



PD-L1 Testing and Assessment: Practical Considerations for Oncologist and Pathologist

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Introduction

Recently, immunotherapy with anti-PD-1 (programmed cell death protein 1) or anti-PD-L1 (programmed cell death ligand 1) antibodies has shown both favorable and durable responses in a subset of patients with metastatic and advanced cancers. Although no robust predictive biomarker for immune checkpoint inhibitors (ICI) has been established till date, PD-L1 immunohistochemistry (IHC) testing has emerged with a passable utility. However, PD-L1 is still far from being a perfect biomarker. Nevertheless, PD-L1 testing by IHC to evaluate the immunoexpression of PD-L1 protein in tumor cells and/or immune cells is a useful predictive biomarker for predicting response to ICI.^{1–4}

Types of PD-L1 IHC Assays and Scoring

In oncology practice, the three most commonly used PD-L1 IHC assays, their respective PD-L1 antibodies, and associated IHC platforms are 22C3 (Dako), SP142 (Ventana), and SP263 (Ventana). A particular PD-L1 antibody clone and its associated platform have been approved by U.S. Food and Drug Administration (FDA) for respective ICI (PD-1 and PD-L1 inhibitor) intended for a particular malignancy type. Moreover, the approval also takes into account the type of cells expressing PD-L1, based on which the following three types of scoring (►Figs. 1–8) have been developed:

1. **Tumor Proportion Score (TPS):** It is scored as percentage of tumor cells showing distinct membranous staining. TPS is frequently utilized for metastatic non-small cell lung carcinoma (NSCLC). A potential misinterpretation can occur due to known membranous immunostaining of native pneumocytes or reactive histiocytes, which can be erroneously included in TPS (►Fig. 7). Hence, correlation with histomorphology is prudent for accurate scoring.

TPS (%) = $\frac{\text{PD-L1 positive tumor cells} \times 100}{\text{Total tumor cells (PD-L1 positive + PD-L1 negative tumor cells)}}$

2. **Immune Cells Staining (ICS):** It is scored as the percentage of tumor area that is occupied by PD-L1-stained immune cells of any intensity. ICS is commonly utilized for metastatic triple negative breast cancer and urothelial carcinoma. The scoring is done on immune cells only within tumor micro environment (►Fig. 3). Areas of necrosis and granulation tissue should not be considered or sampled for assessment.

3. **Combined Positive Score (CPS):** It is scored as number of PD-L1-stained cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. It is expressed in numbers and not in percentage, as it may exceed 100. CPS is frequently utilized for metastatic and recurrent head and neck squamous cell carcinoma as well as metastatic gastric/gastroesophageal adenocarcinoma (►Fig. 4).

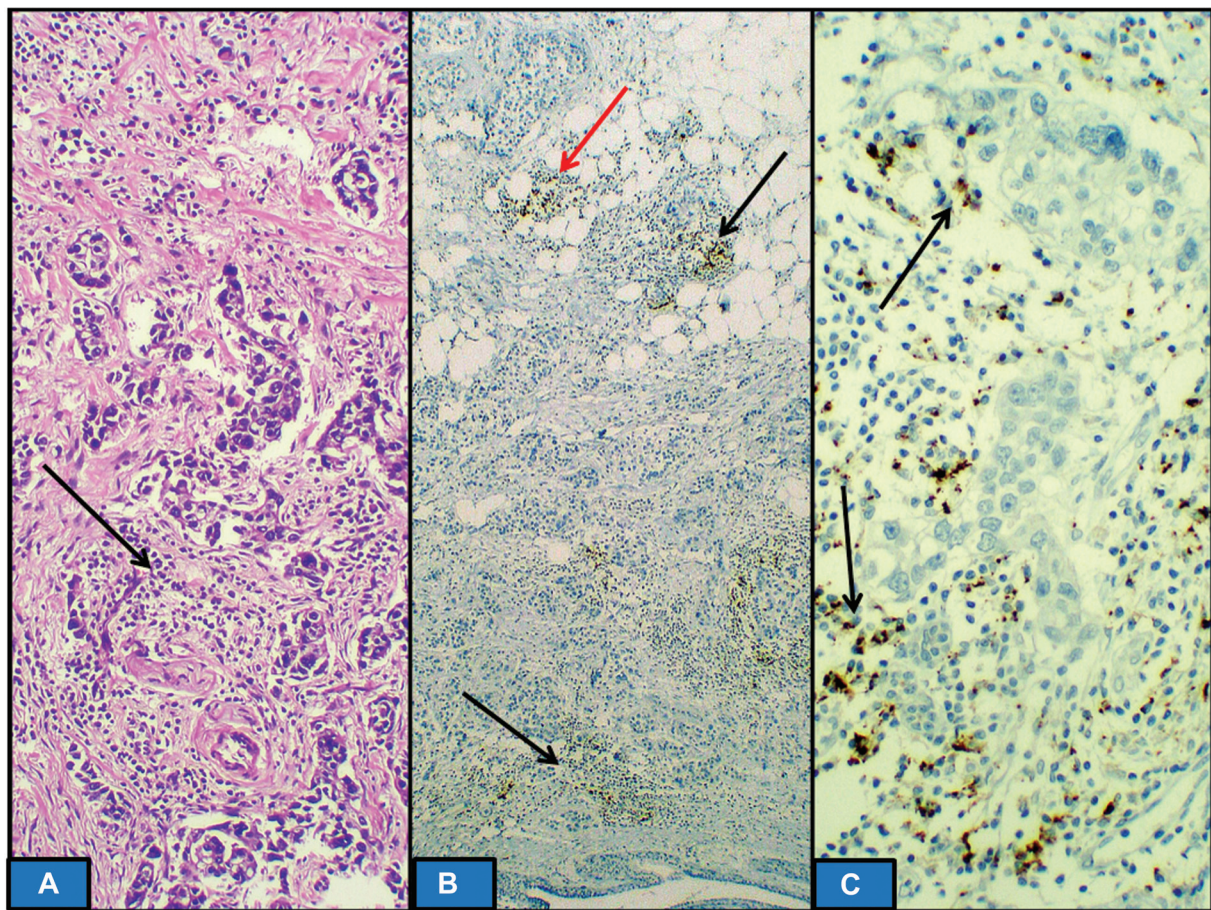


Fig. 1 (A) Hematoxylin and eosin sections showing metastatic grade III, triple negative breast cancer in axillary lymph node with immune cells in tumor microenvironment (TME) (black arrow). (B and C) Programmed cell death protein ligand 1 (PD-L1) (SP142) immunohistochemistry showing immune cells staining (ICS) score of 5%. The ICS in TME only should be taken into consideration. PD-L1 staining in immune cells away from TME (red arrow) not in contact with tumor cells is not to be considered.

CPS= PD-L1 immunostained cells (tumor cells, lymphocytes, macrophages) \times 100
Total viable tumor cells

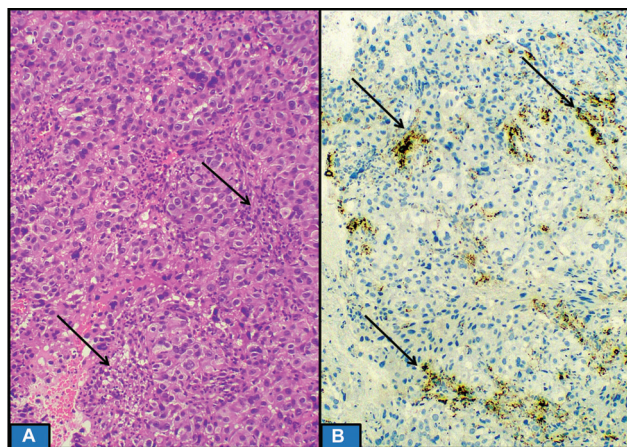


Fig. 2 (A) Hematoxylin and eosin sections showing primary breast invasive duct carcinoma, grade III, (triple negative breast cancer) with immune cells (TILs) in tumor microenvironment (arrow). (B) Programmed cell death protein ligand 1 (SP142) immunohistochemistry showing immune cells staining score of 20% (arrow). TILs: tumor infiltrating lymphocytes.

Clinical Setting for PD-L1 IHC Testing

1. **Companion Diagnostic Test:** It is a prerequisite or mandatory test that provides information for the effective and safe use of an intended therapeutic drug. The various companion diagnostic PD-L1 assays with details are listed in ►Table 1.
2. **Complementary Diagnostic Tests:** It is not a mandatory test before initiating the treatment with intended drug; however, it aids in the therapeutic decision. For example, Ventana SP142 PD-L1 assay is used as a complementary diagnostic test for intended treatment with Atezolizumab in previously treated NSCLC if TPS \geq 50% or IC score \geq 10%.

Laboratory Developed Tests (Interconvertibility of Assays)

FDA-approved/CE-marked PD-L1 assays are validated assays in clinical trials. Any assay/test other than these assays are known as laboratory developed tests (LDT), also known as “Fit for purpose” testing. This is advocated, as a single laboratory cannot establish multiple IHC platforms. LDTs are difficult to achieve as they require adequate validation

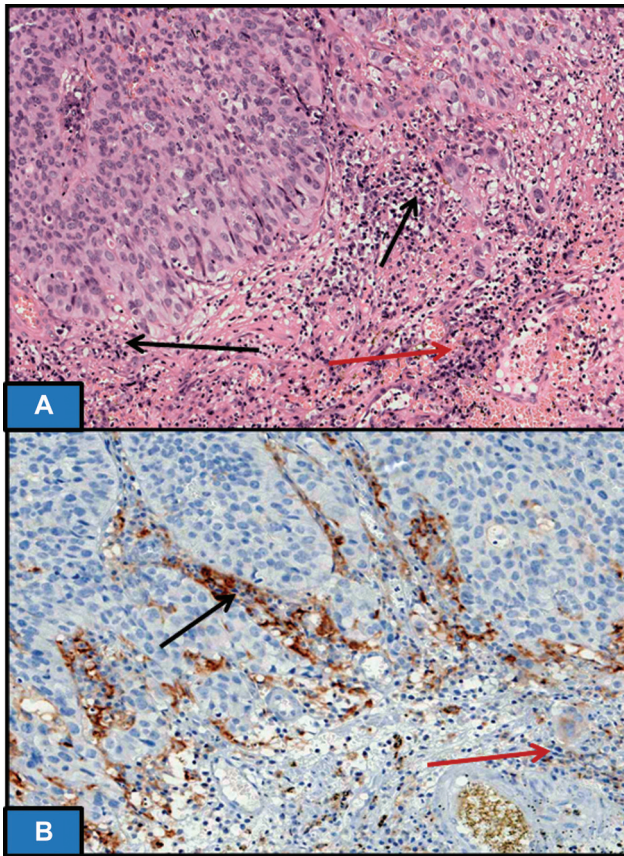


Fig. 3 (A) Hematoxylin and eosin section showing high-grade urothelial carcinoma of bladder with immune cells in tumor micro-environment (TME) (arrow). (B) Programmed cell death protein ligand 1 (SP142) immunohistochemistry showing immune cells staining score of 25% (black arrow). The immune cells away from TME (red arrow) will not be taken into consideration. No membranous staining seen in tumor cells.

against an appropriate standard. LDT is developed by the laboratory with FDA-approved tests and concordance of >90% is required as validation.^{1,2}

Specimen Type, Adequacy, and Factors Affecting Accuracy of Test

- The PDL-1 immunoexpression results vary spatiotemporally, and hence the most recent tumor specimen, whenever available and feasible, may be utilized for testing for patient selection.
- The tissue should be fixed in 10% neutral buffered formalin for optimal results.
- The storage time for paraffin blocks used for testing should preferably be less than 3 years.
- There should be no diffidence in using cell blocks for the evaluation of TPS in NSCLC, as several cases of NSCLC are diagnosed on malignant pleural effusion. Minimum 100 viable tumor cells are required for TPS evaluation in both cell block and tru-cut biopsy. Blueprint study (Phase 2b)

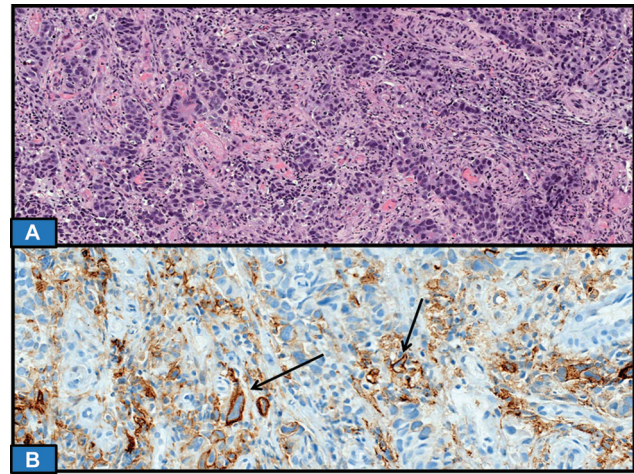


Fig. 4 (A) Hematoxylin and eosin section showing poorly differentiated adenocarcinoma of stomach. (B) Programmed cell death protein ligand 1 (PD-L1) immunohistochemistry showing CPS of 40. The high score is attributed to immune cells displaying PD-L1 staining, while very few tumor cells show membranous positivity (arrow).

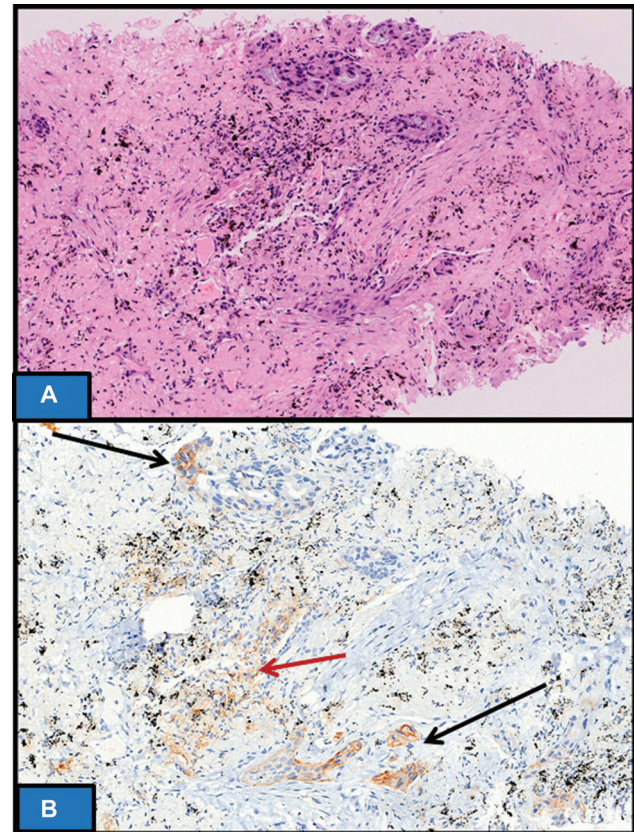


Fig. 5 (A) Hematoxylin and eosin section showing non-small cell lung carcinoma in a tru-cut biopsy. (B) Distinct membranous programmed cell death protein ligand 1 immunoexpression (tumor proportion score) in 5% of tumor cells of weak intensity (black arrow). Immune cells staining (red arrow) will not be taken into consideration.

has proven the harmonization of TPS in tru-cut biopsy, cell blocks, and resection specimens for NSCLC. However, cell blocks are not suitable for ICS and CPS evaluation.⁵

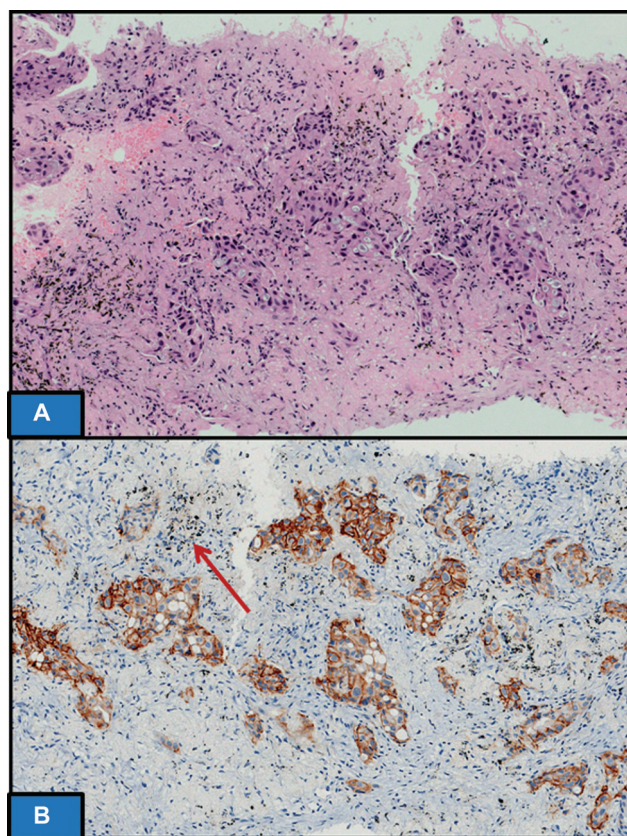


Fig. 6 (A) Hematoxylin and eosin section showing non-small cell lung carcinoma in a tru-cut biopsy. (B) Distinct membranous programmed cell death protein ligand 1 immunoreexpression (tumor proportion score) in 90% of tumor cells of moderate to strong intensity. Immune cells staining (red arrow) will not be taken into consideration.

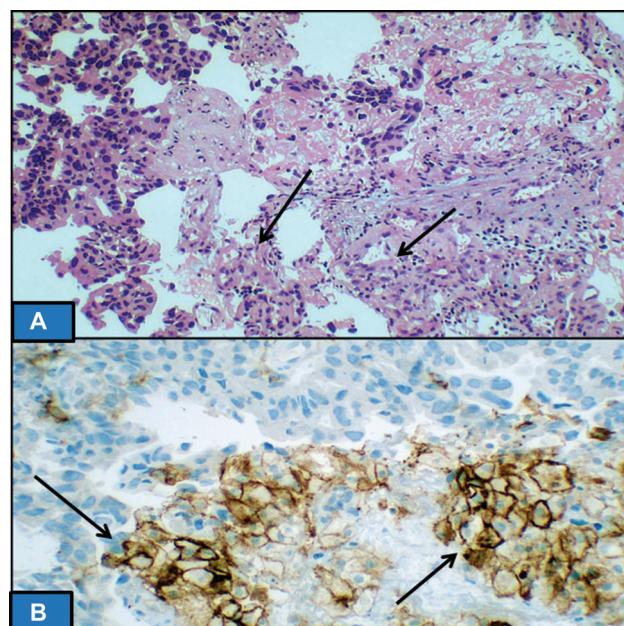


Fig. 7 (A) Hematoxylin and eosin section showing tru-cut biopsy with non-small cell lung carcinoma with adjoining areas of histiocytes (arrow). (B) Distinct membranous programmed cell death protein ligand 1 immunoreexpression in histiocytes that can be erroneously taken as positive tumor cell staining. Note that the size of nucleus of histiocytes is significantly smaller than the tumor nucleus (upper half unstained), and this serves as a clue to differentiate between the two.

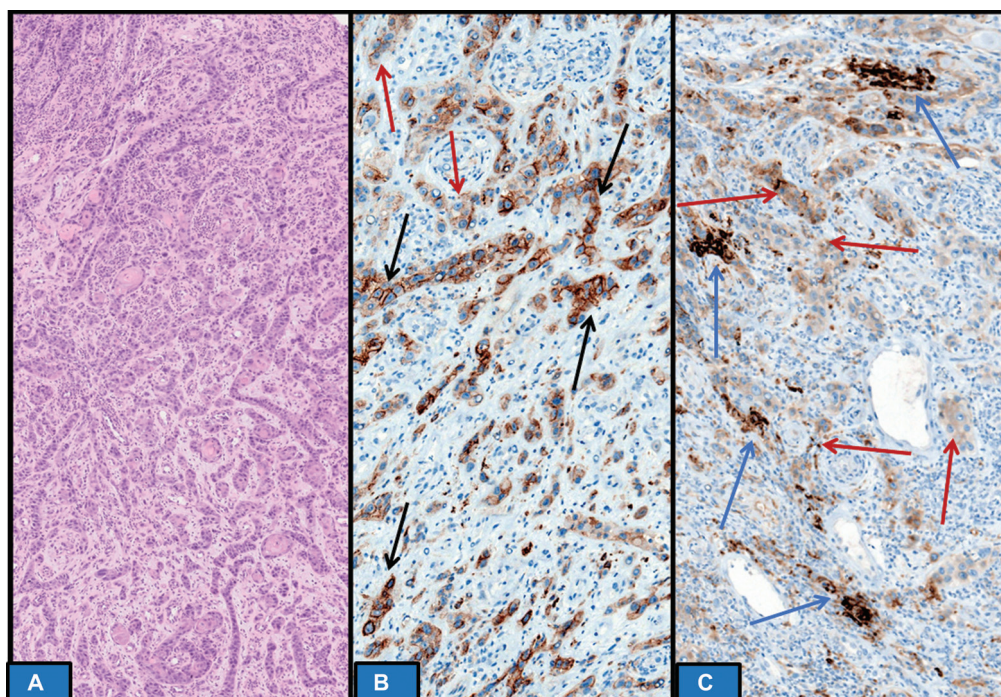


Fig. 8 (A) Hematoxylin and eosin section showing recurrent locally advanced head and neck squamous cell carcinoma. (B and C) Programmed cell death protein ligand 1 immunoreexpression is heterogenous. Few areas show partial and complete distinct membranous staining (black arrow) in tumor cells to be included in scoring. Significant tumor cells show cytoplasmic staining without membranous accentuation (red arrow), which is not to be counted in scoring. Immune cells staining is very less (blue arrow); however, it will be included in scoring. The final CPS was 25.

Table 1 List of FDA/CE marked approved companion of diagnostic PD-L1 assay for intended use of ICI in various malignancies and their respective immunoexpression with their cutoff threshold

Type of malignancy and affected organ	Intended ICI and line of therapy	Companion of diagnostic PD-L1 assay	Type of scoring for PD-L1 immunoexpression	Approving agency
NSCLC (metastatic/UR stage III, NE for definite CT/RT)	Pembrolizumab (anti-PD-1) 1st line monotherapy	22C3 (Dako)	TPS; $\geq 50\%$	FDA
NSCLC (metastatic/UR stage III, NE for definite CT/RT)	Pembrolizumab (anti-PD-1) 1st line monotherapy	SP263 (Ventana)	TPS; $\geq 50\%$	CE marked
NSCLC (Metastatic)	Pembrolizumab (anti-PD-1) 2nd line monotherapy	22C3 (Dako)	TPS; $\geq 01\%$	FDA
Urothelial carcinoma (LA/metastatic NE for CT)	Atezolizumab 1st line monotherapy	SP142 (Ventana)	ICS; $\geq 05\%$	FDA
Urothelial carcinoma (LA/metastatic NE for CT)	Pembrolizumab 1st line monotherapy	22C3 (Dako)	CPS; ≥ 10	FDA
TNBC (recurrent LA/metastatic)	Atezolizumab 1st line, in combination with nab-paclitaxel	SP142 (Ventana)	ICS; $\geq 01\%$	FDA
TNBC (recurrent LA/metastatic)	Pembrolizumab (anti-PD-1) 1st line, in combination with nab-paclitaxel	22C3 (Dako)	CPS; ≥ 10	FDA
Gastric/GEJ adenocarcinoma (recurrent LA/metastatic)	Pembrolizumab (anti-PD-1) 3rd line monotherapy	22C3 (Dako)	CPS; ≥ 01	FDA
Cervical carcinoma (recurrent LA/metastatic)	Pembrolizumab (anti-PD-1) 2nd line monotherapy	22C3 (Dako)	CPS; ≥ 01	FDA
Esophagus SCC (recurrent LA/metastatic)	Pembrolizumab (anti-PD-1) 2nd line /3rd line monotherapy	22C3 (Dako)	CPS; ≥ 10	FDA
HNSCC (metastatic/recurrent/UR)	Pembrolizumab (anti-PD-1) 1st line monotherapy	22C3 (Dako)	CPS; ≥ 01	FDA

Abbreviations: CE, European Conformity; CT, chemotherapy; CPS, combined positive score; FDA, U.S. Food and Drug Administration; HNSCC, head and neck SCC; ICI, immune checkpoint inhibitor; LA, locally advanced; NE, not eligible; NSCLC, nonsmall cell lung carcinoma; PD-L1, programmed cell death protein ligand 1; RT, radiation therapy; SCC, squamous cell carcinoma; TNBC, triple-negative breast cancer; UR, unresectable.

- Decalcified tissues (bone metastatic site) are also not recommended for PD-L1 evaluation.
- Satisfactory positive and negative PD-L1 controls should be taken on the same slide before interpretation of test (► Fig. 9).
- Regular participation in External Quality Assurance Scheme and Proficiency testing ensures accuracy and reproducibility of test results.^{1,5}

Limitations of PD-L1 Testing and Future Perspectives

Although PD-L1 testing remains the most common predictive biomarker in current oncology practice, it is still

an imperfect biomarker as some patients who are PD-L1 negative may still respond to ICI while those who are positive may not respond to ICI. The other challenge is intra- and intertumoral heterogeneity for PD-L1 immunoexpression that has implications in scoring and PD-L1 results. Moreover, with recent strategies to combine ICI with chemotherapy, it may further limit the precise significance of predictive utility of PD-L1 testing. A close collaboration between oncologist and pathologist is essential and further prospective large randomized trials are required to establish the precise role of biomarkers, especially PD-L1 for predicting response to ICI.^{3,4}

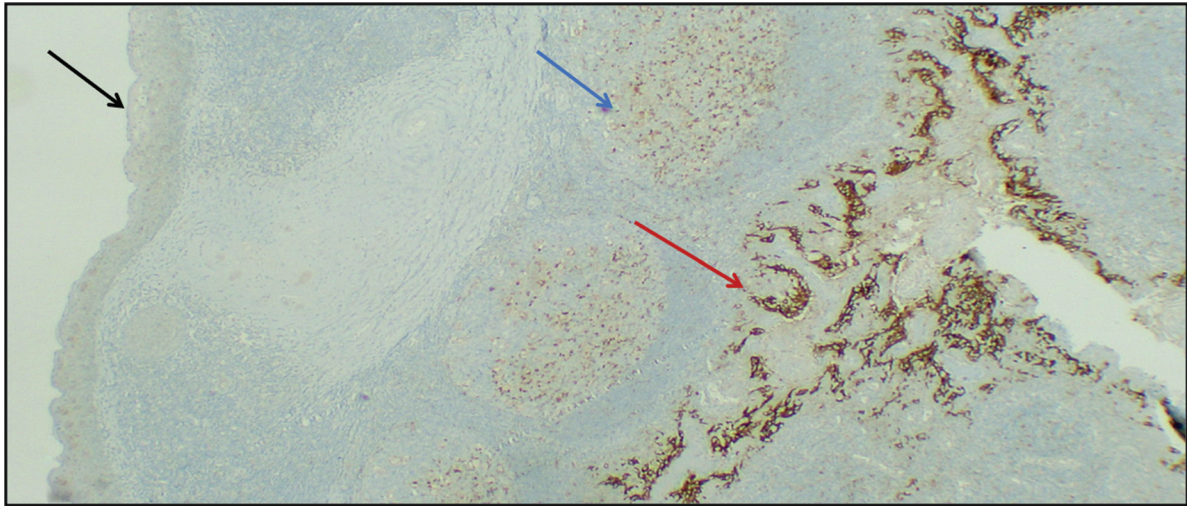


Fig. 9 On-slide positive and negative control (tonsillar tissue). The cryptic epithelium showing strong membranous positivity (red arrow), the surface epithelium is negative (black arrow), and the germinal centers show granular staining of moderate intensity (blue arrow).

Source of Support

None declared.

Conflict of Interest

None declared.

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