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PROGNOSTIC IMPLICATIONS OF PD-L1 EXPRESSION AND LOSS OF PTEN IN PATIENTS WITH RHABDOMYOSARCOMA, EWING'S SARCOMA AND OSTEOSARCOMA

Background. In children, osteosarcoma (OS), Ewing's sarcoma (ES), and rhabdomyosarcoma (RMS) are the most common sarcomas. A link between the anti-programmed death ligand-1 PD-L1 and the tumor suppressor phosphatase and tensin homologue (PTEN) expression has been described in many tumors. The **aim** of this work is to determine clinicopathological relationships and the possible prognostic significance of PD-L1 and PTEN expression in rhabdomyosarcoma (RMS), Ewing's sarcoma (ES), and osteosarcoma (OS). **Materials and Methods.** Expression of PD-L1 and PTEN were examined by immunohistochemistry in 45 archival RMS, ES, and OS cases. **Results.** The positive expression of PD-L1 was found in 16.7% and 31.6% of ES and OS, respectively. The negative PD-L1 was related to a substantially longer survival in ES cases ($p = 0.045$), but positive PD-L1 expression was significantly associated with the increased tumor stage and vascular invasion in the OS cases ($p = 0.005$ and $p = 0.002$), respectively. On the other hand, PTEN loss was strongly associated with deep tumor, high tumor grade, and recurrence in RMS ($p = 0.002$, $p = 0.045$, and $p = 0.026$, respectively). However, PTEN loss was significantly absent in ES as tumor grade increased ($p = 0.031$). It is noteworthy that tumor recurrence, the loss of PTEN, and positive PD-L1 were all considered predictive factors in OS patients ($p = 0.045$, $p = 0.032$, and $p = 0.02$, respectively). **Conclusions.** In children, OS and ES have positive PD-L1 expression, which has an independent unfavorable prognostic effect and raises the possibility of using PD-L1 as a therapeutic target. OS, ES, and RMS prognosis are all predicted by PTEN loss.

Keywords: PD-L1, PTEN, Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma.

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Pediatric sarcomas are the aggressive tumors with poor outcomes. Sarcomas are broadly categorized into soft tissue sarcoma (STS) and bone sarcomas. In children, osteosarcoma (OS), Ewing's sarcoma (ES) and rhabdomyosarcoma (RMS) are the most common sarcomas [1, 2]. A conventional therapeutic approach for these sarcomas showed no significant improvement in patient outcome [3]. It is crucial to look for new biomarkers and efficient therapies since STS and OS are highly heterogeneous [4, 5]. Therefore, a novel treatment approach, like immunotherapy, is urgently needed. Anti-programmed death ligand-1 (PD-L1), one of these immunotherapies, has demonstrated considerable success in the treatment of several cancers, including melanoma, renal cell carcinoma, and non-small cell lung cancer [6, 7]. Unfortunately, pediatric sarcomas often show a very limited number of genetic incongruities, which are frequently caused by a single translocation. As a result, these tumors are probably less immunogenic, which restricts the use of immunotherapy as an effective therapeutic modality. However, there are encouraging, ongoing clinical trials using immunotherapy for advanced pediatric malignancies [8]. Additionally, earlier research showed that PD-L1 is expressed in a variety of STSs, supporting the possibility of employing it as a successful therapeutic target in pediatric sarcomas [9, 10]. PD-L1 is one of the immune checkpoint inhibitor [6], and there is a correlation between the expression of PD-L1 and the tumor suppressor phosphatase and tensin homologue (PTEN), with PTEN loss or repression leading to an increase in PD-L1 on the membrane surface of tumor cells [11—13]. PTEN is a tumor suppressor that plays an important role in carcinogenesis by adversely regulating the protein kinase B (AKT) and phosphoinositide 3-kinase (PI3K) signaling pathways, both of which are important for the growth and survival of cancer cells. A loss of PTEN function is thought to be a key factor in carcinogenesis and has been linked to the majority of cancer types [14]. PTEN abrogation frequently resulted in changes to the tumor microenvironment, including an increase in a non-inflamed tumor due to the production of anti-inflammatory cytokines and a notable decrease in T-cell activity [15, 16].

In the current study, we looked at the possible predictive usefulness and clinicopathological correlations of PD-L1 and PTEN expression in pediatric sarcomas as RMS, ES, and OS. This is the first research to look at the expression and function of PTEN and PD-L1 in pediatric sarcomas.

Materials and Methods

Tumor samples. The archival formalin fixed-paraffin embedded blocks were obtained from 54 patients with RMS, OS, and ES from Pathology Department, Umm Al-Qura University. The blocks were retrieved from the pathology files for patients diagnosed between December 2011 and January 2021. The study procedure was commended by the Institutional Review Board of Umm Al-Qura University (Ethical approval no. HAPO-02-k-012-10-790). Data follow-up started upon diagnosis, and a 60-month median follow-up was used. All archive blocks that were admitted for the first diagnosis were chosen, while the archival blocks that belonged to patients with missing or incomplete clinical data were eliminated. To determine the sarcoma's histological diagnosis and subtype in accordance with the World Health Organization classification, all H&E-stained slides from all instances were examined [5]. The clinicopathological data are shown in Table 1.

Immunohistochemistry procedure. The formalin-fixed paraffin-embedded blocks underwent immunohistochemical (IHC) staining. 4-m sections were cut, dewaxed with xylene, and rehydrated with graded ethanol at corresponding concentrations of 100%, 90%, and 70%. For antigen retrieval, the sections were microwave-irra-

diated in the EDTA buffer. The sections underwent endogenous peroxidase suppression before analysis by a monoclonal mouse anti-human PTEN antibody (clone 6H2.1, dilution 1/1000, DakoCytomation, Denmark) and monoclonal mouse anti-human PD-L1 antibody (clone 22C3, dilution 1/200, Dako, Denmark) at 4 °C overnight. Then they were stained with a streptavidin-biotin-peroxidase kit (Dako, Denmark). Finally, the sections were immersed in 3,3'-diaminobenzidine, counterstained, and mounted. For each run, positive controls for PTEN (melanoma) and PD-L1 (normal placenta) were used. Negative controls were also included without the primary antibody.

Scoring system. The intensity of staining and percentage of tumor cells that were positive were used to calculate the IHC score in a blind semi-

Table 1. **Clinicopathological characteristics of patients**

Clinicopathological characteristics		RMS		ES		OS	
		No	%	No	%	No	%
Age	1—10	6	35.3	10	55.6	6	31.6
	10—19	11	64.7	8	44.4	13	68.4
Gender	Male	10	58.8	10	55.6	10	52.6
	Female	7	41.2	8	44.4	9	47.4
Site	Trunk	8	47.1	6	33.3	8	42.1
	Extremities	9	52.9	12	66.7	11	57.9
Size	< 5 cm	4	23.5	4	22.2	7	36.8
	≥ 5 cm	13	76.5	14	77.8	12	63.2
Depth	Superficial	6	35.3	5	27.8	5	26.3
	Deep	11	64.7	13	72.2	14	73.7
Grade	Grade 1	3	17.6	3	16.7	3	15.8
	Grade 2	5	29.4	6	33.3	5	26.3
	Grade 3	9	52.9	9	50.0	11	57.9
Stage	Stage 1	3	17.6	4	22.2	3	15.8
	Stage 2	4	23.5	6	33.3	6	31.6
	Stage 3	10	58.8	8	44.4	10	52.6
Vascular invasion	Absent	10	58.8	13	72.2	12	63.2
	Present	7	41.2	5	27.8	7	36.8
Metastasis	Absent	8	47.1	10	55.6	9	47.4
	Present	9	52.9	8	44.4	10	52.6
Survival	Survival	12	70.6	14	77.8	13	68.4
	Death	5	29.4	4	22.2	6	31.6
Recurrence	Absent	11	64.7	12	66.7	11	57.9
	Present	6	35.3	6	33.3	8	42.1
PTEN	Loss	8	47.1	11	61.1	8	42.1
	Positive	9	52.9	7	38.9	11	57.9
PD-L1	Negative	17	100.0	15	83.3	13	68.4
	Positive	0	0	3	16.7	6	31.6

quantitative investigation, which produced a rating system. A semi-quantitative scoring identified the tumor cells' positive PD-L1 expression as 0—3+, based on weak, moderate, and strong membranous staining, respectively, and the percentage of tumor cells expressing the protein was recorded. When full or incomplete membranous expression was found in ≥1% of the tumor cells, regardless of the intensity, PD-L1 expression was considered positive. Once discovered, PD-L1 expression on immune cells (lymphocytes and/or plasma cells) was also reported [17].

Although the nucleus also displayed positive PTEN staining, most frequently it was seen in the cytoplasm. A score of less than 25% was referred to as PTEN negative or PTEN loss [18].

Statistical methods. The data were analyzed using the Statistical Package for Social Sciences (SPSS: An IBM Company, Version 22.0, IBM Corporation, Armonk, NY, USA). The chi-square test was performed to assess the relationship between the marker expression and the clinicopathological parameters as well as gene mutations. Kaplan — Meier plots and log-rank tests were used to evaluate the relationship between PDL overexpression and the overall survival. The significant results were recognized at $p < 0.05$. The prognosis of RMS, ES, and OS patients was assessed via univariate and multivariate Cox regression. Hazard risk (HR) and relative 95% confidence interval (CI) were analyzed.

Results

PTEN expression in RMS, ES, and OS. There was a loss of PTEN expression in 47.1% of RMS, 61.1% of ES, and 42.1% of OS cases in this study (Fig. 1). The loss of PTEN was significantly associated with deep tumor, increased tumor grade, and tumor recurrence in RMS ($p = 0.002$, $p = 0.045$, $p = 0.026$, respectively) (Table 2). There was, however, significant absence of PTEN loss with increasing tumor grade in cases of ES ($p = 0.031$) (Table 3). In OS, however, PTEN expression was lost as tumor stage increased, but this association was insignificant ($p = 0.06$) (Table 4).

PD-L1 expression in RMS, ES, and OS. Only 3 (16.7%) and 6 (31.6%) cases, respectively, with positive expression of PD-L1 in ES and OS were found. Additionally, the negative PD-L1 expression was significantly associated with a long survival in ES cases ($p = 0.045$), whereas high PD-L1 expression was strongly linked with increased tumor stage and vascular invasion in cases of OS ($p = 0.005$ and $p = 0.002$, respectively). In cases of ES and OS, the PD-L1 expression did not significantly correlate with any other clinicopathological factors (Table 3 and 4). Surprisingly, all cases of RMS were found to have low or negative PD-L1 expression (Fig. 2).

Prognostic factors for RMS, ES, and OS. In univariate regression analysis, site of the tumor (in the extremities), small size of tumor (less than 5 cm), deep seated tumors, tumor of higher grade and higher stage, vascularized tumors, absence of metastasis, expression of PTEN and PD-L1, and being RMS compared to ES were significantly associated with recurrence in all examined cases ($p < 0.05$).

A multivariate regression analysis identified each site of the tumor (in the extremities), positive for PD-L1 and being RMS compared to ES to be independently associated with tumor recurrence. As compared to tumor location in the trunk, tumor site in the extremities had 73 % lower odd's ratio (OR 0.29, 95% CI 0.09—0.96, $p = 0.04$). Patients with tumors positive for PD-L1 had 4.9 times higher risk of recurrence, and compared to RMS, ES had 71 % lower OR (OR 0.27, 95% CI 0.07—1.0, $p = 0.05$) (Table 5).

Survival analysis for RMS, ES, and OS. Regarding PD-L1, a significant association was found between negative expression of PD-L1 and long survival of Ewing sarcoma patients ($p = = 0.045$). In OS patients with low PD-L1 expression, the overall survival rate was significantly higher than in the patients with high PD-L1

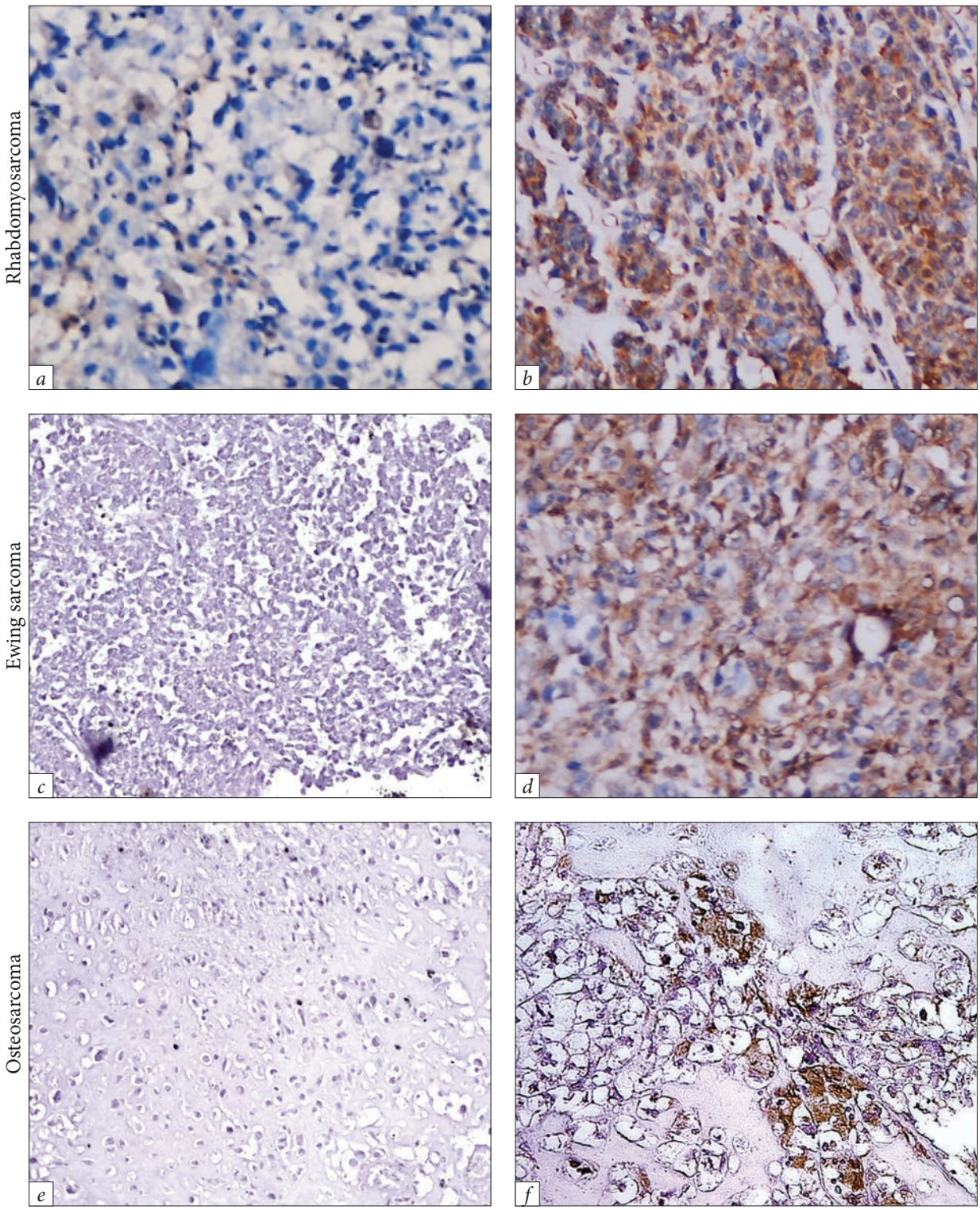

Fig. 1. PTEN expression in pediatric sarcomas: *a*) negative PTEN in RMS; *b*) positive PTEN in RMS; *c*) negative PTEN in ES; *d*) positive PTEN in ES; *e*) negative PTEN in OS; *f*) positive PTEN in OS; 100x

expression. The 5-year overall survival rate was 23.1% for patients with high PD-L1 expression and 76.9% for patients with low PD-L1 expression. The difference between the PD-L1-positive and PD-L1-negative groups was not statistically significant ($p = 0.2$).

Regarding PTEN expression and survival analysis, there was no significant difference in the overall survival between the PTEN high expression group and PTEN low expression group in all sarcomas.

Discussion

Pediatric sarcomas are highly aggressive malignant mesenchymal tumors. There are limitations in sarcoma studies due to their rarity and morphological variability. That is why a mainstay of sarcoma treatment and the management regimen has not changed for decades [19, 20]. In this study, we evaluated the expression of PD-L1 and PTEN in the most frequent pediatric sarcomas (RMS, ES, and OS). Recently, PD-L1

Table 2. **Association between PTEN expression and different clinicopathological factors of RMS cases**

Clinicopathological characteristics		PTEN		
		Loss No. (%)	Positive No. (%)	*p*
Age	1-10	1(16.7)	5(83.3)	0.070
	10-19	7(63.6)	4(36.4)	
Gender	Male	5(50.0)	5(50.0)	0.788
	Female	3(42.9)	4(57.1)	
Site	Trunk	2(25.0)	6(75.0)	0.096
	Extremities	6(66.7)	3(33.3)	
Size	< 5 cm	2(50.0)	2(50.0)	0.901
	≥ 5 cm	6(46.2)	7(53.8)	
Depth	Superficial	0(0.0)	6(100.0)	0.002
	Deep	8(72.7)	3(27.3)	
Grade	Grade 1	0(0.0)	3(100.0)	0.045
	Grade 2	2(40.0)	3(60.0)	
	Grade 3	6(66.7)	3(33.3)	
Stage	Stage 1	1(33.3)	2(66.7)	0.680
	Stage 2	2(50.0)	2(50.0)	
	Stage 3	5(50.0)	5(50.0)	
Vascular invasion	Absent	4(40.0)	6(60.0)	0.517
	Present	4(57.1)	3(42.9)	
Metastasis	Absent	3(37.5)	5(62.5)	0.488
	Present	5(55.6)	4(44.4)	
Recurrence	Absent	3(27.3)	8(72.7)	0.026
	Present	5(83.3)	1(16.7)	
Survival	Survival	4(33.3)	8(66.7)	0.088
	Death	4(80.0)	1(20.0)	

Notes: Test of significance: Chi-square
$p < 0.05$ is considered significant.

has become a successful therapeutic target and has shown long term remissions in patients with late stage cancer such as melanoma, lung, renal, and bladder carcinoma [21]. In addition, PTEN was found to have a therapeutic potential in the advanced OS [22]. Therefore, in this study, we investigate the potential prognostic value and clinicopathological correlations of PD-L1 and PTEN expressions in RMS, ES, and OS cases.

Regarding PD-L1, we found negative expression in all RMS cases. Consistent with our findings, the previous studies reported that all cases of RMS showed negative PD-L1 expression [9, 23]. This finding may explain why no responses have been reported to anti-PD-L1 when it was used as a single therapeutic regimen in RMS [23]. Our finding is in contrast to Kim et al. [10], who showed the positive expression of PD-L1 in tumor cells in 37% (12/32) samples. However, the discordant observations in the expression of PD-L1 can be explained by the use of a different clone of anti-PD-L1 antibodies and the antigen

Table 3. **Association between PTEN and PD-L1 expression and different clinicopathological factors of ES cases**

Clinicopathological characteristics		PTEN			PD-L1		
		Loss No. (%)	Positive No. (%)	*p*	Loss No. (%)	Positive No. (%)	*p*
Age	1-10	6(60.0)	4(40.0)	0.920	9(90.0)	1(10.0)	0.426
	10-19	5(62.5)	3(37.5)		6(75.0)	2(25.0)	
Gender	Male	6(60.0)	4(40.0)	0.920	8(80.0)	2(20.0)	0.693
	Female	5(62.5)	3(37.5)		7(87.5)	1(12.5)	
Site	Trunk	4(66.7)	2(33.3)	0.751	5(83.3)	1(16.7)	1.000
	Extremities	7(58.3)	5(41.7)		10(83.3)	2(16.7)	
Size	< 5 cm	1(25.0)	3(75.0)	0.104	3(75.0)	1(25.0)	0.637
	≥ 5 cm	10(71.4)	4(28.6)		12(85.7)	2(14.3)	
Depth	Superficial	3(60.0)	2(40.0)	0.956	5(100.0)	0(0.0)	0.265
	Deep	8(61.5)	5(38.5)		10(76.9)	3(23.1)	
Grade	Grade 1	0(0.0)	3(100.0)	0.031	3(100.0)	0(0.0)	0.079
	Grade 2	4(66.7)	2(33.3)		6(100.0)	0(0.0)	
	Grade 3	7(77.8)	2(22.2)		6(66.7)	3(33.3)	
Stage	Stage 1	1(25.0)	3(75.0)	0.130	4(100.0)	0(0.0)	0.311
	Stage 2	4(66.7)	2(33.3)		5(83.3)	1(16.7)	
	Stage 3	6(75.0)	2(25.0)		6(75.0)	2(25.0)	
Vascular invasion	Absent	8(61.5)	5(38.5)	0.956	10(76.9)	3(23.1)	0.265
	Present	3(60.0)	2(40.0)		5(100.0)	0(0.0)	
Metastasis	Absent	6(60.0)	4(40.0)	0.920	8(80.0)	2(20.0)	0.693
	Present	5(62.5)	3(37.5)		7(87.5)	1(12.5)	
Recurrence	Absent	8(66.7)	4(33.3)	0.523	11(91.7)	1(8.3)	0.201
	Present	3(50.0)	3(50.0)		4(66.7)	2(33.3)	
Survival	Survival	8(57.1)	6(42.9)	0.546	13(92.9)	1(7.1)	0.045
	Death	3(75.0)	1(25.0)		2(50.0)	2(50.0)	

Notes: Test of significance: Chi-square
$p < 0.05$ is considered significant.

retrieval system. In ES, PD-L1 expression was detected in only 16% of our cases. A previous study reported that PD-L1 immunoreactivity was detected in 19% of ES cases, this result was similar to our findings [24]. Furthermore, our study revealed a significant association between high PD-L1 expression and short overall survival in ES. The same result was also reported by another study [10]. In OS, we found that expression of PD-L1 was detected in about 31% of cases. Supporting this result, Shen et al. [25] reported an increased PD-L1 mRNA in 24% of OS cases. Alike with our findings, the previous studies described that about 30% of OS cases showed positive PD-L1 [26, 27]. In contrast, Torabi et al. [28] and Park et al. [29] demonstrated a positive PD-L1 immunoreactivity in 0% and 3% respectively in their studies. This controversy may be attributed to the different PD-L1 antibodies used and different variants of OS such as high-grade spindle cell morphology as mentioned in Park's study [29]. Moreover, an increased PD-L1 im-

Table 4. **Association between PTEN and PDL1 expression and different clinicopathological factors of OS cases**

Clinicopathological characteristics		PTEN			PD-L1		
		Loss No. (%)	Positive No. (%)	*p*	Loss No. (%)	Positive No. (%)	*p*
Age	1-10	1(16.7)	5(83.3)	0.142	1(16.7)	5(83.3)	0.370[c]
	10-19	7(53.8)	6(46.2)		5(38.5)	8(61.5)	
Gender	Male	5(50.0)	5(50.0)	0.490	3(33.3)	6(66.7)	0.630
	Female	3(33.3)	6(66.7)		3(30.0)	7(70.0)	
Site	Trunk	4(50.0)	4(50.0)	0.578	3(37.5)	5(62.5)	0. 658[c]
	Extremities	4(36.4)	7(63.6)		3(27.3)	8(72.7)	
Size	< 5 cm	2(28.6)	5(71.4)	0.390	1(14.3)	6(85.7)	0.238
	≥ 5 cm	6(50.0)	6(50.0)		5(41.7)	7(58.3)	
Depth	Superficial	1(20.0)	4(80.0)	0.268	2(40.0)	3(60.0)	0.520
	Deep	7(50.0)	7(50.0)		4(28.6)	10(71.4)	
Grade	Grade 1	0(0.0)	3(100.0)	0.114	0(0.0)	3(100.0)	0.114[c]
	Grade 2	2(40.0)	3(60.0)		1(20.0)	4(80.0)	
	Grade 3	6(54.5)	5(45.5)		5(45.5)	6(54.5)	
Stage	Stage 1	0(0.0)	3(100.0)	0.060	0(0.0)	3(100.0)	0.005
	Stage 2	2(33.3)	4(66.7)		0(0.0)	6(100.0)	
	Stage 3	6(60.0)	4(40.0)		6(60.0)	4(40.0)	
Vascular invasion	Absent	4(33.3)	8(66.7)	0.338	1(8.3)	11(91.7)	0.002
	Present	4(57.1)	3(42.9)		5(71.4)	2(28.6)	
Metastasis	Absent	2(22.2)	7(77.8)	0.106	1(11.1)	8(88.9)	0.071
	Present	6(60.0)	4(40.0)		5(50.0)	5(50.0)	
Recurrence	Absent	4(36.4)	7(63.6)	0.578	3(27.3)	8(72.7)	0.506
	Present	4(50.0)	4(50.0)		3(37.5)	5(62.5)	
Survival	Survival	5(38.5)	8(61.5)	0.658	3(23.1)	10(76.9)	0.257
	Death	3(50.0)	3(50.0)		3(50.0)	3(50.0)	

Notes: Test of significance: Chi-square
$p < 0.05$ is considered significant.

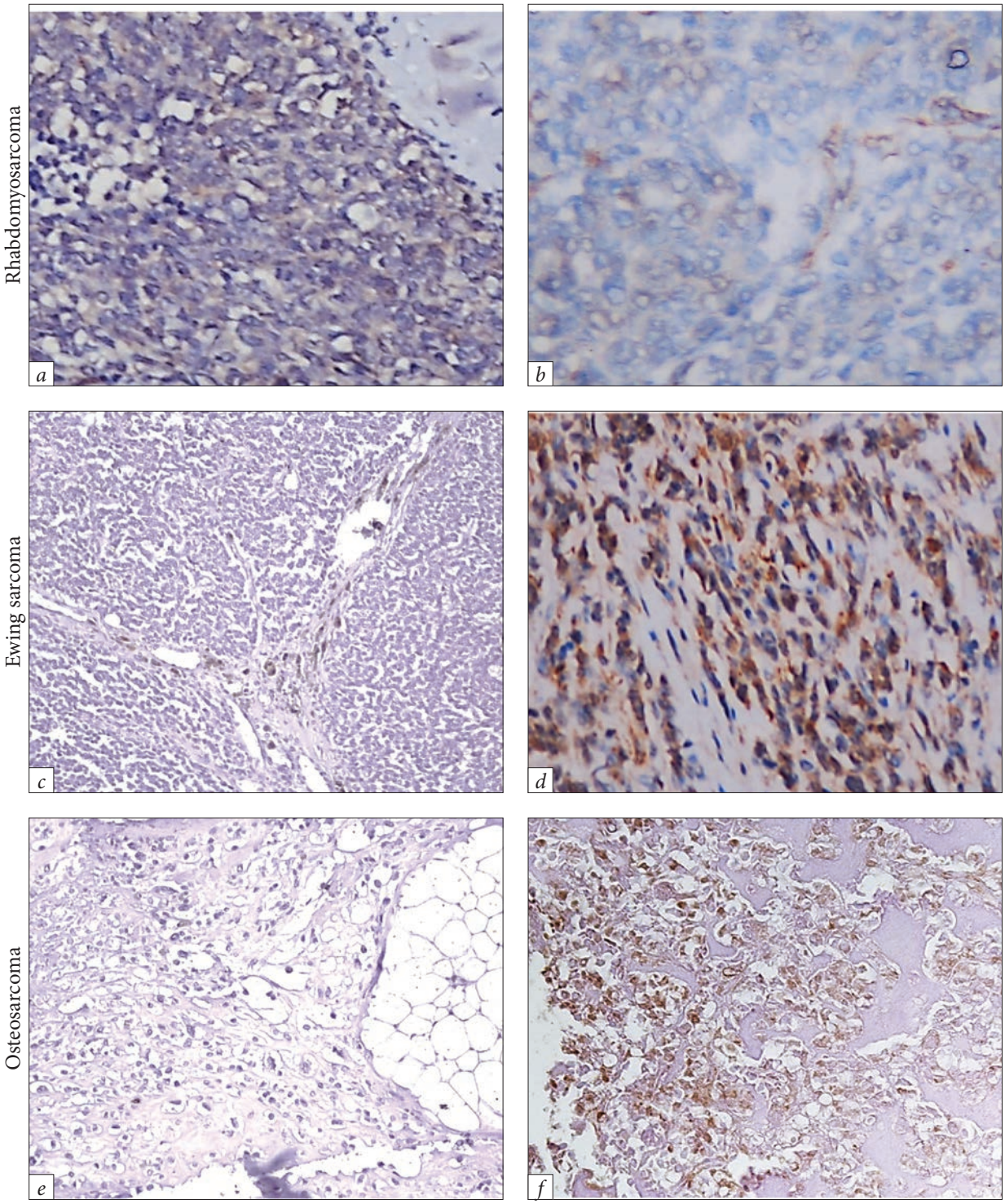

Fig. 2. PD-L1 expression in pediatric sarcomas: *a*) negative PD-L1 in RMS; *b*) negative PD-L1 in RMS at higher magnification (200x); *c*) negative PD-L1 in ES; *d*) positive PD-L1 in ES; *e*) negative PD-L1 in OS; *f*) positive PD-L1 in OS; 100x except for (**b**)

munoreactivity was significantly associated with increased OS stage and the existence of vascular invasion. Alike with this result, Liu et al. [27] reported a significant increase in the tumor stage associated with the positive PD-L1. The aforementioned data showed a reduction of the PD-L1 expression in most of the studied pediatric sarcoma. A recent study has shown that PD-L1 down-regulation is linked to increased T-cell mediated cytotoxicity and increased PTEN expression [11]. Moreover, the PTEN loss was associated with the increased PD-L1 expression in colorectal carcinoma [30]. Thus, we investigated the expression of PTEN in our cases.

In RMS, we found a PTEN loss in about 48% of cases comparable to this result, and the PTEN loss was revealed in 41% of leiomyosarcoma cases [31]. Furthermore, the PTEN loss was significantly associated with tumor aggressiveness parameters such as the increased tumor depth, increased tumor grade, and recurrence. It led to activation of the PI3K/AKT/mTOR pathway, a well-known pathway that is critical for tumor growth [32, 33]. Hence, the PTEN loss induces impairment of myogenic differentiation in a tumor derived from a RMS mouse model [34].

The majority of ES cases showed a PTEN loss, and its depletion was significantly associated with the increased tumor grade. This finding could be explained by a study reporting that PTEN activates the PI3K pathway, which in turn controls an activity and oncogenic phenotypes in ES, which promotes tumor proliferation [35].

Although there was no significant association in the overall survival related to the PTEN loss, there was a trend of increased aberration with

Table 5. **Multi-regression model for predictors of recurrence**

Test name	Univariate analysis	Multivariate analysis		
Variables	*p*	Odd's ratio	95% confidence interval	*p*
Site of Tumor (*vs.* Trunk)	0.05	0.29	0.09—0.96	0.04
Size of tumor (*vs.* < 5 cm)≥5 cm	0.05	0.76	0.22—2.57	0.66
Depth (*vs.* superficial)				
Deep	0.001	1.08	0.22—5.43	0.93
Grade				
Grade I	Ref	Ref		
Grade II	0.006	0.39	0.07—2.33	0.30
Grade III	0.003	3.18	0.45—22.57	0.25
Stage				
Stage I	Ref	Ref		
Stage II	0.002	0.59	0.12—2.89	0.51
Stage III	0.004	4.81	0.86—26.81	0.07
Vascularization (*vs.* none)	0.001	5.06	0.09—292.2	0.43
Metastasis (*vs.* none)	0.001	0.23	0.01—10.45	0.45
PTEN (*vs.* none)	0.04	1.25	0.42—3.75	0.69
PDL1 (*vs.* none)	<0.0001	4.90	1.41—17.0	0.01
Type of tumor				
RMS	Ref	Ref		
ES	0.03	0.27	0.07—1.0	0.05
OS	0.12	0.91	0.23—3.52	0.89

tumor stage progression; the small number of cases in each group may explain the non-significant result. Other studies reported that PTEN expression was found in cases without metastasis, and SIX1 reduced PTEN expression to induce tumor proliferation and OS tumorigenesis [36, 37]. It is clear from our results that tumor recurrence and the loss of PTEN were prognostic factors in RMS. However, in ES tumor depth and recurrence were considered prognostic factors. Interestingly, increased tumor stage, tumor recurrence, PTEN loss, and the positive PD-L1 were considered prognostic factors in OS.

PD-L1 expression may serve a prognosis indicator for a poor outcome; therefore, the independent prognostic contribution of PD-L1 was assessed using the Cox multivariate survival analysis while adjusting for known clinical-pathologic variables. Even in low-stage sarcomas, patients with STSs who showed a positive PD-L1 phenotype had a shorter survival time and a more advanced sarcoma phenotype [38]. Lack of PD-L1 expression in ES cells was associated with both poor and progression-free survival [24]. Numerous investigations, however, have shown that the predictive significance of PD-L1 expression in sarcoma is uncertain. It was discovered that the expression of PD-L1 mRNA varied among STS subtypes and was a poor predictor of prognosis. Favorable survival was predicted by PD-L1 positivity found by immunohistochemistry on tissue microarrays from formalin-fixed paraffin-embedded tissues [4]. However, in another investigation, it was discovered to be a negative prognostic factor [39]. PD-L1 was not associated with clinical features of STS but was associated with poorer clinical OS characteristics in complete tissue sections. Disagreements in PD-L1 expression studies in sarcomas may be related to variances in detection methods, the lack of a gold standard for quantification of expression, the use of various antibodies, and the use of tissue microarrays *vs.* the entire tissue sections. Furthermore, variations in the number of cases of particular subtypes, inclusion of tissues taken before or after therapy, and tumor heterogeneity may have contributed to the disparities in outcomes.

The 5-year relapse-free survival rate in PDL1 positive patients was much higher, but this was most likely due to more frequent PD-L1 expression in early-stage RMS. Although PD-L1 expression was related to improved survival, this was due to the trend of more frequent positive PD-L1 expression in the early stages. Surprisingly, the clinical effects of PD-L1 expression in STSs vary with the subtype [8].

In conclusion, the significant fraction of PD-L1 positivity and the association of PD-L1 with worse clinical outcome give justifications for immune checkpoint inhibition in patients with PD-L1-positive sarcoma. Tumor PD-L1 expression was linked to a considerably worse 5-year overall survival rate [40]. Furthermore, a substantial relationship between PD-L1 and metastasis has been documented. These findings imply that the link between PD-L1 and cancer development is merely a minor side effect of PTEN loss [15]. The PTEN activity affects PI3K-induced PIP3, which plays a vital role in modulating subsequent signaling pathways involved in cell survival, proliferation, and migration [11]. Patients with PTEN-deficient castration-resistant prostate cancer who were treated with abiraterone plus an AKT inhibitor showed better radiographic progression-free survival [41]. PTEN protein expression is linked to better disease-specific and disease-free survival. Its loss is mostly related to high-risk/metastatic cancers and has a significant detrimental influence on OS [42].

PD-L1 expression is found in children's OS and ES, has an independent poor prognostic effect and raises the possibility of using PD-L1 as a therapeutic target. PTEN deficiency predicts the development of OS, ES, and RMS. However, there was a limitation in our study regarding the number of cases, and further research on larger cohorts is required.

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ПРОГНОСТИЧНЕ ЗНАЧЕННЯ ЕКСПРЕСІЇ PD-L1 ТА ВІДСУТНОСТІ ЕКСПРЕСІЇ PTEN У ПАЦІЄНТІВ З РАБДОМІОСАРКОМОЮ, САРКОМОЮ ЮЇНГА ТА ОСТЕОСАРКОМОЮ

Стан питання. Остеосаркома (ОС), саркома Юїнга (СЮ) і рабдоміосаркома (РМС) є найпоширенішими саркомами в дітей. Зв'язок між експресією PD-L1 (ліганда-1 рецептора програмованої загибелі клітин — 1) та супресора пухлинного росту PTEN (фосфатази та гомолога тензину) був описаний для багатьох пухлин. **Метою** цієї роботи було визначити клінікопатологічні зв'язки та можливе прогностичне значення експресії PD-L1 та PTEN при найпоширеніших саркомах дітей. **Матеріали та методи.** Ретроспективне дослідження експресії PD-L1 і PTEN проведено імуногістохімічним методом на зразках пухлинної тканини 45 хворих на ОС, СЮ та РМС. **Результати.** Експресію PD-L1 виявлено в 16,7% і 31,6% випадків ЮС та ОС, відповідно. Показано, що відсутність експресії PD-L1 асоціюється з кращими показниками виживаності в пацієнтів із СЮ (p = 0,045), тоді як її наявність пов'язана з більшим поширенням пухлинного процесу та проростанням до кровоносних судин у хворих на ОС (відповідно p = 0,005 та p = 0,002). З іншого боку, відсутність експресії PTEN була характерною для інвазивних новоутворень, низького ступеня диференціювання та рецидивом у хворих на РМС (p = 0,002, p = 0,045 і p = 0,026, відповідно). Встановлено, що відсутність експресії PTEN характерна для СЮ низького ступеня диференціювання (p = 0,031). Слід зазначити, що рецидив новоутворення, відсутність експресії PTEN та наявність PD-L1 можна вважати прогностичними факторами в пацієнтів з ОС (p = 0,045, p = 0,032 і p = 0,02, відповідно). **Висновки.** У дітей з ОС та СЮ наявність експресії PD-L1 є негативним прогностичним фактором, що вказує на можливість використання PD-L1 як терапевтичної мішені. Відсутність експресії PTEN є прогностичним фактором у хворих на ОС, СЮ та РМС.

Ключові слова: PD-L1, PTEN, саркома Юїнга, остеосаркома, рабдоміосаркома.