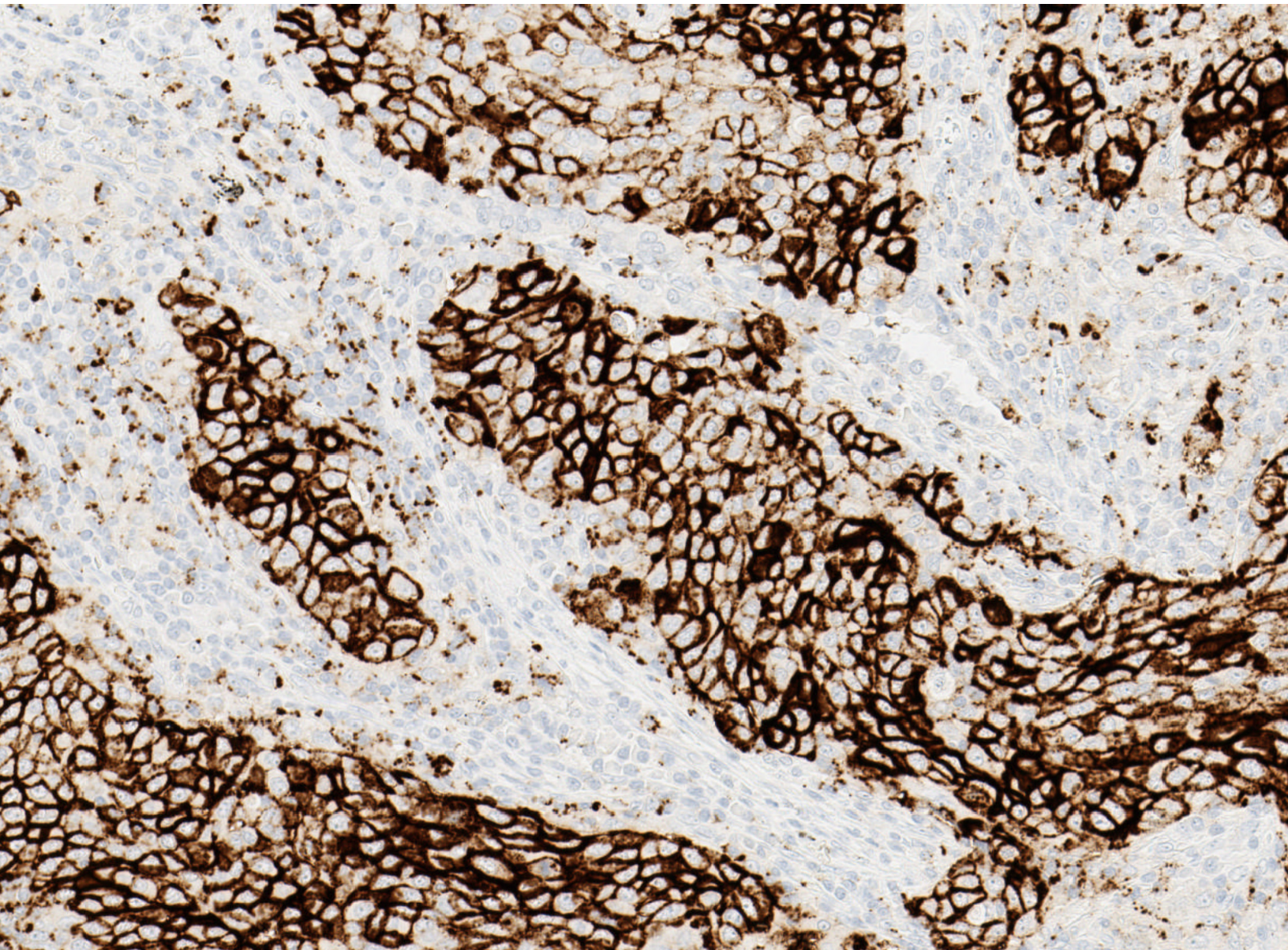
VENTANA PD-L1 (SP142) Assay

Interpretation Guide for Non-Small Cell Lung Cancer

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



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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,

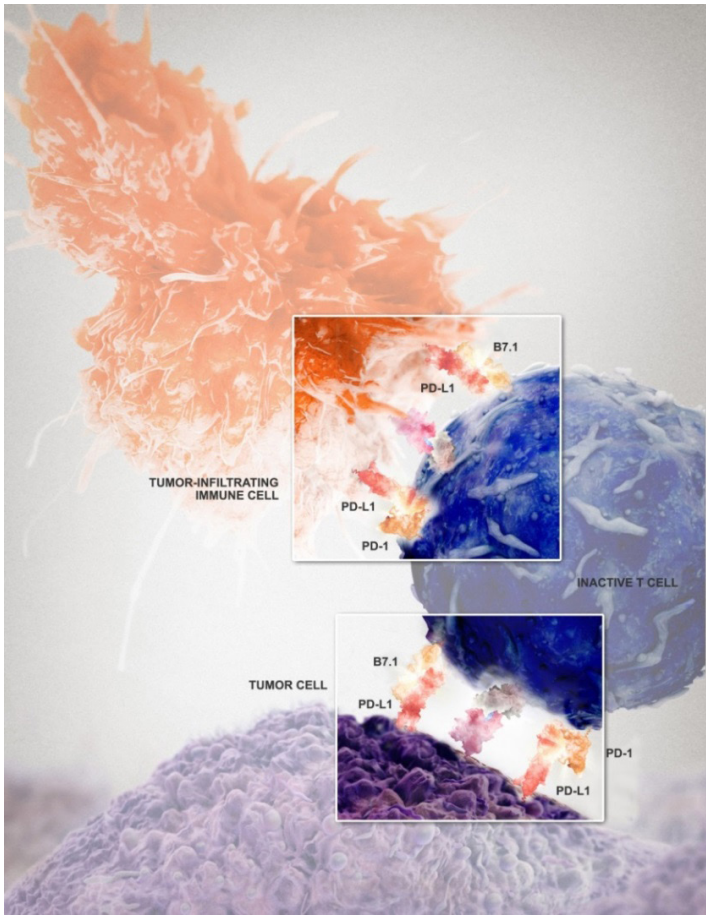


Figure 1: PD-1, PD-L1 pathway.

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

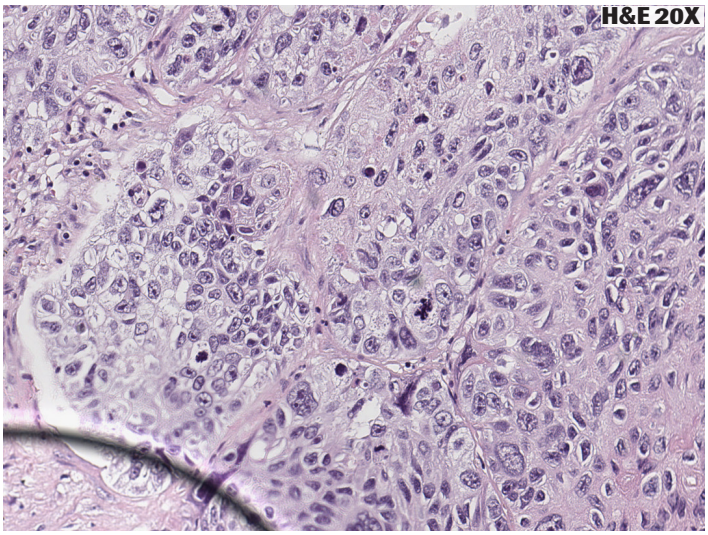
The VENTANA PD-L1 (SP142) Assay interpretation guide is designed to assist pathologists in interpreting and scoring NSCLC tissues stained with VENTANA PD-L1 (SP142) Assay.

- 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.



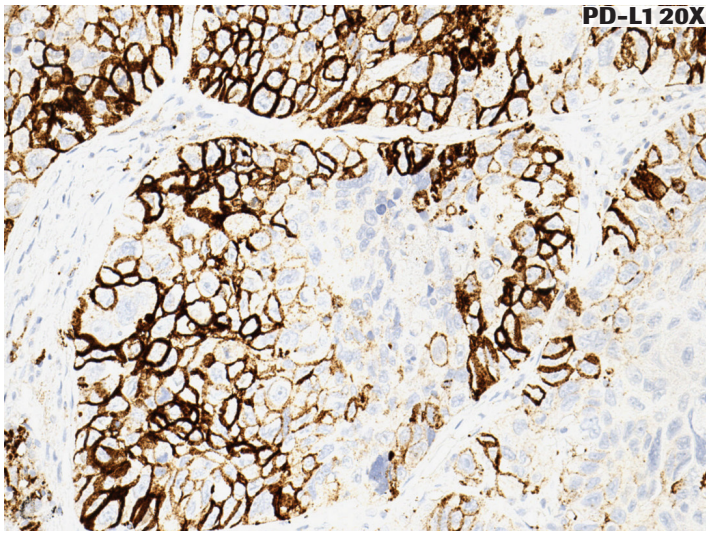
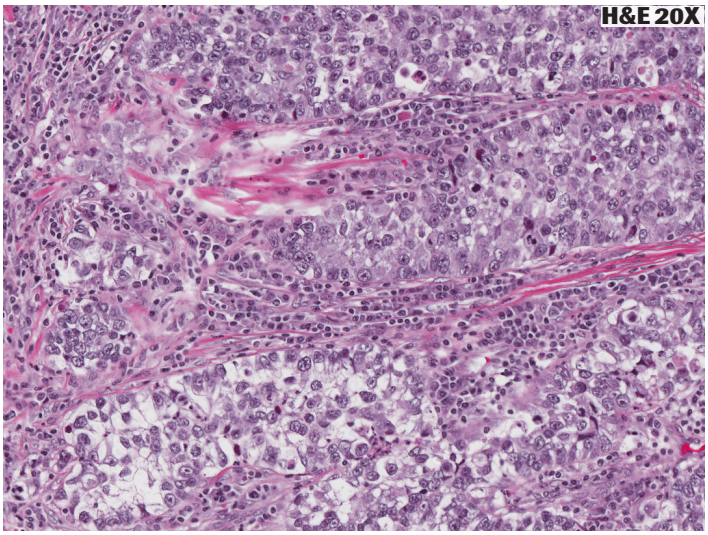


Figure 2: NSCLC tissue showing moderate to strong circumferential TC membrane staining.



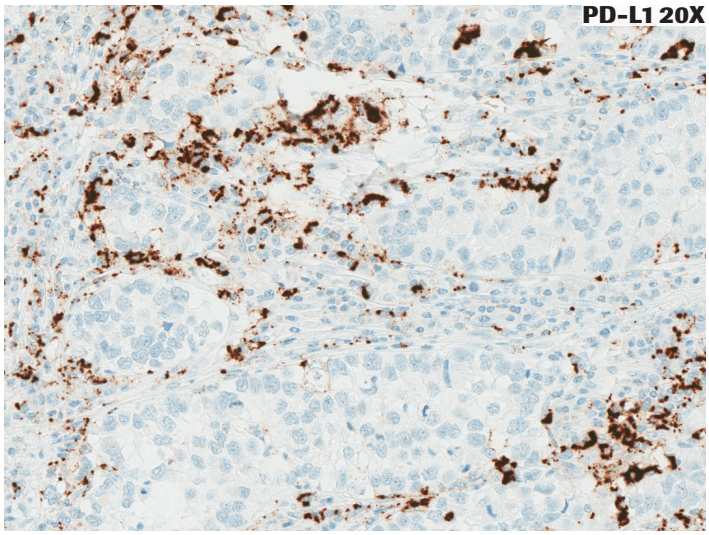


Figure 3: NSCLC tissue showing dark brown punctate and linear IC staining.

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

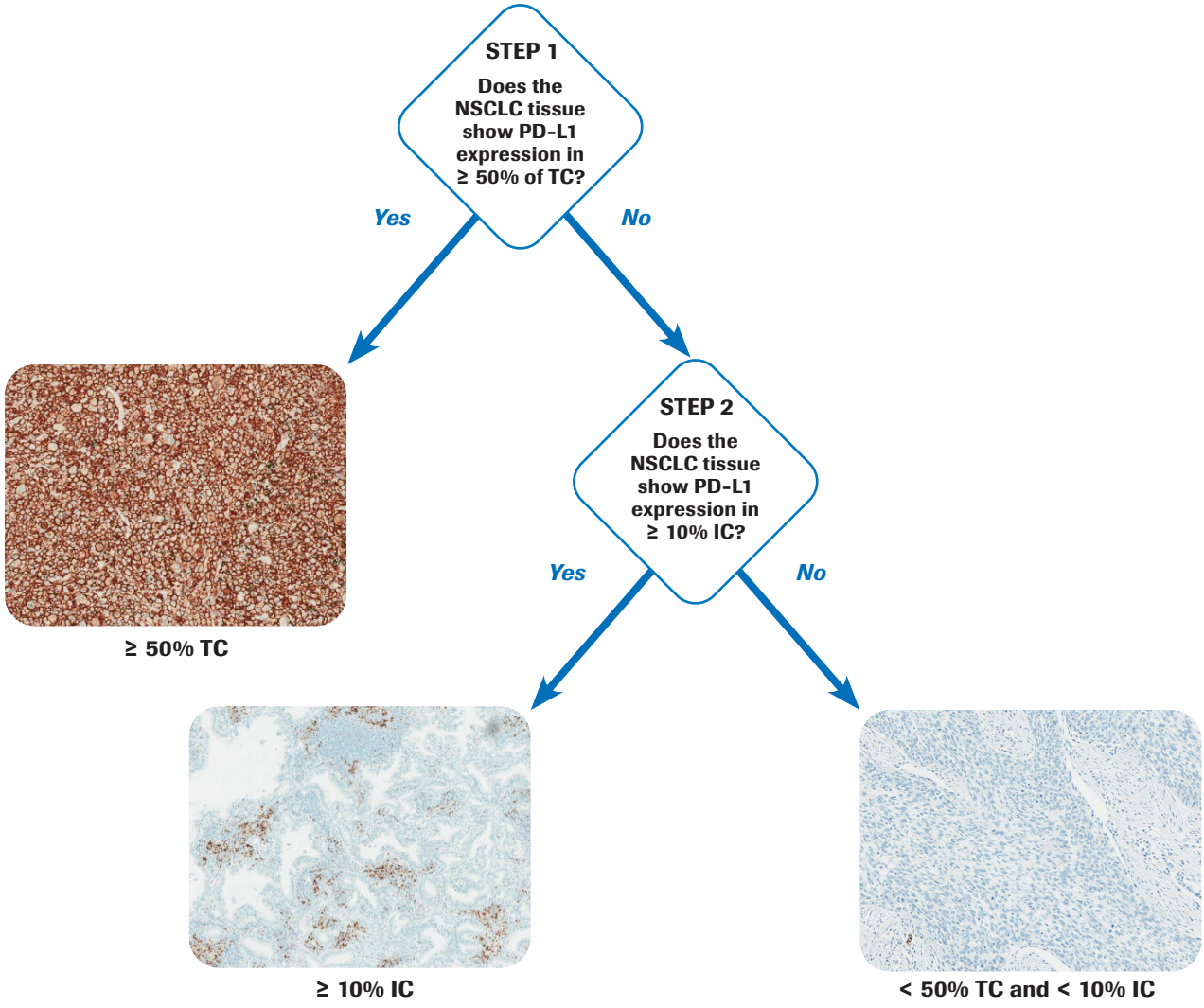
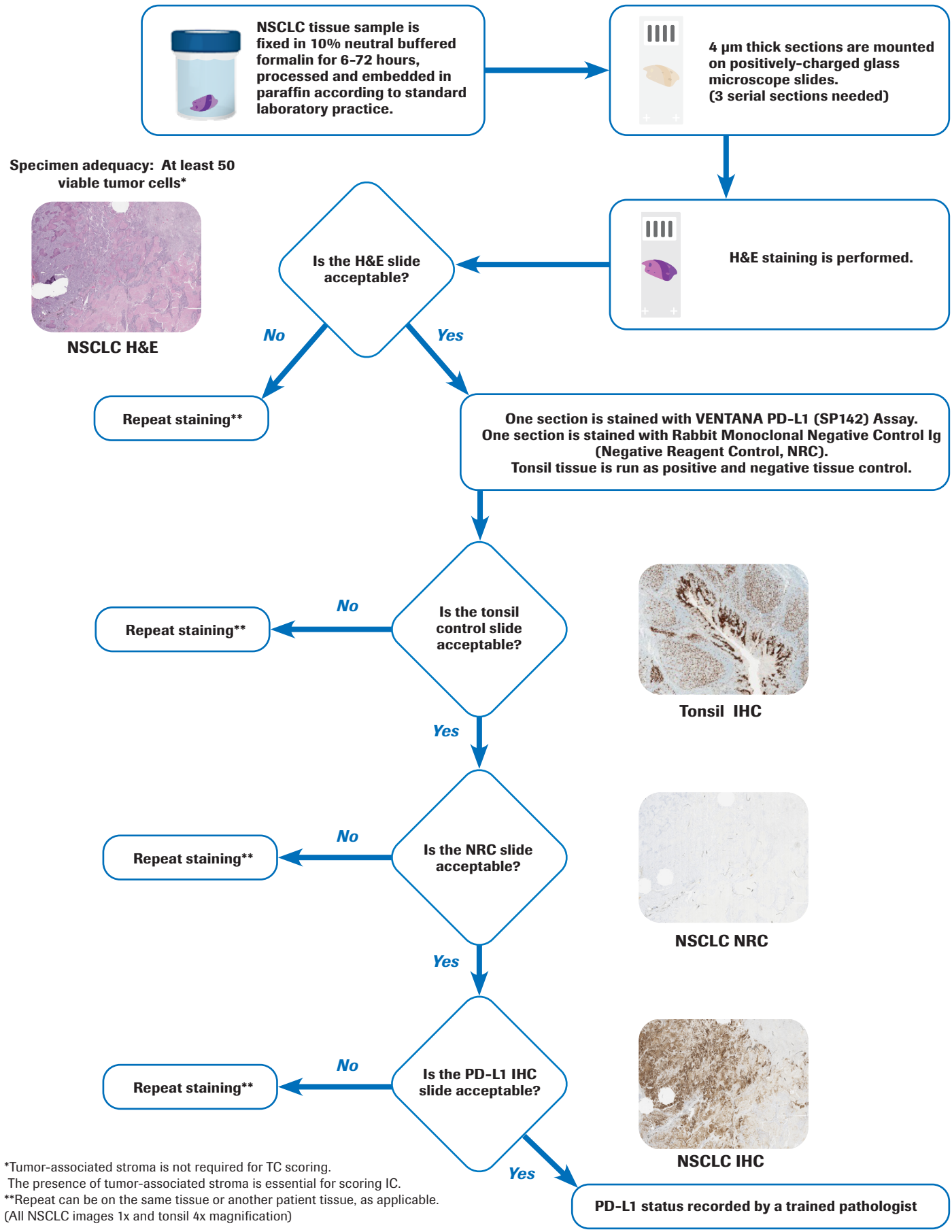


Figure 4: Stepwise scoring process – VENTANA PD-L1 (SP142) Assay in NSCLC tissues (images 10x magnification).

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)

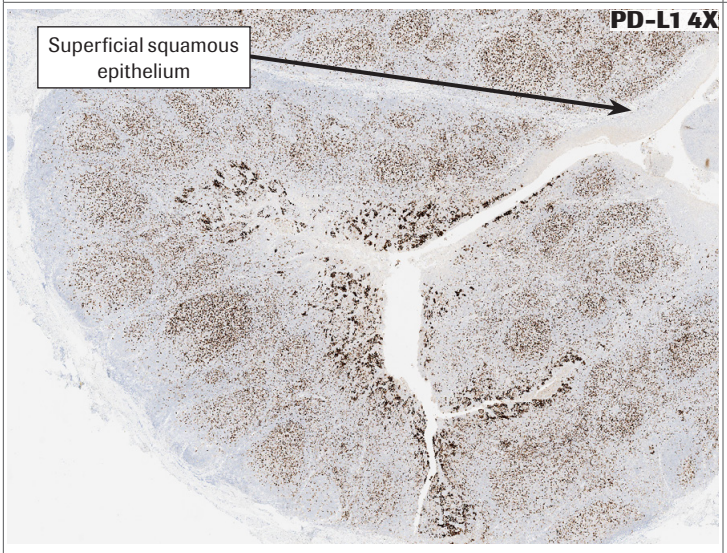
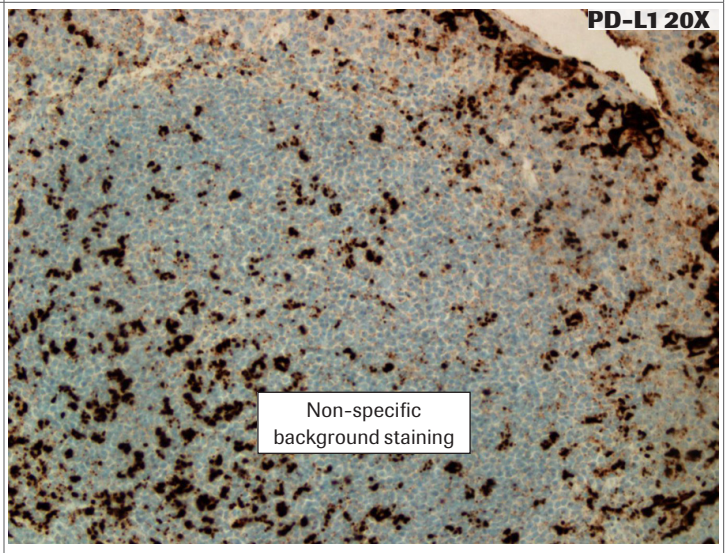
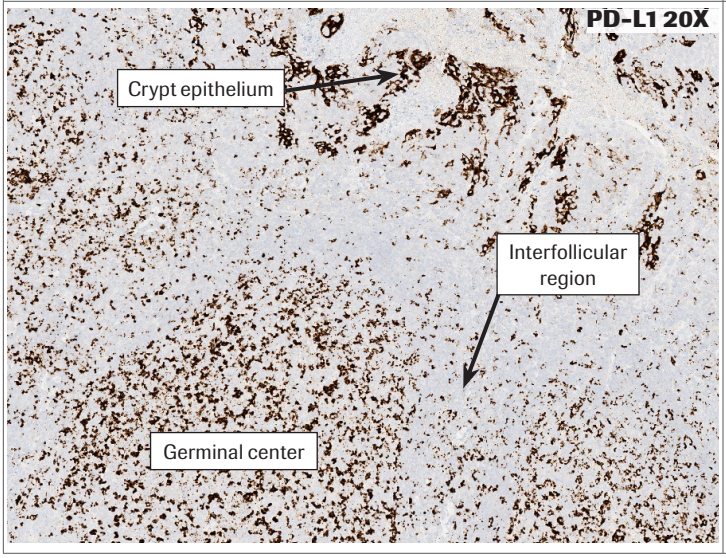
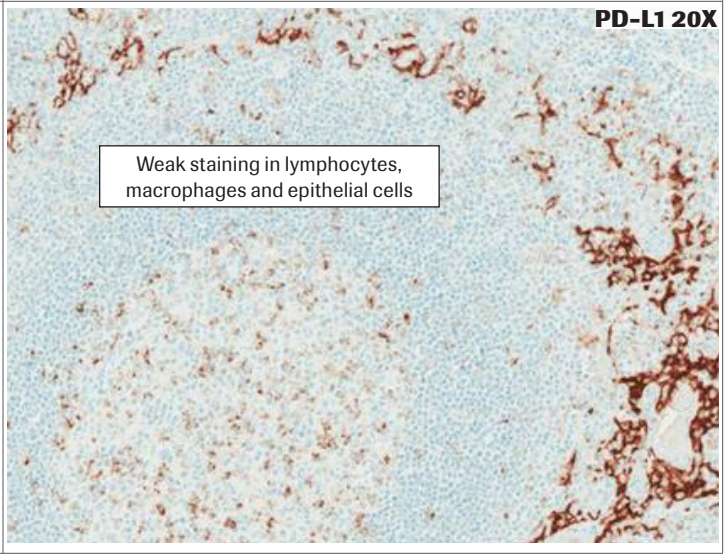
Controls

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:

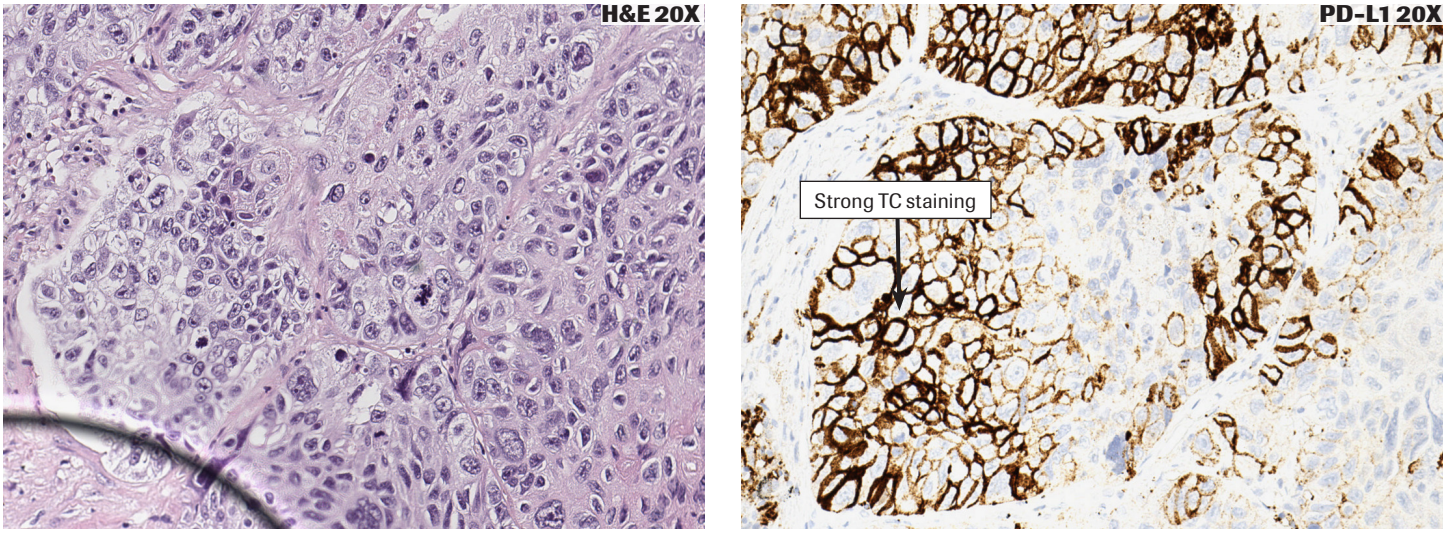


Figure 5: TC often exhibit moderate to strong, partial or complete circumferential membrane staining with or without cytoplasmic component.

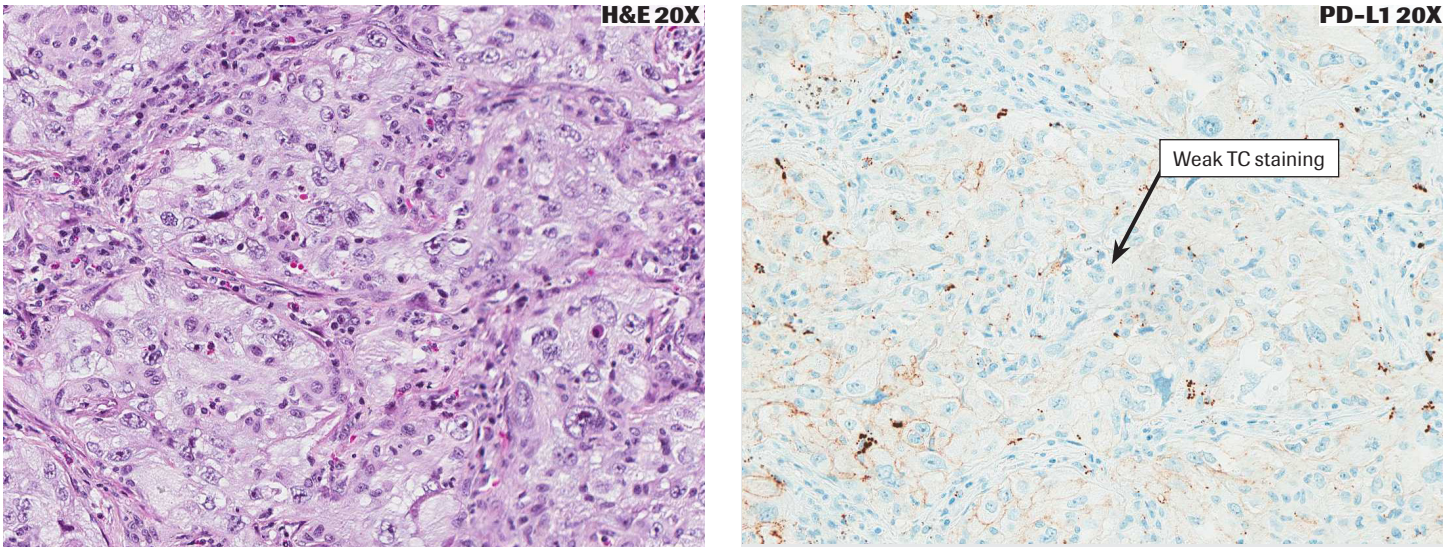


Figure 6: Weak membrane staining is sometimes observed, which requires high magnification visualization for confirmation.

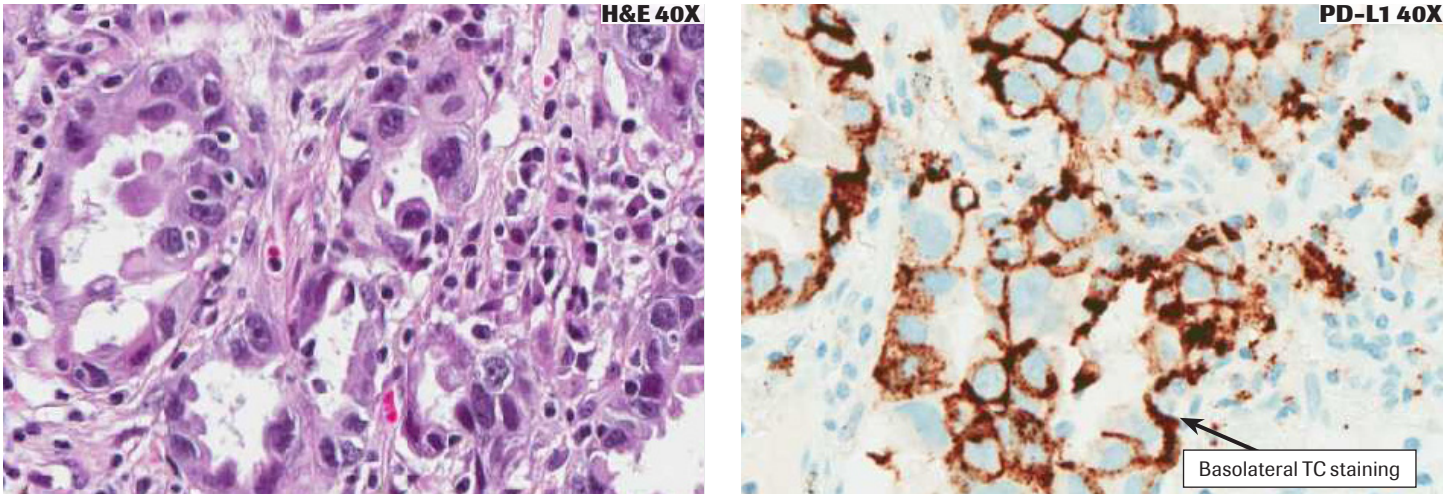


Figure 7: Basolateral membrane staining can be observed in adenocarcinomas.

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.

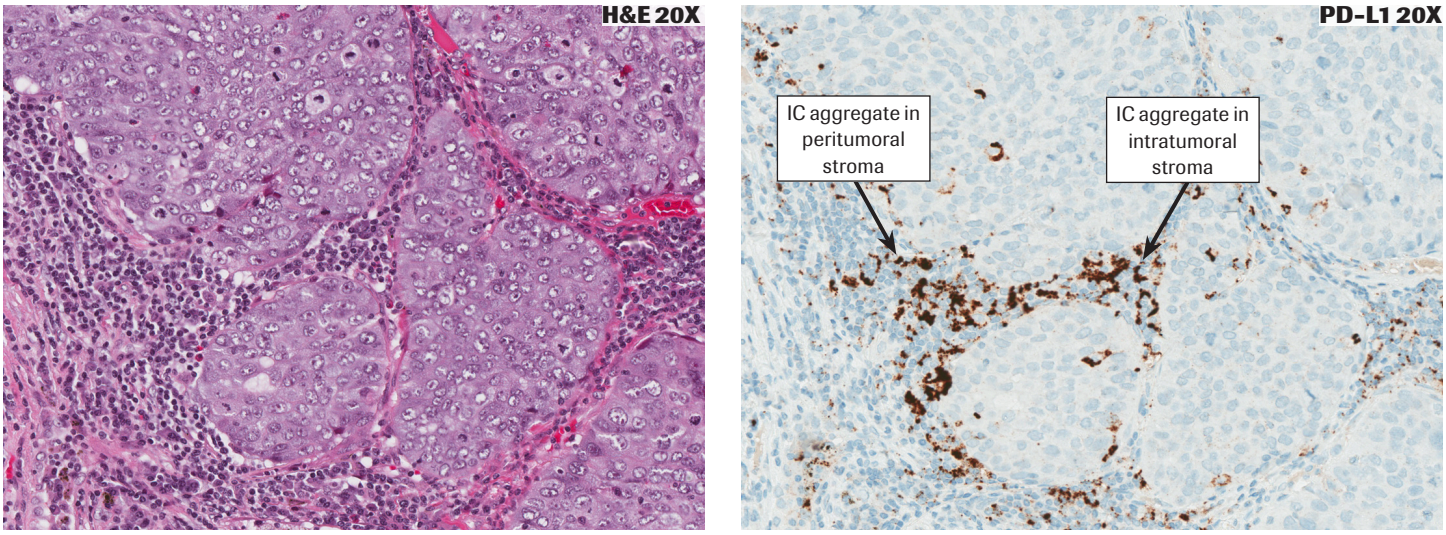
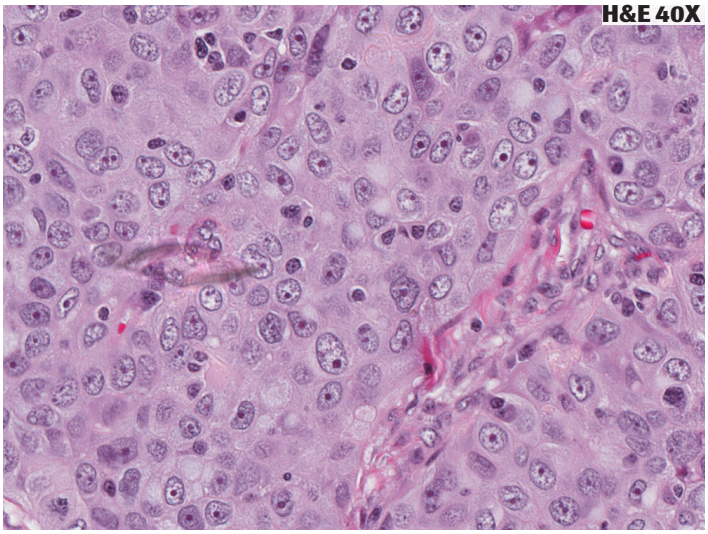


Figure 8: IC often show dark brown punctate or linear staining, which is the predominant IC staining pattern observed in the majority of tissues. IC staining is often seen as aggregates either in intratumoral or peritumoral stroma (tumor-stroma interface) or in both locations.



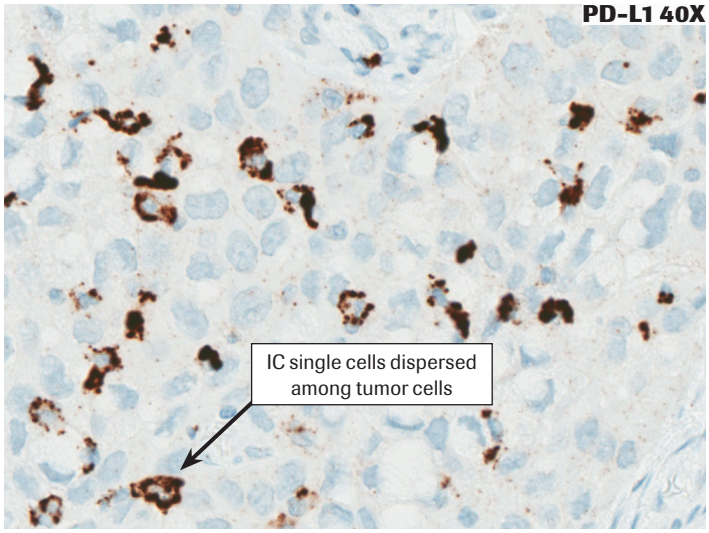
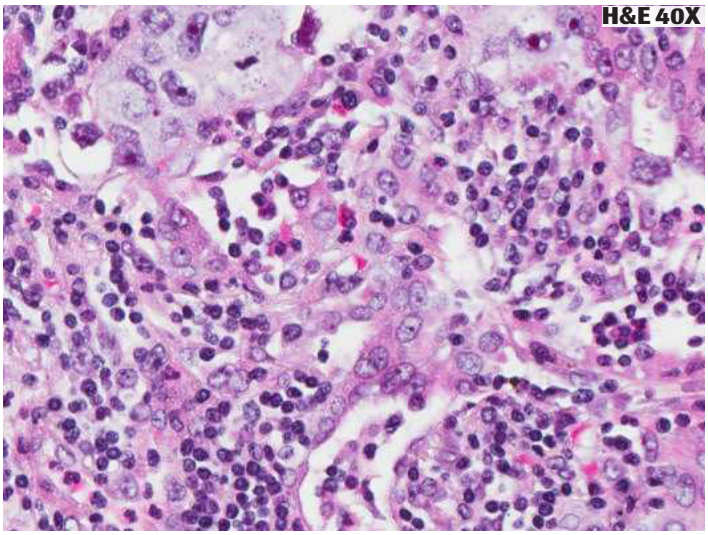
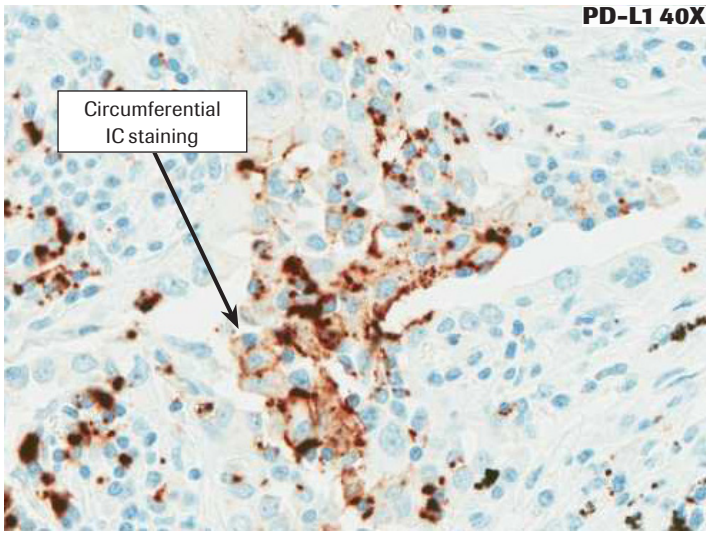
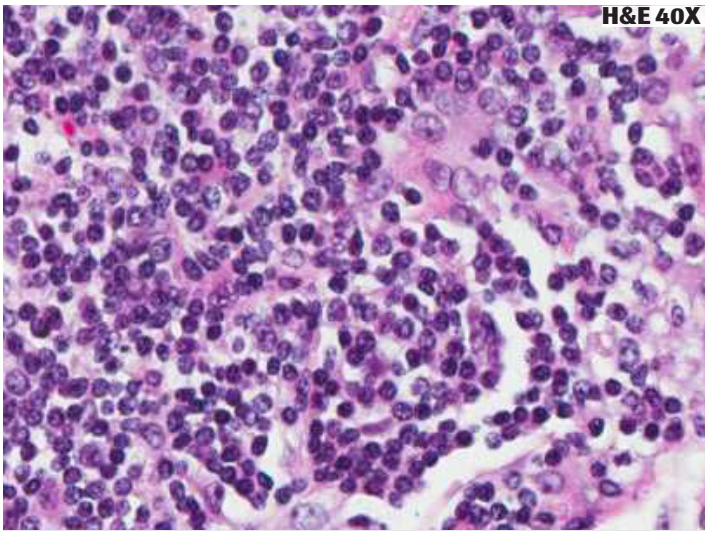


Figure 9: Occasionally, IC staining can also be observed in the form of focal or diffuse scattered single cells or small aggregates (single cell spread) dispersed among tumor cells. This pattern may be seen in association with aggregates in tumor stroma. IC staining corresponds to the immune cells among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







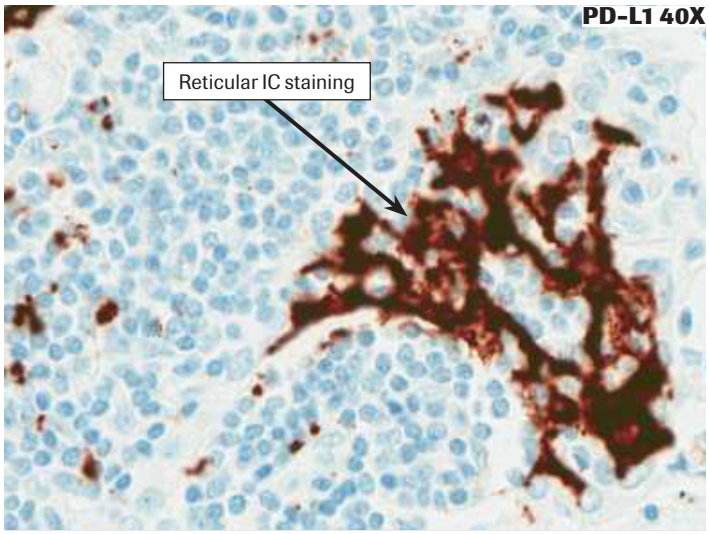
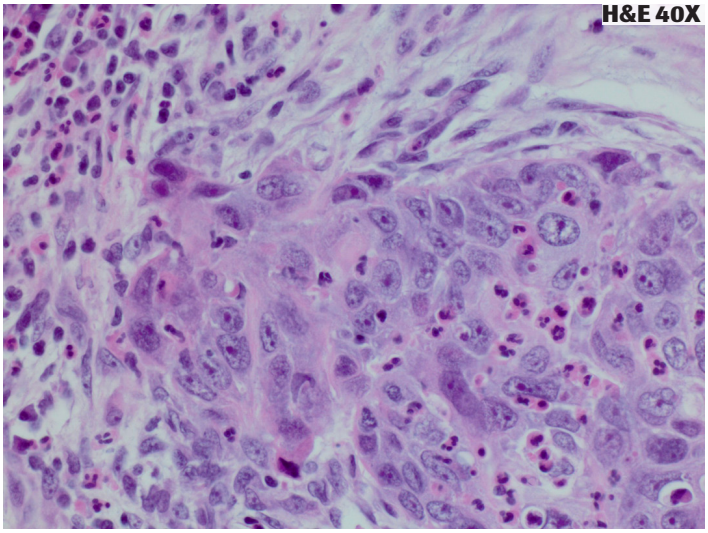


Figure 10: Circumferential membranous or reticular pattern of PD-L1 staining may be observed in IC with macrophage and/or dendritic cells, respectively.



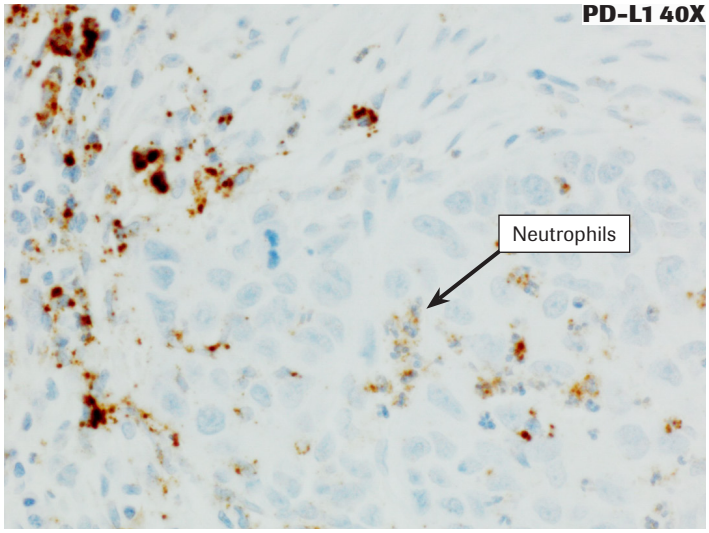
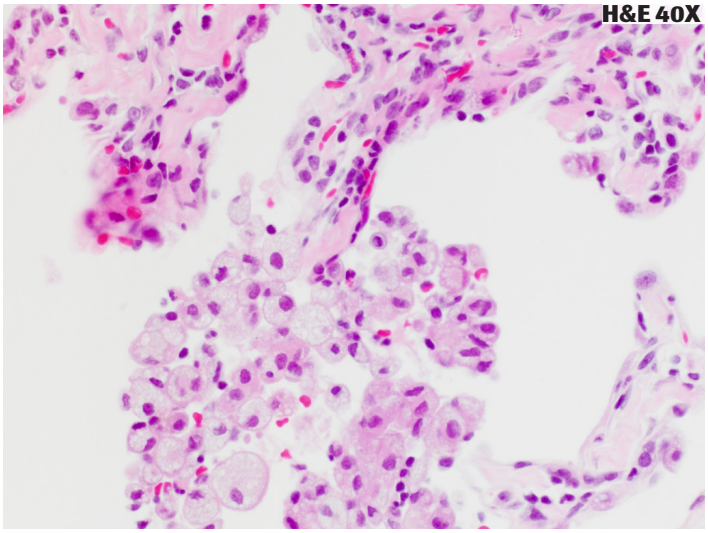


Figure 11: Rarely, IC staining can be observed in neutrophils, as fine punctate staining along with diffuse granular staining. Neutrophil staining can be seen dispersed among tumor cells, in the intratumoral or peritumoral stroma, or as aggregates.



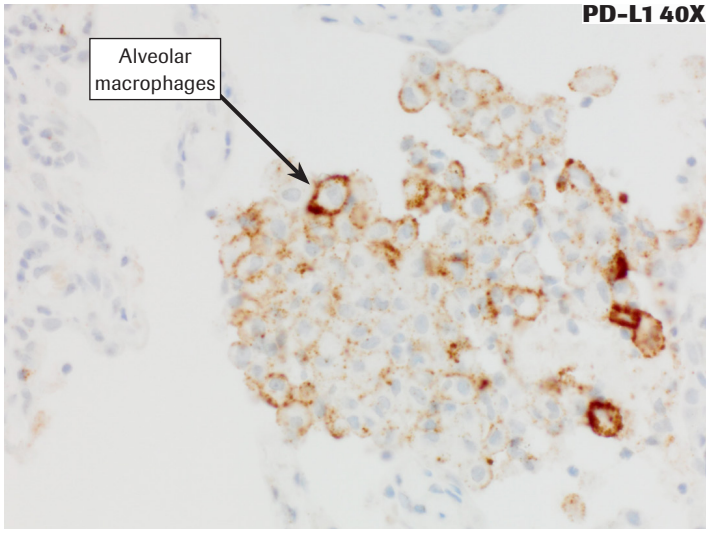


Figure 12: Alveolar macrophages can exhibit circumferential membrane staining, which can be of moderate or strong intensity. Review of corresponding H&E slide would help differentiate this from TC staining.

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**).

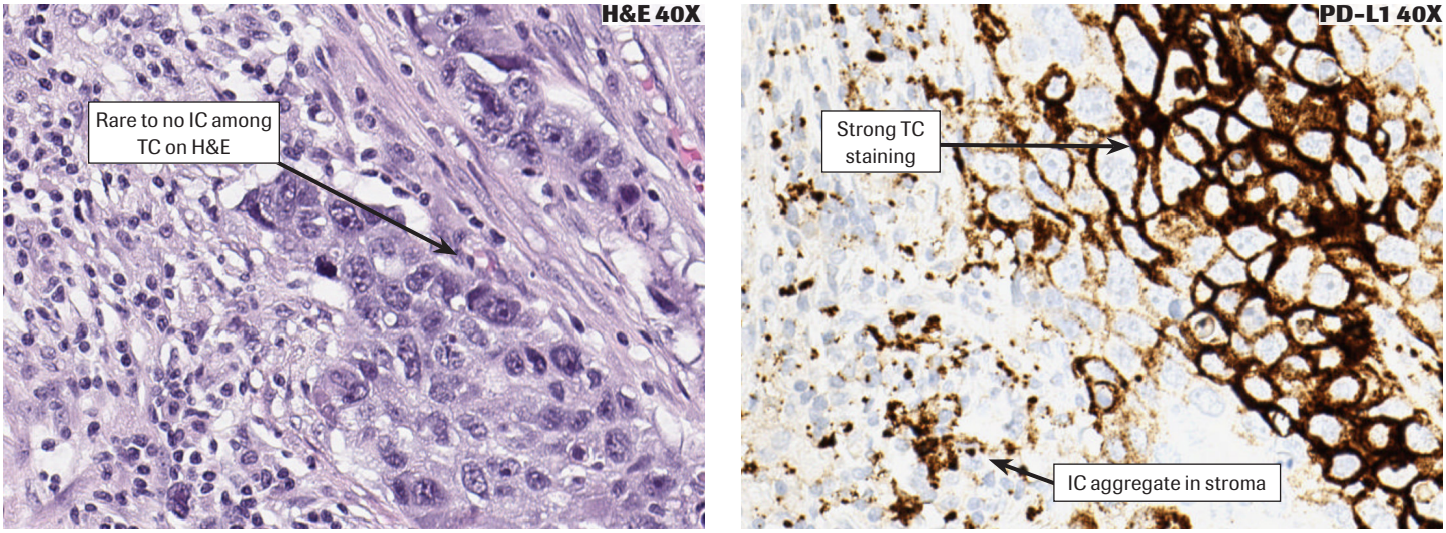


Figure 13: TC show strong membrane staining, with rare IC among the tumor cells identified on H&E.

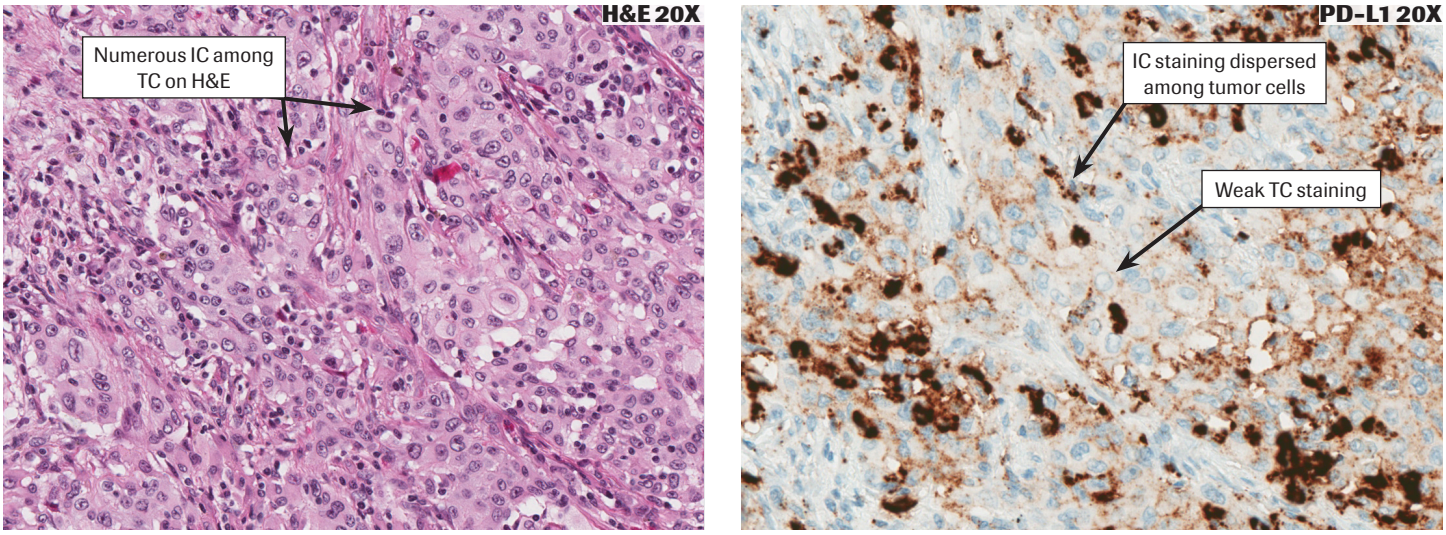


Figure 14: TC show weak to moderate membrane staining, with many IC among TC identified on H&E. Note the presence of strong punctate IC staining among the TC.

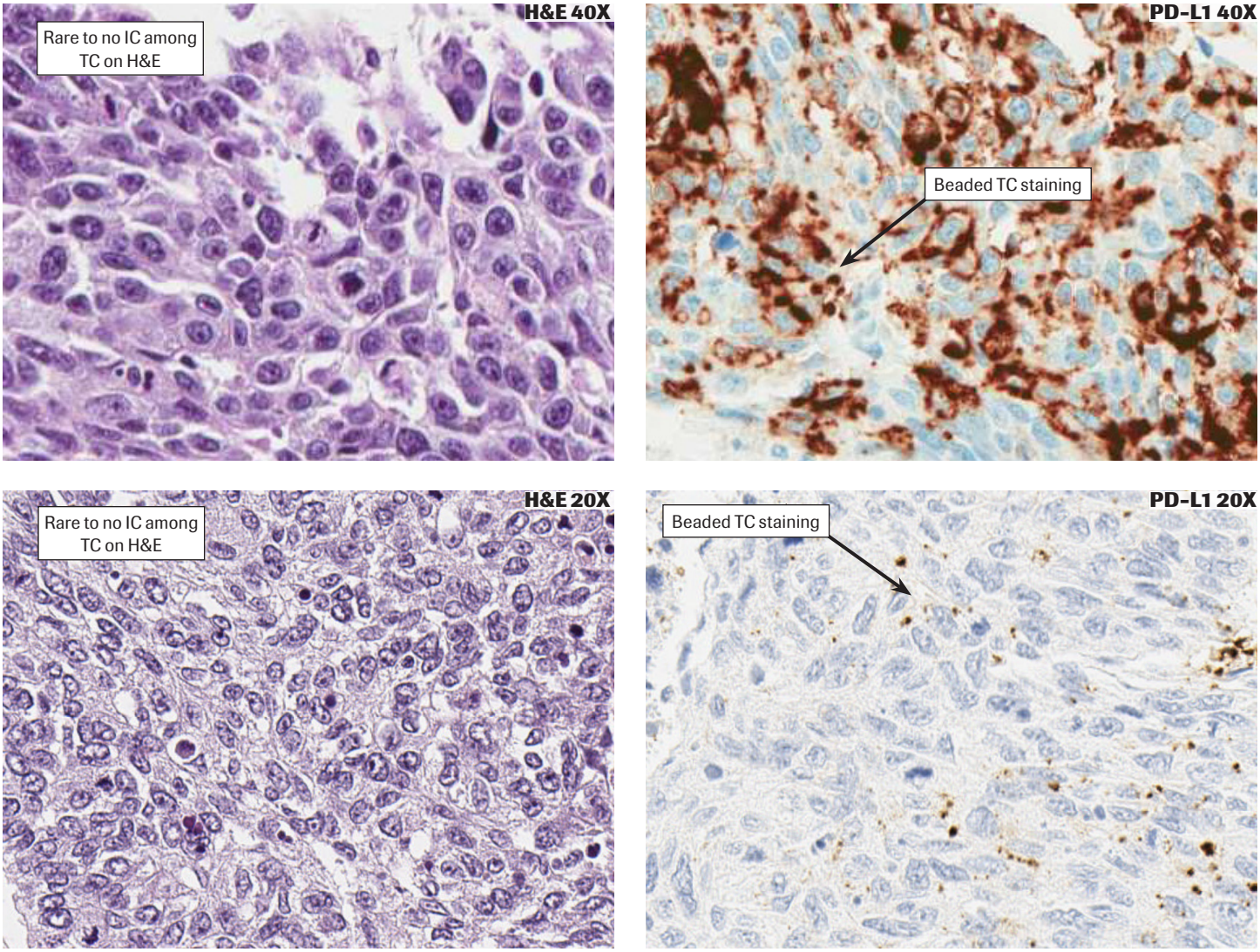


Figure 15: If H&E does not show many identifiable IC, and a granular or beaded staining pattern is observed among TC, then this staining should be attributed to TC rather than IC.

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**).

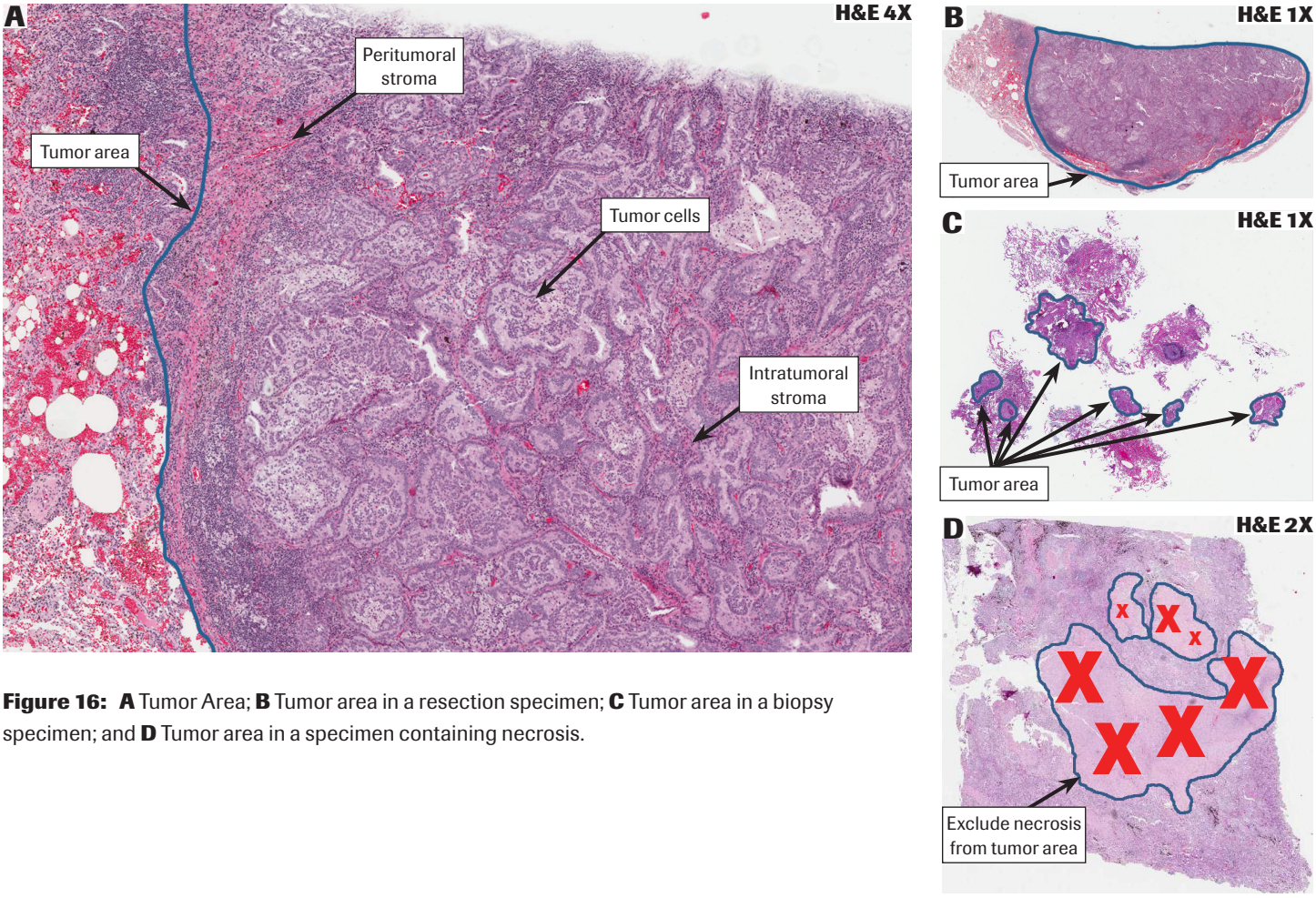
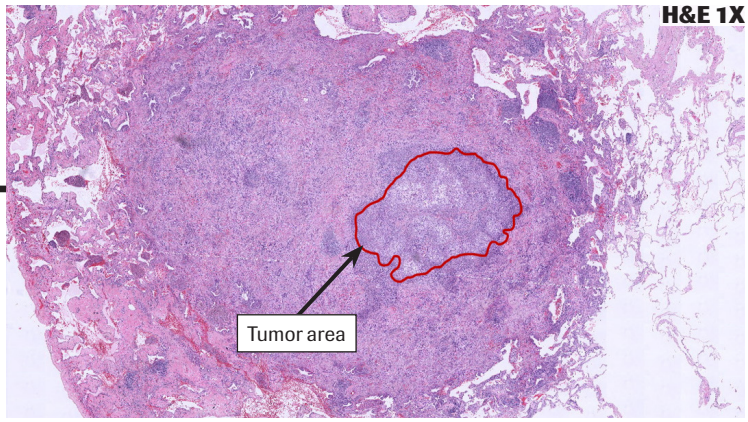


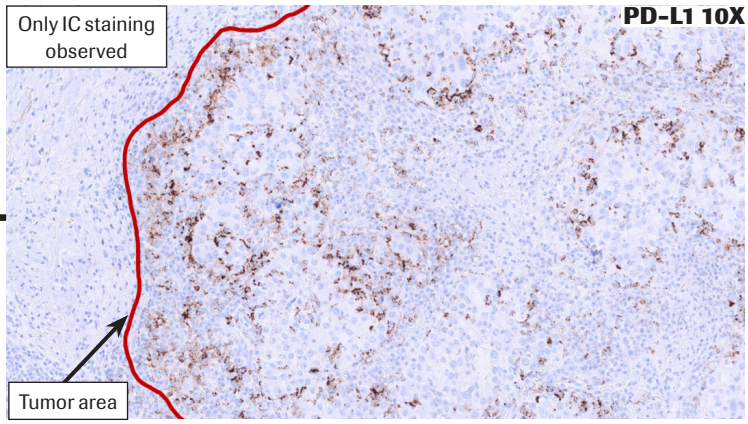
Figure 16: **A** Tumor Area; **B** Tumor area in a resection specimen; **C** Tumor area in a biopsy specimen; and **D** Tumor area in a specimen containing necrosis.

Scoring of PD-L1 IC aggregate staining:

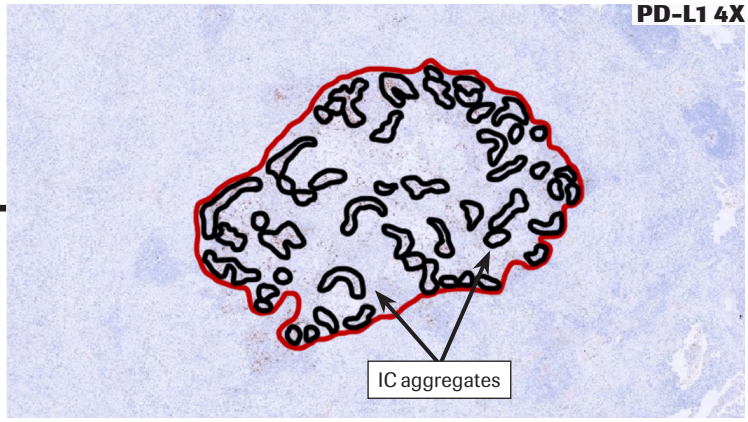
Review the H&E-stained slide for the presence of tumor, necrosis, adequacy (at least 50 viable tumor cells*), and assessment of tumor area.



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



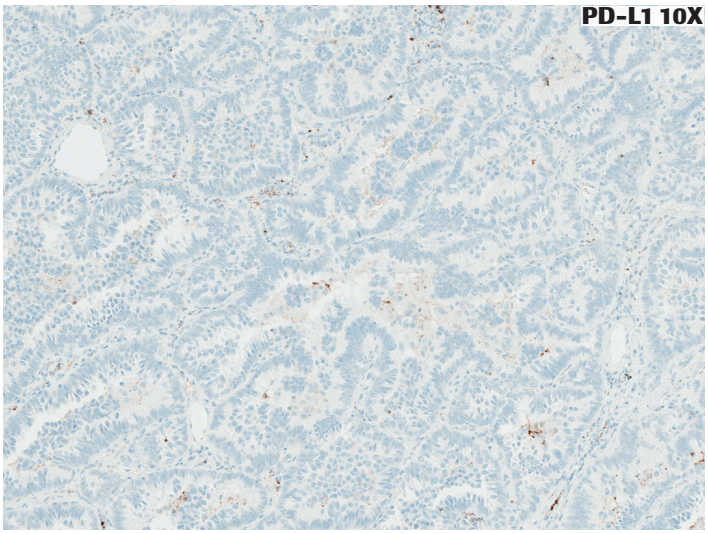
Visually encircle IC aggregates as closely as possible using 2x or 4x magnification.



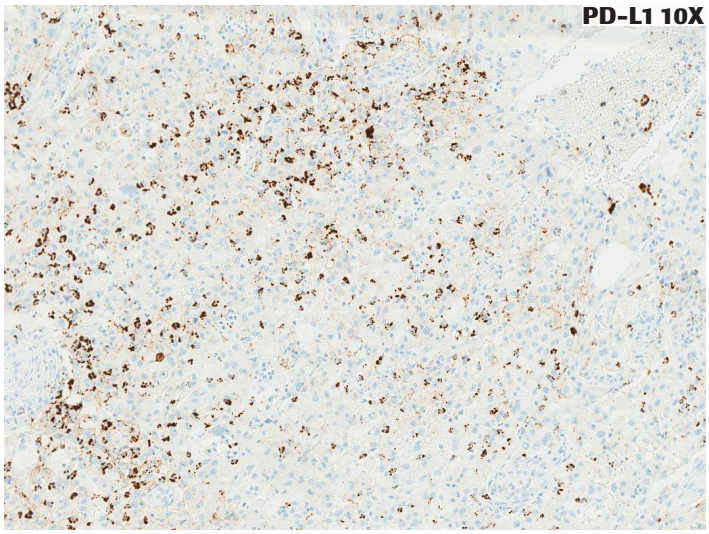
Combine the aggregate staining and estimate the proportion of tumor area occupied by IC aggregates using 2x or 4x magnification. PD-L1 expression for this case is ≥ 10% IC. The Reference Images section should be used as an aid in scoring.

*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**).

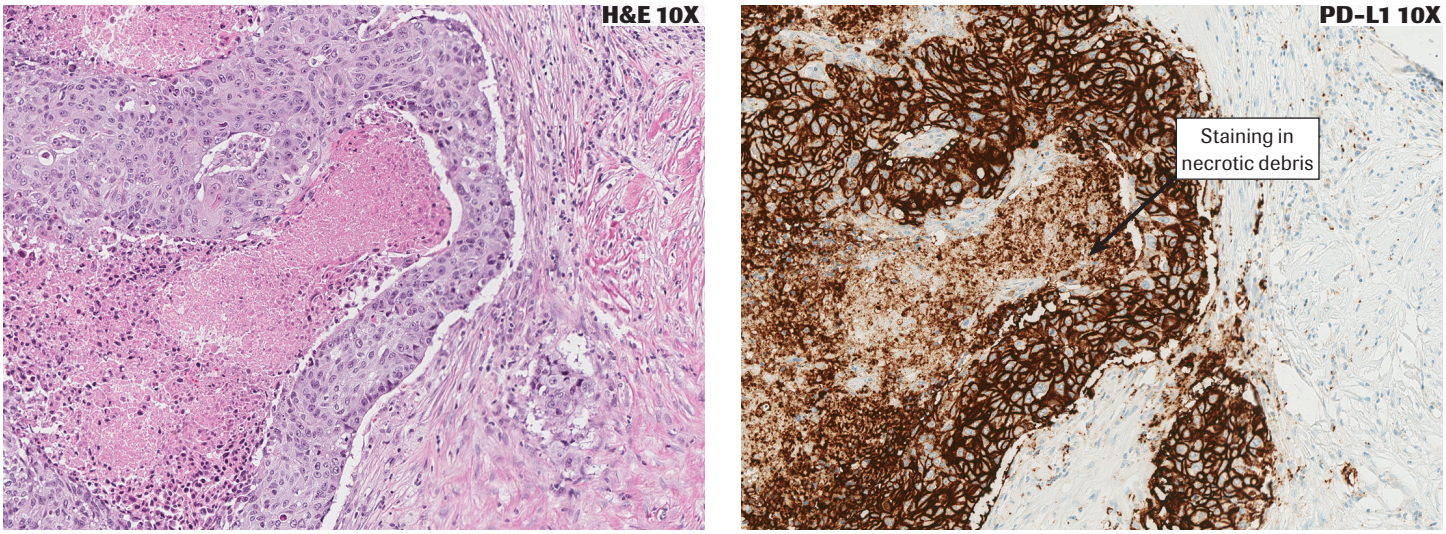


Figure 17: Necrotic debris showing PD-L1 staining. Necrotic debris staining should not be included in scoring.

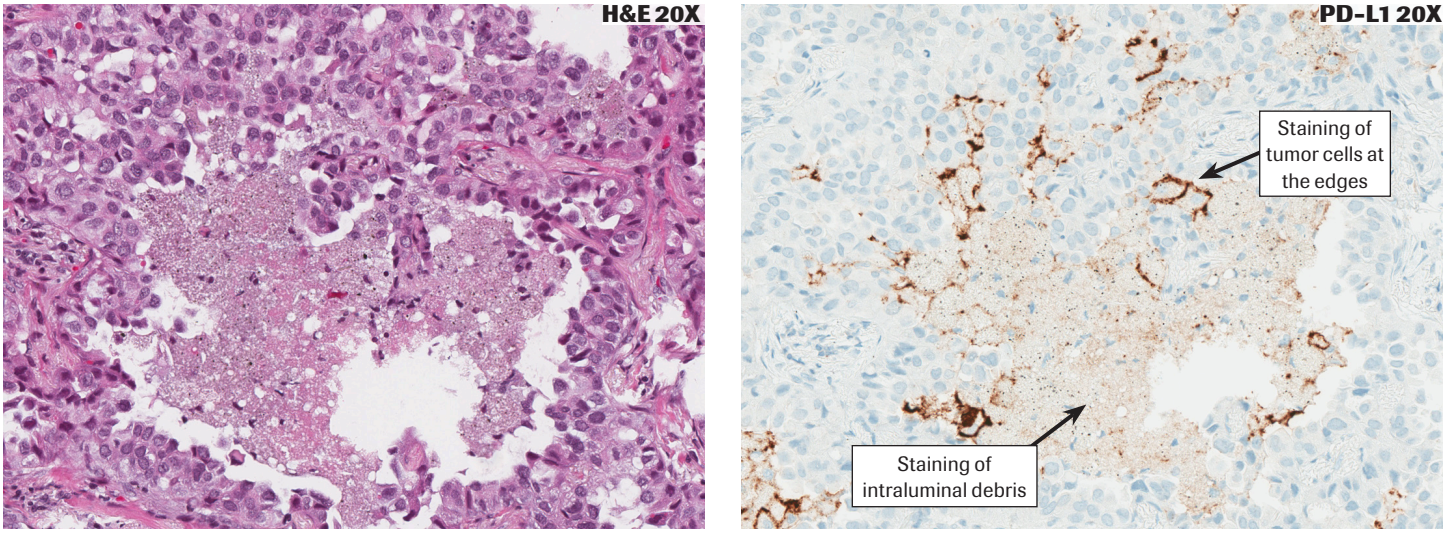


Figure 18: Intraluminal debris showing PD-L1 staining. This should not be included in the scoring. A few viable tumor cells at the edges stain for PD-L1 and these should be included in TC scoring.

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**).

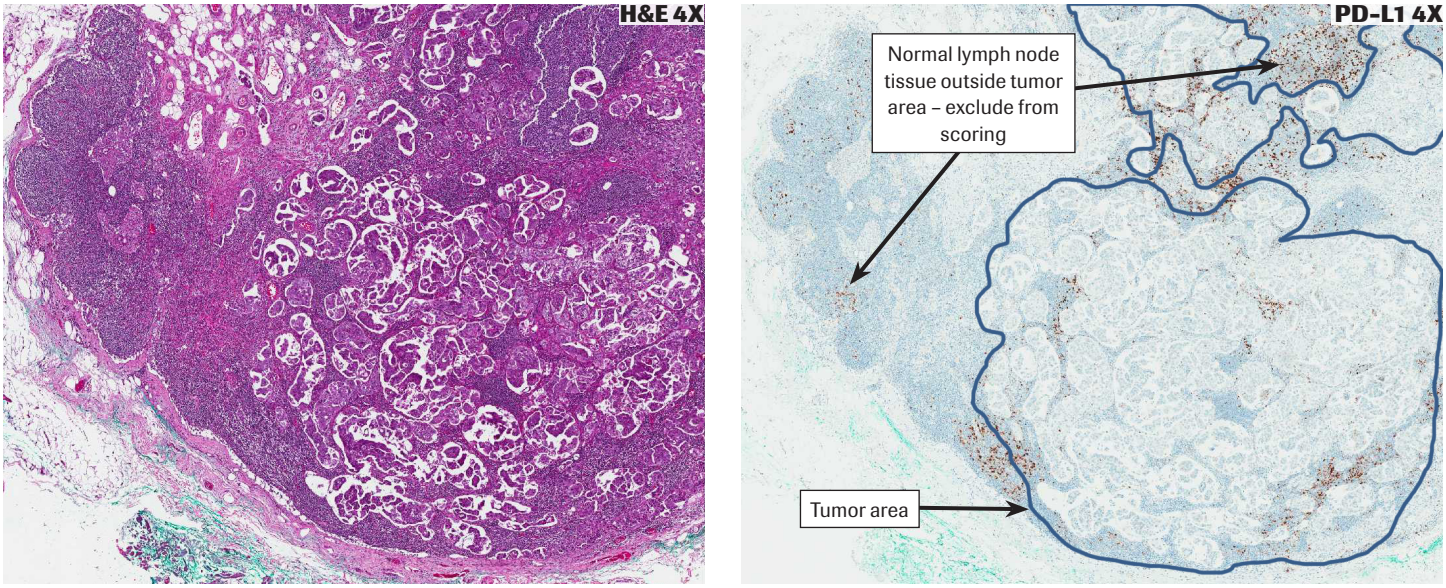


Figure 19: PD-L1 staining in a NSCLC metastatic to a lymph node

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.

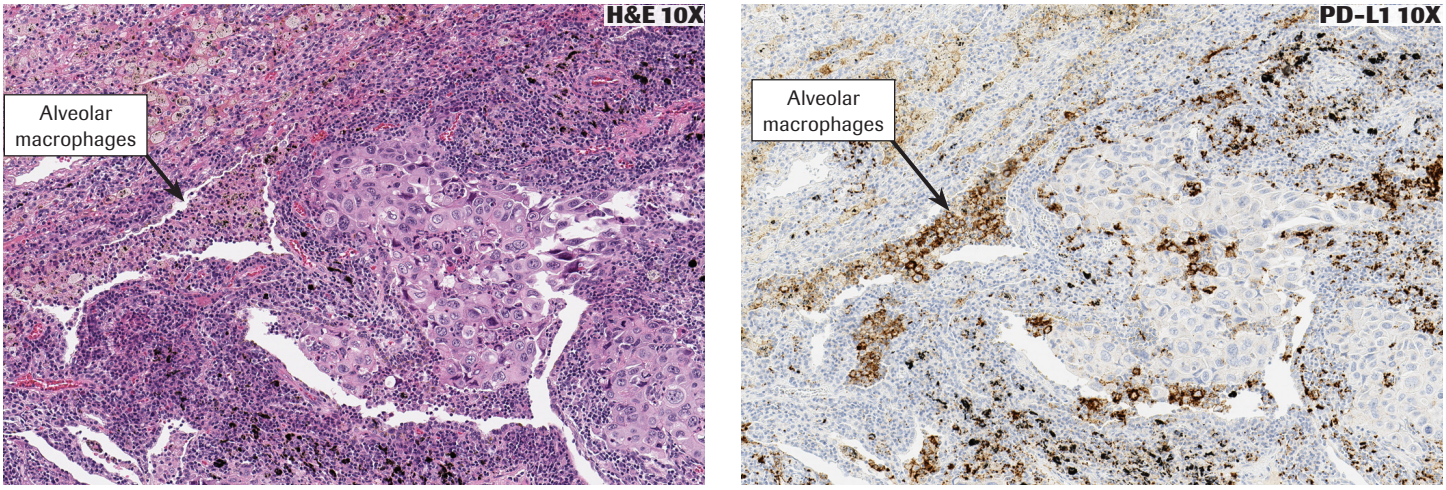


Figure 20: PD-L1 staining of alveolar macrophages at the edge of the tumor.

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

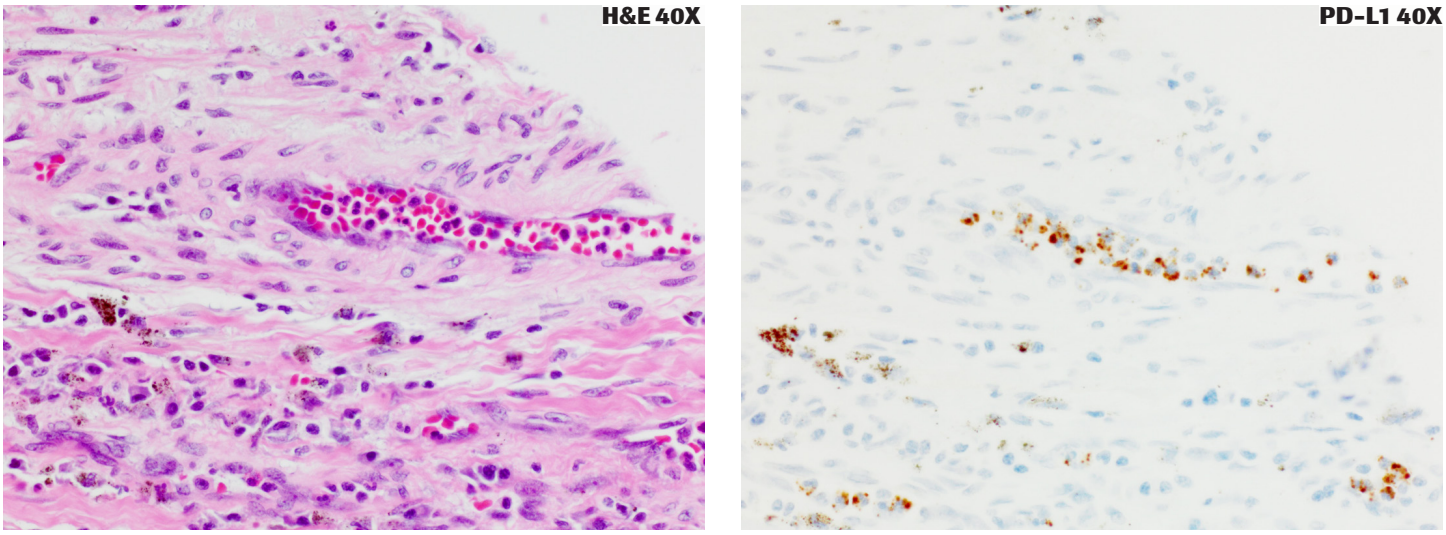


Figure 21: Intravascular immune cells (neutrophils in this case) are not counted toward IC scoring.

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**.

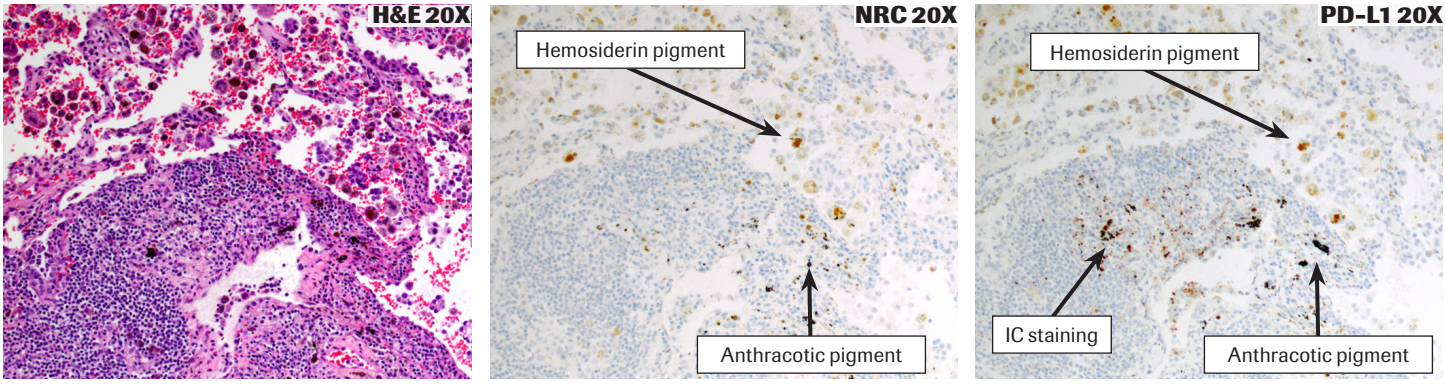
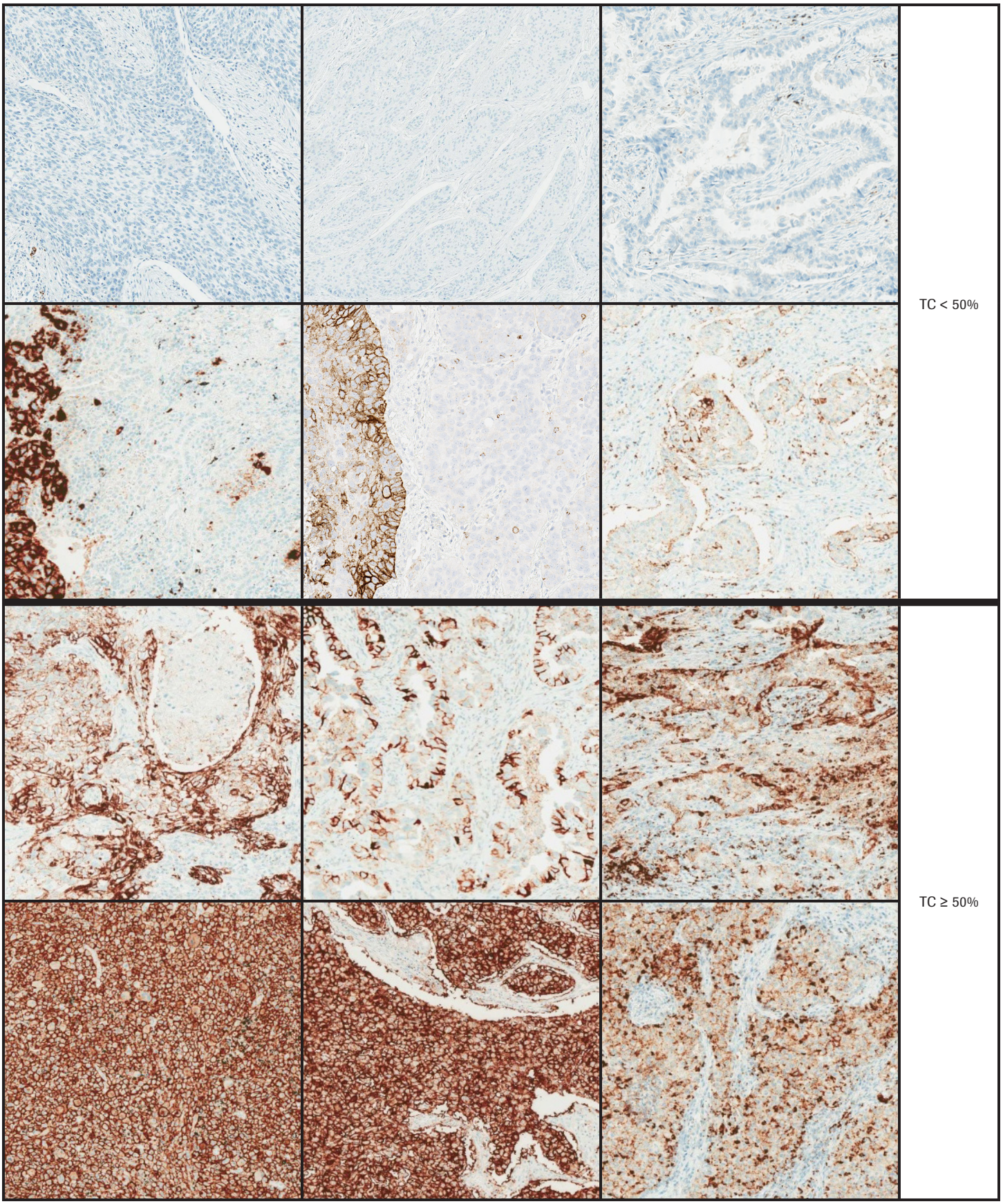


Figure 22: PD-L1 IC staining adjacent to anthracotic pigment and corresponding tissue section stained with negative reagent control and H&E.

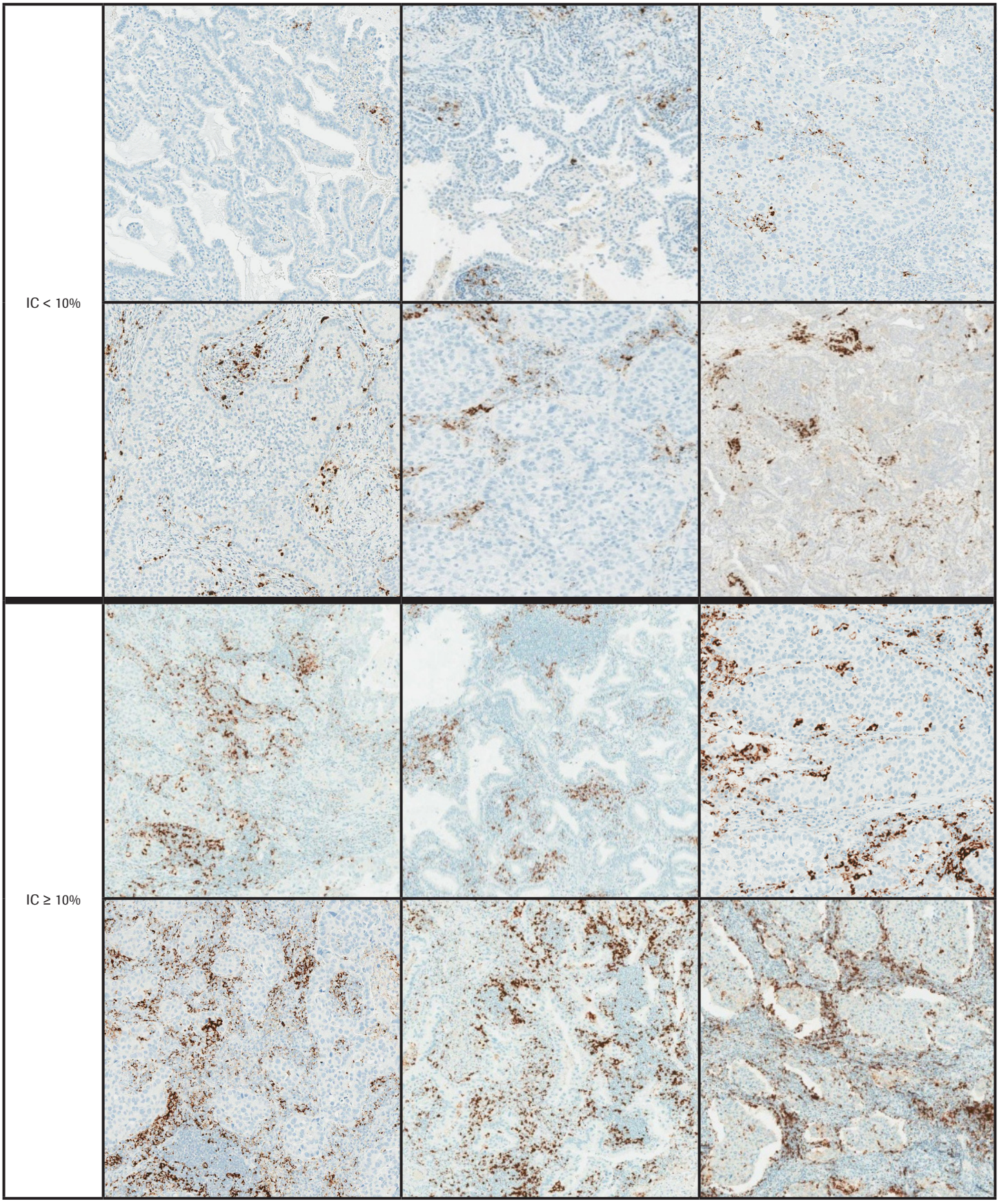
Reference Images

TC Expression



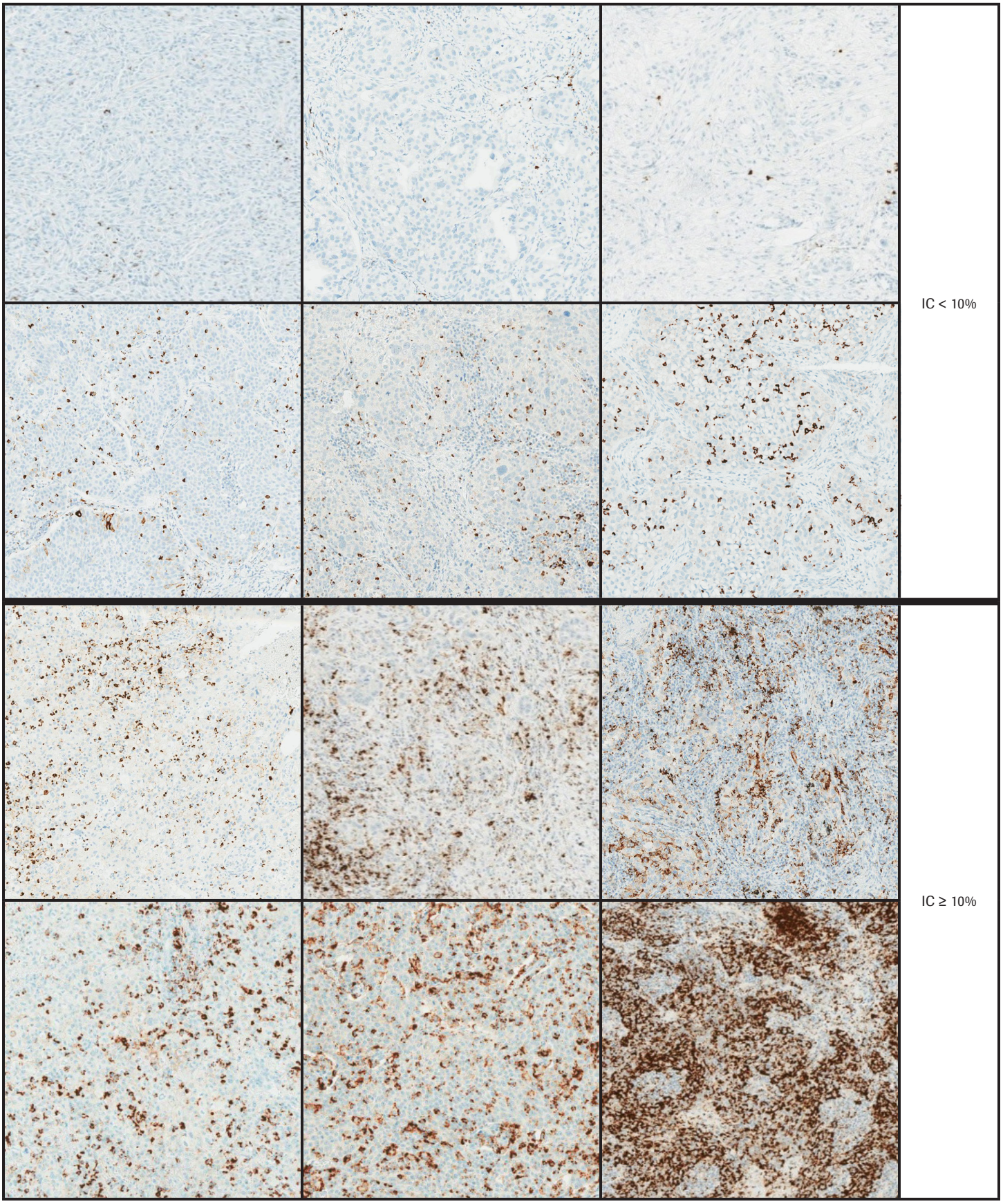
All images 10x magnification

IC Expression – Aggregates



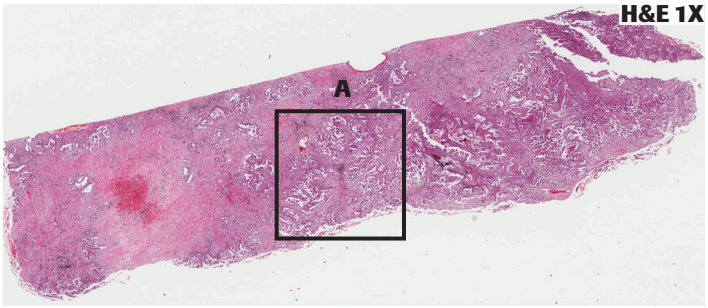
All images 10x magnification

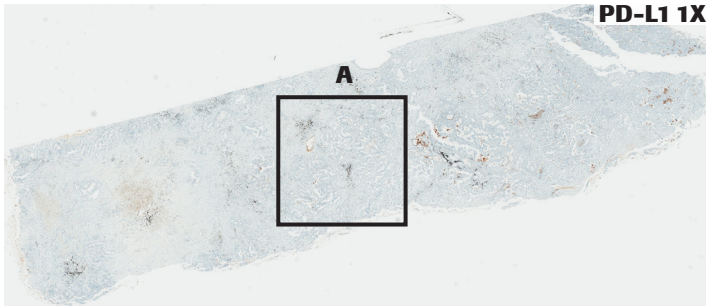
IC Expression – Single-Cell Spread

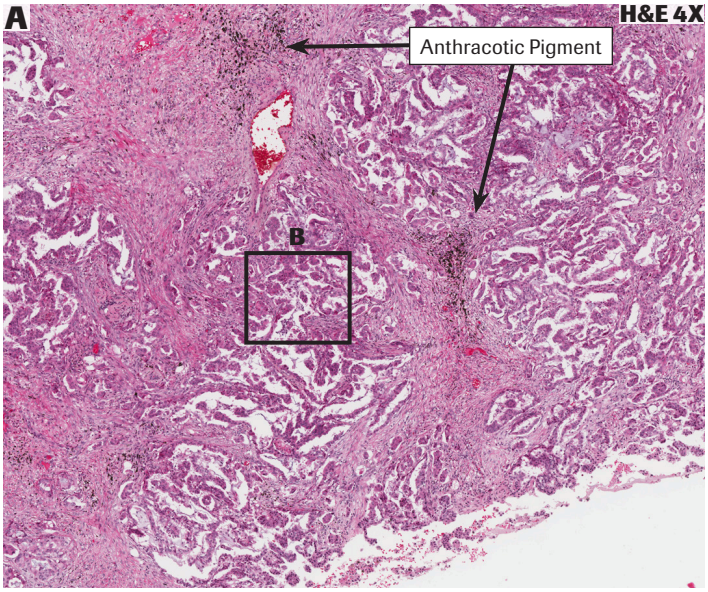


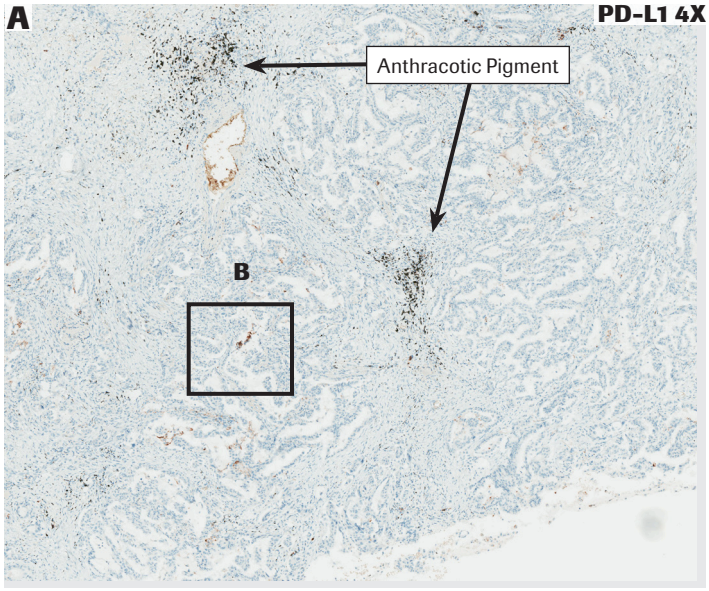
All images 10x magnification

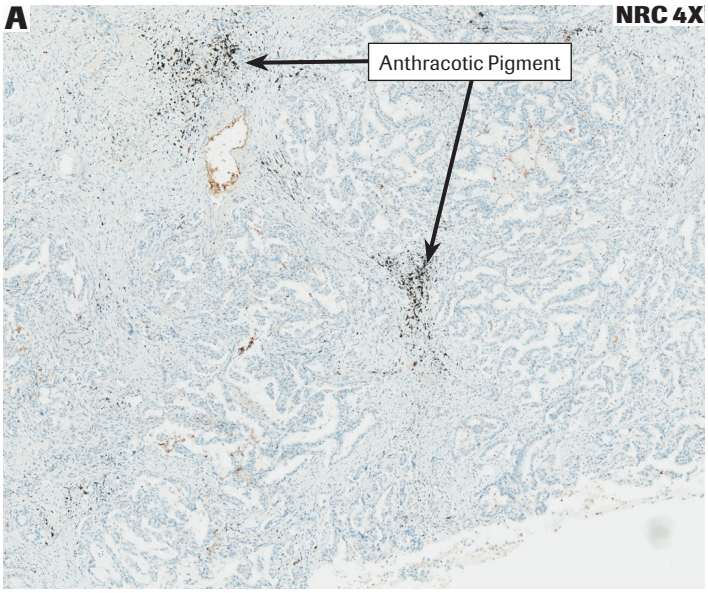
Example Cases: TC < 50% and IC < 10%

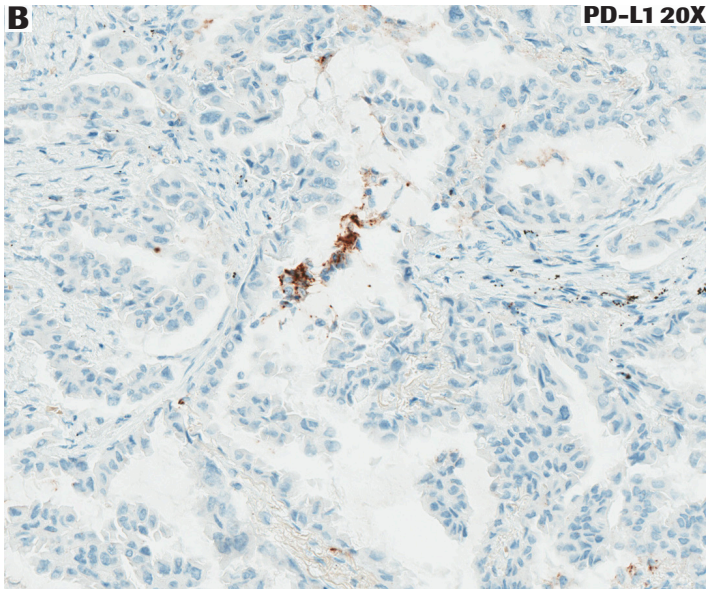




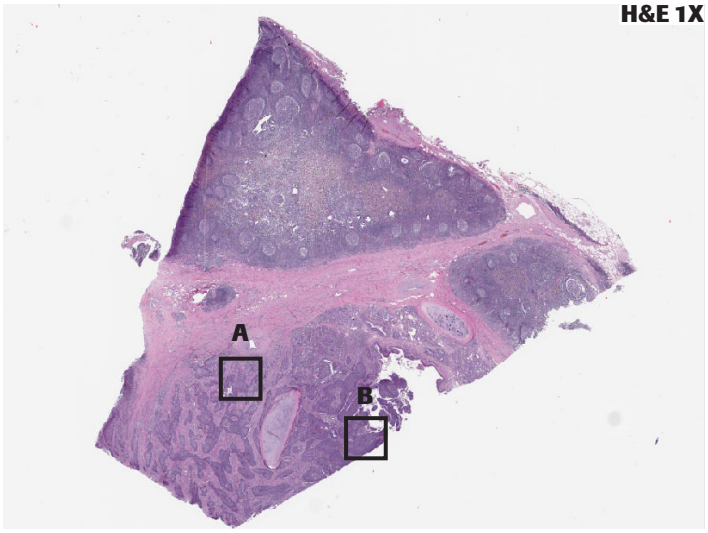


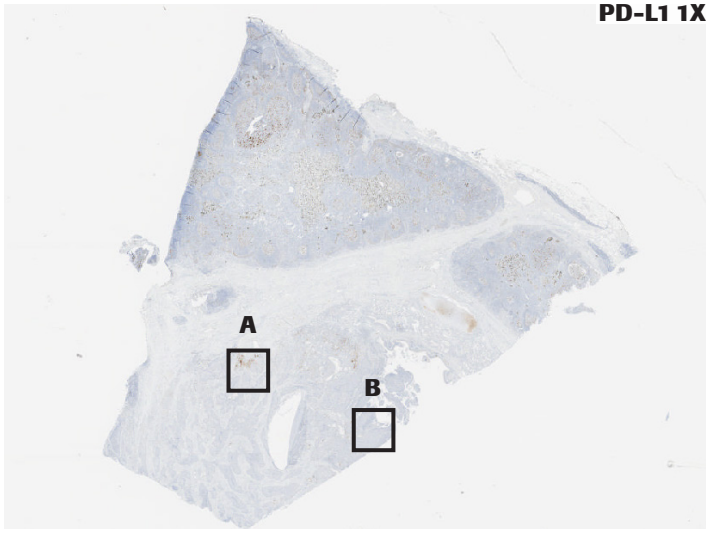


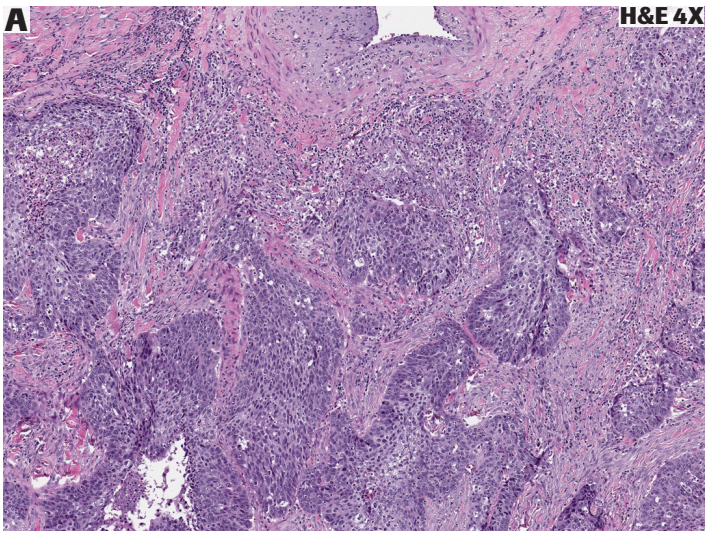


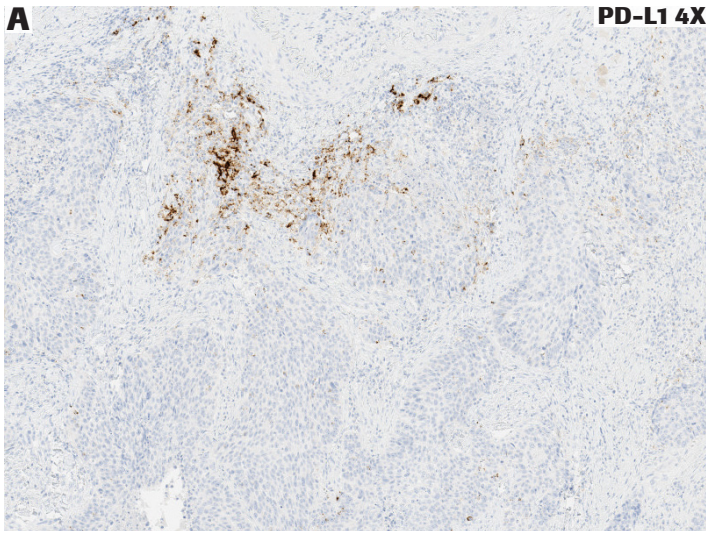


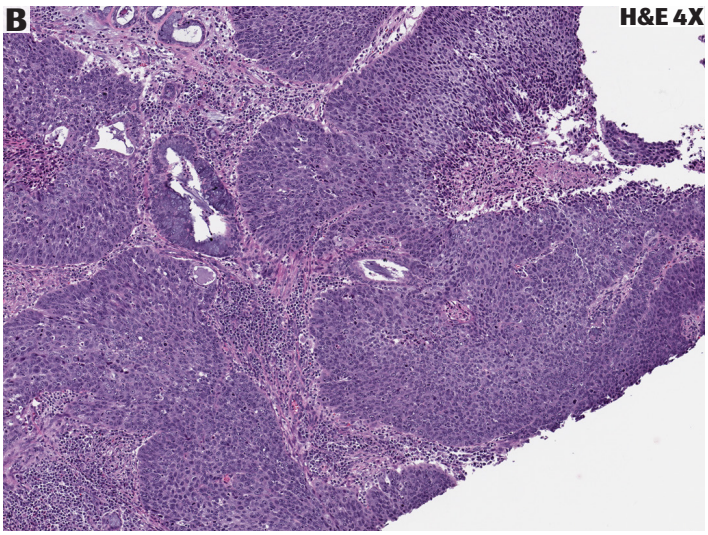
Case 1: This case is TC < 50% and IC < 10%. Scores: TC: 0%; IC: < 1%. This case shows staining of intraluminal debris which should be discounted while scoring IC. Also note the presence of anthracotic pigment.

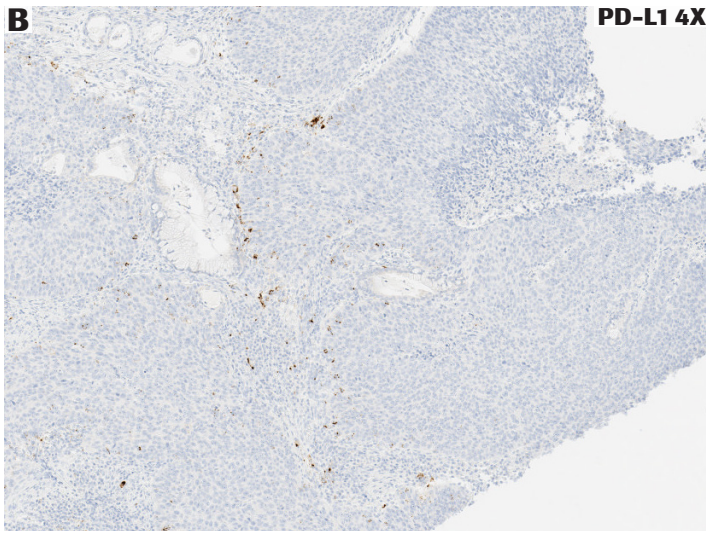




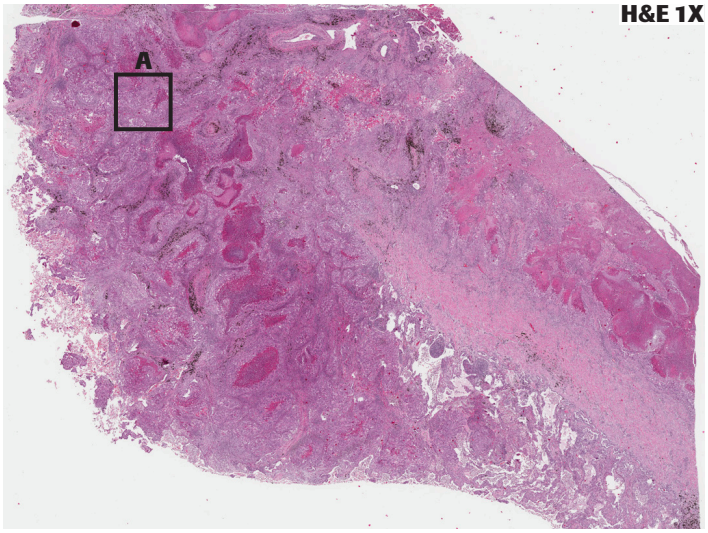


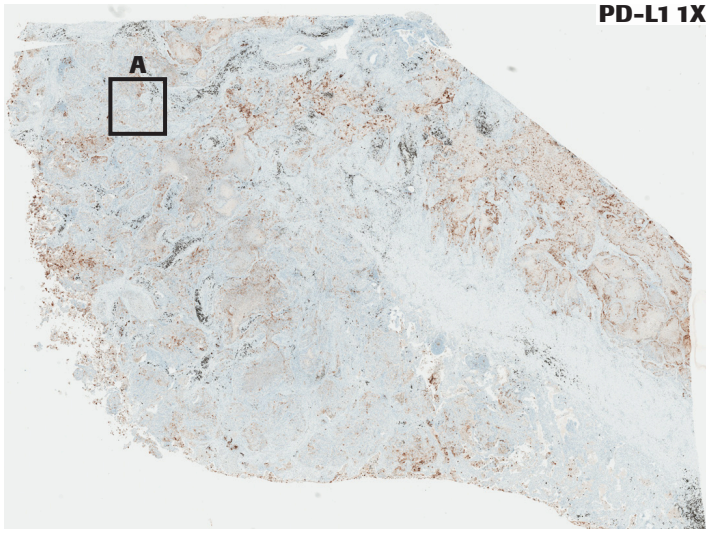


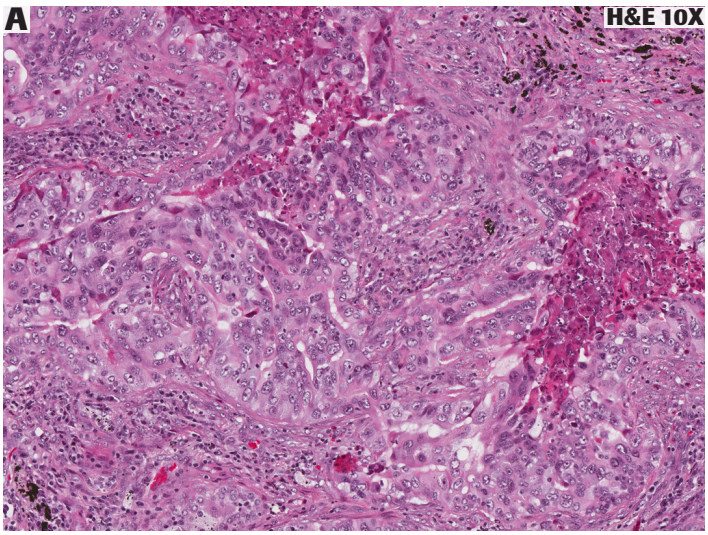


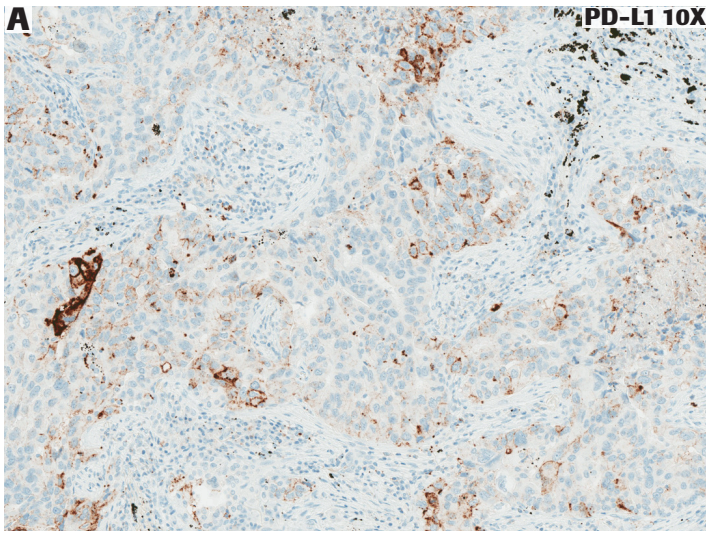


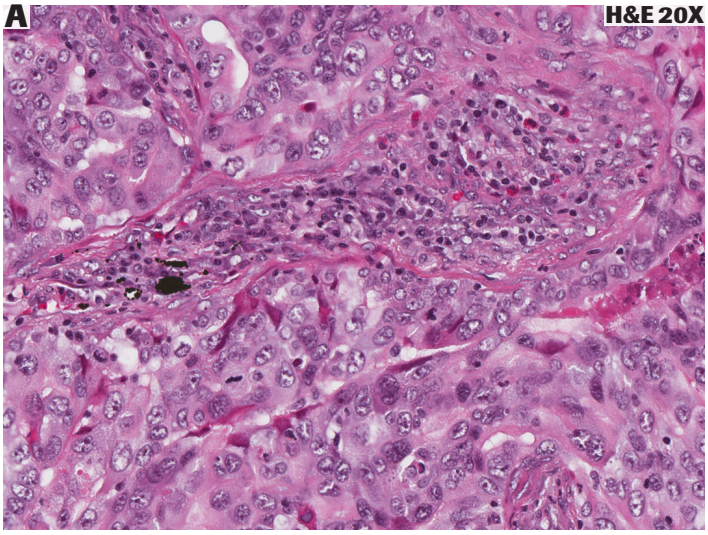
Case 2: This case is TC < 50% and IC < 10%. Scores: TC: 0% and IC: 2%. This case is a lymph node metastasis showing focal interface staining for immune cells. Note that tumor is clearly separated from lymph node tissue, hence making delineation of tumor area easier.

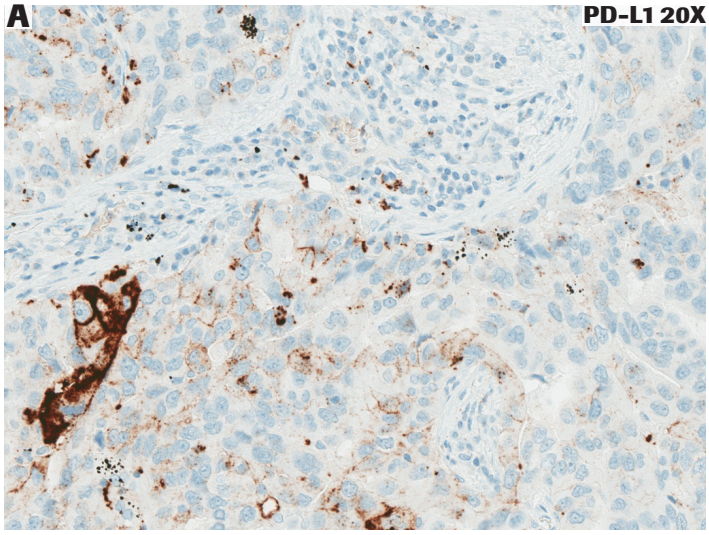




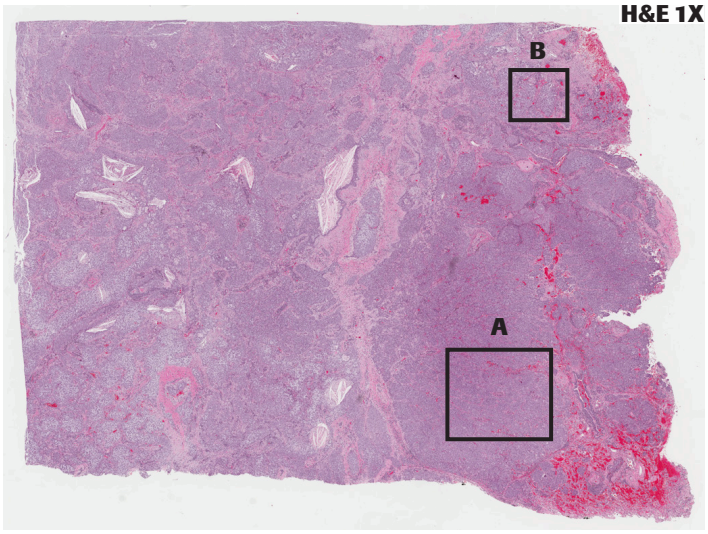


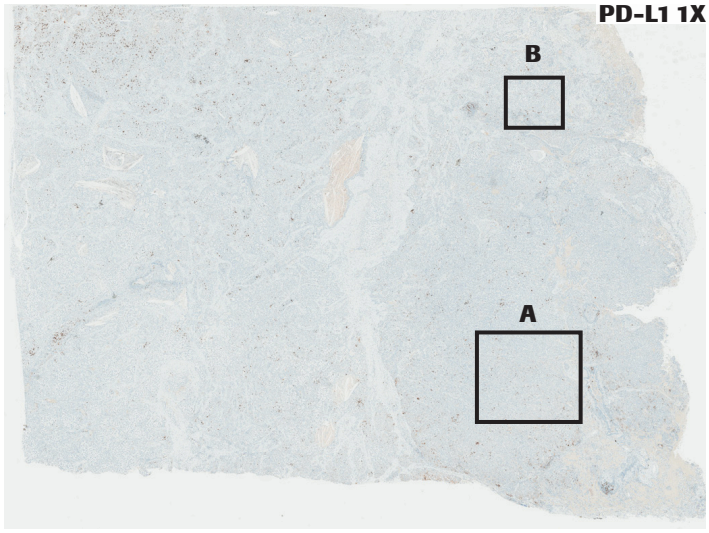


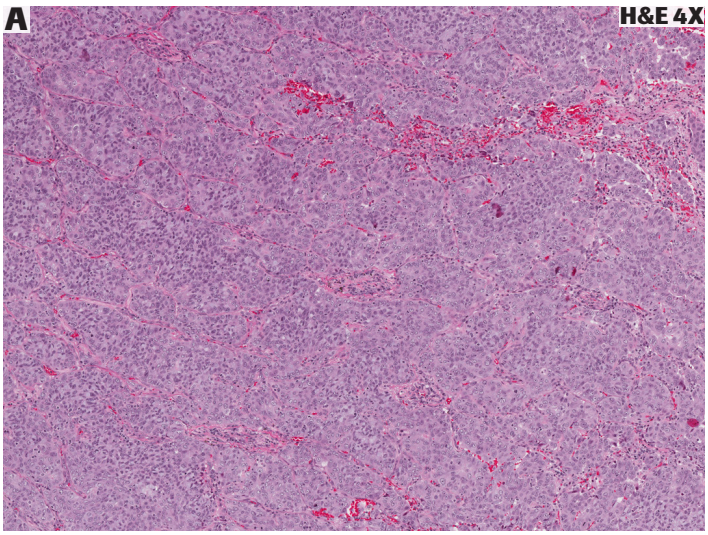


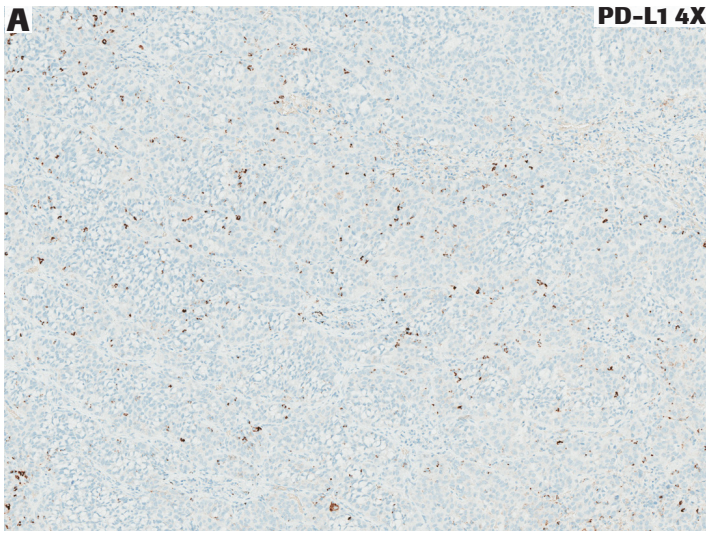


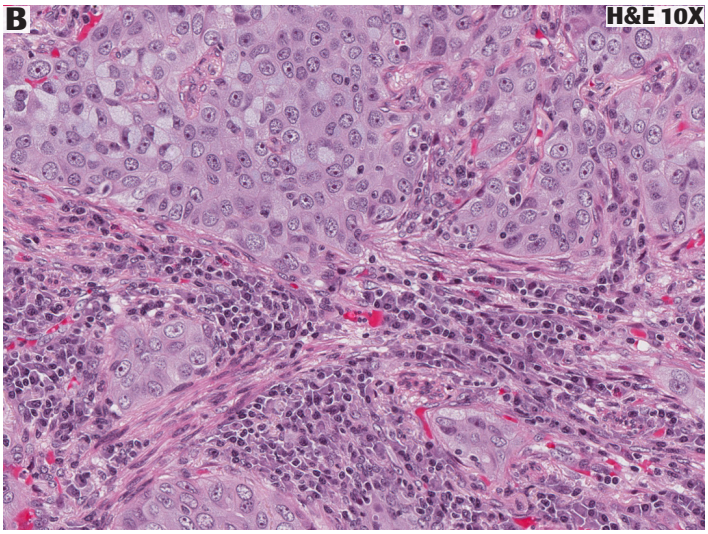
Case 3: This case is TC < 50% and IC < 10%. Scores: TC: 15% and IC: 1%. Note the presence of primarily weak TC staining.

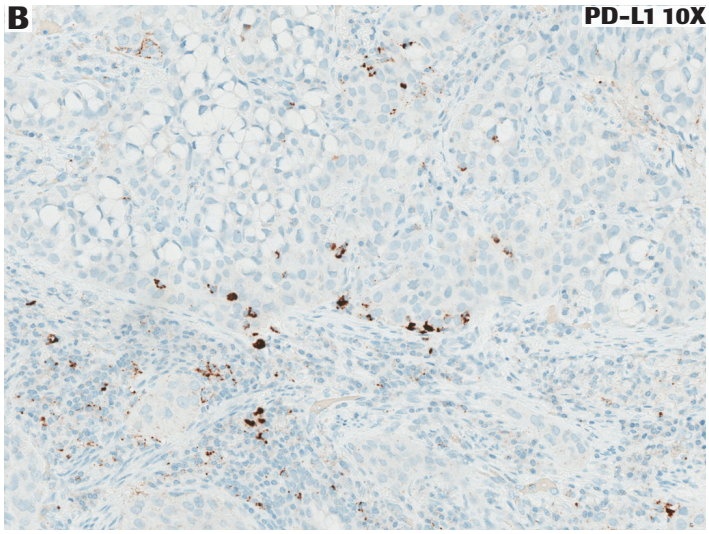






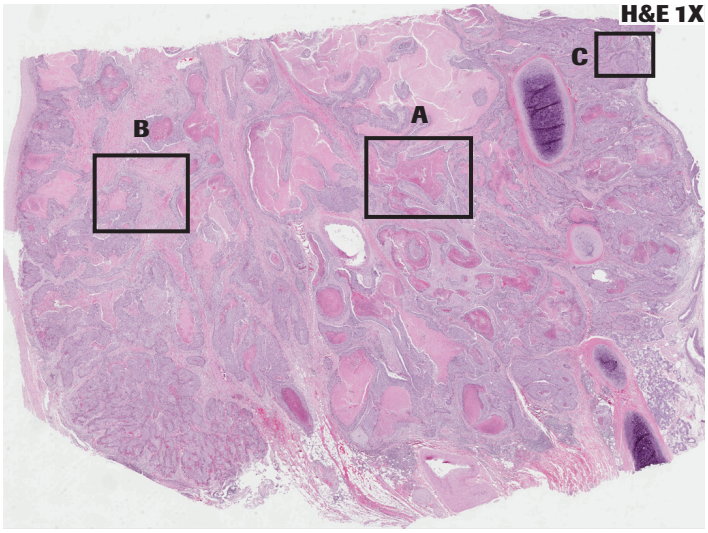


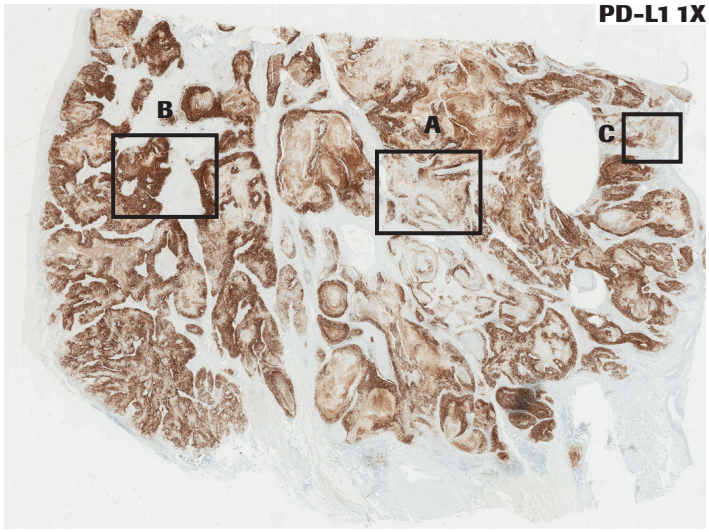


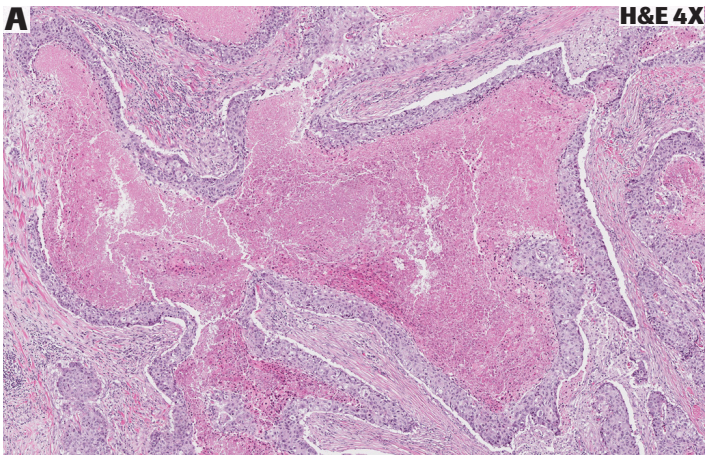


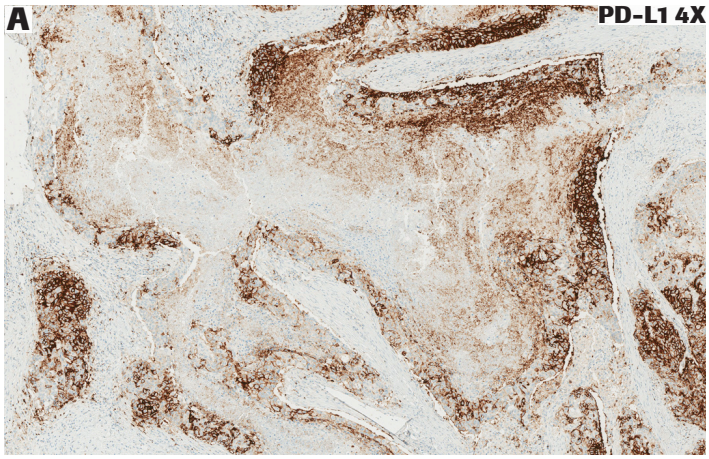
Case 4: This case is TC < 50% and IC < 10%. Scores: TC: 0% and IC: 5%. This case has relatively uniform single cell spread. Estimation of IC percentage should be performed using the **IC Expression – Single-Cell Spread** reference images.

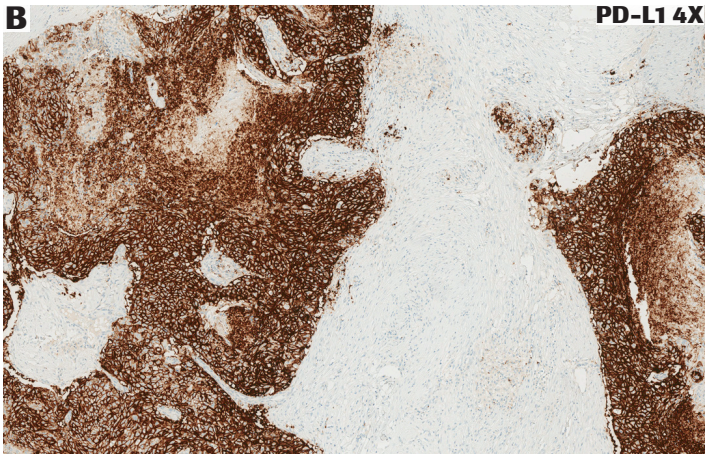
Example Cases: TC ≥ 50%

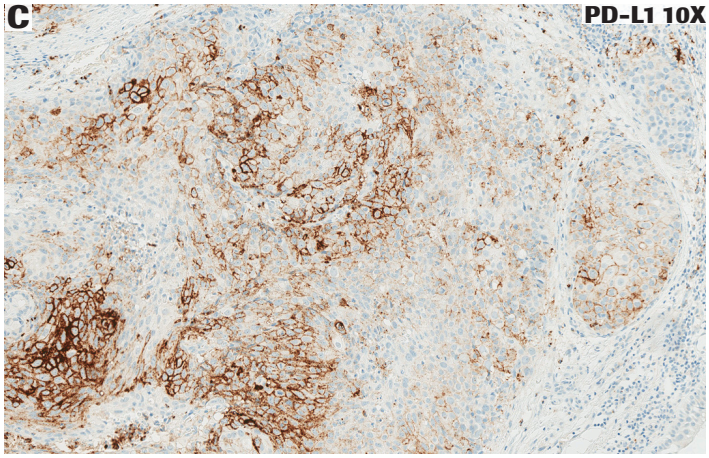




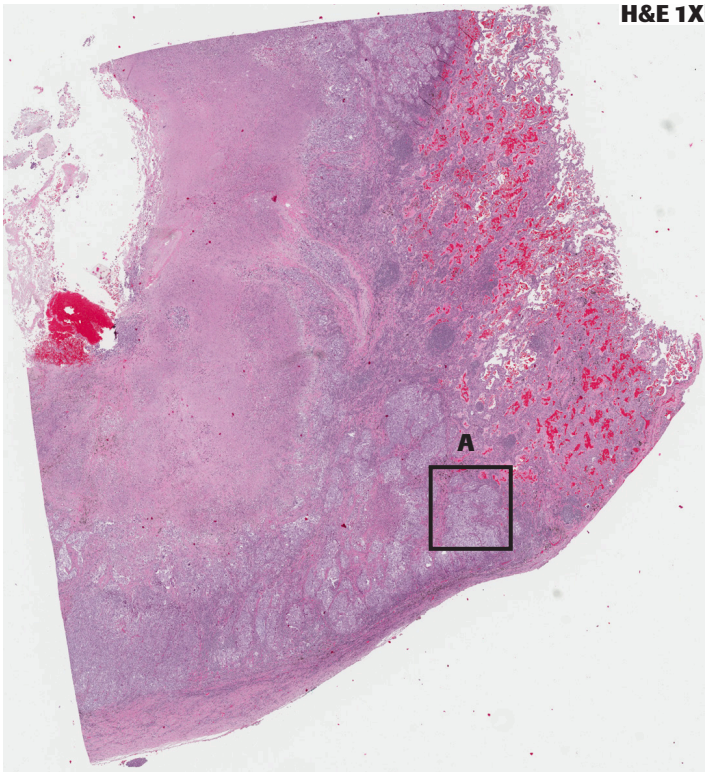


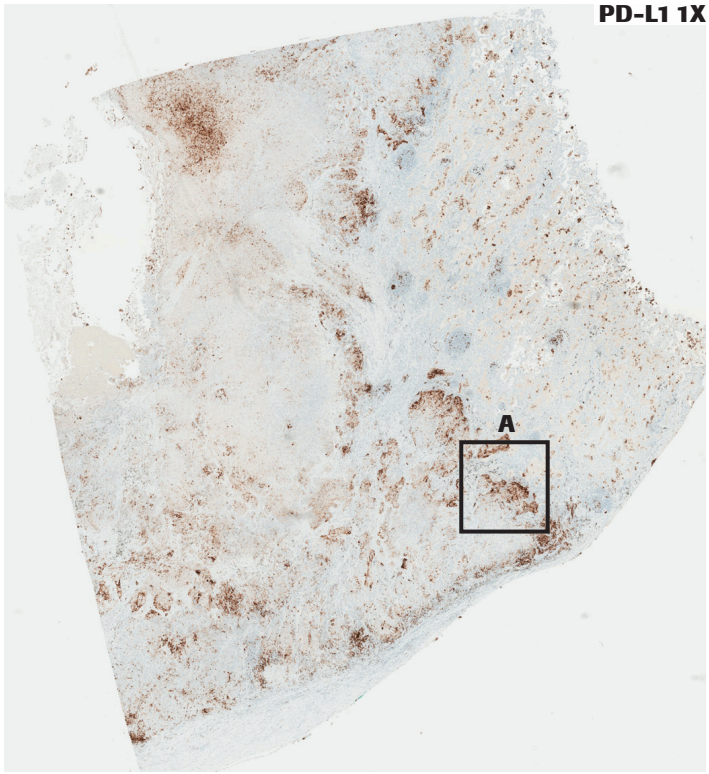


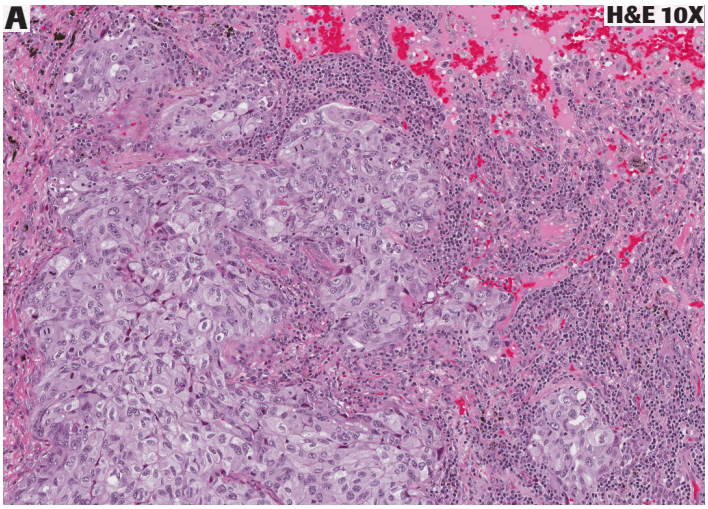


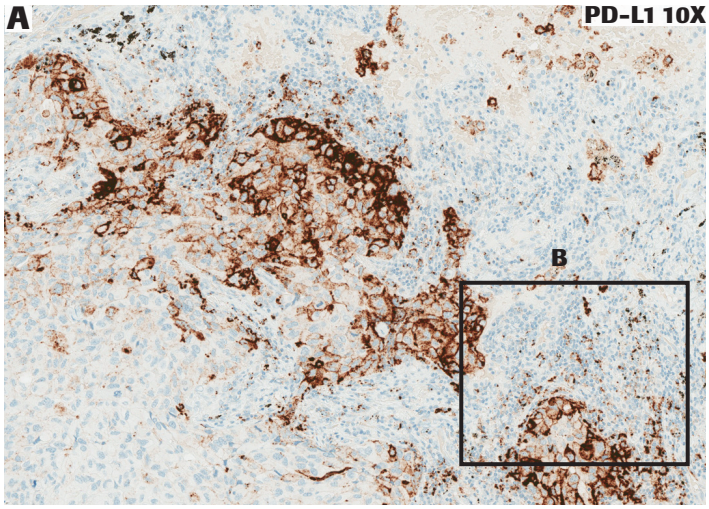


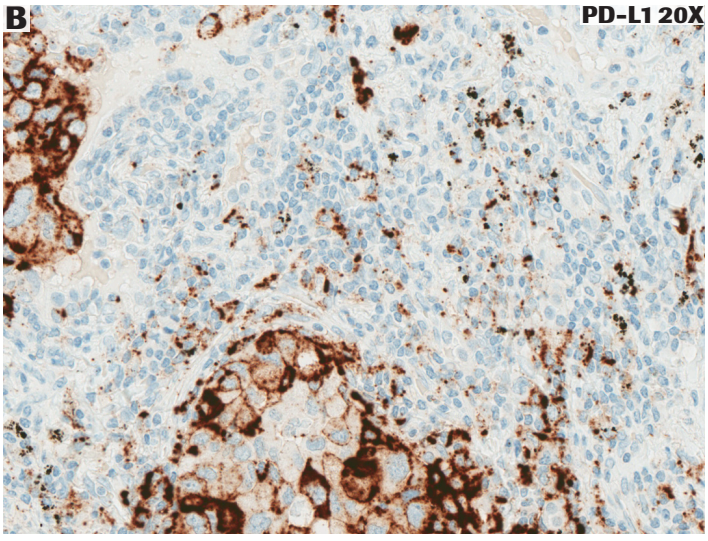
Case 5: This case is TC ≥ 50%. Scores: TC: 80% and IC: 2%. This case illustrates TC staining of variable intensity associated with IC staining. In regions with strong TC staining careful attention to the intratumoral stroma is necessary. In regions with weak to moderate TC staining IC can be assessed 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.





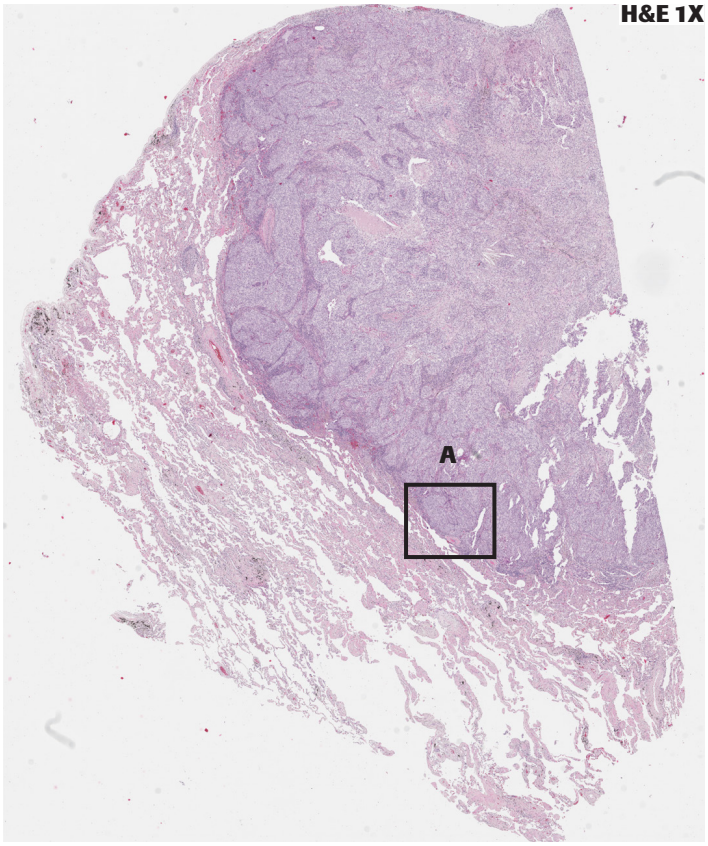


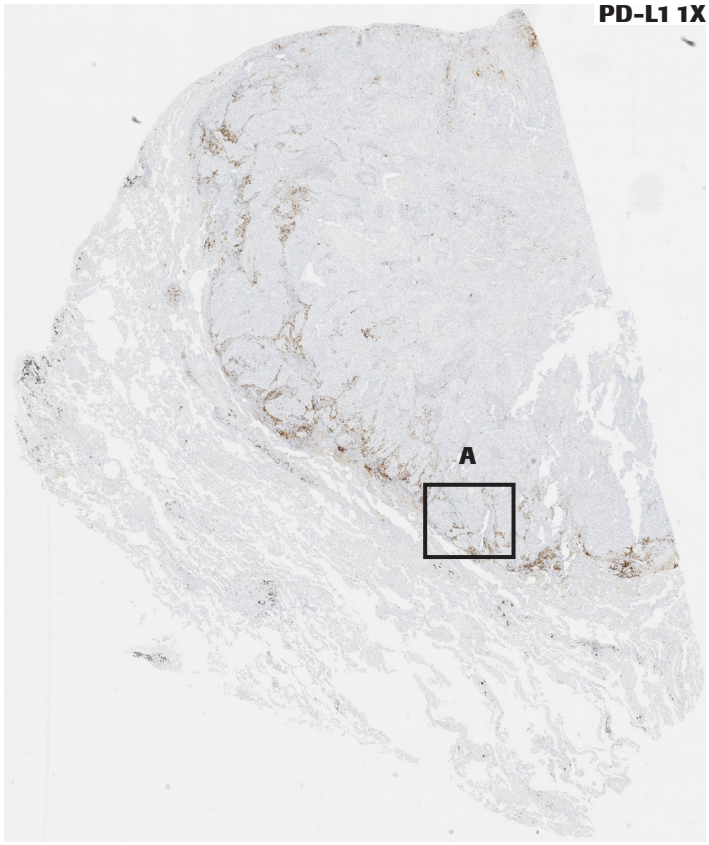


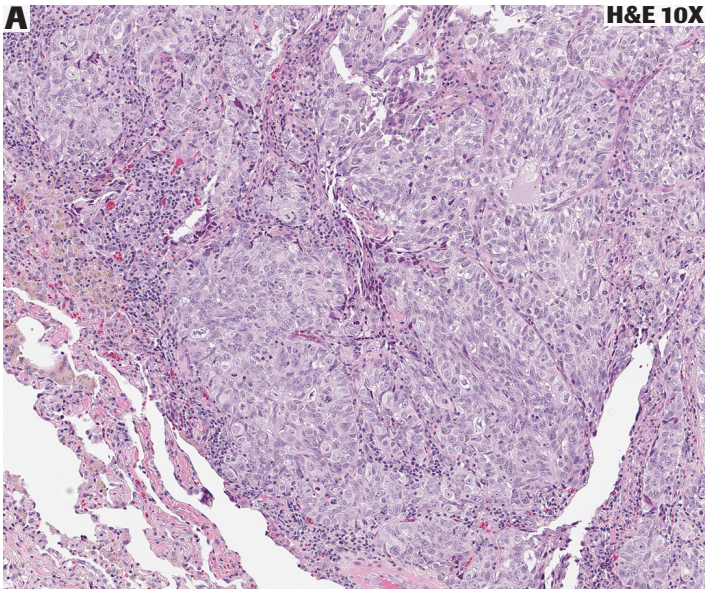


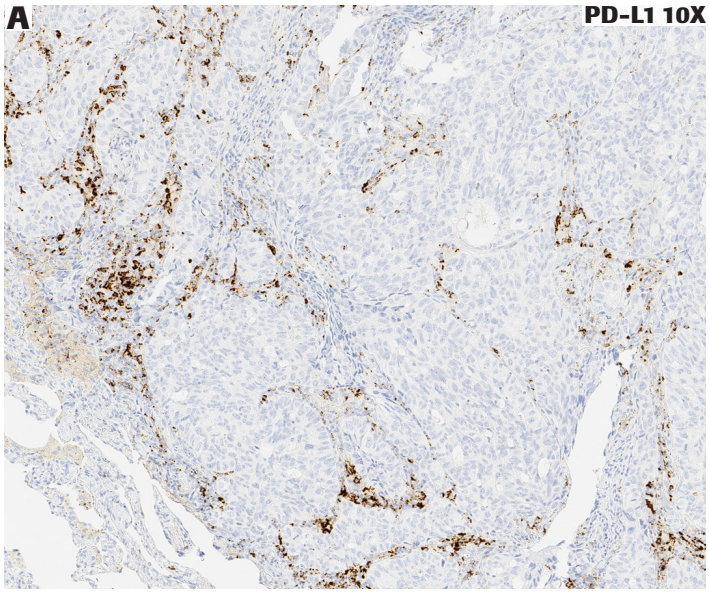
Case 6: This case is TC ≥ 50%. Scores: TC: 60%; IC: 5% . This case shows a combination of TC and IC staining requiring high magnification examination and review of the corresponding H&E to distinguish TC staining from IC staining.

Example Cases: IC ≥ 10%

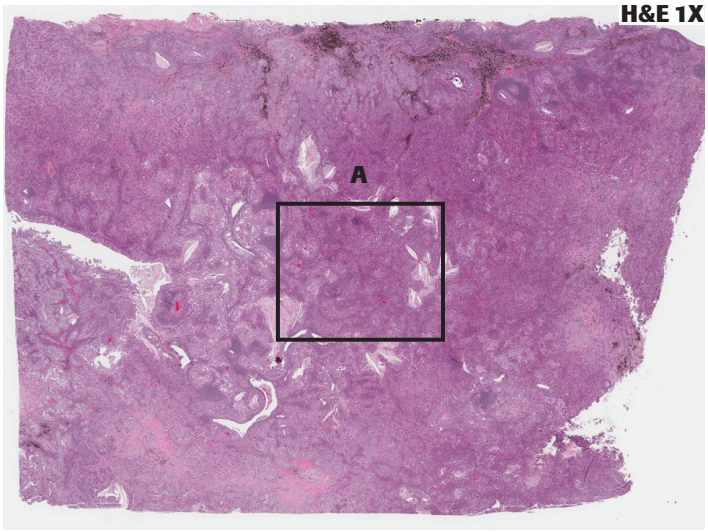


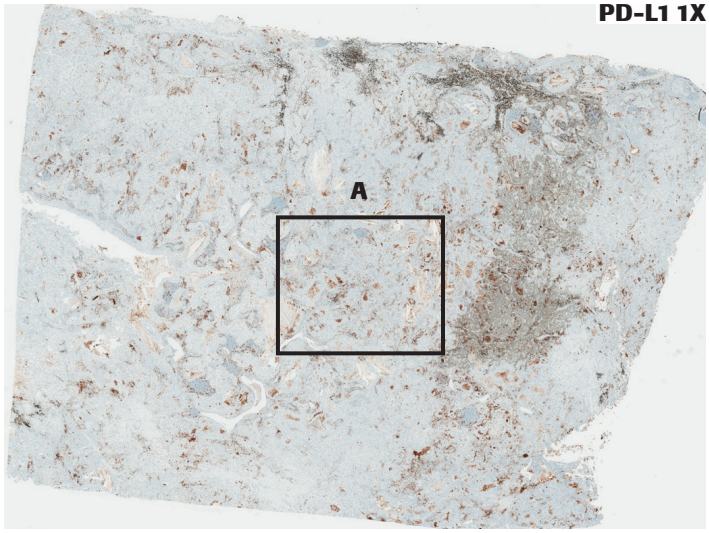


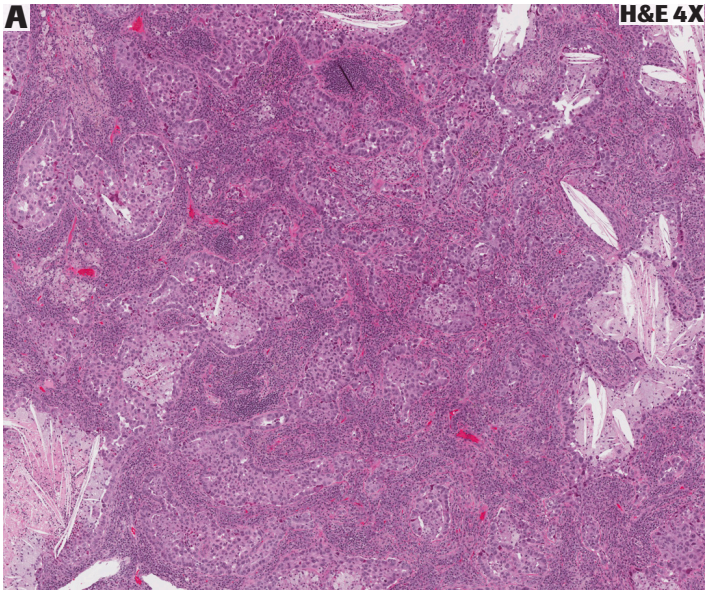


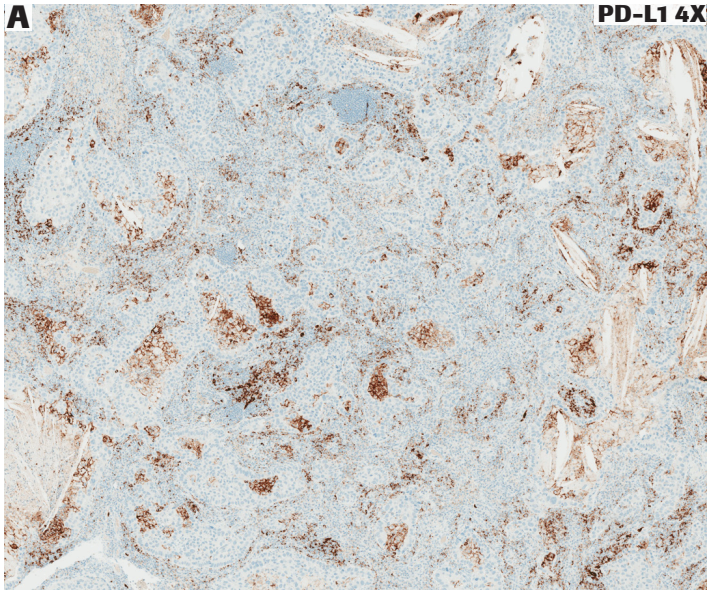


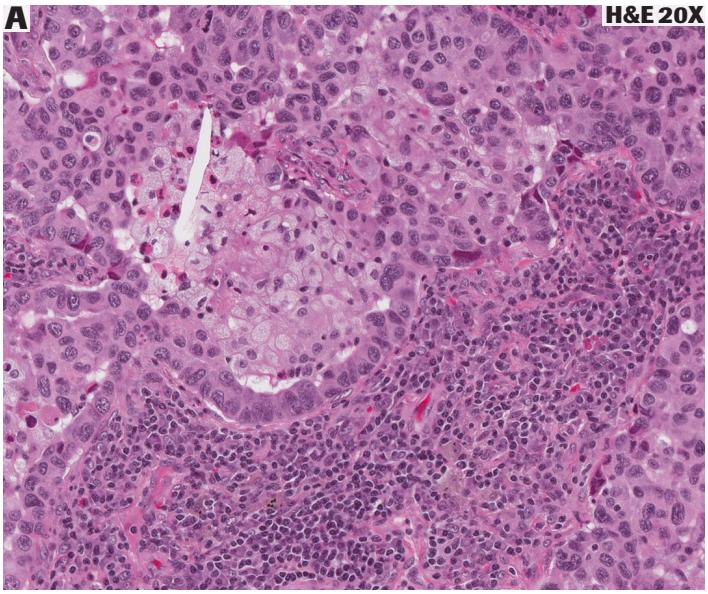
Case 7: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0% and IC: 10%. Note the concentration of PD-L1 IC along the edges of the tumor in the stroma.

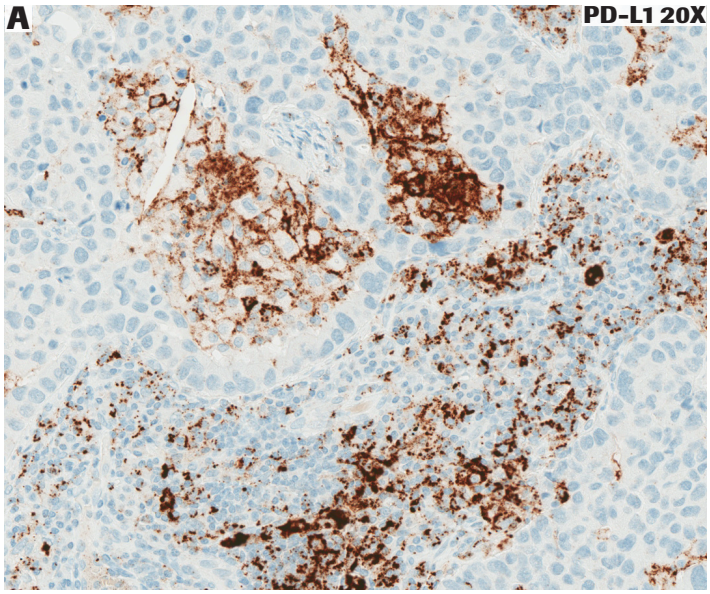




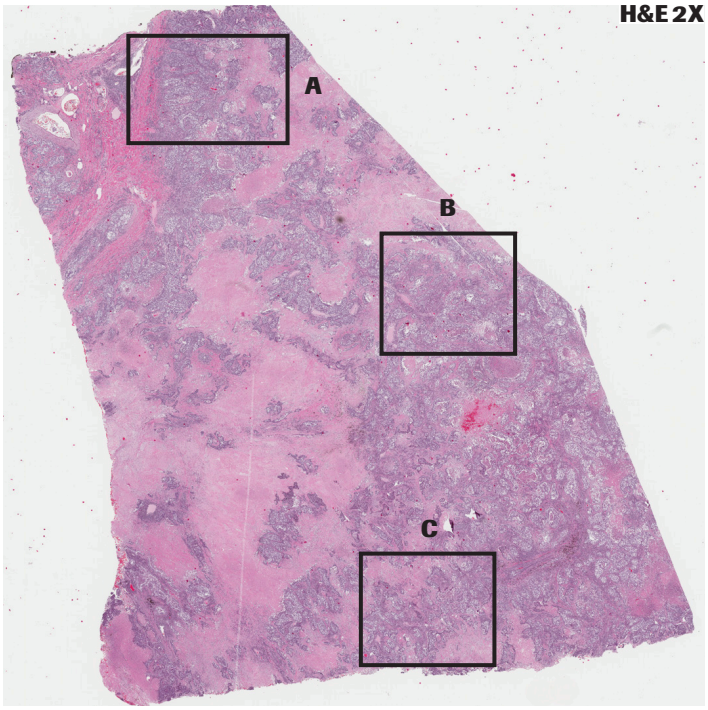


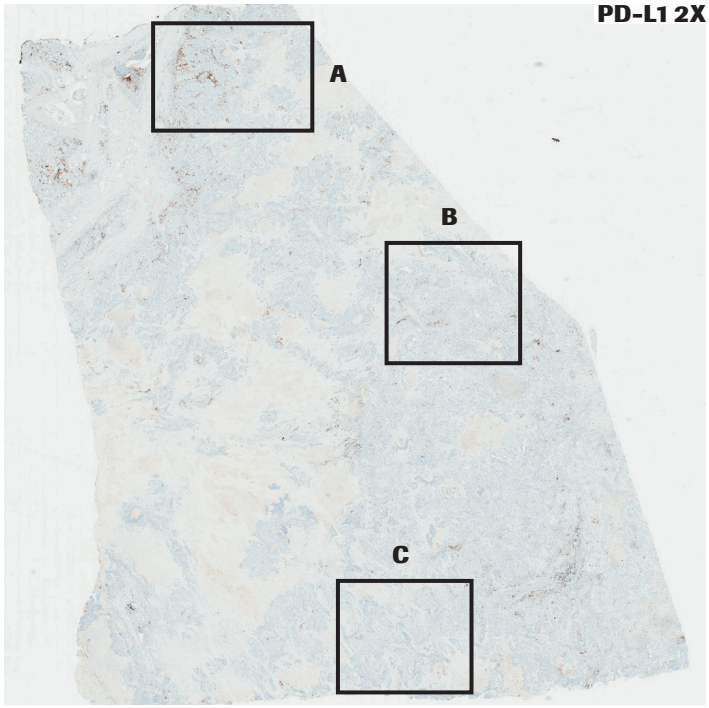


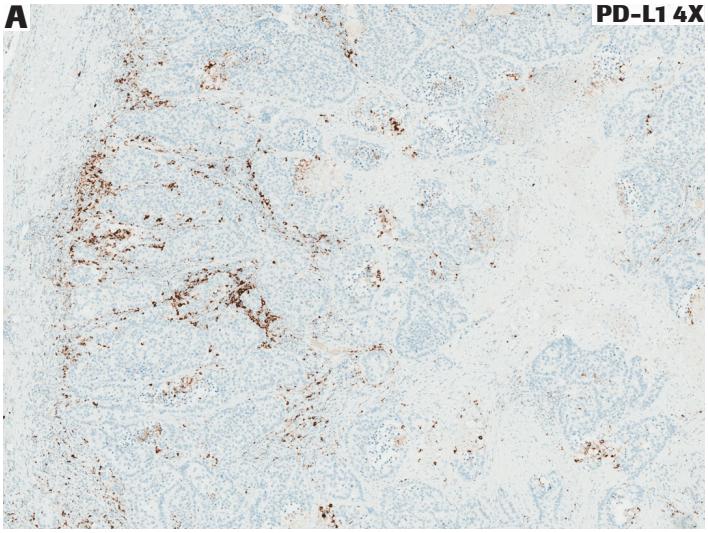


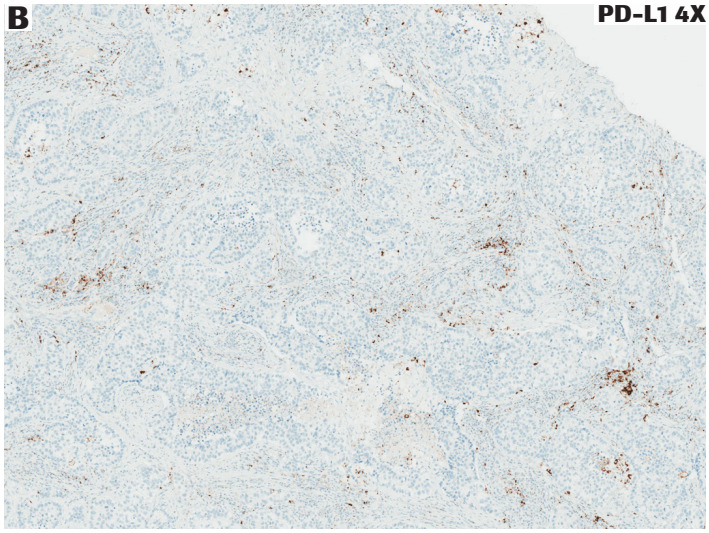


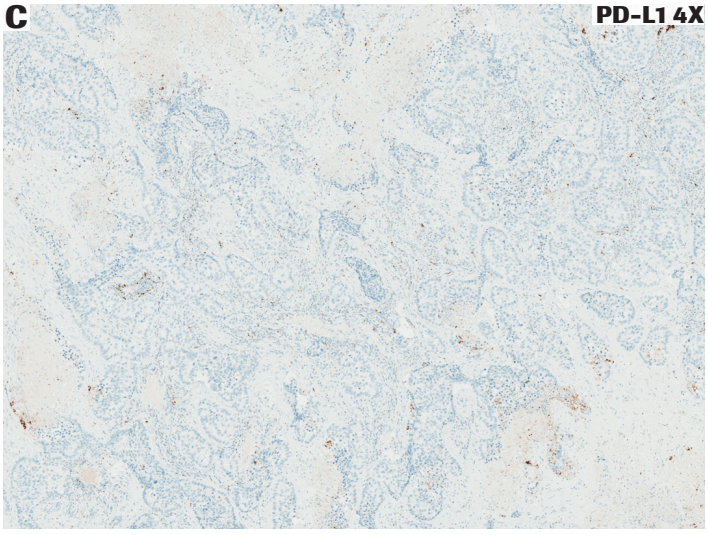
Case 8: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0%; IC: 20%. Note the presence of PD-L1 staining viable intraluminal IC (macrophages) contiguous to TC. These can be mistaken for TC staining and require review of corresponding H&E. These are counted towards IC percentage.





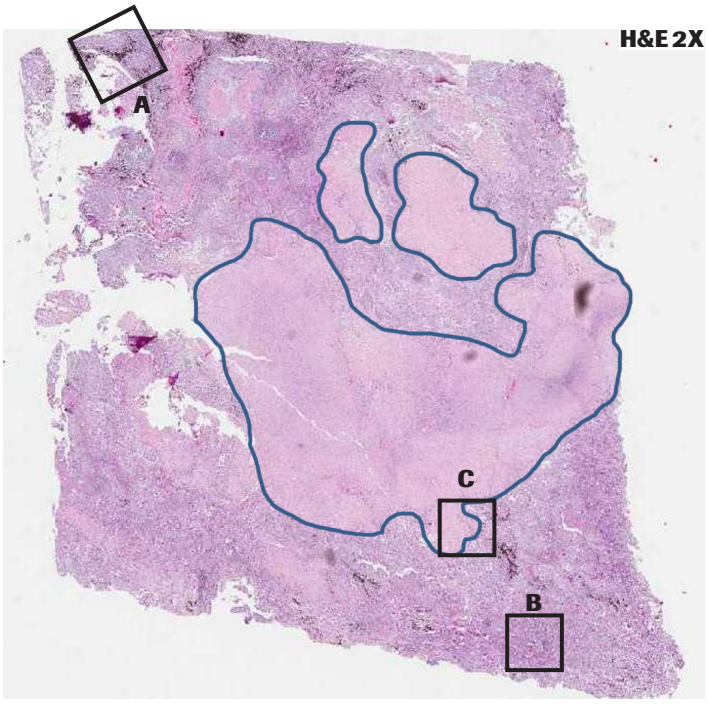


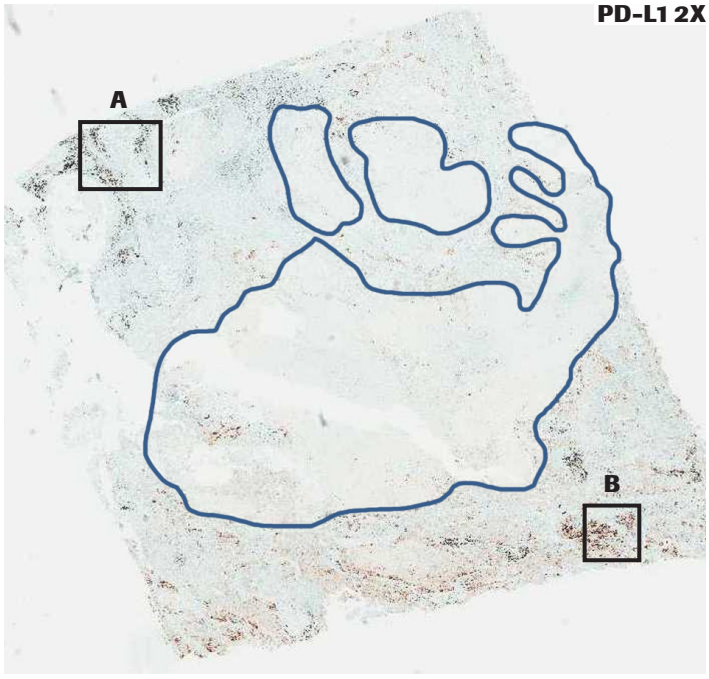


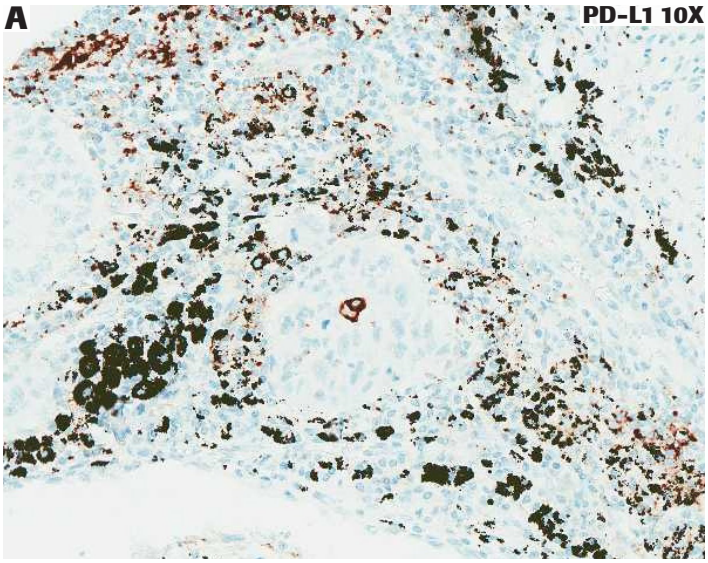


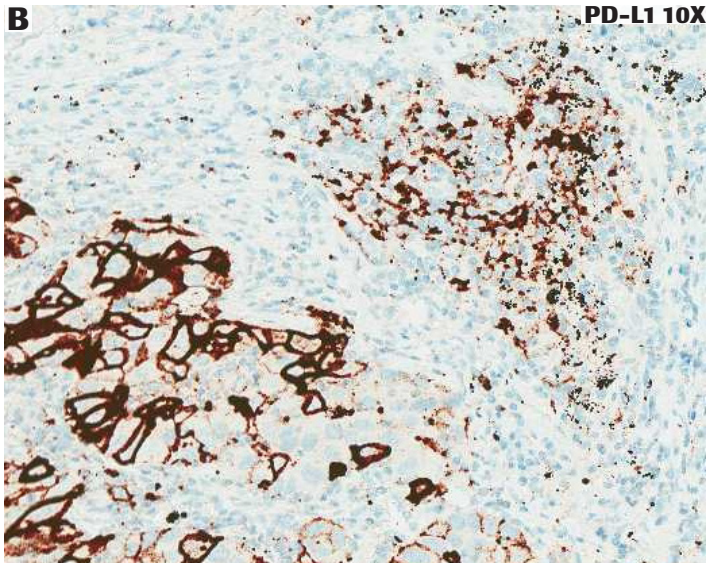
Case 9: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 0%; IC: 10%. Three representative regions of IC staining are shown in this case with different ranges of PD-L1 IC staining in each. Overall, the case has 10% IC. Note the presence of geographic necrosis which should be excluded from tumor area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

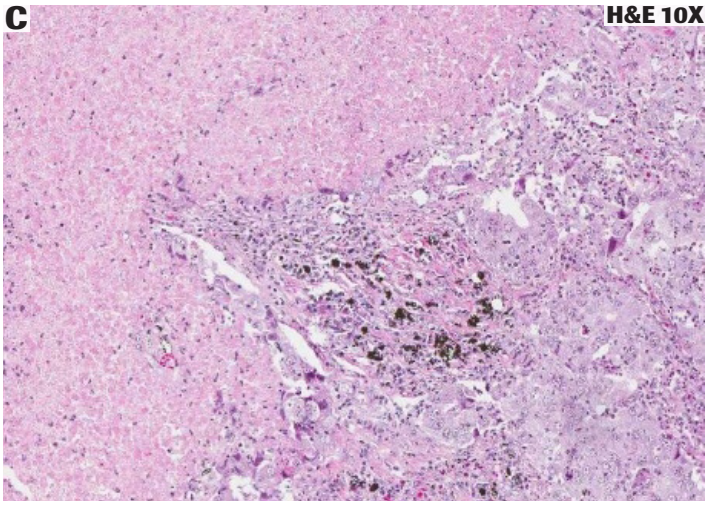
Challenging Cases



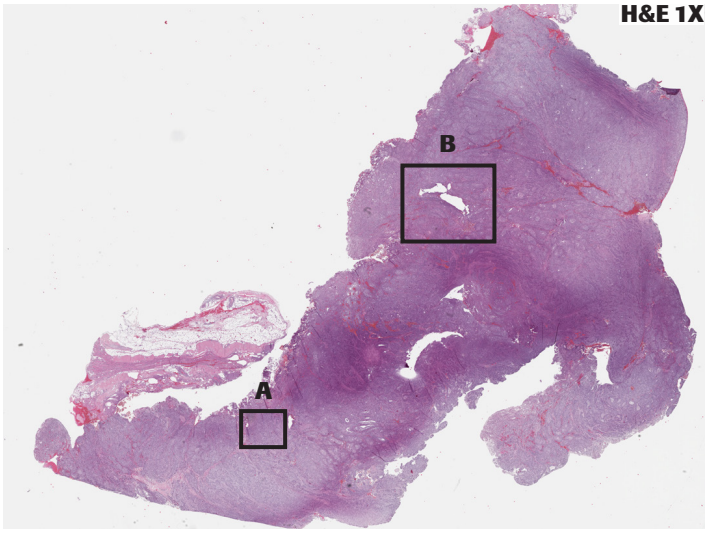


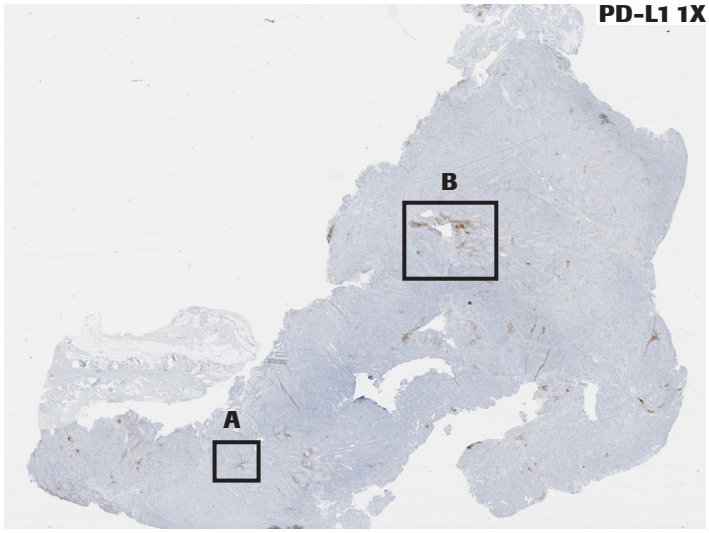


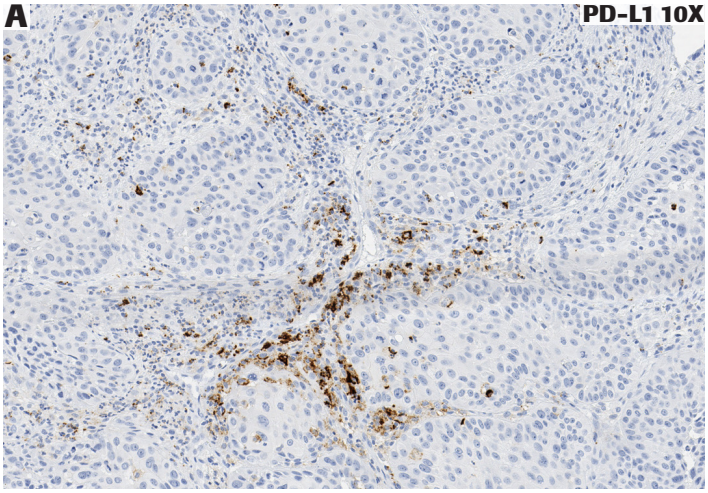


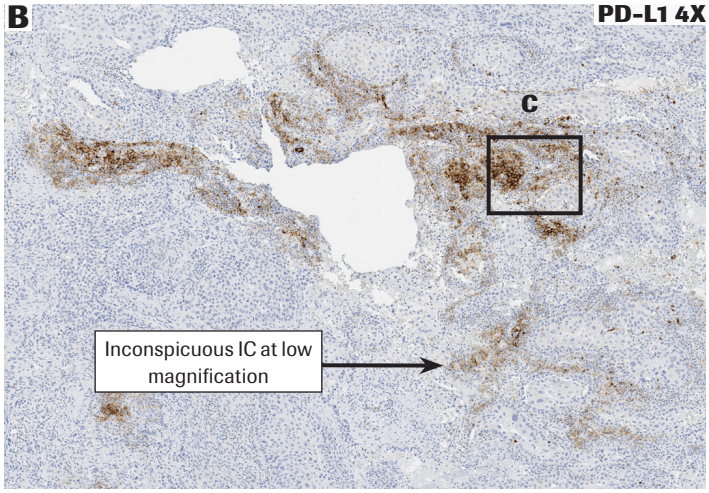


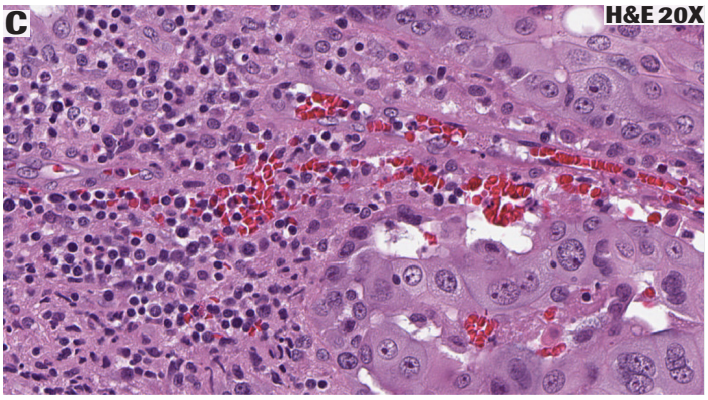
Case 10: This case is TC < 50% and IC < 10%. Scores: TC: 20%; IC: 5%. This case has geographic necrosis which should be excluded from tumor area definition. High magnification images of **Regions A** and **B** show IC aggregates in the midst of anthracotic pigment and next to TC staining. When necrotic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

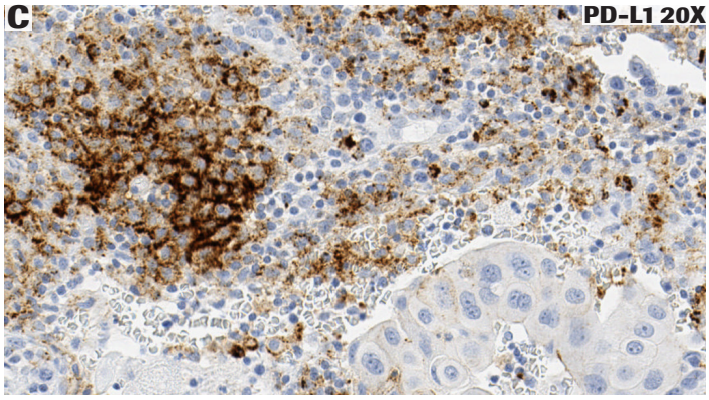




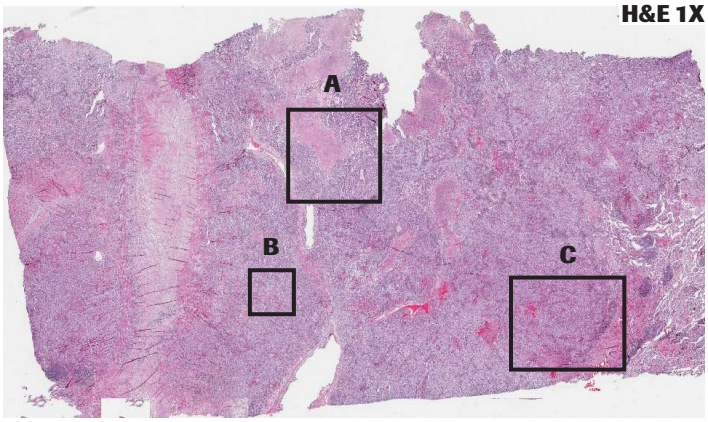


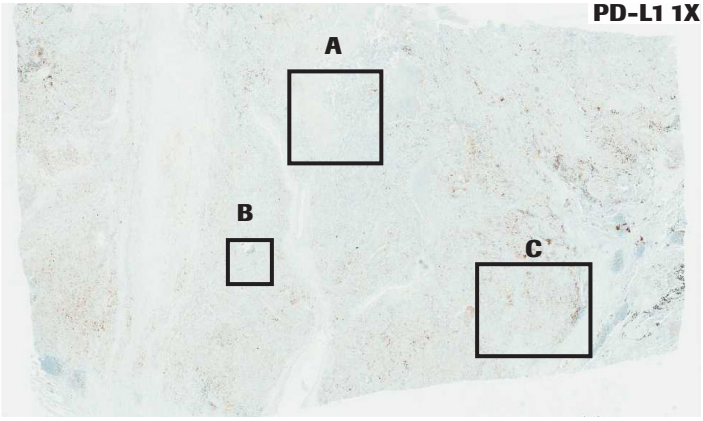


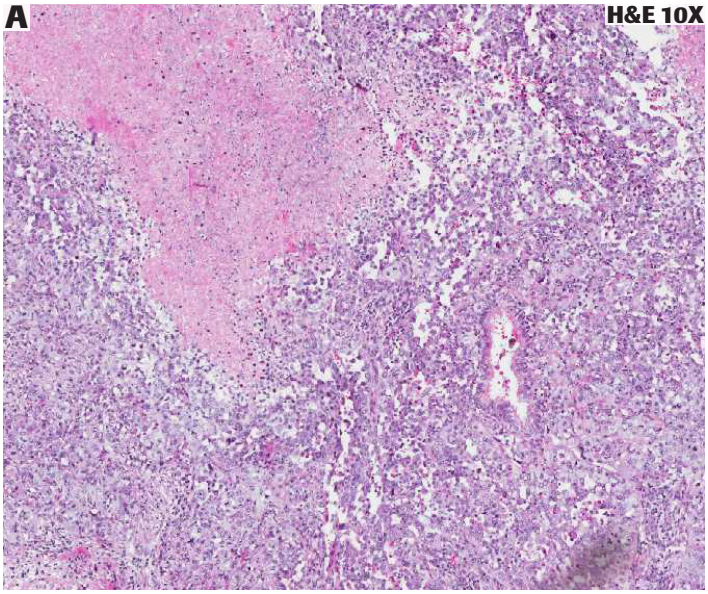


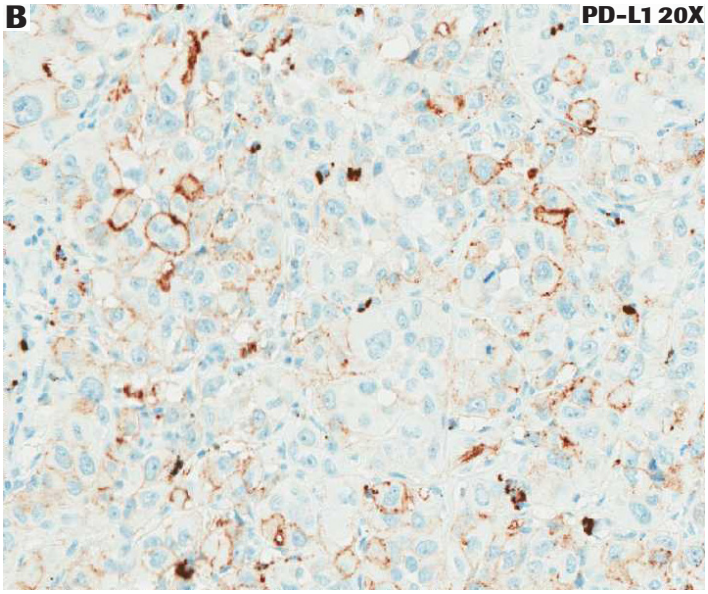


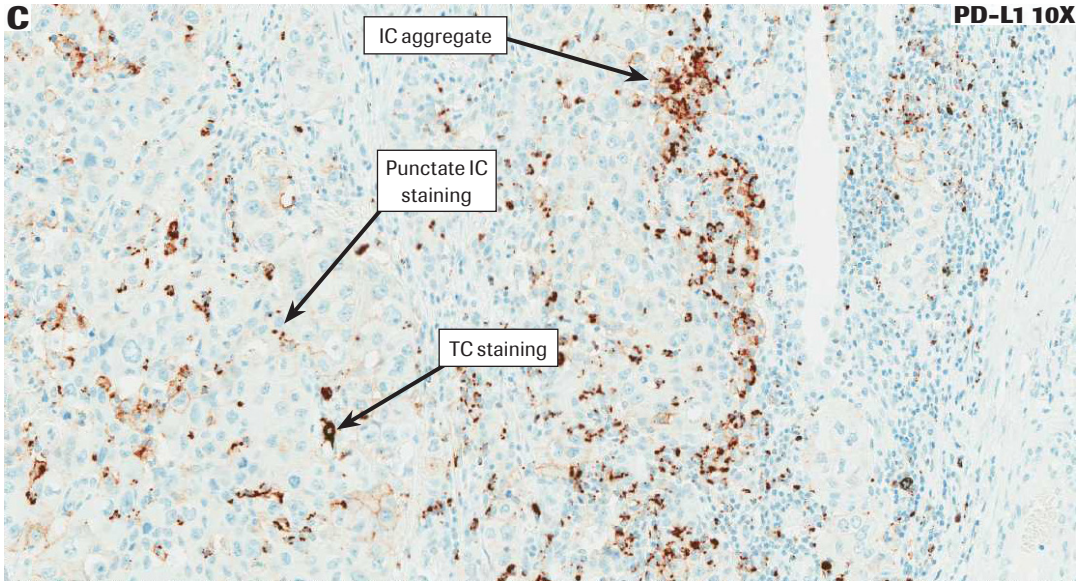
Case 11: This case is TC < 50% and IC < 10%. Scores: TC: 0%; IC: 5%. This tissue illustrates focal IC staining and presence of macrophage staining, which often can be misinterpreted as TC given the circumferential staining (**Region B**). Also note the presence of light staining IC which is not readily 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**).



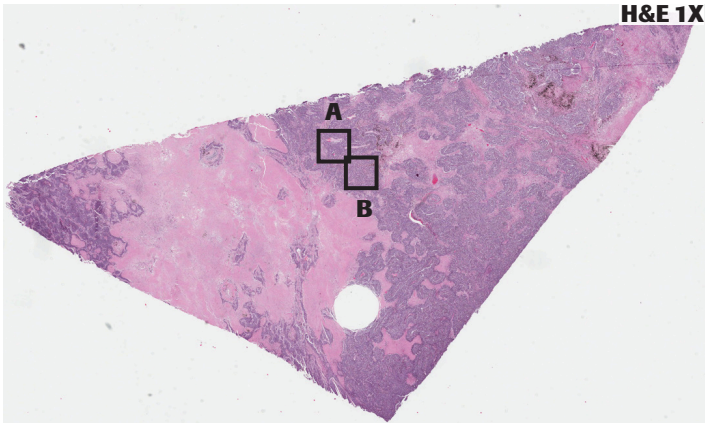


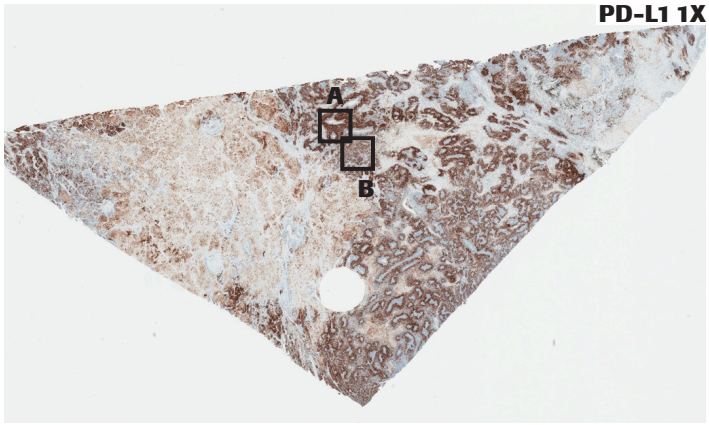


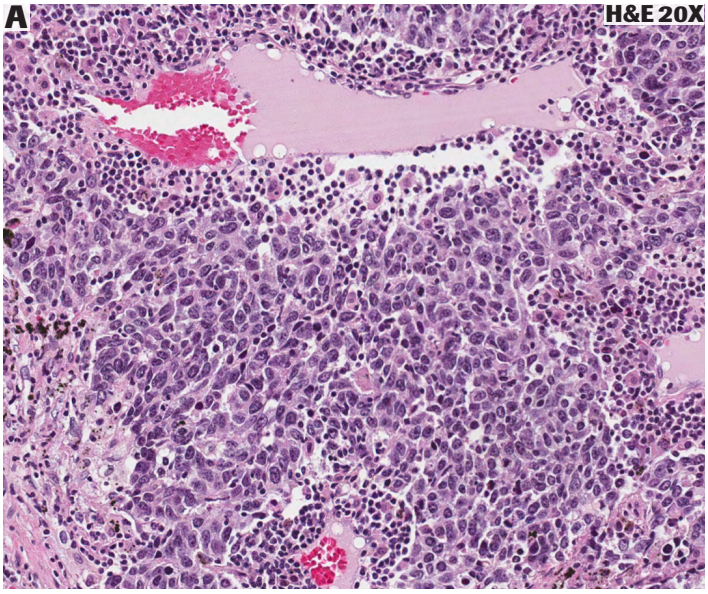


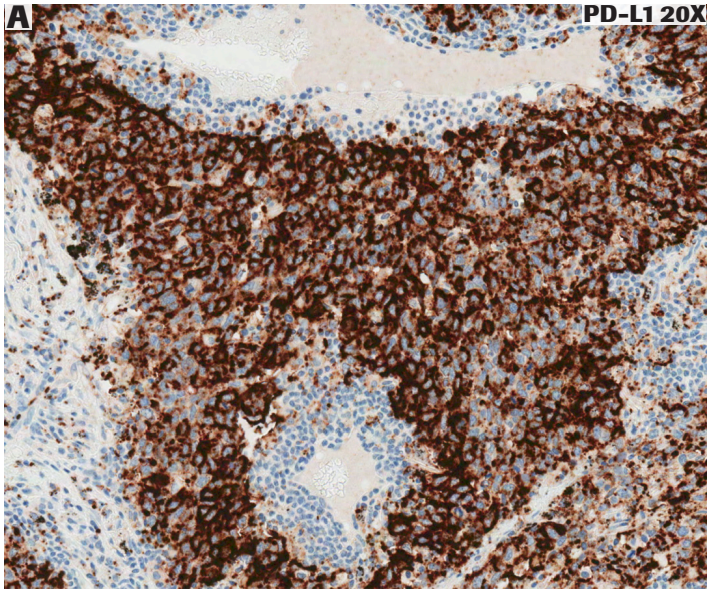


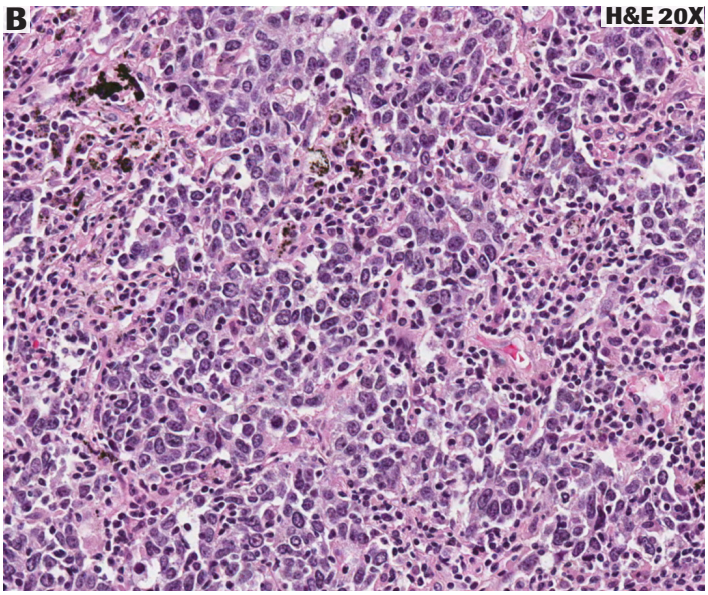
Case 12: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 10%; IC: 15%. On high magnification notice weak membrane staining of tumor cells (**Region B**) and strong punctate IC staining interspersed amongst tumor cells (**Region C**). This case illustrates the importance of using high magnification to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

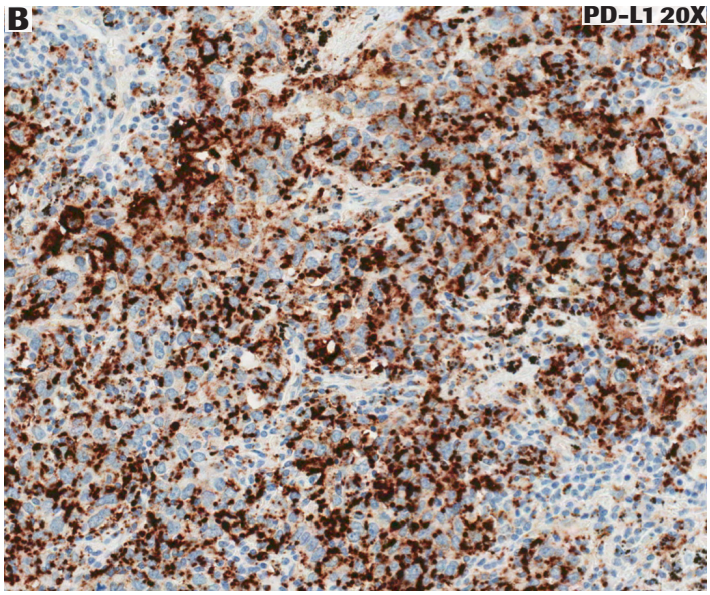








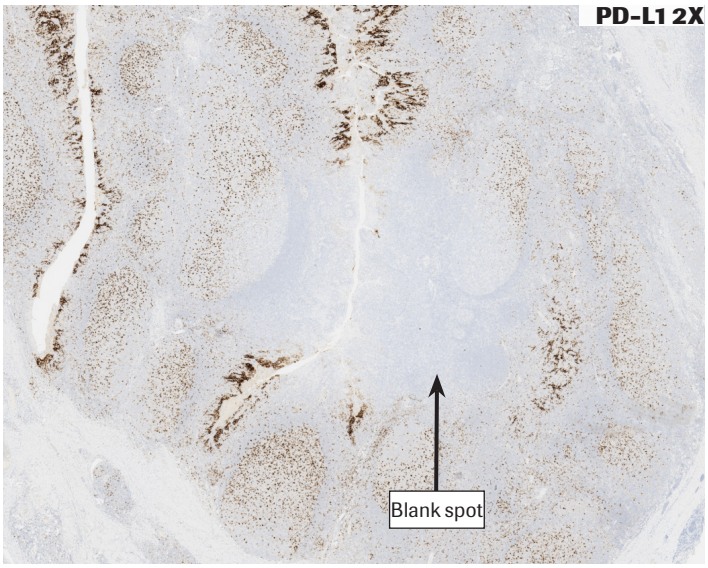


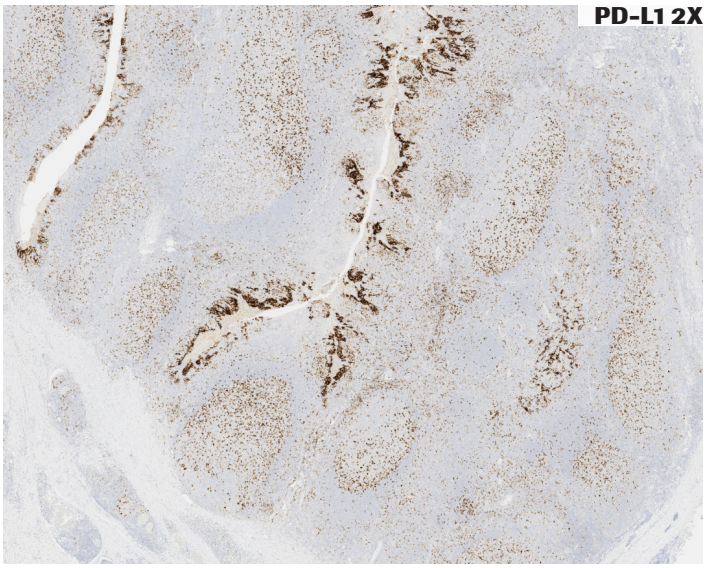


Case 13: This case is TC < 50%, but IC ≥ 10%. Scores: TC: 40%; IC: 50%. Note the presence of TC with more linear membrane staining (**Region A**) and IC as granular staining (**Region B**). This case shows strong staining in both TC and IC with dense immune infiltrate on H&E. This scenario requires careful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

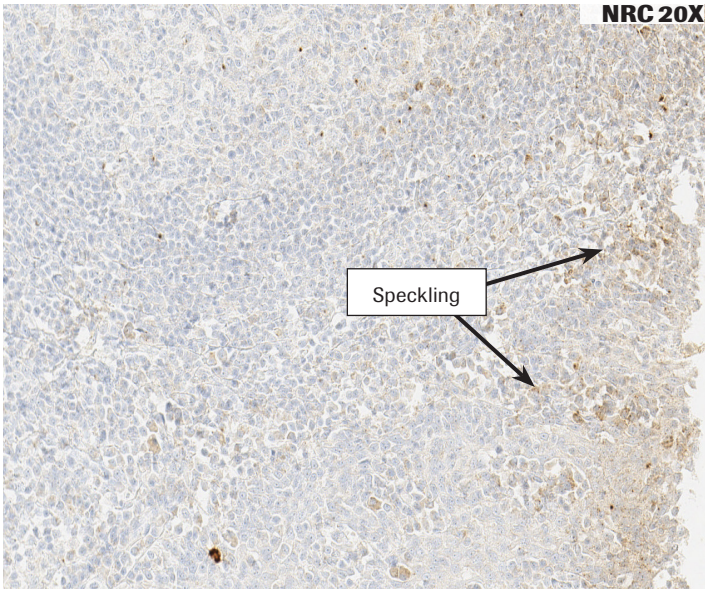
Staining Artifacts

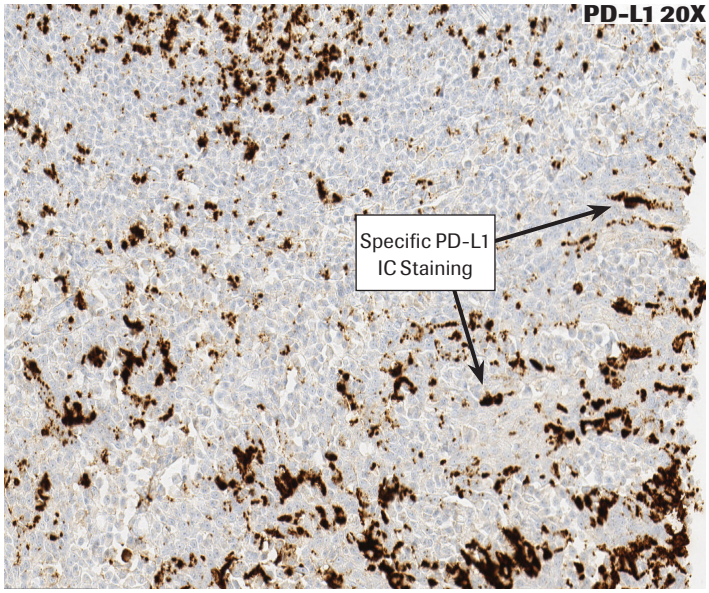
Artifacts noted in this section can be observed on Negative Reagent Control and VENTANA PD-L1 (SP142) Assay-stained slides. The presence of these artifacts may require repeat staining if they interfere with interpretation of VENTANA PD-L1 (SP142) Assay staining. Always review the corresponding Negative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



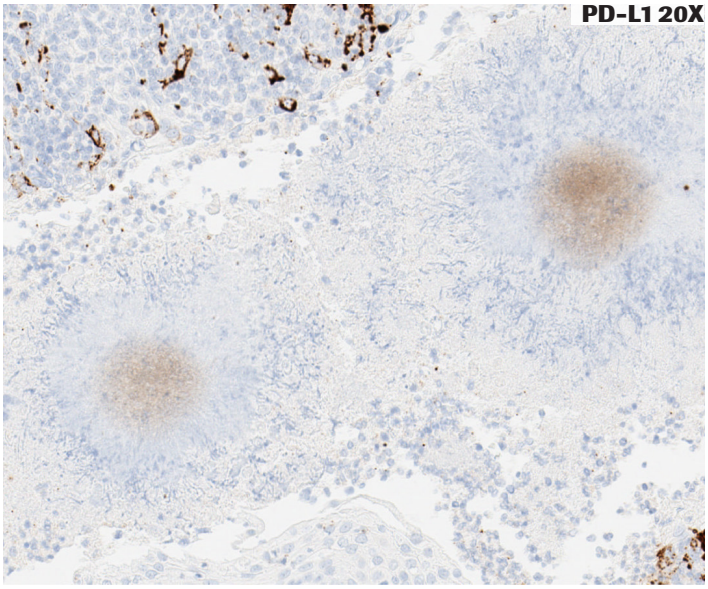


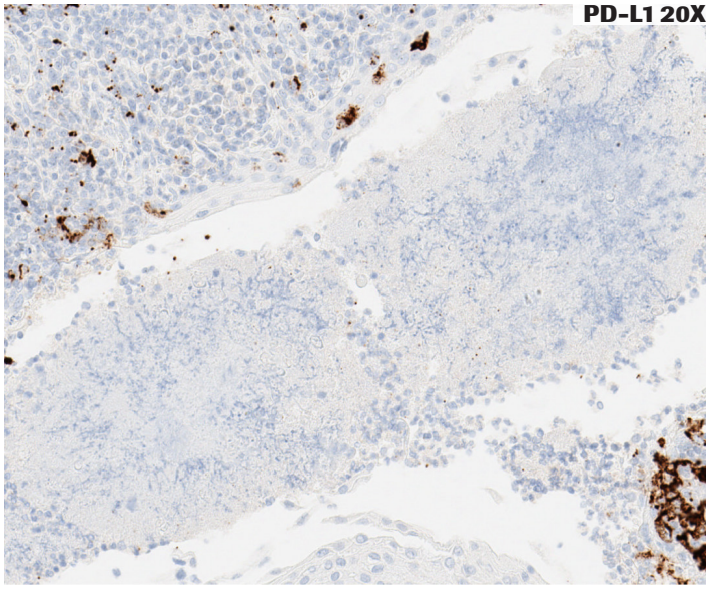
Blank Spots: Blank spots are light to non-staining areas that are typically circular and are due to a static bubble formed during the staining procedure. The image on the left depicts an example of a blank spot opposed to the appropriate staining depicted in the image on the right. If the blank spot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



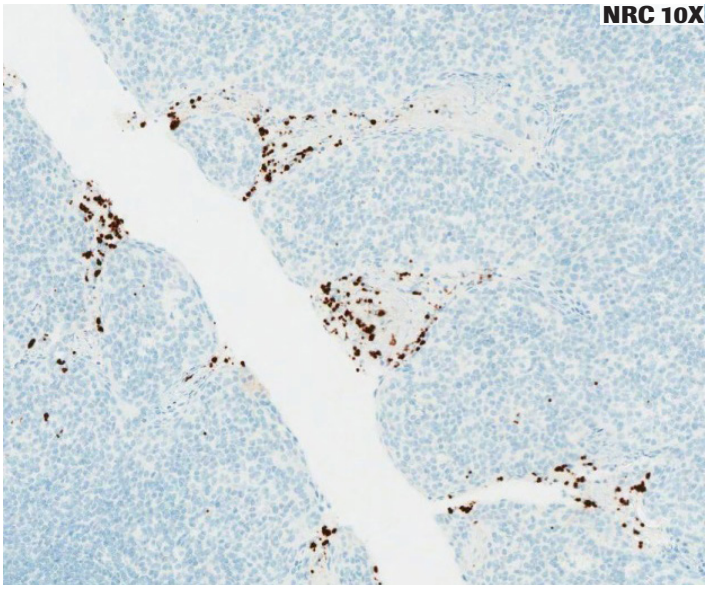


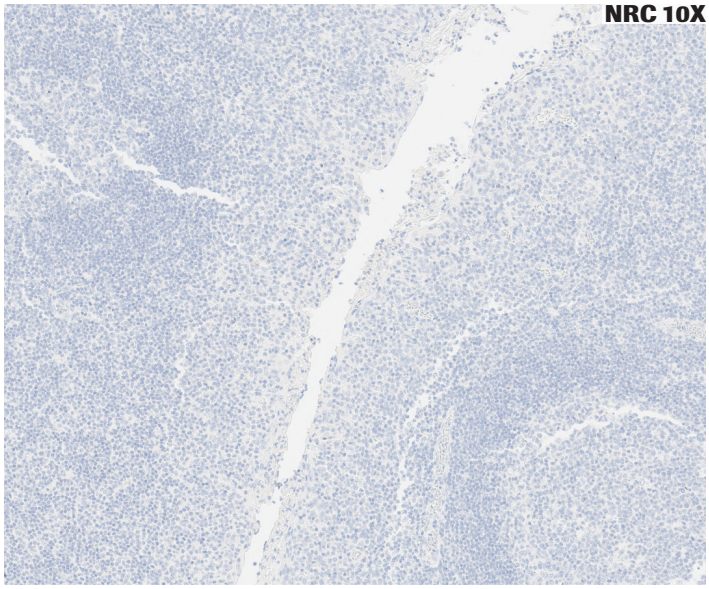
Speckling: Speckling, depicted in the image to the left, is weak to moderate non-specific staining that appears as a uniformly distributed fine granular precipitate most often in the cytoplasm. Speckling does not conform to either IC or TC staining characteristics. This artifact should not be confused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



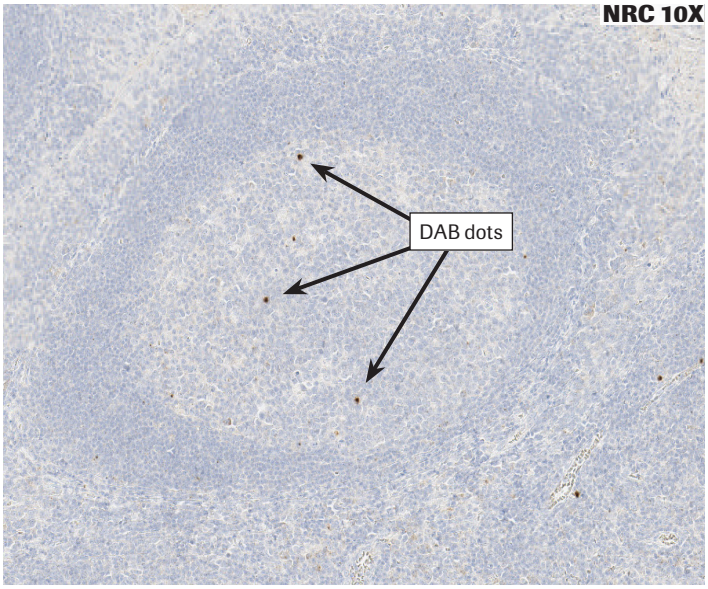


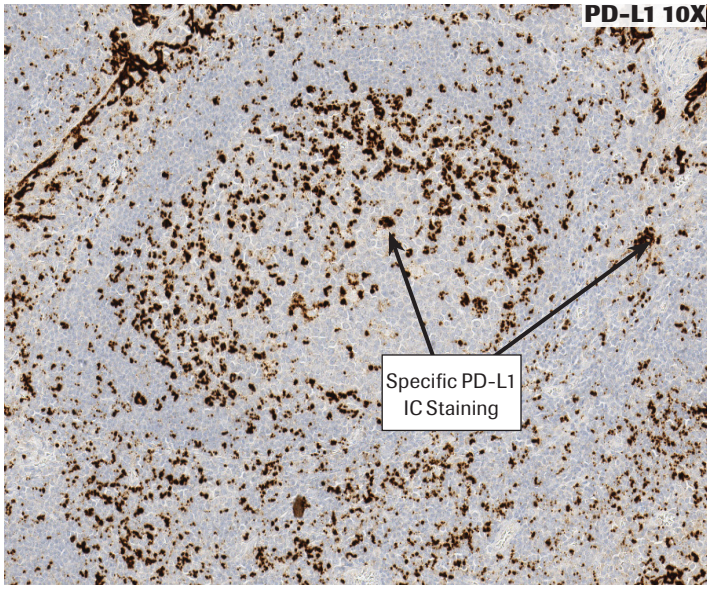
DAB Spots: DAB spots are circular spots that may form due to trapped DAB underneath the tissue section during the staining procedure. If this artifact interferes with the interpretation of PD-L1 stained slide, repeat the stain with fresh unstained slides. In the image to the right, the DAB spot is not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



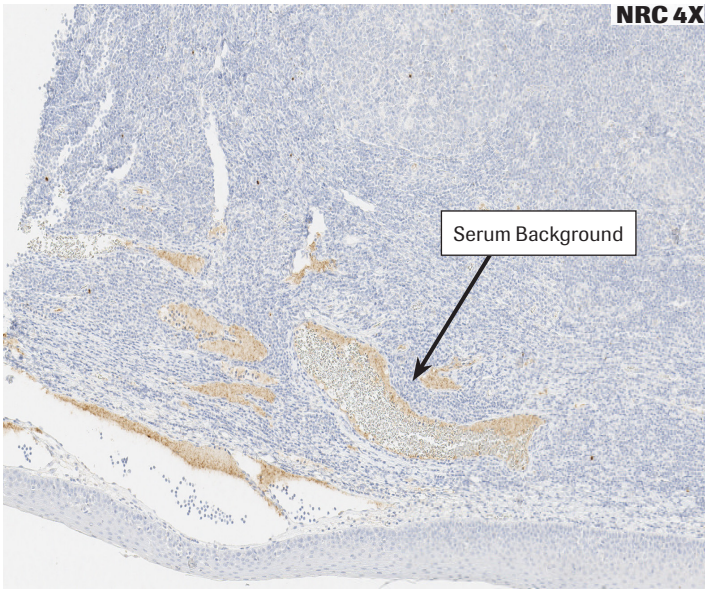


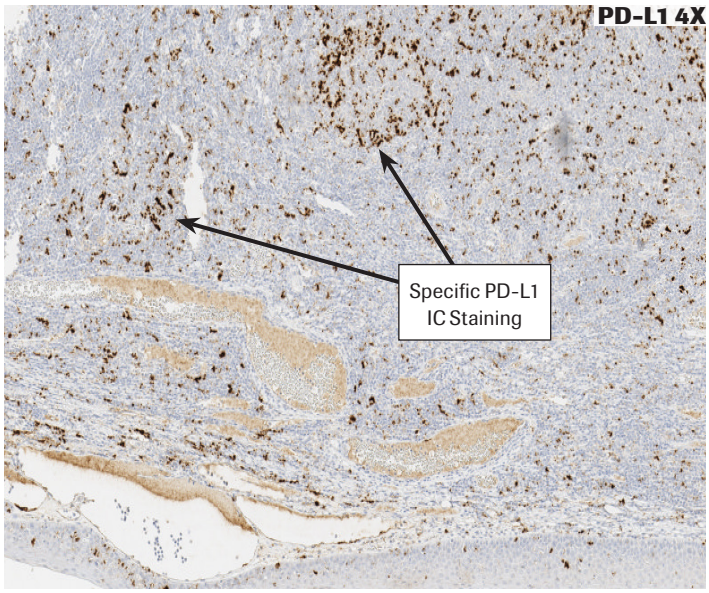
Luminal Debris: Tonsil stained with VENTANA PD-L1 (SP142) Assay can serve as both a positive and negative tissue control due to positive and negative staining elements being present. Luminal staining due to cross reactivity with an unknown antigen can be observed in the image of a tonsil stained with 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.





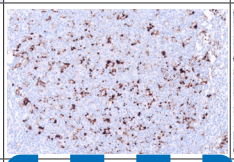
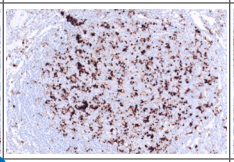
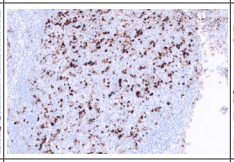
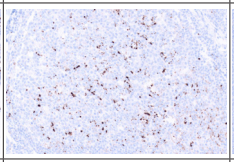
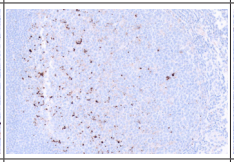
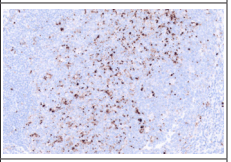
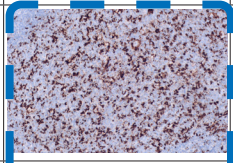
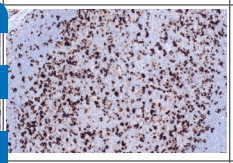
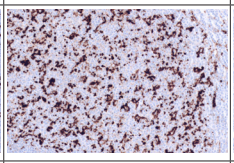
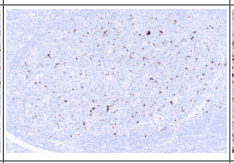
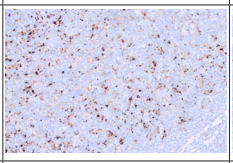
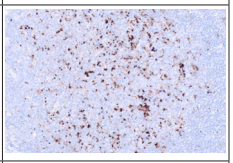
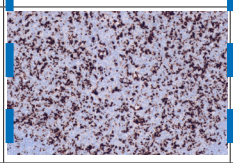
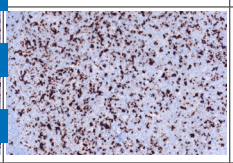
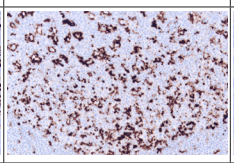
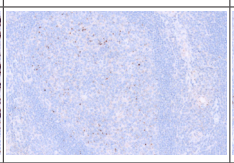
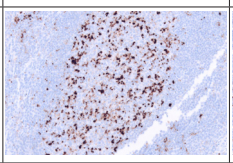
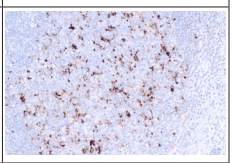
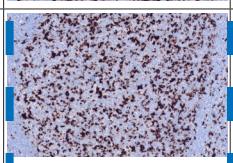
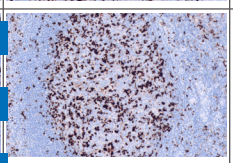
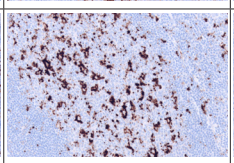
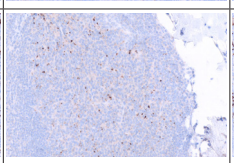
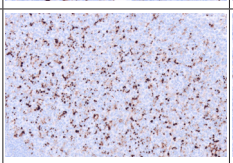
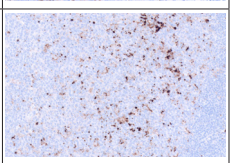
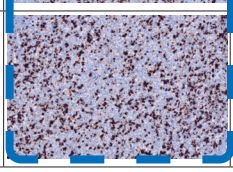
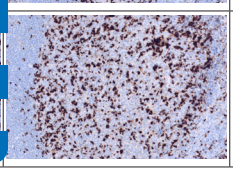
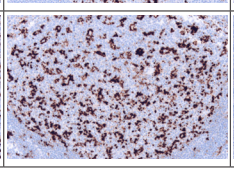
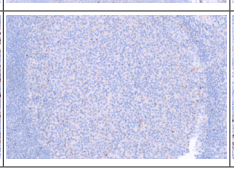
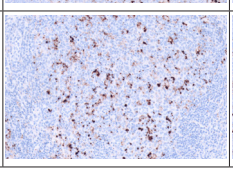
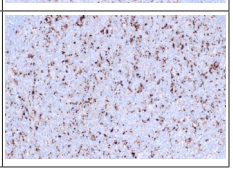
Serum Background: Serum background is non-specific staining in vascular spaces and serum extravasates. This is depicted in the bottom of each image above. It should not be confused with specific PD-L1 IC staining as depicted in the image to the right.

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**).

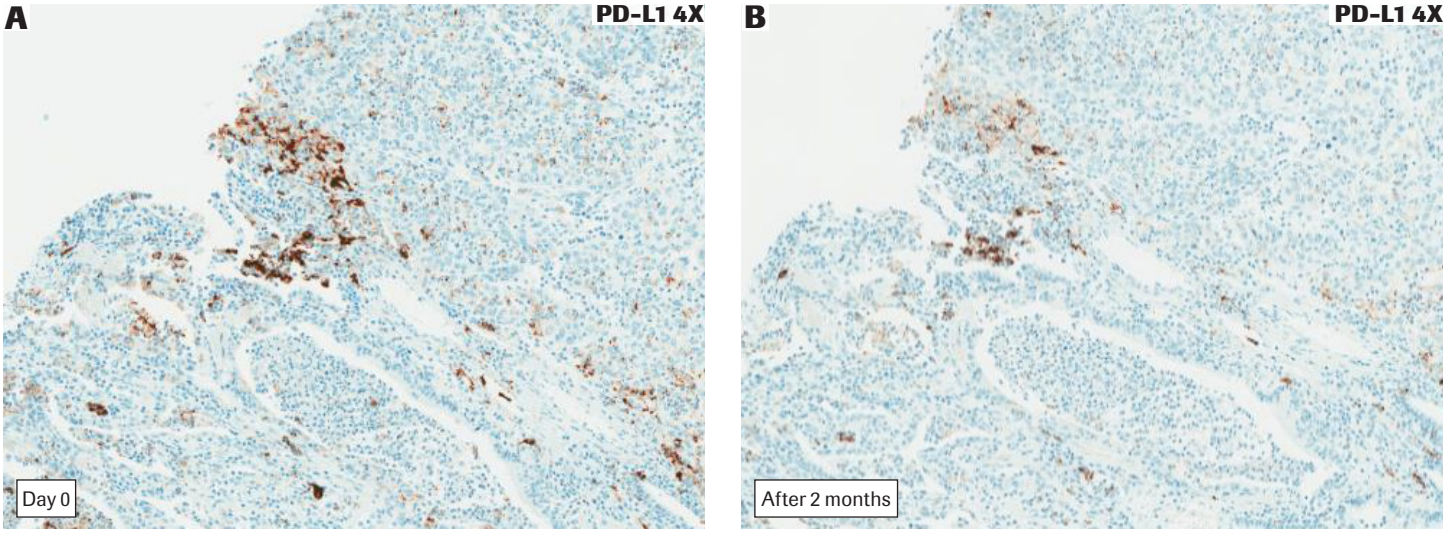


Figure 23: Serial sections of NSCLC tissue stained at Day 0 (**A**) and after two months storage at ambient temperature (**B**).

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

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