



Association of PD-L1 expression by immunohistochemistry and gene microarray with molecular subtypes of ovarian tumors

Curtis David Chin¹ · Charlene Marie Fares¹  · Maira Campos² · Hsiao-Wang Chen² · Itsushi Peter Shintaku¹ · Gottfried Ewald Konecny² · Jianyu Rao¹

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Abstract

Identifying patients who respond to immune checkpoint blockade (ICB) is a significant challenge in oncology. While PD-L1 expression by immunohistochemistry (IHC) is the current diagnostic gold standard for patient selection, it nevertheless does not capture all patients who may respond to ICB. Recent gene expression studies in high-grade serous ovarian carcinoma have defined an immunoreactive molecular subtype that has a measurable difference in patient survival compared with non-immunoreactive subtypes, but no studies have yet demonstrated its impact on predicting response to ICB. As a step toward establishing the predictive value of gene expression classifiers in ICB, we assessed the relationship between PD-L1 IHC and molecular subtypes of ovarian epithelial cancer. This was done by analyzing a total of 93 tissue specimens from patients with stage III and IV disease, and comparing PD-L1 IHC with gene expression by Agilent microarrays using TCGA-defined subtypes. We showed that ovarian tumors with elevated IHC PD-L1 expression are most strongly associated with immunoreactive subtype as compared with other molecular subtypes, reaching statistical significance against differentiated (Dunn's test, 33.39, $p = 0.0003$) and mesenchymal (39.63, $p < 0.0001$) subtypes. Comparing PD-L1 scoring with CPS vs. TPS showed similar trends, but with stronger correlation strength when using CPS (Kruskal–Wallis, $H = 27.52$, $p < 0.0001$), as opposed to TPS ($H = 25.04$, $p < 0.0001$). Interestingly, while PD-L1 gene expression by microarray was significantly increased in the immunoreactive subtype ($H = 20.25$, $p = 0.0002$), it showed a positive but relatively poor correlation to IHC. Overall, the results demonstrate potential value in use of the molecular classifier to select patients for ICB, pending further studies that assess its ability to predict treatment outcomes. In the future, integration of cellular, protein, and genomic biomarkers in the tumor and tumor microenvironment may improve current methods of predicting treatment response.

These authors contributed equally: Curtis David Chin, Charlene Marie Fares

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- ✉ Gottfried Ewald Konecny
gkonecny@mednet.ucla.edu
- ✉ Jianyu Rao
jrao@mednet.ucla.edu

¹ Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

² Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Introduction

Ovarian cancer is the fifth leading cause of cancer death, and is the deadliest gynecologic malignancy among women in the United States [1]. Despite standard radical surgery and initial high response rates to platinum/taxane-based chemotherapy, nearly all patients experience a relapse with median progression-free survival of only 18 months [2, 3]. Therapy for recurrent disease remains limited, and patients often develop chemotherapy resistance [4–8]. Immune checkpoint blockade (ICB) with antibodies directed against programmed cell death protein 1 (PD-1) and its ligand (PD-L1) have been widely applied in oncology, with several clinical trials underway in ovarian cancer [9–12]. While no FDA-approved PD-L1 immunotherapy exists for ovarian cancer, interim results of a phase II trial of pembrolizumab monotherapy showed

modest antitumor efficacy across all patients, with an optimal PD-L1 expression combined positive score (CPS) cutoff point of 10 [13, 14].

Although durable responses to ICB have been observed across several tumor types, most patients fail initial therapy [15]. Therefore, determining which patients will benefit from anti-PD-1/PD-L1 therapy remains an important clinical question. PD-L1 expression by immunohistochemistry (IHC) is used as a biomarker to select patients for ICB, and remains the gold standard as a companion/complementary diagnostic for anti-PD-1/PD-L1 therapy given association with higher response rates [16–18]. However, many challenges exist with using PD-L1 IHC as a biomarker for immunotherapy due to issues with subjectivity and non-standardization. Challenges include variation in staining intensity and proportion of tumor/immune cells positive for PD-L1 across tumor types and within individual tumors (i.e., intratumoral heterogeneity) [19, 20], inconsistencies in antibody clonal affinity and specificity [21, 22], and selection of optimal cutoffs for predicting response to ICB [23]. As such, identification of additional biomarkers that could supplement or replace IHC in predicting response to ICB is needed.

Recent large-scale genomic studies of multiple tumor histologies have investigated whether gene expression-based molecular subtypes associated with distinct tumor biology are predictive of clinical prognosis [24]. The most common histological subtype of ovarian cancer, high-grade serous ovarian carcinoma (HGSOC), has four distinct gene transcriptional subtypes—immunoreactive, differentiated, proliferative, and mesenchymal—validated by The Cancer Genome Atlas (TCGA) Research Network study [25]. When applied to well-annotated patient cohorts, the transcriptional subtypes demonstrated measurable differences in clinical outcomes, with the immunoreactive subtype being most favorable with longest overall survival [26].

Currently, there is no data to support that a gene expression-based molecular classifier has predictive value in determining response to ICB in ovarian cancer. As PD-L1 is an important component of the immunoreactive subtype, determining if an association exists between PD-L1 IHC positivity and immunoreactive subtype is an important step toward developing better measures for predicting response to immunotherapy and assessing if molecular classifier has therapeutic relevance in ICB. In this study, we assessed the relationship between PD-L1 expression by IHC and gene expression profiles of ovarian tumors using TCGA-defined subtypes across a cohort of patients with varying clinical and tumor histopathologic characteristics.

Methods

Tubo-ovarian cancer cohort, clinical and histopathologic data, sample collection and storage

Fresh-frozen tissue was obtained across a cohort of 170 women enrolled in a phase II trial for primary ovarian cancer with well-characterized clinical and histopathologic data (TRIO14 trial) as previously described [27]. Briefly, TRIO14 explored efficacy and safety of ganitumumab, a monoclonal antibody inhibitor of insulin-like growth factor 1 receptor vs. placebo, in addition to front line carboplatin/paclitaxel in patients with optimally debulked (<1 cm) FIGO stage III and IV ovarian epithelial cancers, including fallopian tube and primary peritoneal disease. Samples were collected during surgery, snap frozen within 30 min, and stored at -80°C until RNA isolation and quantification as described previously [26]. Testing of tissue specimens was performed after approval by UCLA institutional review board. A total of 93 specimens were selected based on availability of adequate tissue for both IHC and molecular subtyping analysis by Agilent gene expression microarrays. A subset of these specimens ($n = 86$) also had PD-L1 gene expression data available. Microarray raw data is available with GEO accession number GSE53963.

PD-L1 IHC staining and pathology interpretation

Freshly cut 4- μm -thick sections obtained from archival formalin-fixed paraffin-embedded tissue were mounted on positively charged glass microscope slides, with at least five serial sections available for each specimen. For IHC, staining was performed with PD-L1 monoclonal antibody (SP142 clone, Spring Bio) using a protocol optimized and validated at UCLA Pathology for research specimen testing. Most of the immunostaining procedure was performed on a Leica Bond III autostainer, using Bond-associated reagents (including Bond Refine DAB Detection system); antigen retrieval was performed using a pressure cooker/Leica high pH retrieval buffer as described below. Briefly, 150 μL of rabbit monoclonal anti-PD-L1 antibody at 200 \times dilution was incubated for 60 min, washed, subsequently incubated with HRP- and anti-rabbit antibody-conjugated polymer for 8 min, washed, incubated with hydrogen peroxide reagent, washed, and finally incubated with DAB substrate for signal development. Manual components of this IHC procedure included (1) deparaffinization of sections through xylenes and graded ethanol solutions and antigen retrieval in a pressure cooker set to 5 min/22.5 psi (Biocare Medical) using Bond ER2 (a high pH epitope retrieval solution) prior to primary antibody application on Bond III, and (2) post-DAB intensification with cupric sulfate (5 + 8.5 g NaCl in

1000 ml deionized water) for 10 min subsequent to application of DAB substrate wash step on the Bond III, wash, hematoxylin counterstaining, and dehydration/cover-slipping. A subset of tissue specimens was stained in parallel with another PD-L1 antibody (22C3 clone, Dako/Agilent), following kit instructions provided by Dako/Agilent, for comparison of staining intensity and specificity. In addition, stability of paraffin sections was assessed by staining in parallel a subset of tissue specimens on archived slides.

Two pathologists (CDC and JR) scored all slides for percentage of tumor and immune cells staining positive for PD-L1 as per interpretation guidelines set by Ventana/Roche. H&E slides were used to differentiate areas of tumor vs. normal tissue, and verify histological pattern and grade. PD-L1 expression consensus scores were reported using CPS and tumor proportion score (TPS):

$$\text{CPS} = \frac{\# \text{PD-L1 staining tumor cells and associated infiltrating immune cells}}{\# \text{tumor cells}} \times 100$$

$$\text{TPS} = \frac{\# \text{PD-L1 staining viable tumor cells}}{\# \text{tumor cells}} \times 100$$

Gene expression profiles and molecular subtype classification

Procedures used for establishing gene expression profiles and application of TCGA classifiers for assigning molecular subtypes (immunoreactive, differentiated, proliferative, and mesenchymal) have been described previously [26]. Briefly, 825 ng of total RNA was purified with RNeasy kits (Qiagen inc.) and hybridized to Agilent Whole Human Genome 4 × 44K Expression Arrays, using a mixed reference containing equal amounts of each ovarian tumor sample. Slides were scanned and data were exported into Rosetta Resolver (Rosetta Inpharmatics LLC), with log ratios of signal from individual tumor to signal from the reference mix used for analysis. Starting from 2500 probe-sets, subclasses were computed by reducing the dimensionality of the expression data using a consensus non-negative matrix factorization clustering method [28]. Differentially expressed marker genes for each subclass were determined using significance analysis of microarrays [29]. A minimized gene set was derived for validation of subclass assignment, using a prediction analysis of microarray classification strategy, resulting in 633 unique probe-sets to distinguish the four subtypes [30].

Statistical methods

Comparison of PD-L1 staining between two antibody clones, SP142 and 22C3, was performed using Spearman correlation analysis. Kruskal–Wallis testing and Dunn's

testing on multiple sample pairs assessed for correlation between PD-L1 IHC and molecular/histological subtypes as well as between PD-L1 IHC and gene expression. Mann–Whitney testing compared PD-L1 expression with histological grade, level of CA-125, and extent of residual disease.

Results

A total of 93 patients had adequate tissue for both IHC and molecular subtyping. Patient characteristics are presented in Table 1. TPS of ≥10% was observed in 11.8% of patients, while 23.7% of patients had CPS ≥ 10. Differentiated and mesenchymal subtypes were most common (29.0% each), followed by proliferative (22.6%) and immunoreactive (19.4%). The majority of patients had serous histological subtype (86.0%) and high-grade histology (68.8%); high-grade serous carcinomas represented 59% of all included cases (Supplementary Table 1). The distribution of high-grade serous carcinomas was even across the four molecular subtypes, whereas the distribution of other histologic types and grades contained greater representation from differentiated and mesenchymal molecular subtypes (Supplementary Fig. 1). In addition, the primary site was ovarian in 88.2% and most patients had stage IIIC disease (82.8%); only advanced stage (FIGO III and IV) tumors were included in this study.

Comparison of SP142 and 22C3 antibody clones showed stronger PD-L1 staining intensity with SP142, for both tumor cells and tumor-associated immune cells in this ovarian tumor cohort (Fig. 1). There was a statistically significant positive correlation in PD-L1 expression between the two antibody clones for tumor cells ($\rho = 0.789$, $p = 0.0004$) and immune cells ($\rho = 0.795$, $p = 0.0004$), using Spearman correlation (Fig. 1c, d). In addition, there was minimal difference in staining between archived and freshly cut slides, showing stability of staining over time (Supplementary Fig. 2).

Tumors characterized as having an immunoreactive molecular subtype were associated with relatively higher levels of PD-L1 expression by IHC as compared with other molecular subtypes, reaching statistical significance against differentiated and mesenchymal tumors (Fig. 2). Comparison of PD-L1 scoring with CPS vs. TPS showed similar trends, but with more robust correlation strength when using CPS (Kruskal–Wallis, $H = 27.52$, $p < 0.0001$), as opposed to when using TPS ($H = 25.04$, $p < 0.0001$) (Fig. 2i, j). Higher CPS and TPS cutoffs resulted in enrichment of tumors classified with immunoreactive molecular signature, notably at ≥10 (Fig. 2k, l).

PD-L1 gene expression determined by microarray was elevated at statistically significant levels in the

Table 1 Patient characteristics ($n = 93$).

Characteristic	No. of patients (%)
Tumor proportion score (TPS)	
0	16 (17.2)
$\geq 1\%$	77 (82.8)
$\geq 10\%$	11 (11.8)
Combined positive score (CPS)	
0	3 (3.2)
≥ 1	90 (96.8)
≥ 10	22 (23.7)
Molecular subtype	
Differentiated	27 (29.0)
Immunoreactive	18 (19.4)
Mesenchymal	27 (29.0)
Proliferative	21 (22.6)
Histological subtype	
Serous	80 (86.0)
Endometrioid	5 (5.4)
Clear cell	1 (1.1)
Mixed	3 (3.2)
Other	4 (4.3)
Histological grade	
Low	25 (26.9)
High	64 (68.8)
Unknown	4 (4.3)
Primary site	
Ovarian	82 (88.2)
Fallopian tube	6 (6.5)
Primary peritoneal	4 (4.3)
Unknown	1 (1.1)
Stage	
IIIA/IIIB	8 (8.6)
IIIC	77 (82.8)
IV	8 (8.6)
Elevated CA-125	
Yes	82 (88.2)
No	11 (11.8)
Residual disease	
No or microscopic	67 (72.0)
<1 cm	26 (28.0)

Clinical, immunohistopathologic, and molecular characteristics of 93 ovarian tumors.

immunoreactive subtype against all other subtypes (Kruskal–Wallis, $H = 20.25$, $p = 0.0002$), specifically proliferative subtype (Dunn's, 29.42 , $p = 0.0036$), differentiated subtype (34.32 , $p < 0.0001$), and mesenchymal subtype (23.45 , $p = 0.0201$) (Fig. 3a). In addition, while higher gene expression for PD-L1 generally corresponded with higher CPS values (Fig. 3b and Supplementary Fig. 3),

only the differentiated subtype showed a positive correlation between the two biomarkers that reached statistical significance ($\rho = 0.4996$, $p = 0.0080$) (Fig. 3e).

There was no statistically significant correlation between PD-L1 IHC and histological type of ovarian cancer (Fig. 4a). However, there was a significant difference in PD-L1 expression between low- and high-grade tumors (Mann–Whitney, $U = 501$, $p = 0.0056$) (Fig. 4b). In addition, PD-L1 expression was not associated with presence or absence of elevated CA-125 or extent of residual disease (Fig. 4c, d).

Discussion

Gene expression-based subtyping of tumors as a prognostic and/or predictive marker has been widely explored in many cancers [31]. In HGSOC, subtyping identified four signatures across several studies: immunoreactive, differentiated, proliferative, and mesenchymal [32]. Given that the immunoreactive subtype is enriched for genes associated with immune response, this group could be a promising target for ICB. To date, modest antitumor responses to ICB have been reported in ovarian cancer, largely in patients who express PD-L1 on both cancer and tumor infiltrating immune cells [13]. Although PD-L1 expression by IHC shows association with response to immunotherapy, its negative predictive value in regards to therapy response remains problematic. Thus, additional biomarkers are needed and molecular subtype may serve this purpose. Gene signature in ovarian cancer has not been evaluated in the context of immunotherapy, however a T-cell-inflamed gene expression profile was recently shown to predict response to ICB across several tumor types [33–35]. To glean a better understanding of the role that molecular classifier may play in predicting response to ICB, we sought to investigate if an association exists between ovarian cancer subtype and PD-L1 IHC.

We have shown that FIGO stage III–IV ovarian tumors with increased PD-L1 expression by IHC are most strongly associated with immunoreactive molecular subtype. In addition, higher cutoffs for both CPS and TPS enrich for immunoreactive tumors. Although the majority of immunoreactive tumors shows elevated expression of PD-L1, not all patients with this subtype are captured by IHC, raising the question that perhaps additional patients who may benefit from ICB could be identified using a molecular classifier. Furthermore, although PD-L1 is differentially enriched in the immunoreactive subtype, proliferative tumors which are not primed for immune activation also demonstrated relatively increased levels of PD-L1 staining, possibly consistent with overall moderate specificity of IHC in predicting response to immunotherapy [36, 37].

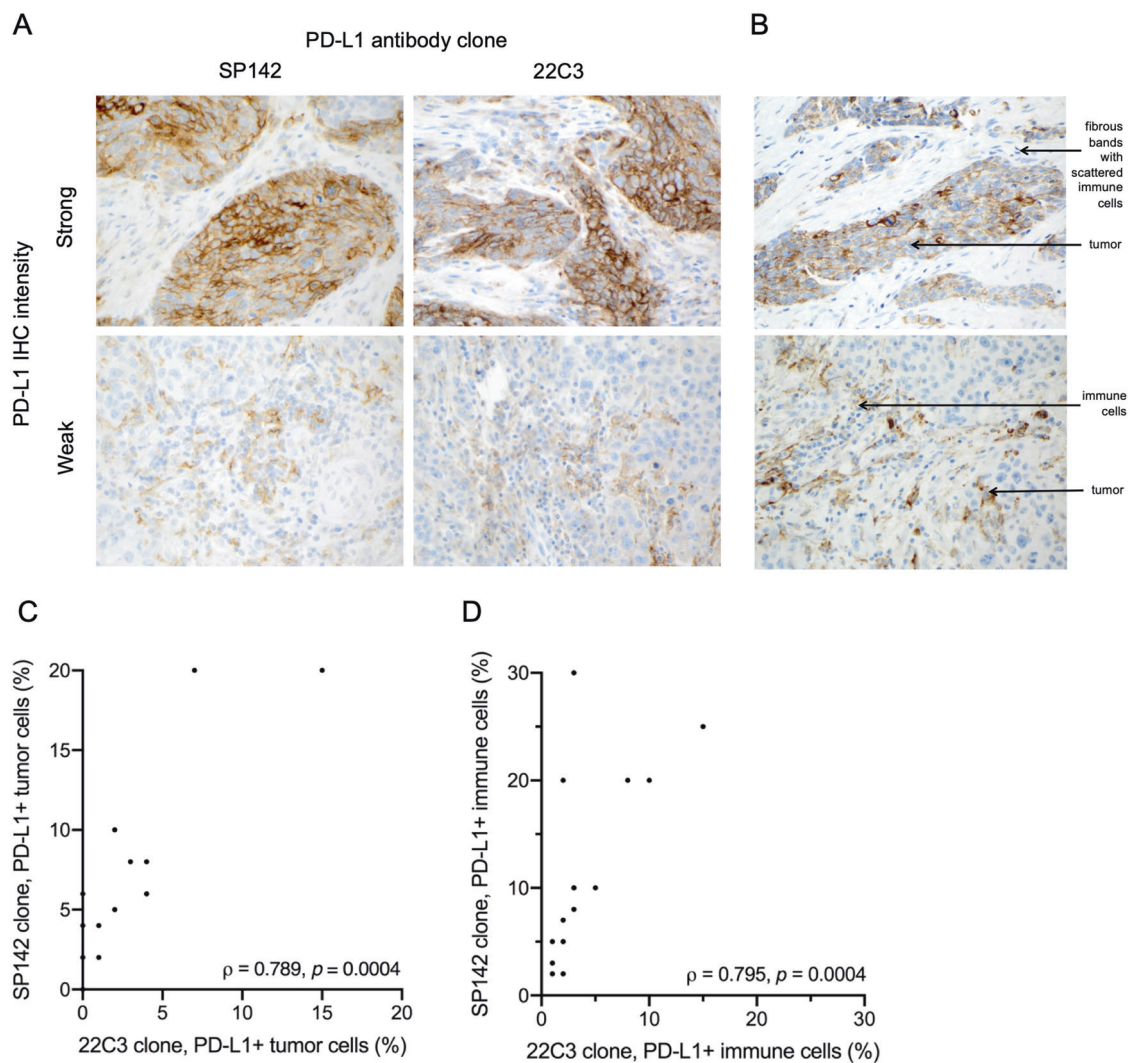


Fig. 1 Comparison of PD-L1 IHC staining with different antibody clones. a Representative high-magnification images (400 \times) of differential PD-L1 IHC staining with SP142 and 22C3 clones. **b** Representative high-magnification images (400 \times) of PD-L1 IHC staining of

tumor and immune cells using SP142 clone. **c, d** Scatterplots showing correlation of IHC PD-L1 expression of tumor and immune cells between SP142 and 22C3 antibody clones, using the Spearman correlation test.

In line with prior studies, PD-L1 RNA expression was strongly associated with immunoreactive subtype. However, when comparing PD-L1 RNA expression with IHC, results showed poor correlation, although there was a positive trend overall. Notably, some tumors demonstrated increased RNA expression but low staining, possibly related to discordance between transcriptome and proteome, secondary to posttranscriptional and post-translation changes. In addition, tissue handling and degradation of product may contribute. Even though PD-L1 IHC and RNA did not show strong concordance, the immunoreactive subtype incorporates additional genes and pathways related to immune activation and tumor microenvironment, which could be predictive. Enrichment of genes involved in T- and B-cell activation, cytokine/chemokine signaling, interferon-gamma, JAK/STAT signaling, and MHC levels amongst

others is seen in the immunoreactive subtype (Supplementary Fig. 3).

In contrast to published results showing inferior staining with SP142 using the Ventana reagent and platform as compared with DAKO 22C3, our results showed stronger intensity of staining with SP142 for both tumor cells and immune cells [38]. The PD-L1 SP142 procedure, as developed at UCLA Pathology—using probe obtained from Spring Bio, antigen retrieval with a pressure cooker, and Leica high pH retrieval buffer—has been validated against the 22C3 DAKO kit and has consistently produced results reflected in this study [39, 40]. Our experience has been that the superior sensitivity of SP142 in detecting tumor cell PD-L1 expression might be related to this procedure, which utilizes Leica instead of Ventana staining platform. Although a direct comparison of the SP142 clone IHC by

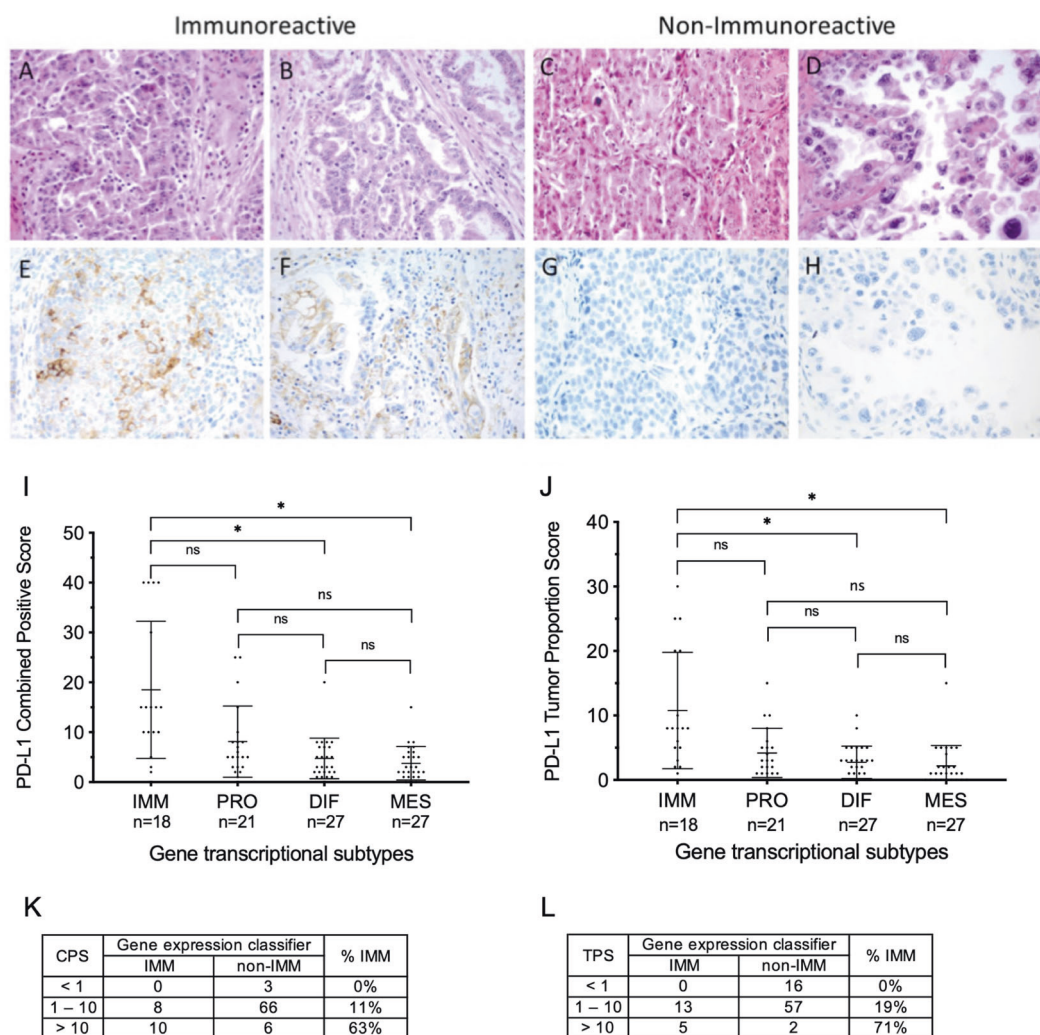


Fig. 2 Association of PD-L1 expression by IHC with molecular subtypes of ovarian tumors. **a–h** Representative H&E and corresponding PD-L1 IHC images of ovarian tumors classified as immunoreactive (left) and non-immunoreactive (right), using SP142 PD-L1 antibody clone. Histological subtypes of tumors were serous carcinoma (**a**, **c**, **e**, **g**), endometrioid carcinoma (**b**, **f**), and clear cell carcinoma (**d**, **h**). Scatter dot plots of PD-L1 expression using CPS (**i**) and TPS (**j**) for tumors characterized as immunoreactive (IMM), differentiated (DIF), mesenchymal (MES), or proliferative (POS) molecular

subtypes based on TCGA classifiers. Kruskal–Wallis test $H = 27.52$, $p < 0.0001$ (**i**) and $H = 25.04$, $p < 0.0001$ (**j**). Significant Dunn's tests in **i** between IMM vs. DIF (33.39, $p = 0.0003$) and IMM vs. MES (39.63, $p < 0.0001$); and in **j** between IMM vs. DIF (30.52, $p = 0.0010$) and IMM vs. MES (39.24, $p < 0.0001$). Short line indicates mean value; error bars indicate one standard deviation; * indicates $p < 0.05$; ns indicates no significance. **k**, **l** Percentage of tumors classified as immunoreactive at different CPS and TPS cutoffs.

the method described above and on the Ventana autostainer using their SP142 kit has not been done on adjacent sections of the same tumors, some differences in the two methods are present that might explain the reported disparity. Perhaps the most likely explanation is the use of a pressure cooker vs. onboard epitope retrieval on slide plate, as pressure cooker epitope retrieval is generally acknowledged as the most rigorous heat-induced epitope retrieval method. The primary antibody concentration and length of primary antibody incubation could influence comparative staining specificity and intensity as well. Previously we performed an on-site, side-by-side comparison of the Ventana Opti-View detection system vs. the Leica Bond Refine detection

system on the same primary antibodies and tissues and showed that the Bond Refine system produced stronger staining at the same primary antibody concentrations. In addition, SP142 has been validated for immune cell detection and may be more sensitive at staining immune cells [21, 41]. As CPS was shown to predict modest response to ICB in ovarian cancer, the detection of PD-L1 expressing immune cells is important in this histology [13, 14].

There are limitations to this study; most notable is the application of molecular classifiers to non-serous histology, as subtyping was developed and validated in HGSOc. The majority of samples used in this study was high-grade serous histology, however it is unclear if this TCGA-defined

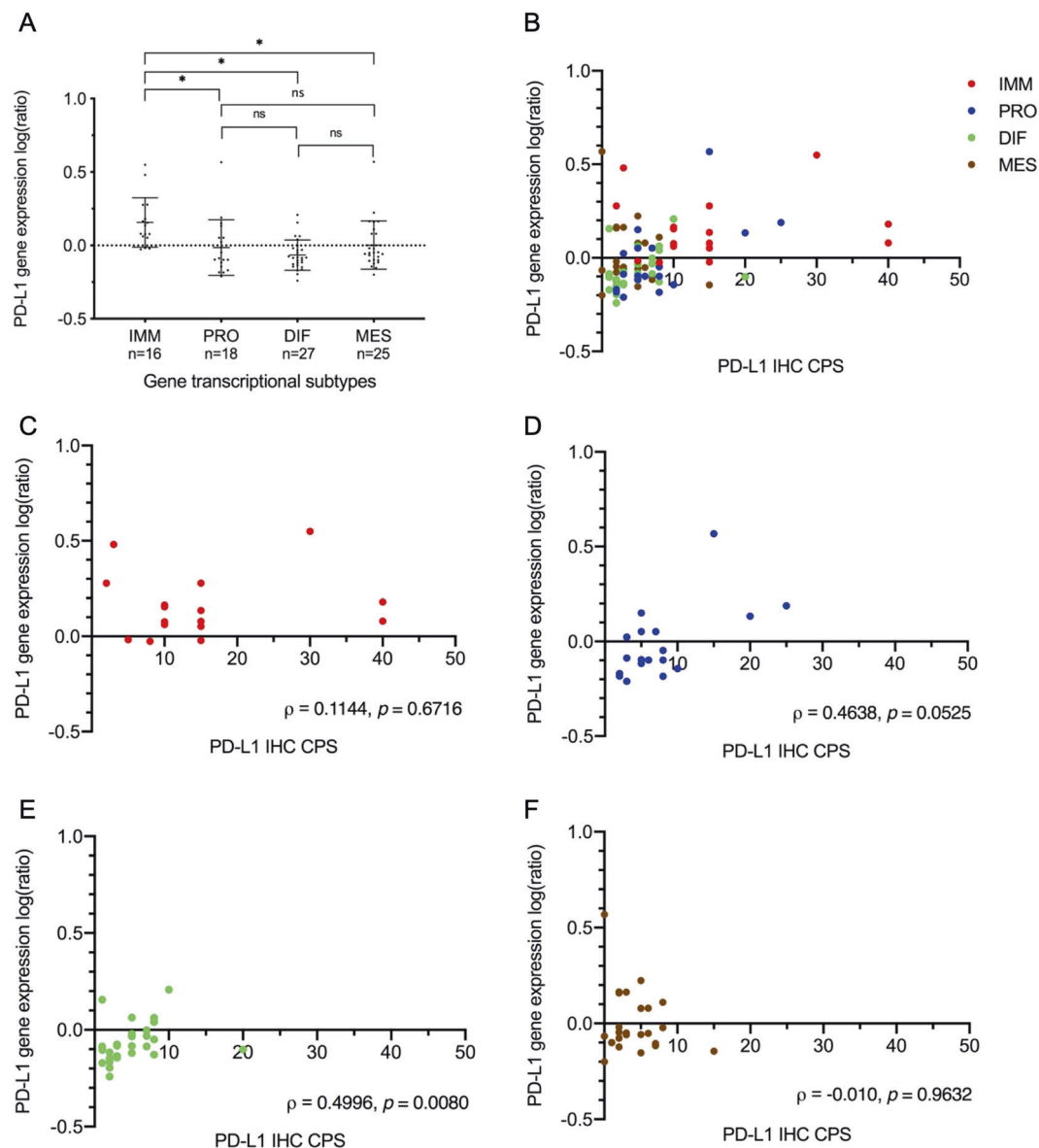


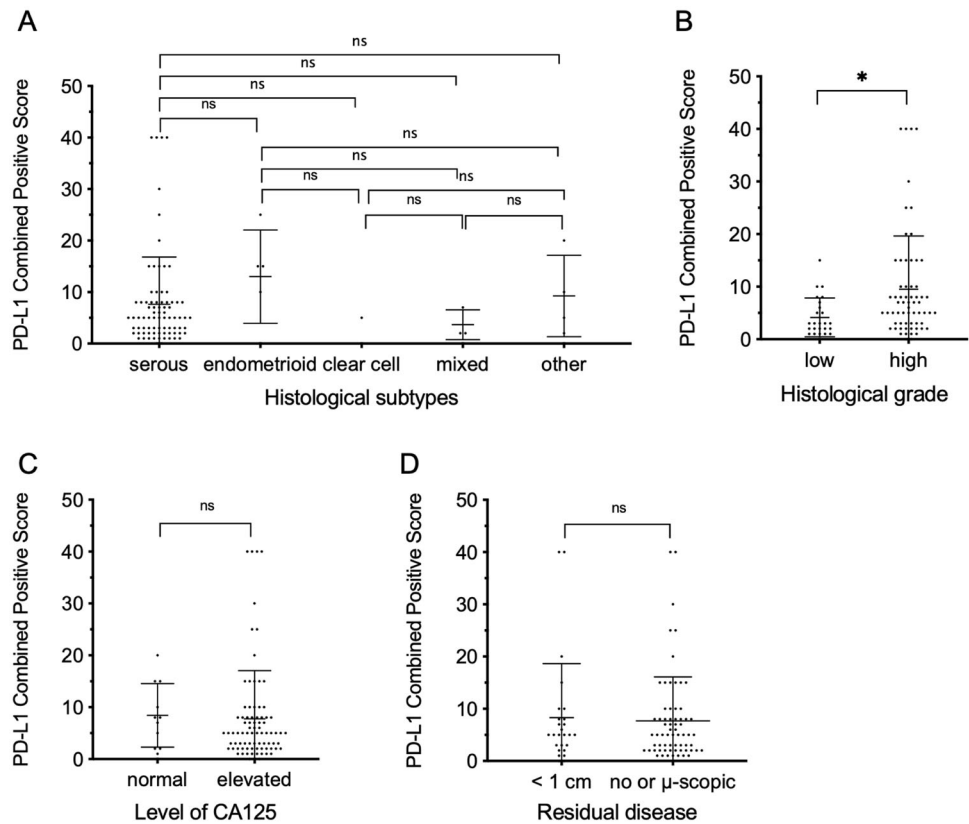
Fig. 3 Association of PD-L1 gene expression levels determined by microarray with TCGA classifiers and IHC. **a** Scatter dot plots of PD-L1 gene expression for tumors characterized as immunoreactive (IMM), differentiated (DIF), mesenchymal (MES), or proliferative (POS) molecular subtypes based on TCGA classifiers. Kruskal–Wallis test $H = 20.25$ ($p = 0.0002$), with significant Dunn’s tests between IMM vs. PRO (29.42, $p = 0.0036$), IMM vs. DIF (34.32, $p < 0.0001$),

and IMM vs. MES (23.45, $p = 0.0201$). Short line indicates mean value; error bars indicate one standard deviation; * indicates $p < 0.05$; ns indicates no significance. **b–f** PD-L1 gene expression levels vs. PD-L1 IHC CPS across TCGA classifiers, with IMM in red, PRO in blue, DIF in green, and MES in brown; strength of correlation determined using the Spearman correlation test.

molecular subtyping has prognostic or predictive relevance in non-serous tumors, which have different clinical prognosis and pathophysiology. For example, non-HGSOC epithelial ovarian tumors have more indolent courses and are more often characterized by mutations in regulators of mitogen-activated protein kinase (MAPK) such as *KRAS* and *BRAF*, whereas HGSOC is characterized by aggressive clinical course and genomic/chromosomal instability with *TP53* mutations [42]. It is possible that the findings from this study are relevant only for the high-grade serous

histology. That said, as PD-L1 IHC is currently being performed and evaluated across all tubo-ovarian cancer histologies and grades as a biomarker for immunotherapy, we believe that applying this molecular classifier in a manner similar to PD-L1 IHC would be a step toward evaluating its potential value in the clinical setting. The lack of any statistically significant relationship between PD-L1 IHC and tumor histology or grade (Fig. 4), along with a trend of greater CPS and TPS with immunoreactive subtype (Fig. 2), suggests that the association between PD-L1 IHC

Fig. 4 Association of PD-L1 IHC with clinical and histopathological characteristics. PD-L1 CPS compared with histological subtypes (a), degree of histological differentiation (b, Mann–Whitney $U = 501$, $p = 0.0056$), level of CA-125 (c), and residual disease (d). * indicates $p < 0.05$; ns indicates no significance, using Kruskal–Wallis testing (a) and Mann–Whitney testing (b–d).



and molecular expression is independent of histology or grade. Further investigation of the applicability of the TCGA classifier in non-HGSOC with greater numbers of cases is warranted, along with exploration of other molecular classifications.

Currently it is unclear if molecular classifier could replace IHC or be used to enhance prediction of response in conjunction with staining. This study was a step toward determining whether or not a gene expression-based molecular classifier has any predictive value in determining response to ICB; it was limited by lack of data on clinical outcomes needed for comparing PD-L1 IHC to molecular grouping in predicting response to ICB. Further studies are needed to fully assess its ability to predict treatment outcomes.

Gene expression profiling may share similarities with another biomarker predictive of response to ICB—mismatch repair (MMR) deficiency—in that additional mechanisms outside of PD-L1 upregulation could sensitize tumors to therapy. It is thought that microsatellite instability (MSI) increases genomic instability, resulting in additional neoantigens that are recognized by immune effector cells following ICB. These findings led to the first tissue/site-agnostic approval for pembrolizumab [43]. MMR-deficient tumors can be interrogated with either MMR IHC or MSI status using multiplex polymerase-chain reaction or next-generation sequencing. The National Comprehensive Cancer Network guidelines endorse universal IHC or MSI

testing for all individuals diagnosed with endometrial and colorectal cancers. As the FDA did not specify which assay should be used, the College of American Pathologists is currently completing guidelines for MMR and MSI testing [44]. Beyond predictive accuracy, cost and technical demands must be considered in clinical adoption of any test. IHC currently is more cost-effective than molecular subtyping by gene expression microarrays, but adoption of the latter may be facilitated if cost and complexity diminish over time.

The immunoreactive molecular subtype in HGSOC may serve as a superior biomarker in selecting patients for ICB, given there are multiple determinants of response other than PD-L1 status. Tumors may be classified as having low PD-L1 by IHC, but could have additional immune processes occurring that play an important role in response to immunotherapy. It is thought that by integrating multiple cellular, protein, and genomic biomarkers that provide information on both tumor and tumor microenvironment, current prediction techniques could be enhanced [45]. Recently, a clinical-grade consensus classifier for molecular subtypes of HGSOC was developed using Nanostring technology (unpublished data), which can be readily applied into clinical trials to determine if the immunoreactive subtype has predictive relevance in immunotherapy.

In conclusion, we showed that ovarian tumors with elevated PD-L1 expression by IHC are most strongly

associated with immunoreactive molecular subtype. Interestingly, while PD-L1 RNA expression was strongly associated with immunoreactive molecular subtype, PD-L1 RNA expression and IHC CPS showed poor correlation, perhaps due to posttranscriptional and post-translation changes in the latter. Future work is needed to show whether or not a gene expression-based molecular classifier has predictive value in determining response to ICB in ovarian cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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