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Association of PD-L1 Expression with Immunohistochemical Molecular Profile in Endometrial Carcinomas

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Abstract

Objective:

PD-L1 expression is a potential biomarker for predicting immunotherapy response, but its role in endometrial carcinoma (EC) is unclear. This study evaluated PD-L1 expression in tumor cells (TC), immune cells (IC), and by the combined positive score (CPS), and examined associations with mismatch repair (MMR) status, p53 immunophenotype, and clinicopathological features.

Methods:

A total of 299 EC cases were retrospectively analyzed. PD-L1 was assessed by immunohistochemistry using TC, IC, and CPS scoring. MMR proteins (MLH1, PMS2, MSH2,

MSH6) and p53 expression were also evaluated, and correlations with histological subtype, grade, and clinical parameters were examined.

Results:

PD-L1 positivity was observed in 33.9% of IC, 16.2% of TC, and 35.7% of CPS cases. IC and CPS expression were significantly associated with low-grade tumors ($p<0.001$). MMR-deficient tumors demonstrated higher PD-L1 expression across all scoring methods ($p<0.001$ for IC/CPS; $p=0.020$ for TC). In addition, p53 mutation correlated with MMR stability ($p=0.014$) and with PD-L1 TC positivity ($p=0.044$).

Conclusion:

PD-L1 expression in TC correlated with p53 status and was linked to MMR deficiency. Combined evaluation of PD-L1, MMR, and p53 may aid stratification and guide immunotherapy in EC.

Keywords: Endometrial cancer, PD-L1, mismatch repair, tumor cells, immune cells

INTRODUCTION

Endometrial cancer (EC) is the most common gynecologic malignancy worldwide, with approximately 420,368 new cases and 97,723 deaths reported annually [1]. It ranks as the fourth most prevalent cancer overall, following breast, lung, and colorectal malignancies. While the majority of EC cases are sporadic (approximately 90%), a minority are familial, often associated with hereditary syndromes such as Lynch syndrome [2].

In recent years, molecular biomarkers have gained prominence in EC classification and treatment planning. Among these, mismatch repair (MMR) protein status and p53 expression have emerged as clinically relevant parameters with both prognostic and predictive significance, particularly in the context of immunotherapy. The Cancer Genome Atlas (TCGA) proposes a four-tier molecular classification system, including POLE-ultramutated, MMR-deficient (MMRd), p53-mutant (p53mut), and no specific molecular profile (NSMP) subtypes [3].

Programmed death-ligand 1 (PD-L1), a surface protein expressed by tumor and immune cells, plays a critical role in immune evasion by binding to the PD-1 receptor and inhibiting T-

cell activation. Its expression has been associated with high-grade and advanced-stage EC and may predict favorable responses to immune checkpoint inhibitors [4]. MMR deficiency, commonly caused by inactivation of MLH1, MSH2, MSH6, or PMS2, is present in approximately 20–35% of EC cases and is often linked to increased immune activation and a better response to immunotherapy. In support of this, Le et al. (2015) demonstrated that MMR-d ECs are more sensitive to PD-1/PD-L1 blockade therapies [5].

The originality of the present work lies in its inclusion of a large cohort (n=299) and the comprehensive evaluation of PD-L1 expression in tumor cells, immune cells, and by using the CPS method. While previous studies have examined the relationship between PD-L1 and MMR or between PD-L1 and p53 separately, the simultaneous assessment of all three parameters (PD-L1, MMR, and p53) within the same cohort, along with their clinicopathological correlations, is rarely reported. In this context, our study not only provides extensive data from Turkey but also offers a holistic analysis of the interplay between PD-L1, MMR, and p53 in relation to clinicopathological and prognostic features, thereby making a distinctive contribution to the existing literature.

This study aims to evaluate PD-L1 expression in EC in relation to MMR protein status and p53 immunoexpression, together with various clinicopathological parameters, as these markers may help predict immunotherapy responses and guide individualized treatment strategies.

MATERIALS AND METHODS

Selection of Study Group:

Between 2017 and 2024, a total of 299 patients with primary epithelial endometrial carcinoma were retrospectively included in the study, based on biopsy and surgical resection specimens obtained at the Department of Medical Pathology, Pamukkale University Faculty of Medicine. Only cases with available formalin-fixed paraffin-embedded tissue blocks collected at the time of initial diagnosis were eligible. To eliminate potential treatment-related alterations in histopathological and immunohistochemical assessments, patients who had received neoadjuvant chemotherapy, radiotherapy, or immunotherapy prior to tissue sampling were excluded. Secondary malignant neoplasms (metastases) and primary non-epithelial uterine tumors were likewise excluded from the cohort. The cases were retrospectively re-evaluated.

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Pamukkale University during the meeting held on February 22, 2022, with Decision No. 04. As this was a retrospective study using archived pathology specimens and anonymized patient data, the requirement for obtaining informed consent was waived by the Ethics Committee.

EC cases were classified according to the WHO 2020 classification [6]. Tumor staging was determined based on pTNM classification (AJCC 8th Edition) and the International Federation of Gynecology and Obstetrics [FIGO 2023] staging criteria for EC. The Cancer Genome Atlas (TCGA) proposes a four-tier molecular classification system, including POLE-ultramutated, MMR-deficient (MMRd), p53-mutant (p53mut), and no specific molecular profile (NSMP) subtypes [3]. However, due to the unavailability of sequencing facilities, POLE mutation analysis could not be performed in our study. Therefore, our molecular classification focuses on the remaining three subgroups: MMRd, p53mut, and NSMP.

Histopathological data, including patient age, tumor type, histological grade, nuclear grade, tumor size, depth of invasion, lymphovascular invasion, perineural invasion, cervical stromal invasion, endocervical gland involvement, and uterine serosa involvement, were retrieved from pathology reports. Information on treatment details, disease-free survival, and overall survival was collected from the hospital system.

Immunohistochemistry

For IHC analysis, tumor-rich paraffin blocks were selected from specimens. A 0.6 mm core was extracted from the selected paraffin blocks to create tissue microarray paraffin blocks. From the selected paraffin blocks, 3–5 μm thick sections were prepared on lysine-coated slides and subjected to IHC staining using the Ventana Benchmark XT™ fully automated staining system. The following antibodies were used: PD-L1 (rabbit monoclonal antibody, dilution: 1/100, clone: SP142, catalog no: 790-4860, Ventana, USA), MLH1 (Ventana, clone M1, undiluted), MSH2 (Ventana, clone G219-1129, undiluted), MSH6 (Ventana, clone SP93, undiluted), PMS2 (Ventana, clone A16-4), and p53 (Ventana, clone DO-7, undiluted). The ultraView Universal DAB detection kit was used for all stainings.

For IHC staining, a negative control was performed by omitting the primary antibody, while placental chorionic villi served as the positive control for PD-L1. The stained slides were evaluated by two pathologists (YAK, NAV), considering cytoplasmic and/or membranous

staining of tumor-infiltrating lymphocytes (TILs) and stromal lymphocytes for PD-L1 immunoreactivity. PD-L1 expression was categorized as negative (<1%) and positive (≥1%). PD-L1 expression was assessed separately in tumor cells (TCs) and immune cells (ICs) infiltrating the tumor stroma and was also evaluated using the Combined Positive Score (CPS). PD-L1 positivity was defined as at least 1% membranous and/or cytoplasmic staining in either TCs or ICs. CPS was calculated by dividing the total number of PD-L1-stained cells (tumor cells, lymphocytes, and macrophages) by the number of tumor cells and multiplying by 100. A CPS ≥1 was considered positive PD-L1 expression [3].

DNA mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6) were defined as lost in tumor cells when nuclear staining was entirely absent, while external and internal non-neoplastic controls (lymphoid, endothelial, and stromal cells) exhibited positive nuclear staining [7,8]. MMR protein status was classified as MMR-deficient (MMRd) if the nuclear expression of any MMR protein was completely lost in the presence of an intact internal control. Conversely, tumors were considered MMR-proficient (MMRp) when all four MMR proteins (MLH1, PMS2, MSH2, and MSH6) showed preserved nuclear expression in tumor cells. The mutant-type p53 staining pattern was defined as either strong and diffuse nuclear staining in more than 70% of tumor cell nuclei, cytoplasmic staining, or complete absence of nuclear staining (null pattern). In contrast, wild-type expression was characterized by weak and heterogeneous staining [9].

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 25.0 software (Armonk, NY: IBM Corp.). Descriptive statistics for continuous quantitative variables (e.g., age) were analyzed using standard descriptive statistical methods, including arithmetic mean, standard deviation, and median. Categorical variables (presence frequencies) were reported with their frequencies and percentages within the total sample. Comparisons of categorical variables were conducted using the Chi-square test or Fisher's Exact Test, depending on the distribution of the cases. Disease-free survival and overall survival were analyzed using the Kaplan-Meier method, and prognostic factors were further evaluated using multivariate Cox regression analysis. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Clinicopathological Findings

This study included 299 patients diagnosed with epithelial EC. Among them, 210 cases (70.2%) underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO) and lymph node dissection, while 89 cases (29.8%) underwent biopsy. The mean age of the patients was 61 years (range: 31–89). Of the cases, 60.8% were younger than 65 years, while 39.2% were 65 years or older. Regarding histological subtypes, 257 cases (86%) were endometrioid carcinoma, 20 cases (6.7%) were serous carcinoma, 8 cases (2.7%) were clear cell carcinoma, 8 cases (2.7%) were mixed type (endometrioid carcinoma+ serous carcinoma), and 6 cases (2%) were classified as other types (4 carcinosarcomas and 2 dedifferentiated carcinomas). A total of 79 cases (26.4%) were high-grade tumors. The mean tumor size was 3.61 cm (range: 0–10 cm). Tumor size was <3 cm in 101 cases (37.8%) and ≥3 cm in 166 cases (62.2%). IHC analysis revealed positive expression in 33 out of 277 cases (11.8%) for mutant-type p53. Myometrial invasion beyond the inner half was observed in 83 cases (32.2%). Serosal invasion was present in 4 cases (1.3%), lymphovascular invasion in 34 cases (11.4%), and perineural invasion in 4 cases (1.3%) (Table 1). Malignant peritoneal cytology was detected in 4 cases (1.3%). A total of 124 cases (41.5%) were classified as early-stage (stage I–II), while 13 cases (4.3%) were advanced-stage (stage III–IV). Adjuvant/postoperative immunotherapy was administered in only four cases (1.3%), whereas the vast majority of patients (98.7%; n=295) had no history of immunotherapy. Of the four patients who received immunotherapy—all of whom belonged to the high-grade and MMRd subgroups none had died at last follow-up. Regarding adjuvant/postoperative chemotherapy, 64 patients (21.4%) received treatment, 229 patients (76.6%) did not, and treatment information was unavailable for six patients (2.0%). With respect to adjuvant/postoperative radiotherapy, 107 patients (35.8%) underwent treatment, 186 patients (62.2%) did not, and treatment status was not recorded in six patients (2.0%). Recurrence was observed in 2 cases (0.7%), with a mean disease-free survival of 34.32±21.20 months. A total of 21 cases (7%) had died, with an overall survival duration of 34.8±30.8 months.

Table 1. Clinicopathologic Data

Clinicopathologic Data	**n (%)**
Age groups	
<65	180 (60.8%)
≥65	116 (39.2%)
Tumor Histological Type	
Endometrioid	257 (86.0%)
Serous	20 (6.7%)
Clear Cell	8 (2.7%)
Mixed Type	8 (2.7%)
Other(e.g.,carcinosarcoma, dedifferentiated)	6 (2.0%)
Specimen type	
Curettage	89 (29.8%)
TAH+BSO	210 (70.2%)
Tumor diameter	
< 3 cm	101 (37.8%)
≥ 3 cm	166 (62.2%)
Tumor grade	
Low grade	195 (65.2%)
High grade	80 (26.8%)
Lymphovascular invasion	
No	255 (85.3%)
Yes	34 (11.4%)
Perineural invasion	
No	285 (98.6%)
Yes	4 (1.4%)
Cervical stromal invasion	
No	252 (84.3%)
Yes	8 (2.7%)

Uterine serosa involvement	
No	255 (98.5%)
Yes	4 (1.5%)
Lower uterine segment involvement	
No	232 (89.2%)
Yes	28 (10.8%)
Endocervical gland involvement	
No	245 (94.5%)
Yes	15 (5.5%)
Myometrial invasion	
<%50	174 (67.8%)
≥%50	83 (32.2%)
Survival	
Alive	278 (93.0%)
Deceased	21 (7.0%)

PD-L1 Expression in Endometrial Cancer

PD-L1 was performed on 280 cases. Among these, PD-L1 positivity was observed in 95 cases (33.9%) for IC, in 45 cases (16.2%) for TC, and in 100 cases (35.7%) based on the CPS (Figure 1,2,3). PD-L1 and Its Association with Clinicopathological Features are summarized in Table 2.

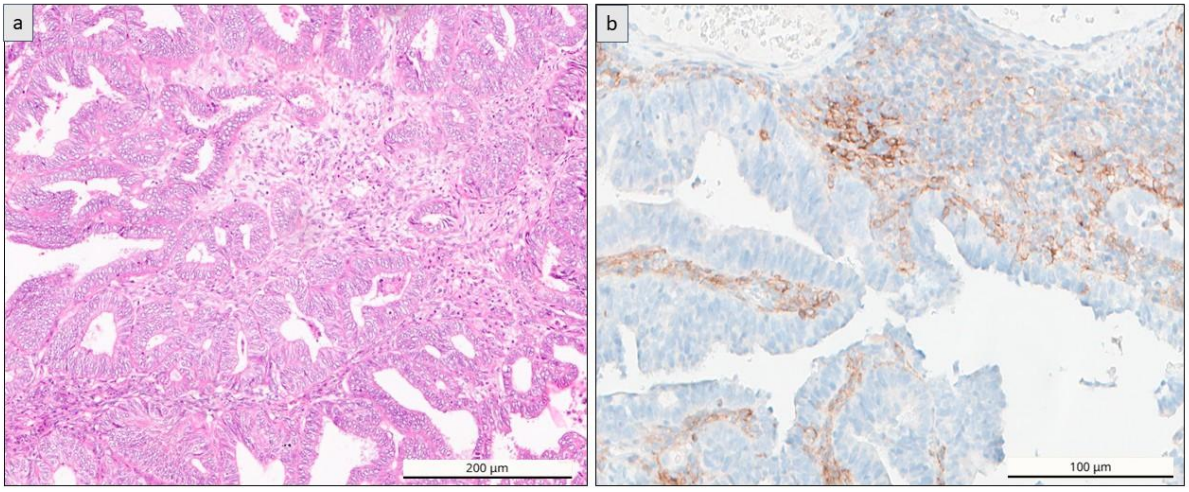


Figure 1. (a) Low-grade endometrioid carcinoma (H&E, x100). (b) PD-L1: Intense staining in immune cells (IC)

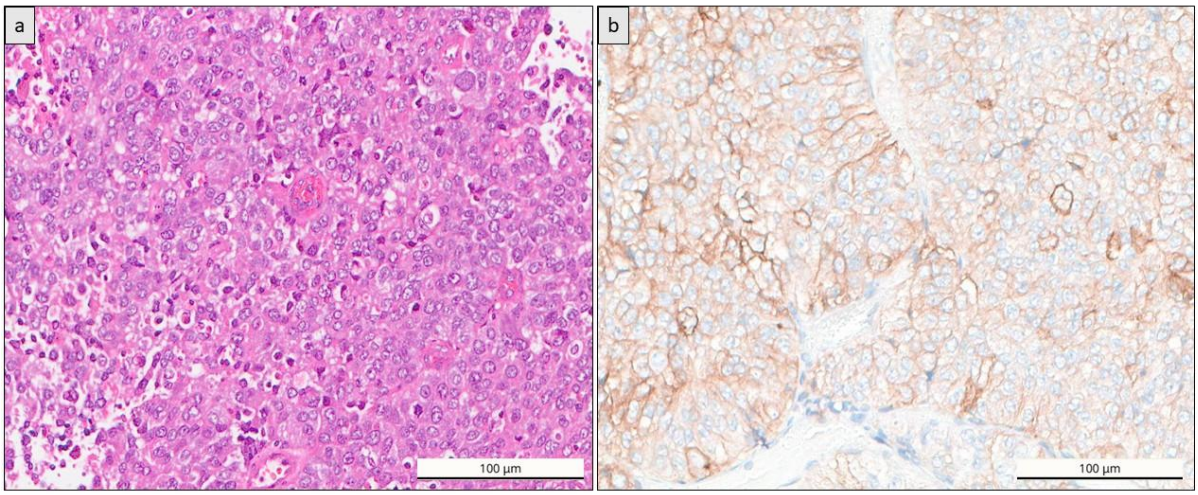


Figure 2. (a) High-grade serous carcinoma (H&E, ×200). (b) PD-L1: Intense staining in tumor cells (TC) and immune cells (IC) (IHC, ×200).

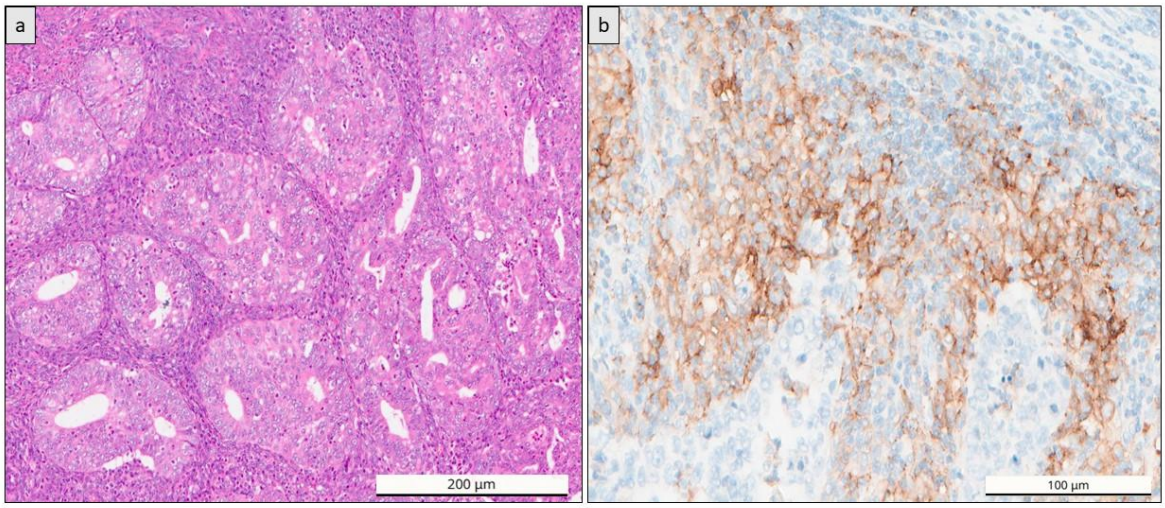


Figure 3. (a) High-grade endometrial carcinoma (H&E, ×100). (b) Immunohistochemical analysis (IHC, ×200) showing diffuse membranous and cytoplasmic PD-L1 expression in both tumor and immune cells.

Table 2. PD-L1 and Its Association with Clinicopathological Features

Clinicopathological Features	**PD-L1 + TC**			**PD-L1 + IC**			**PD-L1+ CPS**		
	N	%	**p ***	n	%	**p ***	n	%	**p ***
Tumor Histological Type									
Endometrioid	7	11	**0.000**	76	27.1	**0.041**	80	28.5	0.075
Serous	6	2.1		11	3.9		11	3.9	
Clear Cell	4	1.4		3	1.1		1	1.4	
Mixed Type	3	1.1		3	1.1		3	1.1	
Other	1	0.4		2	0.7		2	0.7	

Tumor Size			0.291			0.981		
< 3cm	18	7.1		34	13.4		38	15
≥ 3 cm	21	8.3		54	21.3			
							54	21.3
Lymphovascular Invasion			0.743			0.225		
No	37	13.2		81	28.9		83	29.5
Yes	6	2.1		13	4.6		14	5
Stage			0.493			0.702		
Early Stage	13	10.2		36	28.1		36	28.3
Late Stage	2	1.6		4	3.1		4	3.1
Histological Grade			**0.000**			**0.001**		
Low	18	6.9		53	20.5		57	21.9
High	22	8.5		34	13.1		35	13.5
Myometrial invasion			0.903			0.186		
<%50	26	10.7		56	23		59	24.2
≥%50	12	4.9		32	13.1		75	30.7
Recurrence			0.525			0.630		
No	45	16		94	33.6		99	35.2
Yes	0	0		1	0.4		1	0.4
MSI Status			**0.020**			**0.000**		
MMR-deficient	22	7.9		47	17		49	17.6
MMR-proficient	23	8.3		47	17		51	17.3
P53			0.044			0.310		
Wild type	33	12.5		76	28.9		79	29.9

Mutant	9	3.4		13	4.9		15	5.7
Specimen type			0.668			**0.011**		**0.039**
Curettage	31	11		77	27.5		79	28.1
TAH+BSO	14	5		18	6.4		21	7.5

*p: p-values were calculated using Chi-square or Fisher's exact test, as appropriate. A p-value < 0.05 was considered statistically significant.

Association of PD-L1 with Molecular and Pathological Subgroups

When comparing PD-L1 expression in IC across histological subtypes, positivity was observed in 3.9% of serous carcinoma, 1.1% of mixed-type, and 1.1% of other tumors (including carcinosarcoma and dedifferentiated carcinoma). In contrast, higher rates were noted in endometrioid carcinoma (31.3%) and clear cell carcinoma (37.5%) (p = 0.047). PD-L1 IC expression was also significantly more frequent in MMRd tumors compared with MMRp cases (49.5% vs. 25.8%). A weak to moderate inverse correlation was found between MMR status and PD-L1 IC expression (Spearman's rho = –0.237, p < 0.001), indicating that MMRd tumors are more likely to show immune cell-associated PD-L1 positivity. These findings support the hypothesis that MMRd tumors display increased immunogenicity and may respond more favorably to immune checkpoint blockade targeting the PD-1/PD-L1 axis.

PD-L1 expression in TC was more frequently observed in mixed-type and clear cell carcinomas compared with endometrioid, serous, and other subtypes (p=0.001), and this latter finding should be interpreted with caution due to the small number of cases in these rare subtypes. No significant association was observed between PD-L1 expression in immune cells (IC) and p53 mutation status (p=0.310). A statistically significant association was found between PD-L1 expression in TC and MMR status ($p = 0.020$, Pearson χ^2; $p = 0.025$, Fisher's exact test). PD-L1 TC positivity was observed in 23.4% of MMRd tumors and 12.5% of MMRp tumors. A weak but significant inverse correlation was identified between MMR status and PD-L1 TC expression (Spearman's $\rho = –0.140$, $p = 0.019$), indicating that MMRp tumors tend to lack PD-L1 expression in tumor cells. These findings suggest that MMRd tumors may be more likely to display tumor cell PD-L1 expression, potentially enhancing their visibility to immune surveillance and supporting their candidacy for immunotherapeutic approaches targeting the PD-1/PD-L1 axis.

A statistically significant association was found between p53 immunophenotype and PD-L1 TC ($\chi^2 = 4.062, p = 0.044$). PD-L1 TC positivity was more frequently observed in tumors with mutant-type p53 expression (9/32, 28.1%) than in those with wild-type p53 (33/232, 14.2%). Although Fisher's exact test yielded borderline significance ($p = 0.067$, two-sided), the linear-by-linear association and Spearman correlation supported a weak but statistically significant positive correlation (Spearman's $\rho = 0.124$, $p = 0.044$). These findings suggest that PD-L1 expression in tumor cells may be more commonly associated with mutant-type p53 in endometrial carcinomas. No statistically significant relationship was found between PD-L1 CPS positivity and tumor histological subtypes (p=0.075). Similarly, no significant association was observed between PD-L1 CPS and p53 mutation status (p=0.156). A statistically significant association was observed between MMR status and PD-L1 CPS ($\chi^2 = 16.096$, $p < 0.001$). PD-L1 CPS positivity was more frequently detected in MMRd tumors compared to MMRp ones. Fisher's exact test confirmed the robustness of this association ($p < 0.001$). Moreover, a weak to moderate inverse correlation was found between MMR status and PD-L1 CPS expression (Spearman's $\rho = -0.241$, $p < 0.001$), indicating that MMRp tumors are more likely to exhibit CPS negativity.

Comparison of PD-L1, MMR Proteins and p53 with Clinicopathological Findings

PD-L1 IC positivity was more frequently observed in low-grade tumors (p=0.001). Additionally, uterine serosa involvement was more common in PD-L1 IC-positive cases (p=0.011). PD-L1 TC positivity was more frequently observed in non-endometrioid subtypes ($p < 0.001$). Among PD-L1 TC-positive cases, p53 was more often of the mutant-type (p=0.044), and MMR status was more stable (p=0.020). PD-L1 TC positivity was also significantly associated with high-grade tumors ($p < 0.001$).

Association of PD-L1 CPS, p53 Mutation, and MMR with Clinicopathological Findings

PD-L1 CPS positivity was more frequently observed in low-grade tumors (p=0.003). Among 279 cases, p53 mutation was detected in 11.8% of cases. P53 mutation was more frequently observed in endometrioid and serous carcinoma compared to other subtypes ($p < 0.001$). Additionally, p53 mutation was more prevalent in high-grade tumors ($p < 0.001$). In the p53 mutant group, myometrial invasion beyond the inner half of the myometrium was observed at a higher rate (p=0.016). Additionally, p53 mutation was found to be associated with MMR stability (p=0.014) and PD-L1 TC positivity (p=0.044).

MMRd tumors were detected in 33.7% of 294 cases (Figure 4). MMRp tumors were more frequently observed in patients younger than 65 years (p=0.002). Moreover, MMRd tumors demonstrated significantly higher PD-L1 positivity in IC and CPS (p<0.001 for both) and a weaker but significant association in TC (p=0.020).

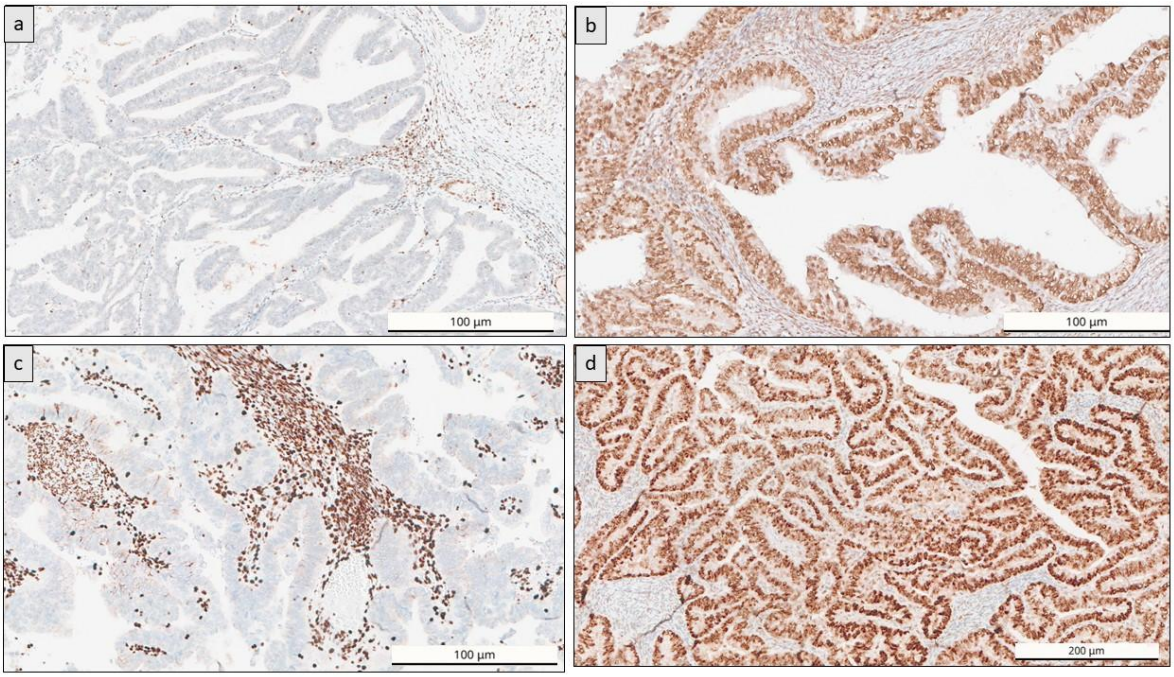


Figure 4. (a) MSH-2: Loss of nuclear expression in tumor cells (IHC, ×200). (b) MLH-1: Strong nuclear staining (IHC, ×200). (c) MSH-6: Loss of nuclear staining in tumor cells; immune cell positivity used as internal control (IHC, ×200). (d) PMS-2: Diffuse nuclear positivity (IHC, ×100).

Association of PD-L1, MMR Proteins, and p53 with Survival

PD-L1 IC, PD-L1 TC, PD-L1 CPS positivity, and MMRp tumors were not found to be significantly associated with survival (p>0.05). However, p53 mutation positivity was associated with shorter survival (p < 0.001) (Figure 5). In the multivariate Cox regression analysis adjusted for stage, grade, and treatment variables, the overall model was statistically significant (χ^2=23.514; p=0.015). However, none of the individual variables, including PD-L1 expression, stage, grade, or treatment modalities, retained independent prognostic significance. A total of 21 deaths were recorded during follow-up. Given that only four patients received immunotherapy, no statistical survival analysis specific to immunotherapy could be performed; all four of these patients were alive at the last follow-up.

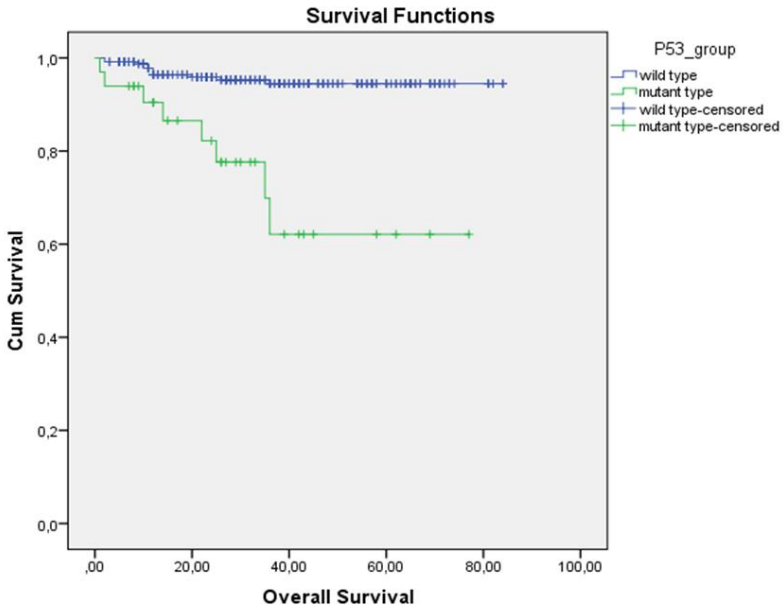


Figure 5. Kaplan–Meier survival analysis showed that p53 mutation positivity was associated with shorter survival ($p < 0.001$)

DISCUSSION

Molecular alterations within the tumor and its microenvironment can contribute to targeted therapies, treatment resistance, or favorable therapeutic responses. In our study, we examined PD-L1 expression in tumor and immune cells, its relationship with MMR status and p53 mutation, and its association with prognostic factors.

PD-L1

In the study by Annukka Pasanen et al., PD-L1 (SP263) expression was detected in 8.6% of TCs and 27.7% of ICs, while the CPS was ≥1 in 19.4% of cases [10]. In the study by Miaksi Chev, PD-L1 (28-8) expression was evaluated in both TCs and ICs, with PD-L1 positivity observed in 62.7% (37/59) of ICs and 28.8% (17/59) of TCs. Additionally, PD-L1 expression in ICs was found to be significantly higher in endometrial carcinoma compared to non-neoplastic endometrium ($p<0.001$), highlighting the potential role of PD-L1 in the tumor microenvironment of EC [11]. Furthermore, to better elucidate the divergent PD-L1 expression patterns observed between high-grade and low-grade endometrial carcinomas, tumor microenvironmental characteristics were considered. High-grade tumors are known to exhibit a more immunologically active yet concurrently immunosuppressive microenvironment,

characterized by abundant TILs, frequent mismatch repair deficiency, and upregulation of immune checkpoint molecules such as PD-L1 [10,12,13]. In contrast, low-grade tumors typically display reduced immune cell infiltration, lower interferon-γ signaling, and diminished checkpoint molecule expression, which may contribute to their relatively lower PD-L1 levels. These microenvironmental differences may help explain the heterogeneity of PD-L1 expression across histological grades and underscore the complex interplay between tumor biology and immune regulation in endometrial carcinoma [14,15,16].

In the study by Shuang Zhang et al., PD-L1 (SP142) was highly expressed in 70% of TC and 16% of IC [17]. A recent meta-analysis reported that the prevalence of PD-L1 expression in EC patients was approximately 34.26% in TCs and 51.39% in ICs (17). The prevalence of PD-L1 expression in TC among EC patients varies widely, ranging from 5.3% to 76.5% [17, 3]. On the other hand, the reported prevalence of PD-L1 expression in IC ranges from a minimum of 16.29% [17] to a maximum of 72.44% [18].

In our study, PD-L1 expression was observed in 33.9% of IC cases, 16.2% of TC cases, and 35.7% of cases based on CPS. PD-L1 expression was found to be higher in ICs compared to TCs, which is consistent with previous literature [17,19,20,21,22,13,23]. Our findings align with existing studies, further supporting that PD-L1 expression is more prevalent in ICs than in TCs. PD-L1-positive EC have been found to be more prevalent, particularly in high-grade serous and mixed serous/endometrioid tumors, as well as in advanced-stage cancers [10,17]. Although some studies have suggested that PD-L1 positivity may be associated with poor prognosis and an increased risk of recurrence [4,21], no statistically significant association between PD-L1 expression and survival outcomes was observed in our cohort. This discrepancy may be related to the limited number of survival events in our study and indicates that the prognostic relevance of PD-L1 expression requires validation in larger series. Consistent with the literature, our study also found that PD-L1 expression in TC was more frequently observed in high-grade tumors. These findings support the notion that immune cells do not merely restrict T-cell activity through PD-L1 expression but also facilitate the initial intratumoral expansion of T cells [14,24,25]. Activated T cells secrete interferon-γ, which increases PD-L1 expression in both tumor and immune cells; however, immune cells may be more sensitive to this cytokine-mediated induction [15,16]. In addition, PD-L1 expression on immune cells has been suggested to function both as a mechanism to limit excessive inflammation and as a contributor to tumor immune evasion [24]. PD-L1 expression is therefore expected to be higher in advanced-stage

endometrial carcinoma, which is often associated with poorer clinical outcomes [26,27]. Collectively, these results further reinforce the interaction between tumor cells and the inflammatory tumor microenvironment [13], and support the rationale for further investigation of PD-L1 immune checkpoint inhibitors in selected patient populations.

These results further reinforce the idea of an interaction between the tumor and its surrounding inflammatory microenvironment [13]. Therefore, PD-L1 immune checkpoint inhibitors may have potential as a treatment strategy for patients with advanced-stage disease.

Association Between PD-L1 and MMR

The association between MMRd and high PD-L1 expression cannot be explained solely by increased tumor mutational burden, but involves several overlapping molecular pathways. In MMRd tumors, the accumulation of mutations generates numerous neoantigens, leading to enhanced $CD8^+$ T-cell infiltration. Activated T cells release interferon-γ, which upregulates PD-L1 expression on tumor and immune cells via the JAK/STAT1/IRF1 signaling axis. In addition, cytosolic DNA fragments resulting from mismatch repair deficiency activate the cGAS–STING pathway, triggering type I interferon responses and further induction of PD-L1 [15,16]. Finally, aberrant activation of the PI3K/AKT/mTOR pathway, often due to PTEN loss or other oncogenic events, has also been implicated in PD-L1 regulation [15,28]. Together, these mechanisms suggest that the MMRd–PD-L1 relationship extends beyond mutational burden alone and is mediated by multiple converging molecular signals.

MMRd tumor phenotype is observed in approximately 17–33% of all EC [13]. In our study, MMRd was detected in 33.7% of tumors. PD-L1 positivity has been linked to T-cell density, with POLE-mutant and MMRd tumors exhibiting higher PD-L1 expression and greater tumor-infiltrating lymphocyte presence compared to other TCGA subgroups [10]. The majority (53%) of MMRd tumors have been reported to be PD-L1 positive [13]. In our study, PD-L1 IC positivity was observed in 49.5% of MMRd tumors, PD-L1 TC positivity in 23.4%, and PD-L1 CPS positivity in 41.8%. MMRd EC have gained special interest due to their distinct immunological characteristics compared to MMRp tumors. These include a higher number of TILs and increased neoantigen production resulting from frameshift mutations caused by MMR [26,29]. Although increased cytotoxic T-cell infiltration is typically considered a positive prognostic marker in many cancers, including MMRd colorectal carcinoma, its ability to restrict tumor growth may be inhibited by tumoral PD-L1 expression [13,29].

This tumor-induced inhibition may explain why elevated levels of tumor-associated cytotoxic T cells are not uniformly correlated with improved survival in EC. Studies have shown that MMRd EC exhibit significantly increased PD-L1 expression in both tumor and immune stromal cells compared to MMRp carcinomas. PD-L1 is frequently expressed in both tumor and immune cells at the periphery of MMRd tumors, suggesting an ongoing interaction between the tumor and the inflammatory microenvironment. The MMR status in EC may be a crucial determinant of the success of immune checkpoint inhibitor therapy [13,30].

Consistent with the literature, our study found that MMRp tumors were associated with early-stage disease ($p=0.020$) and better survival outcomes ($p=0.014$). When comparing PD-L1 IC and CPS with MMR status, PD-L1 positivity was significantly associated with MMRd ($p < 0.001$). The significant association between PD-L1 positivity and MMRd suggests that tumors become more visible to the immune system and exhibit increased immunogenicity. MMRd is linked to a high tumor mutation burden, which may facilitate immune cell recognition of the tumor, leading to increased PD-L1 expression. This finding supports previous studies indicating that patients with MMRd may respond better to immunotherapies, particularly PD-1/PD-L1 inhibitors.

Association Between PD-L1 and p53

An abnormal p53 pattern is a reliable IHC marker for TP53 mutations and defines the high copy-number/p53-abnormal EC subgroup, which has the poorest prognosis among the TCGA/ProMisE subtypes [31]. This subgroup is most common in high-risk histotypes, including serous carcinoma (nearly all cases), carcinosarcoma (>70%), and clear cell carcinoma (~50%) [31]. P53 mutations occur in 10–40% of type I EC and ~90% of type II EC [32]. Amarin et al. reported p53 mutations in 46.5% of cases, significantly correlating with MMR stability ($p < 0.001$) but not with PD-L1 expression ($p = 0.78$) [33]. Similarly, our study found p53 mutations associated with MMRp ($p = 0.014$).

In the present study, the association between PD-L1 expression and p53 status was confined to TC–based evaluation, while no significant correlation was observed when PD-L1 was assessed using the CPS. This finding indicates that the PD-L1–p53 relationship is limited in strength and dependent on the PD-L1 scoring methodology. This discrepancy may be explained by the fact that TC-based PD-L1 assessment primarily reflects tumor-intrinsic

immune evasion mechanisms, whereas CPS incorporates immune cell components of the tumor microenvironment.

Accordingly, the PD-L1–p53 association should be interpreted with caution. Although variations in PD-L1 TC expression were observed among certain histological subtypes, these findings are limited by small subgroup sizes and do not support a consistent or robust interaction across different pathological contexts. Therefore, combined evaluation of PD-L1 and p53 alone is insufficient for definitive clinical stratification and should be interpreted in conjunction with other molecular biomarkers, particularly mismatch repair status.

This study has some limitations. First, due to its retrospective and single-center design, the findings primarily reflect regional data and may not be fully generalizable to broader populations. An important limitation is the absence of POLE mutation analysis, which precluded the full application of the TCGA four-tier molecular classification. POLE-ultramutated endometrial carcinomas have been shown to exhibit an exceptionally high tumor mutational burden, prominent immune cell infiltration, and increased expression of immune checkpoint molecules, including PD-L1 [19,20]. Therefore, the lack of POLE testing may have influenced the distribution of molecular subgroups in our cohort and could have resulted in an underestimation or alteration of PD-L1 positivity rates, as well as affected the strength of some observed associations, including those related to survival outcomes. Future studies incorporating POLE mutation analysis will enable more comprehensive molecular stratification and may further refine the relationship between PD-L1 expression, molecular subtypes, and immunotherapy response.

In addition, the limited number of cases in rare histological subtypes, such as clear cell and mixed carcinomas, restricts the interpretation of subgroup-specific findings. The relatively low number of survival events also limited the ability to demonstrate independent prognostic significance for the evaluated variables. Moreover, the very small number of patients who received immunotherapy (n = 4) precluded meaningful survival analysis for this subgroup, particularly since all treated patients were alive at the last follow-up. Therefore, our findings should be interpreted with caution, and larger, multicenter, prospective studies are warranted to validate and refine these conclusions.

CONCLUSION

In conclusion, PD-L1 IC and CPS expression were more frequently observed in low-grade tumors, whereas PD-L1 tumor cell positivity was more common in non-endometrioid subtypes. PD-L1 expression was not independently associated with survival. Nevertheless, evaluation of PD-L1 in both tumor and immune cell compartments, in conjunction with other molecular biomarkers, may remain relevant when considering immunotherapy in endometrial carcinoma. Mismatch repair status continues to be a key determinant in treatment planning. Although PD-L1 expression alone does not constitute a robust prognostic or predictive biomarker, its assessment alongside MMR and p53 status may provide complementary information for identifying patients who could potentially benefit from immunotherapy. Given the limited and tumor cell–restricted association between PD-L1 and p53, these findings should be interpreted with caution. Further large-scale, prospective studies are needed to define the clinical utility of integrated biomarker approaches.

Declarations

Ethics approval and consent to participate: The ethics approval of this study was accepted by the Non-Interventional Clinical Research Ethics Committee of Pamukkale University at the meeting dated February 22, 2022, with decision number 04.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest

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Availability of data and materials:

This study data was obtained from patient records and materials of Pamukkale University Faculty of Medicine Department of Pathology. Ethics committee approval was received. Additional details regarding the data extraction process can be obtained from the relevant

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All authors have read and agreed to the final version of the manuscript.

Supplementary material

None.

AI Disclosure

During the preparation of this work we used AI GPT-5.2 in order to improve language and readability. After using this tool/service, we reviewed and edited the content as needed and take full responsibility for the content of the publication.

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