



## Hot Topic

## The evolving role of PD-L1 testing in patients with metastatic urothelial carcinoma

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## ABSTRACT

Immune checkpoint inhibitors targeting the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway improve clinical outcomes in patients with locally advanced/metastatic urothelial carcinoma (UC). PD-L1 complementary or companion diagnostic assays are now available for anti-PD-1 and anti-PD-L1 antibodies and these assays enable testing at diagnosis. The role of PD-L1 testing in UC is, however, the subject of much discussion within the medical community, particularly in light of recent restrictions on recruitment of PD-L1-low patients in clinical trials of atezolizumab and pembrolizumab as first-line therapy, and the European Medicines Agency and US Food and Drug Administration limiting use of these agents as first-line therapy in cisplatin-ineligible patients to those with high PD-L1 expression. We explore the evolving evidence for PD-L1 expression testing in UC and the role of PD-L1 expression in both tumor cells and tumor-infiltrating immune cells. We review clinical data on the prognostic and predictive value of PD-L1 expression in response to anti-PD-1/PD-L1 agents as first- and second-line therapy, considering issues such as the differences among complementary diagnostic assays in terms of the type of cells scored, antibodies used, and cutoff values. We consider how PD-L1 testing fits into decision-making and the potential of emerging biomarkers in UC. We conclude that, based on the scientific rationale for its use and evidence from clinical trials, PD-L1 testing provides enriched information on the patients most likely to benefit from immune checkpoint blockade and should be routinely offered to patients with metastatic UC.

## Background and introduction

Urothelial carcinomas (UC) are immunogenic tumors against which immunotherapeutic approaches have shown substantial clinical benefit [1]. Despite their immunogenicity, tumors can evade the immune system by a variety of means, including altered expression of proteins that act within the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway to suppress T-cell antitumor activity [1–3]. PD-L1 is highly expressed on the surface of activated T cells in response to inflammation or infection, acting as an “immune checkpoint” to maintain self-tolerance and modulate physiological immune responses. Tumors co-opt this pathway as a major mechanism of immune resistance, such that PD-L1 expression on tumor cells (TCs) and/or tumor-infiltrating immune cells (ICs) can play a critical role in immunosuppression and evasion of host immune responses by tumors

[1–3].

Understanding the role of immune checkpoints in immune evasion has led to the development of therapies that target the PD-1/PD-L1 pathway. Five immune checkpoint inhibitors shown to improve outcomes in patients with locally advanced/metastatic UC have been approved [4–11]. Indications for anti-PD-L1 (durvalumab, atezolizumab, and avelumab) and anti-PD-1 (nivolumab, pembrolizumab) antibodies in UC are shown in Table 1 [12–25].

To minimize the tendency to administer questionable treatments (eg, vinflunine in UC), regulatory agencies have approved these five immune-oncology (IO) agents, often based on a small effect size, signal of activity from earlier trials, and surrogate endpoints, including the objective response rate (ORR) stratified per PD-L1. Each of these IO agents has been approved based on data from single-arm phase 2 trials. Only two have been tested in randomized trials: atezolizumab and

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**Table 1**  
Indications for anti-PD-1/PD-L1 agents in the United States and Europe and complementary or companion diagnostic assays.

	Assay			
	US, FDA	Europe, EMA		
	UC	UC		
	Other indications <sup>a</sup>	Other indications <sup>b</sup>		
<b>PD-1</b>				
Nivolumab	Patients with locally advanced or metastatic UC who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy [18]	Locally advanced unresectable or metastatic UC in adults after failure of prior platinum-containing therapy [19]	Melanoma; NSCLC; renal cell carcinoma; classical Hodgkin lymphoma; HNSCC [19]	Complementary Dx (US, FDA) PD-L1 IHC 28-8 pharmDx [21]
Pembrolizumab	Patients with locally advanced or metastatic UC who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 (CPS ≥ 10), or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status, patients who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy [16]	NSCLC; melanoma; renal cell carcinoma; classical Hodgkin lymphoma; HNSCC; colorectal cancer; hepatocellular carcinoma [18]	Locally advanced or metastatic UC in adults who have received prior platinum-containing chemotherapy and in adults who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 with a combined positive score ≥ 10 [17]	Companion Dx (US, FDA) PD-L1 IHC 22C3 pharmDx <sup>c</sup> [20]
<b>PD-L1</b>				
Atezolizumab	Patients with locally advanced or metastatic UC who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 (PD-L1 stained IC covering ≥ 5% of the tumor area), as determined by an FDA-approved test, or are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status, or who have disease progression during or following any platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant chemotherapy [23]	NSCLC [23]	Patients with locally advanced or metastatic UC after prior platinum-containing chemotherapy or who are considered cisplatin ineligible and whose tumors have PD-L1 expression ≥ 5% [22]	Complementary Dx (2L) VENTANA PD-L1 (SP142) [24]
Avelumab	Patients with locally advanced or metastatic UC who have disease progression during or following platinum-containing chemotherapy or who have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy [13]	Merkel cell carcinoma [13]	Merkel cell carcinoma [12]	No FDA-approved assay
Durvalumab	Patients with locally advanced or metastatic UC who have disease progression during or following platinum-containing chemotherapy or who have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy [15]	NSCLC	NSCLC [14]	VENTANA PD-L1 (SP263) [25]

EMA, European Medicines Agency; FDA, US Food and Drug Administration; HNSCC, squamous cell carcinoma of the head and neck; NSCLC, non-small cell lung cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand-1; UC, urothelial carcinoma.

<sup>a</sup> See Prescribing Information for details of [13,15,16,18,23].

<sup>b</sup> See Summary of Product Characteristics for details of indications [12,14,17,19,22].

<sup>c</sup> PD-L1 IHC 22C3 pharmDx is an FDA-approved companion diagnostic used to select patients with NSCLC and gastric cancer for pembrolizumab treatment.

pembrolizumab. The approval of multiple immune checkpoint inhibitors has represented a significant advance in this setting, as substantial unmet needs are present for pretreated or cisplatin-ineligible patients, where options are narrow and the possibility to complete a full course of treatment is quite low.

However, response to anti-PD-L1 and anti-PD-1 agents is not observed in all patients, such that novel (including molecular) predictors of response would be beneficial to inform therapeutic choices [1,26]. PD-L1 is increasingly being used as a biomarker to identify patients likely to benefit most from immune checkpoint blockade. For a biomarker to be of value, three criteria need to be met. First, it is important to understand what the biomarker is measuring, and how it is being measured. Second, clinical utility must be demonstrated. Finally, the biomarker must be practicable (ie, it should be reliable; reproducible; avoid additional, invasive procedures; be cost-effective; usable by different laboratories in different countries) [27,28]. Based on the mechanism of action of anti-PD-L1 therapies and their observed benefit, PD-L1 expression is a logical biomarker for response in UC. Investigations into the clinical utility of PD-L1 in UC have followed a similar path in non-small cell lung cancer (NSCLC), where early studies investigated the impact of PD-L1 expression on objective response to IO therapy in all-comer populations, with later phase 3 studies using stratification or prospective selection to confirm clinical utility [29].

The value of understanding PD-L1 expression has been reinforced by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) restrictions to PD-L1-high patients based on emerging data from ongoing trials [30].

Two open-label phase 3 trials are evaluating first-line anti-PD-1/PD-L1 agents with or without chemotherapy versus chemotherapy alone in previously untreated patients with locally advanced or metastatic UC: the IMvigor130 trial of atezolizumab and the KEYNOTE-361 trial of pembrolizumab [31,32]. Early reviews by the studies' independent data monitoring committees suggest that patients in the checkpoint inhibitor monotherapy arms of both trials with PD-L1 low status had adverse outcomes [33,34]. The FDA issued an alert to health care professionals that they should be aware that the populations enrolled in the ongoing clinical trials were eligible for platinum-containing chemotherapy, and therefore differ from those enrolled in the trials that led to the accelerated approvals of the two agents [34]. The FDA has also revised the indication of these two agents in the first-line setting. Pembrolizumab and atezolizumab are indicated for the treatment of patients with locally advanced/metastatic UC who are not eligible for cisplatin-containing chemotherapy and who have high PD-L1 expression. For pembrolizumab, this is defined as combined positive score (CPS: the percentage of PD-L1-expressing TCs and ICs relative to the total number of TCs)  $\geq 10$  as measured by the PD-L1 immunohistochemical (IHC) 22C3 pharmDx assay. For atezolizumab, this is defined as ICs  $\geq 5\%$  of the tumor area as measured by the VENTANA PD-L1 SP142 assay [35]. Similarly, the EMA has restricted approval of pembrolizumab and atezolizumab as first-line treatment of cisplatin-ineligible patients with metastatic UC to those with high PD-L1 expression (CPS  $\geq 10$  for pembrolizumab and ICs  $\geq 5\%$  for atezolizumab) [33].

The ability of PD-L1 to predict response to therapy has been investigated in various tumor types using different PD-L1 complementary or companion diagnostic assays (Table 1), antibodies, scoring algorithms, and cutoffs to measure PD-L1 expression in TCs or ICs, or both (Fig. 1), which can lead to variability in results [36,37]. For example, in advanced NSCLC, high versus low PD-L1 expression is associated with greater likelihood of treatment benefit with anti-PD-1/PD-L1 therapy, and requirements for testing for PD-L1 expression using a companion diagnostic are included in indications for pembrolizumab (expression on  $\geq 50\%$  of TCs for first-line therapy and  $\geq 1\%$  TCs for patients who have received at least one prior chemotherapy regimen) [16,17,38]. In contrast, in advanced renal cell carcinoma, the benefit of nivolumab in patients previously treated with anti-angiogenic agents is not linked to

TC PD-L1 expression ( $\geq 1\%$ ), but it does appear to be of some predictive value in terms of response to combination therapy with ipilimumab in the frontline setting [18,39,40]. Thus, the predictive value of PD-L1 expression in UC cannot be extrapolated from data from other tumors; findings must be carefully evaluated in the tumor of interest.

In this review, we evaluate the prognostic value of PD-L1 expression levels in UC, and the rationale and evidence for PD-L1 expression testing to predict response to anti-PD-1/PD-L1 therapy. We aim to explore the relative roles of TC versus IC versus CPS PD-L1 expression in suppression of antitumor immune responses, as this may be an important consideration in understanding PD-L1 as a predictive biomarker in metastatic UC. We also consider emerging biomarkers in UC, and how PD-L1 testing might fit into future decision-making matrices.

### PD-L1 expression in UC

PD-L1 is expressed in TCs as an adaptive response to inflammatory signals, interferon- $\gamma$  (IFN- $\gamma$ ) in particular, produced by an active antitumor immune response; correlations between TC PD-L1 expression and lymphocytic infiltration suggest the occurrence of a negative feedback loop in which IFN- $\gamma$  induces TC PD-L1 expression, which then suppresses the activity of PD-1-positive T cells [3].

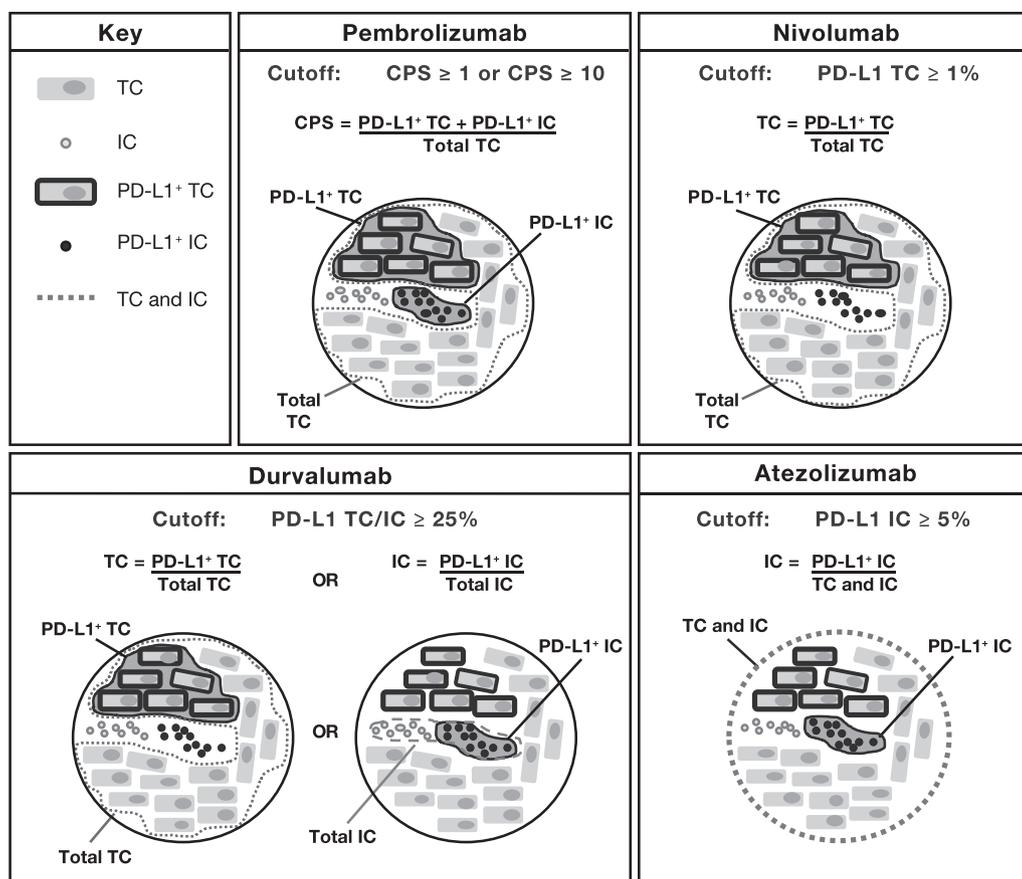
PD-L1 may also be expressed by ICs in UC [6,41]. PD-L1 expression on ICs appears to be induced by two extrinsic pathways involving CD4+ T cells, one IFN- $\gamma$ -dependent and one IFN- $\gamma$ -independent [42]. The relative importance of TC versus IC PD-L1 expression may be greater in highly immunogenic tumors [43], such as UC, which is associated with a high mutational load [1,2]. Preclinical data suggest that while TC PD-L1 expression is transient, IC expression is prolonged, so that the majority of PD-L1 in the immunosuppressive tumor microenvironment may be provided by ICs [42].

### Prognostic value of PD-L1 expression in UC

Several observational studies have examined TC PD-L1 and/or IC PD-L1 expression in UC, using different antibodies and cutoffs (Table 2) [6,44–55]. Most of these studies have assessed TC PD-L1, and, although some found no association between TC PD-L1 expression level and survival [44,46], the majority of observational studies indicate worse prognosis in patients with high TC PD-L1 expression (Table 2) [45,47–49,52,54,55]. Studies looking at IC PD-L1 expression appear to support a positive prognostic association (Table 2) [44,55]. While observational studies play an important role in research, there are major methodological issues in the design and analytical phases of these studies including selection bias and confounding [56]. Furthermore, variations in the timing, origin, and quality of the UC tissue collected are likely to be relevant.

Meta-analyses of studies in solid tumors including studies in metastatic UC that recurred or progressed after platinum-based therapy have provided mixed results with regard to the prognostic value of tumor PD-L1 expression in UC, with association between worse overall survival (OS) but no association with progression-free/disease-free survival in one analysis, and association with worse 3-year but not 5-year OS in another (Table 2) [51,53].

Randomized, stratified clinical studies remain the gold standard when conducting research [57]. Analysis of data from such trials of anti-PD-1/PD-L1 therapy can provide information about the prognostic value of PD-L1 expression. Two studies that were conducted more recently than the meta-analyses referred to above provide somewhat contradictory information about prognostic value. In KEYNOTE-045, in both pembrolizumab- and chemotherapy-treated patients, PD-L1 expression with CPS  $\geq 10$  was associated with worse OS in comparison with the overall population (Table 2) [6]. In contrast, data from IMvigor211 suggest that IC PD-L1 expression  $\geq 5\%$  is associated with improved ORR in comparison with the intent-to-treat population, in both atezolizumab and chemotherapy groups (Table 2) [50]. Although



**Fig. 1.** Comparison of different PD-L1 scoring algorithms used in UC. PD-L1 testing in conjunction with pembrolizumab in UC (PharmDx 22C3) is based on the CPS of TCs and ICs with PD-L1 staining as a proportion of total TC area; with nivolumab (PharmDx 28-8), the proportion of TCs with PD-L1 staining is assessed; with durvalumab (VENTANA SP263), the proportion of ICs with PD-L1 staining within the total IC area and the proportion of TCs with PD-L1 staining is used; with atezolizumab (VENTANA SP142), the proportion of the tumor area occupied by PD-L1 stained ICs is measured. CPS, combined positive score; IC, tumor-infiltrating immune cell; PD-L1, programmed cell death ligand 1; TC, tumor cell.

these findings are seemingly contradictory, it is important to note that differing PD-L1 testing approaches were used in the two trials (TCs and ICs in KEYNOTE-045 and ICs only in IMvigor211).

Although the current evidence from these observational studies, meta-analyses, and randomized clinical trials is far from clear, it does suggest that higher IC PD-L1 expression may be linked to better prognosis in metastatic UC, potentially as a reflection of an active antitumor immune response. Higher TC PD-L1 expression appears to be linked to worse prognosis, a conclusion supported by meta-analyses including other tumor types [51,53].

**PD-L1 expression in prediction of response to anti-PD-1/PD-L1 monotherapy in metastatic UC**

Data on the correlations between clinical outcomes and TC and/or IC PD-L1 expression in metastatic UC are available from numerous clinical trials (Table 3) [4–11,20,21,24,25,50], with most data coming from trials of therapies in the second-line setting. As noted earlier, interpretation of the results of these studies is complicated by the use of unique assay antibodies and particular assay formats for specific agents. Despite this, several trials have suggested that patients with PD-L1-expressing TCs and/or ICs have a greater response to PD-1/PD-L1 inhibition, while others have not demonstrated a correlation between expression and outcomes (Table 3) [1,2].

**Atezolizumab**

In IMvigor210, a phase 2 trial of atezolizumab, higher levels of IC PD-L1 expression (VENTANA SP142 assay), ICs ≥ 5% versus ICs < 1%, were associated with ORRs of 26% versus 8% (Table 3). In contrast, TC PD-L1 expression was low, and did not show an association with objective response [9].

In the phase 3, randomized controlled trial, IMvigor211, enrolling more than 900 patients (Table 3), the study design was based on the hypothesis that the efficacy of atezolizumab would be associated with IC PD-L1 expression; the primary endpoint of improvement in OS versus chemotherapy in patients with ≥ 5% IC PD-L1 expression was not met (median OS [95% confidence interval (CI)] was 11.1 [8.6–15.5] months with atezolizumab and 10.6 [8.4–12.2] months with chemotherapy, hazard ratio (HR) [95% CI] 0.87 [0.63–1.21], P = 0.41), precluding further statistical analysis. Overexpression of PD-L1 on ICs was associated with a more favorable outcome with both atezolizumab and chemotherapy, consistent with a positive prognostic value of higher IC PD-L1 expression, as discussed above [44]. However, the IC PD-L1 biomarker enriched for responses regardless of treatment arm, negating predictive value for response to anti-PD-L1 therapy [50].

More recently, the IMvigor130 trial, which investigated atezolizumab alone or in combination with standard chemotherapy showed a progression-free survival (PFS) advantage for the chemotherapy + immune therapy regardless of biomarker expression. However, the monotherapy arm appeared to out-perform chemotherapy in biomarker positives. As no formal statistical testing has occurred owing to the trial design, conclusions cannot be drawn. Atezolizumab is, however, still FDA-approved for the indication of IMvigor211 (which was intended to be a confirmatory trial).

**Avelumab**

In a pooled analysis of patients with metastatic UC (post-platinum or cisplatin-ineligible) treated with avelumab in the phase 1 JAVELIN solid tumor study, TC PD-L1 expression ≥ 5% (PharmDx 73–10 assay) was associated with an ORR of 24%, versus 13% with TC PD-L1 < 5% (Table 3) [7]. This association was weaker than in an earlier analysis of 44 patients from this study, in which ORR was 54% with TC PD-

**Table 2**  
PD-L1 expression as a prognostic biomarker in UC.

	Study population	n	Assay	TC/IC cutoff	Key findings	Prognostic value
<b>Individual studies</b>						
Bellmunt et al. [44]	Metastatic UC treated with platinum-based chemotherapy	100	IHC, 405.9A11 mAb	TC ≥ 5% ICs scored as absent (0), focal (1), mild (2), moderate (3), or severe (4), with cutoff 0/1 vs 2-4	Median OS not correlated with TC PD-L1 expression ( $P = 0.45$ ) IC PD-L1 expression score 2-4 vs 0/1 correlated with longer median OS (95% CI, 23 months (12 - not reached) vs 12 months (9-16) ( $P = 0.04$ univariate, $P = 0.0007$ multivariate))	No association TC PD-L1 and median Positively prognostic association IC PD-L1 and median OS
Boorjian et al. [45]	UC with radical cystectomy, followed for > 10 years	318	IHC, 5H1	TC ≥ 5%	TC PD-L1 ≥ 5% vs < 5% associated with increased risk of death (HR 3.18, 95% CI, 1.74-5.79, $P < 0.001$ ) in patients with organ-confined disease	Negatively prognostic association TC PD-L1 and death
Faraj et al. [46]	UC with cystectomy, median follow-up 31.5 months	56	IHC, 5H1	TC ≥ 5%	No association	No association PD-L1 TC and OS
Inman et al. [47]	High-risk UC	280	IHC, 5H1	TC ≥ 1%	TC PD-L1 expression prognostic factor of stage progression (OR = 2.20, $P = 0.012$ )	Negatively prognostic association TC PD-L1 and stage progression
Krabbe et al. [48]	High-grade upper tract UC, median follow-up 37.0 months	423	IHC, E1L3N	TC ≥ 1%	TC PD-L1 expression independent prognostic factor (multivariate analysis) in organ-confined disease for longer recurrence-free survival (HR = 0.2, 95% CI, 0.06-0.98; $P = 0.046$ ) and OS (HR = 0.3, 95% CI, 0.11-0.63; $P = 0.003$ )	Negatively prognostic association TC PD-L1 and recurrence-free survival and OS
Nakanishi et al. [49]	UC	65	IHC, MIH1	TC ≥ 12.2%	TC PD-L1 expression associated with higher frequency of post-operative recurrence (42% vs 81%, $P = 0.026$ ) and worse 5-year survival (55% vs 100%, $P = 0.041$ )	Negatively prognostic association TC PD-L1 and post-operative recurrence and 5-year survival
Wang et al. [52]	Bladder cancer	50	IHC	TC > 10%	Negative independent prognostic factor in multivariate analysis ( $P = 0.021$ ) PD-L1 expression associated with pathological grade, clinical stage and recurrence ( $P < 0.05$ )	Negative prognostic association TC PD-L1 expression and pathological grade, clinical stage and recurrence
Xylinas et al. [54]	UC with radical cystectomy and lymphadenectomy	302	IHC	TC ≥ 5%	OS lower in PD-L1 positive than PD-L1 negative ( $P = 0.02$ ); independent prognostic factor on multivariate analysis	Negatively prognostic association TC PD-L1 positive and OS
Zhang et al. [55]	Upper-tract UC	162	IHC, E1L3N	TC ≥ 5% IC scored 1-3; 2 or 3 considered positive	TC PD-L1 expression associated with increased risk of mortality in organ-confined disease (HR = 1.93, 95% CI, 1.09-3.43; $P = 0.02$ univariate analysis) TC PD-L1 expression independent prognostic factor of shorter cancer-specific survival (multivariate analysis, HR = 2.572, 95% CI, 1.233-5.364; $P = 0.012$ )	Negatively prognostic association TC PD-L1 and mortality risk Negatively prognostic association TC PD-L1 and cancer-specific survival
<b>Meta-analyses</b>						
Wang et al. [51]	Four studies in UC	402	-	TC	IC PD-L1 expression independent prognostic factor of longer cancer-specific survival (multivariate analysis, HR = 0.324, 95% CI, 0.114-0.921; $P = 0.034$ ) Worse OS: HR = 2.24 (95% CI, 1.61-3.12); $P < 0.0009$	Positively prognostic association IC PD-L1 and cancer-specific survival Negatively prognostic association
Wu et al. [53]	Two studies in UC Four studies in UC	161 378	- -	TC TC	No association with PFS/DfS: HR = 1.27 (95% CI, 0.58-2.77); $P = 0.548$ Worse 3-year OS: OR = 3.74 (95% CI, 1.14-12.32); $P = 0.03$	TC and OS No association TC and PFS/DfS Negatively prognostic association
<b>Clinical trial data</b>						
		542	PharmDx 22C3	CPS ≥ 10 <sup>a</sup>	No association with 5-year OS: OR = 2.61 (95% CI, 1.00-6.85); $P = 0.05$	PD-L1 and 3-year OS No association PD-L1 and 5-year OS

(continued on next page)

Table 2 (continued)

Study population	n	Assay	TC/IC cutoff	Key findings	Prognostic value
<p>Bellmunt et al. (KEYNOTE-045) [6]</p> <p>Metastatic UC that recurred or progressed after platinum-based chemotherapy</p>				<p>PD-L1 expression appeared to be associated with poorer prognosis relative to overall population in pembrolizumab- and chemotherapy-treated patients: OS (95% CI) (months), pembrolizumab, overall 10.3 (8.0–11.8) vs 8.0 (5.0–12.3) in CPS <math>\geq</math> 10 group; chemotherapy, overall 7.4 (6.1–8.3) vs 5.2 (4.0–7.4) in CPS <math>\geq</math> 10 group</p>	<p>Negatively prognostic association PD-L1 and OS</p>
<p>Powles et al. (IMvigor211) [50]</p> <p>Metastatic UC with progression after platinum-based therapy</p>	931	VENTANA SP142	<p>IC0, &lt; 1%</p> <p>IC1, <math>\geq</math> 1% &lt; 5%</p> <p>IC2/3, <math>\geq</math> 5%</p>	<p>IC2/3 PD-L1 expression appeared to be associated with improved ORR in comparison with ITT population in atezolizumab and chemotherapy groups: ORR (95% CI) %: atezolizumab, IC2/3, 23.0 (15.6–31.9) vs ITT 13.4 (10.5–16.9) Chemotherapy, IC2/3 21.6 (14.5–30.2) vs ITT 13.4 (10.5–16.9)</p>	<p>Positively prognostic association IC2/3 PD-L1 and ORR</p>

CI, confidence interval; CPS, combined positive score; DFS, disease-free survival, HR, hazard ratio; IC, tumor-infiltrating immune cell; IHC, immunohistochemistry; ITT, intent-to-treat; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death ligand-1; PFS, progression-free survival; TC, tumor cell; UC, urothelial carcinoma.

Quality criteria for inclusion: minimum number, must use a validated PD-L1 test.

<sup>a</sup> Combined positive score, % of PD-L1-expressing TCs and ICs expressed relative to the total number of TCs.

L1  $\geq$  5% versus 4% with TC PD-L1 < 5% [58].

*Durvalumab*

In a planned analysis of the UC cohort in a phase 1/2 study of durvalumab in solid tumors (Study 1108), where PD-L1 expression status was based on a cutoff of expression on  $\geq$  25% of TCs or ICs (VENTANA SP263 assay), ORR was 17.8% overall (95% CI, 12.7–24.0), 27.6% (95% CI, 19.0–37.5) in the subgroup with high PD-L1 expression, and 5.1% (95% CI, 1.4–12.5) in the subgroup with PD-L1 expression on < 25% of TCs and ICs (Table 3) [8].

*Nivolumab*

In CheckMate 032, a phase 1/2, trial of nivolumab in patients with metastatic UC with progression or recurrence after platinum-based therapy, with a cutoff of TC PD-L1 expression  $\geq$  1% (PharmDx 28-8 assay), no association of ORR with PD-L1 was observed (Table 3) [10].

In the phase 2 CheckMate 275 trial of nivolumab as second-line therapy, TC PD-L1 expression  $\geq$  5% was associated with a numerically higher ORR of 28% more than the 16% rate in patients with TC PD-L1 < 1% (Table 3). Nevertheless, the authors concluded that ORR in all PD-L1 subgroups compared favorably with historical ORRs (10%) in this setting [11].

*Pembrolizumab*

Trials of pembrolizumab have used the PD-L1 IHC 223 pharmDX assay, with cutoffs based on CPS [4,6]. In KEYNOTE-045, a phase 3 trial in which pembrolizumab was compared with chemotherapy in post-platinum metastatic UC, pembrolizumab was associated with significantly improved median OS versus chemotherapy (10.3 vs 7.4 months;  $P = 0.002$ ). Among patients with CPS  $\geq$  10, median OS was significantly longer in patients treated with pembrolizumab (8.0 months, 95% CI, 5.0–12.3) than in patients treated with chemotherapy (5.2 months, 95% CI, 4.0–7.4; HR 0.57,  $P = 0.005$ ) [6]. Pembrolizumab monotherapy with the 22C3 biomarker has transformed first-line therapy in UC.

*First-line therapy*

Data on the predictive value of PD-L1 testing in patients treated with anti-PD-1/PD-L1 agents as first-line therapy for metastatic UC are available from two completed single-arm, phase 2 trials, IMvigor210 and KEYNOTE-052. In these trials, PD-L1 testing was with the same assay as that used for each agent in the second-line setting [4,5]. In the IMvigor210 trial of atezolizumab in 119 cisplatin-ineligible patients, overall ORR was 23%, but responses occurred in all IC PD-L1 expression groups (Table 3). Median OS was 12.3 months (95% CI 6.0–not estimable) in patients with IC PD-L1 expression  $\geq$  5%, and 19.1 months (95% CI 9.8–not estimable) in patients with IC PD-L1 expression < 5%. Unlike in the second-line setting, no statistically significant enrichment of response according to PD-L1 expression was observed [5]. However, the small sample size and exploratory nature of biomarker analysis were limitations of this analysis. In the KEYNOTE-052 trial of pembrolizumab in 370 patients, the highest ORR was in patients with CPS  $\geq$  10 (Table 3). However, responses were observed across all categories of PD-L1 expression [4]. Thus, PD-L1 expression cutoff of CPS  $\geq$  10 had differing value in predicting response to pembrolizumab in the first- and second-line settings. Although CPS  $\geq$  10 enriched for response to pembrolizumab when used as first line, benefit of pembrolizumab as second-line therapy was independent of PD-L1 expression in terms of CPS  $\geq$  10 in patients treated post-platinum therapy.

As described above, early review of data from the ongoing phase 3 trials of pembrolizumab (KEYNOTE-361) and atezolizumab (IMvigor130) evaluated these agents with or without chemotherapy

**Table 3**

PD-L1 expression as a biomarker predictive of response to anti-PD-1/anti-PD-L1 therapy in clinical trials in metastatic UC.

	Phase	n	Assay	PD-L1 cutoff	Group (n)	ORR (95% CI), %	Predictive of response: Yes/No
<b>Second-line</b>							
Atezolizumab	2	310	VENTANA SP142 [24]	IC ≥ 5% IC ≥ 1%– < 5% IC < 1%	Overall (310)	15 (11–19)	Yes
IMvigor210					IC ≥ 5% (1 0 0)	26 (18–36)	Higher IC PD-L1 expression associated with response
NCT02108652 [9]					IC ≥ 1% (2 0 7) IC < 1% (103)	18 (13–24) 8 (3–15)	
Atezolizumab	3	931	VENTANA SP142 [24]	IC ≥ 5% IC ≥ 1%– < 5% IC < 1%	Overall (116)	23.0	No
IMvigor211					IC ≥ 5% Atezolizumab	(15.6–31.9)	Higher IC PD-L1 expression associated with response to both atezolizumab and chemotherapy
NCT02302807 [50]					IC ≥ 5% Chemotherapy	21.6	
Avelumab	1b	249 <sup>a</sup>	PharmDx 73–10	TC ≥ 5%	Overall (161)	17 (11–24)	Yes
JAVELIN					TC ≥ 5% (63)	24.0 (14–36)	Higher TC PD-L1 expression associated with response
NCT01772004 [7]					TC < 5% (76)	13 (7–23)	
Durvalumab	1/2	191 <sup>b</sup>	VENTANA SP263 [25]	TC or IC ≥ 25%	Overall (191)	17.8	Yes
NCT01693562 [8]					TC/IC ≥ 25% (98)	27.6	Lower TC/IC PD-L1 expression associated with reduced response
					TC&IC < 25% (79)	5.1 (1.4–12.5)	
Nivolumab	1/2	78	PharmDx 28-8 [21]	TC ≥ 1%	Overall (78)	24.4	No
CheckMate 032					TC ≥ 1% (25)	24.0 (9.4–45.1)	Response not associated with higher TC PD-L1 expression
NCT01928394 [10]					TC < 1% (42)	26.2	
					(13.9–42.0)		
Nivolumab	2	265	PharmDx 28-8 [21]	TC ≥ 1% or TC ≥ 5%	Overall (265)	19.6	No
CheckMate 275 [11]					TC ≥ 5% (81)	28.4	Response not associated with TC PD-L1 expression
					TC ≥ 1% (122)	23.8	Therapy provided meaningful benefit irrespective of PD-L1 expression
					TC < 1% (143)	16.1	No optimal PD-L1 expression cutoff
					(10.5–23.1)		
Pembrolizumab	3	542	PharmDx 22C3 [20]	CPS ≥ 10 <sup>c</sup>	Pembrolizumab (270) <sup>d</sup>	21.1	No
KEYNOTE-045					Chemotherapy (272)	11.4 (7.9–15.8)	Benefit of pembrolizumab over chemotherapy was not associated with CPS PD-L1 expression level
NCT02256436 [6]							
<b>First-line</b>							
Atezolizumab	2	119	VENTANA SP142 [24]	IC ≥ 5% IC ≥ 1%– < 5% IC < 1%	Overall (119)	23 (16–31)	No
IMvigor210					IC ≥ 5% (32)	28 (14–47)	No statistically significant enrichment of response by IC PD-L1 expression
NCT02108652 [5]					IC ≥ 1% (80) IC ≥ 1%– < 5% (48) IC < 1% (39)	24 (15–35) 21 (10–35) 21 (9–36)	
Pembrolizumab	2	370	PharmDx 22C3 [20]	CPS ≥ 10	Overall (370) <sup>e</sup>	24 (20–29)	Yes
KEYNOTE-052					CPS ≥ 10 (110)	38 (29–48)	CPS ≥ 10 associated with higher frequency of response
NCT02335424 [4]							

CI, confidence interval; CPS, combined positive score; DFS, disease-free survival; HR, hazard ratio; IC, tumor-infiltrating immune cell; IHC, immunohistochemistry; ITT, intent-to-treat; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death ligand-1; TC, tumor cell; UC, urothelial carcinoma.

Ordered according to trial design: from control arms of randomized trials (eg, IMvigor211, KN045, to single arm or observational studies).

<sup>a</sup> 242 patients with progression after platinum-based chemotherapy, seven cisplatin-ineligible patients.

<sup>b</sup> 182 patients with progression after platinum-based chemotherapy, nine cisplatin-ineligible patients.

<sup>c</sup> Combined positive score, % of PD-L1-expressing TCs and ICs expressed relative to the total number of TCs.

<sup>d</sup> ORR by PD-L1 expression status is not provided. Among patients with CPS ≥ 10, pembrolizumab offered significant benefits over chemotherapy in terms of median OS (see text for details).

<sup>e</sup> Centrally assessed objective response; in the validation set (n = 265), ORR (95% CI) was 39% (28–50) in 80 patients with CPS ≥ 10, 20 (14–28) in 139 patients with a CPS of ≥ 1– < 10, and 11 (4–24) in 46 patients with a CPS < 1.

versus chemotherapy alone in patients with locally advanced or metastatic UC. Unpublished results have indicated that patients in the checkpoint inhibitor monotherapy arms of both trials with PD-L1 low status (CPS < 10 for pembrolizumab or IC PD-L1 < 5% for atezolizumab) struggled compared with patients who received cisplatin- or carboplatin-based chemotherapy [31–34]. Published data from these trials will be needed to establish any predictive value of high PD-L1 expression, but these early results suggest that low PD-L1 expression is associated with lack of response to anti-PD-1/PD-L1 monotherapy [33,34].

In summary, in phase 2 studies in the first-line setting, high PD-L1 expression in terms of CPS ≥ 10 was associated with better outcomes [4], while the predictive value of higher IC PD-L1 expression remains

unclear (note that the number of patients in IMvigor 210 was small [119]) [5]. Current ongoing phase 3 studies [31,32] suggest low PD-L1 expression (CPS < 10 or IC ≥ 5%) is associated with adverse outcomes to anti-PD-1/PD-L1 agents in comparison with chemotherapy [33,34].

There appear to be differences in the clinical utility of PD-L1 as a biomarker in untreated and platinum-pretreated metastatic UC. It is possible that PD-L1 expression is changed after first-line treatment, with chemotherapy acting as an immunomodulator that increases PD-L1 expression in ICs and priming for response to anti-PD-1/PD-L1 therapy [2,59].

Although questions remain about the predictive value of PD-L1 expression, the available knowledge from PD-L1 testing can inform treatment choices. In the second-line setting, anti-PD-1/PD-L1 therapy

may still be an appropriate option in PD-L1–low patients, due to the low efficacy of other treatment options. In the first-line setting, chemotherapy may, however, be a better option in PD-L1–low patients [31,32]

### PD-L1 as a biomarker: issues for consideration

Biomarker expression in general may change owing to the dynamic nature of the tumor microenvironment [1]; as IFN- $\gamma$  induces PD-L1 expression, PD-L1, is a dynamic biomarker that is present at sites of active inflammation, such that biopsy may miss the relevant over-expression [60]. As with other biopsy biomarkers, intratumoral heterogeneity of PD-L1 expression may also affect PD-L1 testing due to incomplete sampling and differential expression. This is another potential reason why response to immunotherapy is seen in some apparently PD-L1–low patients [60,61]. PD-L1 expression status may differ in primary tumor and metastatic sites [62]. In addition, there may be intra-patient heterogeneity of expression between different metastatic locations, as has been observed in melanoma and NSCLC [63–65]. The timing of sampling in relation to treatment is likely to be important, given that chemotherapy may affect PD-L1 expression in UC [59]. Clinical trials have used varying samples for PD-L1 assay. For example, in the KEYNOTE-045 trial of pembrolizumab, archival tumor samples and newly obtained core or excisional biopsy samples were permitted, with no restrictions on the age of the archival samples or the number of intervening therapies received after the sample was obtained [6], whereas in CheckMate 032, biopsy specimens were fresh or archived within 3 months of the start of treatment with nivolumab [10]. The differing expression of PD-L1 in TCs and ICs, dynamic changes, and intratumoral heterogeneity may mean that there are benefits to using both TCs and ICs to obtain the most comprehensive picture of PD-L1 status.

As evident in Table 2 and Fig. 1, there is a lack of standardization between available PD-L1 assays. As noted previously, some measure PD-L1 in TCs, some in ICs, and some use both, while different complementary assays use different antibodies. This lack of standardization is an obstacle to evaluating the strength of PD-L1 as a biomarker [2]. There is evidence, however, for concordance and reproducibility among assays, indicating that PD-L1 expression can be reliably scored in UC. In a study of 235 UC samples comparing four antibody clones (ie, 22C3, 28-8, SP142, E1L3N), the overall results were highly concordant despite some heterogeneity in staining, suggesting diagnostic equivalence between these assays [66]. Similar analytical performance was found with VENTANA SP263, pharmDx 22C3, and pharmDx28-8 assays in a study of 335 tumor biopsy samples from patients with UC, but although the VENTANA SP142 assay had similar performance to the other assays in terms of IC staining, it was less sensitive for TC staining [67]. The Blueprint study in lung cancer has shown similar results [68,69].

Different assays and different algorithms may lead to differences (ie, limited overlap) in the patient populations that would be classified as PD-L1 high versus PD-L1 low/negative and impact treatment results (Fig. 2). In a study staining archival UC tumor samples, the VENTANA SP263 (TC/IC  $\geq$  25%) and pharmDx 22C3 (CPS  $\geq$  10) assays classified 35% and 52% of samples, respectively, as PD-L1 high, with an overlap of 32% (ie, samples were classified as PD-L1 high by both assays). In contrast, the VENTANA SP142 assay (ICs  $\geq$  5% tumor area) classified only 5.7% of samples as PD-L1 high, with an overlap with the VENTANA SP263 assay of 5.4% [70].

### Emerging biomarkers in metastatic UC

#### Tumor mutation burden

PD-L1 has been extensively studied in UC, but emerging biomarkers may be of value independently or in combination with PD-L1 [61,71]. Tumor mutation burden (TMB; reflective of increased neoantigen

burden) is a potential predictive biomarker in UC independent of PD-L1; TMB was associated with response to atezolizumab as first- and second-line therapy, but was not prognostic, and selected a different patient population from IHC PD-L1 assay [5,9,50]. Combining TMB and PD-L1 assessment appeared to increase predictive value: in IMvigor211, median OS in patients with high TMB and IC PD-L1 expression  $\geq$  5% was 17.8 months (95% CI 9.7–not estimable) in those treated with atezolizumab in comparison with 10.6 months (8.2–14.3) in those treated with chemotherapy [50]. Challenges to the use of TMB include difficulties in standardization, tumor evolution over time, and lack of assessment of the immune microenvironment [61].

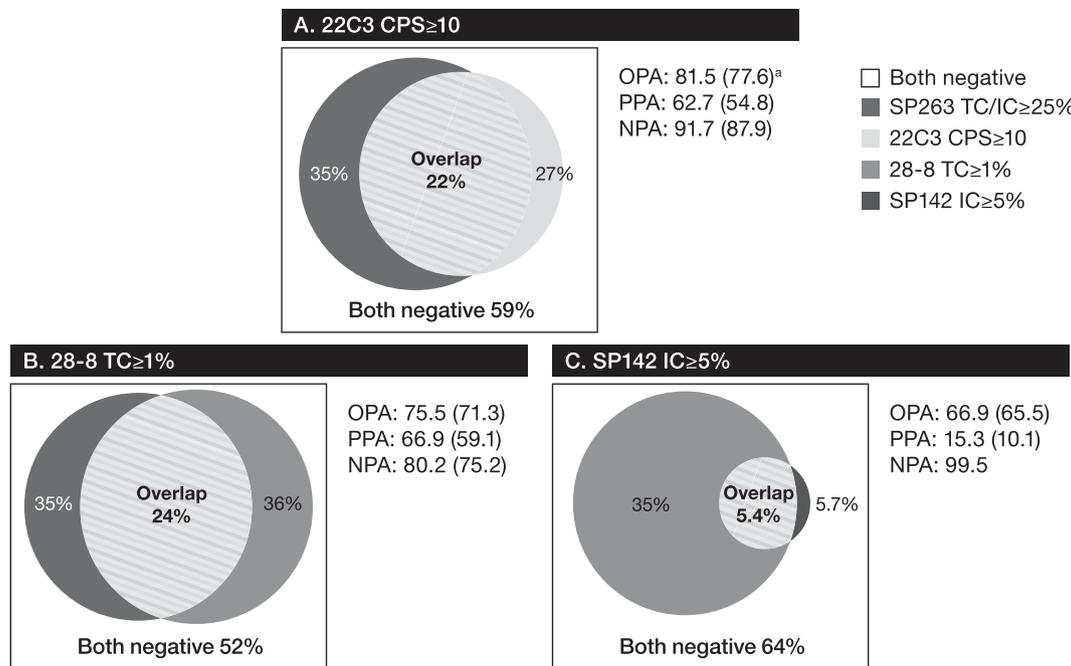
#### Gene expression profiles

Targeted IC gene expression profiles, quantifying chemokines, cytokines, or cell surface proteins may delineate an inflamed tumor microenvironment more fully than a single marker such as PD-L1 [61]. In CheckMate 275, higher values in a 25-gene IFN- $\gamma$  signature were associated with response to nivolumab and higher TC PD-L1 expression [11]. Similarly, a four-gene IFN- $\gamma$  signature was associated with response to durvalumab in the UC cohort of Study 1108 [72]. Multiple gene panels are available, but there is no standardized commercially available panel, and their utility will need to be validated in prospective clinical trials; cost may also be an issue [61,71].

Examination of tumors from patients with metastatic UC treated with atezolizumab has shown that lack of response to this agent is associated with a signature of transforming growth factor (TGF)- $\beta$  signaling in fibroblasts, particularly in tumors showing exclusion of CD8+ T cells from the tumor (a common phenotype in metastatic UC). In a non-clinical model of this immune-excluded phenotype, combination treatment with anti-PD-L1 and TGF- $\beta$ -blocking antibodies reduced stromal TGF- $\beta$  signaling, facilitated T-cell penetration into the tumor center, and provoked anti-tumor immunity and tumor regression [73]. More studies will be needed to demonstrate the utility and practicality of using TGF- $\beta$  as a biomarker.

The Cancer Genome Atlas (TCGA) RNA sequencing of muscle-invasive UC has identified five expression subtypes, luminal, luminal-papillary, luminal infiltrated, basal/squamous, and a poor-survival neuronal type, that may stratify response to different treatments [74]. As with IFN- $\gamma$  gene panels, multiple gene cluster assays have been used for TCGA subtyping, making standardization an issue. Additionally, TCGA subtyping in patients treated with immunotherapy has been limited to small patient numbers. As a result, TCGA subtyping appears to have low negative predictive value for immunotherapy [61]. However, individual TCGA subtypes, in particular the luminal or luminal papillary subtypes, may be most suitable for targeted therapies. For example, the luminal papillary subtype is characterized by aberrations in FGFR3, and several ongoing studies are investigating the efficacy of FGFR inhibitors in patients with mutations or translocations in FGFR3 [75]. Erdafitinib (a pan-FGFR tyrosine kinase inhibitor) has been tested in a phase 2 trial (n = 99) in patients with FGFR DNA alterations (FGFR2 or 3). Confirmed response rates were 40% with median PFS and OS of 5.5 months (95% CI 4.2–6.0) and 13.8 months (95% CI 9.8–NA), respectively (median follow-up: 11 months) [76]. In these select patients, erdafitinib was approved by the FDA. Data in combination with immune therapy are awaited.

Given the limitations of evaluation of biopsies derived from primary tumors and on clinical staging, interest is growing in non-invasive liquid biopsies, which may be more reflective of the current phenotypic/genotypic status of disease, an example is circulating tumor (ct) DNA [77]. Detection of specific mutations in ctDNA has the potential to identify patients likely to respond to certain therapeutic approaches [78]. Changes in variant allele frequencies (VAFs) in ctDNA are being investigated as an early marker of response to treatment: in an analysis of patients with metastatic UC treated with durvalumab, decrease in VAF was correlated with response while patients with progressive



**Fig. 2.** Selected differences between populations by assay/algorithm in UC specimens. Figure shows differences between the populations selected as PD-L1 high between VENTANA SP263 (TC/IC  $\geq$  25%) versus: (A) PharmDx 22C3 (CPS  $\geq$  10); (B) PharmDx 28–8 (TC  $\geq$  1%); (C) VENTANA SP142 (IC  $\geq$  5%). CPS, combined positive score; IC, tumor-infiltrating immune cell; OPA, overall percentage agreement; NPA, negative percentage agreement; PPA, positive percentage agreement; TC, tumor cell. <sup>a</sup>OPA, PPA, NPA (95% confidence interval [CI]). For each metric, lower boundary of 95% CI was calculated with no upper bound using the Clopper-Pearson method.

disease showed no changes in VAF [78,79]. Blood-based TMB is another emerging biomarker that has shown promise in predicting response to second-line atezolizumab in NSCLC [80].

### Discussion points

For a biomarker to be of value, it is important to understand what it is measuring, and how it is being measured; clinical utility must be demonstrated; and the biomarker must be practicable. For PD-L1, there is a clearly defined target and strong rationale for its use. At present, it is not clear whether TCs or ICs represent the best cells to test, but there may be justification for measuring expression levels of PD-L1 in both. In terms of clinical utility, PD-L1 expression, in general, appears to correlate with improved response in patients with UC treated with anti-PD-1/PD-L1 monotherapy [2]. Low-PD-L1 expression may help to identify patients who are more likely to have poor response to second-line monotherapy and who might benefit from the opportunity to receive novel therapies or combination therapy [2]. PD-L1 expression testing may be particularly important in the first-line setting to select the most appropriate therapy, although at present the data are preliminary. Overall, the results in the platinum refractory and previously untreated setting are, at best, inconclusive and inconsistent. However, results from first-line clinical trials should be able to provide clarification.

The efficacy of immunotherapy combinations is also under investigation. The role of PD-L1 expression in prediction of efficacy for such combinations is, at present, unclear. The data supporting the PD-L1 biomarker in immune combinations (with cytotoxic T-lymphocyte-associated antigen 4) were expected to show that the combinations would be effective in biomarker negatives, but, this does not appear to be the case [81]. Data on ipilimumab and nivolumab do show enrichment in biomarker positives in single-arm trials. Additional research is ongoing.

There is currently a lack of standardization among available PD-L1 assays and cutoffs, such that patient populations identified as “PD-L1 high” can differ when different assays and algorithms are used; this

represents an obstacle to evaluating the strength of PD-L1 as a biomarker. There is, however, also concordance across some assays, which is important where multiple assays are available, and indicates that PD-L1 expression can be reproducibly scored in UC. With strong evidence of practicability and utility in predicting response, PD-L1 is currently the most advanced biomarker, and should be routinely offered to patients with metastatic UC. However, PD-L1 is far from a perfect biomarker, and, as new biomarkers emerge, they are likely to be added into a matrix system to more accurately define which patients are most likely to respond to immunotherapies. For example, additional tests may identify patients who respond to IO therapy, regardless of PD-L1 expression, refining its role as a selective biomarker. In the future, monitoring of PD-L1 expression on circulating tumor cells could provide a minimally invasive means of longitudinal monitoring of real-time, on-treatment PD-L1 status that may overcome some of the limitations of current PD-L1 assay methods that rely on archival samples that do not reflect the current state of the cancer or require fresh, invasive tissue biopsy [82].

As the science of biomarkers progresses, PD-L1 testing may become embedded within the testing paradigm in UC alongside other emerging biomarkers, especially given the recent FDA and EMA restrictions on the first-line use of atezolizumab in combination with chemotherapy in “unfit” patients with low levels of PD-L1 expression as based on the recent IMvigor 130 trial. However, in the same trial, PD-L1 testing was shown to be important in monotherapy in the first-line use [83]. The role of biomarkers is unknown as drugs are moved earlier in the disease and contradictory results are sometimes observed from small data sets. Randomized trials are awaited.

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