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PD-L1 Expression in Urothelial Carcinoma With Predominant or Pure Variant Histology

Concordance Among 3 Commonly Used and Commercially Available Antibodies

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BACKGROUND

- Bladder cancer (BC)

  Invasive carcinoma

  - Conventional urothelial carcinoma
  - Urothelial carcinoma variants
    - Urothelial carcinoma with divergent differentiation (squamous, glandular and/other)
    - Nested urothelial carcinoma (including large nested carcinoma)
    - Microcystic urothelial carcinoma
    - Micropapillary urothelial carcinoma
    - Lymphoepithelioma-like urothelial carcinoma

BACKGROUND

- Bladder cancer (BC)
  - Plasmacytoid urothelial carcinoma
  - Giant cell urothelial carcinoma
  - Lipid-rich urothelial carcinoma
  - Clear cell (glycogen-rich) urothelial carcinoma
  - Sarcomatoid urothelial carcinoma
  - Poorly differentiated urothelial carcinoma (including those with osteoclast-like giant cells)
- Squamous cell carcinoma
- Adenocarcinoma
- Small cell neuroendocrine carcinoma

BACKGROUND

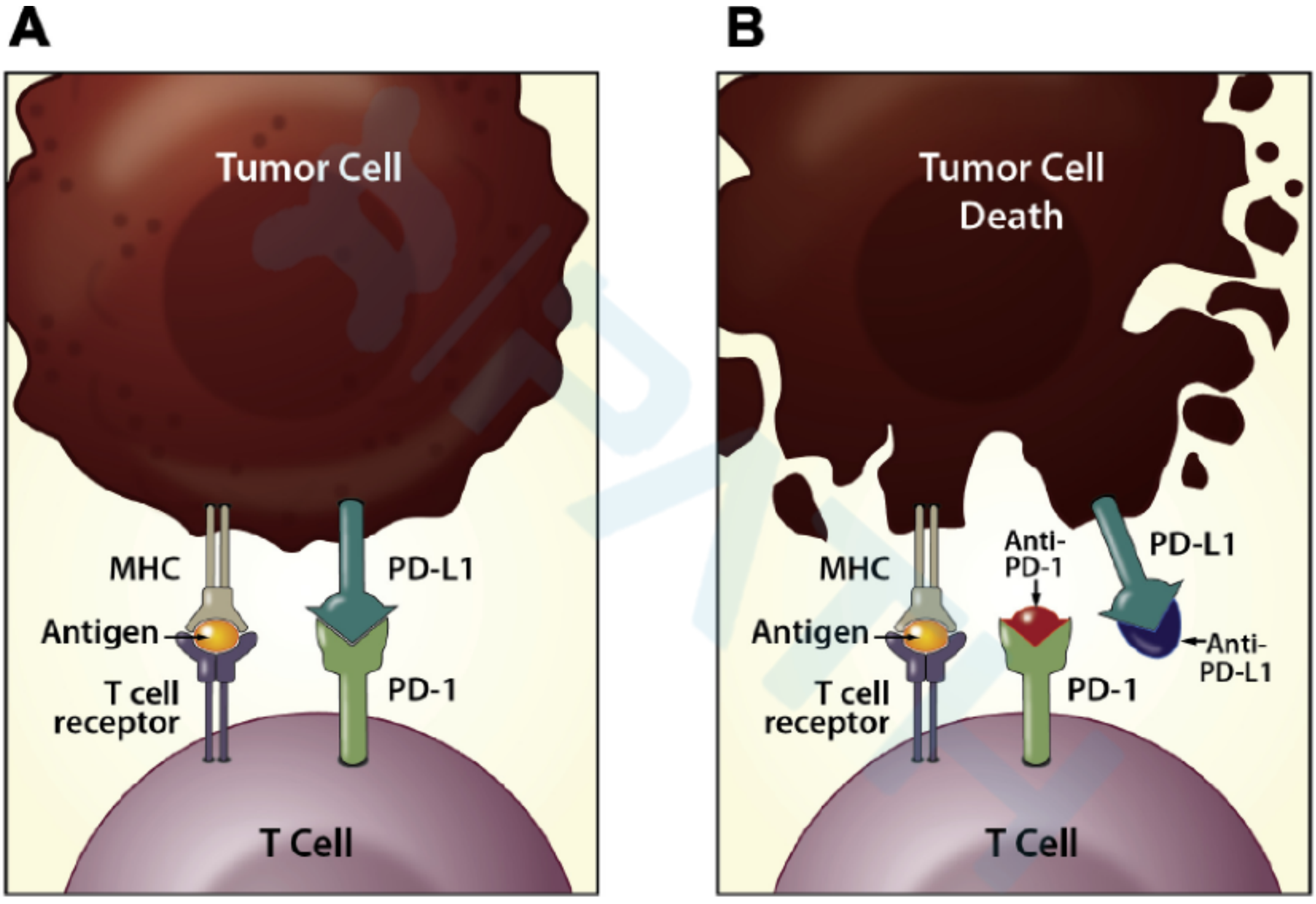
- Bladder cancer (BC)
- The fifth most common cancer the United States and the ninth most frequent in worldwide
- 90% of reported deaths are associated with advanced or metastatic disease
- Cisplatin-based combination chemotherapy has been the gold standard for the treatment of advanced or metastatic BC
  - Associated with serious toxicities
  - Complete and/or durable remissions are rare
  - ~ 50% of patients are cisplatin-ineligible

BACKGROUND

- Immune checkpoint inhibitors have been shown to induce durable responses
  - Patients with progressive disease following chemotherapy
  - Those who are cisplatin-ineligible
- FDA approval of 5 immune checkpoint inhibitors targeting either the programmed cell death protein 1 (PD-1, CD279) or programmed death-ligand 1 (PD-L1, CD274)
  - Only 20% to 25% of treated patients with advanced or metastatic BC respond to ICB
  - Predictive biomarkers of response are clearly needed to select those patients most likely to derive clinical benefit

BACKGROUND

- Programmed death 1 (PD-1)
  - A member of B7 family
  - Plays a key role in mediating tumor-induced immune suppression
  - Expressed in tumor-infiltrating CD8+ T cells
- PD-L1
  - A ligand of PD-1 that inhibits immune responses
  - Tumor cell apoptosis induced by antigenspecific CD8+ T cells
  - Reverse signaling through PD-L1 in T cells regulates cytokine production and inhibits survival of activated T cells



Mechanism of action of PD-1 and PD-L1 inhibitors. A. PD-L1 binds to PD-1 and inhibits T-cell killing of tumor cells. B. Blocking PD-L1 or PD-1 allows T-cell killing of tumor cells. MHC = major histocompatibility complex; PD-1 = programmed cell death protein-1; PD-L1 = programmed death ligand-1.

FDA批准适应症的顺序

Iplimumab

黑色素瘤 一线/二线 → 黑色素瘤辅助

Pembrolizumab

黑色素瘤 二线 → NSCLC 二线 → 黑色素瘤一线 → 头颈癌 二线 → NSCLC 一线 → 霍奇金淋巴瘤二线及以上 → 非鳞癌NSCLC 一线 → 尿路上皮癌 二线 → MSI-H/dMMR实体瘤二线 → 黑色素瘤辅助治疗

Nivolumab

黑色素瘤 二线 → 鳞癌NSCLC 二线 → 黑色素瘤一线 → 非鳞癌NSCLC 二线 → 肾细胞癌二线 → 霍奇金淋巴瘤二线 → 头颈鳞癌二线 → 尿路上皮癌 二线

Atezolizumab

尿路上皮癌 二线 → NSCLC 二线

Avelumab

尿路上皮癌 二线 → Merke细胞癌I

Durvalumab

尿路上皮癌 二线 → NSCLC 同步放化疗后

Cemiplimab

转移性皮肤鳞状细胞癌

BACKGROUND

- Markers that have been associated with ICB response
  - PD-L1 expression, as assessed by immunohistochemistry (IHC)
  - Tumor mutational burden (TMB)
  - Molecular subtyping, as defined by The Cancer Genome Atlas (TCGA) classification, and immune cell (IC) profiling
  - None alone is a robust predictive biomarker
- PD-L1 IHC
  - A practical and rapid assay
  - Higher PD-L1 expression is enriched in responders to ICB in most BC-related studies
  - A predictive biomarker of antiPD-L1/PD1 therapy

	Advantages	Disadvantages
PD-L1 Immunohistochemistry	• Most well-characterized biomarker to date • Rapid turn around time from biopsy • IHC assays are standardized specific to each therapy • Relatively inexpensive	• Discordant results across studies • Poor negative predictive value: responses seen in PD-L1 negative tumors • Multiple antibodies in use to detect PD-L1 • Unclear if composite score or tumor cell score is more reflective of the tumor microenvironment • Biomarker is dynamic over time and does not reflect PD-1/PD-L1 interactions in tumor draining lymph nodes • Does not assess status of the immune microenvironment
TCGA Subtyping	• Evidence of increased immunotherapy response in luminal cluster II subtypes with atezolizumab • Basal cluster I subtype demonstrated increased ORR with nivolumab therapy • Distinct classifications based on tumor gene signatures (i.e. few patients with gene signatures between groups)	• Multiple gene cluster assays used, difficult to standardize • TCGA subtyping in patients treated with immunotherapy is limited to small numbers in each cohort (<60 patients in IMVigor study) • May require deep sequencing to appropriately identify the TCGA subtype • Responses are achieved in all 4 TCGA clusters, suggesting a low negative predictive value • Does not assess status of the immune microenvironment
Tumor Mutational Burden	• Clear examples of durable responses (> 6 months) in patients with high mutation burden • Correlation demonstrated in subgroup analyses between tumor mutation burden and overall response rates with atezolizumab and pembrolizumab	• Difficult to standardize between sequencing assays • Relative weight of SNPs and translocations not yet elucidated • Relationship between tumor mutation burden and neoantigen burden is still undefined • Depth of sequencing required to predict responders vs nonresponders undetermined • Evolution of tumor over time may change the relative mutation burden • Does not assess status of the immune microenvironment
Immune Cell Gene Expression Profiling	• Higher reproducibility relative to PD-L1 IHC to predict immunotherapy responses • Only biomarker assessing immune cell status rather than tumor characteristics • Correlated with response to therapy in subgroup analyses of nivolumab and pembrolizumab trials	• No standardized commercially available gene panel as of yet. Multiple gene panels currently available (T-cell panel, combined T-cell tumor cell panel, IFN-γ specific) • Insufficient negative predictive value: responders seen in all groups • Cost

Advantages and disadvantages of potential biomarkers for immunotherapy

J Immunother Cancer. 2017;5:94.

BACKGROUND

- Urothelial Carcinoma With Predominant or Pure Variant Histology (UCV)
  - In up to one-third of invasive UC
  - A worse prognosis
  - Few studies have explored PD-L1 expression
  - Largely excluded from most ICB studies
  - Higher TMB in certain subtypes (eg, plasmacytoid and small cell carcinoma)
  - Some patients with variant histologies might benefit from ICB
- Assessing PD-L1 expression in UCV may become more relevant

BACKGROUND

- Evaluated PD-L1 expression of both tumor cells (TC) and IC in a cohort of UCV tumors
- Compared the results of 3 different PDL1 antibodies commonly used in BC
  - SP263: durvalumab,22C3: pembrolizumab, SP142: atezolizumab

MATERIAL AND METHODS

- 84 cases of UCV
  - Micropapillary UC (n = 19)
  - UC with squamous differentiation (n = 16)
  - Nested UC (n = 14)
  - Plasmacytoid UC (n = 14)
  - Small cell carcinoma (n = 12)
  - UC with glandular differentiation (n = 9)
- Classic UC component was present in 17 of 84 cases on the same slide
  - 10 micropapillary UC
  - 4 UC with squamous differentiation
  - 2 UC with glandular differentiation
  - 1 small cell carcinoma

MATERIAL AND METHODS

- Immunohistochemistry
  - PD-L1 SP263, 22C3, and SP142
  - Expression on tumor cells (TC) and tumor-infiltrating immune cells (IC) (leukocytes and/or macrophages)
  - H-Score (the sum of the percentage of strong (×3), moderate (×2), and weak (×1) immunoreactivity)
- Statistical analyses
  - SPSS
  - Nonparametrical Spearman rank correlation analyses

MATERIAL AND METHODS

- Various cutoff points as used in previous studies in BC
  - FDA approval of anti-PD-1/PD-L1 agents in BC used cutoff values of 1% or 5%
  - Further cutoff criteria adopted from studies
  - Atezolizumab (IC0, <1%,IC1, 1% to <5%, IC2/3, ⩾ 5%)
  - Durvalumab (TC ⩾ 25% or IC ⩾ 25%)
  - Pembrolizumab (a combined positive score of TC +IC ⩾ 10%)

TABLE 1. Rates of PD-L1 Expression in UCV Using Cutoff Values to Define Positivity as 1% of TC (A) or 5% (B)

	SP263				SP142	
Histologic Differentiation (n)	**Tumors Pos. (n [%])**	**TC (%; Mean)**	**TC (H-score, Mean)**	**IC (%; Mean)**	**Tumors Pos. (n [%])**	**TC (%; Mean)**
A						
Micropapillary (19)	13 (69)	5	7	12	6 (32)	2
Squamous differentiation (16)	14 (88)	42	86	12	14 (88)	20
Nested (14)	5 (36)	4	7	10	1 (7)	3
Plasmacytoid (14)	5 (36)	5	8	9	3 (21)	1
Small cell carcinoma (12)	2 (17)	9	24	7	2 (17)	7
Glandular differentiation (9)	6 (67)	6	9	14	5 (56)	2
Total (84)	45 (54)	13	25	11	31 (37)	6
B						
Micropapillary (19)	6 (32)	5	7	12	2 (11)	2
Squamous differentiation (16)	14 (88)	42	86	12	13 (81)	20
Nested (14)	1 (7)	4	7	10	1 (7)	3
Plasmacytoid (14)	3 (21)	5	8	9	0 (0)	1
Small cell carcinoma (12)	2 (17)	9	24	7	2 (17)	7
Glandular differentiation (9)	5 (56)	6	9	14	1 (11)	2
Total (84)	31 (37)	13	25	11	19 (23)	6

TABLE 1. (*Continued*)

SP142			22C3		
TC (H-score, Mean)	IC (%; Mean)	Tumors Pos. (n [%])	TC (%; Mean)	TC (H-score, Mean)	IC (%; Mean)
4	5	7 (37)	2	4	8
43	9	15 (94)	30	50	14
8	4	4 (29)	4	7	8
1	3	2 (14)	8	3	4
19	3	2 (17)	3	18	6
2	5	6 (67)	9	4	10
13	5	36 (43)	9	15	8
4	5	3 (16)	2	4	8
43	9	15 (94)	30	50	14
8	4	1 (7)	4	7	8
1	3	2 (14)	8	3	4
19	3	2 (17)	3	18	6
2	5	2 (22)	9	4	10
13	5	25 (30)	9	15	8

The highest expression in both TC and IC was observed with clone SP263, followed by 22C3 and SP142

PD-L1 was expressed in a significant percentage of UCV cases at different cutoff points (cutoff 1% TC: 37% to 54%, cutoff 5% TC: 23% to 37%)

The highest expression in UC with squamous differentiation

Patients with UCV may benefit from anti-PD-1/PDL1 therapy and argue against the exclusion of UC with predominant or pure variant histology from clinical ICB studies

RESULTS

- Staining and Assay Differences Between the SP263, 22C3, and SP142 Clones
  - SP263 and 22C3 showed finely dispersed membranous and circumferential expression
  - The SP142 clone showed a coarse and clumpy pattern of expression in TC and IC with mostly strong intensity
  - The staining characteristics were identical in the NOS and variant histology components
  - In UC with squamous differentiation, stronger areas of PD-L1 expression in TC were noted at the periphery/invasive front of tumor

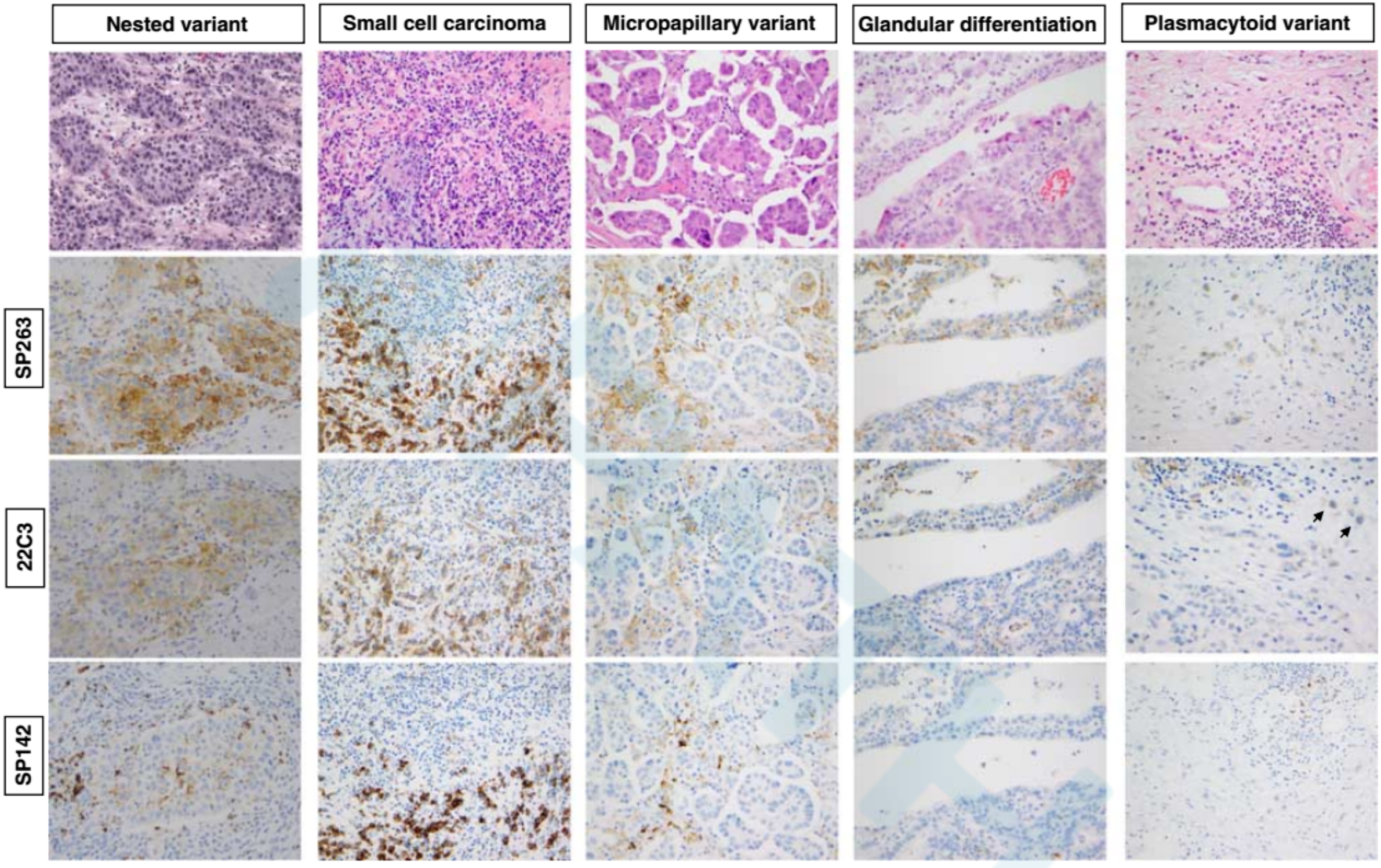


FIGURE 1. PD-L1 immunoreactivity in UC variants. An example of UC nested variant with diffuse PD-L1 expression on TC and IC. The extent of expression is highest with clone SP263 followed by 22C3 and least by SP142. Similar findings in an example of small cell carcinoma but with less obvious expression on immune cells. In examples of micropapillary UC and UC with glandular differentiation, there is weak PD-L1 expression on TC but more prominent expression on IC, which also decreased from SP263 to 22C3 to SP142. An example of plasmacytoid UC with weak expression on TC by clone SP263, nearly absent expression with 22C3 (short arrows), and no expression with SP142.

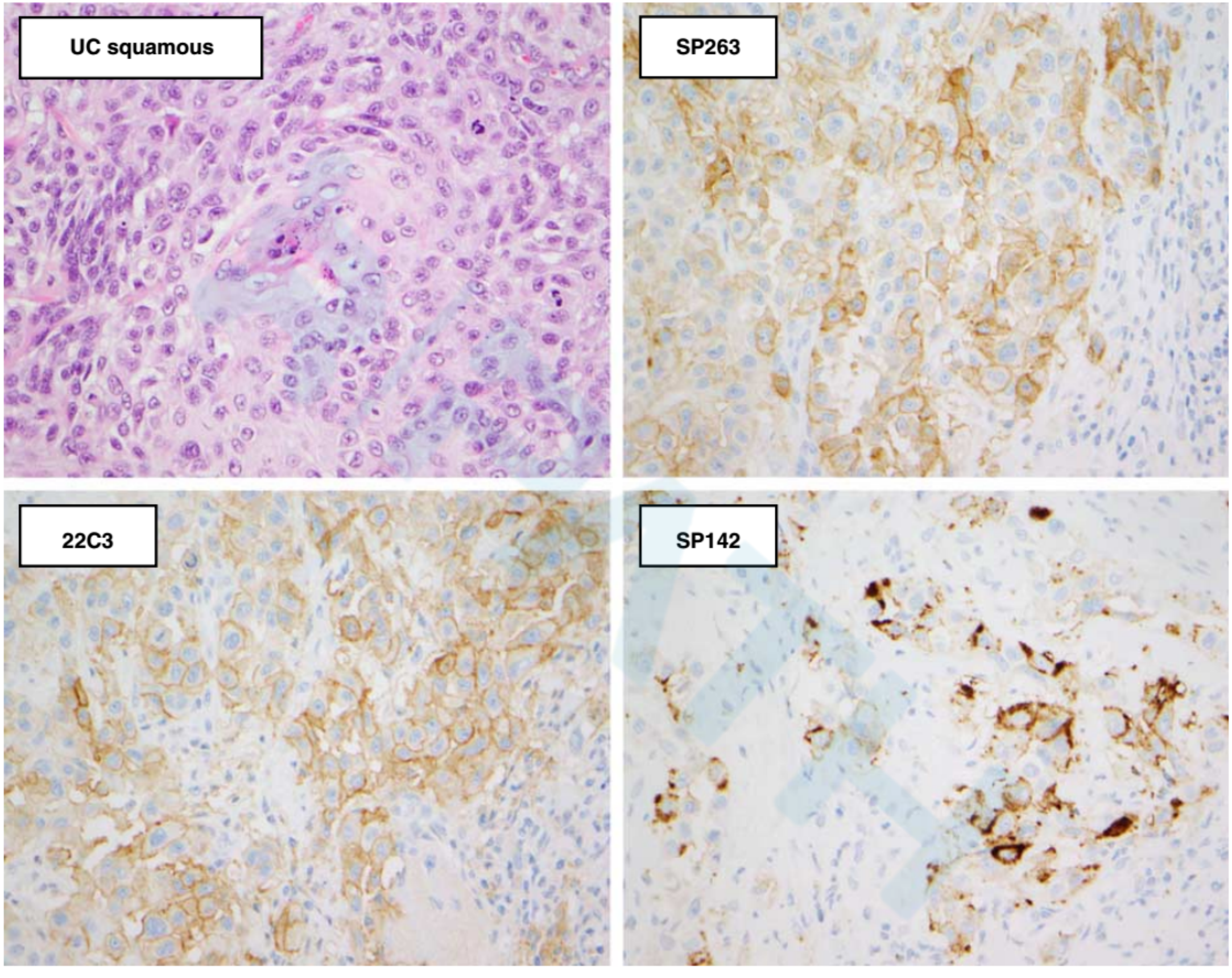


FIGURE 2. Staining characteristics of three PD-L1 clones in UC with squamous differentiation. In this example, membranous and focally circumferential staining of the SP263 and 22C3 PD-L1 clones in TC is evident. Clone SP142 shows a coarser and more granular TC immunoreactivity, which in some cases was difficult to discriminate from IC reactivity.

TABLE 2. Rates of Positive UCV Cases Using (Adapted) Criteria of Clinical Atezolizumab, Pembrolizumab, and Durvalumab Trials

Histologic Differentiation (n)	Atezolizumab Criteria* (n [%])			Pembrolizumab Criteria* (n [%])			Durvalumab Criteria (n [%])		
	IC2/3 (SP263)	IC2/3 (SP142)	IC2/3 (22C3)	TC +IC ≥ 10% (SP263)	TC +IC ≥ 10% (SP142)	TC +IC ≥ 10% (22C3)	TC or IC ≥ 25% (SP263)	TC or IC ≥ 25% (SP142)	TC or IC ≥ 25% (22C3)
Micropapillary (19)	13 (68)	6 (32)	8 (42)	11 (58)	6 (32)	7 (37)	6 (32)	1 (5)	3 (16)
Squamous differentiation (16)	14 (88)	12 (75)	14 (88)	14 (88)	12 (75)	14 (88)	1 (6)	1 (6)	2 (13)
Nested (14)	8 (57)	3 (21)	4 (29)	5 (36)	2 (14)	3 (21)	4 (29)	1 (7)	2 (14)
Plasmacytoid (14)	12 (86)	3 (21)	7 (50)	7 (50)	1 (7)	2 (14)	2 (14)	0 (0)	0 (0)
Small cell carcinoma (12)	6 (50)	2 (17)	5 (42)	4 (33)	2 (17)	3 (25)	0 (0)	0 (0)	0 (0)
Glandular differentiation (9)	8 (89)	5 (56)	8 (89)	7 (78)	2 (22)	4 (44)	2 (22)	2 (22)	1 (11)
Total (84)	61 (73)	31 (37)	46 (55)	48 (57)	25 (30)	33 (39)	15 (18)	5 (6%)	8 (10%)

IC2/3: immune cell reactivity in ≥ 5% of tumor-associated IC (*adapted IC score), TC+IC ≥ 10%: combined positive score as a sum of tumor cell and immune cell reactivity in ≥ 10% (*adapted positive score), TC or IC ≥ 25%: immunoreactivity in tumor cells or immune cells in ≥ 25%.

A significant proportion of UCV expresses PD-L1, as assessed by different antibodies, at different cutoff levels and methods of evaluation (TC and/or IC)

TABLE 3. Pairwise Correlation Analyses of the Different PD-L1 Antibody Clones

Pairwise Comparison in UCV			Pairwise Comparison UCV/NOS Same Case		
TC		**R**	**TC**		**R**
SP263	SP142	0.886	SP263 UCV	SP263 NOS	0.631
SP263	22C3	0.886	SP142 UCV	SP142 NOS	0.538
SP142	22C3	0.898	22C3 UCV	22C3 NOS	0.650
TC H-score			TC H-score		
SP263	SP142	0.887	SP263 UCV	SP263 NOS	0.632
SP263	22C3	0.886	SP142 UCV	SP142 NOS	0.491
SP142	22C3	0.901	22C3 UCV	22C3 NOS	0.654
IC			IC		
SP263	SP142	0.780	SP263 UCV	SP263 NOS	0.876
SP263	22C3	0.809	SP142 UCV	SP142 NOS	0.888
SP142	22C3	0.855	22C3 UCV	22C3 NOS	0.919

All clones showed strong agreement in a pairwise comparison, both in TC and IC (R-values: 0.780 to 0.901), which indicates that all 3 clones are potentially useful in the evaluation of PD-L1 expression in UCV

Moderate Agreement in UCV and UC NOS in the Same Cases

DISCUSSION

- A significant proportion of UCV expresses PD-L1
  - Different antibodies
  - Different cutoff levels
  - Differen methods of evaluation (TC and/or IC)
- UCV exhibited equal or higher PD-L1 expression on TC compared with that reported in the literature for classic/pure UC (4% to 30%)

DISCUSSION

- UC with squamous differentiation exhibited significantly higher mean TC reactivity rates
  - Consistent with those of a recent report on high rates of PD-L1 expression detected in pure squamous cell carcinoma of the bladder
  - The second line atezolizumab study (IMvigor210) that found PD-L1 expression on TC predominantly in tumors of the basal subtype according to the TCGA classification
- Staining characteristics of the SP142 clone are unique and less comparable
  - Consistent finding in different cancers
  - Lower mean number of positive TC detected
  - Expression pattern is coarsely granular “clumpy”

DISCUSSION

- Strong agreement (R-values: 0.780 to 0.901) among all 3 clones that were tested
  - Similar to findings from other cancers such as non–small cell lung carcinoma and malignant melanoma
  - As well as other reports in BC
  - Potentially useful expression details can be gleaned from any of the 3 PD-L1 antibody clones used in the evaluation of UCV
- The present study had some limitations
  - TMB
  - Other genes commonly mutated in BCs like FGFR3, TP53, and others
  - Not tested all available PD-L1 clones
  - Tested those clones that are most relevant in BC

CONCLUSION

- PD-L1 expression in a high percentage of UCV
- Comparable results using three different and readily available clones
- Provide rationale and further support to include UCV in clinical anti-PD-1/PD-L1 trials

THANK YOU