Review Article

PD-L1 assessment in urothelial carcinoma: a practical approach

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Abstract: Five programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors are currently approved for treatment of locally advanced or metastatic urothelial carcinoma of the bladder and the upper urinary tract. Due to restrictions by the FDA and EMA first-line treatment with Atezolizumab and Pembrolizumab in platinum-ineligible patients requires immunohistochemical PD-L1 testing. In the second-line setting all drugs are approved without PD-L1 testing. Used PD-L1 assays in clinical trials include the 28-8 pharmDx (Nivolumab), the 22C3 pharmDx (Pembrolizumab), Ventana SP142 (Atezolizumab), and the Ventana PD-L1 SP263 assays (Durvalumab). Differences in antibodies, needed platforms and testing algorithms have raised questions about interchangeability and comparability among these assays and their diagnostic use. We provide a practical review about the current recommendations, used assays and algorithms of PD-L1 testing in urothelial carcinoma to help oncologists, urologists and pathologists to understand analytical features, differences in antibody assays, differences in scoring algorithms and comparability of various PD-L1 assays. We reviewed and summarized published studies from the last four years (2016–2019) on PD-L1 testing in bladder cancer and present a condensed practical guideline including pre-analytical, analytical and test-specific issues.

Keywords: Bladder cancer; immunotherapy; programmed death-ligand 1 (PD-L1); PD-L1 testing; immunohistochemistry

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Introduction

Inhibitory checkpoint proteins such as programmed death-1 (PD-1), programmed death ligand-1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) can suppress anti-tumour T-cell responses (1,2). Enhancement of these checkpoint proteins is a common immune-evasive strategy of several solid tumours such as non-small cell lung cancer (NSCLC), malignant melanoma or urothelial carcinoma (UC) (1-3). Immune checkpoint blockade (ICB) drugs interfere with these tumour-related immune-evasive strategies, and several immunotherapeutic drugs targeting PD-1 and PD-L1 are emerging in the treatment of UC (4-11). Some of these drugs have now been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of advanced/metastatic UC (12-14).

Although PD-L1 testing was not prescribed in UC, one

year ago the FDA and EMA restricted the first-line use of the anti-PD-1/PD-L1 drugs Keytruda (Pembrolizumab) and Tecentriq (Atezolizumab) in cisplatin ineligible patients based on still unpublished outcome data of the ongoing phase three trials (15). Both drugs are only indicated as monotherapy in adult patients with locally advanced or metastatic UC who are not eligible for cisplatin containing chemotherapy and whose tumours are PD-L1 positive, as assessed by immunohistochemistry (IHC) (15). Due to this restriction, PD-L1 IHC testing is now required in selected UC patient populations.

The parameters for PD-L1 testing are very complex, which makes it a tempting task for pathology labs to deliver reliable test results. Major biological issues to overcome are significant intra-tumor heterogeneity and therapy-induced changes in expression (16). The main technical obstacles to overcome lie in the different FDA/EMA approved diagnostic PD-L1 antibody assays and the subsequent specific scoring algorithm which is unique for each companion diagnostic assay.

The aim of the contribution is to provide a detailed overview about approved complementary/companion in-vitro diagnostics (CIVDs) and scoring algorithms (with special emphasis on the currently needed cut-off systems in first-line therapy). Furthermore, we want to provide pathologists and clinicians with practical guidelines on how to report and interpret PD-L1 assessment results in UC. In December 2018, a group of experts was brought together in Milan to discuss current knowledge about PD-L1 testing in UC and to assess the research that is still required as a priority, in order for reliable and accurate testing to be ensured. This paper is based on the discussions and conclusions of that meeting.

Pre-analytics

Test request form

Each ICB drug is approved in conjunction with a specific staining assay (e.g., Atezolizumab/Tecentriq – Ventana SP142 assay) and scoring algorithm. According to the current recommendations and requirements of the FDA and EMA, PD-L1 assessment should be carried out with the specifically approved PD-L1 assay ("companion diagnostics"). This means that PD-L1 testing for a specific ICB (e.g., Atezolizumab) has to be carried out with its specific PD-L1 assay, in the case of Atezolizumab with the Ventana SP142 assay (15).

Therefore the type of test request form is crucial since it has major organizational, technical and financial implications for the pathology laboratories. There are two major options for the clinician to request for PD-L1 testing in UC: a general testing for eligibility for all approved drugs or a specific PD-L1 testing for a specific ICB drug. With a generic request form the clinician asks for a PD-L1 test without specification of the intended ICB drug, while a specific request form goes with specification of the ICB drug. Since all PD-1/PD-L1 targeting agents are considered to be similarly efficient, it lays in the oncologists/urologists choice which drug is intended to be applied. In our opinion a specific request form is preferable since it enables the labs to provide optimal testing conditions. If the clinician gives no specification we would encourage the diagnosing pathologist to proof the eligibility for both biomarker-restricted ICBs. Therefore a fluid communication between clinicians and pathologists is vital to deliver a fast and high-quality test result which matches the clinician's request.

Sample-type

Since UC is a frequently recurrent and steadily progressive disease, pathologists will often have multiple tissue samples per patient available. The most frequent sample type is the (often multiple) trans-urethral resection of the bladder (TURB), and others including cystectomy-specimens, lymph nodes and metastatic tissues. In an ideal world PD-L1 IHC could be performed on multiple specimens per patient, but in general the pathologist will need to select one specific tissue sample.

Regarding sample selection criteria, the access data of the FDA approved anti PD-L1 antibody clones for UC (Dako 22C3, Ventana SP263 and Ventana SP142) are conflicting and often vague regarding to sample selection criteria: it is only clearly stated that samples or smears as well as samples of bone metastases which have been decalcified should not be used due to a lack of validation. Since anti-PD-L1 IHC is only indicated in patients with locally advanced or metastatic UC, it is common sense to select a tissue sample with at least invasive UC. If no tissue samples with invasive UC are available, the pathologist should ask the clinician for a fresh biopsy with invasive UC—ideally from a metastatic site—to ensure a reliable PD-L1 assessment. When multiple tissue samples with invasive UC are available, we propose a pragmatic approach, with selection of the latest available tissue block with a sufficient amount of invasive UC (at least 100 tumor cells) and the

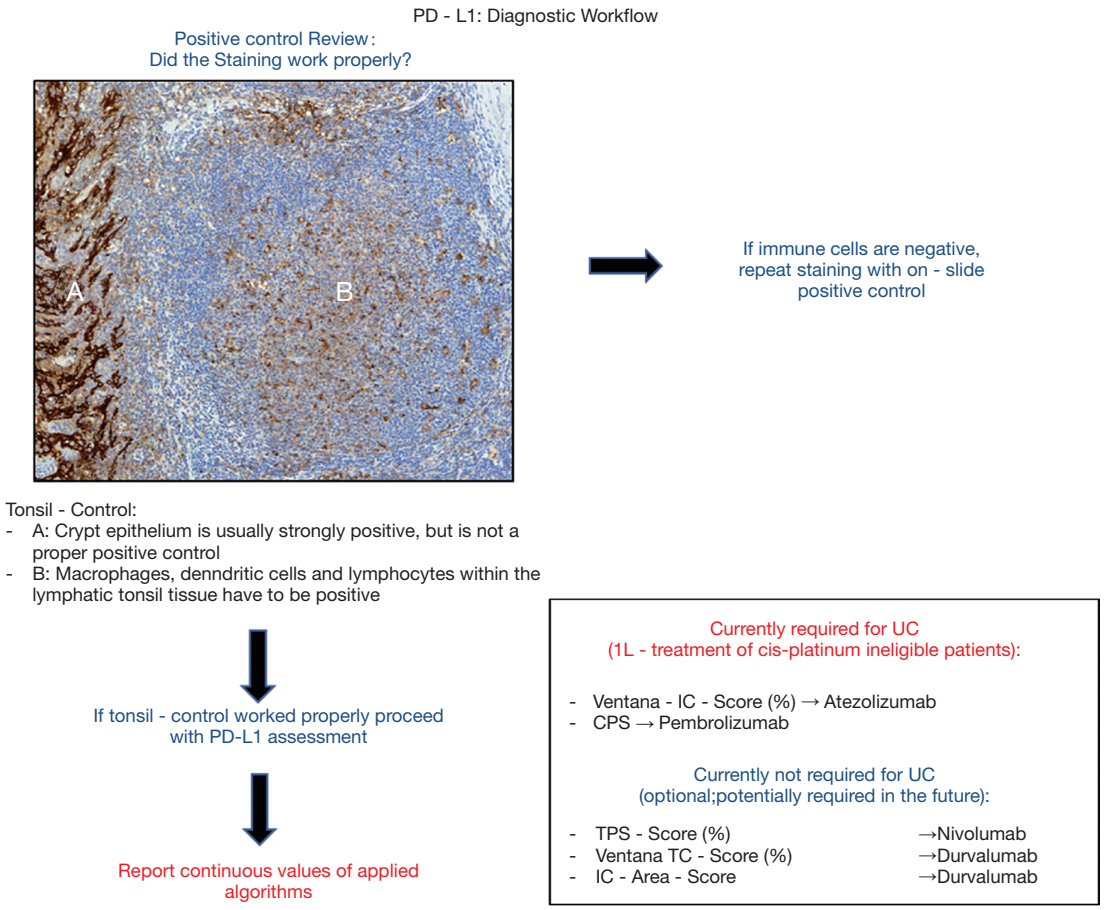

Figure 1 PD-L1 assessment—potential workflow.

lowest amount of cauterization artifacts or necrotic areas. To overcome heterogeneity effects PD-L1 staining in multiple samples (e.g., two or more blocks of the same TURB) could be useful in selected cases, although recent data have shown rather high concordance rates between smaller biopsy samples (TURB) and corresponding cystectomy specimens (17,18). We further recommend using biopsies of metastatic settlements if available, since recent data showed huge discrepancies of the PD-L1 status between primary UC and corresponding liver metastasis (known to respond the worst of all metastatic settlements to checkpoint inhibition) (19). Sample selection in patients who received neo-adjuvant chemo- or immunotherapy should be done with particular attention, since it is known that such treatments can alter PD-L1 expression (16,17,20). We therefore recommend not to use specimens which have been obtained immediately after chemotherapy or immunotherapy (e.g., cystectomy specimens of patients who underwent neoadjuvant chemotherapy).

Other pre-analytical considerations

To ensure the reliability of the test results it is essential to pay attention to pre-analytical considerations, including fixation and sample processing. Tissue for PD-L1 testing does not require any special preparation. The key pre-analytical steps are similar to those for other immunohistochemical and molecular tests, and include sample tracking, adequate and rapid fixation, tissue processing, sectioning, and tissue prioritization.

Beside adequate fixation in neutral buffered formalin it is highly recommended to generate fresh tissue cuts for PD-L1 testing due to known storage damage effects with subsequent loss of antigenicity. We further strongly recommend the use of on-slide positive controls (*Figure 1*). As on-slide positive control we recommend the usage of tonsil tissue

Table 1 US Food and Drug Administration and European Medical Agency approved PD-L1 assays

Diagnostic assay	Staining platform	Staining characteristics	Approved assay for
Ventana SP142	Ventana	• Dot-/ant-like staining pattern • Low tumor cell staining • Developed for immune cell scoring	Atezolizumab [Tecentriq©]
Ventana SP263	Ventana	• Homogenous tumor cell staining • Homogenous tumor cell staining • Mostly strong staining intensity	Durvalumab [Imfinzi©]
Dako 22c3	Dako Link 48*	• Homogenous tumor cell staining • Homogenous tumor cell staining • Mostly weak staining intensity	Pembrolizumab [Keytruda©]
Dako 28-8	Dako Link 48*	• Homogenous tumor cell staining • Homogenous tumor cell staining • Moderate-strong staining intensity	Nivolumab [Opdivo©]

*Currently exclusively approved for the Dako Link 48 platform—approval process for Omnis-platform ongoing.

with an adequate amount of lymphatic tissue since the crypt epithelium shows strong but only unspecific PD-L1 staining (*Figure 1*). Macrophages, dendritic cells and lymphocytes within the lymphatic tonsil tissue should exhibit an intermediate to strong cytoplasmic or membranous staining to be considered as positive (*Figure 1*). Due to only few or absent immune cells placental tissue is not recommended as positive control. We further recommend to run a negative control for each tested tissue sample to prevent misinterpretation due to unspecific staining artifacts. We refer to the excellent work by Cree *et al.* on PD-L1 testing in NSCLC for more detailed generic pre-analytical considerations (20,21).

Analytics

Assays

Although we would still encourage the use of closed-assays at this stage [as required by the FDA and recommended by the EMA (22)], the implementation of LDT's is likely to be already common practice in a substantial amount of pathology laboratories. Recent studies indicated that several assays (Dako 22c3, Dako 28-8, Ventana SP263) could be used interchangeably in multiple indications with the exception of the Ventana SP142 assay which has been shown to detect significantly less tumor cells than the other assays (18,23-29). For (partially) tumor cell-based PD-L1 assessment algorithms like the combined positive score (CPS) we do not recommend the use of the Ventana SP142 assay (*Tables 1*,*2*). If LDTs are utilized, we recommend that these assays are carefully validated with internal and external controls in accordance with the FDA and EMA approved PD-L1 CVIDs. Furthermore, we recommend steadily participation in ring trials to validate the own LDT performance in comparison to other institutions and a reference standard.

Beside differences in the detection of tumor cells, the currently approved PD-L1 assays differ also in staining characteristics. While both Ventana assays (SP263, SP142) show a very strong staining intensity, especially the Dako 22c3 often presents with a very weak staining intensity (*Figure 2A*). The SP142 shows a typical "ant-/dot-like" staining pattern (*Figure 2A*). Tumor heterogeneity (*Figure 2B*) can be a further challenge which should be taken into consideration especially in small biopsies or TURB specimens.

Scoring algorithms and cut-off systems

Although different PD-L1 antibody assays have been approved for each specific ICB drug, the main difference between PD-L1 CVIDs lays primarily in their different scoring algorithms. While the algorithm for Atezolizumab [Ventana IC-Score (developed with SP142); *Figure 3A*]

Table 2 Scoring algorithms, cut-off systems and pit-falls

IO-Drug	Approved PD-L1 assay	Algorithm based on	Pitfalls
Atezolizumab (Tecentriq©)	Ventana SP142	Ventana IC-algorithm	Plasma cells have to be excluded from scoring
		Currently necessary for first-line therapy stratification for Atezolizumab: → Cut-off: 5%-IC	All immune cells are included (incl. neutrophil granulocytes)
Durvalumab (Imfinzi©)	Ventana SP263	Ventana IC-Area-algorithm; Ventana TC-algorithm	Immune cell positivity is scored according to the area occupied by all immune cells (IC-"Area"-score)
		→ Cut-offs: 25% IC or/and 25% TC	No combined positive score (!): Patients are positive when exceeding one of the two cut-offs or both
		This algorithm is currently not prescribed and only explored in ongoing clinical trials	Plasma cells have to be excluded from scoring
			All immune cells are included (incl. neutrophil granulocytes)
Pembrolizumab (Keytruda©)	Dako 22c3	Combined positive score (CPS)	Combined positive score including immune cells and tumor cells
		Currently necessary for first-line therapy stratification for Atezolizumab: → Cut-off: CPS 10	Plasma cells have to be excluded from scoring
			Neutrophil granulocytes not included
Nivolumab (Opdivo©)	Dako 28-8	Tumor proportion score (TPS)	–
		→ Cut-offs: 5%-TC	
		This algorithm is currently not prescribed and only explored in ongoing clinical trials	

is supposed to refer to the area of positive immune cells covering the entire tumor area including the desmoplastic tumor stroma and the tumor cell area, CPS (developed with 22c3) focusses on the total amount of PD-L1 positive immune cells (IC) and tumor cells (TC) in proportion to the total number of TC (*Figure 3B*). The tumor cell score (TC-Score; *Figure 3C*) has been explored in UC Checkmate-trials but is currently not used for Nivolumab (*Figure 3C*). However, the TC-Score is also part of the "Durvalumab"-algorithm which differs greatly from the Ventana IC-score or the CPS: the "Durvalumab"-algorithm bases on separate scoring of tumor cells (TC-Score; *Figure 3C*) and immune cells (IC-Area-Score; *Figure 3D*). Especially the immune cell scoring of the "Durvalumab"-algorithm is different: while the "Atezolizumab"-algorithm bases on positive IC scoring per tumor area (Ventana-IC-score), the "Durvalumab"-IC-algorithm bases on the area occupied by positive immune cells in proportion to the total area occupied by all present tumor associated immune cells (IC-Area-Score; *Figure 3D*). In cases with very low overall count of immune cells, the positive cut-off can only be exceeded if all immune cells are positive. Furthermore, it is important to note that the "Durvalumab"-algorithm is not a combined positive score like the CPS: Tumor samples are supposed to be positive if one of the both or both cut-offs are exceeded while it is not possible to exceed the cut-off through a combination of the IC and TC scoring values.

In addition to the different scoring algorithms, each algorithm bases on different positivity cut-offs (depicted in *Table 2*). A recent study demonstrated that this inter-algorithm variability can lead to critical scenarios where the same patient could receive e.g., first-line treatment with Atezolizumab due to exceeding the 5%-IC cut-off but not Pembrolizumab due to an insufficient amount of additional TC/IC to exceed the CPS10 cut-off (23). Discordant classifications between the Atezolizumab and Pembrolizumab algorithm occurred in approximately 42% of patients which were positive for at least one algorithm (23). Such discordances could further increase if PD-L1 testing will become obligate for treatment with

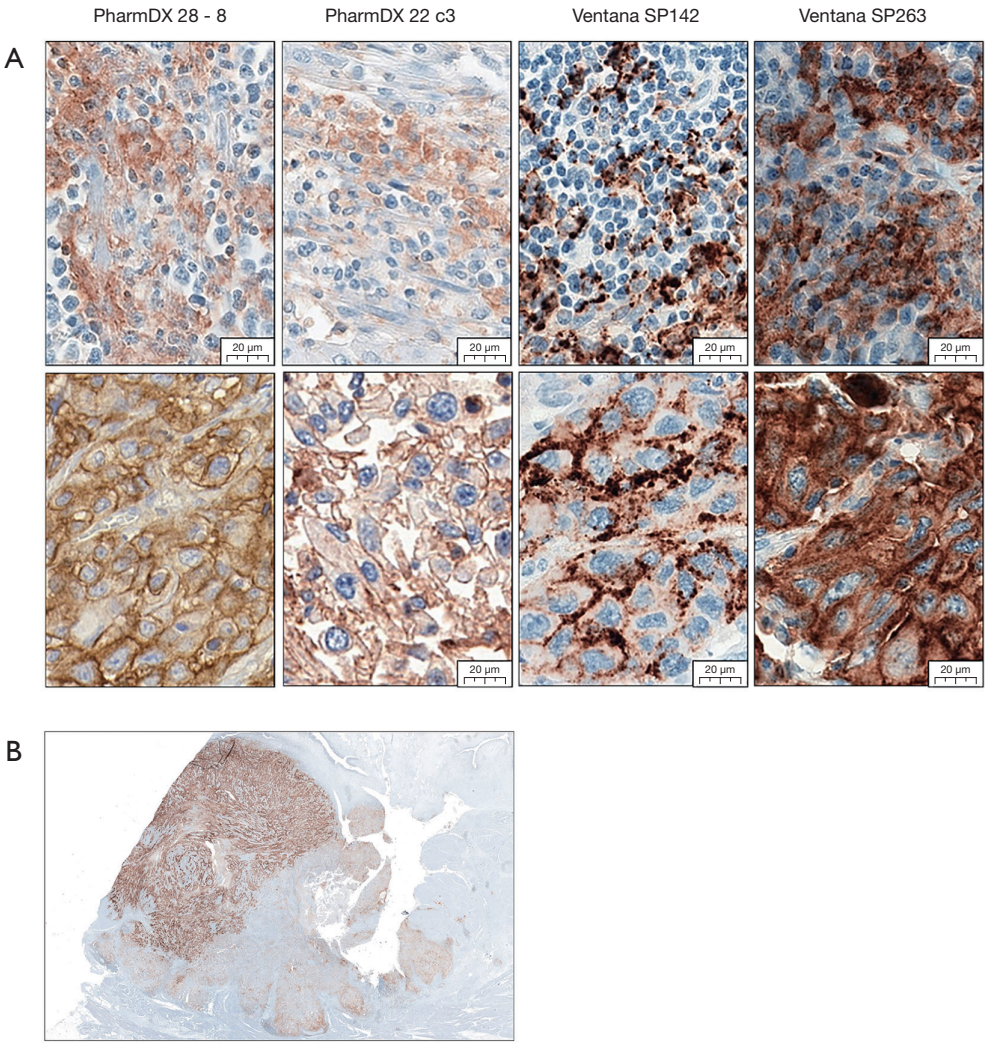

Figure 2 PD-L1 assays. (A) Staining characteristics of different Food and Drug Administration and European Medical Agency approved companion diagnostic PD-L1 assays in muscle-invasive bladder cancer (Magnification: 400×). (B) Illustration of heterogeneous PD-L1 expression on tumor and immune cells in a case of muscle-invasive bladder cancer. The staining was performed with the Ventana SP263 assay (Magnification: 20×).

Durvalumab. Therefore, we encourage PD-L1 scoring in UC according to all currently needed scoring algorithms (such as Ventana-IC-Score and CPS).

Post-analytics (reporting of the results)

It is preferable that PD-L1 IHC results are part of an integrated report, including histopathological diagnosis, the results of IHC to type the tumor and molecular data (if available). Essential parameters of a PD-L1 IHC test result include the anti PD-L1 antibody clone used, the type of IHC platform, the scoring algorithm and the final PD-L1 score. The use of an LDT should be explicitly mentioned in the report (*Table 3*).

PD-L1 scoring results should be reported for all relevant scoring algorithms in accordance with the clinician's request. We suggest to report the absolute scoring values of each applied scoring algorithm since a simple positive/negative report does not reflect the rapid changes in immune oncology. To facilitate the interpretation of the report we encourage all pathologists to report whether the patient is eligible or ineligible for checkpoint inhibition

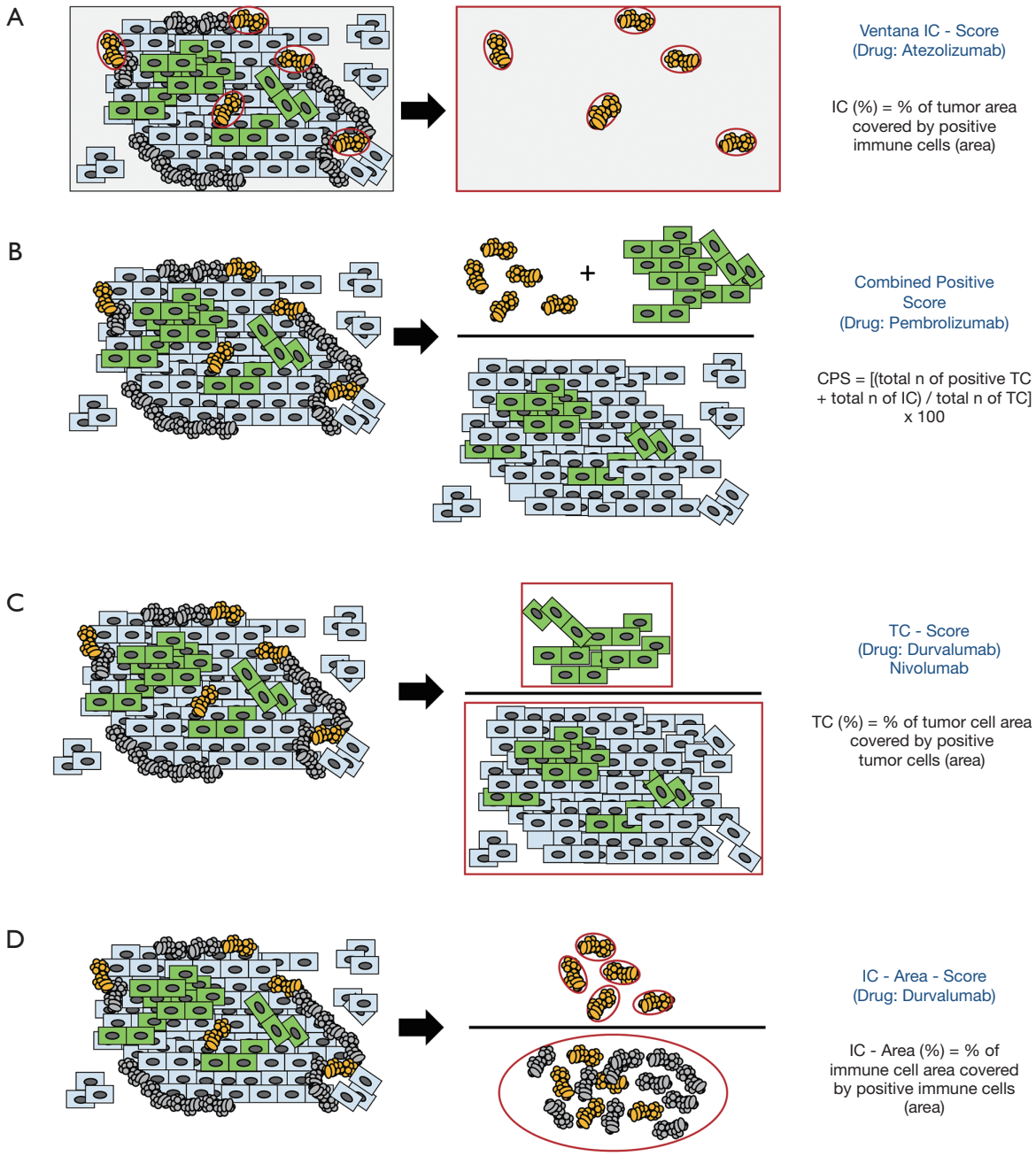

Figure 3 Illustrations of PD-L1 scoring algorithms applied in UC and other cancer types. (A) Ventana immune cell score (IC-Score; %): This score is required to assess first line treatment eligibility with Atezolizumab of platinum-based chemotherapy ineligible patients with metastasized or locally advanced urothelial carcinomas of the bladder and upper urinary tract. Patients are eligible for first-line Atezolizumab treatment if the cut-off of 5%-IC is exceeded. (B) Combined Positive Score (CPS): This score is required to assess first line treatment eligibility with Pembrolizumab of platinum-based chemotherapy ineligible patients with metastasized or locally advanced urothelial carcinomas of the bladder and upper urinary tract. Patients are eligible for first-line Pembrolizumab treatment if the cut-off of 10 is exceeded. Cave: The CPS is capped at 100 (although it could theoretically reach values above 100) and has no dimension. (C) Tumor cell score (TC-Score; %): This score is currently not required for PD-L1 assessment of urothelial carcinomas but it has been explored within the IMvigor trials (Atezolizumab). Furthermore, this score is currently under exploration in the Durvalumab trials. (D) IC-area score (%): This score is currently not required for PD-L1 assessment in urothelial carcinomas, but is currently explored in Durvalumab trials.

Table 3 Example of a standardized PD-L1 test result

PD-L1 IHC reporting template
PD-L1 IHC parameters:
(I) Antibody Clone: e.g., Dako 22C3
(II) Staining Platform: e.g., Dako Omnis
(III) LDT: No (closed assay)/Yes (please specify)
PD-L1 IHC score:
(I) Algorithm: e.g., Combined Positivity Score (CPS)
(II) Exact score: e.g., [#PD-L1 staining cells (tumor cells, lymphocytes, macrophages)/Total # viable tumor cells] ×100
(III) According to the derived combined positive score, the above referenced patient is ELIGIBLE/NOT ELIGIBLE (CPS ≥/<10) for first-line treatment with Pembrolizumab in case of cis-platinum ineligibility

based on the current approvals by the FDA and EMA.

As mentioned above, we encourage pathologic reporting of PD-L1 scoring in UC according to all currently needed scoring algorithms (such as Ventana-IC-Score and CPS). In case of discordant scoring results between different algorithms (with a respective below and above cut-off result), we advise to perform a follow-up test with the appropriate anti-PD-L1 clone in a closed assay setting (companion diagnostic assay/CVIVD), to confirm the positive result. In such cases an explanatory note should guide the clinician towards the possibility of applying an alternative ICB-drug which has not be intended initially (e.g., pembrolizumab instead of atezolizumab or vice versa).

It is up to each pathology lab to compose the integrated report with the PD-L1 IHC results, although several studies have shown that standardised structured reporting (SSR) using agreed published datasets significantly improves the quality of individual pathology reports (30,31).

Conclusions and future directions

Many aspects of PD-L1 IHC for advanced UC remain unclear or unfinished and should be refined. Prospective harmonization studies should provide further insight in the exchangeability of diagnostic PD-L1 antibody clones, algorithms and assays. Well-designed concordance studies are required to validate the implementation of LDT's for PD-L1 IHC. More specific data on tumor heterogeneity, cut-off values and interplay between immune and tumor cell IHC are needed to guide the pathologists towards optimal scoring.

It is becoming obvious that the predictive utility of PD-L1 alone for ICB in advanced UC might be insufficient (32). The combination of PD-L1 with other new biomarkers like tumor mutational burden (TMB) or immune cell infiltration will be required for an optimal personalized patient selection. Meanwhile, pathologists should focus on well validated PD-L1 IHC assays and appropriate reporting of the PD-L1 assessment.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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