Original paper

Sarcoma – correlation between CD73 and PD-L1 and their relationship with prognosis

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This study aimed to evaluate CD73 and PD-L1 and determine their relationship with each other and with overall survival (OS) in sarcoma patients.
The paraffin blocks of 101 patients were analysed. 56.4% were female, and the mean age was 51.39 years. The mean OS was 20.73 months, and the Ki-67 proliferative index was 41.45. A positive correlation was found between CD73 tumour and CD73 tumour-infiltrating lymphocyte (TIL) findings. CD73 tumour and TIL findings were also positively correlated with PD-L1 percentages and PD-L1 intensity.
An inverse correlation was detected between OS and CD73 tumour and TIL groups of 5–25%, 25–50%, 50–75%, 75–90%, and > 90%, but no such correlation was found for the ≤ 5% group. There was an inverse correlation between OS and the PD-L1 percentages of <10% and > 50% and the PD-L1 intensity of weak-moderate and strong, but no correlation was found for the negative values. Lastly, an inverse correlation was found between OS and the Ki-67 proliferative index.
We found CD73 and PD-L1 positivity to be associated with decreased OS in sarcoma patients and determined a significant correlation between these parameters. This result is promising in terms of achieving better survival and disease control with anti-CD73 and anti-PD-L1 therapy in selected patients.

Key words: sarcoma, CD73, PD-L1, overall survival.

Introduction

Sarcomas refer to a heterogeneous group of tumours with a mesenchymal origin and constitute 1% of adult malignant tumours. More than 50 histological subtypes have been defined according to the World Health Organization classification. In adults, the most common soft tissue sarcomas are malignant fibrous histiocytoma, liposarcoma, fibrosarcoma, leiomyosarcoma, and synovial sarcoma, while primary bone sarcomas comprise osteosarcoma, Ewing's sarcoma, and chondrosarcoma. Although they often tend to be located on the extremities, they can emerge anywhere on the body. The diagnosis and treatment of sarcomas require a multidisciplinary approach, with systemic therapy, including cytotoxic chemotherapy and molecular targeted agents, being the main treatment modalities in advanced or metastatic disease. The five-year survival rate is between 55 and 65% [1–6].

CD73 is a cell surface 5'-nucleotidase that converts monophosphate to adenosine. The released adenosine has an effect on immune suppression by binding to the adenosine A2A receptor on T and natural killer cells, limiting interferon gamma production and cytocidal activity [7]. It has been found that CD73 is overexpressed in various solid malignant tumours, and its overexpression in high-stage serous ovarian cancers is associated with reduced overall survival (OS) and poor prognosis [8]. Programmed death 1 (PD-1), a member of the CD28 family, is an inhibitory receptor that plays a major role in the immune escape of the tumour. PD-L1 is expressed on T, B, and dendritic cells and macrophages and acts as a receptor for PD-1 binding. PD-L1 expression has been associated with poor prognosis in some malignant tumours, while no such relationship has been reported in others [9, 10]. In light of these studies in the literature, our aim in the current study was to investigate CD73 and PD-L1, which are reported to be poor prognostic factors in many malignancies, and to evaluate the conflicting results on the correlation of CD73 with PD-L1 and their relationship with OS in patients diagnosed with sarcoma.

Material and methods

In this study, the data of 101 patients diagnosed with sarcoma and who died between 2011 and 2019 were retrospectively analysed. Gender, age, diagnosis, Ki-67 proliferative index (PI), OS time, PD-L1 percentage, PD-L1 intensity, and CD73 tumour- and CD73 tumour-infiltrating lymphocyte (TIL) status of the patients were recorded. The study was approved by the local Ethics Committee of the university (approval number: 10.06.2016-54).

Haematoxylin and eosin-stained sections of the cases were re-examined, and paraffin blocks containing tumour areas were selected. Five-micron-thick sections were taken from these blocks and stained with PD-L1 and CD73 antibodies in a fully automated immunohistochemistry stainer. A semi-quantitative assessment was used to evaluate the applied stains. PD-L1 intensity was classified as negative, weak-medium, and strong, and tumour cell staining was noted as a percentage (negative, $< 10\%$, 10–50%, and $> 50\%$) in the most intense area. Tumour cells and TILs were also evaluated separately with the CD73 antibody. The rates of $< 5\%$, 5–25%, 25–50%, 50–75%, 75–90%, and $> 90\%$ were used in the evaluation.

IBM SPSS Statistics version 23.0 was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements as mean and standard deviation (median, minimum, and maximum where necessary). χ^2 and Fisher's exact test statistics were used to compare categorical measurements between the groups. Normality of distribution was evaluated with the Kolmogorov-Smirnov test. For binary variables, an independent Student's *t*-test was used when the assumptions were met and the Mann-Whitney *U* test otherwise. One-way analysis of variance and the Kruskal-Wallis test were employed for more than 2 variables. Pearson's and Spearman's methods were used for the correlation analysis between the data. The statistical significance level was taken as < 0.05 in all tests.

Results

Of the 101 patients included in the study, 56.4% were female and 43.6% were male, and the mean age was 51.39 years. Considering the diagnostic status, 15.8% of the patients had leiomyosarcoma, 15.8% liposarcoma, 15.8% Ewing's sarcoma, 14.9% pleomorphic sarcoma, and 9.9% malignant peripheral nerve sheath tumour. The remaining diagnoses and percentages are shown in Table I. The mean OS time was 20.73 months and the mean Ki-67 index was 41.45 (Table I). Table II presents the PD-L1 intensity, PD-L1 percentages, and CD73 tumour and TIL percentages of the cases (Figs. 1–7).

Table I. Demographic characteristics, diagnosis, overall survival time, and Ki-67 index of the patients

Parameters	Number (%)
Gender	
Female	57 (56.4)
Male	44 (43.6)
Age, mean ±SD/median (min–max)	51.39 ±17.27/52 (18–87)
Overall survival, mean ±SD/median (min–max)	20.73 ±17.35/15 (1–120)
Ki-67 proliferative index, mean ±SD/median (min–max)	41.45 ±20.58/40 (6–95)
Diagnosis	
Synovial sarcoma	9 (8.9)
Chondrosarcoma	7 (6.9)
Leiomyosarcoma	16 (15.8)
Liposarcoma	16 (15.8)
Myxosarcoma	3 (3)
Ewing's sarcoma	16 (15.8)
Pleomorphic sarcoma	15 (14.9)
Rhabdomyosarcoma	3 (3)
Kaposi's sarcoma	6 (5.9)
Malignant peripheral nerve sheath tumour	10 (9.9)

Table II. PD-L1 intensity, PD-L1 percentages, and CD73 tumour and tumour-infiltrating lymphocyte percentages

Parameters	Results	Number (%)
PD-L1 intensity	Negative	76 (82.6)
	Weak-moderate	12 (13)
	Strong	4 (4.4)
PD-L1	Negative	76 (82.6)
	< 10	14 (15.2)
	10–50	0 (0)
	> 50	2 (2.2)
CD73 tumour	< 5	42 (45.7)
	5–25	9 (9.8)
	25–50	8 (8.7)
	50–75	8 (8.7)
	75–90	4 (4.3)
	> 90	21 (22.8)
CD73 tumour-infiltrating lymphocyte	< 5	49 (53.3)
	5–25	18 (19.6)
	25–50	15 (16.3)
	50–75	4 (4.3)
	75–90	2 (2.2)
	> 90	4 (4.3)

A positive correlation ($r = 0.541$) was found between the CD73 tumour and TIL findings of the patients ($p = 0.000$). A positive correlation was also detected between the PD-L1 percentages of the patients and their CD73 tumour ($r = 0.220$) and TIL ($r = 0.282$) findings ($p < 0.05$). There was also a positive correlation between the PD-L1 intensity and the CD73 tumour ($r = 0.280$) and TIL ($r = 0.240$) findings ($p < 0.05$) (Table III).

Overall survival was observed to have an inverse correlation with CD73 tumour and TIL groups of 5–25%, 25–50%, 50–75%, 75–90%, and 90% ($p < 0.05$). However, there was no significant correlation between OS and the ≤ 5% CD73 tumour and TIL group. Overall survival was inversely correlated with the PD-L1 percentages of <10% and > 50% and the PD-L1 intensities of weak-moderate and strong

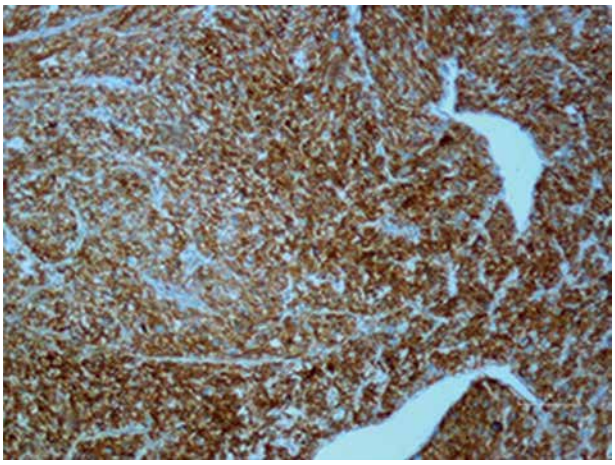

Fig. 1. Immunohistochemical image of PD-L1, revealing intense and diffuse staining (100×)

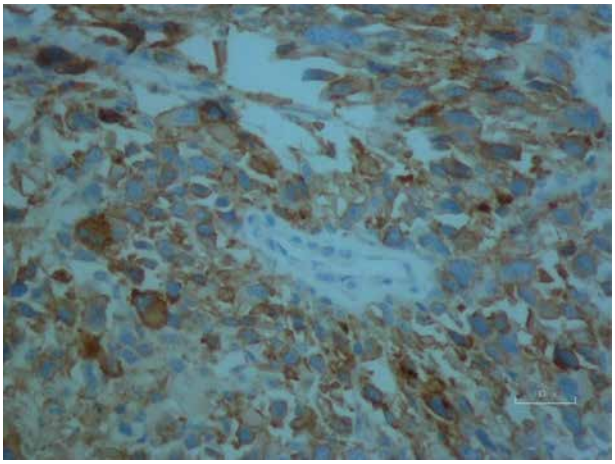

Fig. 2. Immunohistochemical image of PD-L1 (200×), showing moderate staining in tumour cells

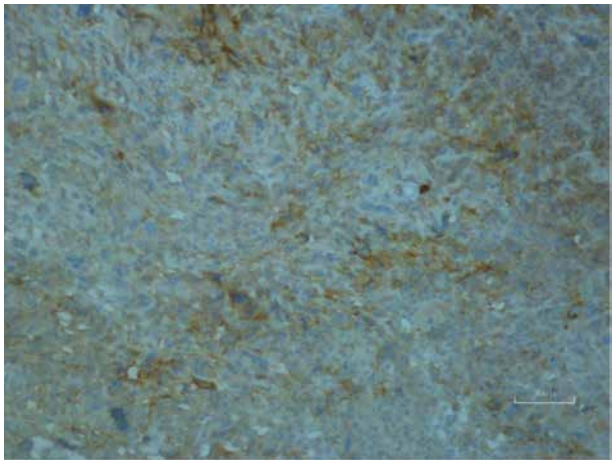

Fig. 3. Immunohistochemical image of PD-L1 (100×), revealing weak positivity in tumor cells

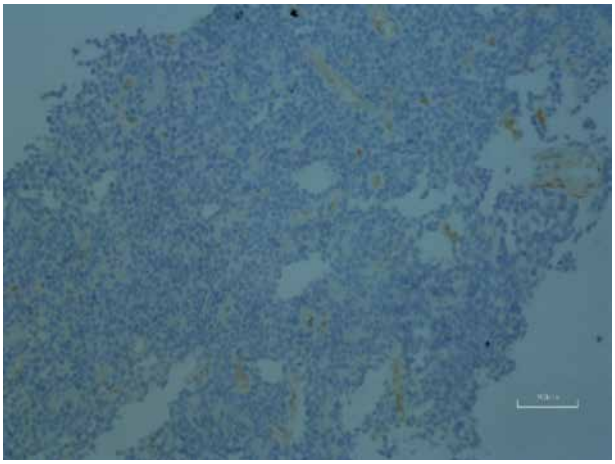

Fig. 4. Immunohistochemical image of PD-L1 (100×), showing negative staining

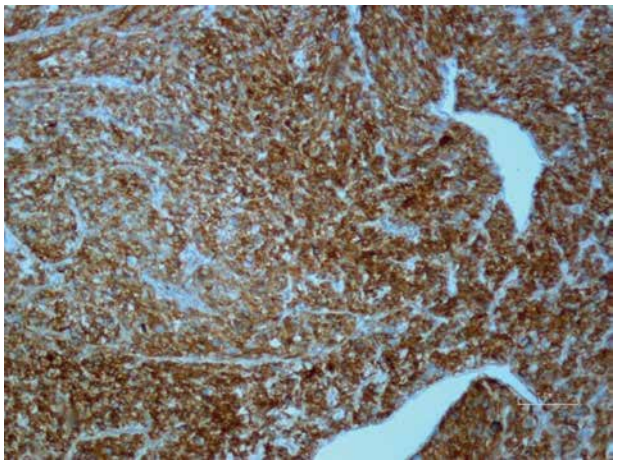

Fig. 5. Immunohistochemical image of CD73 (100×), showing more than 90% positive staining in both tumour cells and lymphocytes

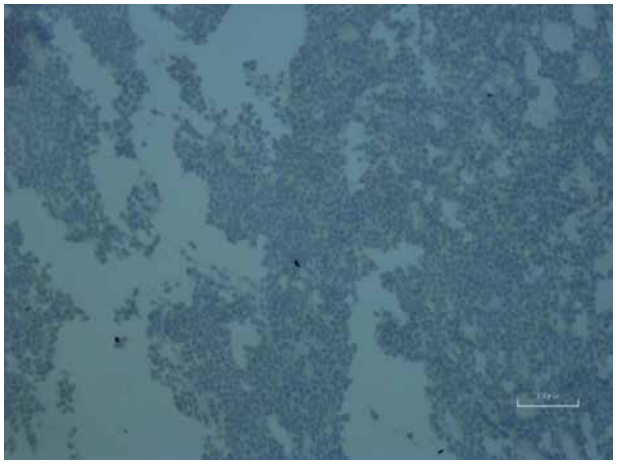

Fig. 6. Immunohistochemical image of CD73 (100×), showing negative staining

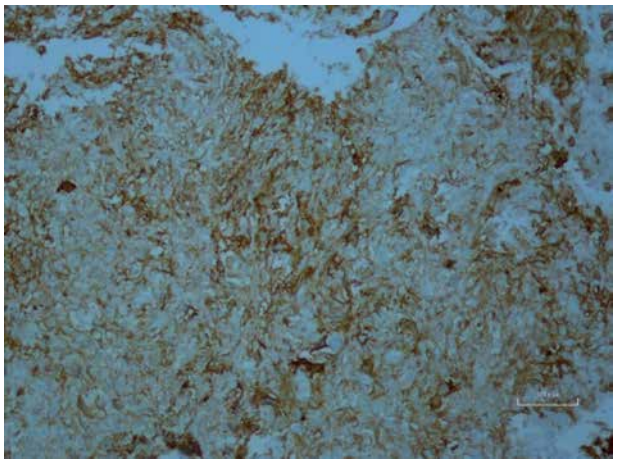

Fig. 7. Immunohistochemical image of CD73 (100×), showing 90% positivity in tumour cells and 5–25% positivity in tumour-infiltrating lymphocytes

($p < 0.05$), but no significant correlation was found between OS and negative staining (Table IV). A reverse correlation ($r = -0.521$) was found between the OS findings and the Ki-67 PI of the patients ($p < 0.05$).

Table III. Relationship of the CD73 tumour and tumour-infiltrating lymphocyte status of the patients with their PD-L1 percentage and intensity

Parameters	PD-L1 percentage		PD-L1 intensity	
	R	*P*	*R*	*P*
CD73 tumour	0.220	0.036	0.280	0.007
CD73 tumour-infiltrating lymphocyte	0.282	0.006	0.240	0.021

Table IV. Relationship of overall survival with the CD73 tumour, CD73 tumour-infiltrating lymphocyte and PD-L1 percentages, and PD-L1 intensity

Parameters	Percentage	Overall survival	
		R	*P*
CD73 tumour	< 5	0.125	0.256
	5–25	–0.103	0.032
	25–50	–0.210	0.041
	50–75	–0.362	0.039
	75–90	–0.238	0.042
	90	–0.018	0.018
CD73 tumour-infiltrating lymphocyte	< 5	0.019	0.898
	5–25	–0.487	0.040
	25–50	–0.211	0.039
	50–75	–0.384	0.041
	75–90	–0.758	0.011
	90	–0.612	0.029
PD-L1 percentage	Negative	0.235	0.411
	< 10 10–50	–0.138	0.019
	> 50	–1.000	0.000
PD-L1 intensity	Negative	0.289	0.172
	Weak-moderate	–0.193	0.035
	Strong	–0.529	0.041

Discussion

Sarcomas of mesenchymal origin are primarily divided into 2 main groups: bone and soft tissue sarcomas. The 2 most common subtypes of soft tissue sarcomas are liposarcoma and leiomyosarcoma. Among bone sarcomas, Ewing's sarcoma is the second most common [11]. In our study, patients diagnosed with liposarcoma, leiomyosarcoma, and pleomorphic sarcoma constituted the majority of soft tissue sarcomas, while Ewing's sarcoma was the most common bone sarcoma diagnosis. In the literature, the median age at the time of diagnosis has been reported as 60 years [3]. The mean age of our cases was 51.39 years. CD73 affects

immunosuppression [7]. It is overexpressed in various solid malignant tumours (colorectal, prostate, ovaries, gall bladder, breast, endometrium, etc.) [8]. In a study examining CD73 in patients with triple-negative breast cancer, CD73 was found to be associated with decreased OS, disease-free survival, and anti-tumour immunity [12]. PD-1 interaction with its ligands PD-L1 and PD-L2 contributes to the suppression of T-cell function and the restriction of tumour cell death. The overexpression of PD-L1 in tumour cells can prevent T-cell cytolysis and facilitate cancer formation. High PD-L1 expression has been associated with poor tumour differentiation and poor prognosis in non-small cell lung cancer and malignant melanoma [9, 10]. In a study evaluating gastrointestinal neuroendocrine neoplasms (GI-NEN), it was found that CD73 expression was significantly correlated with PD-L1 [7].

In a study conducted on patients with metastatic malignant melanoma, high soluble CD73 (sCD73) values in samples taken before anti PD-1 treatment (nivolumab) were found to be correlated with progression-free survival and decreased OS [13]. In our study, a positive correlation was observed between CD73 tumour and TIL findings. In addition, there was a significant (positive) correlation between CD73 tumour and TILs and PD-L1 intensity and PD-L1 percentage. We also determined that CD73 tumour, CD73 TIL, PD-L1 intensity, and PD-L1 percentage positivity were associated with decreased OS. Ki-67 is a nuclear antigen associated with cell proliferation, and its expression is related to the growth fraction of the tumour. The proliferative index determined from Ki-67 expression has been found to have prognostic significance in various types of sarcoma [14]. Various studies have revealed that the Ki-67 level is an independent predictor of survival in sarcomas [15]. In our data, the Ki-67 PI was associated with decreased OS. In the literature, it has been suggested that CD73 can be a biomarker for anti-PD-1 treatment in patients with GI-NEN [7]. The detection of high levels of CD73 in metastatic malignant melanoma patients has been associated with poor response to anti-PD-1 treatment. sCD73 enzyme activity in peripheral blood before treatment has been interpreted as useful in predicting response to nivolumab [13]. In a study examining TJD5, a CD73 antibody, it was stated that TJD5 was expected to suppress the immunosuppressive tumour microenvironment and work in harmony with PD-1 and PD-L1 antibodies.

Conclusions

In brief, sarcomas are rare, malignant tumours of mesenchymal origin. We determined that CD73 and PD-L1 positivity, which was previously associated with poor prognosis and decreased OS in various solid tumours, was also associated with reduced OS in sarcoma patients. Similarly, the Ki-67 PI was associated with decreased OS. There was also a positive correlation between CD73 and PD-L1, and these biomarkers presented as indicators of poor prognosis. In conclusion, the correlation between these 2 parameters is promising in terms of targeting better survival and disease control with anti-CD73 and anti-PD-L1 therapy in selected patients.

The authors declare no conflict of interest.

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