapy, the ORR was 22.0% (95% CI: 12.3%, 34.7%).

Table 11. Summary of efficacy from Cohort 2 of IMvigor210.

	All Patients (N = 310)	PD-L1 Expression Subgroups	
		< 5%[a] (N = 210)	≥ 5%[a] (N = 100)
Number of IRF-assessed Confirmed Responders	46	20	26
ORR % (95% CI)	14.8% (11.1, 19.3)	9.5% (5.9, 14.3)	26.0% (17.7, 35.7)
Complete Response (CR) (%)	5.5%	2.4%	12.0%
Partial Response (PR) (%)	9.4%	7.1%	14.0%
Median DOR, months (range)	NR (2.1+, 13.8+)	12.7 (2.1+, 12.7)	NR (4.2, 13.8+)

NR = Not reached, CI = Confidence Interval, + Denotes a censored value

[a] PD-L1 expression in tumor-infiltrating immune cells (IC)

IMvigor130 (NCT02807636) is an ongoing multicenter, randomized study in previously untreated patients with metastatic urothelial carcinoma who are eligible for platinum-containing chemotherapy. The study contains three arms: TECENTRIQ monotherapy, TECENTRIQ with platinum-based chemotherapy (i.e., cisplatin or carboplatin with gemcitabine), and platinum-based chemotherapy alone (comparator). Both cisplatin-eligible and cisplatin-ineligible patients are included in the study. Tumor specimens were evaluated prospectively using the VENTANA PD-L1 (SP142) Assay at a central laboratory. The independent Data Monitoring Committee (iDMC) for the study conducted a review of early data and found that patients classified as having PD-L1 expression of < 5% when treated with TECENTRIQ monotherapy had decreased survival compared to those who received platinum-based chemotherapy. The iDMC recommended closure of the monotherapy arm to further accrual of patients with low PD-L1 expression; however, no other changes were recommended for the study, including any change of therapy for patients who had already been randomized to and were receiving treatment in the monotherapy arm.

PERFORMANCE CHARACTERISTICS

NSCLC

Scoring Algorithm – NSCLC

NSCLC tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC provided in Table 12. High PD-L1 expression is defined as having PD-L1 expression on ≥ 50% TC or ≥ 10% IC. Refer to the interpretation guide (P/N 1015703EN) for additional instructions and representative images.

Table 12. VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC.

STEP 1 Tumor Cell (TC) Staining Assessment	PD-L1 Expression
Presence of discernible PD-L1 membrane staining of any intensity in ≥ 50% of tumor cells	≥ 50% TC
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 membrane staining of any intensity in < 50% of tumor cells	Proceed to Step 2
STEP 2 Tumor-Infiltrating Immune Cell (IC) Staining Assessment	**PD-L1 Expression**
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 10% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 10% IC
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 10% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 50% TC and < 10% IC

Tissue Thickness – NSCLC

Tissue thickness was evaluated using NSCLC specimens. Duplicate sections at 3, 4, 5, 6, and 7 microns were stained with VENTANA PD-L1 (SP142) Assay and evaluated for PD-L1 TC and IC expression. Sample sets consisted of a minimum of 8 NSCLC specimens with a range of PD-L1 expression for each IC and TC level tested.

All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 TC or IC level within the range of thickness tested. Ventana recommends that NSCLC specimens be cut at 4 microns for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision – NSCLC

Studies for VENTANA PD-L1 Assay staining of NSCLC specimens were completed to demonstrate:

- Intra-day Repeatability - 5 replicate slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument in a single day and evaluated for PD-L1 TC and IC expression. Sample sets consisted of 24 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.
- Inter-day Precision - 10 slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days. Sample sets consisted of 24 NSCLC specimens with a range of PD-L1 expression for each TC and IC expression level tested.
- Instrument, Antibody and Detection Lot Precision - a minimum of 9 slides from each NSCLC specimen were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments. Sample sets consisted of a minimum of 18 NSCLC specimens with a range of PD-L1 expression for each TC and IC level tested.

All slides were blinded and randomized and then evaluated for PD-L1 TC or IC expression level. Repeatability and Intermediate Precision results are summarized in Table 13 and Table 14.

Table 13. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 50% TC).

Repeatability/Intermediate Precision Parameter	Agreement % (95% CI)[a]
Intra-day repeatability (within a single day)	PPA: 100.0 (94.4-100.0) NPA: 100.0 (93.5-100.0) OPA: 100.0 (96.9-100.0)
Inter-day precision (5 non-consecutive days)	PPA: 100.0 (97.1-100.0) NPA: 100.0 (96.5-100.0) OPA: 100.0 (98.4-100.0)
Inter-instrument and Inter-lot precision (compared to case-level mode, across instruments and lots)	PPA: 99.7 (98.1-99.9) NPA: 95.2 (91.2-97.5) OPA: 97.9 (96.2-98.9)

[a] Two-sided Wilson score method CI

Table 14. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens (PD-L1 expression ≥ 10% IC).

Repeatability/Intermediate Precision Parameter	Agreement % (95% CI)
Intra-day repeatability (within a single day)	PPA: 98.3 (91.1-99.7)[a] NPA: 100.0 (94.0-100.0)[a] OPA: 99.2 (95.4-99.9)[a]
Inter-day precision (5 non-consecutive days)	PPA: 96.2 (91.3-98.3)[a] NPA: 98.2 (93.6-99.5)[a] OPA: 97.1 (94.1-98.6)[a]
Inter-antibody and Inter-detection agreement (pairwise-comparison)	APA: 95.1 (91.1-98.1) ANA: 90.2 (82.3-96.2) OPA: 93.4 (88.7-97.5)
Inter-instrument and Inter-detection agreement (pairwise-comparison)	APA: 96.3 (93.2-98.8) ANA: 92.7 (86.0-97.7) OPA: 95.1 (91.2-98.4)
Inter-instrument and Inter-antibody agreement (pairwise-comparison)	APA: 96.3 (93.1-98.8) ANA: 92.6 (85.9-97.8) OPA: 95.1 (91.1-98.4)

[a] Two-sided Wilson score method CI

Reader Precision Study – NSCLC

To assess Inter- and Intra-Reader Precision, three pathologists evaluated 80 unique NSCLC cases, with a range of PD-L1 expression, that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 12). Readers scored all specimens twice, with a minimum of 2 weeks between reads. The agreement rates between the readers and between each pathologist's reads are summarized in Table 15.

Table 15. Reader precision of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens.

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 88.8 (82.0-94.1) ANA: 89.0 (82.2-94.4) OPA: 88.9 (82.8-94.1)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.7 (89.9-96.6) ANA: 93.6 (89.8-96.7) OPA: 93.6 (90.3-96.6)

Inter-laboratory Reproducibility Study – NSCLC

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP142) Assay staining was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in NSCLC tissue specimens. Twenty-eight unique NSCLC specimens with a range of PD-L1 expression were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers) using the VENTANA PD-L1 (SP142) Assay scoring algorithm for NSCLC (Table 12). Results are summarized in Table 16.

Table 16. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of NSCLC specimens.

Inter-laboratory Reproducibility	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 86.6 (83.0-89.5)[a] NPA: 99.8 (98.7-100.0)[a] OPA: 93.2 (91.3-94.7)[a]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 89.5 (80.9-95.5) ANA: 92.1 (84.4-97.1) OPA: 91.0 (90.3-91.6)[a]
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 93.9 (89.3-97.4) ANA: 95.4 (90.6-98.2) OPA: 94.7 (92.2-96.5)[a]

[a] Two-sided Wilson score method CI

CLINICAL PERFORMANCE

NSCLC ≥ 50% TC or ≥ 10% IC

The performance of VENTANA PD-L1 (SP142) Assay as a companion diagnostic was investigated in IMpower110 (NCT02409342), a multicenter, international, randomized, open-label trial in patients with stage IV NSCLC whose tumors express PD-L1 (TC ≥ 1% or IC ≥ 1%), who had received no prior chemotherapy for metastatic disease. The study was designed to evaluate the safety and efficacy of TECENTRIQ relative to chemotherapy consisting of a platinum agent (cisplatin or carboplatin per investigator discretion) in combination with either pemetrexed (non-squamous disease) or gemcitabine (squamous disease).

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression. Patient specimens were FFPE NSCLC tissue from biopsies (66.0%), resections (15.7%), or of other type (18.3%); 72.4% were from primary tumors and 27.6% from metastatic tumors.

Table 17 describes the overall staining acceptability rate for VENTANA PD-L1 (SP142) Assay among all NSCLC subjects screened for the study. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 2909 subjects, specimens for 65 subjects failed the initial staining attempt. When staining was repeated, results for the 15 of the 65 subjects remained unacceptable (14 due to unacceptable negative reagent control and 1 due to unacceptable morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial (i.e., first-pass) and final overall staining acceptability rates: 97.8% and 99.5%, respectively. The initial and final acceptability rates for background staining and morphology were greater than 99%.

Table 17. VENTANA PD-L1 (SP142) Assay NSCLC staining performance characteristics in IMpower110.

Attribute	Acceptability rate % (n/N) (95% CI) [a]	
	Initial[b]	Final[c]
Overall staining acceptability rate	97.8 (2844/2909) (97.2-98.2)	99.5 (2894/2909) (99.2-99.7)
Morphology	99.4 (2844/2860) (99.1-99.7)	100.0 (2894/2895) (99.8-100.0)
Background	100.0 (2844/2844) (99.9-100.0)	100.0 (2894/2894) (99.9-100.0)

[a] Two-sided Wilson score method CI

[b] Initial staining attempt [c] Final staining attempt

The evaluation of efficacy is based on the subgroup of patients with high PD-L1 expression (TC ≥ 50% or IC ≥ 10%), excluding those with EGFR or ALK genomic tumor aberrations. The trial excluded patients with a history of autoimmune disease administration of a live attenuated vaccine within 28 days prior to randomization, active or untreated CNS metastases, administration of systemic immunostimulatory agents within 4 weeks or systemic immunosuppressive medications within 2 weeks prior to randomization.

Randomization was stratified by sex, ECOG performance status, histology (non-squamous vs. squamous) and PD-L1 expression (TC ≥ 1% and any IC vs. TC < 1% and IC ≥ 1%). Patients were randomized (1:1) to receive one of the following treatment arms:

- Arm A: TECENTRIQ 1200 mg every 3 weeks until disease progression or unacceptable toxicity
- Arm B: Platinum-based chemotherapy

Arm B platinum-based chemotherapy regimens for non-squamous NSCLC consisted of cisplatin (75 mg/m²) and pemetrexed (500 mg/m²) OR carboplatin (AUC 6 mg/mL/min) and pemetrexed (500 mg/m²) on Day 1 of each 21-day cycle for a maximum of 4 or 6 cycles followed by pemetrexed 500 mg/m² until disease progression or unacceptable toxicity.

Arm B platinum-based chemotherapy regimens for squamous NSCLC consisted of cisplatin (75 mg/m²) on Day 1 with gemcitabine (1250 mg/m2) on Days 1 and 8 of each 21-day cycle OR carboplatin (AUC 5 mg/mL/min) on Day 1 with gemcitabine (1000 mg/m2) on Days 1 and 8 of each 21-day cycle for a maximum of 4 or 6 cycles followed by best supportive care until disease progression or unacceptable toxicity.

Administration of TECENTRIQ was permitted beyond RECIST-defined disease progression. Tumor assessments were conducted every 6 weeks for the first 48 weeks following Cycle 1, Day 1 and then every 9 weeks thereafter. Tumor specimens were evaluated prospectively using the VENTANA PD-L1 (SP142) Assay at a central laboratory, and the results were used to define subgroups for pre-specified analyses.

The major efficacy outcome measure was overall survival (OS) sequentially tested in the following subgroups of patients, excluding those with EGFR or ALK genomic tumor aberrations: TC ≥ 50% or IC ≥ 10%; TC ≥ 5% or IC ≥ 5%; and TC ≥ 1% or IC ≥ 1%.

Among the 205 chemotherapy-naïve patients with stage IV NSCLC with high PD-L1 expression (TC ≥ 50% or IC ≥ 10%) excluding those with EGFR or ALK genomic tumor aberrations, the median age was 65.0 years (range: 33 to 87), and 70% of patients were male. The majority of patients were White (82%) and Asian (17%). Baseline ECOG performance status was 0 (36%) or 1 (64%); 88% were current or previous smokers; and 76% of patients had non-squamous disease while 24% of patients had squamous disease.

The trial demonstrated a statistically significant improvement in OS for patients with high PD-L1 expression (TC ≥ 50% or IC ≥ 10%) at the time of the OS interim analysis. There was no statistically significant difference in OS for the other two PD-L1 subgroups (TC ≥ 5% or IC ≥ 5%; and TC ≥ 1% or IC ≥ 1%) at the interim or final analyses. Efficacy results for patients with NSCLC with high PD-L1 expression are presented in Table 18 and Figure 2.

Table 18. Efficacy results from IMpower110 in patients with NSCLC with high PD-L1 expression (TC ≥ 50% or IC ≥ 10%) and without EGFR or ALK genomic tumor aberrations.

	Arm A: TECENTRIQ	Arm B: Platinum-Based Chemotherapy
Overall Survival[a]	N = 107	N = 98
Deaths (%)	44 (41%)	57 (58%)
Median, months	20.2	13.1
(95% CI)	(16.5, NE)	(7.4, 16.5)
Hazard ratio[b] (95% CI)	0.59 (0.40, 0.89)	
p-value[c]	0.0106[d]	

[a] Based on OS interim analysis. The median survival follow-up time in patients was 15.7 months.

[b] Stratified by sex and ECOG performance status

[c] Based on the stratified log-rank test compared to Arm A

[d] Compared to the allocated alpha of 0.0413 (two-sided) for this interim analysis.

CI=confidence interval; NE=not estimable

Investigator-assessed PFS showed a HR of 0.63 (95% CI: 0.45, 0.88), with median PFS of 8.1 months (95% CI: 6.8, 11.0) in the TECENTRIQ arm and 5 months (95% CI: 4.2, 5.7) in the platinum-based chemotherapy arm. The investigator-assessed confirmed ORR was 38% (95% CI: 29%, 48%) in the TECENTRIQ arm and 29% (95% CI: 20%, 39%) in the platinum-based chemotherapy arm.

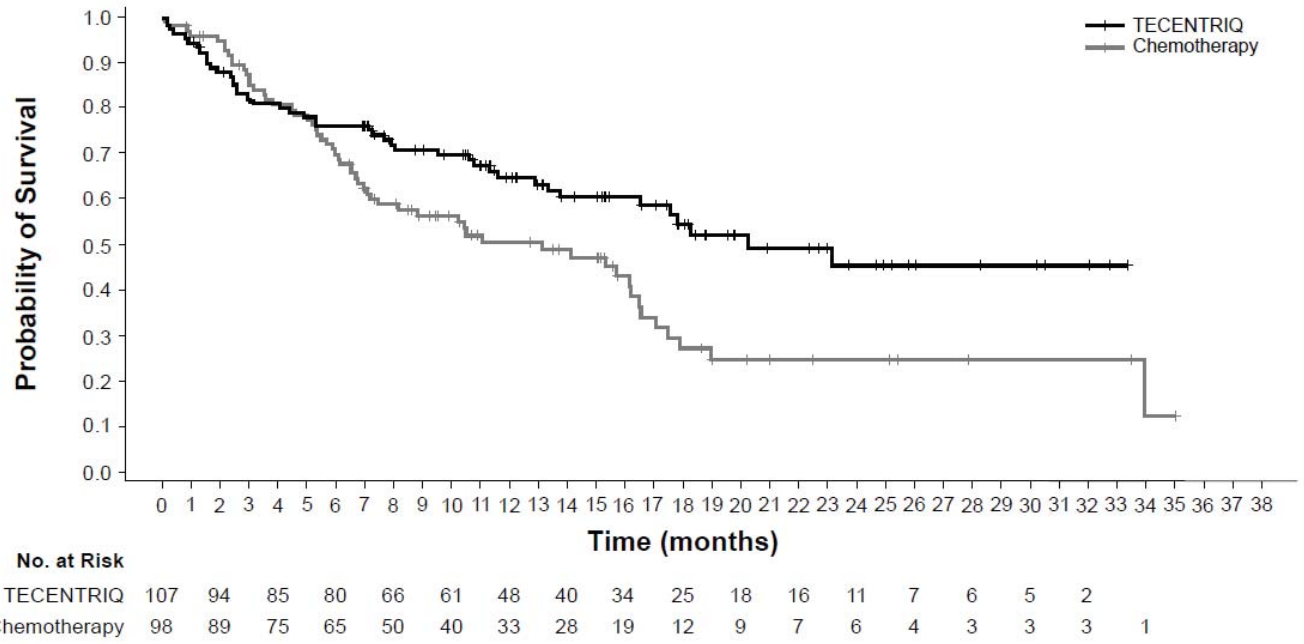


Figure 2: Kaplan-Meier plot of overall survival in IMpower110 in patients with NSCLC with high PD-L1 expression (TC ≥ 50% or IC ≥ 10%) and without EGFR or ALK genomic tumor aberrations

The performance of VENTANA PD-L1 (SP142) Assay was investigated in OAK (NCT02008227), a Phase III multi-center, international, randomized, open-label trial designed to evaluate the efficacy and safety of TECENTRIQ treatment in patients with metastatic NSCLC who progressed during or following a platinum-containing regimen.

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression. Patient specimens were FFPE NSCLC tissue from needle cores (34.5%), punch biopsies (16.3%), resections (28.7%), or of other type (20.5%); 66.6% were from primary tumors and 33.4% from metastatic tumors.

Table 19 describes the overall staining acceptability rate for VENTANA PD-L1 (SP142) Assay among all NSCLC specimens screened for the study. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 1185 specimens, 72 failed the initial staining attempt and staining was repeated. 26 of the 72 samples remained unacceptable (1 due to unacceptable tonsil control, 19 due to unacceptable negative reagent control and 6 due to unacceptable background or morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial (i.e., first-pass) and final overall staining acceptability rates: 93.9% and 97.8%, respectively. Final morphology and background acceptability rates were greater than 99%.

Table 19. VENTANA PD-L1 (SP142) Assay NSCLC staining performance characteristics in OAK.

Attribute	Acceptability rate % (n/N) (95% CI) [a]	
	Initial[b]	Final[c]
Overall staining acceptability rate	93.9 (1113/1185) (92.4-95.1)	97.8 (1159/1185) (96.8-98.5)
Morphology	98.5 (1122/1139) (97.6-99.1)	99.6 (1160/1165) (99.0-99.8)
Background	98.2 (1119/1139) (97.3-98.9)	99.7 (1161/1165) (99.1-99.9)

[a] Two-sided Wilson score method CI

[b] Initial staining attempt [c] Final staining attempt

The OAK study enrolled 1225 patients with the primary analysis population consisting of the first 850 randomized patients; eligible patients were stratified by PD-L1 expression status in IC, by the number of prior chemotherapy regimens, and by histology. Patients were randomized (1:1) to receive either TECENTRIQ administered intravenously at 1200 mg every 3 weeks until unacceptable toxicity or either radiographic or clinical progression or docetaxel administered intravenously at 75 mg/m2 every 3 weeks until unacceptable toxicity or disease progression. Tumor specimens were evaluated prospectively for PD-L1 expression on TC and IC using VENTANA PD-L1 (SP142) Assay and the results were used to define the PD-L1 expression subgroups for pre-specified analyses described below.

In the OAK study, among patients in the primary analysis population, the median age was 64 years (range: 33 to 85), and 61% of patients were male. The majority of patients were white (70%). Approximately three-fourths of patients had non-squamous disease (74%), 10% had known EGFR mutation, 0.2% had known ALK rearrangements, and most patients were current or previous smokers (82%). Baseline ECOG performance status was 0 (37%) or 1 (63%). Seventy-five percent of patients received only one prior platinum-based therapeutic regimen.

The major efficacy outcome measure of the OAK study was overall survival (OS) in the primary analysis population (first 850 randomized patients). The results of the OAK study with a median follow up of 21 months are presented in Table 20 and Figure 3.

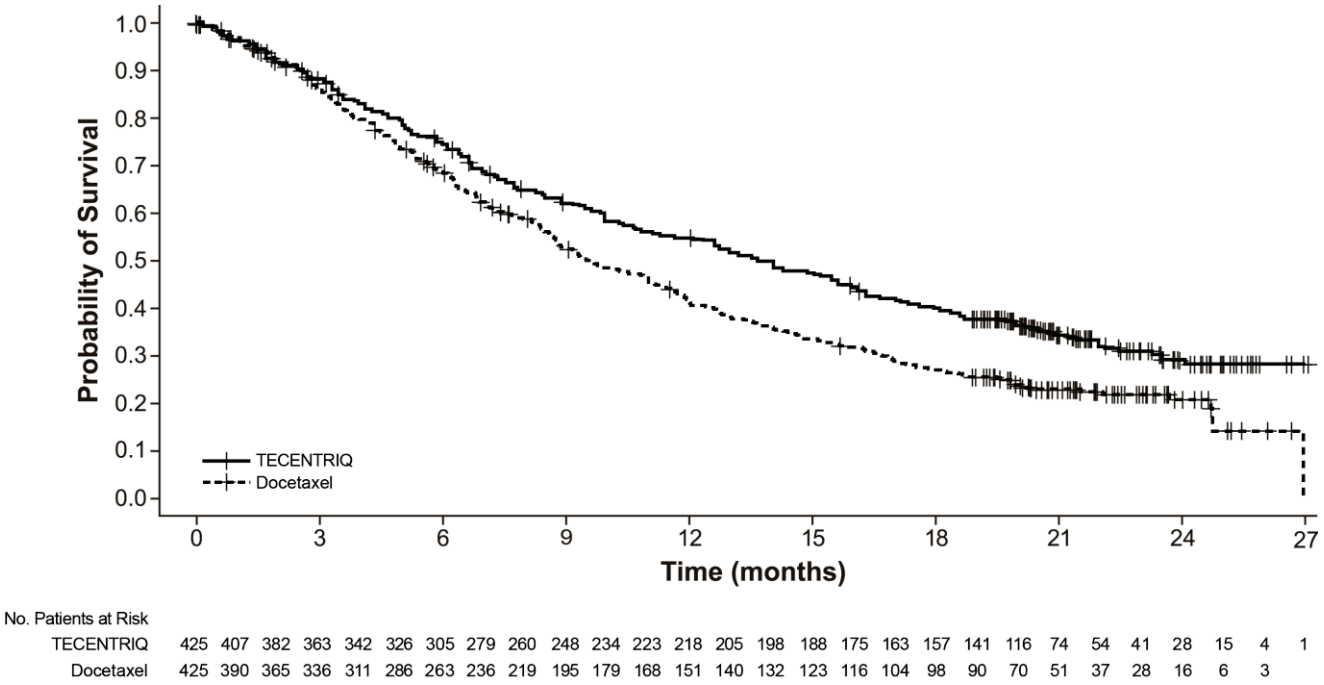
Tumor specimens were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used to define the PD-L1 expression subgroups for pre-specified analyses. Of the 850 patients, 16% were classified as having high PD-L1 expression, defined as having PD-L1 expression on ≥ 50% TC or ≥ 10% IC. In an exploratory efficacy subgroup analysis of OS based on PD-L1 expression, the hazard ratio was 0.41 (95% CI: 0.27, 0.64) in the high PD-L1 expression subgroup and 0.82 (95% CI: 0.68, 0.98) in patients who did not have high PD-L1 expression.

Table 20. Efficacy results in the primary analysis population from the OAK study.

Overall Survival	TECENTRIQ (n=425)	Docetaxel (n=425)
Deaths (%)	271 (64)	298 (70)
Median, months (95% CI)	13.8 (11.8, 15.7)	9.6 (8.6, 11.2)
Hazard ratio[a] (95% CI)	0.74 (0.63, 0.87)	
p-value[b]	0.0004	

[a] Stratified by PD-L1 expression in tumor-infiltrating immune cells, the number of prior chemotherapy regimens, and histology

[b] Based on the stratified log-rank test, CI = confidence interval



No. Patients at Risk

TECENTRIQ	425	407	382	363	342	326	305	279	260	248	234	223	218	205	198	188	175	163	157	141	116	74	54	41	28	15	4	1
Docetaxel	425	390	365	336	311	286	263	236	219	195	179	168	151	140	132	123	116	104	98	90	70	51	37	28	16	6	3	

Figure 3. Kaplan-Meier plot of overall survival in the primary analysis population of OAK (NCT02008227)

PERFORMANCE CHARACTERISTICS

TNBC

Scoring Algorithm – TNBC

TNBC tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC provided in Table 21. Refer to the interpretation guide (P/N 1018231EN) for additional instructions and representative images.

Table 21. VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC.

Immune Cell (IC) Staining Assessment[a]	PD-L1 Expression
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 1% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 1% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 1% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 1% IC

[a] PD-L1 staining in tumor cells should not be included in the scoring determination of TNBC patient tissue.

Tissue Thickness – TNBC

Tissue thickness was evaluated using 10 unique TNBC specimens (5 PD-L1 ≥ 1% IC and 5 PD-L1 < 1% IC). Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 status within the range of thickness tested. Ventana recommends that specimens be cut at 4 microns for staining with VENTANA PD-L1 (SP142) Assay.

Repeatability and Intermediate Precision - TNBC

Studies for VENTANA PD-L1 Assay staining of TNBC specimens were completed to demonstrate:

- Intra-day Repeatability – 5 replicate slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within one day.
- Inter-day Precision – 10 slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days.
- Inter-instrument and Inter-lot Precision – 27 slides each from 24 unique TNBC specimens (12 PD-L1 ≥ 1% IC and 12 PD-L1 < 1% IC) were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC (Table 21). Results are summarized in Table 22.

Table 22. Repeatability and intermediate precision of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Repeatability/Intermediate Precision Parameter	Agreement % (95% CI)
Intra-day repeatability (within a single day)	PPA: 100.0 (94.0-100.0) NPA: 95.0 (87.2-100.0) OPA: 97.5 (93.3-100.0)
Inter-day precision (5 non-consecutive days)	PPA: 100.0 (96.9-100.0) NPA: 96.7 (92.7-100.0) OPA: 98.3 (96.3-100.0)
Inter-instrument and Inter-lot precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	PPA: 98.3 (96.0-100.0) NPA: 99.2 (97.2-100.0) OPA: 98.6 (97.1-99.8)

Reader Precision – TNBC

To assess Inter- and Intra-Reader Precision, three pathologists evaluated 60 unique TNBC specimens (30 PD-L1 ≥ 1% IC and 30 PD-L1 < 1% IC) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm for TNBC (Table 21). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and between each pathologist's reads are summarized in Table 23.

Table 23. Inter- and intra-reader precision of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Reader Precision	Agreement % (95% CI)
Inter-reader precision (average of reader-to-reader pairwise comparisons from first read)	APA: 91.1 (86.0-95.7) ANA: 91.1 (86.1-95.6) OPA: 91.1 (86.7-95.6)
Intra-reader precision (average of all three readers' agreement rates between first and second reads)	APA: 93.8 (89.5-97.1) ANA: 93.9 (89.2-97.3) OPA: 93.9 (89.9-97.2)

Inter-laboratory Reproducibility Study – TNBC

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in TNBC specimens. Twenty-eight unique TNBC specimens (14 PD-L1 ≥ 1% IC and 14 PD-L1 < 1% IC) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The sample set consisted of a total of 419 case slides (140 slides for two sites and 139 slides for the third site) generated from 28 unique TNBC specimens. The final staining acceptability rate for the VENTANA PD-L1 (SP142) Assay was 98.6% in this study. Results are summarized in Table 24.

Table 24. Inter-laboratory reproducibility of VENTANA PD-L1 (SP142) Assay staining of TNBC specimens.

Inter-laboratory Reproducibility[a]	Agreement % (95% CI)
Overall agreement (compared to a consensus score, across sites, days and readers)	PPA: 93.2 (90.4-95.2)[b] NPA: 96.6 (94.4-98.0)[b] OPA: 94.8 (93.1-96.1)[b]
Inter-site agreement (average of site-to-site pairwise comparisons)	APA: 91.5 (85.6-96.0) ANA: 91.3 (86.6-95.7) OPA: 91.4 (86.4-95.9)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	APA: 93.6 (88.2-97.0) ANA: 93.3 (87.8-96.7) OPA: 93.4 (90.6-95.4)[b]

[a] n = 419 evaluable case slides

[b] Two-sided Wilson score method CI

CLINICAL PERFORMANCE

TNBC

The performance of VENTANA PD-L1 (SP142) Assay was investigated in IMpassion130 (NCT02425891), a multicenter, international, double-blinded, placebo-controlled, randomized trial designed to evaluate the efficacy and safety of TECENTRIQ in combination with paclitaxel protein-bound in patients with unresectable locally advanced or metastatic TNBC patients that had not received prior chemotherapy for metastatic disease.

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression. Patient specimens were FFPE TNBC tissue from biopsies (66.8%), resections (29.3%), or of other type (3.9%); 64.7% were from primary tumors and 35.3% from metastatic tumors.

Table 25 describes the overall staining acceptability rate for VENTANA PD-L1 (SP142) Assay among all TNBC specimens screened for the study. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 1284 specimens, 52 failed the initial staining attempt and staining was repeated on 49 samples. There remained 23 unacceptable samples (17 of the 23 due to unacceptable negative reagent control and 6 due to unacceptable background or morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial (i.e., first-pass) and final overall staining acceptability rates: 96.0% and 98.0%, respectively. Final morphology and background acceptability rates were greater than 98%.

Table 25. VENTANA PD-L1 (SP142) Assay TNBC staining performance characteristics.

Attribute	Acceptability rate % (n/N) (95% CI)[a]	
	Initial[b]	Final[c]
Overall staining acceptability rate	96.0 (1232/1284) (94.7-96.9)	98.0 (1258/1284) (97.0-98.6)
Morphology	98.4 (1232/1252) (97.5-99.0)	99.5 (1258/1264) (99.0-99.8)
Background	100.0 (1232/1232) (99.7-100.0)	100.0 (1258/1258) (99.7-100.0)

[a] Two-sided Wilson score method CI

[b] Initial staining attempt [c] Final staining attempt

IMpassion130 enrolled 902 patients; eligible patients were stratified by presence of liver metastases, prior taxane treatment, and by PD-L1 expression status in IC by the VENTANA PD-L1 (SP142) Assay (< 1% IC vs. ≥ 1% IC). Of the 902 patients in the intent to treat population (ITT), 41% (369 patients) were classified as PD-L1 expression ≥ 1% IC. Patients were then randomized (1:1) to receive either TECENTRIQ (840 mg) or placebo intravenous infusions on Days 1 and 15 of every 28-day cycle, plus paclitaxel protein-bound (100 mg/m^2) administered via intravenous infusion on Days 1, 8 and 15 of every 28-day cycle. Patients received treatment until radiographic disease progression per RECIST v1.1, or unacceptable toxicity.

Patients were excluded if they had a history of autoimmune disease, administration of a live attenuated vaccine within 4 weeks prior to randomization, administration of systemic immunostimulatory agents within 4 weeks or systemic immunosuppressive medications within 2 weeks prior to randomization; or untreated or corticosteroid-dependent brain metastases. Tumor assessments were performed every 8 weeks (± 1 week) for the first 12 months after Cycle 1, day 1 and every 12 weeks (± 1 week) thereafter.

In IMpassion130, the median age was 55 years (range: 20-86). Overall, most patients were women (99.6%) and the majority of patients were white (68%), Asian (18%), Black or African American (7%), and American Indian or Alaskan Native (4.4%). The demographic and baseline disease characteristics of the study population were well balanced between the treatment arms. Baseline ECOG performance status was 0 (58%) or 1 (41%). Overall, 41% of enrolled patients had PD-L1 expression ≥ 1% IC, 27% had liver metastases and 7% brain metastases at baseline. Approximately half the patients had received a taxane (51%) or anthracycline (54%) in the (neo)adjuvant setting. Patient demographics and baseline tumor disease in the PD-L1 expressing population were generally representative of the broader study population.

Tumor specimens (archival or fresh) were evaluated prospectively using the VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used as a stratification factor for randomization and to define the PD-L1 expression subgroups for pre-specified analyses.

The major efficacy outcomes were investigator-assessed progression free survival (PFS) in the ITT and PD-L1 expressing patient population per RECIST v1.1 and overall survival (OS) in the ITT population. Overall survival data were immature with 43% deaths in the ITT population. The efficacy results of IMpassion130 for the patient population with PD-L1 expression ≥ 1% IC are presented in Table 26 and Figure 4.

Table 26. Efficacy Results from IMpassion130 in patients with PD-L1 expression ≥ 1% IC.

	PD-L1 Expression ≥ 1%[a]	
	TECENTRIQ in combination with paclitaxel protein-bound	Placebo in combination with paclitaxel protein-bound
Progression-Free Survival[b],[c]	(n=185)	(n=184)
Events (%)	136 (74)	151 (82)
Median, months	7.4 (6.6, 9.2)	4.8 (3.8, 5.5)
Stratified Hazard ratio (95% CI)[d]	0.60 (0.48, 0.77)	
p-value	<0.0001	
Objective Response Rate[b],[c],[e],[f]	n=185	n=183
Number of responders (%)	98 (53)	60 (33)
(95% CI)	(45.5, 60.3)	(26.0, 40.1)
Complete response (%)	17 (9)	1 (<1)
Partial response (%)	81 (44)	59 (32)
Duration of Response[b],[c],[f]	n=98	n=60
Median (months)	9.2	6.2
(95% CI)	(7.5, 11.9)	(5.5, 8.8)

[a] PD-L1 expression in tumor-infiltrating immune cells (IC)

[b] As determined by investigator assessment

[c] per RECIST v1.1 (Response Evaluation Criteria in Solid Tumors v1.1)

[d] Stratified by presence of liver metastases, and by prior taxane treatment

[e] Patients with measurable disease at baseline

[f] Confirmed responses

PFS=Progression-Free Survival; CI=Confidence Interval; ORR=Objective Response Rate; DOR=Duration of Response; NE=Not Estimable

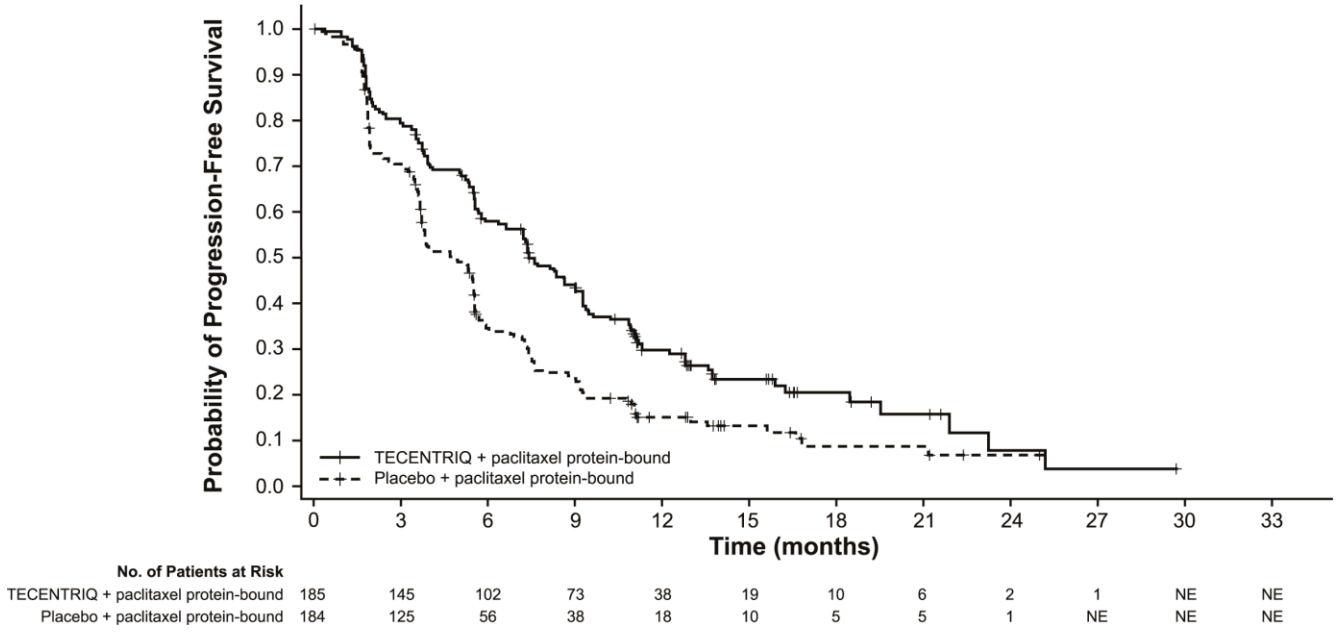


Figure 4. Kaplan-Meier plot of progression-free-survival in IMpassion130 in patients with PD-L1 expression ≥ 1% IC

TROUBLESHOOTING

Troubleshooting guidance is provided in Table 27. If a problem cannot be attributed to any of these causes, or if the suggested corrective action fails to resolve the problem, consult your local support representative.

Table 27. Troubleshooting guidance for VENTANA PD-L1 (SP142) Assay.

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that U OptiV DAB VENTANA PD-L1 (SP142) procedure was used.
		Verify that VENTANA PD-L1 (SP142) was selected for Primary Antibody
	Degradation of tissue	Verify tissue was stained within the recommended time frame following sectioning.
	Dispenser malfunction	Verify nozzle cap is removed.
		Ensure dispenser is primed
		Check the priming chamber for foreign materials or particulates, such as fibers or precipitate
		Refer to inline dispenser package insert associated with P/N 740-4859 located at www.ventana.com
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that U OptiV DAB VENTANA PD-L1 (SP142) procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

REFERENCES

1. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother. 2007;56(5):739-745.
2. Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 2007;27(1):111-122.
3. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-567.
4. Bellmunt J, Mullane SA, Werner L, et al. Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma. Ann Oncol. 2015;26(4):812-817.
5. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-3175.
6. Fehrenbacher L, Spira A, Ballinger M et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;387(10030):1837-1846.
7. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558-562.
8. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909–20.
9. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-2454.
10. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018;379(22):2108-2121.
11. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
12. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
13. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
14. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
15. Anatomic Pathology Checklist. College of American Pathologists. Jul 28, 2015.
16. CLSI. Quality assurance for design control and implementation of immunohistochemistry assay: approved guidelines. 2nd edition Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011.
17. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
18. Hautzer NW, Wittkuhn JF, McCaughey WT. Trypsin digestion in immunoperoxidase staining. J Histochem Cytochem. 1980;28(1):52-53.
19. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. 1980;73(5):626-632.
20. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

GTIN Global Trade Item Number

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)
www.ventana.com

VENTANA PD-L1 (SP142) Assay

Interpretation Guide for Non-Small Cell Lung Cancer

≥ 50% TC or ≥ 10% IC Stepwise Scoring Algorithm

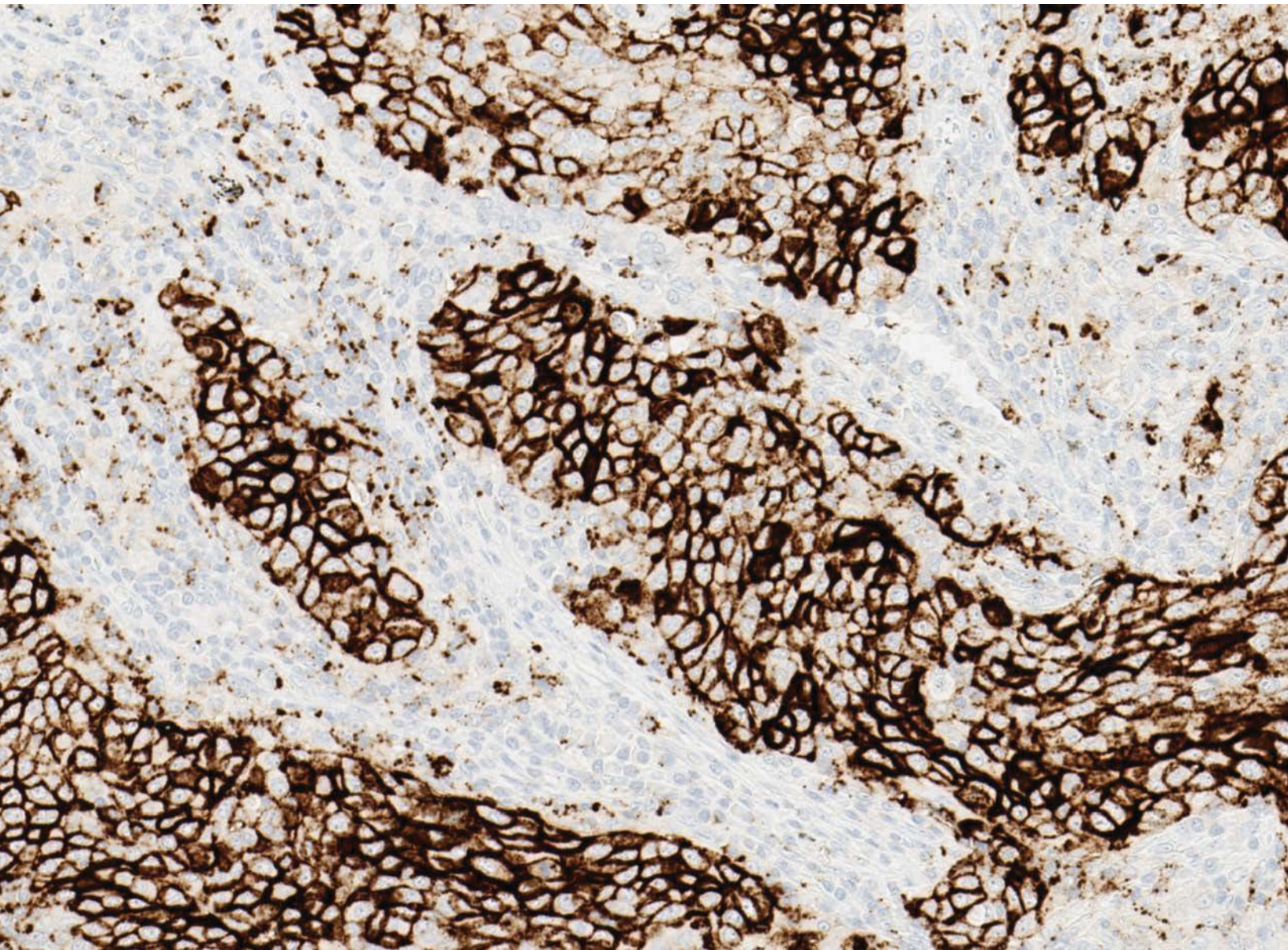


Table of Contents

Introduction 1

Intended Use 2
Intended Use of Product 2
Purpose of Interpretation Guide 2

Clinical Evaluation 3
Staining Overview 3
VENTANA PD-L1 (SP142) Assay Scoring Algorithm – NSCLC 4
Specimen Flow 6
Controls 7
Staining Characteristics – NSCLC 8
Differentiation of TC and IC Staining: 12
Scoring Method 14
Scoring of PD-L1 IC aggregate staining: 15
Scoring of PD-L1 single-cell spread IC staining: 16
Scoring Methods: Challenges and Pitfalls 17

Reference Images 20
TC Expression 20
IC Expression - Aggregates 21
IC Expression – Single-Cell Spread 22
Example Cases: TC < 50% and IC < 10% 23
Example Cases: TC ≥ 50% 27
Example Cases: IC ≥ 10% 29
Challenging Cases 32

Staining Artifacts 36
Impact of Pre-Analytical Conditions on VENTANA PD-L1 (SP142) Assay 39
Acceptable Fixation Conditions to Achieve Optimal Staining Results with VENTANA PD-L1 (SP142) Assay 39
Antigen Stability on Cut Tissue Sections 40

References 41

Introduction

VENTANA PD-L1 (SP142) Assay is an immunohistochemical assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody to recognize the programmed death-ligand 1 (PD-L1) protein. This assay was co-developed by Roche/Ventana Medical Systems, Inc. (Ventana) and Roche/Genentech to identify patients who are most likely to respond to treatment with TECENTRIQ® (atezolizumab).

Lung cancer has been the most common cancer in the world for several decades and remains the leading cause of cancer deaths worldwide. It is estimated to account for 12.9% of all new cancer cases and is responsible for nearly 1.59 million deaths annually worldwide, or approximately one in five cancer-related deaths.[1] In the European Union alone, approximately 274,000 lung cancer-related deaths are predicted for 2016.[2] Although improvements have been made in diagnosis and therapy options, prognosis remains poor with low long-term survival rates for all stages. Over the past three decades, lung cancer has shown among the least improvement in survival rates when compared with other cancers.[3]

Non-small cell lung cancer (NSCLC), one of the two major types of lung cancer, accounts for approximately 85% of all lung cancer cases.[4] In more than half of patients newly diagnosed with NSCLC, the disease has already metastasized, greatly decreasing the likelihood of survival. The 5-year relative survival rate for NSCLC diagnosed as distant disease is 4.7%.[4] The majority of patients with NSCLC present with inoperable, locally advanced disease (Stage IIIB) or metastatic disease (Stage IV), neither of which currently has any curative treatment options; on average, these patients die within a year of diagnosis. Improvement in the clinical outcome of lung cancer is likely to be achieved through better understanding of the molecular events that underlie its pathogenesis, identifying new biomarker targets, and developing new treatment options.

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors, programmed death-1 (PD-1) and B7.1 (**Figure 1**). PD-1 is an inhibitory receptor expressed on T-cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[5] Ligation of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T-cells. B7.1 is a molecule expressed on antigen presenting cells and activated T-cells. PD-L1 binding to B7.1 on T-cells and antigen-presenting cells can mediate downregulation of immune responses,

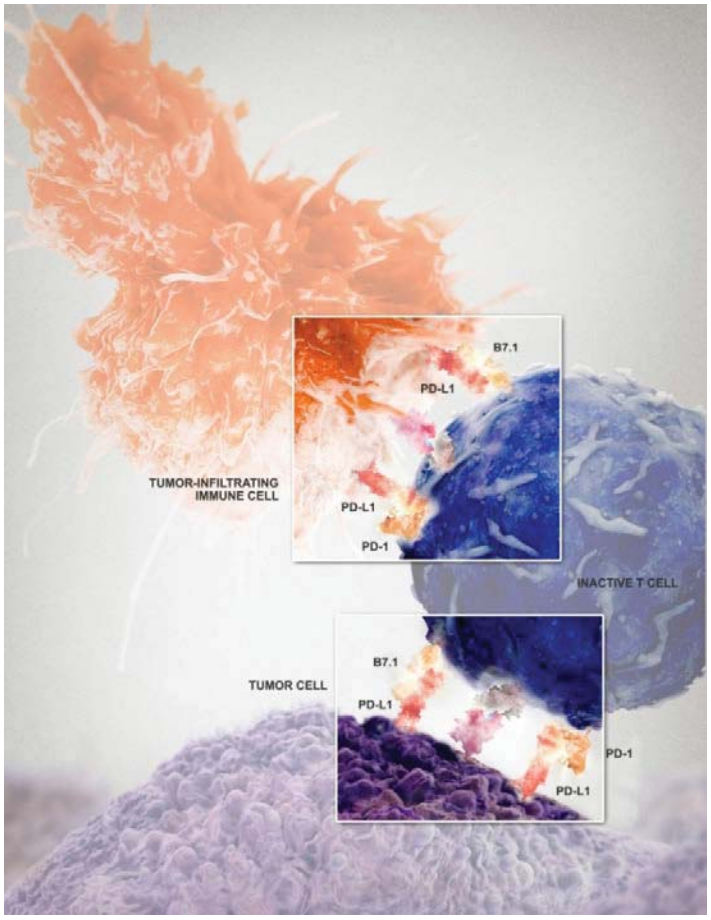


Figure 1: PD-1, PD-L1 pathway.

including inhibition of T-cell activation and cytokine production.[6] PD-L1 expression has been observed in immune cells and malignant cells and aberrant expression of PD-L1 on tumor cells (TC) has been reported to impede anti-tumor immunity, resulting in immune evasion.[5, 7] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment. The association between PD-L1 expression in TC or tumor-infiltrating immune cells (IC) and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported across multiple cancers.[7-10]

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors. Atezolizumab is a non-glycosylated IgG1 kappa immunoglobulin that has a calculated molecular mass of 145 kD.

Intended Use

Intended Use of Product

Refer to the corresponding VENTANA PD-L1 (SP142) Assay package insert for the detailed intended use of this product.

Note: Use of this diagnostic with indicated therapies may not be approved in all countries. Please consult your local Roche representative for local approvals.

Purpose of Interpretation Guide

The VENTANA PD-L1 (SP142) Assay interpretation guide is designed to assist pathologists in interpreting and scoring NSCLC tissues stained with VENTANA PD-L1 (SP142) Assay.

- The photomicrographs included as part of this training guide illustrate the staining patterns, as well as the range of PD-L1 expression, which may be present in NSCLC tissues stained with VENTANA PD-L1 (SP142) Assay.
- The staining criteria in this interpretation guide outlines the scoring of NSCLC tissue stained with VENTANA PD-L1 (SP142) Assay using a ≥ 50% of TC or ≥ 10% of IC stepwise approach.
- The use of tonsil as a tissue control in the context of PD-L1 evaluation, and the associated staining characteristics and performance are addressed.
- Challenging cases, staining artifacts, and the impact of pre-analytic conditions on the assay are also addressed.

Clinical Evaluation

Staining Overview

Immunohistochemical (IHC) staining with VENTANA PD-L1 (SP142) Assay demonstrates staining in tumor cells (TC, **Figure 2**) as well as tumor-infiltrating immune cells (IC, **Figure 3**). Detailed staining characteristics are described in the Staining Characteristics section.

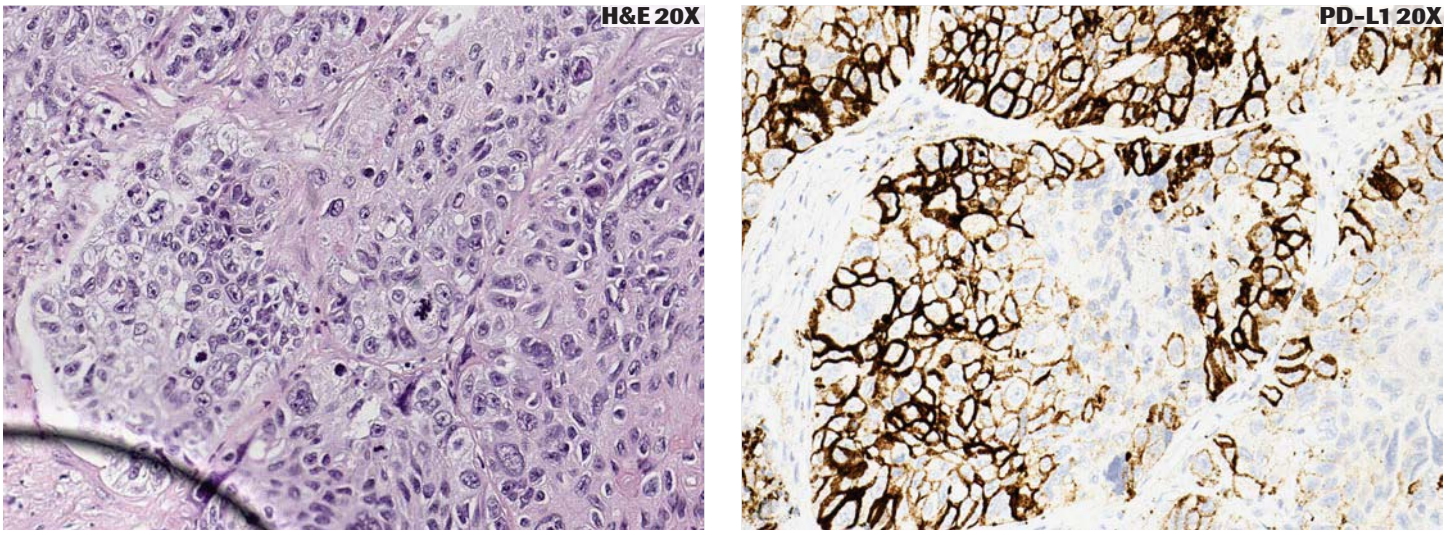


Figure 2: NSCLC tissue showing moderate to strong circumferential TC membrane staining.

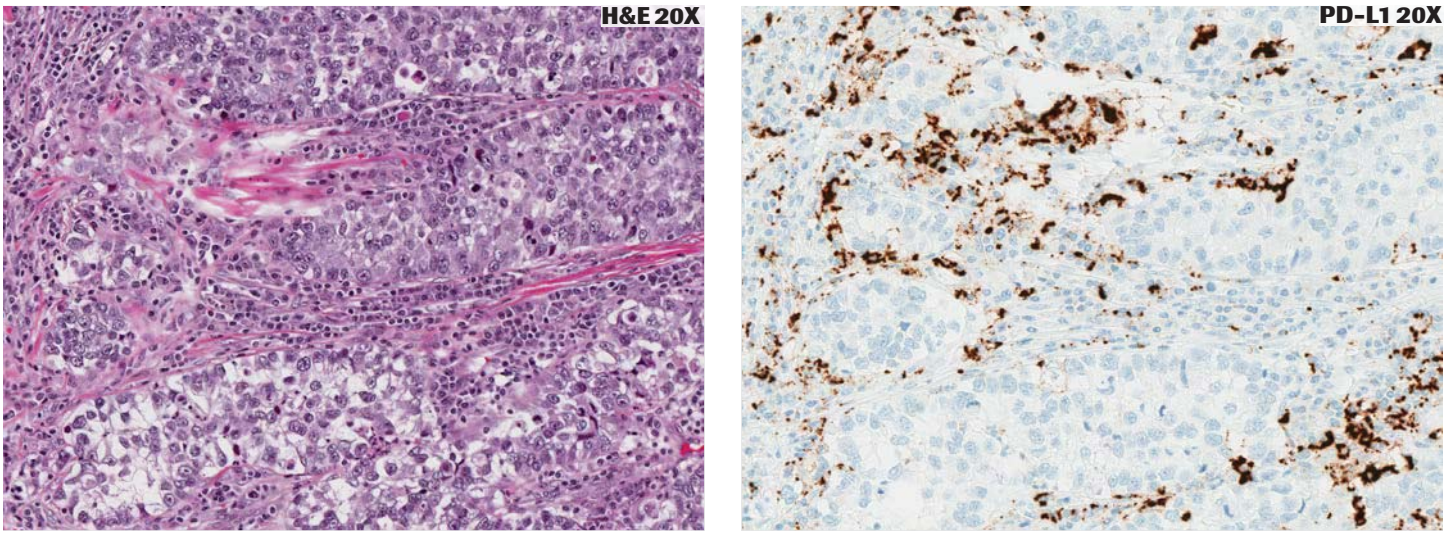


Figure 3: NSCLC tissue showing dark brown punctate and linear IC staining.

VENTANA PD-L1 (SP142) Assay Scoring Algorithm – NSCLC

NSCLC tissue stained with VENTANA PD-L1 (SP142) Assay will be scored using a stepwise approach according to the criteria outlined in **Table 1**. TC are scored as the proportion of viable tumor cells showing PD-L1 membrane staining of any intensity. IC are scored as the proportion of tumor area that is occupied by PD-L1 staining IC of any intensity. High PD-L1 expression is defined as having PD-L1 expression on ≥ 50% of TC or ≥ 10% of IC. VENTANA PD-L1 (SP142) Assay stained slides will first be evaluated for TC staining (Step 1 in **Table 1**). If the specimen contains any discernible PD-L1 membrane staining of any intensity in ≥ 50% TC, the case will be assigned a PD-L1 expression level of ≥ 50% TC. If the specimen contains < 50% TC staining, the slide will then be evaluated for IC staining (Step 2 in **Table 1**). If the specimen contains PD-L1 staining of any intensity in IC occupying ≥ 10% of tumor area, the case will be assigned a PD-L1 expression level of ≥ 10% IC. If the specimen contains PD-L1 staining of any intensity in IC covering < 10% of tumor area, the case will be assigned a PD-L1 expression level of < 50% TC and < 10% IC. The stepwise scoring process is illustrated in **Figure 4**.

NSCLC tissue samples obtained from resections, excisions, core needle and other biopsy procedures from both primary and metastatic sites are acceptable. This assay has not been validated for use with cytology samples or decalcified bone specimens. A tissue is considered adequate for VENTANA PD-L1 (SP142) Assay interpretation if it contains at least 50 viable tumor cells; tumor associated stroma is not required for TC scoring. Presence of tumor associated stroma is essential for scoring IC. Staining requires three sections from each case, one serial section for hematoxylin & eosin (H&E) staining, a second for negative reagent control staining, and a third for VENTANA PD-L1 (SP142) Assay staining. Prequalified benign tonsil tissue should be used as positive and negative tissue control for each staining run. Detailed instructions for control tissue qualification and acceptability are outlined in **Table 3**. Matched patient's tissue should be stained with negative reagent control to assess nonspecific background staining.

Table 1: VENTANA PD-L1 (SP142) Assay Stepwise Scoring Algorithm for NSCLC

Step 1: Tumor Cell (TC) Staining Assessment	**PD-L1 Expression**
Presence of discernible PD-L1 membrane staining of any intensity in ≥ 50% of tumor cells	≥ 50% TC
Absence of any discernible PD-L1 staining (OR) Presence of discernible PD-L1 membrane staining of any intensity in < 50% of tumor cells.	Proceed to Step 2
Step 2: Tumor Infiltrating Immune Cell (IC) Staining Assessment	**PD-L1 Expression**
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 10% of tumor area occupied by tumor cells, associated intratumoral and contiguous peritumoral stroma	≥ 10% IC
Absence of any discernible PD-L1 staining (OR) Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 10% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 50% TC and < 10% IC

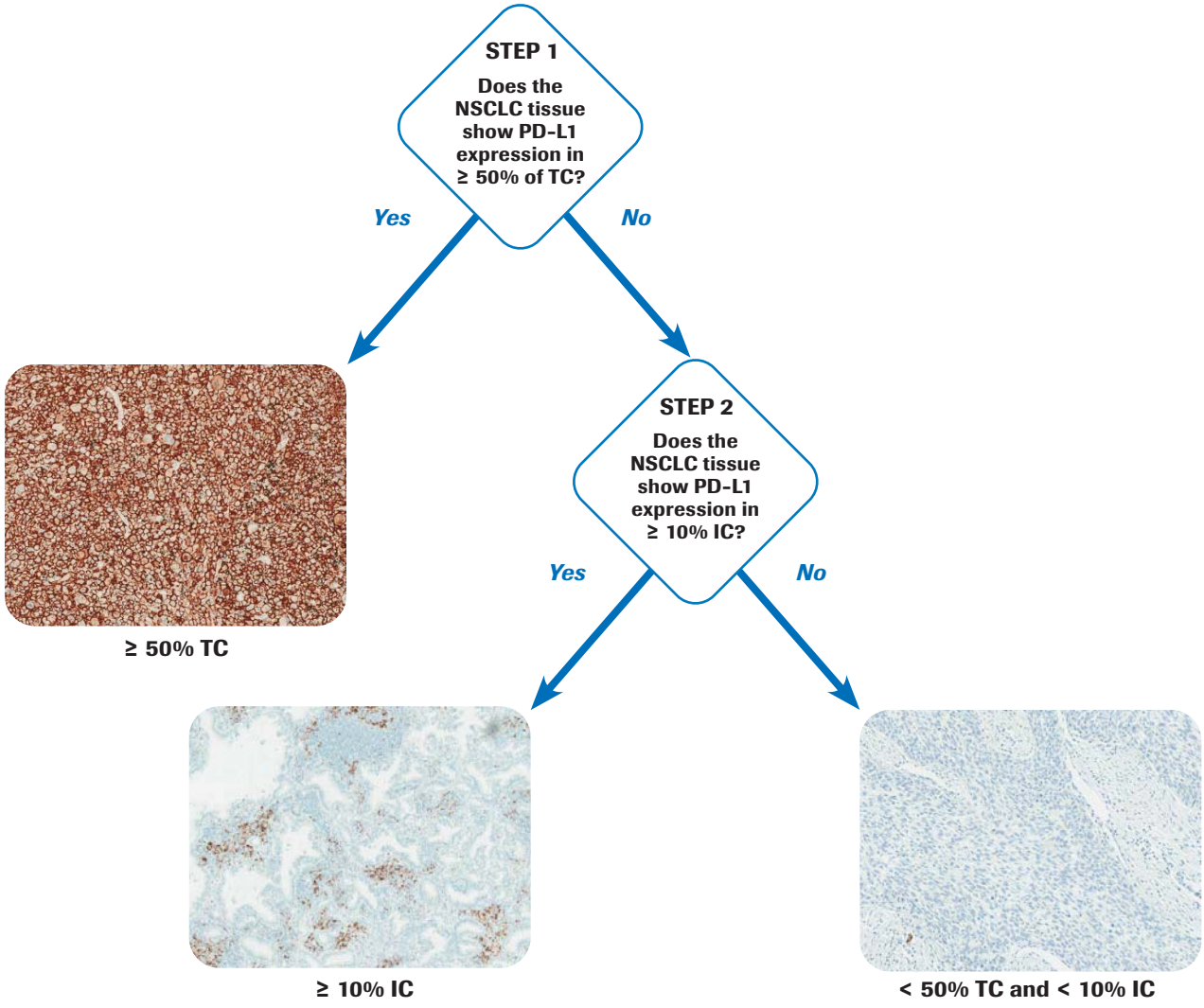
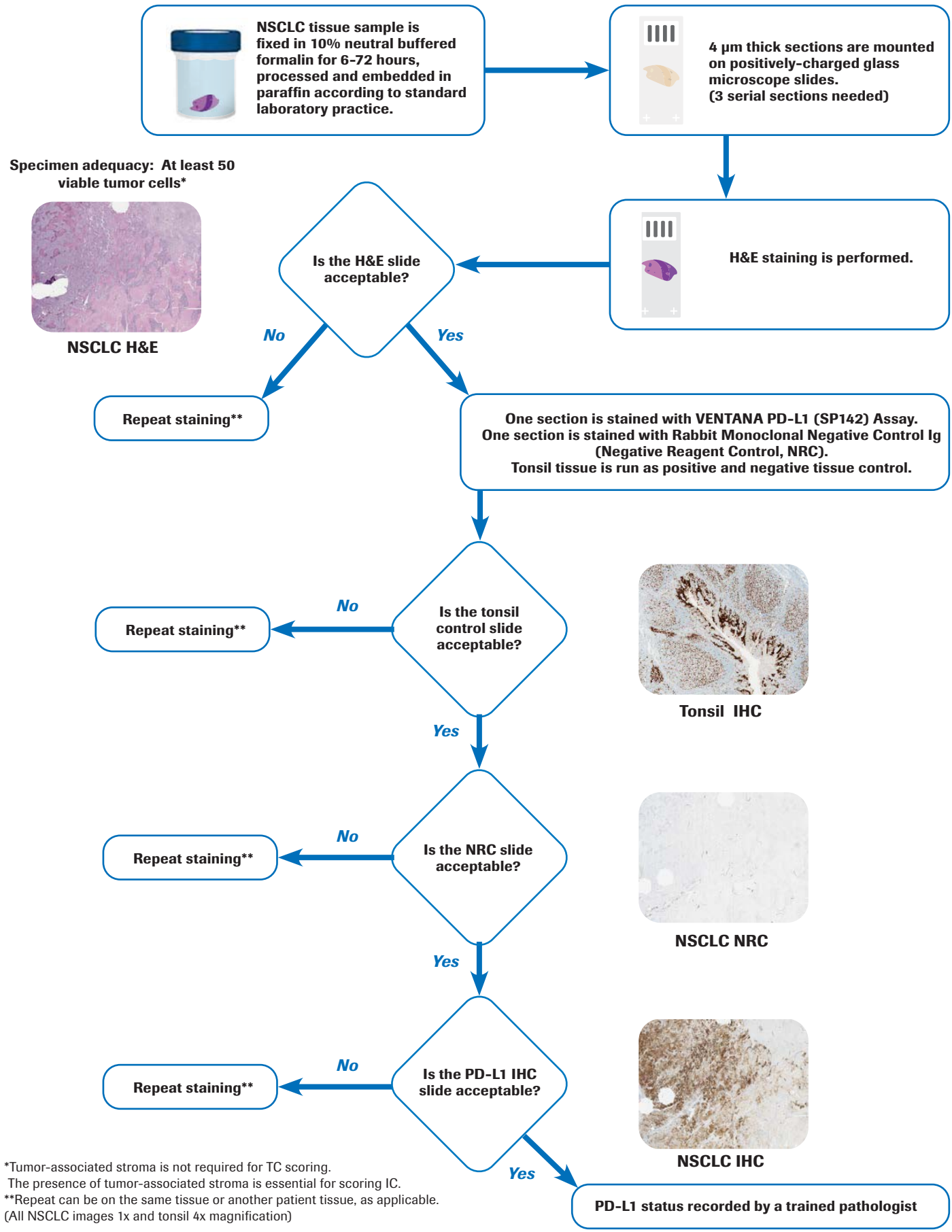


Figure 4: Stepwise scoring process – VENTANA PD-L1 (SP142) Assay in NSCLC tissues (images 10x magnification).

Specimen Flow



*Tumor-associated stroma is not required for TC scoring.
The presence of tumor-associated stroma is essential for scoring IC.
**Repeat can be on the same tissue or another patient tissue, as applicable.
(All NSCLC images 1x and tonsil 4x magnification)

Controls

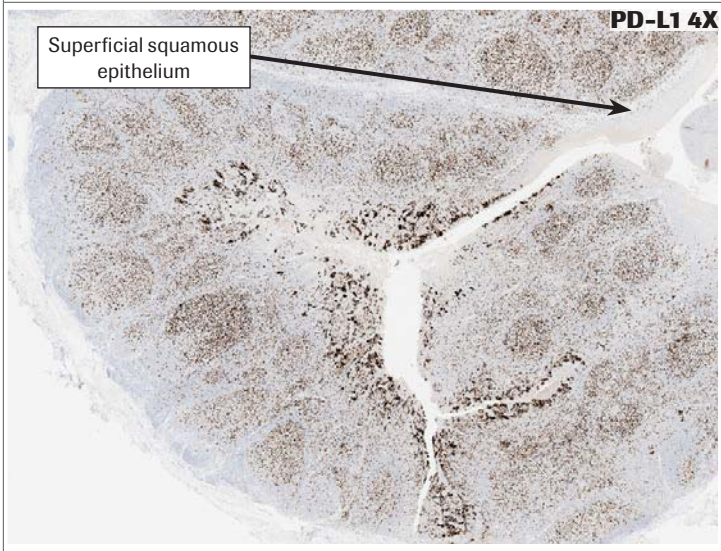
Tissue controls will be used only for monitoring the correct performance of processed tissues, test reagents and instruments, not as an aid in formulating a specific score for patient samples. One tissue control for each set of test conditions is recommended in each staining run (on-slide controls are acceptable).

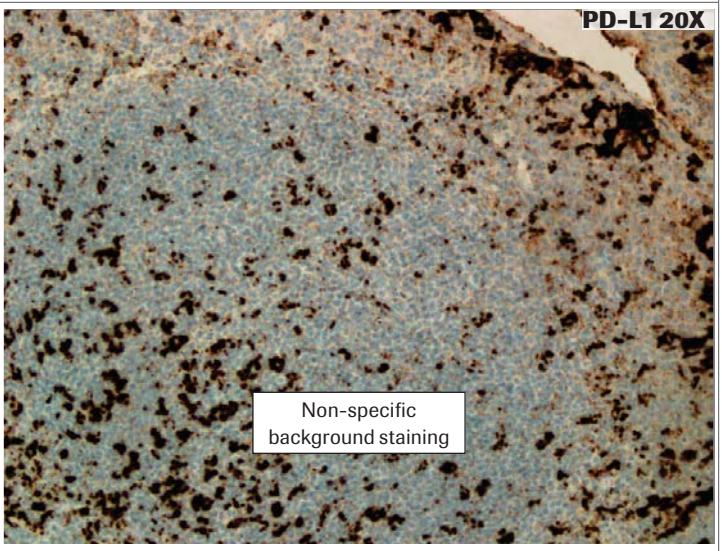
Benign human tonsil is an ideal tissue control as it contains both positive and negative staining epithelial and immune cells and can serve as both a positive and negative tissue control for VENTANA PD-L1 (SP142) Assay. Tonsil tissue stained with VENTANA PD-L1 (SP142) Assay demonstrates staining of lymphocytes and macrophages in germinal centers, with scattered PD-L1 staining immune cells among PD-L1-negative cells in interfollicular regions. Also, diffuse staining is observed in the reticulated crypt epithelial cells with an absence of staining of superficial squamous epithelial cells.

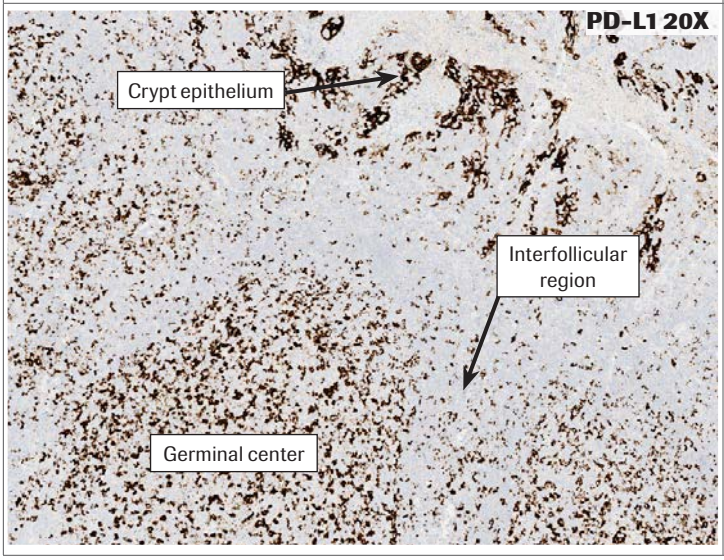
Tonsil tissue fixed in 10% NBF and processed similar to patient tissues should be qualified and used as a tissue control. The tonsil tissue control should show acceptable staining for an assay run to pass. If tonsil tissue shows unacceptable staining, the run is considered invalid and a repeat run, including patient samples, should be performed. Qualification and acceptability criteria for tonsil tissue controls are listed in **Table 2**.

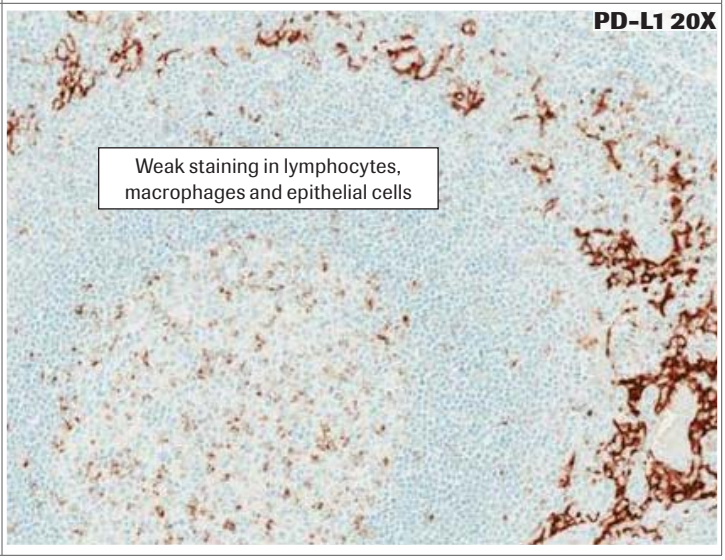
Table 2: Tonsil Qualification and Acceptability Criteria

Acceptable	Unacceptable
Negative tissue elements: PD-L1 negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Excessive non-specific background staining obscuring the identification of PD-L1 positive cells.
Positive tissue elements: Moderate to strong PD-L1 staining noted in lymphocytes, and macrophages in germinal centers, with diffuse staining in reticulated crypt epithelial cells.	Weak to no PD-L1 staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.









Staining Characteristics – NSCLC

PD-L1 staining with VENTANA PD-L1 (SP142) Assay in NSCLC tissues demonstrates staining in TC (**Figure 5–Figure 7**) as well as IC (**Figure 8–Figure 12)**. The images in this interpretation guide are snapshots from scanned slides; magnification is noted for each image.

TC Staining:

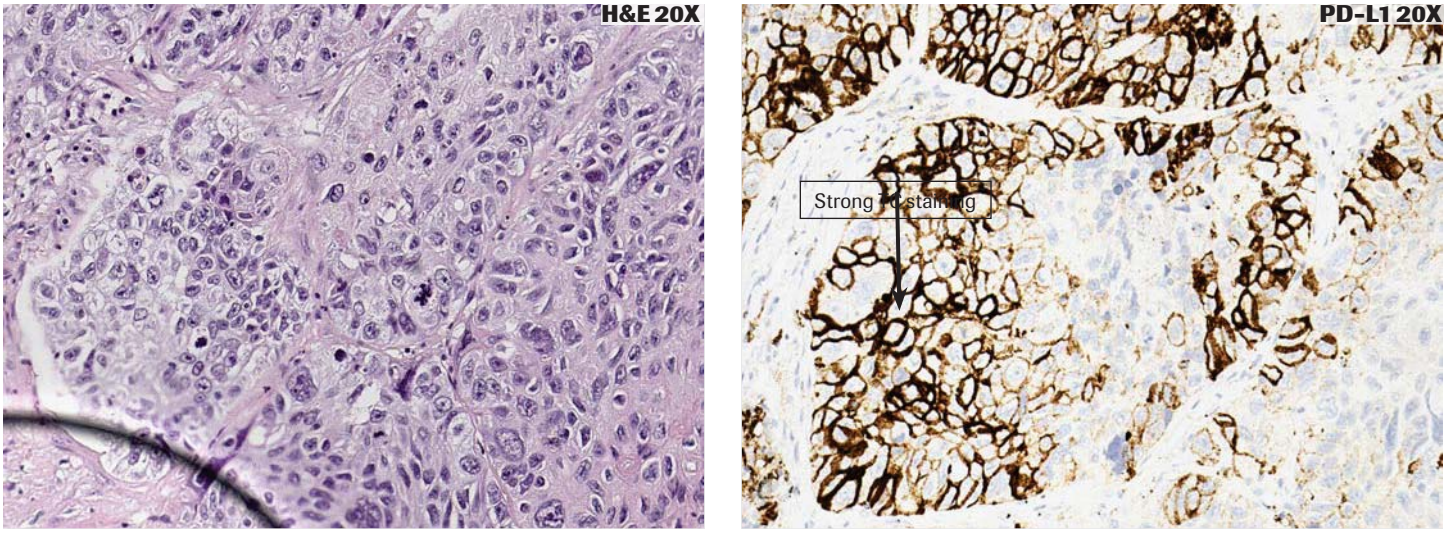


Figure 5: TC often exhibit moderate to strong, partial or complete circumferential membrane staining with or without cytoplasmic component.

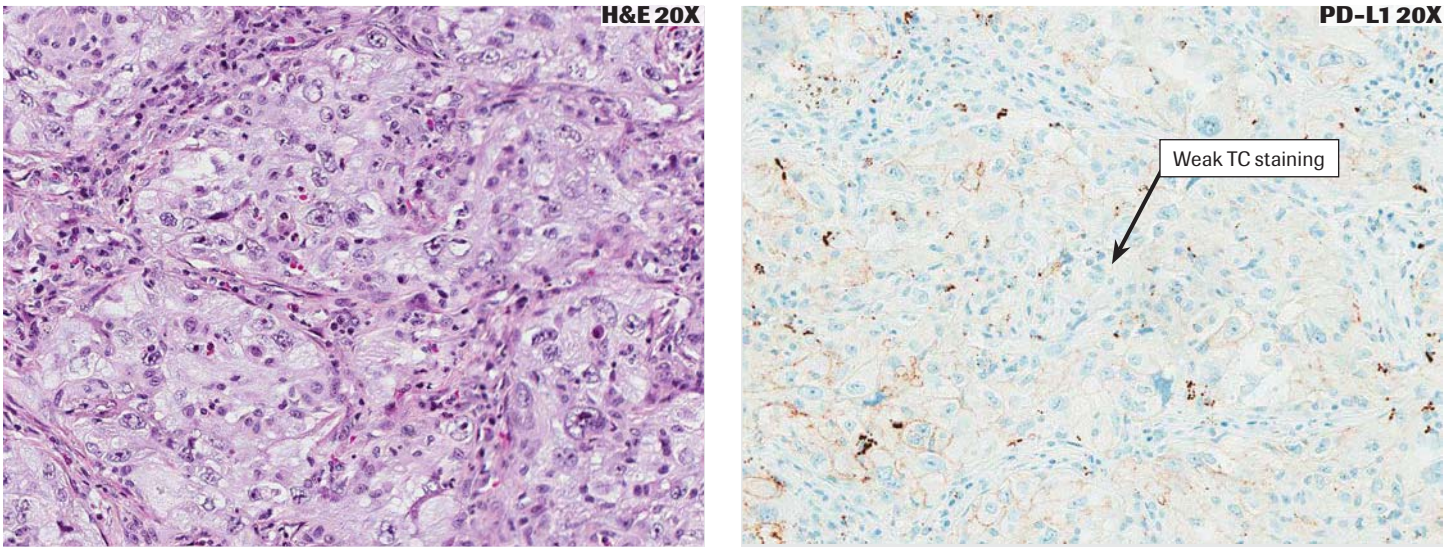


Figure 6: Weak membrane staining is sometimes observed, which requires high magnification visualization for confirmation.

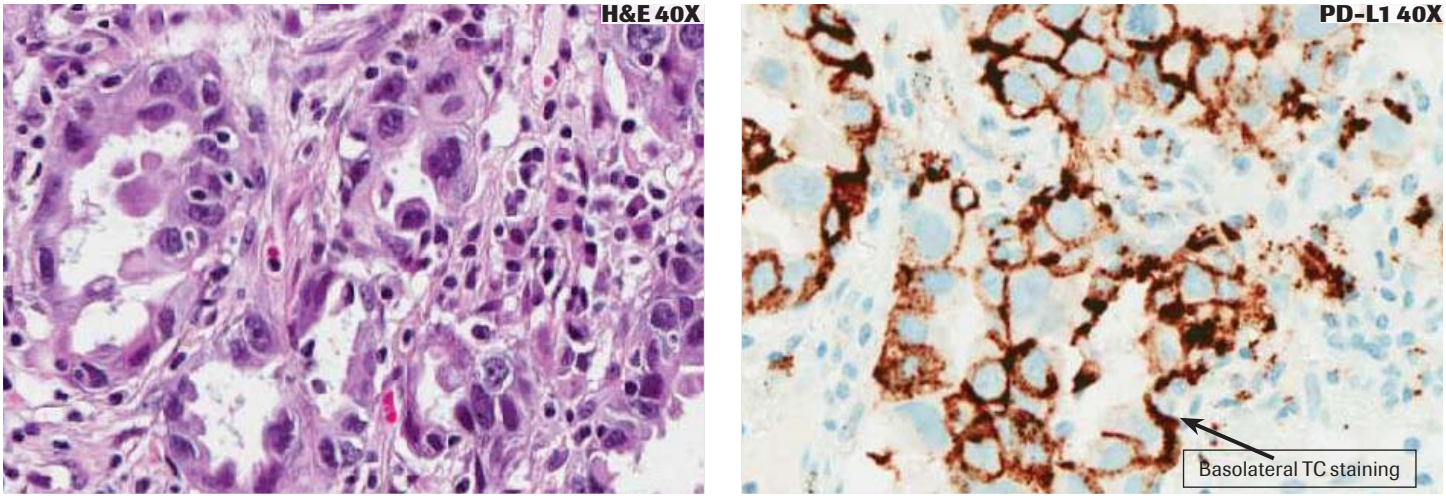


Figure 7: Basolateral membrane staining can be observed in adenocarcinomas.

IC staining:

IC are immune cells present in the intratumoral and contiguous peritumoral stroma. The VENTANA PD-L1 (SP142) Assay stain highlights a heterogeneous population of immune cells; the majority of which is morphologically consistent with lymphocytes, macrophages, dendritic cells, and granulocytes.

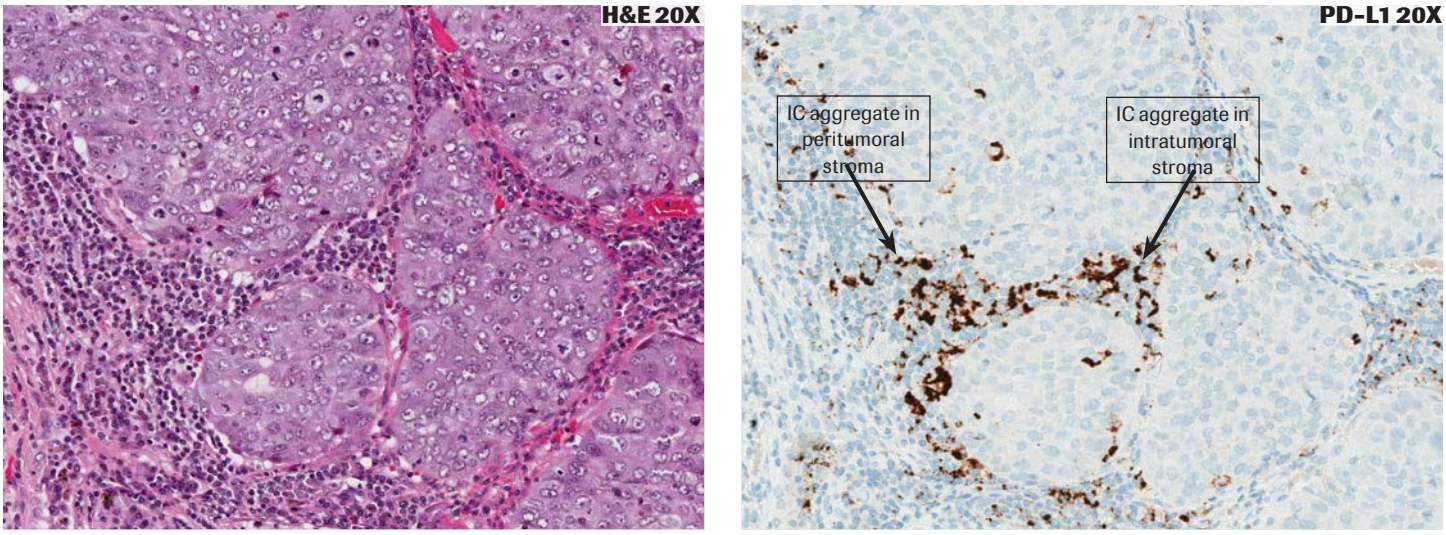


Figure 8: IC often show dark brown punctate or linear staining, which is the predominant IC staining pattern observed in the majority of tissues. IC staining is often seen as aggregates either in intratumoral or peritumoral stroma (tumor-stroma interface) or in both locations.

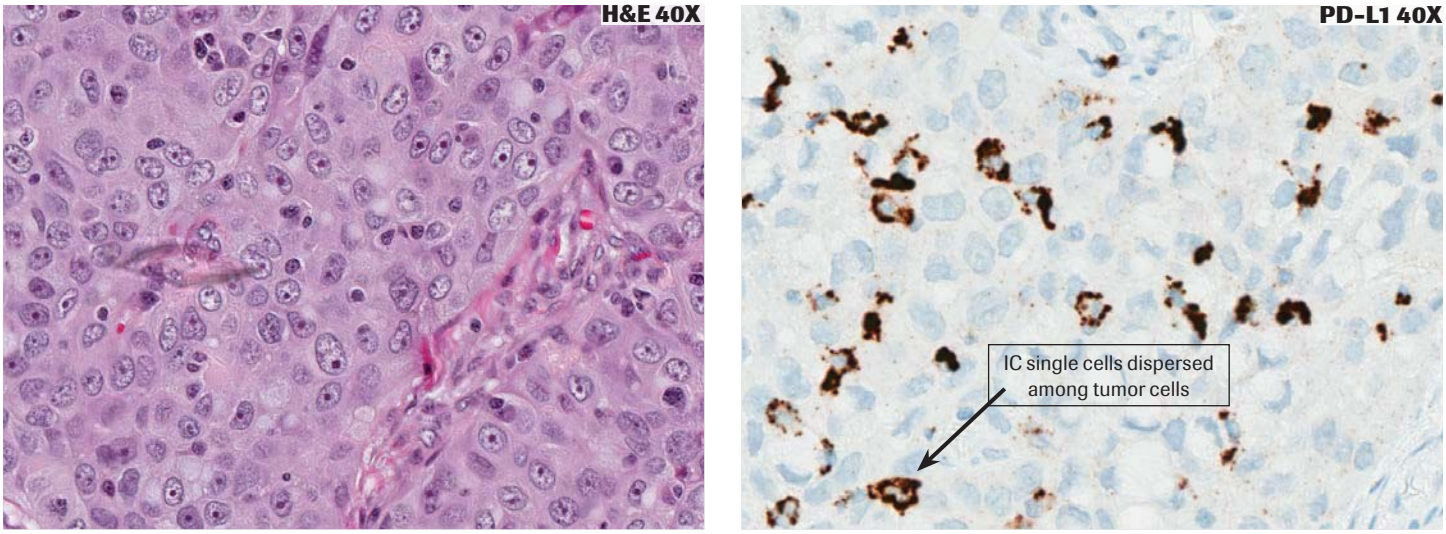


Figure 9: Occasionally, IC staining can also be observed in the form of focal or diffuse scattered single cells or small aggregates (single cell spread) dispersed among tumor cells. This pattern may be seen in association with aggregates in tumor stroma. IC staining corresponds to the immune cells among tumor cells in the H&E image.

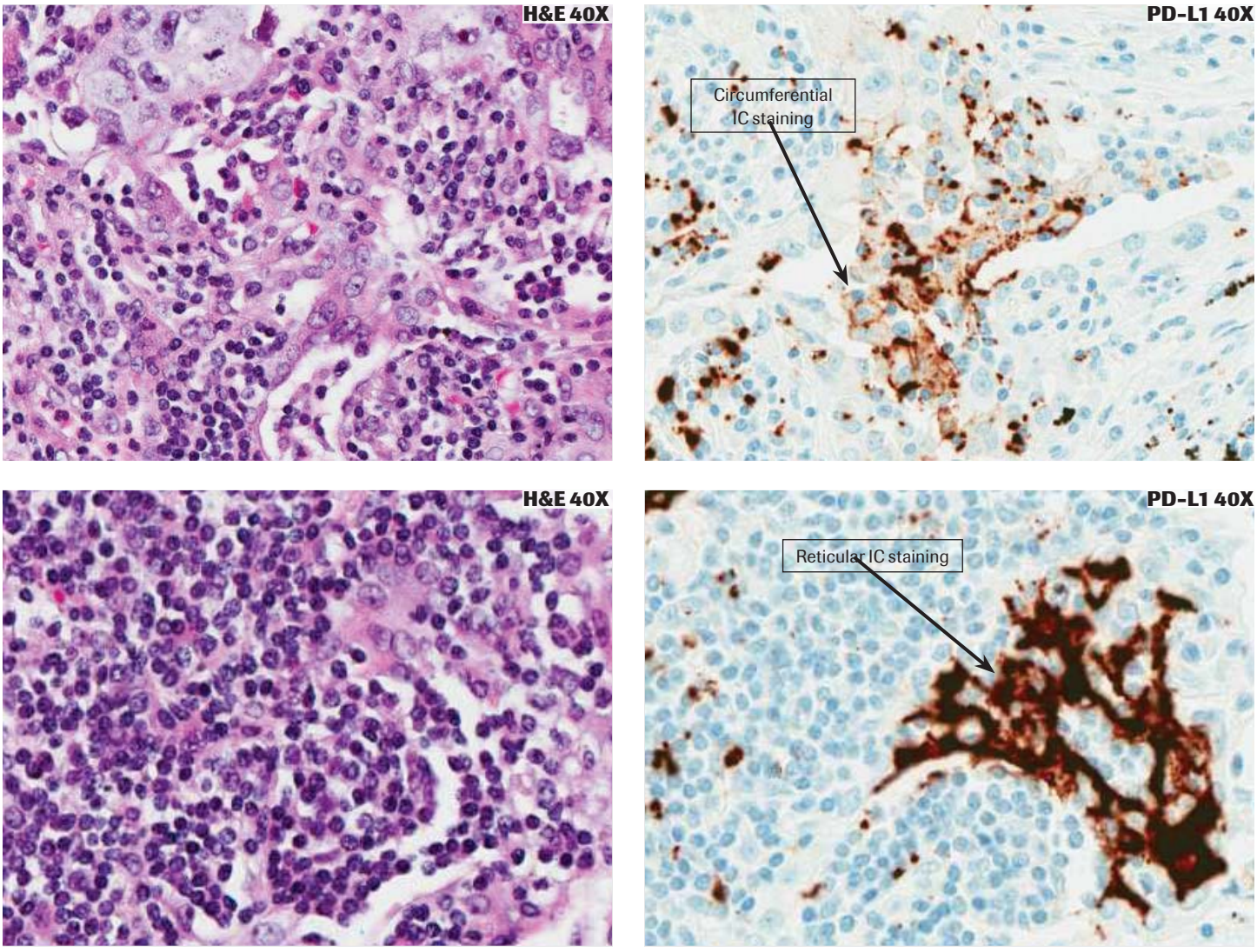
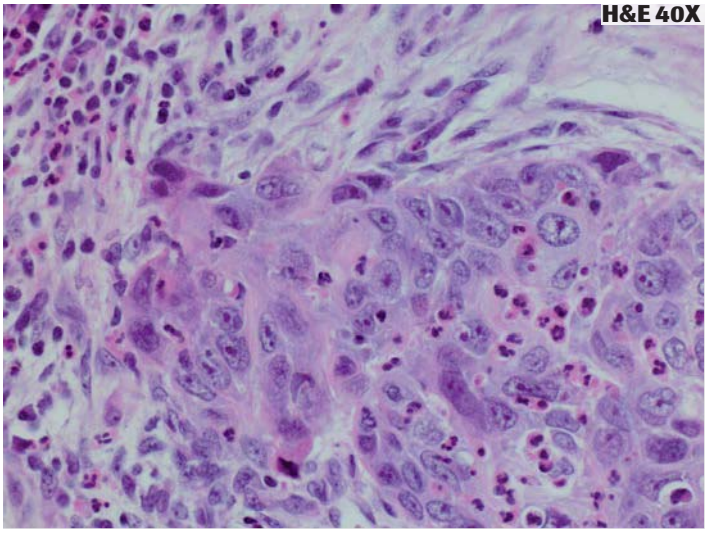


Figure 10: Circumferential membranous or reticular pattern of PD-L1 staining may be observed in IC with macrophage and/or dendritic cells, respectively.



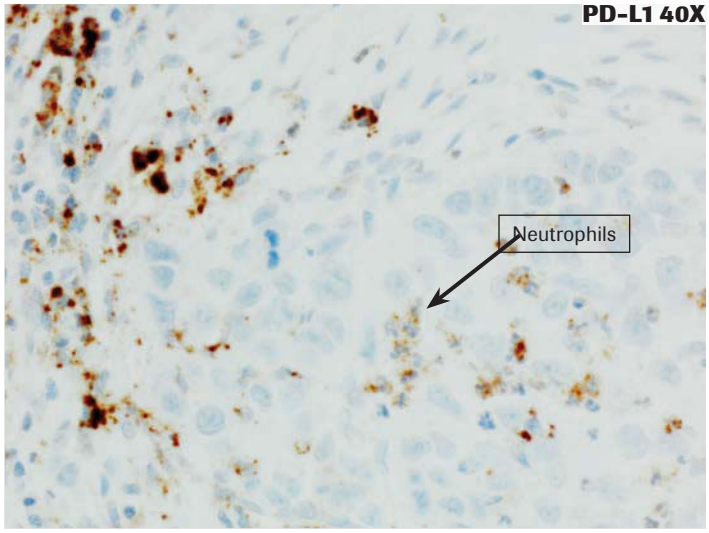
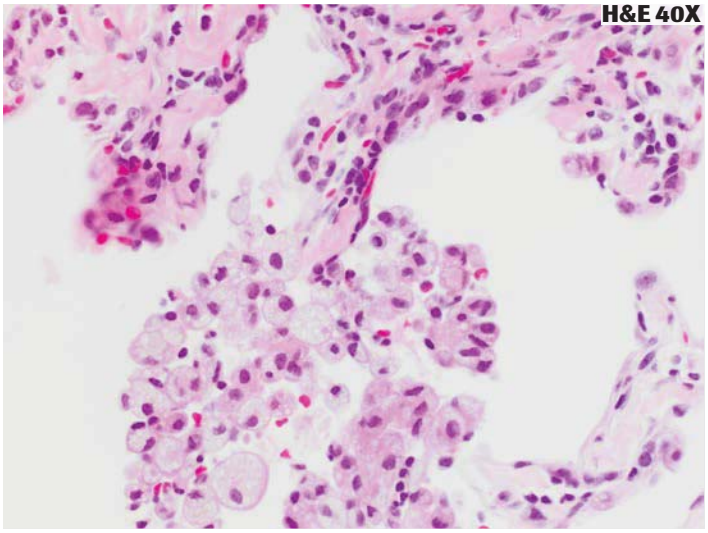


Figure 11: Rarely, IC staining can be observed in neutrophils, as fine punctate staining along with diffuse granular staining. Neutrophil staining can be seen dispersed among tumor cells, in the intratumoral or peritumoral stroma, or as aggregates.



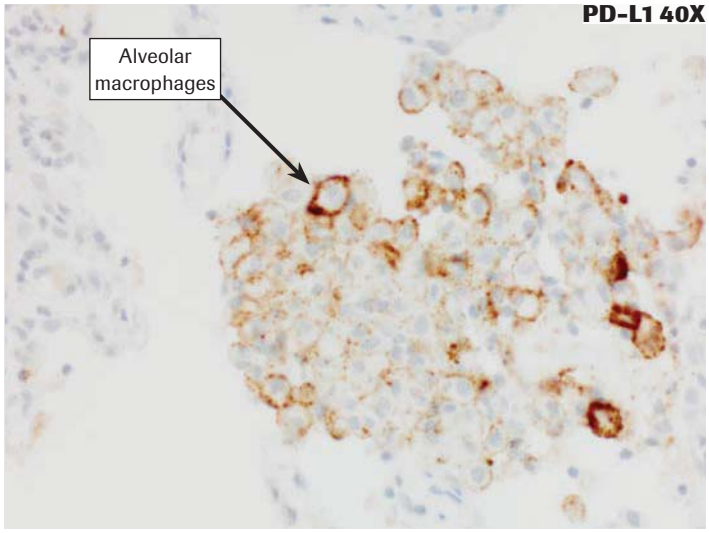


Figure 12: Alveolar macrophages can exhibit circumferential membrane staining, which can be of moderate or strong intensity. Review of corresponding H&E slide would help differentiate this from TC staining.

Differentiation of TC and IC Staining:

TC staining can be observed in association with IC staining. Review of the corresponding H&E slide will help in identifying IC among TC. This along with a high magnification review of the PD-L1 stained slide may aid in differentiating between TC and IC staining. The following images demonstrate different commonly observed patterns encountered in clinical practice, when TC and IC staining is observed together (**Figure 13**-**Figure 15**).

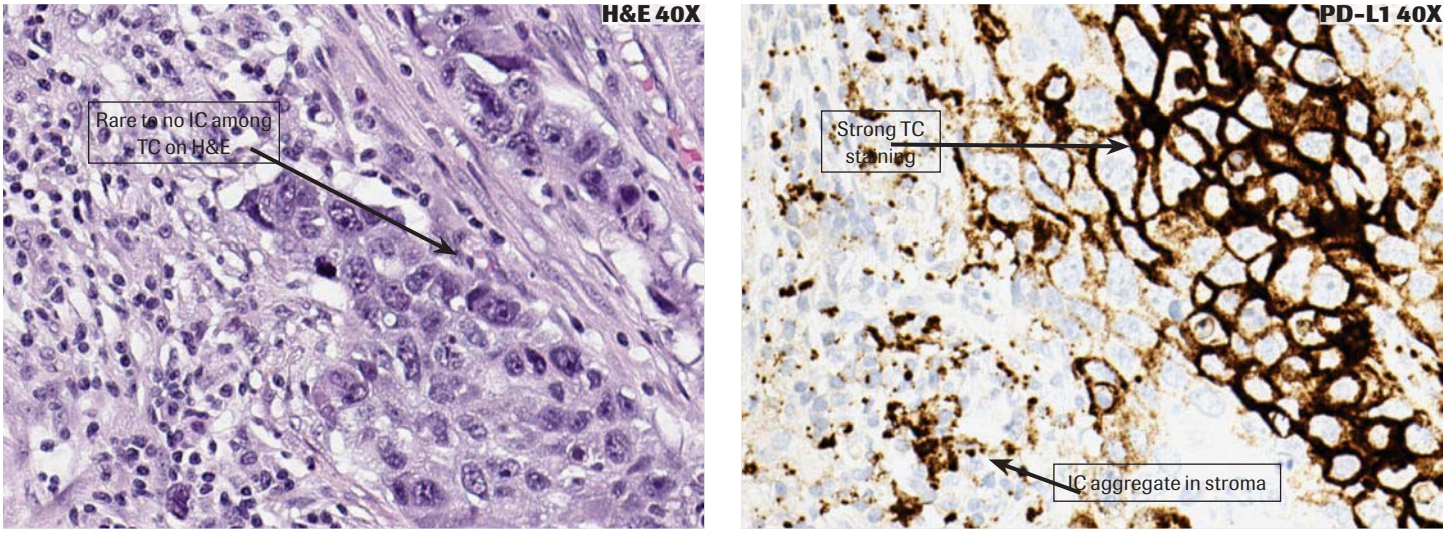


Figure 13: TC show strong membrane staining, with rare IC among the tumor cells identified on H&E.

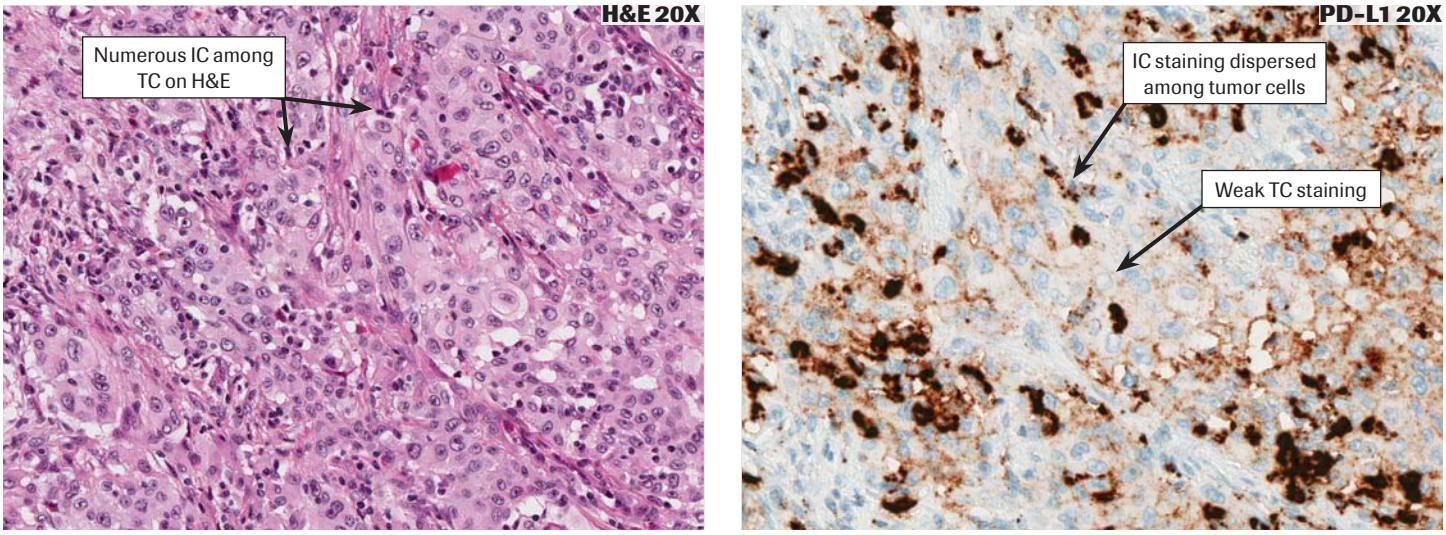


Figure 14: TC show weak to moderate membrane staining, with many IC among TC identified on H&E. Note the presence of strong punctate IC staining among the TC.

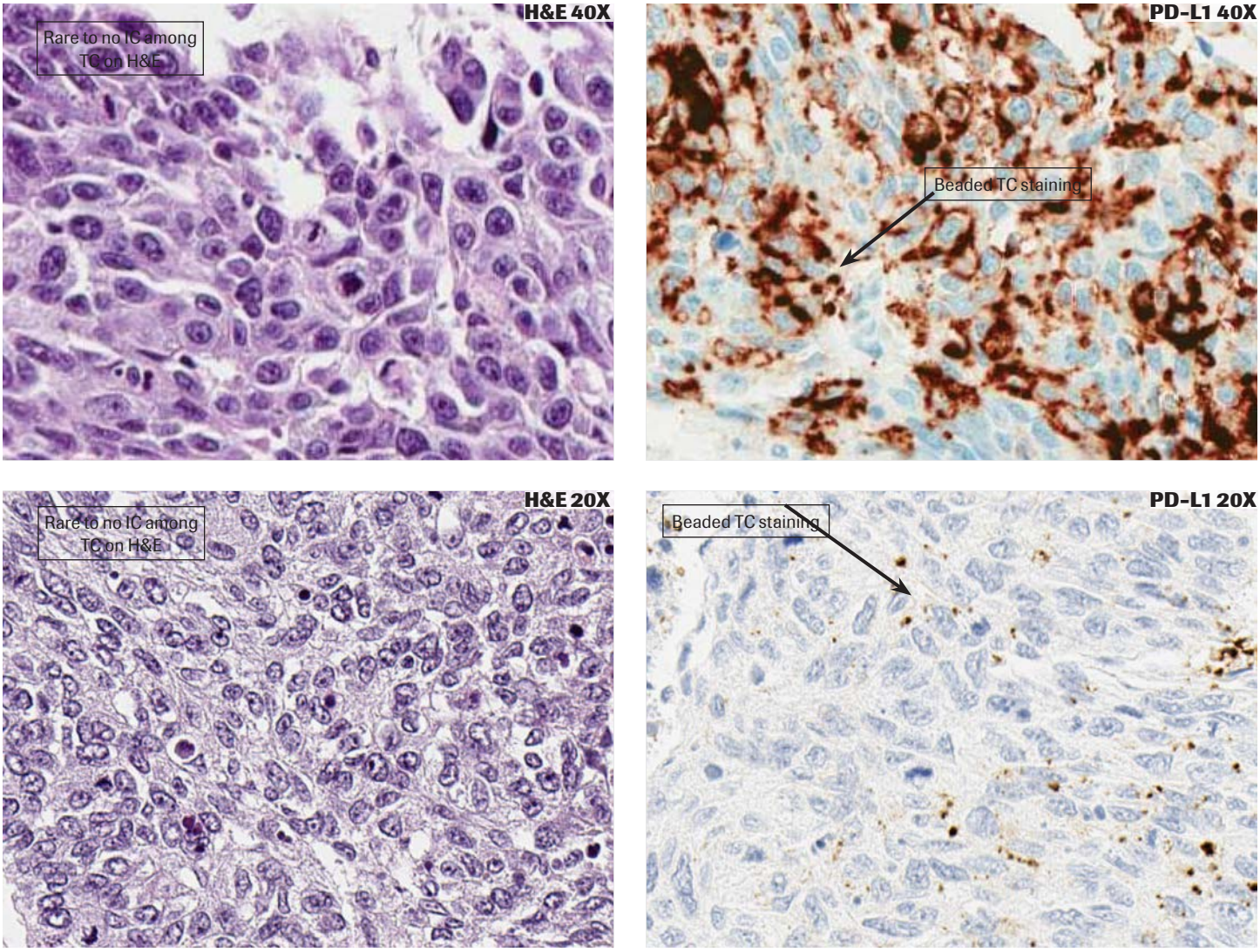


Figure 15: If H&E does not show many identifiable IC, and a granular or beaded staining pattern is observed among TC, then this staining should be attributed to TC rather than IC.

Scoring Method

VENTANA PD-L1 (SP142) Assay-stained NSCLC tissue will be evaluated for both TC and IC staining using a stepwise approach as outlined in **Table 2**.

- **TC scoring**: TC staining is scored as the **percentage of viable tumor cells showing membrane staining of any intensity.** Membrane staining should be visible as curvilinear staining along tumor cell membrane even if associated with granular or beaded quality. Cytoplasmic staining can be observed along with membrane staining, but is not included for tumor cell scoring.
- **IC scoring**: IC are scored as the proportion of tumor area that is occupied by PD-L1 staining immune cells of any intensity. Any IC staining irrespective of type of cells or localization is included.

  - **Tumor Area**: Tumor area for PD-L1 (SP142) interpretation is defined as the area occupied by viable tumor cells, and their associated intra- and contiguous peritumoral stroma (**Figure 16A-C**). Necrotic tumor is excluded from this definition of tumor area (**Figure 16D**).

  - In fragmented tissue samples, including biopsies, where distinction of intra versus peritumoral stroma is difficult, only stroma that is contiguous to individual tumor nests is included in the tumor area definition; stroma that is part of the tissue fragment, but not contiguous to viable tumor, is excluded (**Figure 16B**).

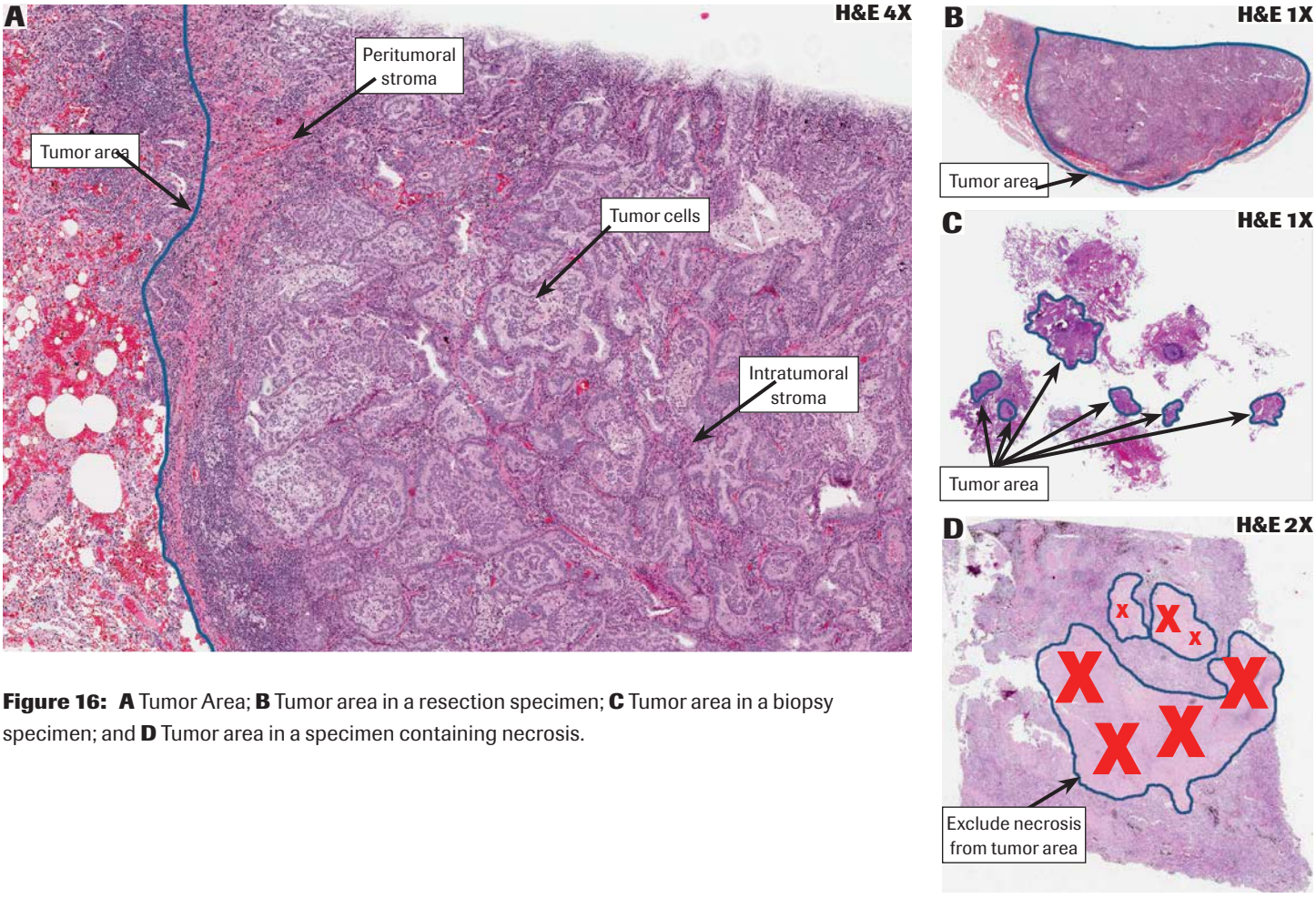
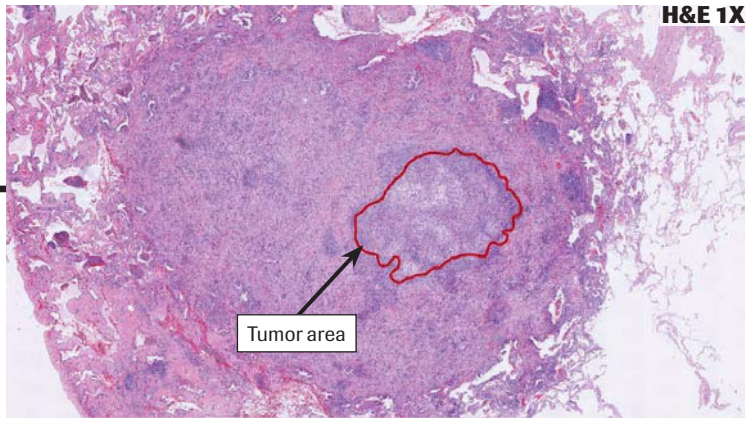


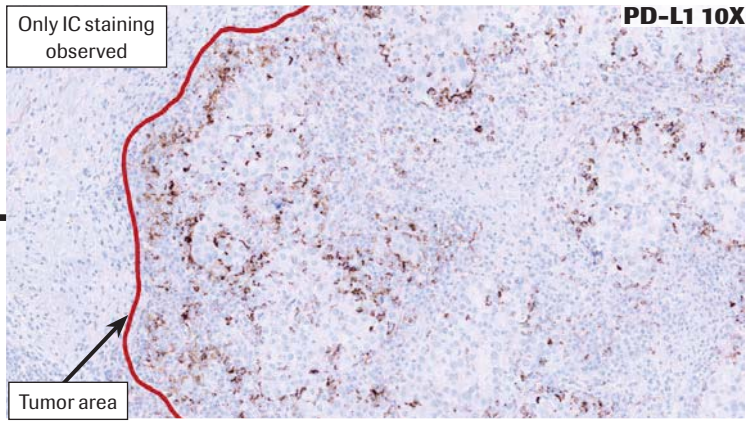
Figure 16: **A** Tumor Area; **B** Tumor area in a resection specimen; **C** Tumor area in a biopsy specimen; and **D** Tumor area in a specimen containing necrosis.

Scoring of PD-L1 IC aggregate staining:

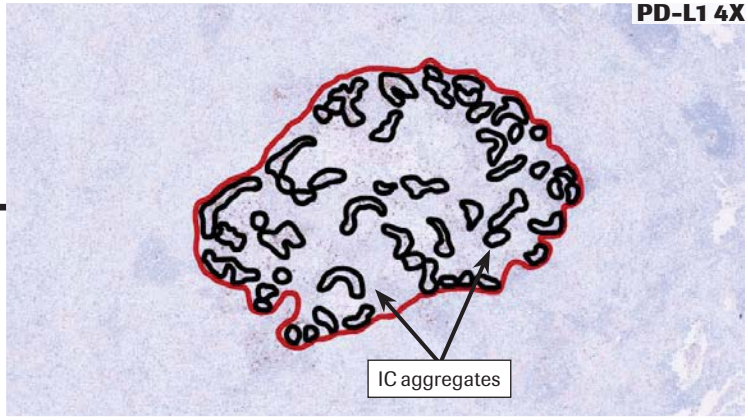
Review the H&E-stained slide for the presence of tumor, necrosis, adequacy (at least 50 viable tumor cells*), and assessment of tumor area.



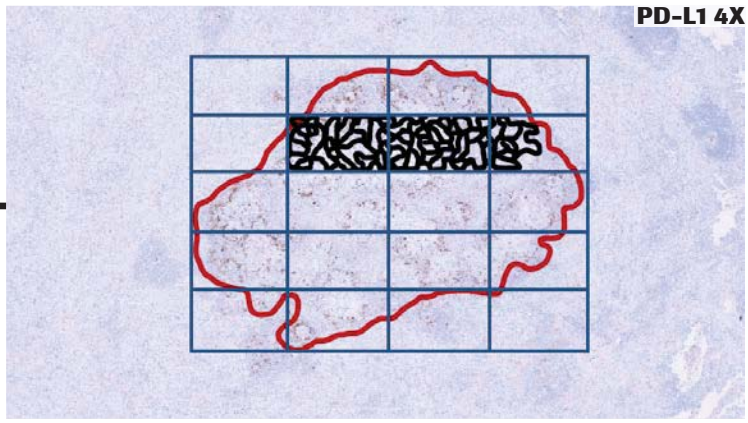
Review the PD-L1 stained slide at 10x or 20x magnification and confirm overall staining pattern as IC, TC or both.



Visually encircle IC aggregates as closely as possible using 2x or 4x magnification.

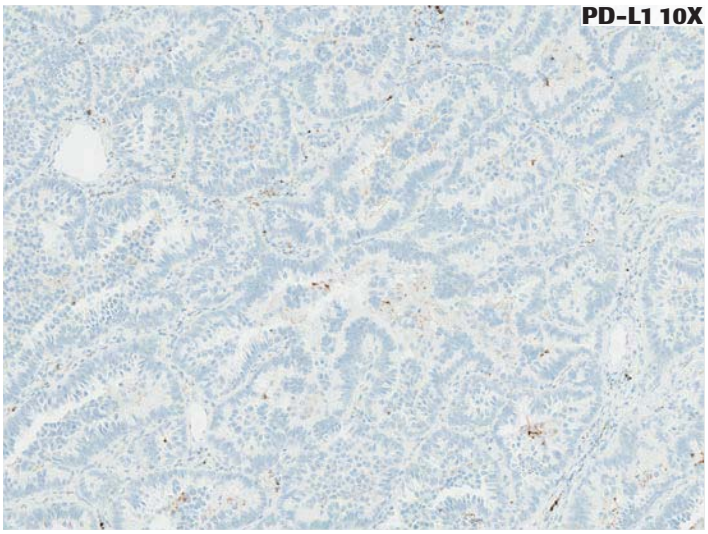


Combine the aggregate staining and estimate the proportion of tumor area occupied by IC aggregates using 2x or 4x magnification. PD-L1 expression for this case is ≥ 10% IC. The Reference Images section should be used as an aid in scoring.

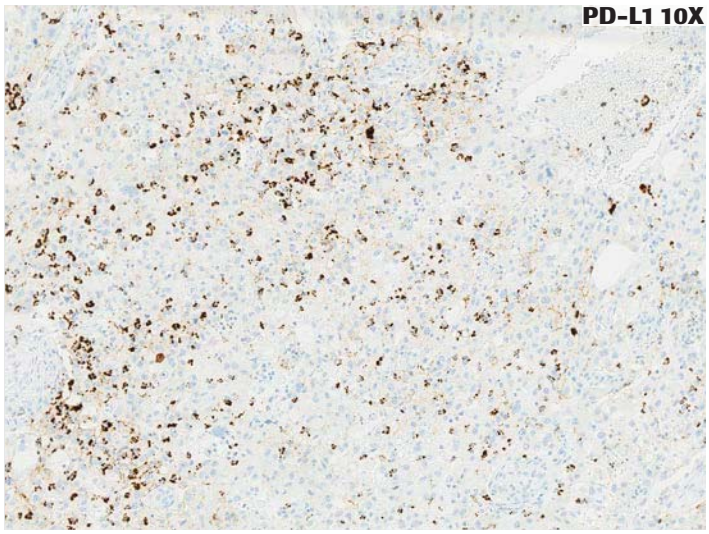


*Tumor associated stroma is not required for TC scoring.
The presence of tumor-associated stroma is essential for scoring IC.

Scoring of PD-L1 single-cell spread IC staining:



Cell density for single-cell spread IC is < 1%



Cell density for single-cell spread IC is ≥ 10%

Single-cell spread IC is scored based on the density of single-cell spread, using the Reference Images section of this guide.

Scoring Methods: Challenges and Pitfalls

1. **Staining in the necrotic debris and glandular intra-luminal debris:** Necrotic debris or immune cells in the periphery of necrotic or apoptotic regions can show PD-L1 staining. This staining may be granular and can be mistaken for IC staining. This staining, as well as the neutrophil staining observed as aggregates, should be excluded from scoring (**Figure 17** and **Figure 18**).

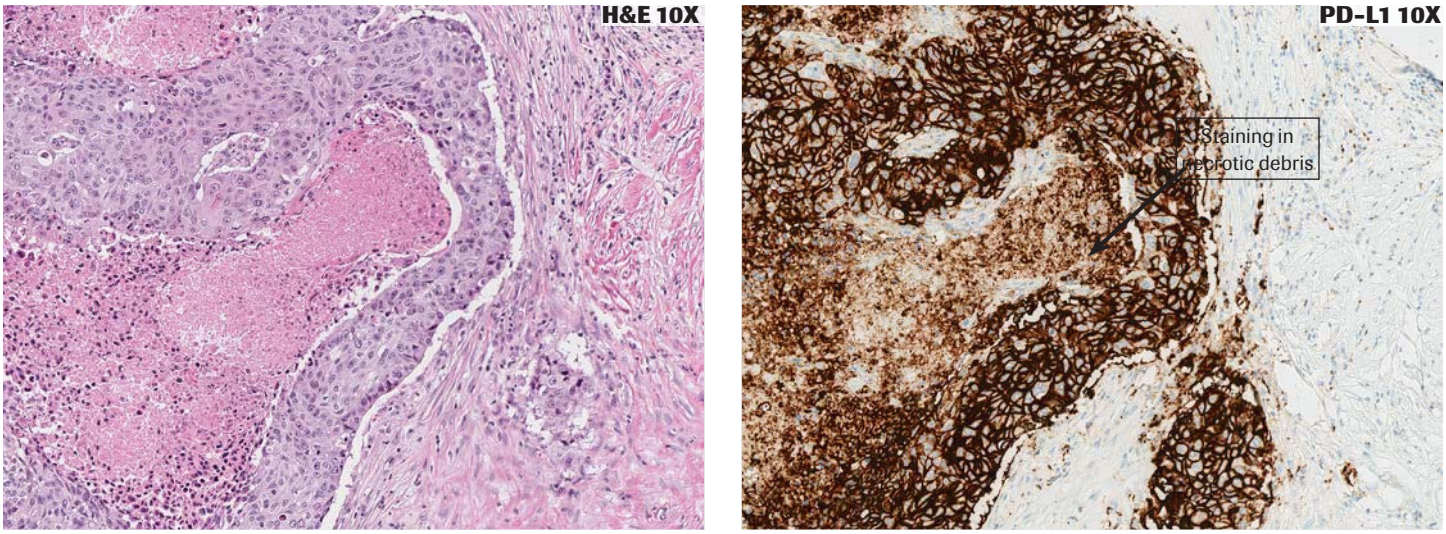


Figure 17: Necrotic debris showing PD-L1 staining. Necrotic debris staining should not be included in scoring.

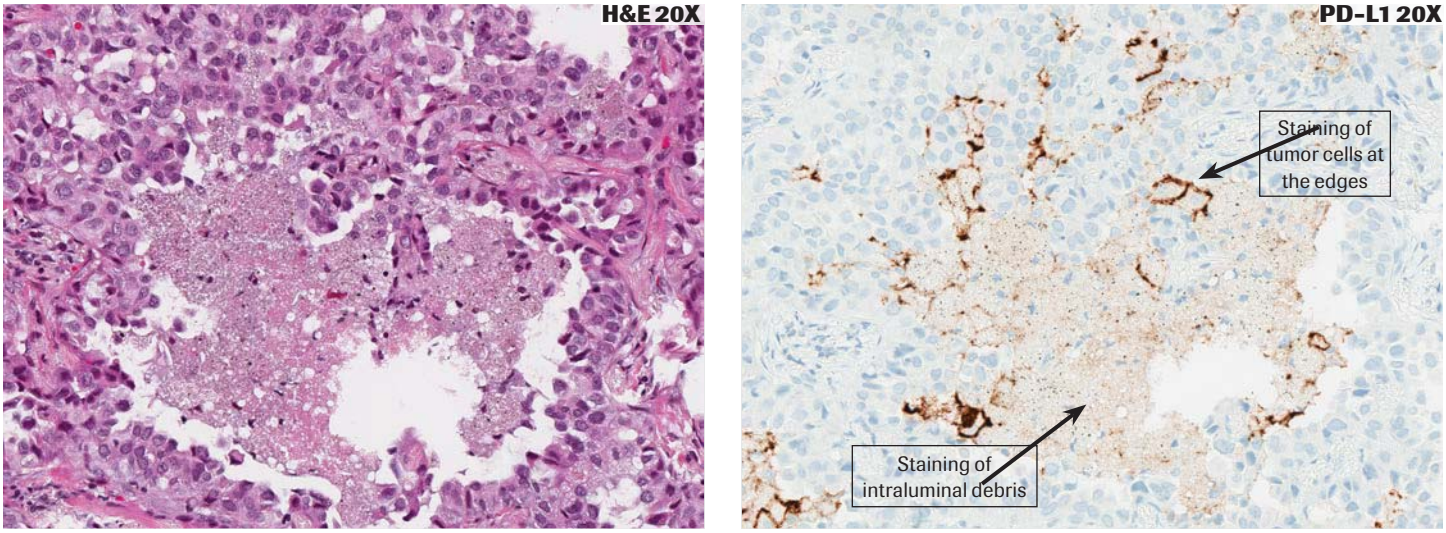


Figure 18: Intraluminal debris showing PD-L1 staining. This should not be included in the scoring. A few viable tumor cells at the edges stain for PD-L1 and these should be included in TC scoring.

2. **Lymph node metastasis:** VENTANA PD-L1 (SP142) Assay can be used to test both primary and metastatic samples. Metastatic samples can originate from various organs which include, but are not limited to, lymph node, liver, adrenal gland, bone, and soft tissue. Metastases from bone are not suitable for staining with VENTANA PD-L1 (SP142) Assay. Lymph node metastases deserve special attention, given the presence of native immune cells which show staining for PD-L1. In tumors metastasizing to lymph nodes only immune infiltrate staining contiguous to the tumor cells should be counted towards the PD-L1 IC percentage (**Figure 19**).

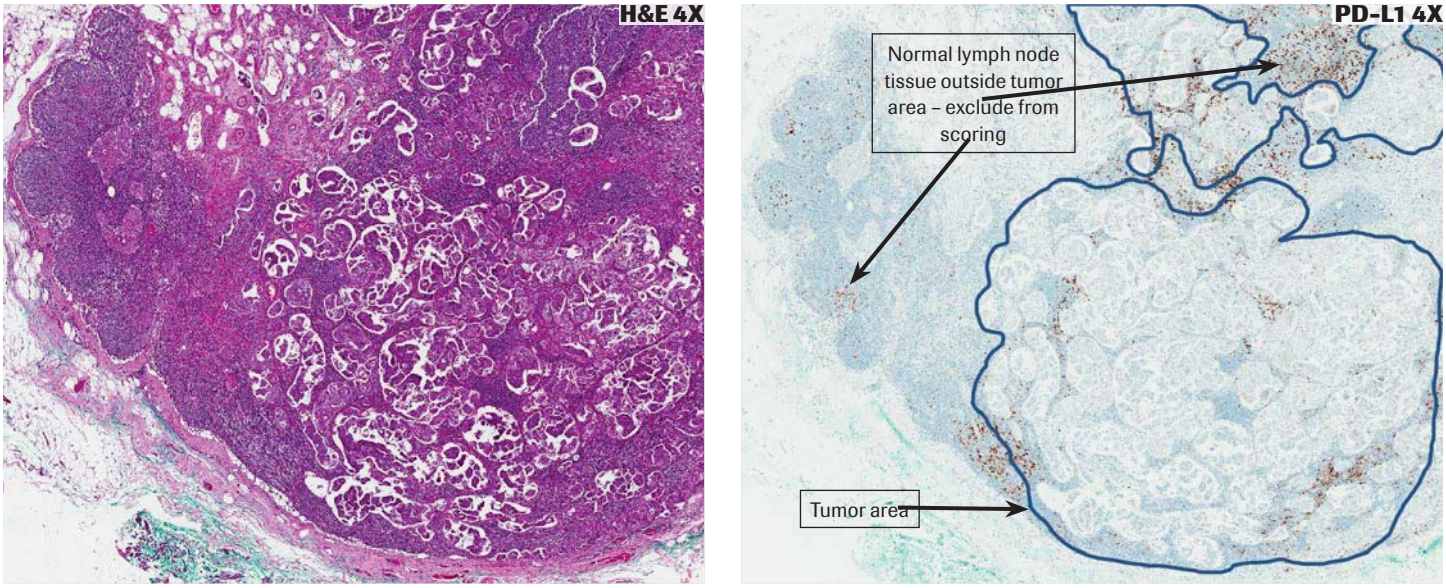


Figure 19: PD-L1 staining in a NSCLC metastatic to a lymph node

3. **Alveolar Macrophage Staining:** Alveolar macrophages stain for PD-L1 (**Figure 20**). PD-L1 staining in alveolar macrophages can be included towards PD-L1 IC percentage only if these are entrapped within the tumor mass and are contiguous to the tumor cells. Strong staining alveolar macrophages can be mistaken for TC and require review of corresponding H&E for confirmation.

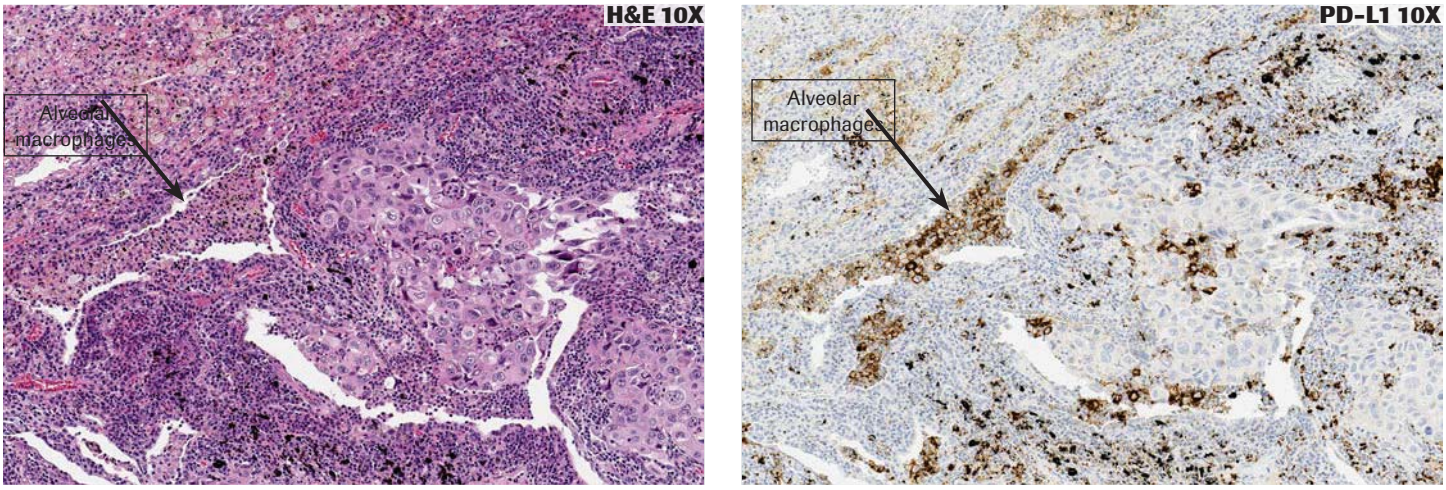


Figure 20: PD-L1 staining of alveolar macrophages at the edge of the tumor.

4. **Intravascular Immune cells:** Vasculature in tumor stroma may show PD-L1 positive immune cells (**Figure 21**). These are not considered towards IC scoring.

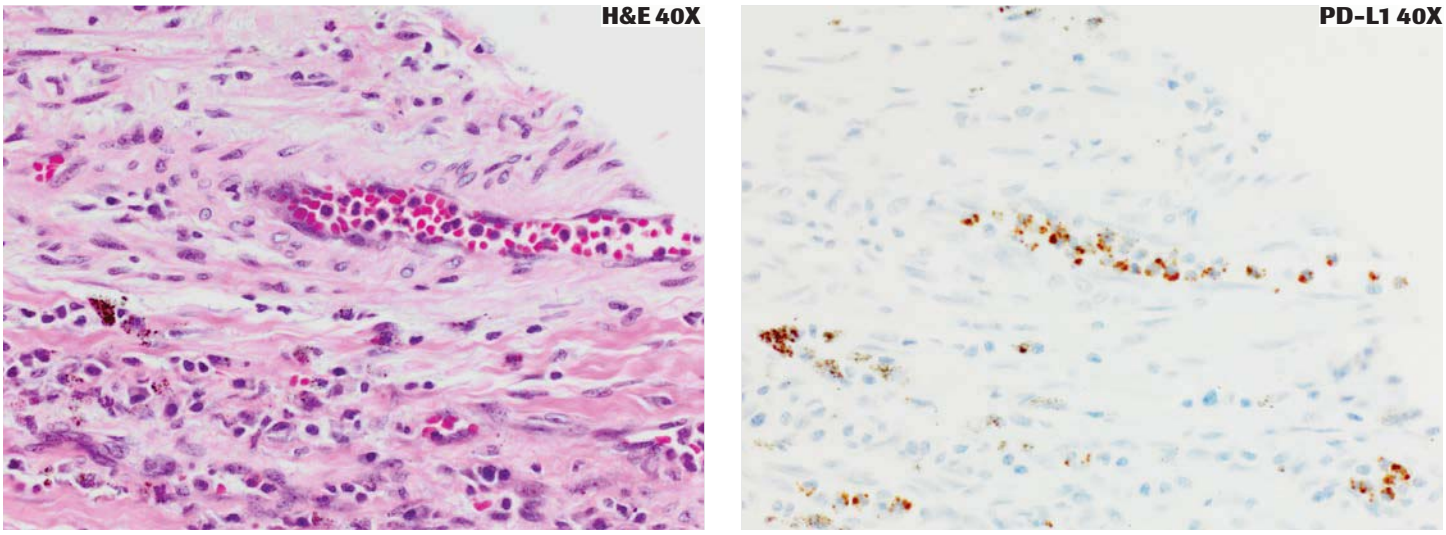


Figure 21: Intravascular immune cells (neutrophils in this case) are not counted toward IC scoring.

5. **Hemosiderin and anthracotic pigments:** Anthracotic pigment and/or hemosiderin pigment may interfere with IC scoring. Examination of matched negative reagent control, as well as review at high magnification, may be required in these situations. This is illustrated in **Figure 22**.

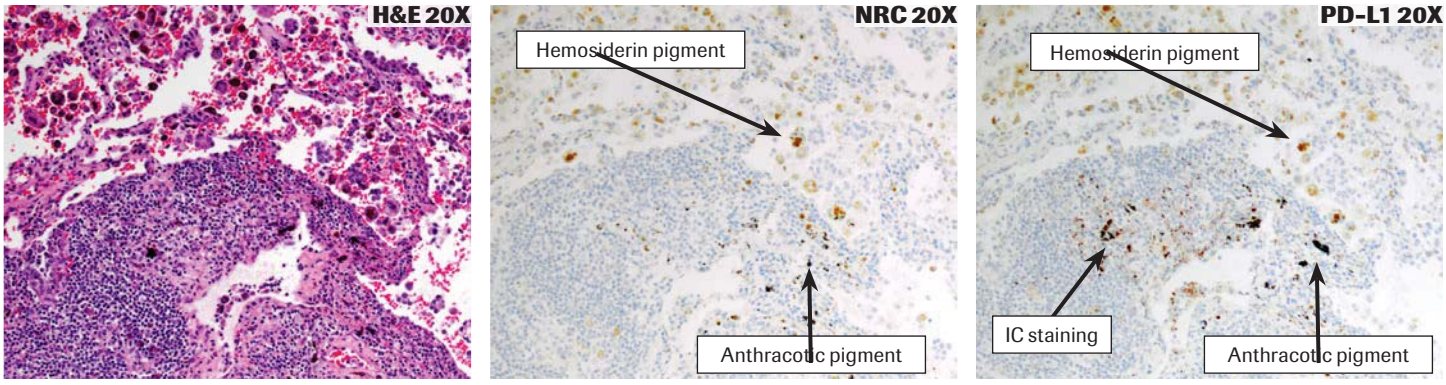
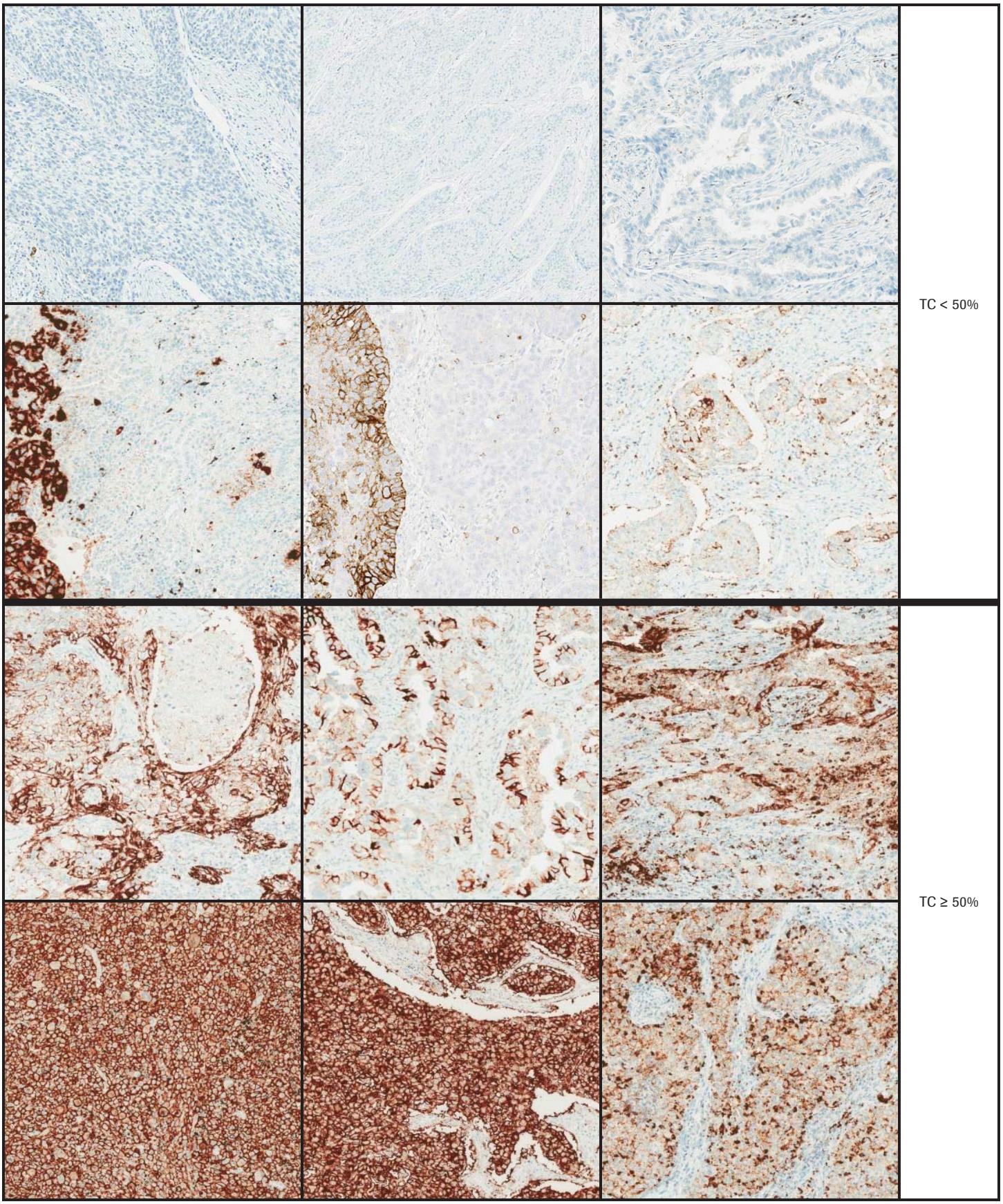


Figure 22: PD-L1 IC staining adjacent to anthracotic pigment and corresponding tissue section stained with negative reagent control and H&E.

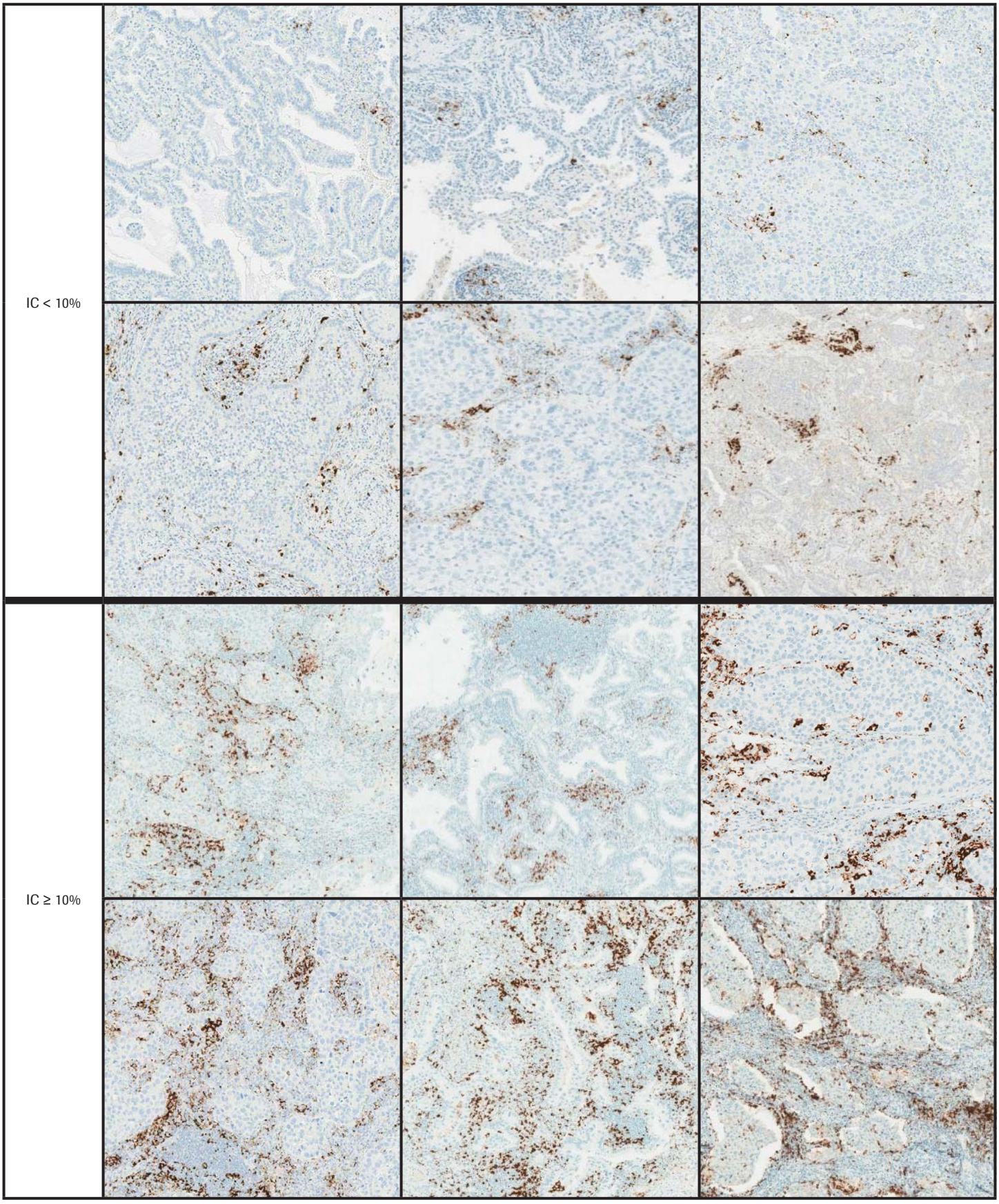
Reference Images

TC Expression



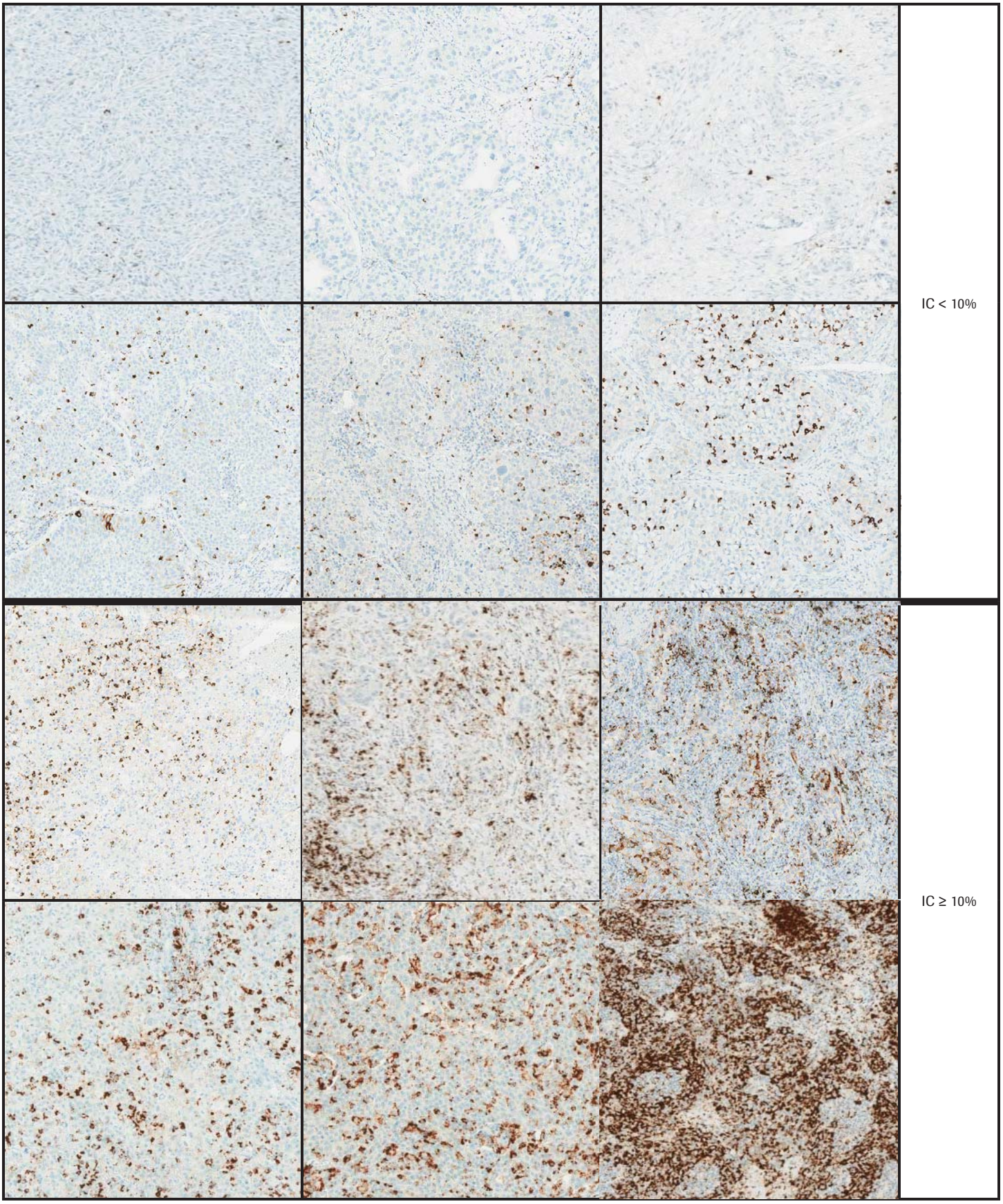
All images 10x magnification

IC Expression – Aggregates



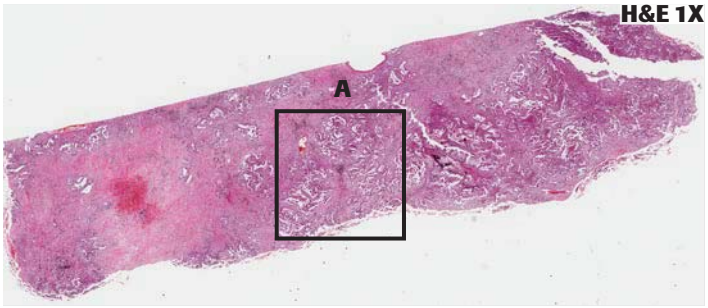
All images 10x magnification

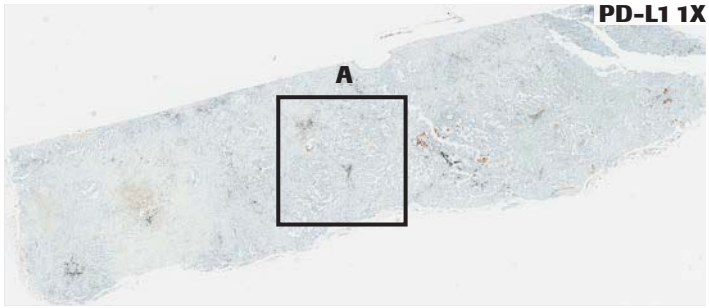
IC Expression – Single-Cell Spread

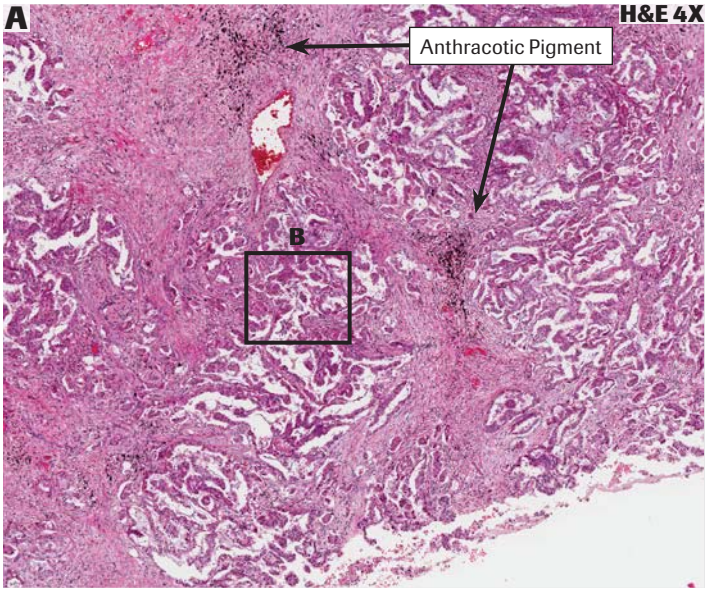


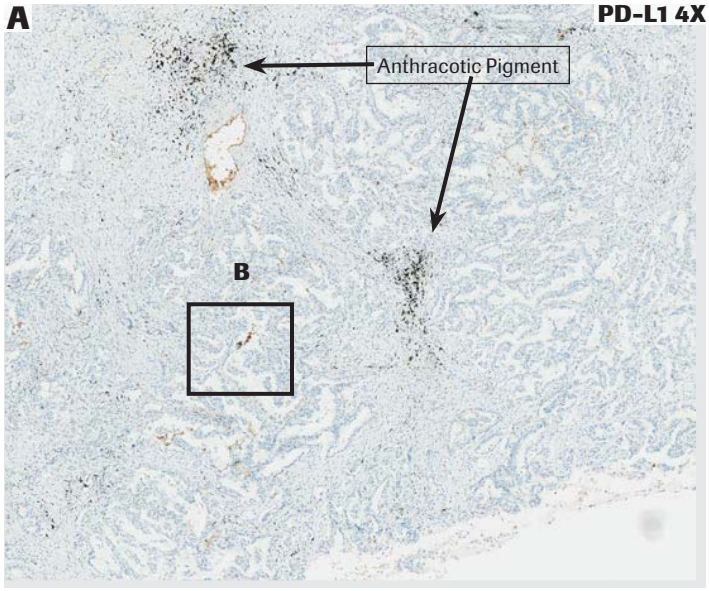
All images 10x magnification

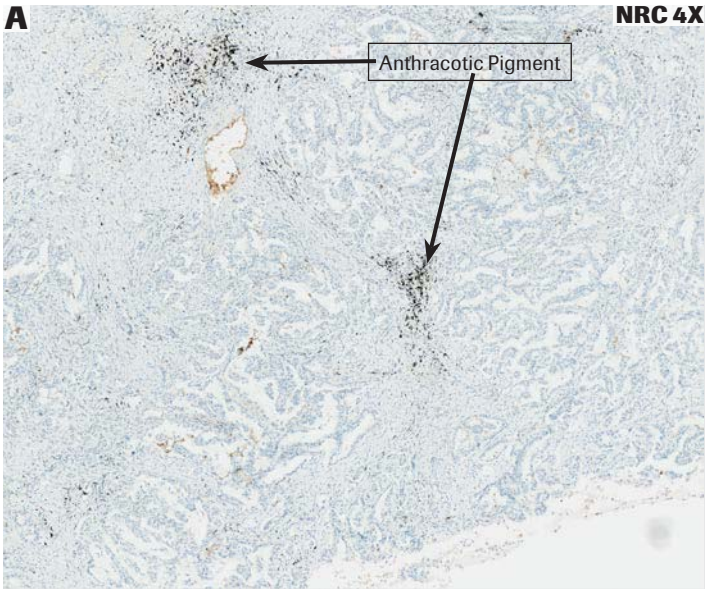
Example Cases: TC < 50% and IC < 10%

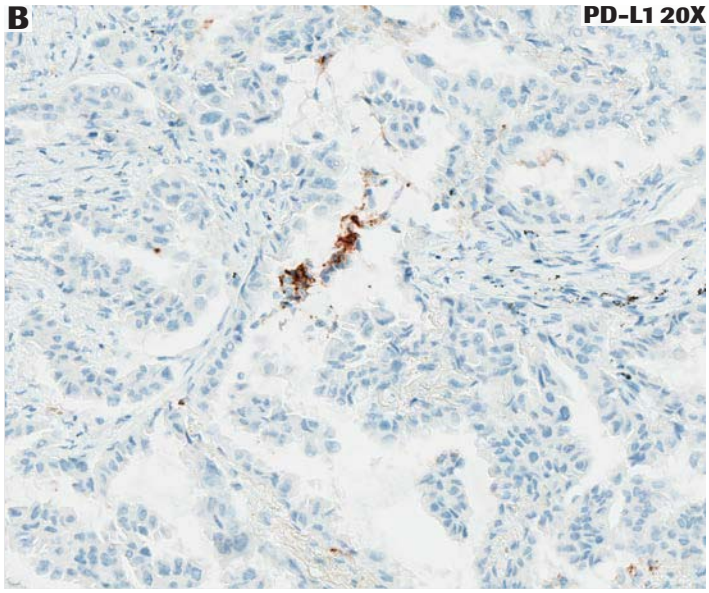




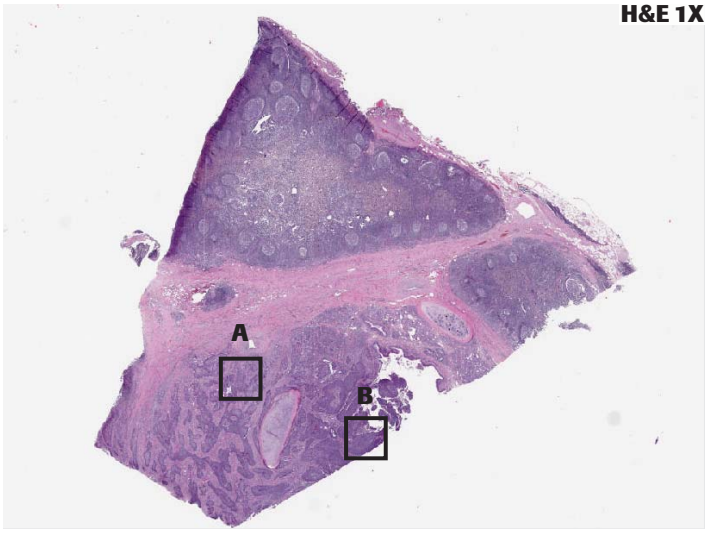


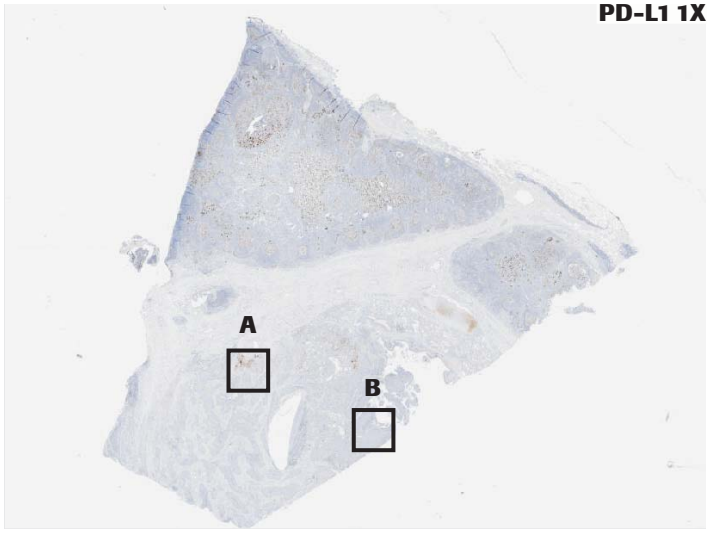


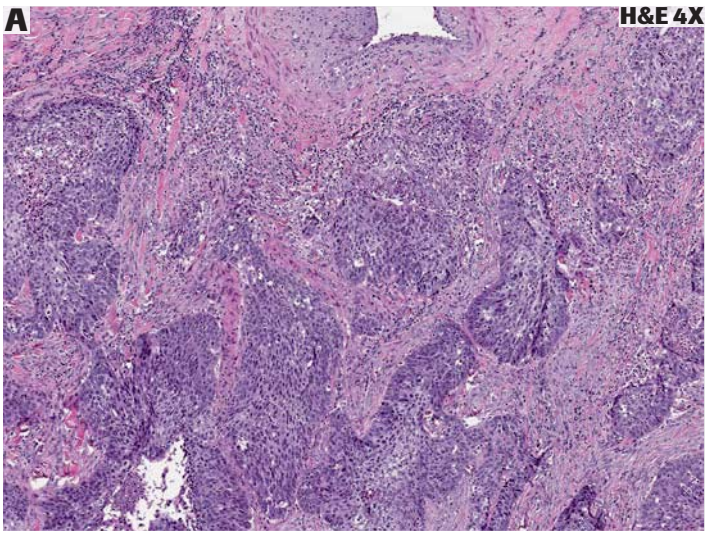


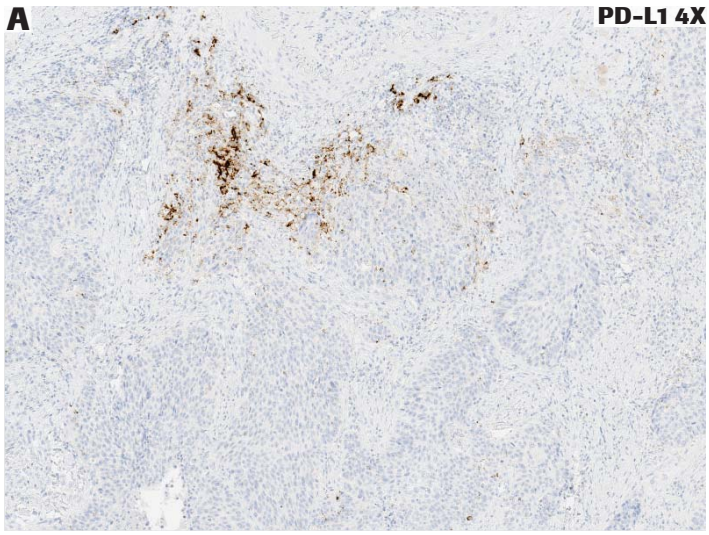


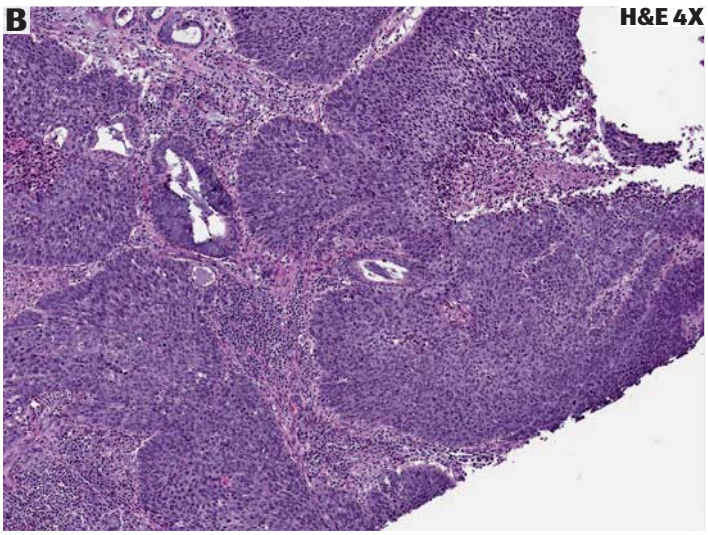
Case 1: This case is TC < 50% and IC < 10%. Scores: TC: 0%; IC: < 1%. This case shows staining of intraluminal debris which should be discounted while scoring IC. Also note the presence of anthracotic pigment.

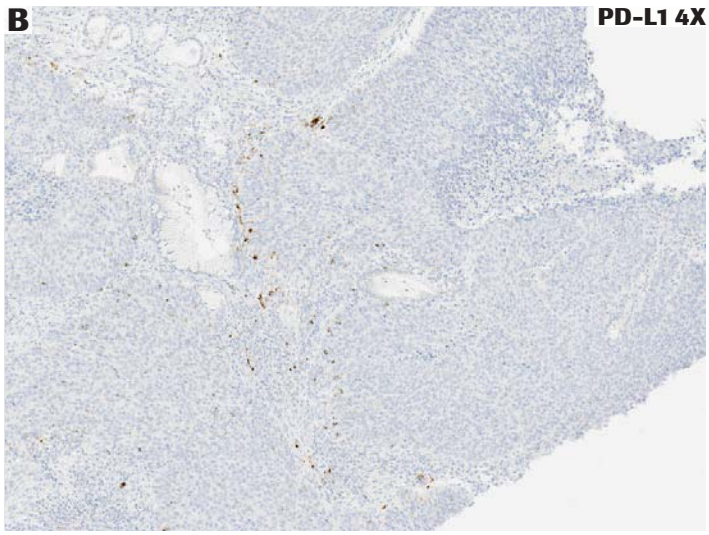




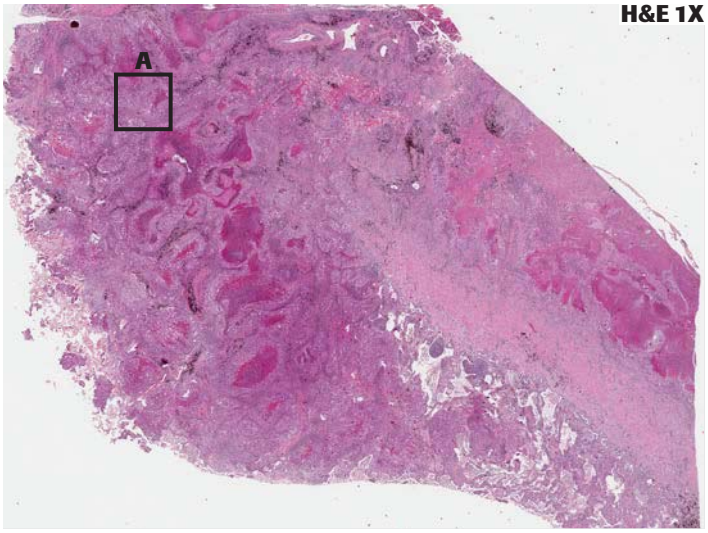


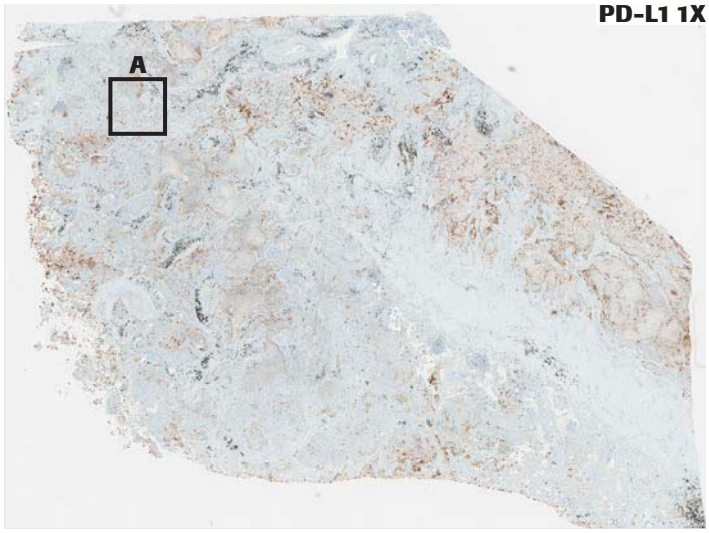


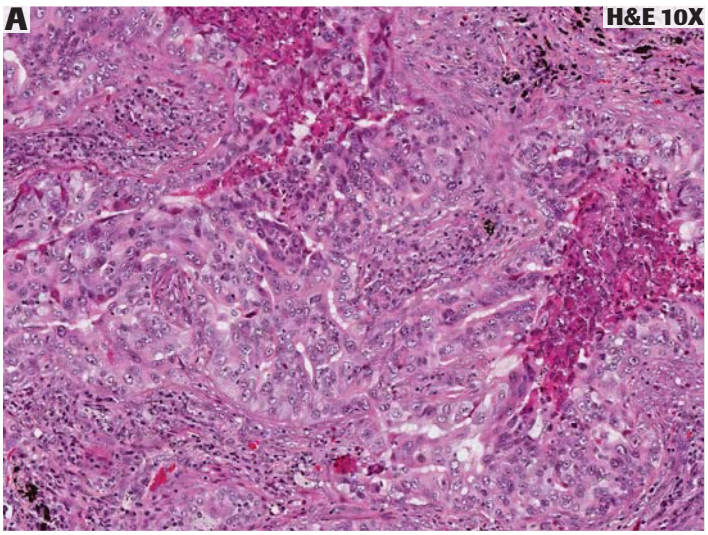


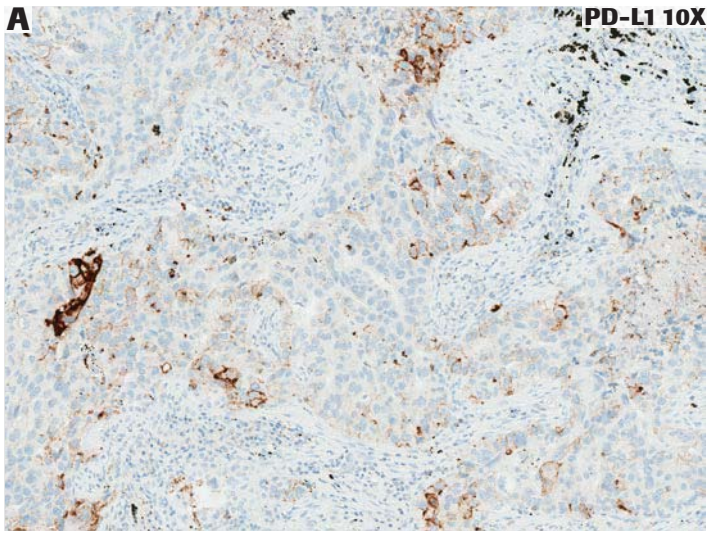


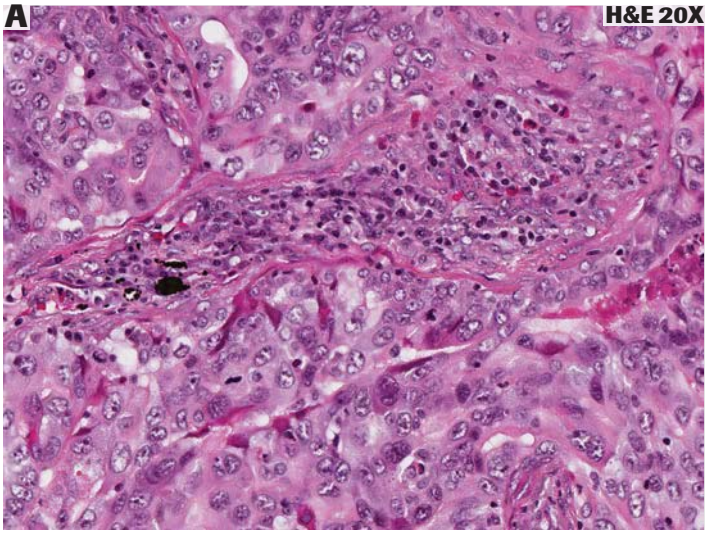
Case 2: This case is TC < 50% and IC < 10%. Scores: TC: 0% and IC: 2%. This case is a lymph node metastasis showing focal interface staining for immune cells. Note that tumor is clearly separated from lymph node tissue, hence making delineation of tumor area easier.

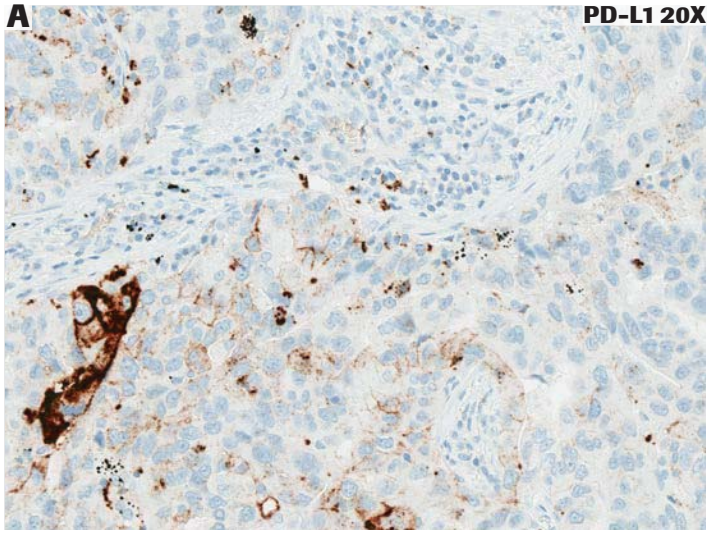




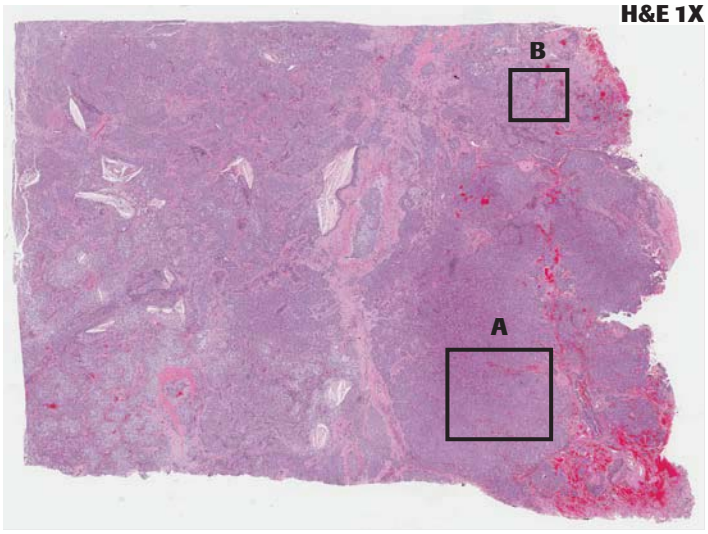


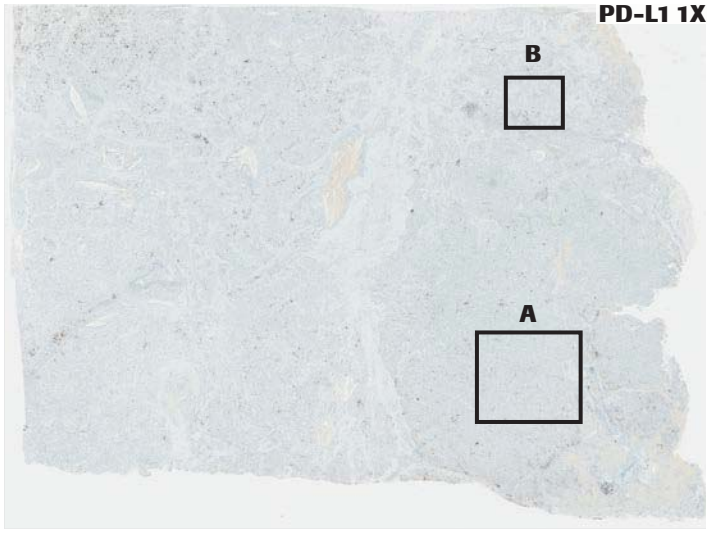


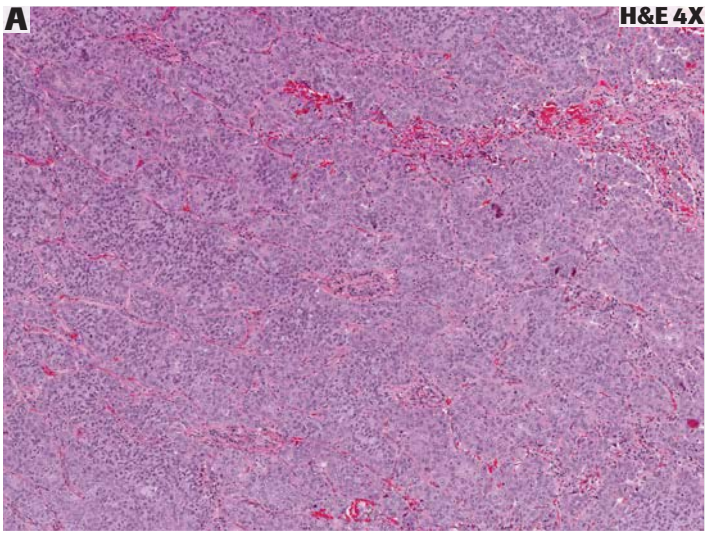


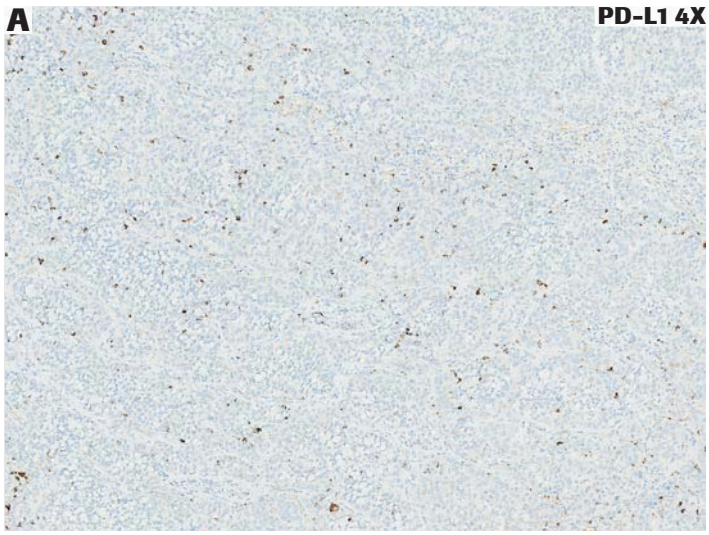


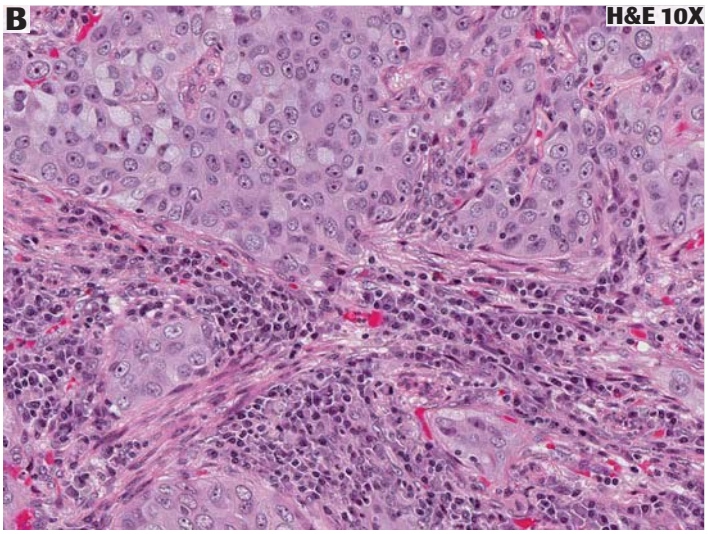
Case 3: This case is TC < 50% and IC < 10%. Scores: TC: 15% and IC: 1%. Note the presence of primarily weak TC staining.

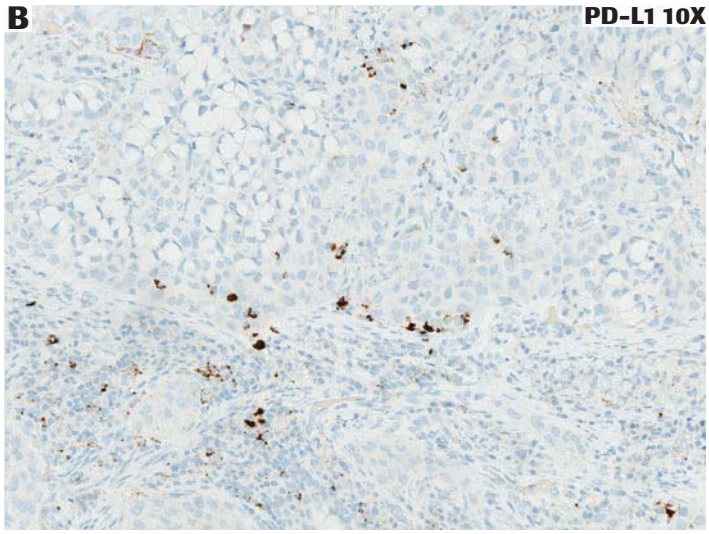






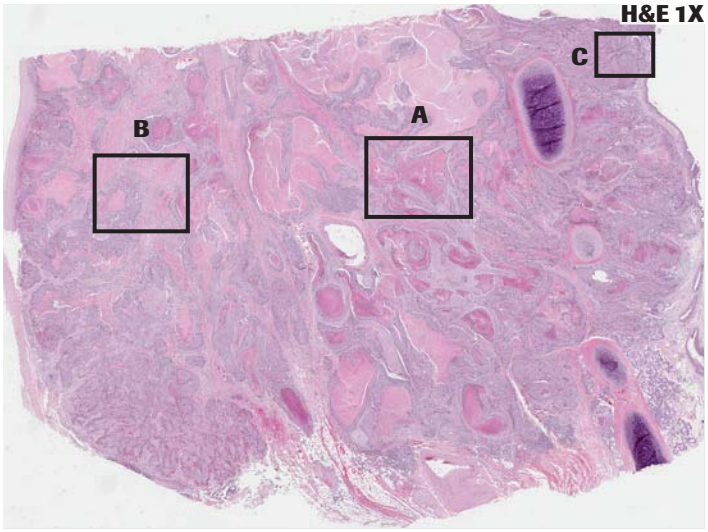


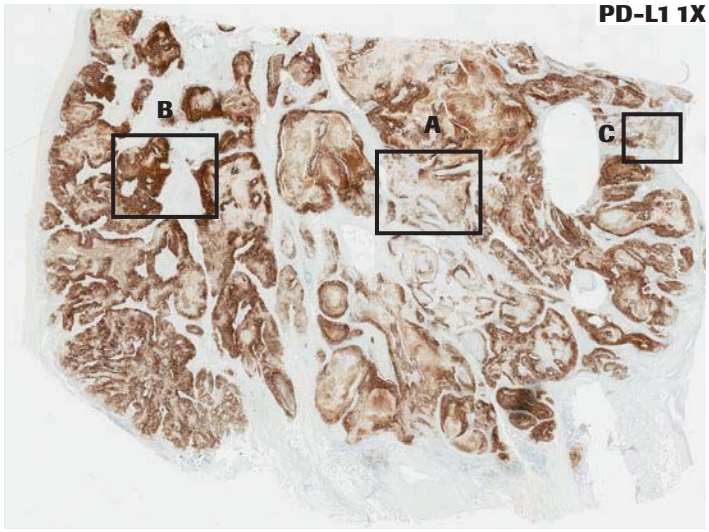


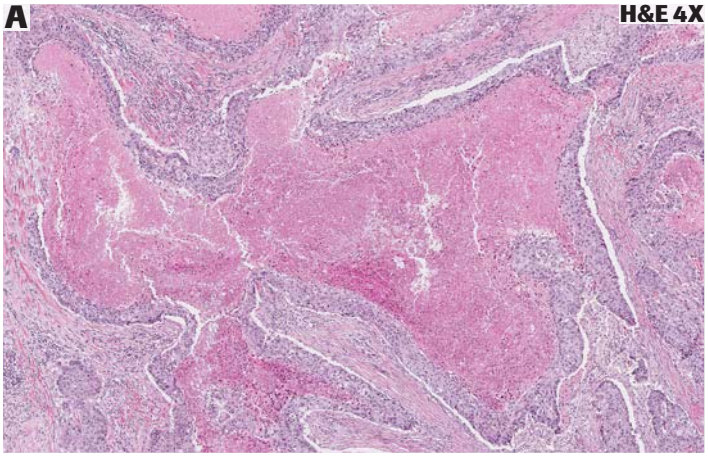


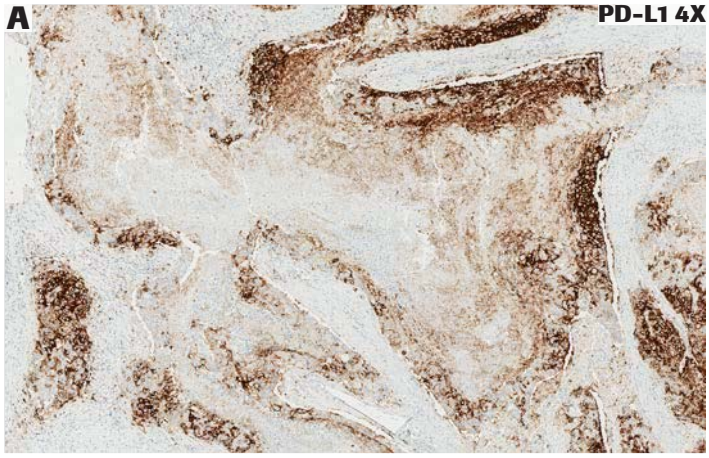
Case 4: This case is TC < 50% and IC < 10%. Scores: TC: 0% and IC: 5%. This case has relatively uniform single cell spread. Estimation of IC percentage should be performed using the **IC Expression – Single-Cell Spread** reference images.

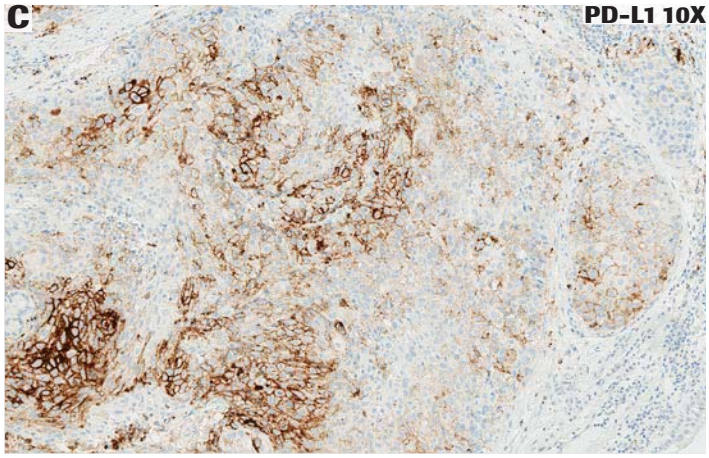
Example Cases: TC ≥ 50%



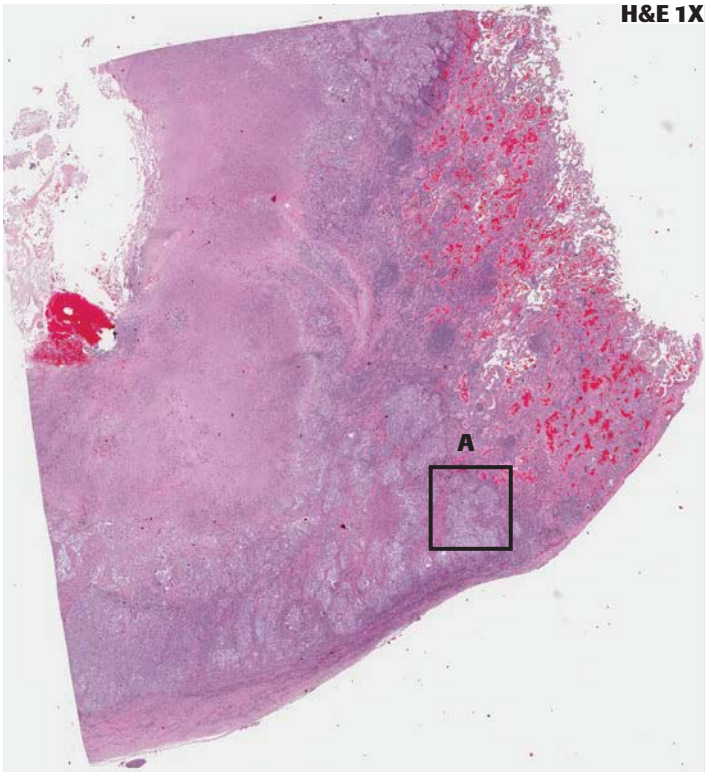


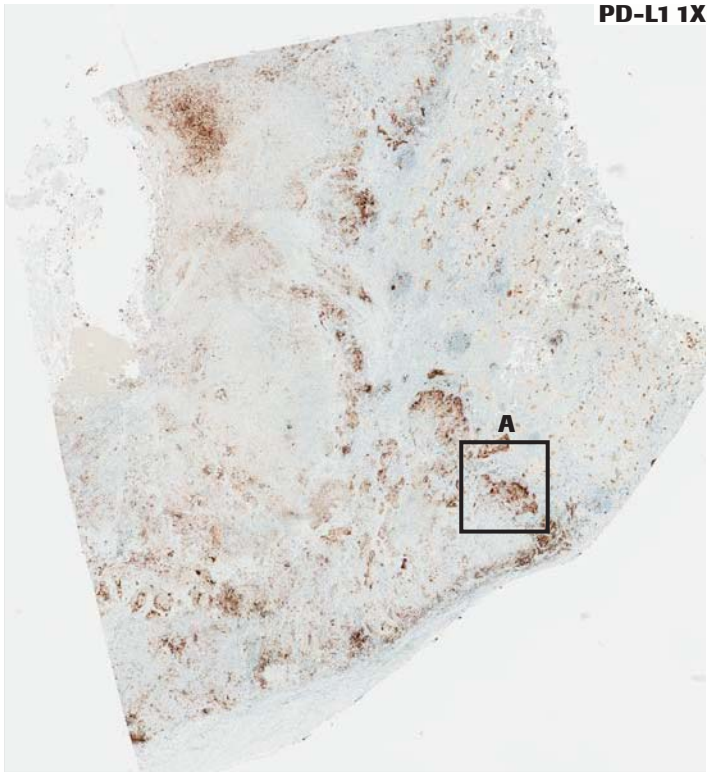


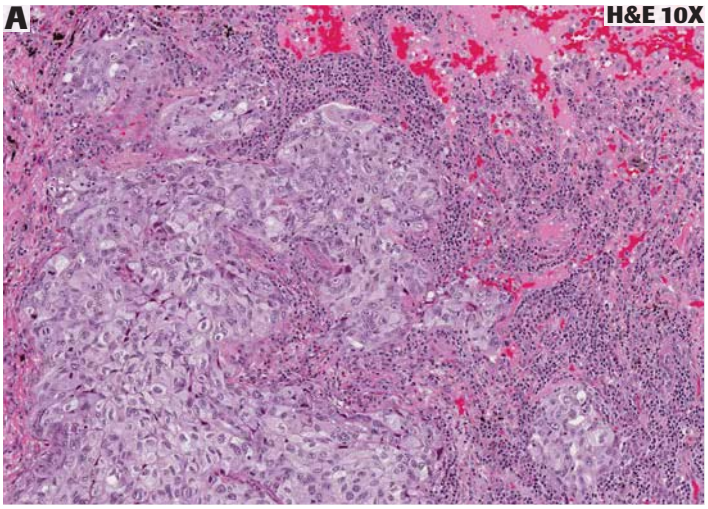


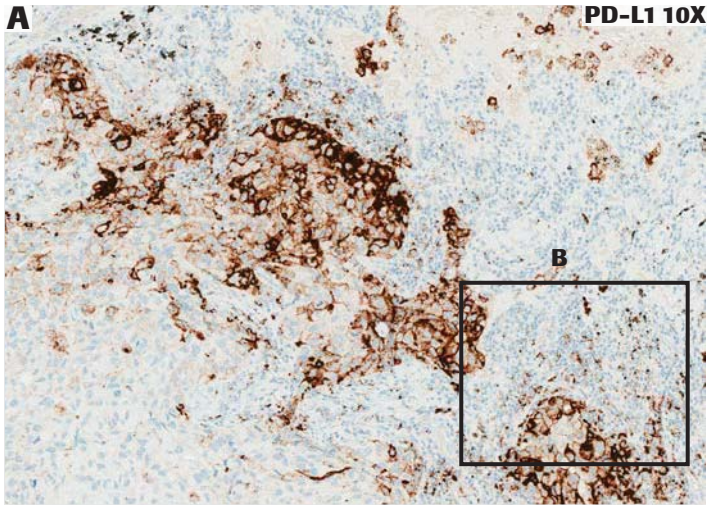


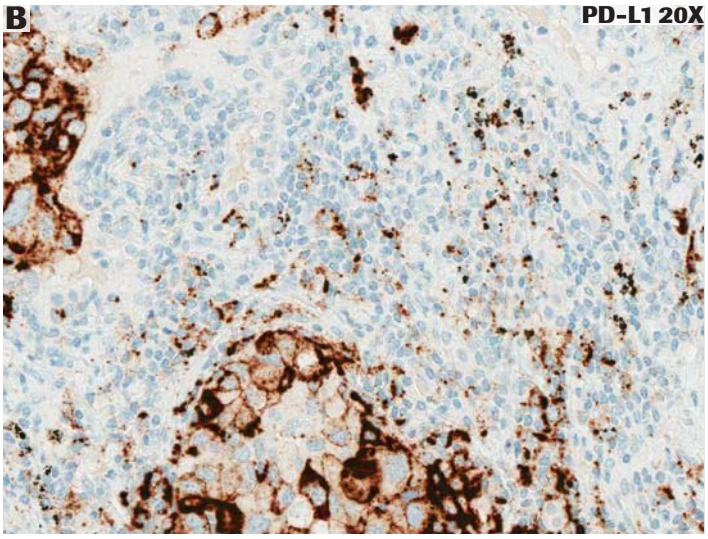
Case 5: This case is TC ≥ 50%. Scores: TC: 80% and IC: 2%. This case illustrates TC staining of variable intensity associated with IC staining. In regions with strong TC staining careful attention to the intratumoral stroma is necessary. In regions with weak to moderate TC staining IC can be assessed relatively easily. Review of the corresponding H&E aids in distinguishing TC and IC staining. Also note: geographic necrosis is not included in the estimation of IC percentage.





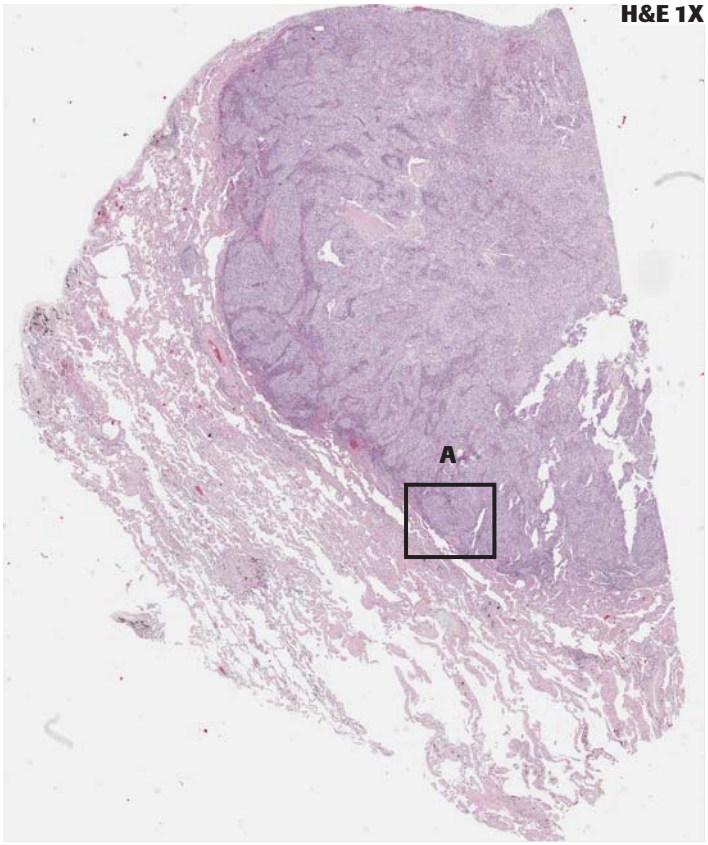


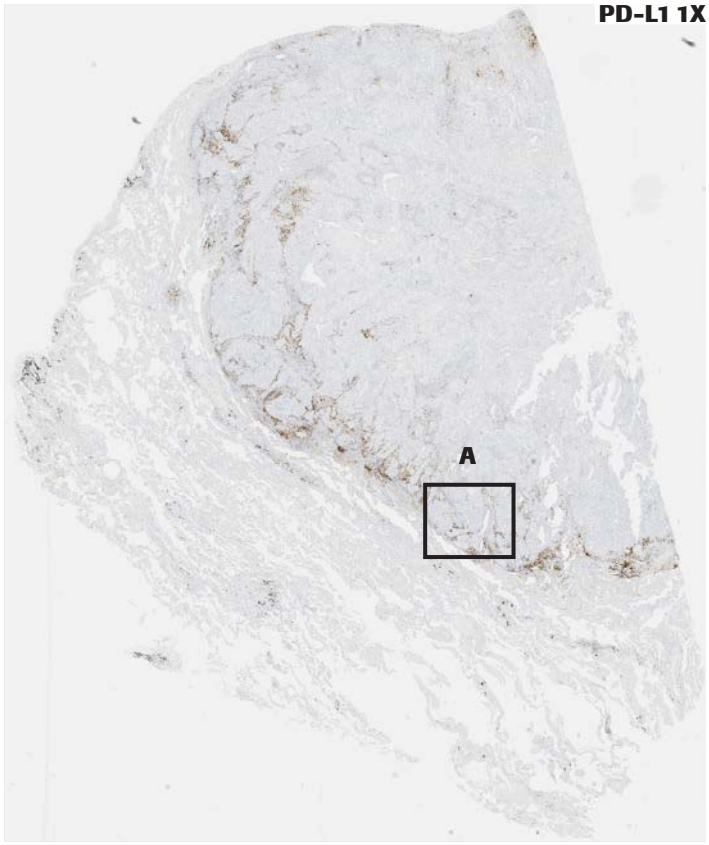


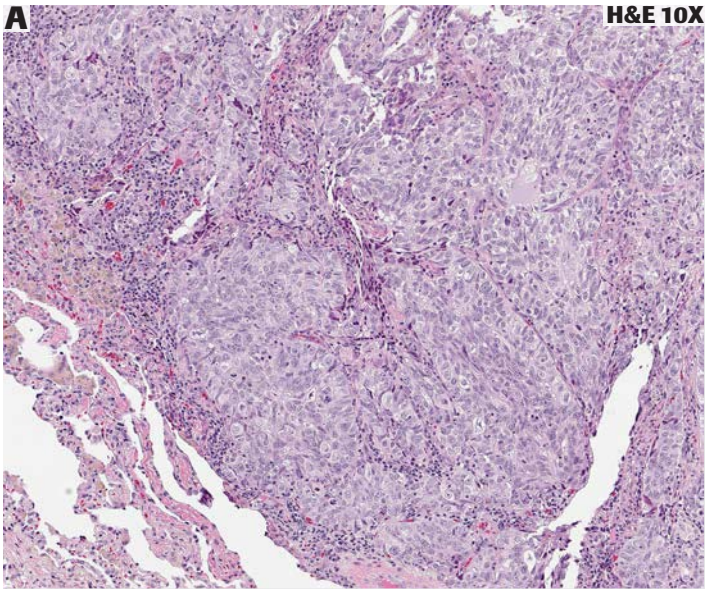


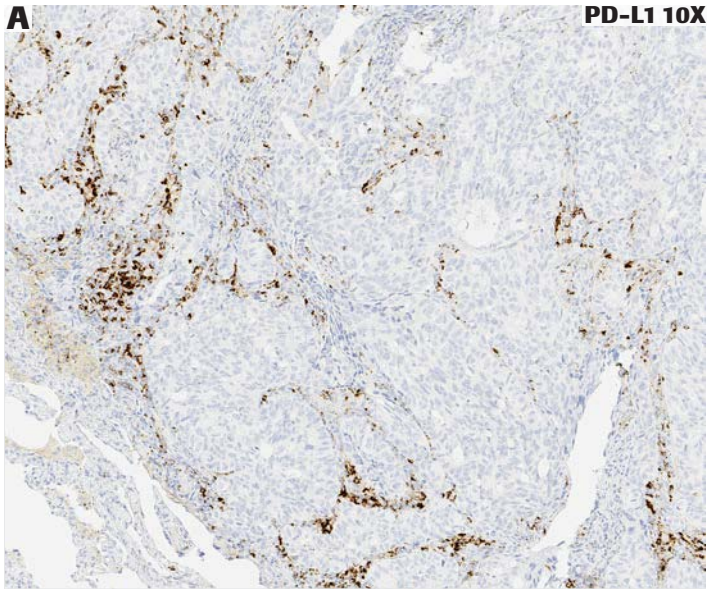
Case 6: This case is TC ≥ 50%. Scores: TC: 60%; IC: 5% . This case shows a combination of TC and IC staining requiring high magnification examination and review of the corresponding H&E to distinguish TC staining from IC staining.

Example Cases: IC ≥ 10%

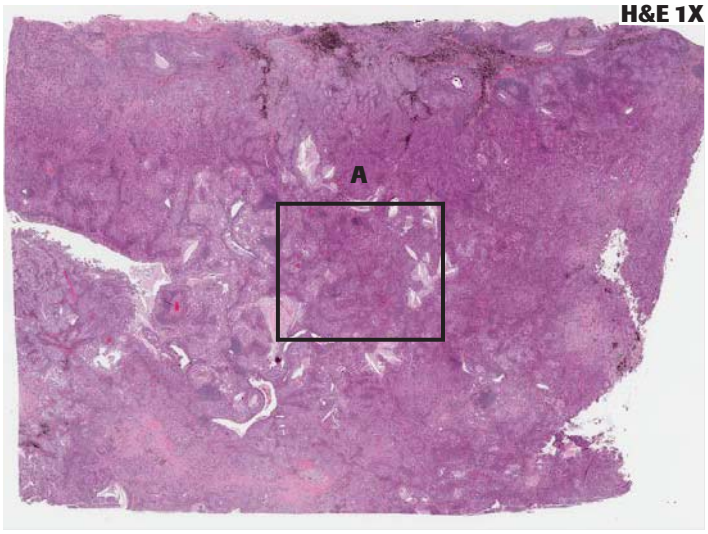


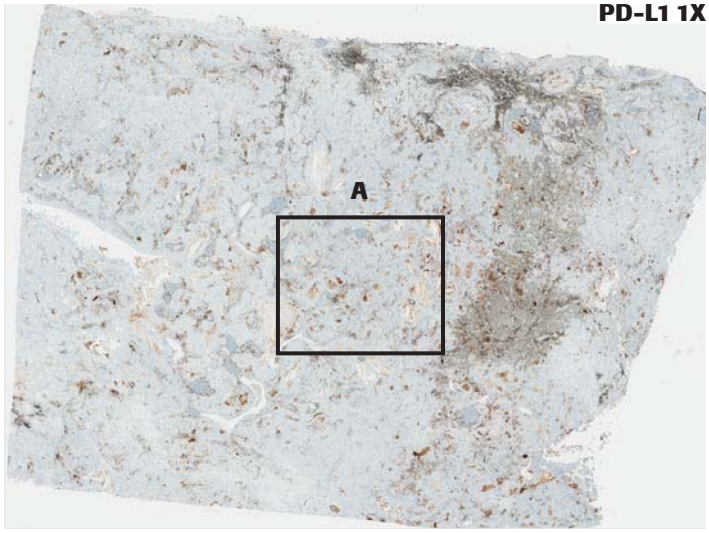


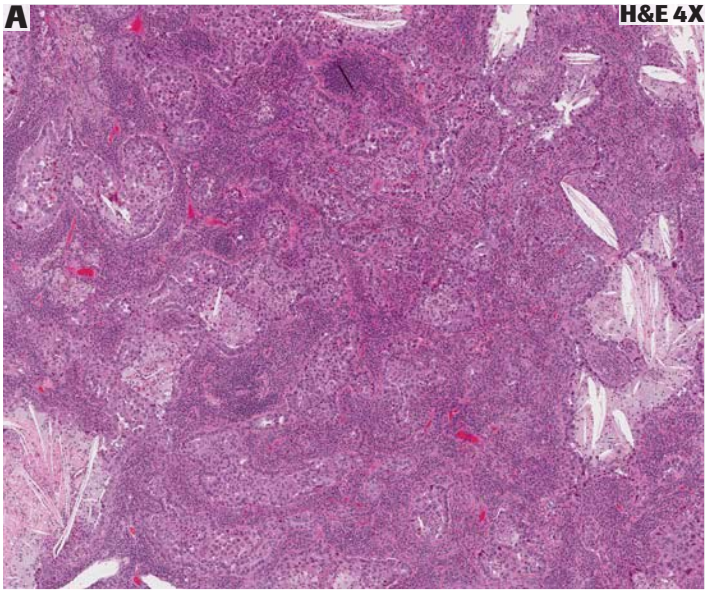


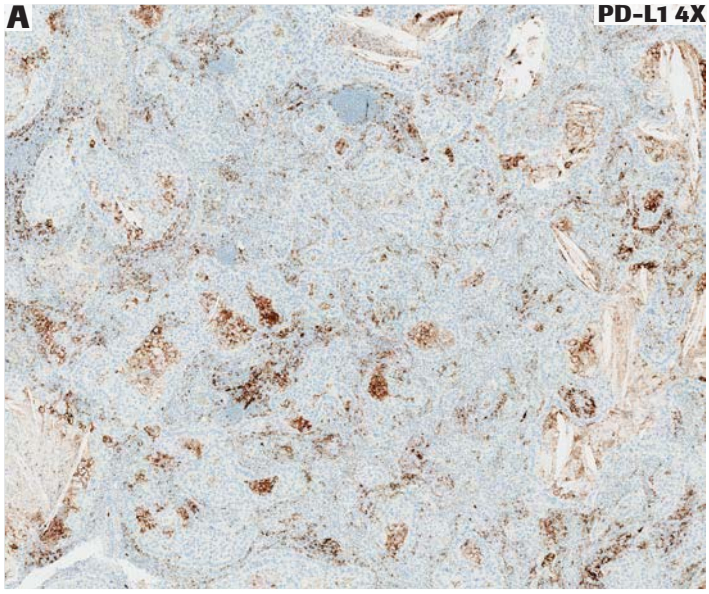


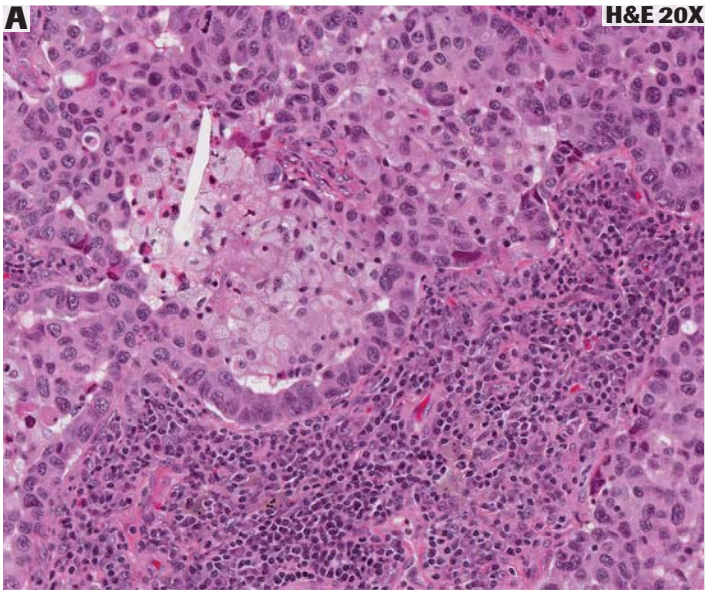
Case 7: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0% and IC: 10%. Note the concentration of PD-L1 IC along the edges of the tumor in the stroma.

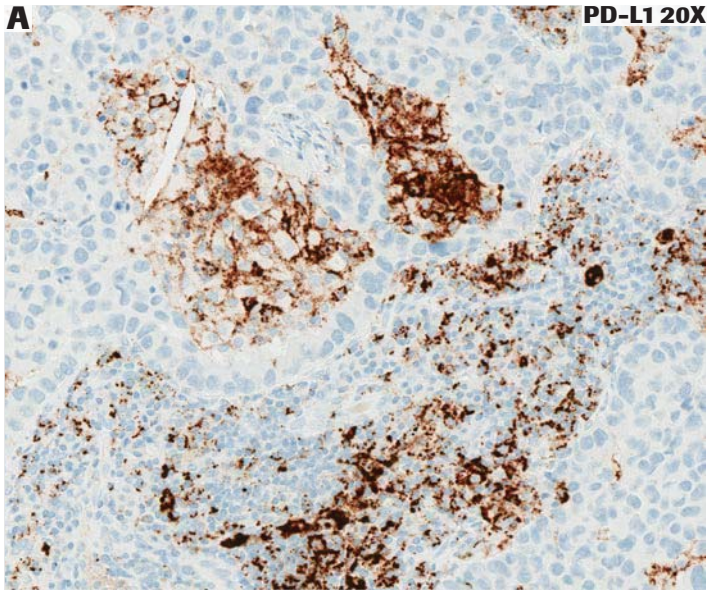




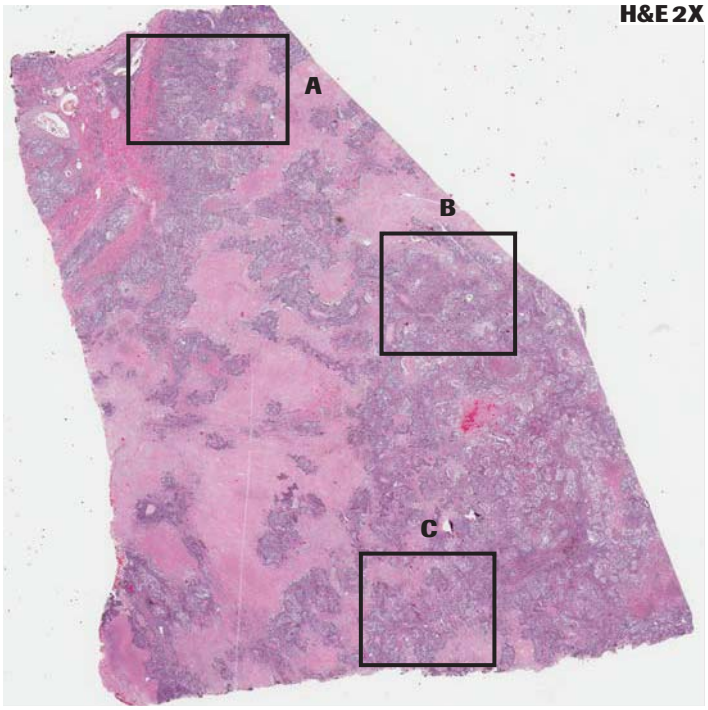


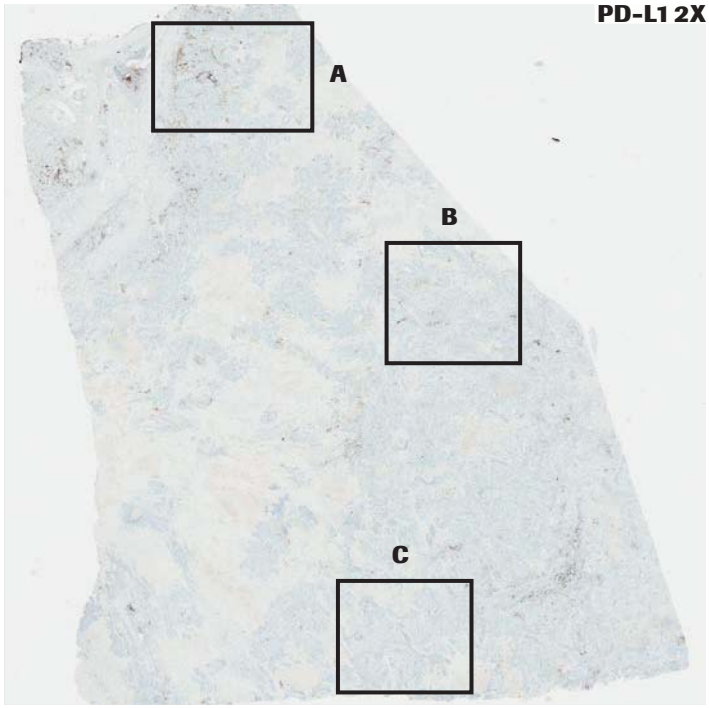


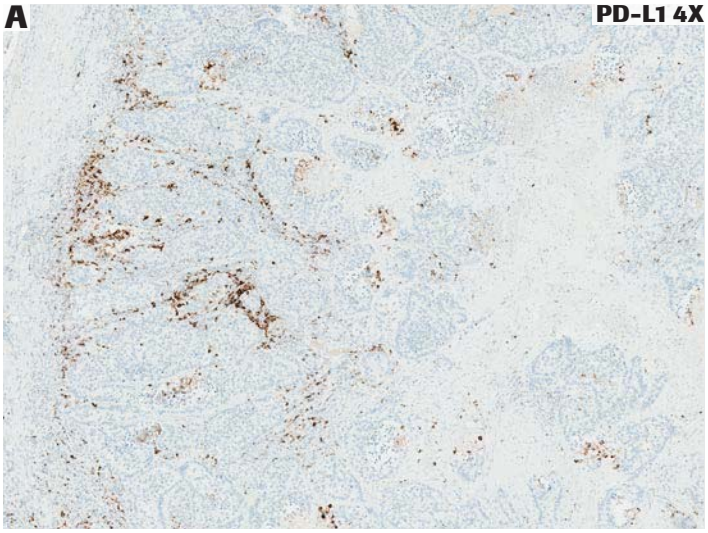


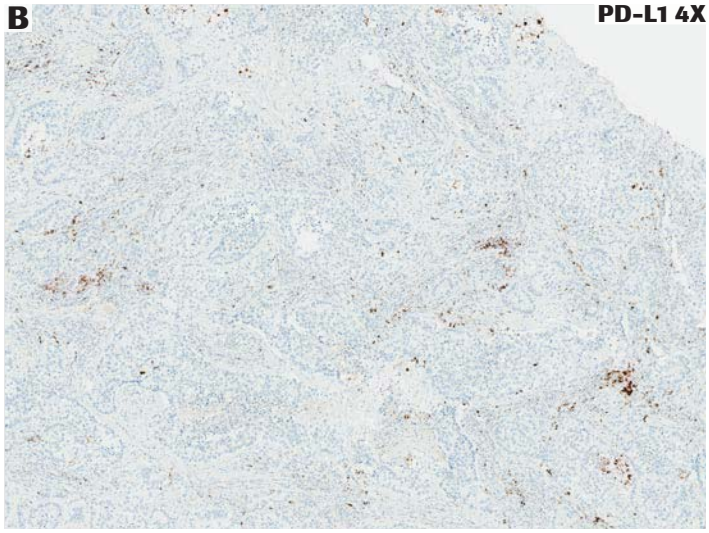


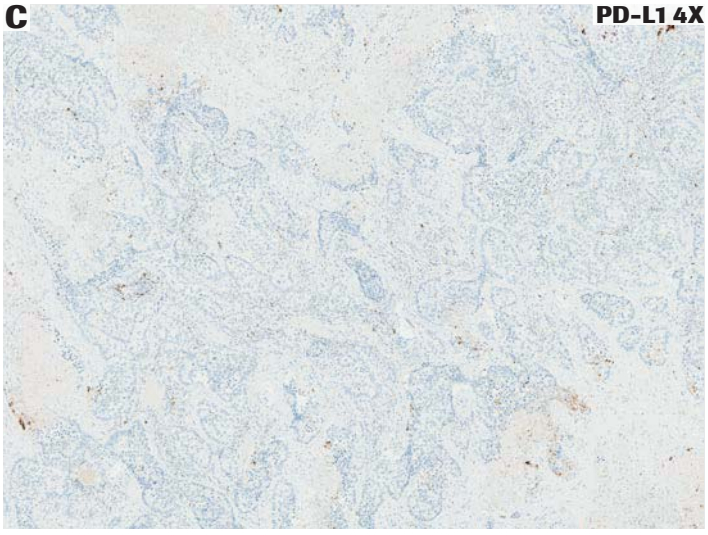
Case 8: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0%; IC: 20%. Note the presence of PD-L1 staining viable intraluminal IC (macrophages) contiguous to TC. These can be mistaken for TC staining and require review of corresponding H&E. These are counted towards IC percentage.





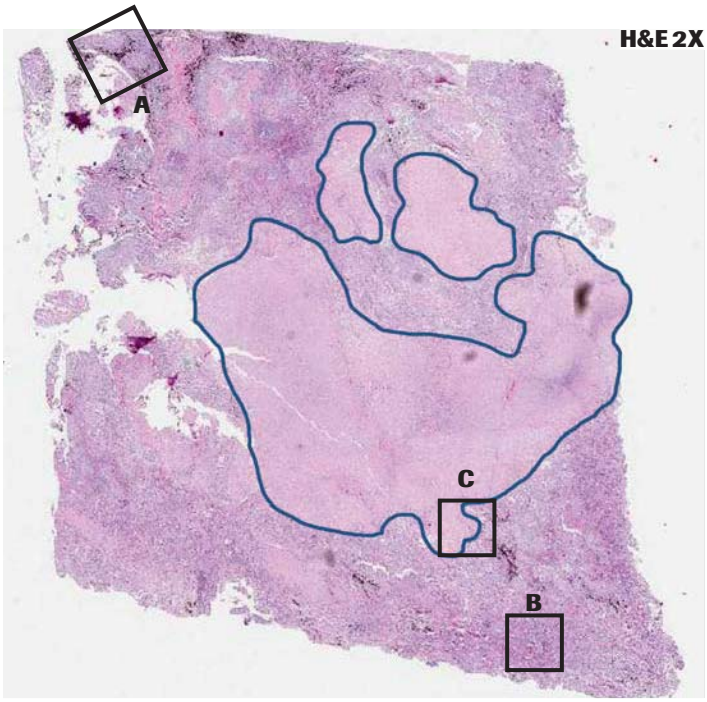


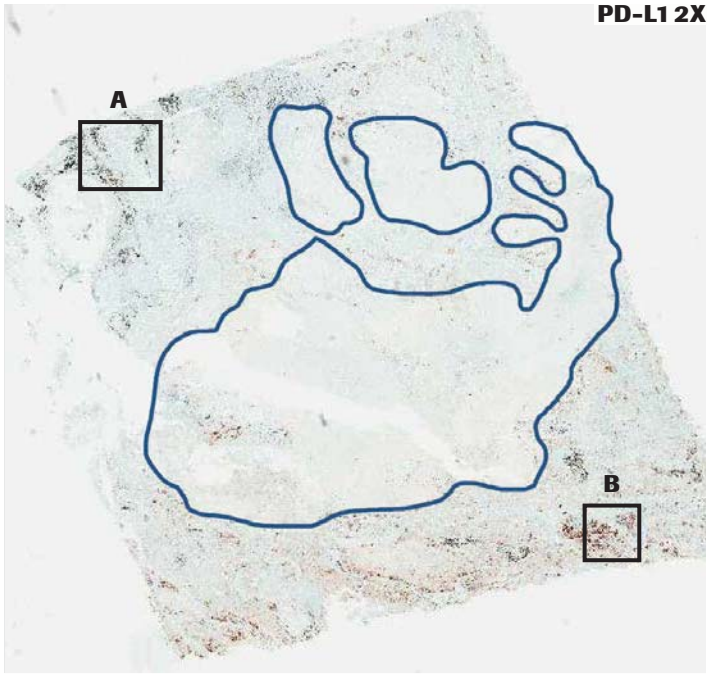


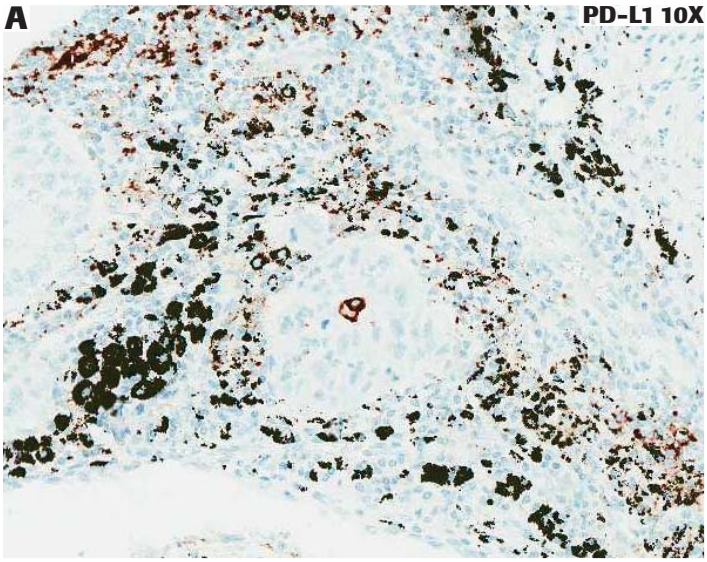


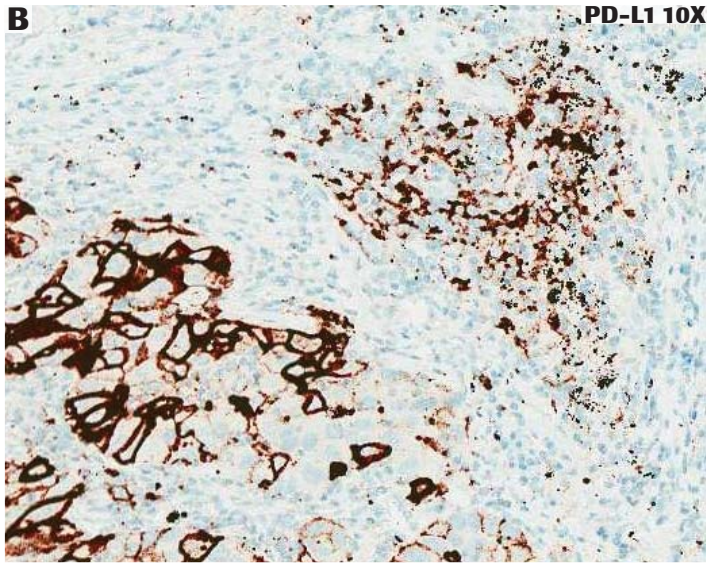
Case 9: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0%; IC: 10%. Three representative regions of IC staining are shown in this case with different ranges of PD-L1 IC staining in each. Overall, the case has 10% IC. Note the presence of geographic necrosis which should be excluded from tumor area definition while scoring IC.

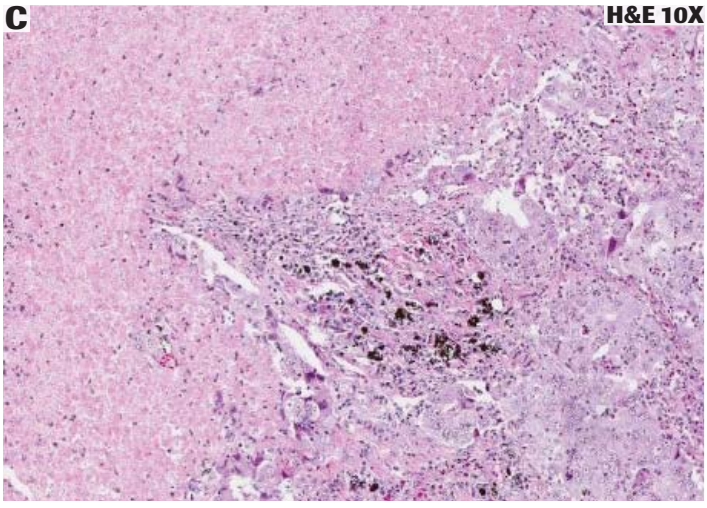
Challenging Cases



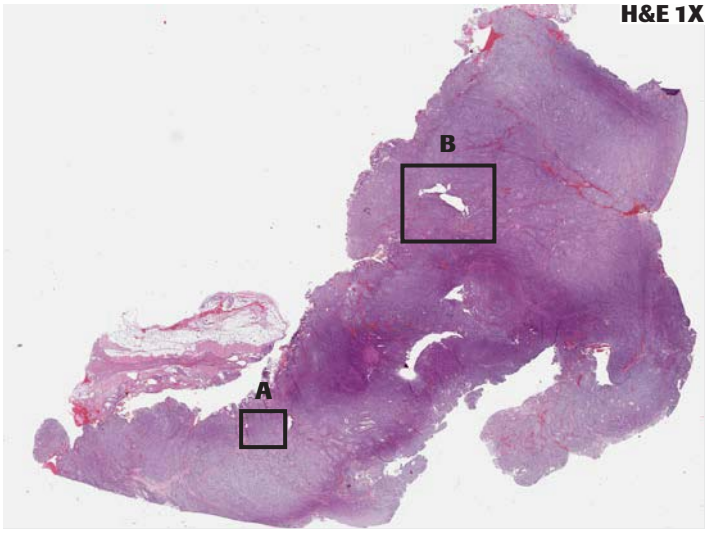


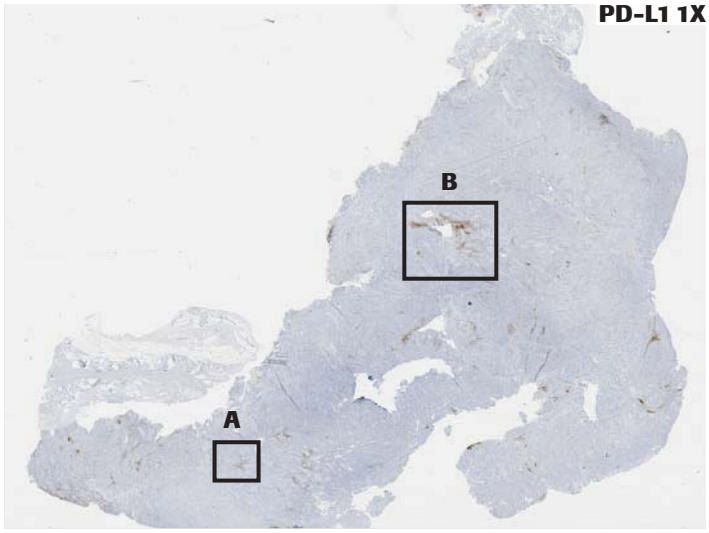


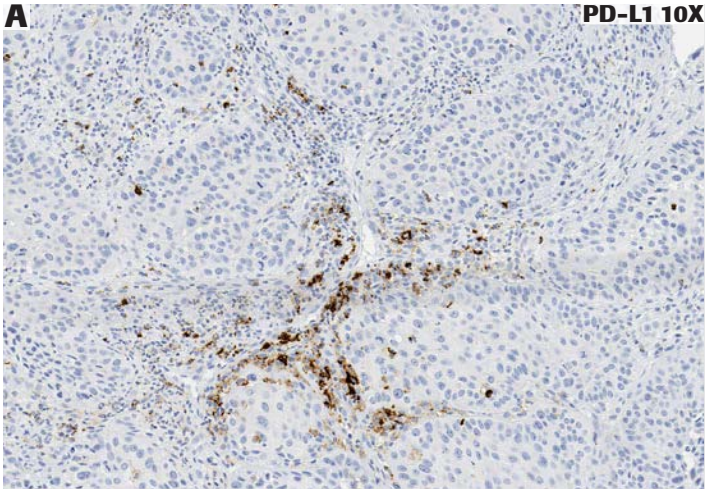


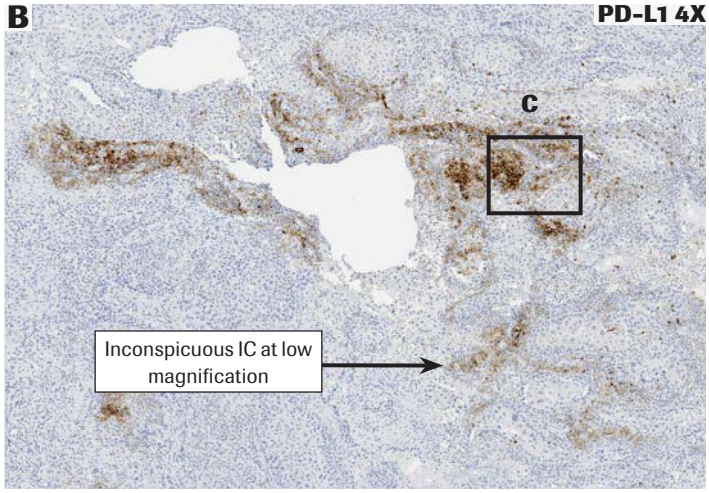


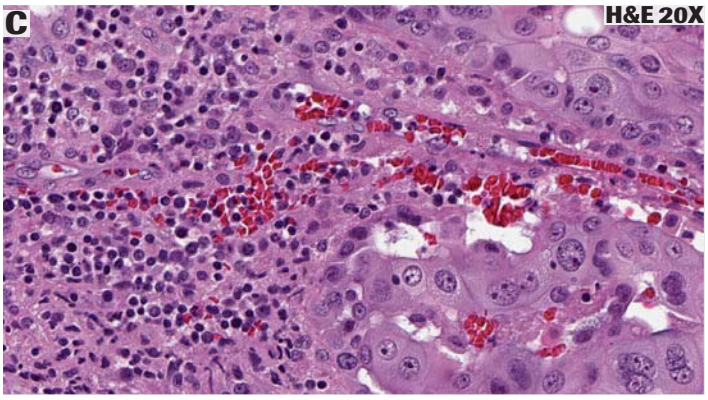
Case 10: This case is TC < 50% and IC < 10%. Scores: TC: 20%; IC: 5%. This case has geographic necrosis which should be excluded from tumor area definition. High magnification images of **Regions A** and **B** show IC aggregates in the midst of anthracotic pigment and next to TC staining. When necrotic region is excluded this case has 5% IC.

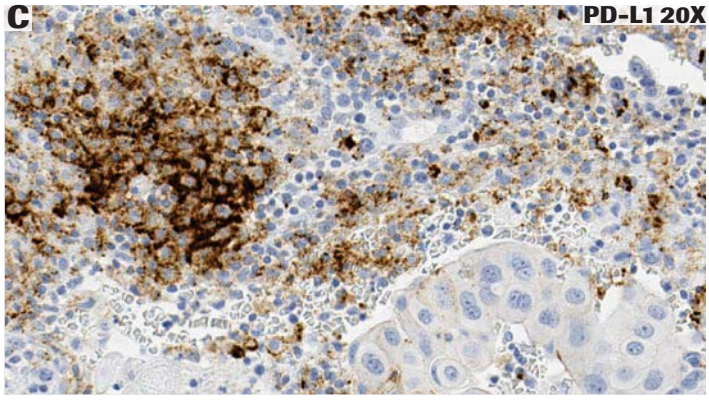




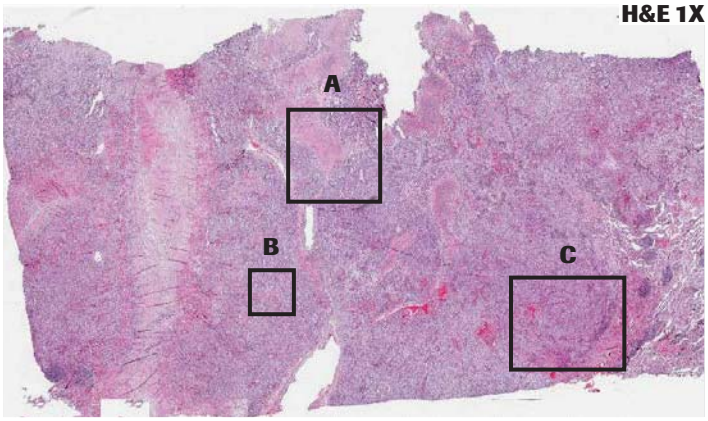


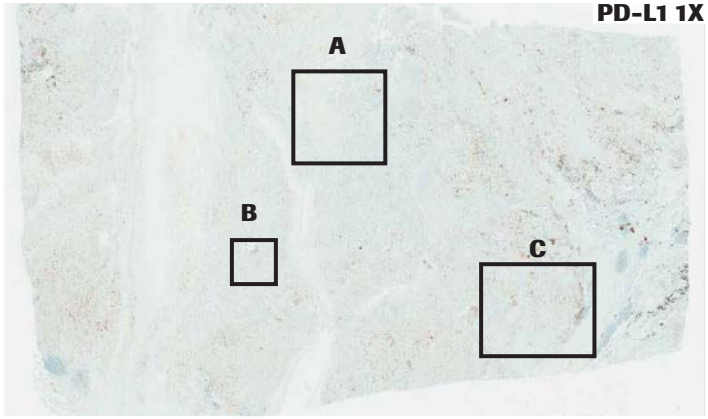


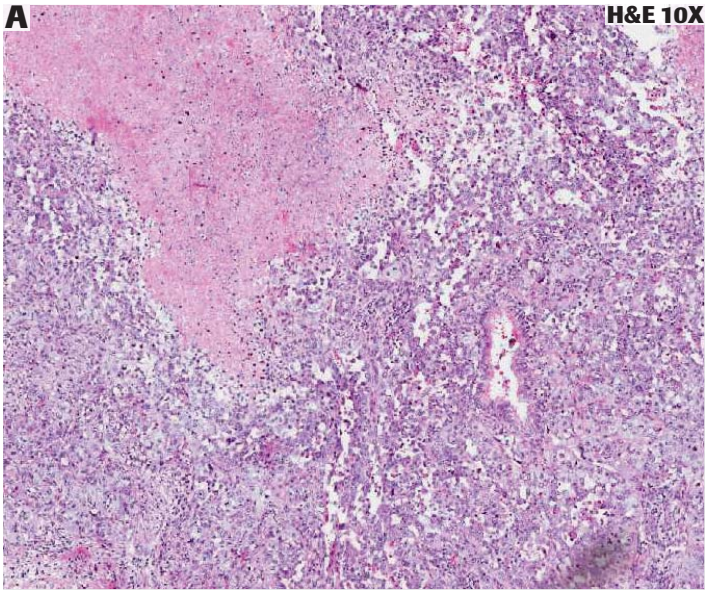


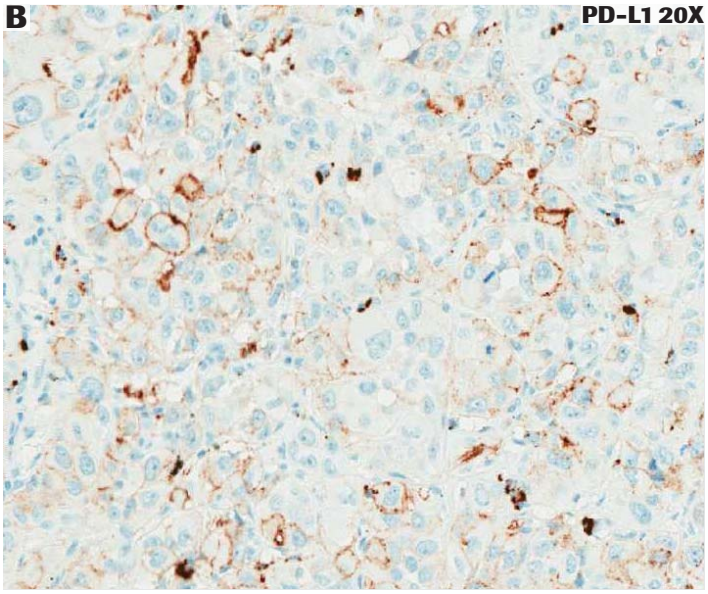


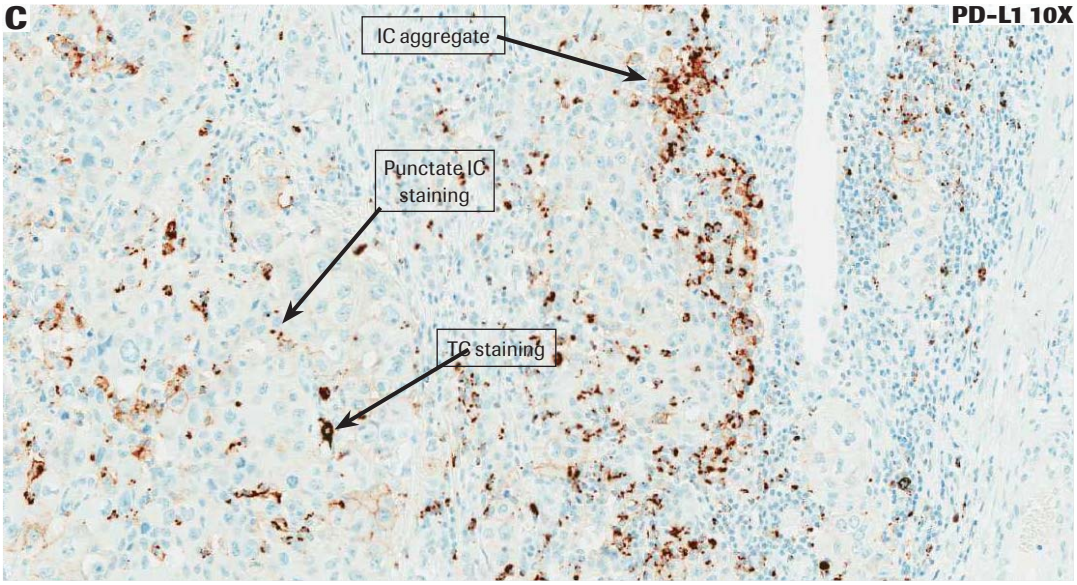
Case 11: This case is TC < 50% and IC < 10%. Scores: TC: 0%; IC: 5%. This tissue illustrates focal IC staining and presence of macrophage staining, which often can be misinterpreted as TC given the circumferential staining (**Region B**). Also note the presence of light staining IC which is not readily apparent at low magnification. This case illustrates the importance of examining PD-L1 stained tissue at high magnification to differentiate macrophage staining from TC staining using the corresponding H&E (**Region C**).



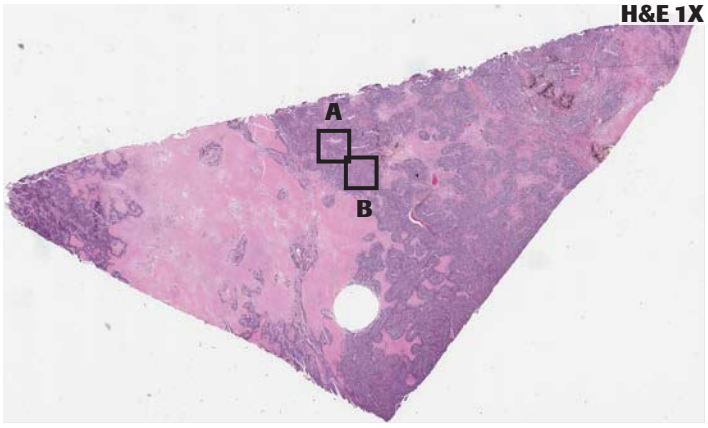


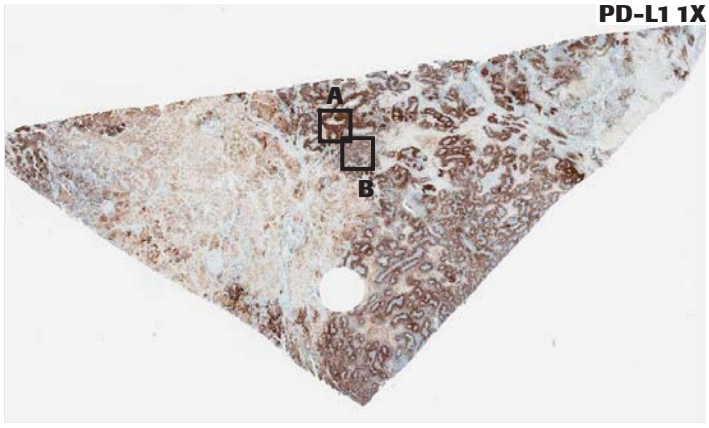


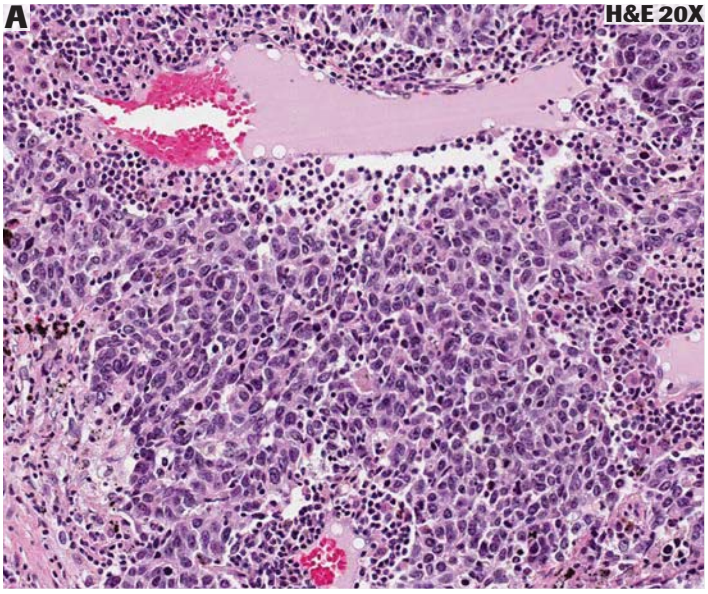


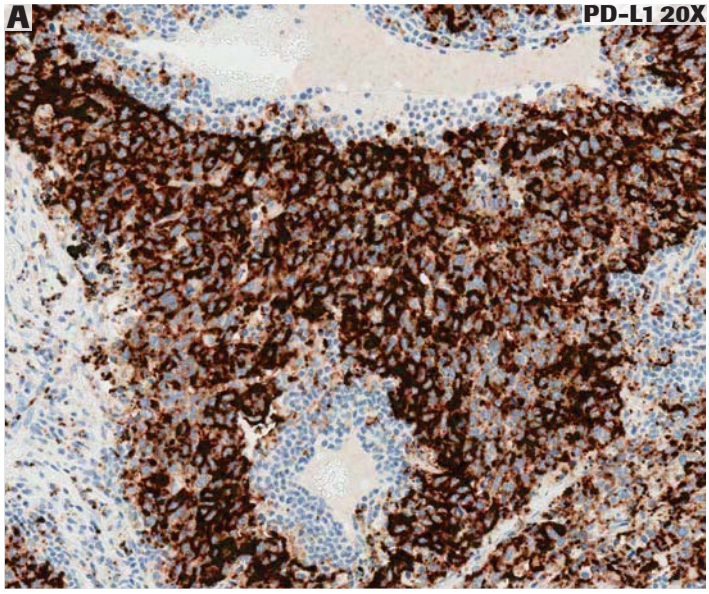


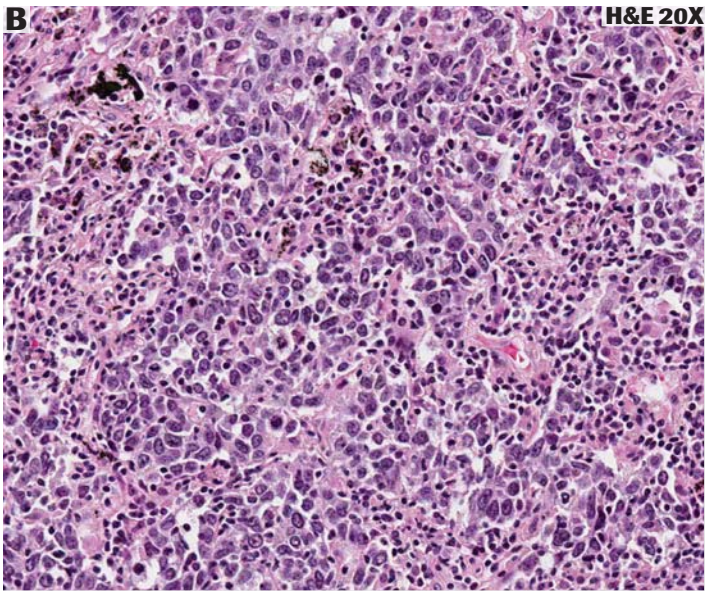
Case 12: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 10%; IC: 15%. On high magnification notice weak membrane staining of tumor cells (**Region B**) and strong punctate IC staining interspersed amongst tumor cells (**Region C**). This case illustrates the importance of using high magnification to differentiate IC from TC staining.

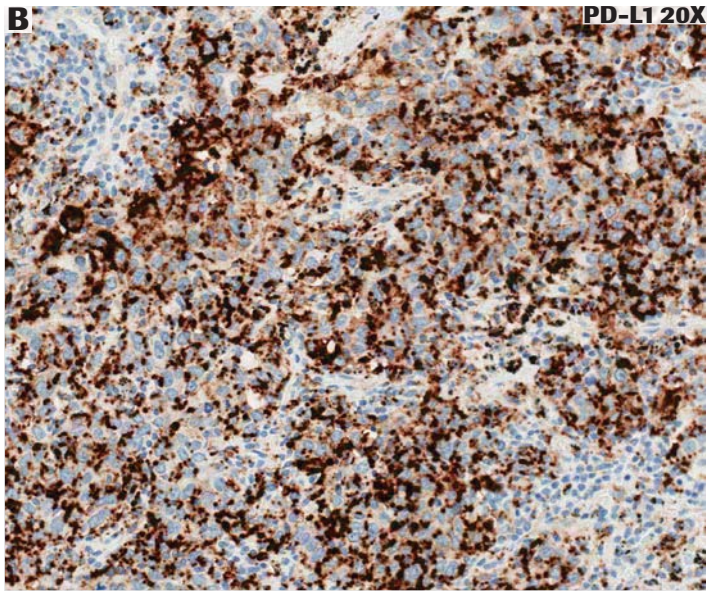








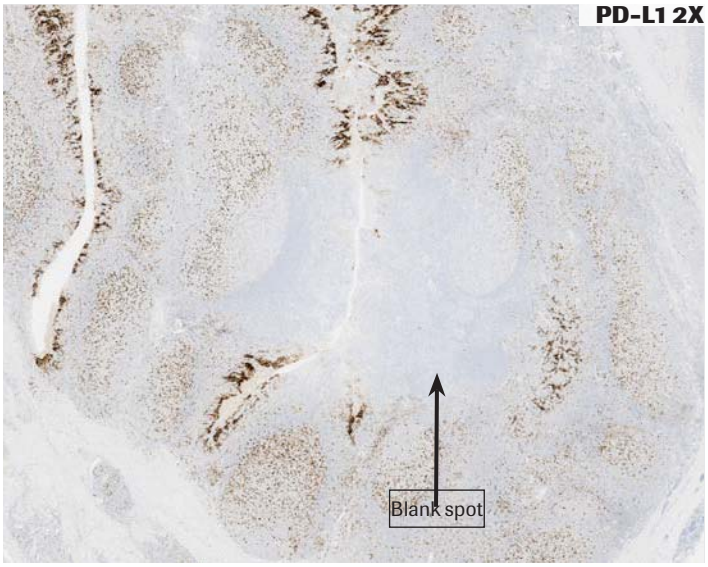


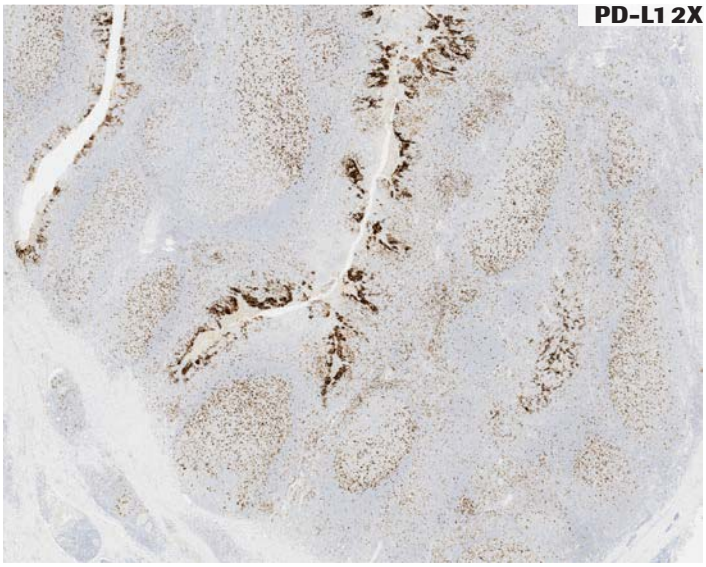


Case 13: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 40%; IC: 50%. Note the presence of TC with more linear membrane staining (**Region A**) and IC as granular staining (**Region B**). This case shows strong staining in both TC and IC with dense immune infiltrate on H&E. This scenario requires careful examination at high magnification to attribute a score for TC and IC. Regions shown here aid in separating TC from IC staining.

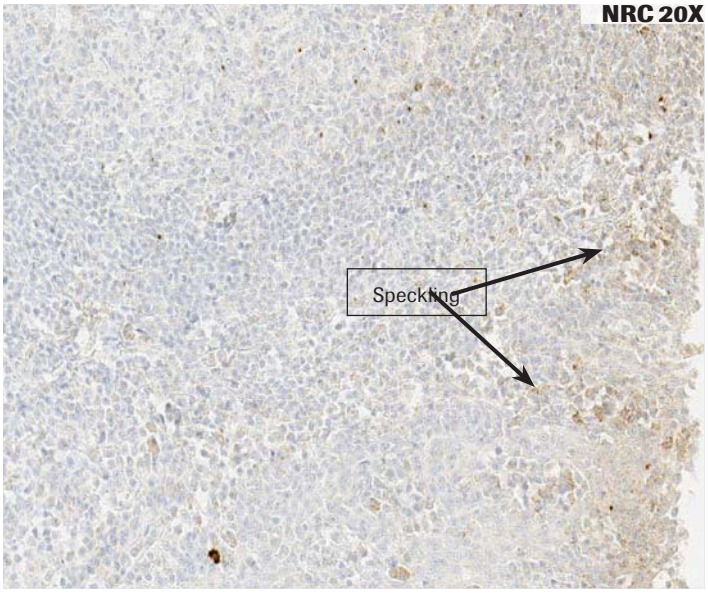
Staining Artifacts

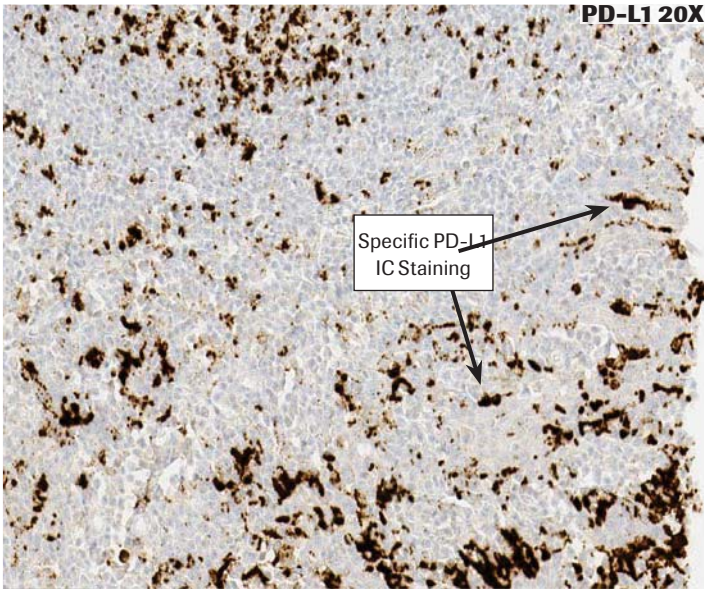
Artifacts noted in this section can be observed on Negative Reagent Control and VENTANA PD-L1 (SP142) Assay-stained slides. The presence of these artifacts may require repeat staining if they interfere with interpretation of VENTANA PD-L1 (SP142) Assay staining. Always review the corresponding Negative Reagent Control slide to ensure that non-specific background staining is within acceptable limits.



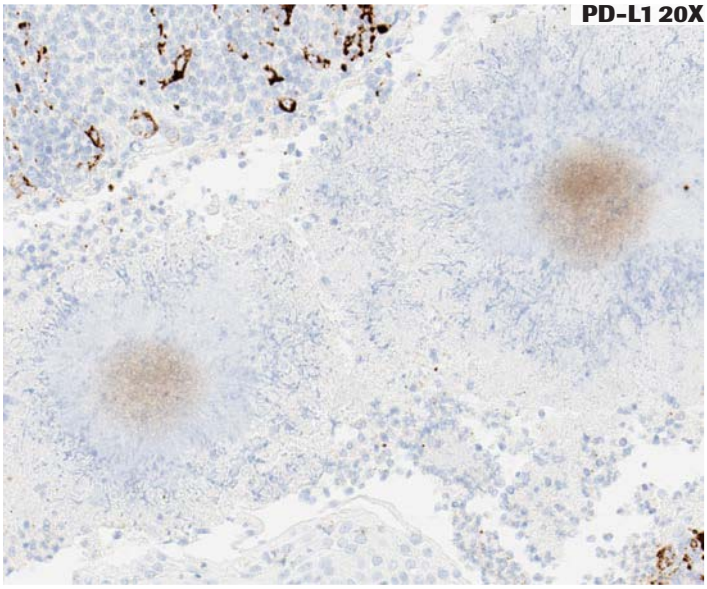


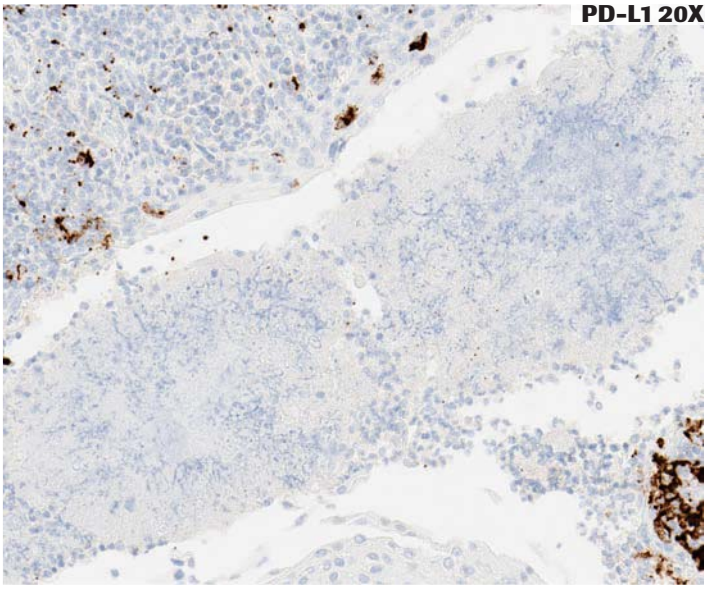
Blank Spots: Blank spots are light to non-staining areas that are typically circular and are due to a static bubble formed during the staining procedure. The image on the left depicts an example of a blank spot opposed to the appropriate staining depicted in the image on the right. If the blank spot interferes with interpretation of the PD-L1-stained slide, a repeat run may be required.



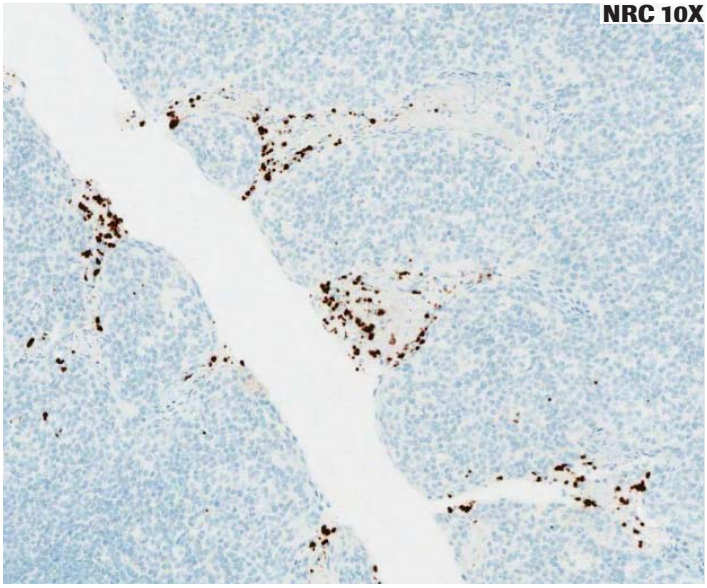


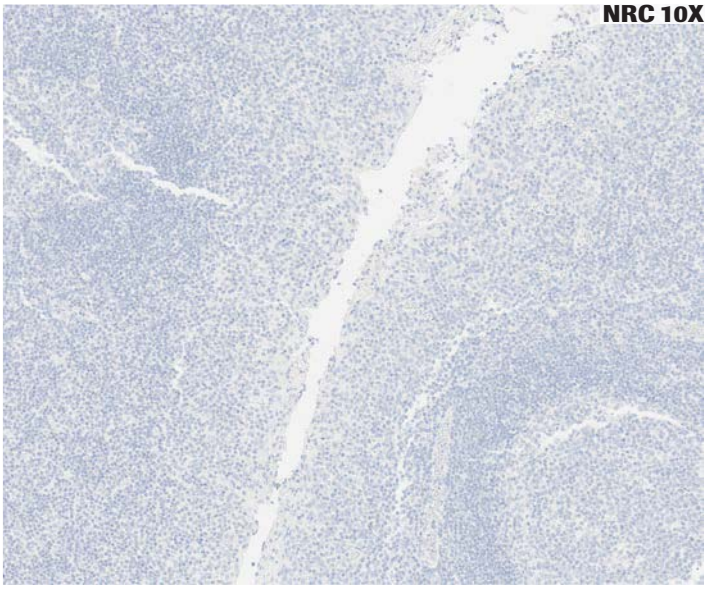
Speckling: Speckling, depicted in the image to the left, is weak to moderate non-specific staining that appears as a uniformly distributed fine granular precipitate most often in the cytoplasm. Speckling does not conform to either IC or TC staining characteristics. This artifact should not be confused with specific staining such as depicted in the image to the right.



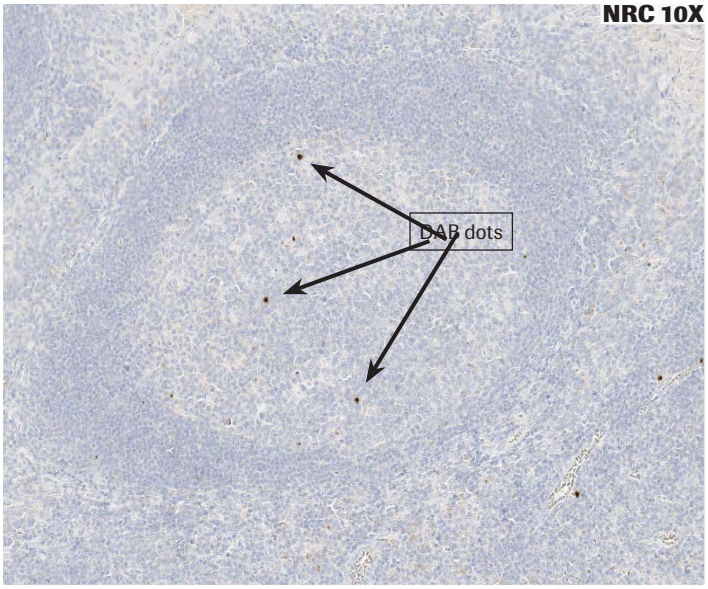


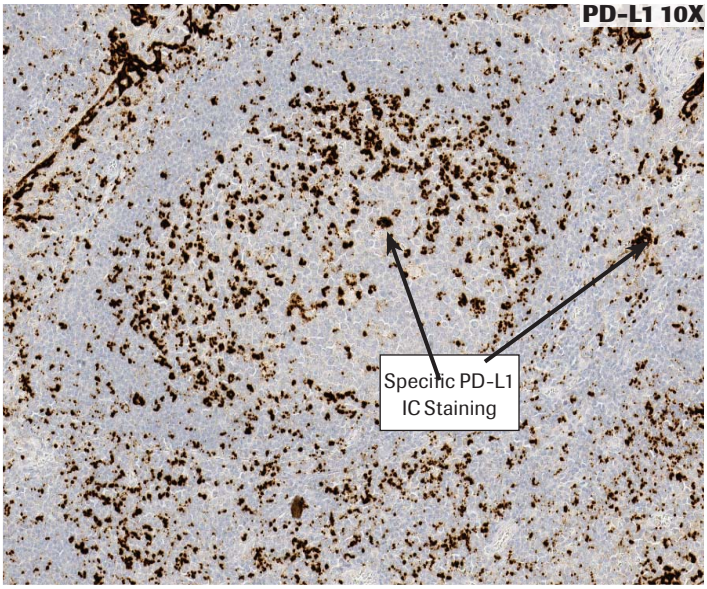
DAB Spots: DAB spots are circular spots that may form due to trapped DAB underneath the tissue section during the staining procedure. If this artifact interferes with the interpretation of PD-L1 stained slide, repeat the stain with fresh unstained slides. In the image to the right, the DAB spot is not present with repeat staining of a serial section.



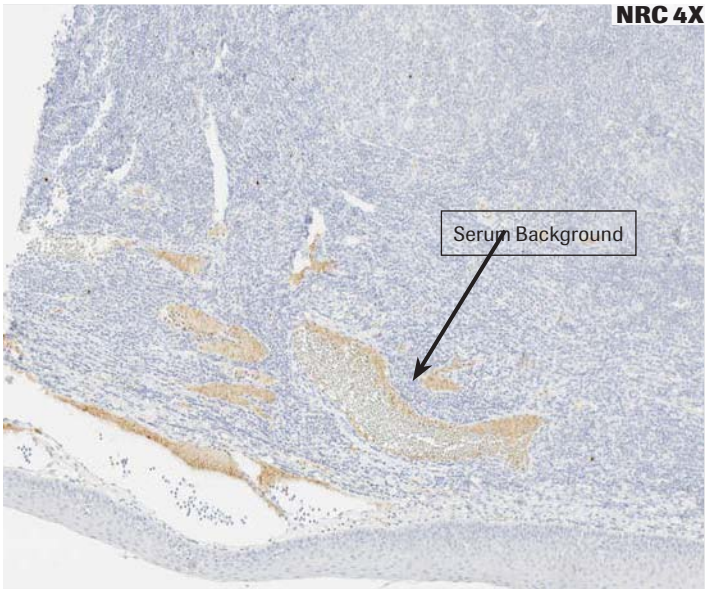


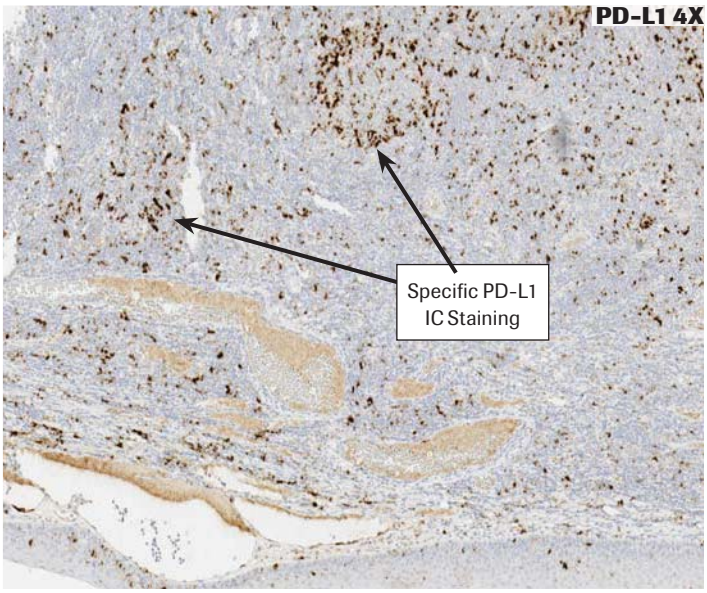
Luminal Debris: Tonsil stained with VENTANA PD-L1 (SP142) Assay can serve as both a positive and negative tissue control due to positive and negative staining elements being present. Luminal staining due to cross reactivity with an unknown antigen can be observed in the image of a tonsil stained with NRC on the left. An appropriate example of tonsil stained with NRC is depicted on the right. If you choose to run NRC on tonsil control tissue and luminal debris is observed, the sample should not be used as control.





DAB Dots: Non-specific punctate background may be observed in tissue of any indication and are small, indiscriminate staining artifacts from the amplification detection system. In comparison to PD-L1 staining of IC, DAB dots are smaller and exhibit a different, crisp morphology outline than the punctate IC staining. Expected immune cell staining can be seen in the image to the right.





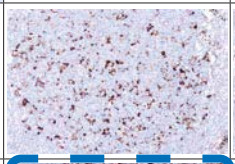
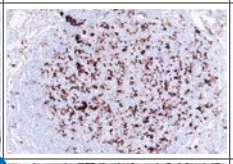
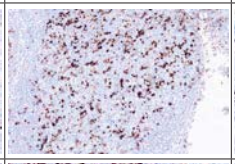
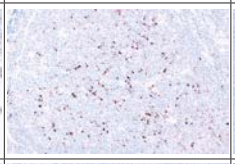
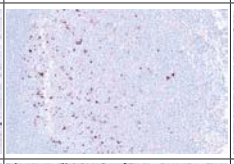
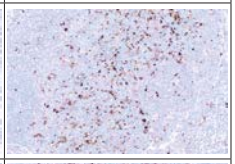
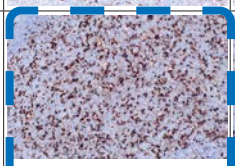
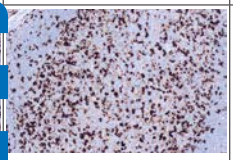
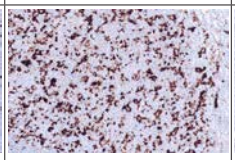
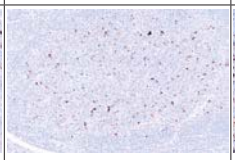
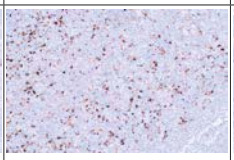
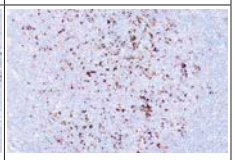
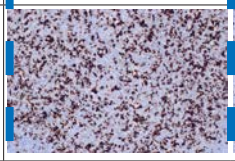
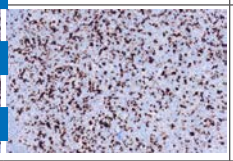
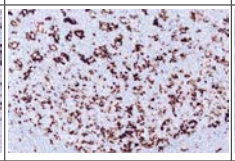
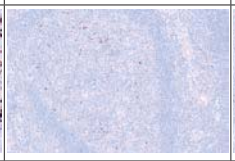
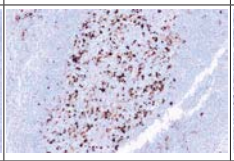
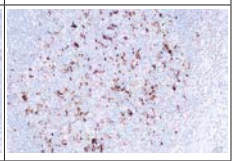
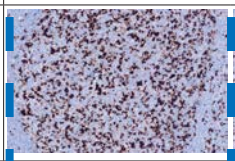
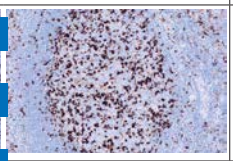
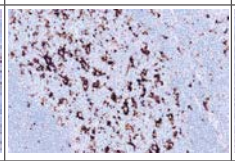
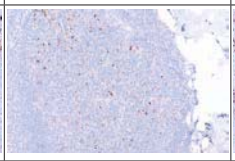
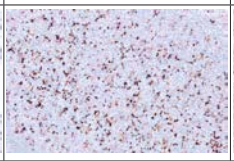
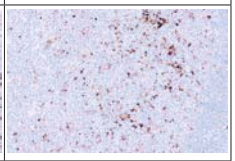
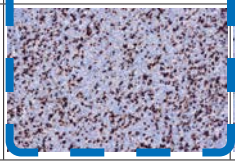
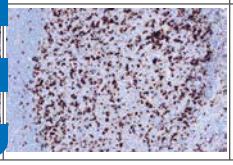
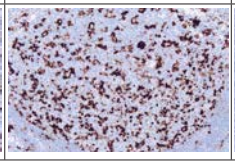
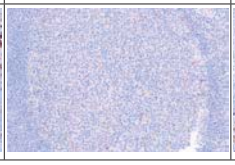
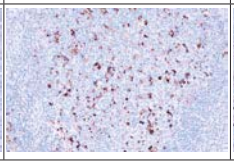
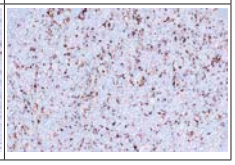
Serum Background: Serum background is non-specific staining in vascular spaces and serum extravasates. This is depicted in the bottom of each image above. It should not be confused with specific PD-L1 IC staining as depicted in the image to the right.

Impact of Pre-Analytical Conditions on VENTANA PD-L1 (SP142) Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results with VENTANA PD-L1 (SP142) Assay

- Ventana recommends fixation in 10% NBF for 6-72 hours.
- Zinc Formalin demonstrates comparable staining to 10% NBF.
- Less than 6 hour fixation is not recommended.
- Samples fixed with Z-5 demonstrate inconsistent staining with those fixed in 10% NBF; Z-5 fixation is not recommended.
- PREFER (Anatech, Ltd.) and alcohol fixatives including AFA (weaker staining) are not recommended.

Table 4: VENTANA PD-L1 (SP142) Assay Staining of Tonsil Tissue Across Fixatives and Fixation Time

Time point (Hrs)	Fixative					
	10% NBF	Zinc Formalin	Z-5*	PREFER* (Anatech, Ltd.)	AFA*	95% Alcohol*
1*						
6						
12						
24						
72						

Recommended

*Not recommended

(all images 20x magnification)

Antigen Stability on Cut Tissue Sections

Cut sections (unstained slides) of NSCLC and human tonsil tissues should be stained within 2 months of sectioning. Tissue cut sections (unstained slides) stored at ambient temperature show a significant loss of staining after this time (**Figure 23**).

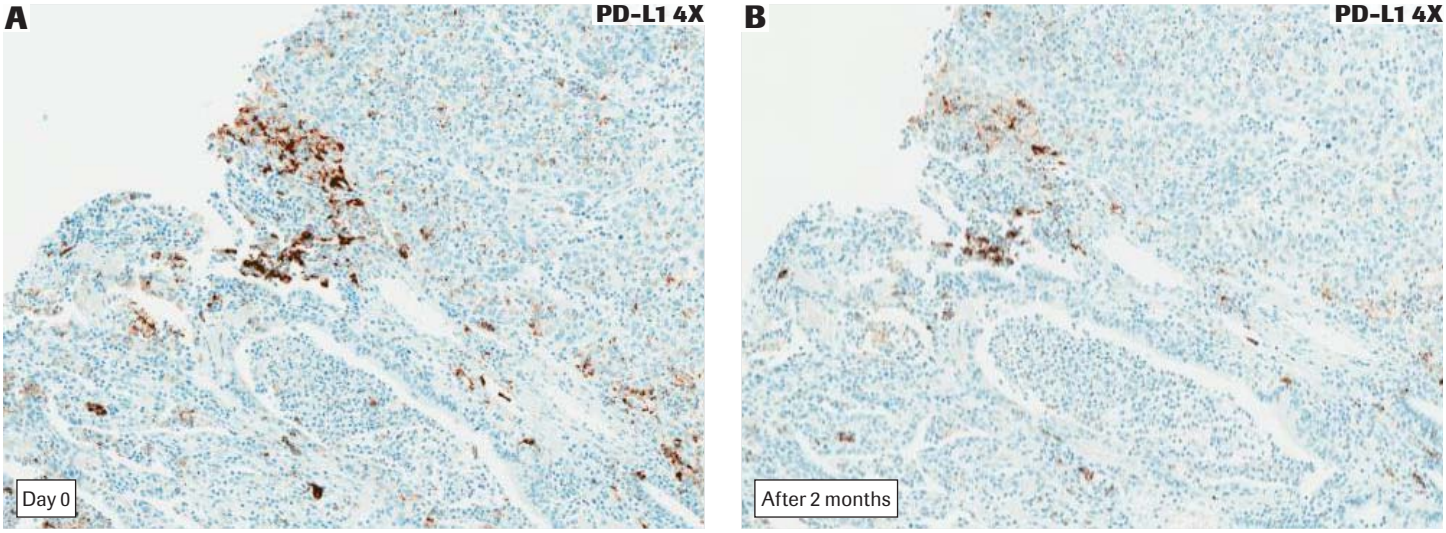


Figure 23: Serial sections of NSCLC tissue stained at Day 0 (**A**) and after two months storage at ambient temperature (**B**).

References

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. http://globocan.iarc.fr. Published 2013-12-12. Updated 2014-01-09. Accessed 2016-02-08.

2. Malvezzi M, Carioli G, Bertuccio P, et al. European cancer mortality predictions for the year 2016 with focus on leukaemias. Ann Oncol. 2016;27(4):725-31

3. Siegel R, Naishadham D, Jemal A. Cancer Statistics, 2013. CA Cancer J Clin. 2013;63(1):11-30.

4. Howlader N, Noone AM, Krapcho M, et al. (eds). SEER Cancer Statistics Review (CSR),1975-2012. National Cancer Institute. http://seer.cancer.gov/csr/1975_2012/. Published 2015-04-23. Updated 2015-11-18. Accessed 2016-02-08.

5. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother. 2007;56(5):739-745.

6. Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 2007;27(1):111-122.

7. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-567.

8. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-3175.

9. Fehrenbacher L, Spira A, Ballinger M et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;387(10030):1837-1846.

10. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-2454.

Refer to the corresponding VENTANA PD-L1 (SP142) Assay package insert for manufacturer contact information.

www.ventana.com

1015703EN Rev C
2020-07-15

IVD