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PD-L1 (22C3) Expression and Molecular Features of Tubo-Ovarian Carcinomas

Chang J[1#], Li P[2#], Harada S[1], Arend RC[3], Diffalha SAL[1], Kahn AG[1], Mackinnon A[1], Pesoli C[1], Siegal GP[1,#] and Huang X[1*,#]*

[1]*Department of Pathology, The University of Alabama at Birmingham, USA*

[2]*School of Nursing, The University of Alabama at Birmingham, USA*

[3]*Department of Obstetrics and Gynecology, The University of Alabama at Birmingham, USA*

[#]*These authors contributed equally to this work*

Abstract

Introduction: Immune checkpoint inhibitors have been used to treat multiple cancers. Programmed Death-Ligand 1 (PD-L1) Immunohistochemistry (IHC) and Tumor Mutational Burden (TMB) are two of the biomarkers relied upon to select patients for immune therapy. Conventional therapy for tubo-ovarian carcinomas cause significant toxic side effect, therefore, it is necessary to investigate other avenues of treatment such as modulation of the immune environment for benefit as potential targets for these types of cancer.

Methods: Of 138 patients diagnosed and treated for tubo-ovarian carcinoma between 2013 and 2021 were identified. We explored several strategies including stromal Tumor-Infiltrating Lymphocyte (sTIL) density, PD-L1 IHC, TMB, and molecular profiling.

Results: Our study showed sTIL density is positively associated with both PD-L1 positivity (P=0.0005) and TMB score (Rho=0.20, p=0.042). No significant association was identified between PD-L1 positivity and TMB score. PD-L1 positivity was found to be positively associated with *PTEN* mutation (p=0.0142).

Conclusion: PD-L1 positivity and TMB score should best be considered as independent biomarkers for anti-PD-1/PD-L1 inhibition in tubo-ovarian carcinoma. Clinical significance of positive association between PD-L1 expression and *PTEN* mutation needs further investigation.

Keywords: Tubo-ovarian carcinoma; sTIL; PD-L1; TMB; *PTEN*

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Correspondence:
Gene P Siegal, Department of Pathology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA,
E-mail: gsiegal@uabmc.edu
Xiao Huang, Department of Pathology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA,
E-mail: xiaohuang@uabmc.edu

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Introduction

The Tumor Immune Microenvironment (TIME) influences cancer initiation and progression [1,2]. New prognostic and predictive biomarkers have been developed building upon TIME characteristics [1,2]. Biomarkers utilized in this pursuit include stromal Tumor-Infiltrating Lymphocyte (TIL) density, Programmed Death-Ligand 1 (PD-L1) expression, Microsatellite Instability (MSI) and Tumor Mutational Burden (TMB) in selected cases, that, guide clinical management. Ovarian cancer is the fifth leading cause of cancer-related deaths among women in the United States [3]. Tubo-Ovarian Carcinomas (TOC) represents the largest subset of all ovarian neoplasms yet the relationship between these tumors and TIME have not been fully explored. To that end, this study investigated selected immune characteristics in this cohort including stromal TIL (sTIL) density, PD-L1 expression, TMB score, and genetic alterations, in TOC.

The presence of increased number of TILs is associated with better survival and elevated PD-L1 expression in subsets of patients with various types of cancers [4,5]. In ovary cancer, a positive correlation between sTILs and PD-L1 expression has previously been reported along with sTILs being a prognostic marker in post-chemotherapy tumors [6]. The association of sTILs with other TOC biomarkers is still unsettled.

The conventional treatment for advanced TOC is a combination of cytoreductive surgery and platinum-based chemotherapy [7,8], which causes significant toxic side effects with a poor 5-year survival of 45% [6]. Immune Checkpoint Inhibitors (ICIs) have been used for targeting various cancer types and PD-L1 Immunohistochemistry (IHC) assays were developed to select patients for such treatment. Confounding the significance of the findings, it has been well established that

a subset of cancer patients responded to ICIs regardless of PD-L1 expression [9,10]. TMB and MSI is additional U.S. Food and Drug Administration (FDA) approved predictive biomarkers for ICIs [10,11]. To date, none of these biomarkers has been prospectively validated in TOC [7,10]. Given limited treatment choices and poor outcome, it is necessary to better define the immune biomarkers in TOC.

Beyond PD-L1, TMB and MSI, there are other genomic biomarkers that have been offered as useful biomarkers to help select patients for ICIs [12]. Dissection of molecular regulatory networks of PD-L1 in various types of cancer has been described [13,14]. Knowledge of genomic regulation of PD-L1 expression in TOC remains limited.

Methods

Study cohort

Patients diagnosed and treated for TOC at our institution between 2013 and 2021 were identified through a UAB Institutional Review Board (IRB)- approved retrospective protocol IRB-300005038. Eligible patients had key demographics along with their PD-L1 IHC studies collected along with targeted Next-Generation Sequencing analysis (NGS).

Clinical features

Patients' demographic and clinical characteristics were obtained from the electronic medical record/tumor registry of our institution, including age, date of birth, date of diagnosis, FIGO stage, date of first course of chemotherapy, date of surgery, and Overall Survival (OS).

Histology

Histopathology slides were reviewed independently by two pathologists (XH and JC). Ovarian carcinomas were classified according to the 5th edition of World Health Organization classification of female genital tumors. Immunophenotyping was used to aid the classification of TOCs. Quantification of sTIL density was assessed on hematoxylin and eosin-stained slides as the area of the tumor stroma occupied by mononuclear inflammatory cells divided by the total tumor stromal area according to the recommendations by the International Tumor-Infiltrating Lymphocytes Working Group, and analyzed as continuous percentage values [15].

PD-L1 22C3 score and next-generation sequencing analysis

Immunohistochemistry (IHC) for PD-L1 was performed on Formalin-Fix, Paraffin-Embedded (FFPE), 4-μm thick tissue section using the FDA-approved PD-L1 IHC 22C3 pharmDx kit (Dako North America Inc., Carpinteria, CA) on the Dako Autostainer Link 48.PD-L1 Tumor Proportion Score (TPS) was defined as the number of viable tumor cells showing membranous staining of any intensity divided by the total number of viable tumor cells. A TPS ≥ 1% was considered as positive. Next-Generation Sequencing (NGS) assay was performed on FFPE biopsy or surgical resection specimen, targeting 309 cancer-related genes. Only pathological genomic alterations were included for analysis. TMB is defined based on counting the total number of all synonymous and non-synonymous variants present at 5% allele frequency or greater and reported as mutations per Megabase (mut/Mb). TMB was analyzed as a continuous value. PD-L1 IHC and NGS assay was performed at Clinical Laboratory Improvement Amendments (CLIA) certified reference laboratory.

Statistical analysis

Patient demographics, clinical and pathologic characteristics were summarized *via* descriptive statistics as appropriate. Spearman correlation coefficient analysis was conducted by two continuous or ordinal variables. The Wilcoxon rank sum test or Kruskal-Wallis Test was used for group comparisons. A Chi-square test or Fisher's exact test was used to explore the association between two categorical variables. The Kaplan-Meier method and the logrank test were used for survival analysis. The hazard ratio was estimated utilizing Cox regression. All the tests were two-tailed at a significance level of 0.05. For exploratory purpose, all p values were not corrected for multiplicity. The statistical analysis was conducted using SAS 9.4 (Cary, NC).

Results

Characteristics of the study cohort

Between 2013 and 2021, 138 patients with a diagnosis of TOC and available PD-L1 and NGS data were identified in our institution. None of the patients received ICI treatment. The median age at TOC diagnosis was 61 years with a range of 24 to 90 years. The most common histological subtype was HGSC (92/138, 66.7%), followed by low grade serous carcinoma (13/138, 9.4%), carcinosarcoma (10/138, 7.2%), clear cell carcinoma (7/138, 5.1%), endometrioid carcinoma (6/138, 4.3%), and mucinous carcinoma (2/138, 1.4%). Eight cases could not be subclassified because of nonspecific morphology or immunoprofiles, and thus defined as "other epithelial malignancy" (5.8%). Ninety of 92 HGSC (97.8%) showed p53 mutation and the other 2 tumors (1%) showed mutant pattern by p53 immunohistochemistry. The majority of patients (105/138, 76.1%) presented as FIGO stage 3 or 4 disease. Association of clinicopathological features with PD-L1 positivity, TMB score and sTIL density in TOC is summarized in Table 1.

Association between clinicopathological features and PD-L1 expression

Forty-five tumors (45/138, 32.6%) were PD-L1-positive. Patients with FIGO stage 3 disease showed a higher PD-L1 expression, but this was not statistically significant (p=0.062). Forty-seven tumors (34.1%) were collected at the time of interval debulking surgery in patients who had received Neoadjuvant Chemotherapy (NACT) with a regimen including one or more of the following: Platinum, taxane, or bevacizumab, and 16 of them (16/47, 34.0%) were PD-L1 positive. Twenty nine of 91 (31.9%) treatment-naïve tumors were PD-L1 positive. There was no significant association between *PD-L1* expression and receipt of Neoadjuvant Chemotherapy (NACT) (p=0.796).

Distribution of PD-L1 expression according to histological subtypes is shown in Figure 1. Endometrioid Carcinoma (EC) showed the highest positive rate, followed by "other epithelial malignancy", HGSC and Low-Grade Serous Carcinoma (LGSC) (p=0.049). In this study, low grade serous carcinoma, carcinosarcoma, clear cell carcinoma, endometrioid carcinoma, mucinous carcinoma, and other epithelial malignancy tumors were considered as Non-High Grade Serous Carcinoma (NHGSC). Thirty one of 92 (33.7%) of HGSC were PD-L1-positive while 14 of 46 (30.4%) NHGSC were positive. Thus, HGSC did not show a significant higher PD-L1 expression than NHGSC (p=0.700).

Association between pathological features and TMB score

One hundred and twenty six of the 138 tumors had an available TMB score for analysis. The association between the TMB score and FIGO stage was analyzed and no statistical significance was

Table 1: Association of clinicopathological characteristics with PD-L1 positivity, TMB score and sTIL density in epithelial ovarian carcinomas.

	Patients N (% of total)	PD-L1-Positive cases N (% of factor)	P value	TMB (Mean ± SD (N))	P value	sTIL (% Mean ± SD (N))	P value
Total	138	45 (32.6)		126			
FIGO Stage 1	21 (15)	6 (28.6)	0.062	2.9 ± 1.9 (21)	0.807	7.9 ± 9.1 (18)	0.157
FIGO Stage 2	12 (9)	8 (66.7)		3.8 ± 3.6 (12)		18.9 ± 16.9 (8)	
FIGO Stage 3	84 (61)	26 (57.9)		3.5 ± 2.9 (74)		13.3 ± 13.5 (69)	
FIGO Stage 4	21 (15)	5 (23.8)		3.4 ± 2.9 (19)		15.0 ± 16.3 (18)	
NACT[a]	47 (34.1)	16 (34.0)	0.796	3.2 ± 2.5 (36)	0.768	17.9 ± 17 (39)	0.015*
Treatment Naive	91 (65.9)	29 (31.9)		3.5 ± 2.9 (90)		10.6 ± 11 (74)	
HGSC[b]	92 (66.7)	31 (33.7)	0.7	3.6 ± 3.0 (81)	0.523	15.8 ± 14.9 (75)	0.0011*
NHGSC	46 (33.3)	14 (30.4)		3.1 ± 2.5 (45)		7.8 ± 9.4 (38)	
PD-L1 Positive	45 (32.6)			3.1 ± 2.8 (41)	0.422	19.1 ± 14.6 (38)	0.0005*
PD-L1 Negative	93 (48.4)			3.5 ± 2.8 (85)		10.1 ± 12.3 (75)	

*A p value ≤ 0.05 is statistically significant
[a]NACT: Neoadjuvant Chemotherapy; [b]HGSC: High Grade Serous Carcinoma; NHGSC: non-HGSC

Table 2: Clinicopathological characteristics of epithelial ovarian carcinomas with a *PTEN* mutation.

Case No.	PTEN Alteration	FIGO Stage	Histological Subtypes[a]	TP53 Mutation	sTIL (%)	PD-L1-TPS[b]	TMB (Muts/Mb)	LOH	MSI Status[c]	Therapy[d]	Treatment Response[e]	Overall Survival (Months)
47	Loss	2	HGSC	P	10	P	0	Low	S	S+ACT	PD	29
73	Loss	3	HGSC	P	20	N	8	High	S	NACT+S+ ACT	PD	18
80	Splice site 1027-2A>G Y68fs	1	EC	N	10	P	3	Low	S	S+ACT	R	21
115	R130G	3	HGSC	P	1	P	1	Low	S	S+ACT	PD	12
122	Missense H93R	2	EC	N	5	P	3	Low	S	S+ACT	PD	21
127	G132fs R130P	2	EC	N	20	P	3	Low	S	NACT	PD	85

a. Histological Subtypes: HGSC: High Grade Serous Carcinoma; EC: Endometrioid Carcinoma
b. PD-L1-TPS: P: Positive; N: Negative
c. Microsatellite status: S: Stable
d. Therapy: S: Surgery; ACT: Adjuvant Chemotherapy; NACT: Neoadjuvant Chemotherapy
e. Treatment Response: PD: Progressive Disease; R: Remission

demonstrated (p=0.807). The distribution of TMB scores according to histological subtypes is shown in Figure 2. Carcinosarcoma (CS) showed the highest mean TMB score of 4.0 Muts/Mb, followed by HGSC with mean score of 3.6 Muts/Mb. Endometrioid Carcinoma (EC) showed the lowest mean TMB score of 2.3 Muts/Mb. There was no significant association between histological subtypes and TMB score (p=0.881). HGSC (81/126, 64.3%) showed a higher mean TMB score (3.6, SD=3.0, Muts/Mb) then NHGSC (45/126, 35.7%) (3.1, SD=2.5, Muts/Mb), but the association was not statistically significant (p=0.523).

Association between TMB score and PD-L1 expression

Among these 126 tumors, 85 (85/126, 67.5%) were PD-L1 positive. The PD-L1-positive tumors showed a mean TMB score of 3.1 (SD=2.8, Muts/Mb), slightly lower than the PD-L1-negative tumors with mean score of 3.5 (SD=2.9, Muts/Mb). However, this difference was again not statistically significant (p=0.422).

Association between clinicopathological features and sTIL density

One hundred and thirteen of 138 (82%) tumors had available hematoxylin and eosin slides for assessment of sTIL density. Thirty-nine (39/113, 34.5%) tumors were post-NACT and showed a significantly higher sTIL density (mean =17.9%, SD=17.0%) when compared to treatment-naive tumors (mean =10.6%, SD=11.0%) (p=0.015). Distribution of sTIL density according to histological subtypes is shown in Figure 3. sTIL density was found to be significantly associated with histological subtypes, with Mucinous Carcinoma (MC) showing highest mean density at 20% (SD=0%) followed by HGSC at 15.8% (SD=14.9%, p=0.002). HGSC showed a significantly higher sTIL density than NHGSC (mean =7.8%, SD=9.4%) (p=0.0011).

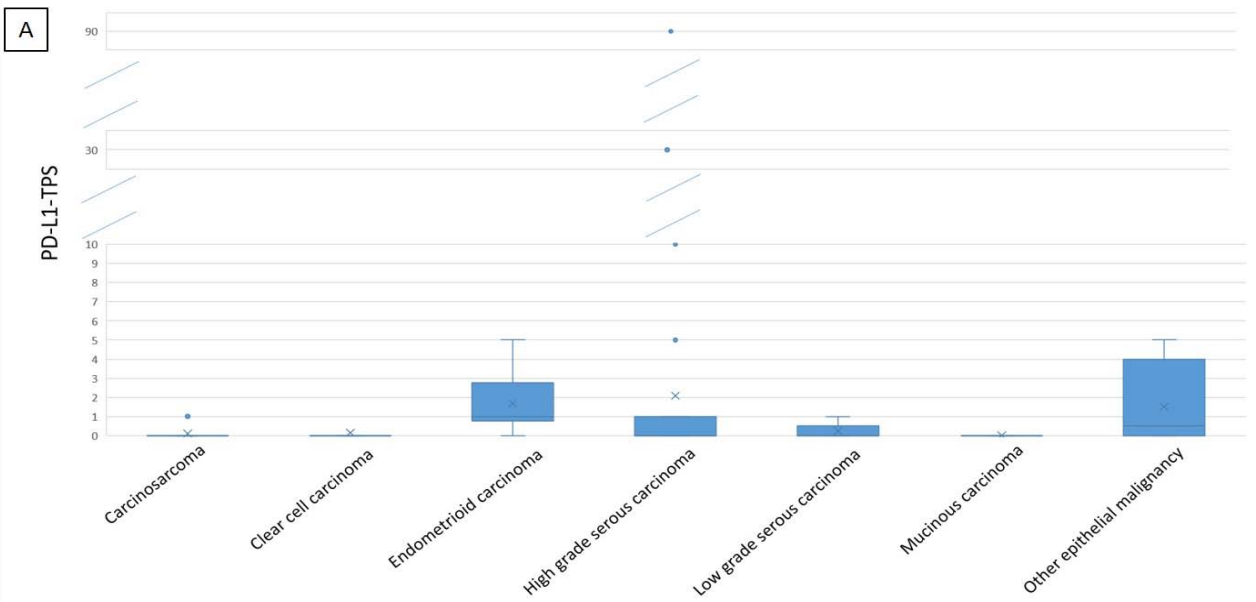
Association of sTIL density with PD-L1 expression and TMB score

Among these 113 tumors, 38 (33.6%) were PD-L1 positive and 75 (66.4%) were negative. PD-L1-positive tumors showed a significantly higher sTIL density (mean =19.1%, SD=14.6%) than PD-L1-negative tumors (mean =10.1%, SD=12.3%) (p=0.0005). The sTIL density also showed a positive correlation with the TMB score (Rho=0.20, p=0.042).

Association between PD-L1 expression and genomic alterations

All 138 patients had available PD-L1 and NGS data for analysis. Comparison of frequency of the genomic alterations between PD-L1-positive and PD-L1-negativeTOC is shown in Figure 4. As expected, TP53 was the most commonly mutated gene in both PD-L1-positive (35/45, 77.8%) and PD-L1-negative tumors (76/93, 81.7%). There was, however, no significant association between PD-L1 positivity and TP53 mutation (p=0.584). Six tumors (6/138, 4.3%) showed a *PTEN* mutation and 5 of them were PD-L1 positive. PD-L1-positive tumors showed a higher rate of *PTEN* mutation (5/45, 11%) than *PD-L1*-negative ones (1/93, 1%) (p=0.0142). Clinicopathological features of the cases with *PTEN* mutation are shown in Table 2. Photomicrographs of representative cases are shown in Figure 5. *BRAF, CHEK2, PIM1, PPP2R1A, MUTYH, CCND3, MET* and *MLH1* were identified in only PD-L1-negative tumors, however, no statistical significance could be achieved due to the limited number of cases.

A

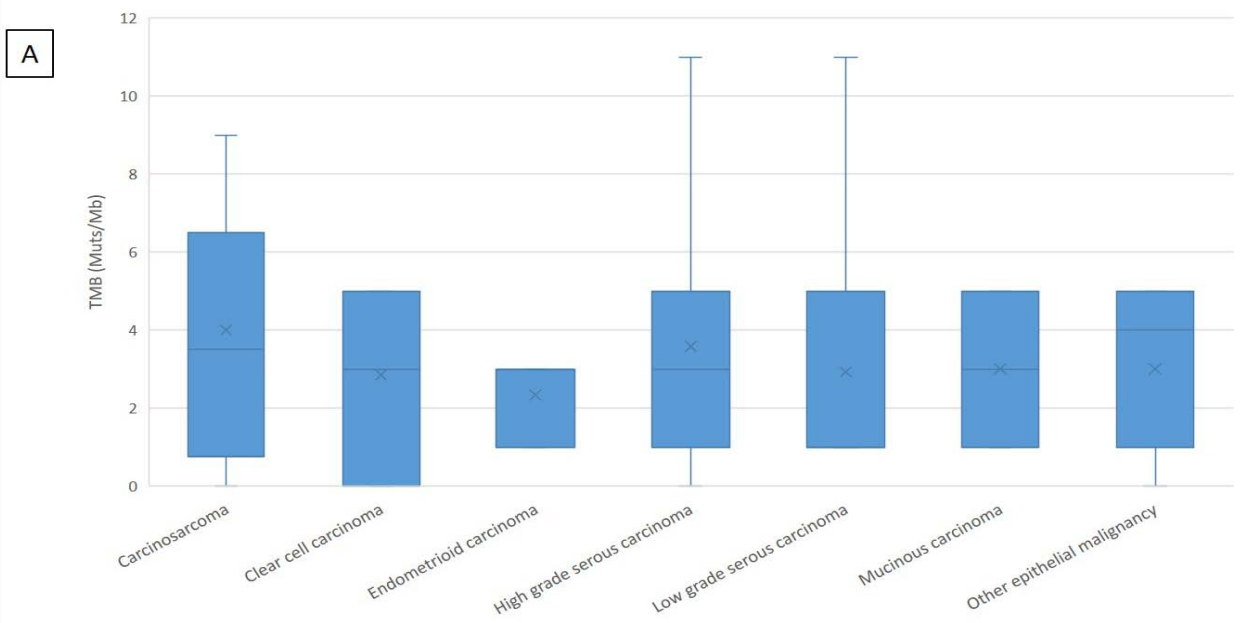


B

PD-L1	Carcinosarcoma n=10	Clear cell carcinoma n=7	Endometrioid carcinoma n=6	High grade serous carcinoma n=92	Low grade serous carcinoma n=13	Mucinous carcinoma n=2	Other epithelial malignancy n=8
Pos. n(%)	1(10%)	1(14%)	5(83%)	31(34%)	3(23%)	0(0%)	4(50%)
Neg. n(%)	9(90%)	6(86%)	1(17%)	61(66%)	10(77%)	2(100%)	4(50%)
P value: 0.0493							

Figure 1: Distribution of PD-L1-TPS according to histological subtypes of tubo-ovarian carcinoma with continuous values (A) and positivity (B).

A



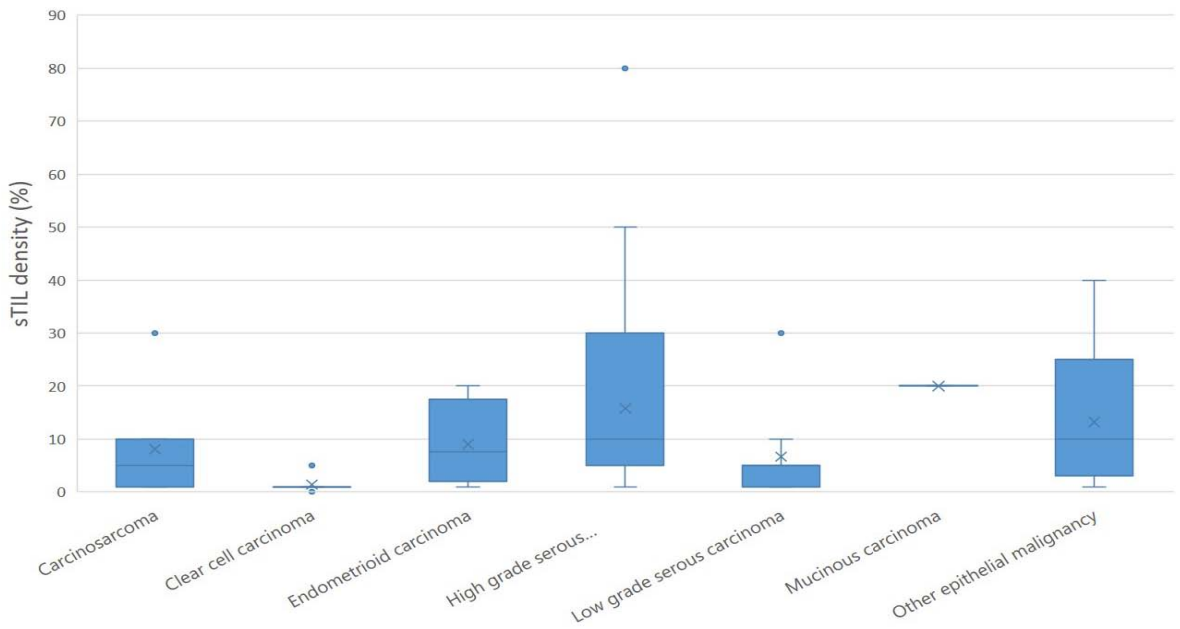
B

TMB score (Mut/Mb)	Carcinosarcoma n=10	Clear cell carcinoma n=7	Endometrioid carcinoma n=6	High grade serous carcinoma n=81	Low grade serous carcinoma n=13	Mucinous carcinoma n=2	Other epithelial malignancy n=7
Mean	4.0	2.9	2.3	3.6	2.9	3.0	3.0
SD*	3.2	2.1	1.0	3.0	3.0	2.8	2.2
P value: 0.881							

* Standard Deviation

Figure 2: Distribution of TMB score according to histological subtypes (A) and statistical data (B).

A



B

sTIL (%)	Carcinosarcoma n=9	Clear cell carcinoma n=7	Endometrioid carcinoma n=4	High grade serous carcinoma n=75	Low grade serous carcinoma n=11	Mucinous carcinoma n=2	Other epithelial malignancy n=5
Mean	8.1	1.4	9.0	15.8	6.6	20.0	13.2
SD*	9.1	1.6	8.2	14.9	8.2	0	15.4
P value: 0.002							

* Standard Deviation

Figure 3: Distribution of sTIL density according to histological subtypes (A) and statistical data (B).

Survival analysis

In the current cohort study, HGSC, sTIL density, PD-L1 expression and TMB score did not demonstrate a significant association with the overall patient survival (p>0.05) (Figure 6) (Table 3).

Discussion

Immune Checkpoint Inhibitors (ICIs) have been used in the treatment of different types of cancers. FDA approved independent biomarkers to test for the utility of such inhibitors include PD-L1 IHC assays for specific tumors along with MSI status and TMB score determinations for agnostic tumors [16-21]. However, the roles of these immune biomarkers in antitumor activity have remained unclear. Our study searched for any associations among the immune parameters and molecular profiling in TOC.

Previous studies showed TILs to be associated with better prognosis in ovarian cancer [22-26]. HGSC is the most common histological subtype of TOC and tends to have worse prognosis [27]. Our study did not show significant correlation between sTIL density and outcome in TOC. Although with a well-known worse outcome, HGSC showed significantly higher sTIL density than NHGSC (p=0.001). Mesnage et al. [6] reported a positive relationship between high sTILs and PD-L1 positivity in TOC. Our data is in agreement with the strong association of high sTIL density and PD-L1 positivity (p=0.0005). Mesnage et al. [6] also compared TILs and PD-L1 expression between post-NACT and treatment-naïve TOC, and concluded that these two parameters increased after NACT. Our study also shows that sTIL density is significantly higher in post-NACT tumors as compared to treatment-naïve tumors. Our study failed to show significant correlation of PD-L1 expression and receipt of NACT. Impact of NACT on the tumor immune environment needs further investigation in larger cohorts and in paired pre- and post-NACT tumors. Our study showed sTIL density and TMB score are positively correlated (p=0.042), which agreed with Fan et al. [28] finding that high-TMB tumors had a higher CD8+ T-cell infiltration pattern than low-TMB groups in Ovarian Cancer (OC) [28]. The potential role of sTIL density as a morphological biomarker for anti-PD-L1 immunotherapy warrant further investigation.

There are few studies which correlate PD-L1 expression with histological subtypes of ovarian cancer. High levels of *PD-L1* expression have been found in Clear Cell Carcinoma (CCC) as reported by Zhu et al. [29] (using the Abacam PD-L1 antibody, positivity threshold ≥ 10%) and Li et al. [30] (using the SP263 clone, semiquantitative immunoreactivity score). Eymerit-Morin et al. reported that HGSC (28% and 42%), grade 3 EC (25% and 50%) and CCC (27% and 30%) showed a higher PD-L1 expression than other histological subtypes (using E1L3N and QR1 clones, respectively). In our study, EC, "other epithelial malignancy", HGSC and LGSC showed higher PD-L1 expression than other subtypes (using 22C3 clones, TPS and a positivity cutoff =1%) (p=0.049). Higher PD-L1 expression in EC and lack of a previously reported correlation in CCC is noted. To date, there are no standardized guidelines for PD-L1 IHC assays, scoring methods and cutoff values. Substantial agreement of PD-L1 expression among different PD-L1 IHC assays and scoring methods has been reported in other cancers, such as lung cancer and breast cancer [31-34]. Given the lack of guidelines and the interpretation challenge of PD-L1 IHC, it is difficult to compare findings among different cohorts. The correlation of PD-L1 expression and histological subtypes still needs further investigation.

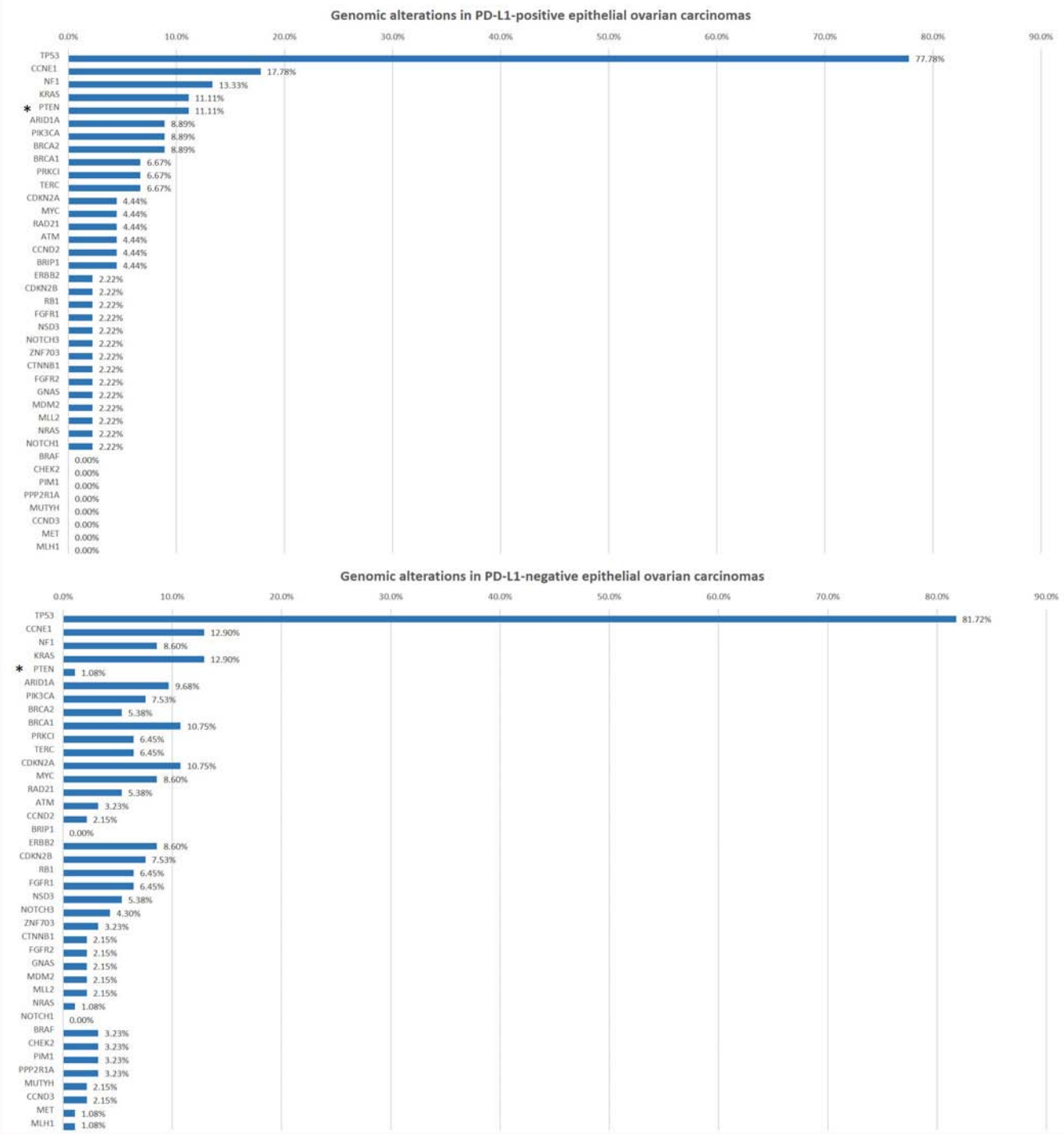


Figure 4: Comparison of frequency of the genomic alterations between PD-L1-positive and PD-L1-negative epithelial ovarian carcinomas.

It has been demonstrated that TMB correlates with clinical outcome and patients with a TMB-high cancer benefit from ICIs [35-37]. Riviere et al. [38] showed intermediate-range TMB correlated with a worse survival, whereas low and very high TMB correlated with better prognosis [38]. Ding et al. [39] investigated the association between TMB and OS in several non-ovarian cancer types and showed conflicting results between the cancer types [39]. Fan et al. [28] showed a higher TMB was associated with better survival in OC [28]. Our study did not, however, show a significant correlation between TMB score and OS in TOC (p=0.330). Wang et al. [40] showed a higher TMB was significantly associated with a higher clinical stage of disease, but there was no significant difference in TMB among different histological subtypes of gynecologic cancers [40]. In our study, neither FIGO stage nor histological subtypes were significantly associated with the TMB score (p=0.807 and 0.880, respectively). The phase 2 KEYNOTE-158 study reported a subgroup of TMB-high advanced stage solid tumors could response to pembrolizumab monotherapy regardless of PD-L1 expression [16]. Yarchoan et al. [41] showed PD-L1 expression and TMB were not significantly correlated in most cancer subtypes, and they suggested

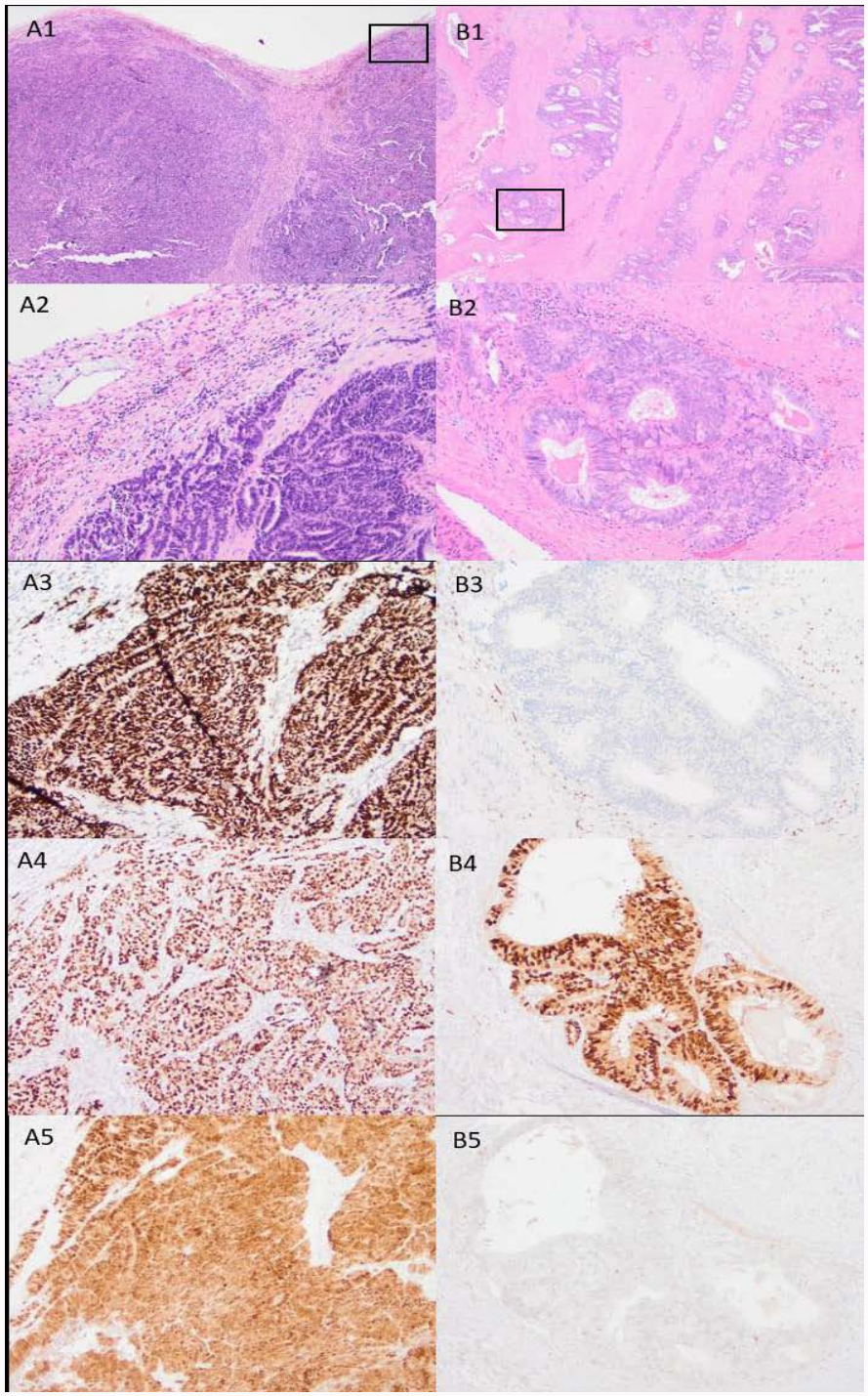


Figure 5: Photomicrographs of two representative cases with *PTEN* mutation. A1-A5, case NO.73, high grade serous carcinoma. B1-B5, case NO. 122, endometrioid carcinoma. A1 and B1, hematoxylin and eosin stain; A2 and B2, higher-magnification images of the boxed areas in A1 and B1, respectively; A3 and B3, Wilms tumor (WT-1) immunohistochemical stain; A4 and B4, estrogen receptor (ER) immunohistochemical stain; A5 and B5, p53 immunohistochemical stain. Original magnification: A1 and B1, x20; A2-A5 and B2-B5, x100.

PD-L1 expression and TMB are independent biomarkers [41]. Our study similarly showed that PD-L1 expression and TMB score were not significantly correlated in TOC (p=0.4216), further supporting PD-L1 and TMB as independent biomarkers for immunotherapy.

Studies showed *PTEN* loss can modify TIME in various cancers [42]. In melanoma, *PTEN* loss was associated with resistance to anti PD-1 immunotherapy [43,44] and decreased T-cell infiltration, but not associated with PD-L1 expression [44]. The association between *PTEN* mutation and immunotherapy resistance was also reported in patients with glioblastoma [45,46]. A negative correlation between *PTEN* loss and PD-L1 positivity was shown in patients with lung cancer [47,48]. In ovarian cancer, *PTEN* loss was highly prevalent in HGSC and associated with poor prognosis and higher TILs count [49,50]. *PTEN* mutation has been reported as a driver in EC and CCC [51,52]. In our study, 6 tumors showed a *PTEN* mutation including 3 HGSC and 3 EC. Five patients initially presented as FIGO stage 2

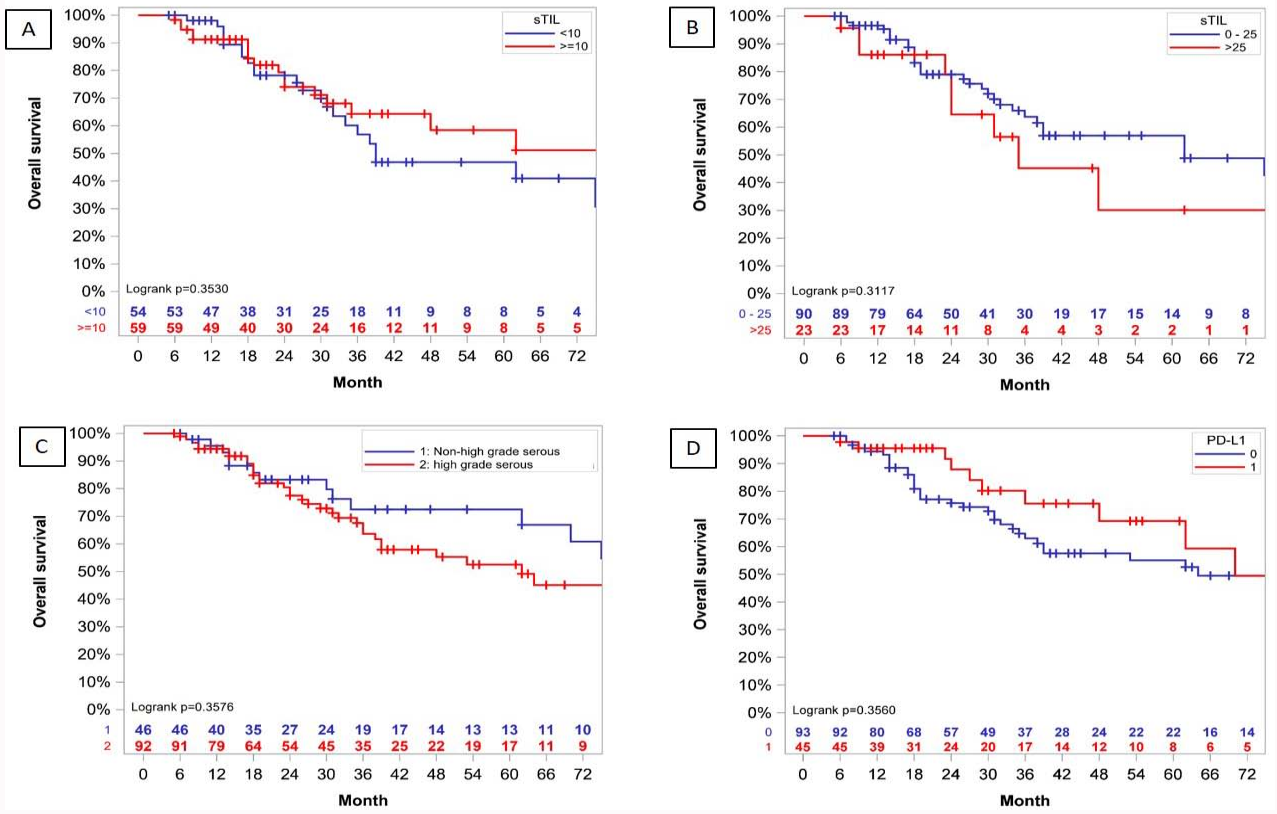


Figure 6: Kaplan-Meier survival curves. A) stromal tumor in iltrating lymphocytes (sTIL) density (<10%) vs. ≥ 10%. B) sTIL density (<25%) *vs.* ≥ 25%. C) high grade serous carcinoma *vs.* non-high grade serous carcinoma. D) PD-L1-positive (red line) *vs.* PD-L1-negative (blue line) tumors.

Table 3: Association of LOH status with TMB score, PD-L1 expression and sTIL density.

Total=87	LOH-high (N=28, 32.2%)	LOH-low (N=59, 67.8%)	P value
PD-L1-positive (N=35, 40.2%) PD-L1-negative (N=52, 59.8%)	15 (42.9%) 13 (25.0%)	20 (57.1%) 39 (75.0%)	0.0804
TMB (Mean ± SD (N))	4.6 ± 3.3 (28)	2.3 ± 2.0 (59)	0.0038*
sTIL (%, Mean ± SD (N))	14.3 ± 11.3 (23)	12.8 ± 15.1 (53)	0.2937

*A p value ≤ 0.05 is statistically significant

or higher disease and developed recurrent/progressive disease with chemotherapy and/or surgery. Only one patient was in remission after 21 months of follow up who presented with FIGO stage 1 disease and received adjuvant therapy after surgery. Interestingly, our study showed that *PTEN* mutation was positively associated with PD-L1 expression in TOC. We believe this is the first clinical study reporting positive correlation between PD-L1 and *PTEN* mutation in TOC. It has been proposed that the *PI3K-AKT* or *STAT3* pathways may induce PD-L1 expression in lung cancer [53]. The first evidence of an oncogenic pathway, *PTEN* loss, causing the induction of PD-L1 expression was reported in glioma [46]. More recently, Ikeda et al. [54] reported upregulation of PD-L1 expression by simultaneous amplification of the PD-L1 and *JAK2* genes in lung cancer [54]. Although some recent studies failed to show direct evidence of *PTEN* loss upregulating PD-L1 expression [44,47], our study again demonstrates this association in TOC. We still believe that losing *PTEN* function in oncogenetic pathways may upregulate PD-L1 expression.

In TOC, our study showed sTIL density to be positively associated with HGSC, PD-L1 positivity and a higher TMB score. No significant association was identified between PD-L1 positivity and TMB score.

We also report PD-L1 expression is positively associated with *PTEN* mutation. Our study is limited by the size and heterogeneity of the cohort and its retrospective nature. In addition, cases included in the current cohort were not a random selection at risk of sampling bias. Furthermore, lack of pembrolizumab treatment precludes further analyses of clinical benefit of ICIs. Association of immune parameters and clinical outcome warrant further study.

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Authors Contribution

JC, SH, CP and XH contributed to study design and data collection. GPS, SH, PL and XH contributed to manuscript editing. PL, SH, GPS, AGK, RA, SAD and AM contributed to manuscript reviewing. PL and XH contributed to statistical analysis. All authors read and approved the final manuscript.

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