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European Association of Urology

Research Letter

PD-L1 Antibody Comparison in Urothelial Carcinoma

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Following the recent recognition that low PD-L1 expression is associated with inferior survival in urothelial carcinoma (UC) patients treated with immune checkpoint inhibitors (ICIs) in the first-line setting, PD-L1 testing is currently required for selection of patients [1,2]. However, the predictive value of PD-L1 in platinum-pretreated patients is controversial [3–5], and discrepancies may be attributed to different antibody clones, staining platforms, and scoring algorithms for determining PD-L1 status (Table 1). To facilitate clinical application of PD-L1 testing, we compared the PD-L1 status of UC patients using five different assays.

Tissue microarrays containing primary tumor samples from 139 patients with muscle-invasive UC (81% male, median age 68 yr; cystectomy, $n = 118$, transurethral resection of bladder tumor $n = 21$; chemotherapy pretreatment, $n = 11$) were stained with five PD-L1 antibodies in strict accordance with the manufacturer guidelines (Table 1). PD-L1 expression was scored on tumor cells (TCs) and infiltrating immune cells (ICs). PD-L1 status was determined in accordance with scoring algorithms and cutoffs used in clinical trials. Since no predefined cutoff was available for E1L3N, we set this at $\geq$25% TC or IC staining (Table 1). Overall and pairwise agreement of PD-L1 status was determined using Krippendorff's α coefficient and Cohen's κ concordance coefficient, respectively.

PD-L1 expression was generally higher on TCs than on ICs for antibodies 22C3, 28-8, SP263, and E1L3N, while SP142 demonstrated less PD-L1 expression on TCs. Pairwise agreement of PD-L1 status varied from 72% to 90% (κ 0.255–0.708) and was lowest for research antibody E1L3N and slightly less between 28-8 and SP142 (Table 1). PD-L1 status was identical for all five PD-L1 assays in 72 out of 117 patients (62%; Krippendorff's $\alpha = 0.513$). Of 30 cases with one dissimilar antibody outcome, 19 (63%) were attributed to discordant E1L3N staining. Considering the four companion diagnostics, agreement in PD-L1 status improved (Krippendorff's $\alpha = 0.630$) with similar PD-L1 status in 91 out of 117 patients (78%). For cases in which one assay was discordant, this was SP142 in five cases (38%), 28-8 in five cases (38%), SP263 in two cases (16%), and 22C3 in one case (8%).

Concordance of PD-L1 status was better for companion diagnostics than for the research antibody E1L3N. Agreement of PD-L1 status between 22C3, 28-8, SP142, and SP263 was substantial (80–90%), implying that these assays may be interchangeable in clinical practice. The 80% pairwise agreement between 28-8 and SP142 is slightly lower than for the other companion diagnostics, which can be explained by the fact that 28-8 scoring only takes TC staining and SP142 only IC staining into account. Similar findings have been reported for non–small cell lung cancer [6,7]. Since none of the study patients had been treated with ICIs, the mutual predictive performance of each assay is not established yet. To date, in view of the modest discriminative value of PD-L1 assays in predicting outcomes in UC, our finding of 80–90% pairwise agreement for the companion diagnostics 22C3, 28-8, SP142, and SP263 may have limited impact on the interpretation of these PD-L1 test results in UC and selection of patients for ICIs targeting PD-1/PD-L1 signaling.

https://doi.org/10.1016/j.eururo.2018.11.002

Table 1 – Assays for determining PD-L1 status in patients with UC

Assay summary					
Antibody clone	22C3	28-8	SP142	SP263	E1L3N
Platform	Autostainer Link 48 (DAKO)	Autostainer Link 48 (DAKO)	Benchmark ULTRA	Benchmark ULTRA	Any
Cutoff for positivity	TCs and ICs ≥10%	TCs ≥5%	ICs ≥5%	TCs or ICs ≥25%	Not defined
PD-L1 expression in UC					
Mean TC staining, % (range)	6.1 (0–90)	7.8 (0–90)	1.1 (0–60)	14.7 (0–100)	18.7 (0–100)
Mean IC staining, % (range)	4.3 (0–70)	2.3 (0–40)	2.3 (0–15)	3.5 (0–20)	2.6 (0–50)
Positive PD-L1 status [a]	35/132 (27%)	30/130 (23%)	28/135 (21%)	28/137 (20%)	35/130 (27%)
PD-L1 status concordance [b]					
	22C3 ≥10%	28-8 ≥5%	SP142 ≥5%	SP263 ≥25%	E1L3N ≥25%
22C3 ≥10%		88% 0.685 (0.538–0.832)	89% 0.697 (0.550–0.844)	88% 0.657 (0.504–0.810)	75% 0.366 (0.184–0.548)
28-8 ≥5%			80% 0.419 (0.229–0.609)	90% 0.708 (0.559–0.857)	72% 0.255 (0.067–0.443)
SP142 ≥5%				86% 0.582 (0.410–0.754)	75% 0.334 (0.150–0.518)
SP263 ≥25%					79% 0.435 (0.257–0.613)
E1L3N ≥25%					

PD-L1 status agreement between all five antibody clones and companion diagnostics only [c]

	All antibody clones	**Companion diagnostics**
Krippendorff's α (95% CI)	0.513 (0.306–0.695)	0.630 (0.432–0.801)
All assays concordant	72/117 (62%)	91/117 (78%)
One assay discordant	30/117 (25%)	13/117 (11%)
22C3	1/30 (3%)	1/13 (8%)
28-8	5/30 (17%)	5/13 (38%)
SP142	4/30 (14%)	5/13 (38%)
SP263	1/30 (3%)	2/13 (16%)
E1L3N	19/30 (63%)	
Two assays discordant	15/117 (13%)	13/117 (11%)

UC = urothelial carcinoma; TCs = tumor cells; ICs = infiltrating immune cells; CI = confidence interval.

[a] Number of patients with a positive PD-L1 status out of the total number of assessable samples per assay. Minor variability in assessable numbers was caused by dropout of tissue cores or the absence of tumor at deeper TMA levels.

[b] Data are presented as percentage agreement and **κ** concordance coefficient (95% CI).

Poor concordance *Excellent concordance*

[c] The companion diagnostics are 22C3, 28-8, SP142, and SP263. Data are presented as the proportion of patients for whom PD-L1 status was concordant for all assays and the proportion of patients for whom one or two assays were discordant. In the case of one discordant assay, the data indicate which antibody clone displayed a different PD-L1 status.

Conflicts of interest: Astrid A.M. van der Veldt has received consultancy fees from MSD, BMS, Pfizer, Eisai, Roche, Novartis, Ipsen, and Pierre Fabre. Tahlita C.M. Zuiverloon has received research funding from Roche. Erik Thunnissen has received consultancy fees from Roche Diagnostics, Histogenex, and Ventana. Ronald de Wit has received consultancy and speaker fees from Merck and Sanofi, consultancy fees from Roche, and research funding Sanofi and Bayer. Geert J.L.H. van Leenders has received consultancy fees from Roche and research funding from Roche and AstraZeneca. Maud Rijnders and Katrien Grünberg have nothing to disclose. This study was funded by a research grant from AstraZeneca.

Acknowledgments: We would like to thank Daan Nieboer (Department of Public Health and Department of Urology, Erasmus MC, University Medical Center Rotterdam) and Esther Oomen-de Hoop (Department of Hematology, Erasmus MC, University Medical Center Rotterdam) for their support in statistical analyses of the data. We would also like to thank Reno Debets (Tumor Immunology Laboratory, Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam).

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[2] U.S. Food and Drug Administration. FDA alerts health care professionals and oncology clinical investigators about an efficacy issue identified in clinical trials for some patients taking Keytruda (pembrolizumab) or Tecentriq (atezolizumab) as monotherapy to treat urothelial cancer with low expression of PD-L1. www.fda.gov/Drugs/DrugSafety/ucm608075.htm.

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November 1, 2018