PD-L1 expression with QR1 and E1L3N antibodies according to histological ovarian cancer subtype: A series of 232 cases

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ABSTRACT

Therapeutic strategies for epithelial ovarian cancers are evolving with the advent of immunotherapy, such as PD-L1 inhibitors, with encouraging results. However, little data are available on PDL-1 expression in ovarian cancers. Thus, we set out to determine the PD-L1 expression according to histological subtype. We evaluated the expression of two PD-L1 clones – QR1 and E1L3N – with two scores, one based on the percentage of labeled tumor cells (tumor proportion score, TPS) and the other on labeled immune cells (combined proportion score, CPS) in a consecutive retrospective series of 232 ovarian cancers. PD-L1 expression was more frequent in high grade serous carcinoma (27.5% with E1L3N clone and 41.5% with QR1 clone), grade 3 endometrioid carcinoma (25% with E1L3N clone and 50% with QR1 clone), and clear-cell carcinomas (27.3% with E1L3N clone and 29.6% with QR1 clone) than other histological subtypes with CPS score. Using the CPS score, 17% of cases were labeled with E1L3N *vs* 28% with QR1. Using the TPS score, 14% of cases were positive to E1L3N *vs* 17% for QR1. For TPS and CPS, respectively, 77% and 78% of the QR1 cases were concordant with E1L3N for the thresholds of 1%. Overall and progression-free survival between PD-L1 positive and PD-L1 negative patients were not different across all histological types, and each subtype in particular for serous carcinomas expressing PD-L1. Expression of PD-L1 is relatively uncommon in epithelium ovarian tumors. When positive, usually <10% of tumor cells are labeled. QR1 clone and CPS appear the best tools to evaluate PD-L1 expression.

Key words: Ovarian cancer; PD-L1 antibody; immunochemistry; histological subtype.

Introduction

Ovarian cancer (OC) is the seventh most frequent cancer diagnosed worldwide, with more than 240,000 new cases per year, and the eighth leading cause of cancer mortality with 152,000 deaths recorded in 2012.[1] In France, OC is the fourth leading cause of cancer mortality in women with more than 3,100 deaths in 2017 (INCa).[2] Recently, Coburn *et al.* evaluated the change in OC incidence worldwide showing an increase in Eastern/Southern Europe and Asia and a decrease in Northern Europe and North America.[1].

Ovarian carcinomas include five major and distinct histological types with different characteristics and prognoses: high-grade serous carcinoma (HGSC, 70%), low-grade serous carcinoma (LGSC, <5%), endometrioid carcinoma (EC, 10%), clear-cell carcinoma (CCC, 10%) and mucinous ovarian carcinoma (MOC, 3%).[1] HGSC and CCC are of poorer prognoses.[3,4] The Cancer Genome Atlas (TCGA) project identified genetic abnormalities or susceptibility alleles for the most common OCs and suggested several subtypes, including an immunoreactive subtype characterized by expression of the T-cell chemokine ligands more specifically identified in HGSC.[5,6] Several studies have focused on inflammatory infiltrate, T cells and tumor-associated macrophage (TAM) expression on both OC cell lines and *in vivo*.[7] PD1 is implicated in programmed cell death and PD1/ PD-L1 is an important immune checkpoint in proliferation and development tumor.[8] Tumor cells with PD-L1 transmembrane protein bind to the PD-1 receptor of T lymphocytes and inactivate them.[8] Treatments that block the PD-1 receptor or the PD-L1 protein (anti-PD-1 or anti-PD-L1) can reverse the inactivation of T lymphocytes. These immune cells can subsequently have a tumor cell action.[9] It has recently been suggested that the presence of intratumoral inflammatory infiltration associated with PD-L1 expression influences survival in HGSC[10,11] with clinical trials using anti-PD-L1 immunotherapy such as pembrolizumab and avelumab showing promising results.[6,12-15] Two recent meta-analysis suggested that PD-L1 expression was not linked to tumor histology, overall survival (OS), and progression-free survival (PFS), but that PD-L1 mRNA expression was closely correlated with poor PFS.[16,17] However, immunohistochemistry evaluation of PD-L1 before treatment was not always performed in these studies. Furthermore, in contrast to non-small-cell lung cancer, there is currently a lack of a consensual interpretation score for PD-L1 in OCs. Moreover, the published threshold of positivity is variable[18] giving rise to extensive debate about the prognostic and predictive values of response to treatment of PD-L1 expression.[19,20] To date, there are no recommendations for evaluating immunohistochemical PD-L1 expression for targeted therapy in first line treatment of OCs (INCa).[2]

So far, no consensus exists on the best antibodies to use in ovarian cancer. Therefore, we decided to use the two PD-L1 antibodies (QR1 clone (Diagomics) and E1L3N (Cell Signaling)), that are consensual for other tumors such as lung cancer and routinely used. Therefore, the aims of this work were to evaluate PD-L1 expression with two antibodies and with two scores, in a large cohort of women with OC and to determine whether PD-L1 expression is correlated with clinicopathological features and prognosis.

Materials and Methods

Patients/population

The pathologic database of the Department of Pathology of Tenon University Hospital (Assistance Publique des Hôpitaux de Paris, Sorbonne University Paris) was searched for all cases of histologically diagnosed OCs (including primary OCs with and without synchronous EC) from January 2005 to July 2017. All the patients had been treated at the ovarian cancer expert center in Tenon University Hospital. Exclusion criteria were tumor samples from other hospitals (cases sent for review) absence of formalin fixed and paraffin embedded (FFPE) tumor block in archives, no primary tissue tumor available, or absence or insufficient FFPE tumor material. Tissue samples were excluded after neoadjuvant chemotherapy. Borderline ovarian tumors were excluded. We recorded the clinicopathologic features, including histologic subtypes, tumor stage and grade, lymph node involvement, recurrence-free survival (RFS) and overall survival (OS) from the medical and pathologic reports. For each case, representative tumor tissue samples of the OC were used all of which had been routinely FFPE.

All the samples (whole slides) were reviewed by a gynecopathologist who reassessed the histologic diagnosis. FFPE blocks corresponding to primary OC were evaluated by routine hematoxylin and eosin (H&E)-stained sections. One to three additional representative blocks were selected in case of contralateral OC.

Tumor stage was classified according to the International Federation of Gynecology and Obstetrics (FIGO 2014). If necessary, the histological type and stage have been reclassified taking into account the 2014 WHO classification. This has sometimes required additional immunohistochemical study, particularly in cases prior to 2010.

Tissue microarrays

The tissue samples were used for the tissue microarrays (TMA). The arrays were constructed with a 1 mm punch on semi-automated Tissue Arrayer MiniCore® (Excilone® Alphelys®). Each selected/donor block was arrayed in triplicate including three tumor cores. The grid layout was designed using TMA designer software® and converted into a Microsoft Excel file. A 3 µm H&E-stained section was reviewed to confirm the presence of tumor sample. A histospot was considered unsuitable for analysis when it was completely absent, contained no tumor tissue (sampling error), or contained too few tumor cells for analysis (less than 10% of the surface area occupied by tumor cells was considered uninformative). Two cores with tumor cells were considered to be available for evaluation.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 5-µm-thick whole tissue sections of TMA blocks in a Bond-III automated immunostainer (Leica Microsystems, Bannockburn, IL). We used two PD-L1 rabbit monoclonal antibodies: QR1 clone (Diagomics®, Berlin, Germany ;1:100) and E1L3N clone (Cell signaling®, , Leiden, Netherlands;1:100). The two antibodies were analyzed by the Ultraview Universal DAB Detection Kit (Roche Diagnostics®). Beforehand, the protocol was finalized using appropriate positive and negative controls for each antibody according to the manufacturer's recommendations (tonsil tissue). Human tonsil tissue was used as a positive and negative control: positive lymphocyte cells and negative epithelial cells.

IHC interpretation - TMA

Two pathologists (CEM and JV) scored the PD-L1 staining independently. In the case of a discordant result between the two observers, the slides were reviewed in a dual-headed microscope and a consensus was established. Two scoring algorithms were used: the combined positive score (CPS) and the tumor proportion score (TPS). CPS is based on the number of PD-L1 stained cells (tumor

cells, lymphocytes and macrophages) related to the number of tumor cells (in percentage). CPS = PD-L1 positive cells (tumor cells+lymphocytes+macrophages)/ (tumors cells) x 100. Positive cells were defined by complete or incomplete circumferential membranous staining whatever the intensity. TPS is based on the percentage of positive tumor cells. TPS = PD-L1 positive tumors cells /tumors cells x100. Tumor cell expression was considered positive when tumor cells showed complete or incomplete circumferential membranous staining whatever the intensity. All the fields of each TMA spot were analyzed at low magnification. We counted the number of labelled and unlabeled tumor cells and labelled immune cells on 10 fields (high power magnification, x400), in the areas most represented in tumor cells. We established an average for each tumor analyzed for CPS and TPS Score.

Statistical analysis

Concordance of IHC expression between the two antibodies

was expressed as a kappa statistic. Kappa statistics measures the agreement between two observers (interobserver).[21] A kappa value of 1 indicates perfect concordance, 0 means agreement at the level of chance, and negative values agreement worse than chance agreement. A kappa value of 0 to 0.2, 0.21 to 0.4, 0.41 to 0.6, 0.61 to 0.8, and 0.81 to 1 was considered slight, fair, moderate, substantial, and almost perfect agreement, respectively. OS and RFS were estimated from log-rank test, Kaplan–Meier curves and, cumulative-incidence methods. A p-value of 0.05 was considered to denote significance. All statistical analysis was performed on Review Manager (RevMan, IOS, version (5.3).

Results

Characteristics of the study population

Between 2005 and 2017, of the 184 patients with OC included in the database, 48 had bilateral OC. Among them, 13 patients had synchronous or metachronous uterine carcinoma. Forty-four tissue samples were excluded after neoadjuvant chemotherapy. Therefore, the study population included 232 ovarian tumors. After reviewed and

Table 1. Epidemiologic and histologic characteristics of the population.

Variable		Patients (percentage)
Median age at diagnosis (year)	56.32	±13 SD
Hormonal status		
Menopausal		118 (63.6%)
Non menopausal		53 (28.8%)
Body mass index (kg/m^2)		19.6±4.1
BRCA mutation		
Absent		88 (47.8%)
Present		22 (12%)
FIGO stage		
I		45 (22.9%)
II		25 (14.4%)
III		92 (52.9%)
IV		12 (6.9%)

Table 2. Distribution of histologic subtype of the 184 patients with ovarian tumors.

Histologic subtype	Number of cases (%) (total n=184)
Serous carcinoma	93 (50.5%)
Low grade	16
High grade	77
Endometrioid carcinoma	37 (20.1%)
Grade G1	12
Grade G2	12
Grade G3	13
Clear cell carcinoma	25 (13.6%)
Mucinous carcinoma	12 (6.5%)
Expansive type	4
Seromucinous carcinoma	6 (3.3%)
Carcinosarcoma	4 (2.2%)
Malignant Brenner tumor	1 (0.5%)
Sex cord and stroma tumor	6 (3.3%)
Or germ cell tumor	
Immature teratoma	1
Granulosa tumor	3
Sertoli-Leydig tumor	1
Embryonal carcinoma	1

Table 3. Distribution of PD-L1 expression in ovarian cancer using E1L3N antibody and QR1 antibody according to CPS and TPS scores.

Score (TPS or CPS)	E1L3N antibody number of cases		QR1 antibody number of cases	
	TPS	CPS	TPS	CPS
0 (negative)	148	142	181	155
0.5	9	7	22	37
1	12	8	7	3
2	3	10	1	9
3	0	2	0	4
5	1	2	3	3
7	0	0	1	2
8	1	0	0	0
10	2	3	0	2
15	2	2	3	0
17		1	0	0
20	1	0	0	2
25	0	1	0	1
30	0	0	1	0
38	0	0	0	1
40	0	1	0	0
50	0	0	1	0
60	0	0	0	1
80	2	0	0	0
85	0	1	0	0
100	0	1	0	0
NR	51	51	12	12
Total	232			

TPS, tumor proportion score = PD-L1 positive tumors cells /tumors cells x100; CPS, combined proportion score = PD-L1+ cells (tumor cells + lymphocytes+ macrophages)/ (tumors cells) x 100; NR, not representative.

additional immunohistochemical study, 23 mixed carcinomas diagnosed before 2014 were reclassified mainly as high-grade serous carcinoma and seromucinous carcinoma according to 2014 WHO classification. Clinical and histological parameters are summarized in Tables 1 and 2. The most common histologic subtypes were serous carcinomas (50.5%) including 82.8% of high-grade serous carcinoma (HGSC) and 17.2 % of low-grade serous carcinoma (LGSC). EC was the second most common subtype (20.1%). CCCs were diagnosed in 13.6% of patients. Finally, sex cord stroma tumor (SCST) and germ cell tumor (GCT) constituted 3.3% of patients.

Qualitative expression of PD-L1 using E1L3N and QR1 antibodies

PD-L1 labeling was mostly weak overall. PD-L1 expression was represented as both complete and incomplete circumferential membranous staining of tumor cells as shown in Figure 1. Labeling was absent for 64% of cases with E1L3N and 78% with QR1 (Figure 1 E,F). A few cases (14% with E1L3N and 17% with QR1) showed expression of PD-L1 with the two antibodies (Figure 1 B,C,G,H). PD-L1 expression was not evaluable in 22% of cases with E3L1N and in 5% of cases with QR1. Immune cells were scarce and rarely labeled (Figure 1C) with no difference for the two antibodies. Interobserver agreement test for qualitative PD-L1

expression was substantial (Kappa = 0.77) for both E1L3N and QR1 antibodies. Interobserver discrepancies corresponded mainly to equivocal cases (6%).

Immunochemistry expression of PD-L1 according to antibodies and histologic subtypes

Distributions of PD-L1 expression according to TPS and CPS with the E1L3N antibody are presented in Table 3.

TPS of PD-L1 expression using the E1L3N antibody

IHC study was inconclusive with the E1L3N antibody in 22% of the OC samples. TPS was negative in 64% of the cases (148/232) and positive in 14% (33/232). Of the positive cases, the staining was mainly weak concerning less than 10% of tumor cells (Table 4). TPS with 10%-50% of labeled cells was observed in five cases (2%). TPS with more than 50% of labeled cells was observed in only two tumors (1% of cases).

CPS of PD-L1 expression using the E1L3N antibody

CPS was negative in 61% of cases (142/232) and positive in 17 % (39/232). The staining was mainly weak concerning less than 10% of tumor cells in 13% (Table 4). CPS with 10%-50% of labeled cells was observed in eight cases (3%) while cases with
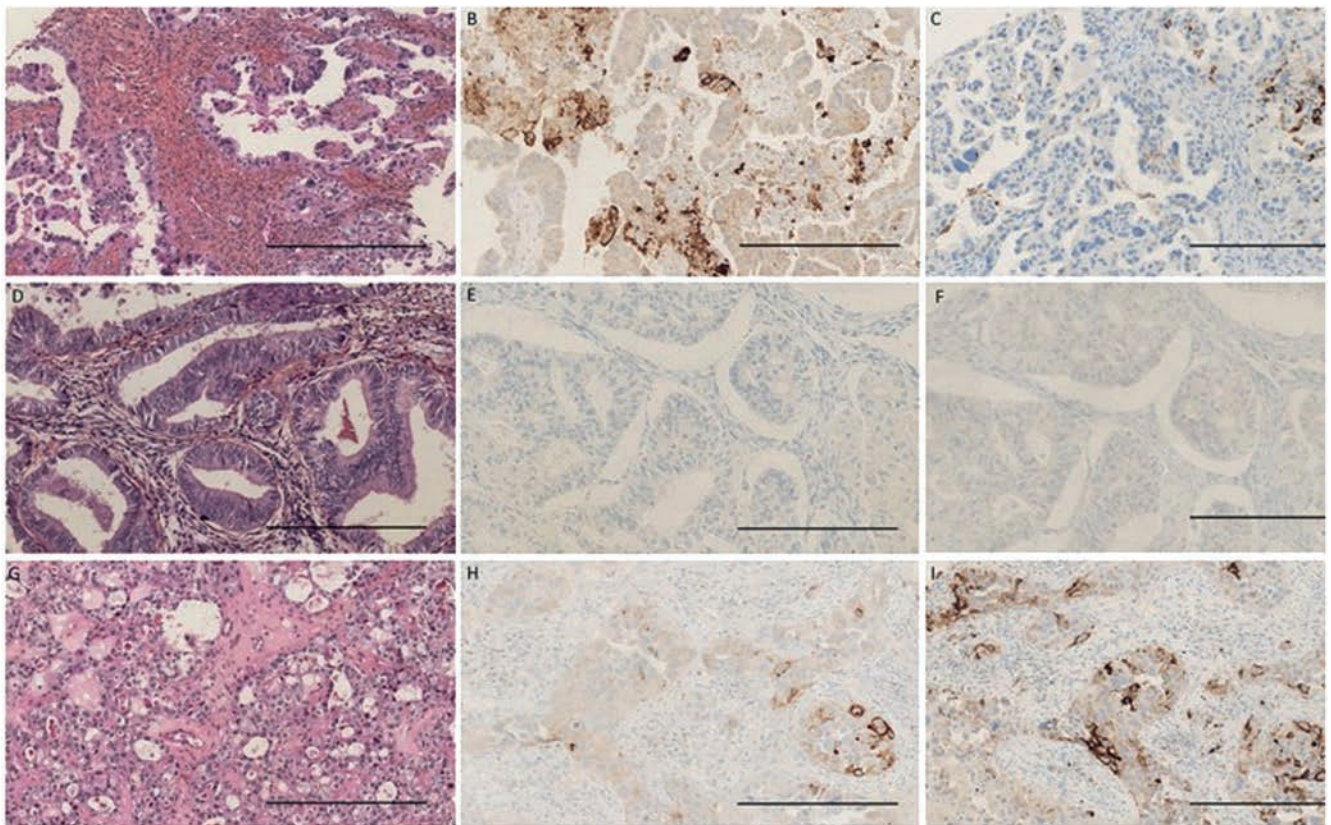

Figure 1. Immunochemical expression of PD-L1 in ovarian carcinomas. The tumors examined show very low peri- or intratumoral inflammatory infiltration. This may explain why the CPS score is lower compared with the TPS score, related to the low proportion of immune cells present in these tumors. A-C) High Grade Serous Carcinoma, magnification x 200. A) Hematoxylin Eosin staining; B) expression of E1L3N: strong and heterogeneous membrane staining of >10% cells (TPS = 15, CPS = 25); C) expression of QR1: focal and rare membrane staining of <10% cells including a majority of immune cells (TPS= 5; CPS = 7). D-F) Grade 2 Endometrioid carcinoma, magnification x200. D) Hematoxylin&Eosin staining (E) lack of expression of E1L3N (F) lack of expression of QR1(TPS = 0, CPS = 0). G-I) Clear cell carcinoma, magnification x 200 (G) Hematoxylin&Eosin staining (H&E) expression of E1L3N <10% tumor cells (TPS = 8; CPS=10) (I) expression of QR1 ≥50% tumors cells (TPS = 30; CPS=38). Interpretation is easy in the majority of cases with the two antibodies used as shown in these pictures. Scale bars: 250 m.

CPS over 50% were observed in two tumors (1% of cases). An excellent correlation was noted between TPS and CPS (Kappa=0.89).

TPS and CPS of PD-L1 expression using the E1L3N antibody according to histologic subtypes

A variation in PD-L1 expression was observed according to histologic subtypes independently of the IHC score used. Using TPS, 27.3% of CCCs expressed PD-L1, 22% of SCs, 14.3% of ECs and 14.3% of seromucinous carcinomas. TPS was absent in MOCs (Table 5). Using CPS, 27.3% of CCCs expressed PD-L1, 23% of SCs, 20% of ECs, and 14.3% of seromucinous carcinomas. CPS was also negative in MOCs. Two of the five carcinosarcomas expressed PD-L1 with TPS or CPS score (Table 5). HGSC expressed significantly more PD-L1 than LGSC (27.5% vs 5% with CPS score and 22.5% vs 5% with TPS score). G2 or G3 grade EC also expressed more PD-L1 than G1 grade endometrioid carcinomas (25% vs 13.3% with CPS score and 27.3% vs 12.5%).

TPS and CPS of PD-L1 expression using the QR1 antibody

Distributions of PD-L1 expression according to TPS and CPS with the QRI antibody are presented in Tables 3 and Table 5.

TPS of PD-L1 expression using the QRI antibody

IHC study was inconclusive with the QR1 antibody in 5.2% of cases. TPS was negative in 78% of the cases (181/232) and positive in 17% (39/232). Of the positive cases, the staining was mainly weak concerning less than 10% of tumor cells in 15% (Table 2). The staining was mainly weak (Table 5). TPS with 10%-50% of labeled cells was observed in four cases (2%). TPS with more than 50% of labeled cells was observed in one tumor (1% of cases).

CPS of PD-L1 expression using the QRI antibody

CPS was negative in 67% of the cases (155/232) and positive in 28 % (65/232). Of the positive cases, the staining was mainly weak concerning less than 10% of tumor cells in 25% (Table 2). CPS with 10%-50% of labeled cells was observed in 6 cases (3%) while only one case showed a staining of more than 50% of the tumor cells.

TPS and CPS of PD-L1 expression using the QR1 antibody according to histologic subtypes

As for the E1L3N antibody, a variation in QR1 expression was observed according to histologic subtypes for both scores. Using the TPS score, 18.5% of CCCs expressed QR1, 22.4% of SCs, 13.35% of ECs, and 8.3% of MCs (1/12) (Table 5. Four cases

(4/27; 15%) of CCCs had TPS score ³10%. With the CPS score, 29.6% of CCCs expressed QR1, 37.9% of SCs, 22.2% of ECs, 8.3% of MOCs (1/12).

TPS and CPS were negative in carcinosarcomas and sero-mucinous carcinomas (Table 5). HGSC expressed significantly more PD-L1 than LGSC (41.5% vs 22.7% with CPS score and 24.5% vs 13.6% with TPS score). G2 or G3 grade EC also expressed more PD-L1 than G1 grade endometrioid carcinomas (50% vs 13.3% with CPS score and 31.3% vs 6.7%).In tumors with a CPS over 10%, 2.2% of ECs were positive for PD-L1 (1/45), 1.7% of SCs (2/116) and 15.4% of CCCs (4/27). The proportion of tissue samples not evaluable for either TPS or CPS was significantly lower for the QR1 than the E1L3N antibody (Table 5). For TPS, the percentage of tumors labeled was similar for both QR1 and E1L3N antibodies taking into account the number of available cases (respectively 17.7% vs 18.2%). For CPS, a higher proportion of tumors was labeled using QR1 (29.5% vs 21.5%) than for E1L3N antibody (p=0.0029).

Disease free survival, overall survival and PDL1 expression

There was no difference in OS between patients expressing PD-L1 (n=43) and those with no PD-L1 expression (n=85); (p=0.16; Figure 2).

In the whole population, no difference in DFS was found according to the PD-L1 status (p=0.25; Figure 3).

Three-year OS was 81.8% vs 81.8%, for PD-L1- and PD-L1+ patients, respectively. Five-year OS was 62% vs 74%, for PD-L1- and PD-L1+ patients, respectively. No difference in OS or DFS was noted according to histologic subtype (Figure 3).

Discussion

The present study demonstrates that a relatively low percentage of OCs express PD-L1 with variations according to histological subtypes. Moreover, the QR1 antibody is associated with a lower rate of non-evaluable tissue samples compared to E1LN3. Finally, CPS appears to be more sensitive than TPS to detect PD-L1 positive tumors.

Using qualitative evaluation of PD-L1, from two-thirds to three-quarters of samples were negative with no difference between the two antibodies. Using semi-quantitative evaluation of PD-L1, the proportion of tissue samples not evaluable was significantly lower when using the QR1 compared with the E1L3N antibody. Moreover, TPS gave a similar percentage of labeled tumors

Table 4. Semi-quantitative distribution of PD-L1 expression in ovarian cancer using E1L3N and QR1 antibodies according to CPS and TPS scores.

Score TPS or CPS	E1L3N antibody Number of cases				QR1 antibody Number of cases			
	TPS (n)	(%)	CPS (n)	(%)	TPS (n)	(%)	CPS (n)	(%)
0	148	64%	142	61%	181	78%	155	67%
0-10	26	11%	29	13%	34	15%	58	25%
10-50	5	2%	8	3%	4	2%	6	3%
50-100	2	1%	2	1%	1	0%	1	0%
NR	51	22%	51	22%	12	5%	12	5%
Total	232							

TPS, tumor proportion score = PD-L1 positive tumors cells /tumors cells x100; CPS, combined proportion score = PD-L1+ cells (tumor cells + lymphocytes+ macrophages)/ (tumors cells) x 100; NR, not representative.

for the two antibodies while CPS gave a higher proportion of tumors labeled by the QR1 antibody (p=0.0029). This suggests that PD-L1 expression in OC is more accurately evaluated with the QR1 antibody and the CPS score. However, specimens expressing PD-L1 in more than 10% of tumor cells were significantly higher with the E1LN3 clone suggesting greater sensitivity. When considering PD-L1 expression according to OC histological subtypes, we found a relatively high expression in HGSCs followed by CCCs and G3 ECs. The comparison of our data with those of previous studies is difficult as other studies mainly focus on one OC histological subtype. Wanq *et al.* confirmed that PD-L1 expression in HGSC was uncommon and often focal in 24.3% of cases (26/81) using a 5% threshold of labeled tumor cells (TPS score).[10] They showed a significant association between PD-L1 and CD8+ tumor infiltrating lymphocyte expression.[10] Another study showed PD-L1 expression in 23.6% of HGSC cases (55/233) with a 1% threshold of labeled tumor cells.[22] Schmoeckel *et al.*'s study involving 288 OCs showed PD-L1 expression (>1% of tumor cells) in 19.5% (55 HGSC and two EC).[22]

Besides the low expression of PD-L1 in OC, another issue is the absence of consensus about how to evaluate PD-L1 expression and the definition of a positive threshold. Several studies use a threshold of 5% while others choose 1% as in pulmonary pathology.[23-26] Some studies use a semi-quantitative evaluation reporting a percentage of positive cells,[24] while others only report the staining of tumor cells, or both tumor and inflammatory cells staining in stroma.[25,26] A meta-analysis showed a wide variation (11% to 88%) in the proportion of ovarian tumors expressing PD-L1.[2] These apparent variations could be explained by differences according to the histological subtypes, the scoring method, and the

Table 5. Antibodies E1L3N and QR1 cell signaling; score according to histological subtype.

E1L3N antibody	CPS immunoscore				NR	TPS score (%)			
	0	0-10	10-50	50-100		0	0-10	10-50	50-100
Serous carcinoma	77	16	6	1		81	15	3	1
High grade serous carcinoma	58	15	6	1	19	62	14	3	1
Low grade serous carcinoma	19	1			3	19	1		
Endometrioid carcinoma	28	7				30	5		
Grade G1	13	2			1	14	1		
Grade G2	6	2			6	7	1		
Grade G3	9	3			5	9	3		
Clear cell carcinoma	16	3	2	1	5	16	3	2	1
Mucinous carcinoma	9				5	9			
Seromucinous carcinoma	6	1			1	6	1		
Carcinosarcoma	3	2			2	3	2		
Malignant Brenner tumor	1					1			
SCST or GCT	2					2			
Granulosa	2				1	2			
Immature teratoma					1				
Embryonal carcinoma					1				
Sertoli-Leydig tumor					1				
Total	142 61%	29 13%	8 3%	2 1%	51 22%	148 64%	26 11%	5 2%	2 1%
QR1 antibody	**0**	**0-10**	**10-50**	**50-100**	**NR**	**0**	**0-10**	**10-50**	**50-100**
Serous carcinoma	72	42	1	1		90	25		1
High grade serous carcinoma	55	37	1	1	5	71	22		1
Low grade serous carcinoma	17	5			1	19	3		
Endometrioid carcinoma	35	9	1			39	6		
Grade G1	13	2			1	14	1		
Grade G2	14					14			
Grade G3	8	7	1		1	11	5		
Clear cell carcinoma	19	4	4	0		22	1	4	0
Mucinous carcinoma	11	1			2	11	1		
Seromucinous carcinoma	8					8			
Carcinosarcoma	5	0			2	5	0		
Malignant Brenner Tumor	1					1			
SCST ot GCT	4	2				5	1		
Granulosa	2	1				2	1		
Immature teratoma	1					1			
Embryonar carcinoma	1					1			
Sertoli-Leydig tumor		1				1			
Total	155 67%	58 25%	6 3%	1 0%	12 5%	181 78%	34 15%	4 2%	1 0%

antibodies used. In the current study, two quantitative scores were used as for lung, head and neck pathologies. PD-L1 expression is well documented (sensitivity, specificity) and used in routine practice in lung pathologies[27] and the International Association for the Study of Lung Cancer (IASLC) has published an atlas to structure and illustrate PD-L1 IHC analysis.[28] Only one study, published in 2020, has compared the use of QR1 with E1L3N, 22C3, and SP263 antibodies in lung cancer and shows no difference for routine analysis.[29] We used QR1 in practice for the evaluation of PD-L1 in lung cancer for several years, and offers good price-quality ratio. Finally, the IASLC indicated that E1L3N has the highest sensitivity for membranous expression when compared with SP142, 9A11, 015, and 7G11 which is why we chose this antibody in the current study. CPS in squamous cell carcinoma of the head and neck is approved by the FDA for the evaluation of PD-L1 (combined positive score ≥1) to indicate treatment with pembrolizumab.[30] However, for breast cancer, the FDA approves atezolizumab treatment when PD-L1 stained tumor-infiltrating immune cells of any intensity covers ≥1% of the tumor area using the SP142 clone.[31] We chose to evaluate QR1 in this study because of data showing that the number of cDNAs (SC1, QR1, hevin) are similar to the secreted protein acidic and rich in cysteine (SPARC) protein, a matricellular protein that regulates cell adhesion, cell cycle, and matrix assembly and remodeling.[32] Moreover, Tumbarello et al. demonstrated that SPARC regulates Transforming Growth Factor Beta Induced (TGFBI) extracellular matrix deposition and paclitaxel response in OC cells.[33] Recently, John et al. found regulation of the bi-directional cross-talk between ovarian cancer cells and adipocytes by SPARC.[34]

Another finding of the present study is the relatively frequent expression of PD-L1 in CCC. Our results are in agreement with those of Li et al. reporting 21.1% of CCC positive for PD-L1(20/95) using both TMA with antibody PD-L1 clone SP263 and

a semiquantitative immunoreactivity score.[35] Zhu et al. found PD-L1 positivity in 48% of CCC (52/122) using a positivity threshold ≥10% (using an Abcam PD-L1 antibody).[19] A recent small series of 30 CCCs showed PD-L1 expression in tumor cells or immune cells (CPS equivalent score) in 44% of CCC with microsatellite stability (MSS) and in all cases of CCC with microsatellite instability (MSI) (3 cases).[36] In addition, the expression of PD-L1 in HGSC appears to be linked to BRCA 1-2 mutations.[37,38] Finally, several studies have underlined that CCC with MSI and HGSC with BRCA1-2 mutations overexpress PD-L1 with a high intratumoral infiltrate suggesting a potential increased sensitivity to PD-1/PD-L1 inhibitors.[37] In our series, patients with documented BRCA status, no significant association was found between the BRCA mutation and PD-L1 expression (p=0.75).

From a clinical point of view, few targeted therapies are available in OC. Recently, anti-PARP has been shown to improve both RFS and OS of patients with somatic and germline BRCA 1-2. Matulonis et al.'s trial targeting OC with positive PD-L1 expression, reported objective response rates of 4.1% for CPS <1, 5.7% for CPS ≥1, and 10% for CPS ≥10.[39] Others recent studies suggested that the presence of PD-1/ PD-L1 positive tumor-infiltrating immune cells was a prognostic value in OC [11.] An original study suggested that higher PD-1 level in the plasma could be a predictive biomarker and predicted poor survival of OC patients.[40] In addition, a recent study by Kim et al. showed that PD-L1 expression levels in tumor cells, intraepithelial tumor-infiltrating lymphocytes, and stromal tumor-infiltrating lymphocytes were correlated with a better prognosis in SC.[41] Interestingly, our results showed a high PD-L1 expression in CCC, implying poor prognosis, supporting the analysis of PD-L1 expression in this specific histological subtype with a potential benefit of targeted therapy. However, PD-L1 expression is not always a marker of response. Xue et al., showed that addition of PARP inhibitor in vitro appears
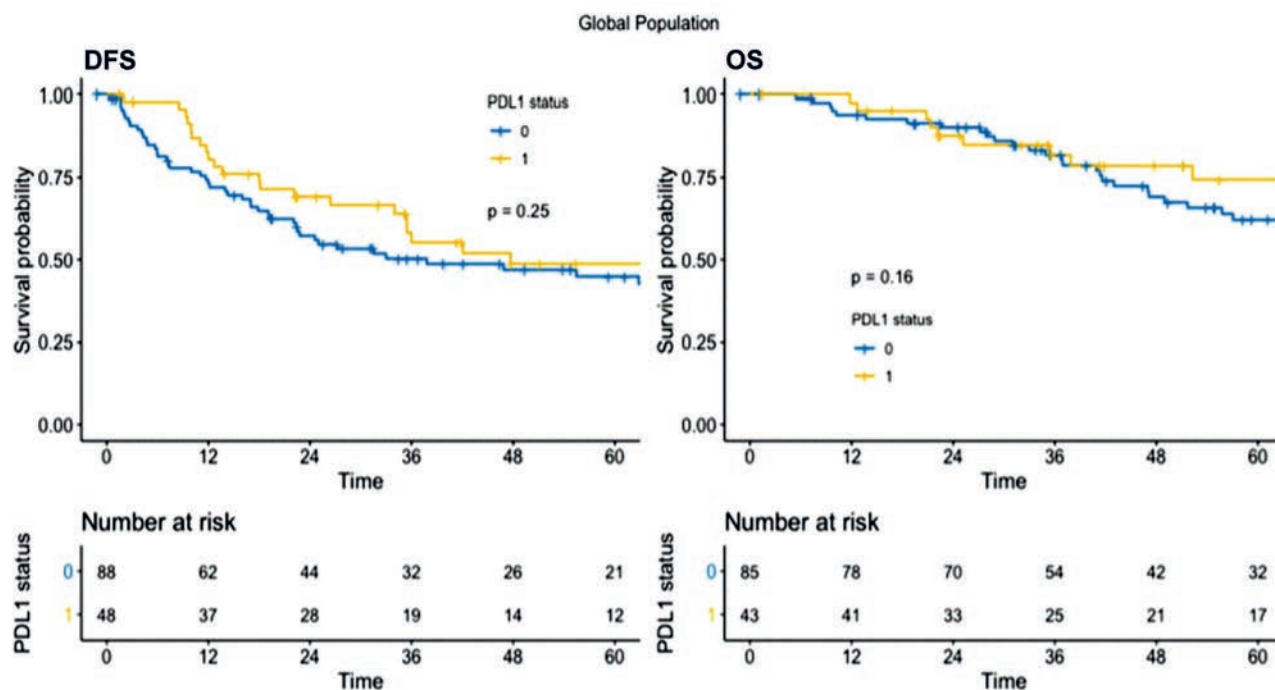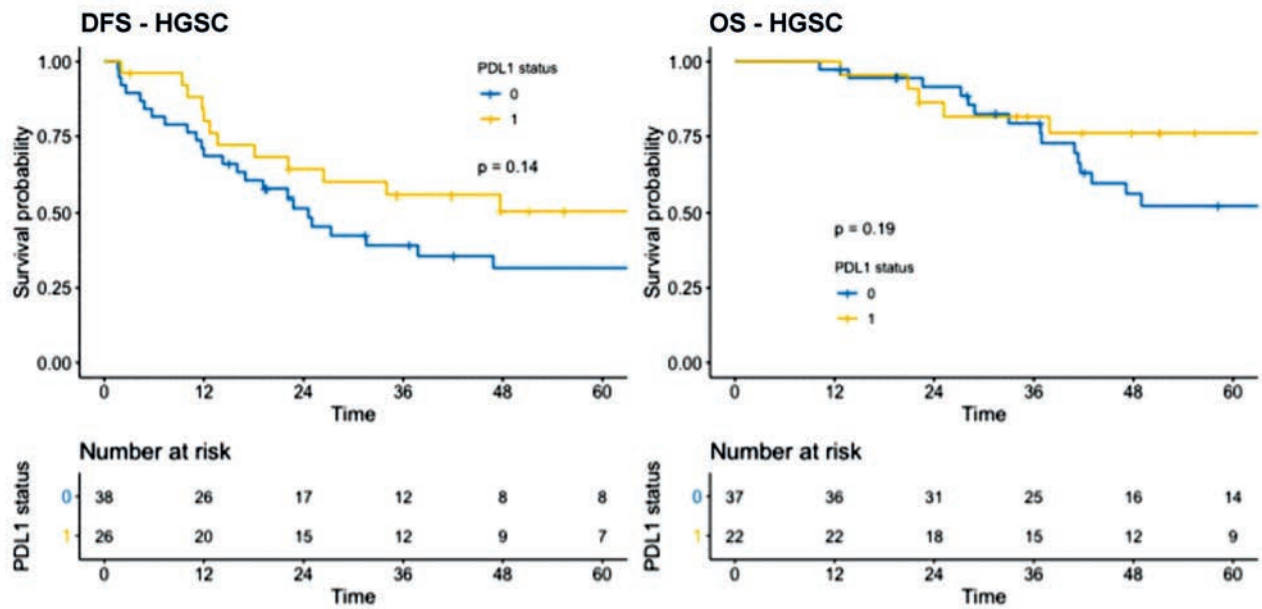

Figure 2. Progression-free survival (PFS) and overall survival (OS) of patients expressing PD-L1 (yellow line) and those with no PD-L1 expression (blue line).
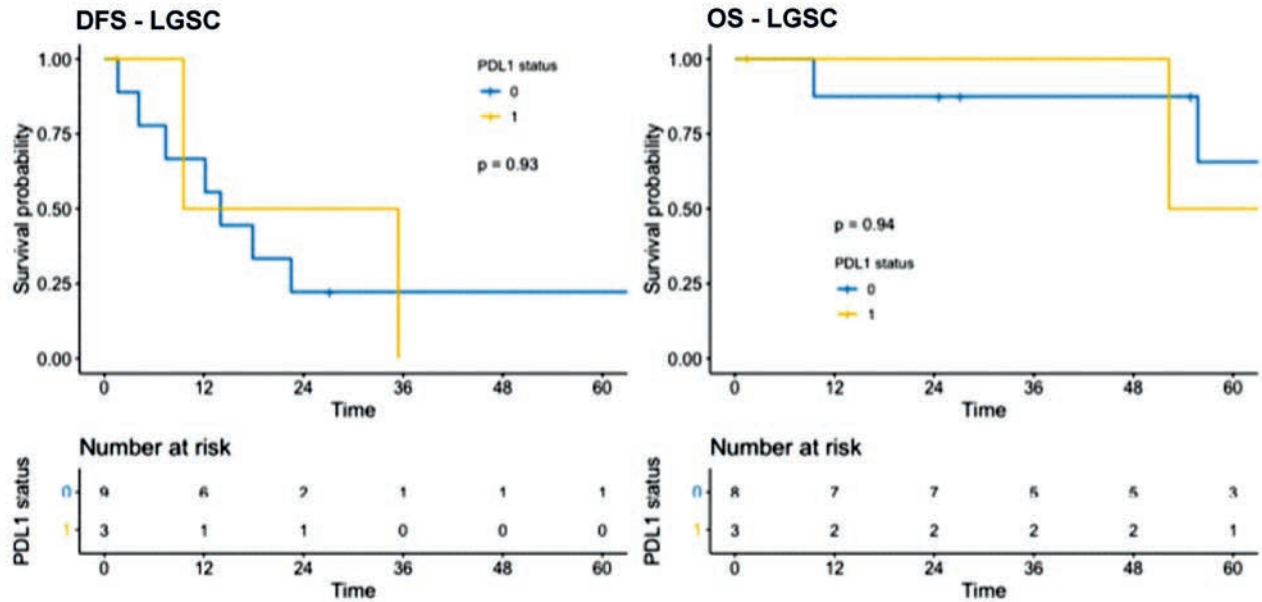
Figure 3. Disease-free survival (DFS) and overall survival (OS) of patients expressing PD-L1 (yellow line) and those with no PD-L1 expression (blue line), according to the histological type.

to increase PD-L1 expression in OC cell lines through the Chk1 pathway. This information could suggest an interest in combining PD1/PD-L1 inhibitor and PARP inhibitor treatment.[42]

In breast cancer, it has been demonstrated that two-thirds of patients with PD-L1 positive tumors have a high response to targeted therapy but also 16.7 % of PD-L1 negative patients.[43]The ongoing "ATALANTE" trial is investigating the efficacy of atezolizumab compared to placebo in patients with PD-L1 positive OC compared to PD-L1 negative patients. Finally, other data found an increase in PD-L1 expression in the tumor or peritumoral stroma after neoadjuvant chemotherapy suggesting that anti-PD-L1 treatment might be administered in this specific setting.[44]Some limits of the present study have to be underlined. First, we only evaluated two antibodies to analyze PD-L1 expression. However, both antibodies were selected based on data supporting their use in other cancers and on the pathogenesis of OC. Second, the low number of OCs expressing PD-L1 in our series might be a potential bias linked to the TMA method and heterogeneous tumor labeling as shown in Figure 1. However this method was validated by Li *et al.* for the evaluation of PD-L1[35] especially in CCC (showing 21% of positive cases). Third, we failed to determine a threshold for PD-L1 expression imposing further studies in large series. Fourth, no relation was evaluated between MSI and PD-L1. Finally, further analysis is required to evaluate the relation between PD-L1 expression and survival in OC especially for CCC.

To conclude, the present study shows that PD-L1 expression is relatively rare in OC with often less than 10% of tumor cells labeled. The QR1 clone and CPS appear to be the best tools to evaluate PD-L1 expression. Further studies are required to evaluate the impact of PD-L1 expression on the management of OC according to histologic subtype.

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