original report

Positive PD-L1 Expression Predicts Worse Outcome in Cutaneous Angiosarcoma

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abstract

Purpose **Programmed death-1 (PD-1) or programmed death ligand-1 (PD-L1) targeted therapies have shown promising survival outcomes in several human neoplasms. However, it is unclear whether the expression of PD-L1 can be correlated to any clinical and pathologic variables in patients with cutaneous angiosarcoma (CA). The aim of this study was to evaluate the clinicopathological significance of PD-L1 expression in CA patients.**

Materials and Methods **Data from 52 patients with CA were retrospectively reviewed. PD-L1 expression, tumor proliferation determined by Ki-67 index, and immunohistochemical evaluation of tumor-infiltrating lymphocytes, CD4+ and CD8+, were used to determine correlation with clinicopathological variables.**

Results **PD-L1 was positively expressed in 40% of all patients. PD-L1 expression was significantly associated with tumor cell proliferation. Multivariate analysis confirmed that high levels of CD8+ tumor-infiltrating lymphocytes were a significant predictor in patients with clinical stage I CA and the positive expression of PD-L1 was an independent prognostic factor in predicting worse outcome.**

Conclusion **PD-L1 expression is a novel pathologic marker for predicting worse outcome in patients with CA.**

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INTRODUCTION

Angiosarcoma is an extremely rare neoplasm that accounts for less than 2% of all soft tissue sarcomas.[1] Cutaneous angiosarcoma (CA) is an aggressive neoplasm arising from vascular or lymphatic endothelial cells and has a dismal outcome. The 5-year overall survival rate of CA has been reported to range from 10% to 30%.[2,3] Although CA can occur anywhere in the body, it is frequently localized to the head and neck regions. It has been challenging to determine the etiological factors for CA of the face and scalp because it affects a small percentage of the population. In Japan, a combined approach with chemotherapy and radiotherapy has been preferred as the first-line treatment of CA, instead of surgical resection. However, there is no established biomarker to predict the prognosis of CA in affected patients. Therefore, the need for a biomarker to define patients selected for surgery should be warranted.

Studies have reported the importance of tumor-infiltrating lymphocytes (TILs) in determining patient outcomes in angiosarcoma.[4-6] Recently, Fujii et al[4] demonstrated that high levels of CD8+ TILs are closely correlated with an improved prognosis and a longer disease-free period of distant metastases in patients with CA, suggesting that immunotherapy using TILs could be a novel treatment approach for angiosarcoma.[4] Recent clinical trials targeting programmed death-1 (PD-1) or programmed death ligand-1 (PD-L1) have exhibited a promising survival outcome in patients with cancers such as lung cancer and malignant melanoma. PD-L1 belongs to the B7 superfamily that down-regulates T-cell activation through the PD-1 receptor and has a negative effect on the immune response.[7-9] Although there are several studies on the expression of PD-L1 and its prognostic significance in different human neoplasms, the clinicopathological significance of PD-L1 expression in soft tissue sarcomas is not well understood. Kim et al[10] reported that PD-1-positive TILs and PD-L1 expression were significantly correlated with the progression and poor survival outcomes in soft tissue sarcomas. In their study, the positive expression of PD-L1 after surgical resection was closely related to poor prognosis in various soft tissue sarcomas. However, less than 5% of patients in the study had angiosarcoma and 80% of samples from those

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patients exhibited positive PD-L1 staining. Therefore, prognostic significance of PD-L1 expression in patients with angiosarcoma could not be clearly determined. Shen et al[11] found that PD-L1 expression positively correlated with TILs in osteosarcoma and thus could be a promising immunotherapeutic approach to treat the disease. When considering the possible clinical importance of immune checkpoint inhibitors targeting PD-L1/PD-1 in a disease associated with poor outcomes, further studies on PD-L1 expression can help understand its potential immunotherapeutic and prognostic roles in treating CA. On the basis of the clinical and therapeutic evidence that correlates PD-L1 expression to predicting outcomes in other human neoplasms, we conducted the current study to evaluate the clinicopathological significance of PD-L1 expression and TILs in CA.

MATERIALS AND METHODS

Patients

We identified and retrospectively examined 52 consecutive patients who were positively diagnosed with CA at the Gunma University and University of the Ryukyus hospitals between October 1987 and September 2014. We obtained 52 paraffin-embedded tissue samples (from biopsy or surgical resection) and patient medical records from the two hospitals. This study was approved by the institutional review board of Gunma University and the ethical committee for clinical studies at University of the Ryukyus. The approach used for the evaluation and resection of these tumors has been described previously.[4]

There is no established method to clinically classify tumor stages in CA. Therefore, as described previously, we tentatively classified patients with CA into three stages: stage I for those with cutaneous tumors, stage II for those with lymph node metastases, and stage III for those with distant metastases, on the basis of the clinical evaluation before treatment.[4]

Immunohistochemical Staining

For PD-L1 and Ki-67, immunohistochemical staining was performed according to the procedures described in previous studies.[12,13] Rabbit monoclonal antibodies against PD-L1 (1:100 dilution; Abcam, Cambridge, MA) and Ki-67 (1:40 dilution; Dako, Glostrup, Denmark) were used. The expression of PD-L1 was considered positive when membrane staining was observed. A semiquantitative scoring method was used for PD-L1: 1, 0% to 5%; 2, 5% to 10%; 3, 10% to 25%; and 4, > 25% of cells were positive. Tumors with score > 2 were graded as positive.

A highly cell-rich area of the immunostained tissue sections was evaluated for Ki-67. Approximately 1,000 nuclei were counted on each slide. Proliferative activity was assessed as the percentage of Ki-67-stained nuclei (Ki-67 labeling index) in the sample. The median value of the Ki-67 labeling index was evaluated, and tumor cells with values greater than the median value were defined as cells with high expression. Immunohistochemical staining was done for CD4+ (1:40 dilution; Dako) and CD8+ (1:200 dilution; Abcam) TILs in the tumor specimens. After evaluating the specimens entirely, the number of CD4+ and CD8+ TILs were counted in the selected hot spot in a $\times$400 magnified field (0.26 mm^2 field area). We determined the median number of CD4+ and CD8+ TILs as the cutoff point for CD4+ and CD8+ TIL density. The tissue sections were examined in a blinded fashion by at least two of the authors, using light microscopy.

Statistical Analyses

Probability values of $<.05$ indicated a statistically significant difference as determined by Fisher's exact test. The correlation between different variables was analyzed using the nonparametric Spearman's rank test. The Kaplan-Meier method was used to estimate survival as a function of time, and survival differences were analyzed by the log-rank test. Overall survival (OS) was determined as the time elapsed from definite diagnosis to death from any cause. Progression-free survival (PFS) was defined as the time elapsed between definite diagnosis and the disease progression or death as a result of disease. Multivariate analyses were performed using a stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analyses were performed using GraphPad Prism 4 (Graph Pad Software, San Diego, CA) and JMP 8 (SAS Institute, Cary, NC) for Windows.

RESULTS

Patient Demographics

Table 1 summarizes patient demographics according to the expression of PD-L1 in patients with CA. The median age was 76 years (range, 57 to 91 years). The primary site of the lesion was the scalp in 34 patients, face in 13, neck in four, and leg in one. Thirty-nine patients were classified as having stage I disease, two as stage II, and 11 as stage III. Surgical resection had been done in 17 patients, radiotherapy in 45, and systemic chemotherapy in 30. As a chemotherapeutic regimen, 26 patients were treated with taxane agents including docetaxel

Table 1. Patients' Demographics According to PD-L1 Expression

Variable	Patients, No. (N = 52)	PD-L1 Expression		
		Positive (n = 21)	Negative (n = 31)	*P*
Age (years)				.259
⩽ 75	24	12	12	
> 75	28	9	19	
Sex				> .999
Male	33	13	20	
Female	19	8	11	
Primary site				> .999
Parietal	26	11	15	
Nonparietal	26	10	16	
Tumor size (mm)				> .999
⩽ 45	27	11	16	
> 45	25	10	16	
Clinical stage				.747
1/2	39	15	24	
3	13	6	7	
Lymph node metastasis				.549
Yes	32*	2	1	
No	18*	17	30	
Distant metastasis				> .999
Yes	29*	4	6	
No	22*	16	25	
Ki-67 expression				.025
High	26	15	11	
Low	26	6	18	
CD4 expression				.781
High	24	9	15	
Low	28	12	16	
CD8 expression				.403
High	24	8	16	
Low	28	13	15	
CD4+CD8 expression				.582
High	25	9	16	
Low	27	12	15	

Abbreviation: PD-L1, programmed death ligand-1.
*These variables lack data from some patients.

(n = 25). The median follow-up period was 12.3 months (range: 1.6 to 92.1 months).

Immunohistochemical Analyses

Fifty-two primary lesions of CA were analyzed using immunohistochemical techniques. Figure 1 shows the representative immunohistostained images of PD-L1 expression (on the basis of a scoring scale of 1 to 4) and CD4+ and CD8+ TILs. PD-L1 immunostaining was observed to be localized predominantly on the plasma membrane in the angiosarcoma cells. The rate of positive PD-L1 expression was 40% (in 21 of 52 patients) and the mean ± SD score for PD-L1 was 1.8 ± 0.8. The percentages of PD-L1 immunohistostains scoring 1, 2, 3, and 4 were 60% (31 of 52), 15% (eight of 52), 13% (seven of 52), and 12% (six of 52), respectively. On the basis of the analyses of CA

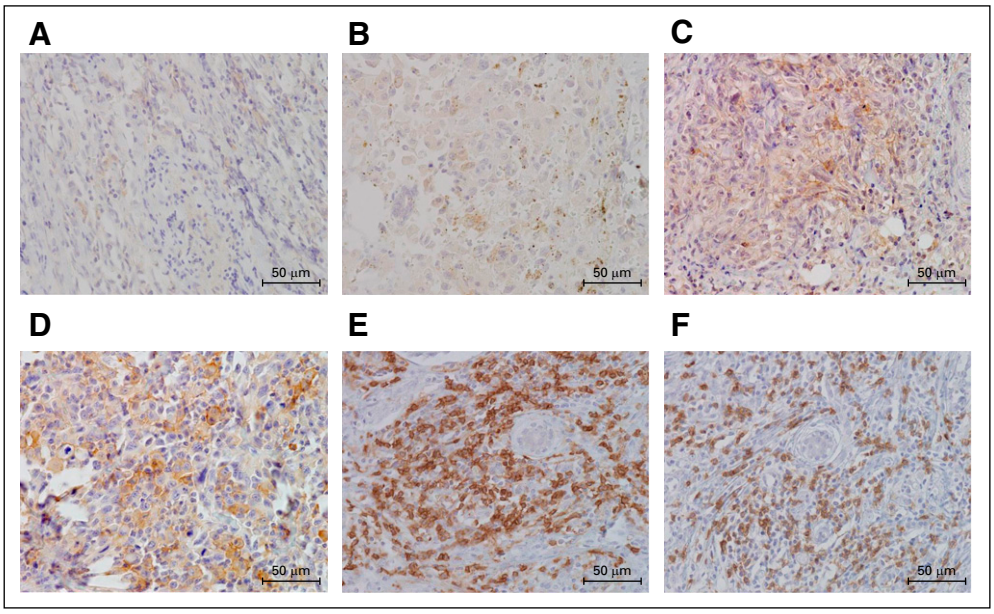

Fig 1. Representative immunohistochemical staining of cutaneous angiosarcoma. Immunohistostaining of PD-L1 reveals the membrane-staining pattern of. (A-D) Semiquantitative scoring of PD-L1 staining: (A) 1; (B) 2; (C) 3; (D) 4. The representative images of (E) CD4 and (F) CD8 as primary tumor-infiltrating lymphocytes are shown.

specimens, cutoff values for the Ki-67 labeling index were determined. The median Ki-67 labeling index was 9% (range, 0% to 51%), and a value of 9% was chosen as the cutoff point. High Ki-67 expression was identified in 50% of the patient samples (26 of 52). The median number of CD4+ and CD8+ TILs was 53 (range, 14 to 158) and 41 (range, 0 to 180), respectively. High CD4+ and CD8+ levels were identified in 46% of the patient samples (24 of 52). The expression of PD-L1 was significantly associated with tumor cell proliferation as determined by the Ki-67 labeling index (Table 1).

Correlation Between PD-L1 Expression and Different Variables

The expression of PD-L1 was significantly correlated with Ki-67 but not CD4+ or CD8+ TILs or tumor size (Table 2).

Univariate and Multivariate Analyses

The median survival times of OS and PFS for all patients were 448 days and 270 days, respectively. The 1-year survival rates of OS and PFS for all patients were 55% and 37%, respectively. Of 52 patients, 40 developed recurrences after the initial treatment and 41 died eventually.

Table 3 shows the results of the univariate and multivariate analyses for all patients. The expression level of PD-L1 was identified as a significant prognostic factor for OS by univariate analysis. Significant prognostic variables for PFS were found to be sex, clinical stage, and the expression levels of PD-L1 and CD8+. The multivariate analysis confirmed that PD-L1 expression was an independent prognostic factor for predicting a worse OS and PFS. Multivariate analysis also confirmed that clinical stage, sex, and the expression level of CD8+ were independent prognostic variables in PFS.

Figure 2A and 2B show the Kaplan-Meier curves for patients with a positive or negative PD-L1 expression for OS and PFS, respectively. For patients with clinical stage I disease, a significant difference in the OS was observed between positive and negative expression of PD-L1 (Fig 2C). There is a significant difference in the OS between high and low numbers of CD8+ TILs (Fig 2D), but not between high and low numbers of CD4+ TILs (Fig 2E).

Survival Analysis According to Different Clinical Parameters

We performed a survival analysis according to the clinical stages classified earlier. Table 4 shows univariate and multivariate analyses in patients with CA who were classified as having clinical stage I disease (n = 39). Univariate analysis confirmed that the expression of PD-L1 and CD8+

Table 2. Correlation With Programmed Death Ligand-1 Expression

Variable	Spearman Rank	95% CI	*P*
Ki-67	0.282	0.001 to 0.512	.043
CD4	−0.121	−0.388 to 0.166	.395
CD8	−0.097	−0.368 to 0.188	.493
Tumor size	0.226	−0.208 to 0.349	.592

Table 3. Univariate and Multivariate Survival Analysis in All Patients

	Overall Survival					Progression-Free Survival				
	Univariate		Multivariate			Univariate		Multivariate		
Variable	MST (days)	*P*	HR	95% CI	*P*	MST	*P*	HR	95% CI	*P*
Age (years)		0.931					.567			
⩽ 75	347					123				
> 75	495					328				
Sex		0.356	1.136	0.782 to 1.706	.511		.011	1.602	1.060 to 2.581	.024
Male	356					165				
Female	583					477				
Primary site		.123					.939			
Parietal	347					200				
Nonparietal	583					235				
Tumor size (mm)		.794					.577			
⩽ 45	495					321				
> 45	387					165				
Clinical stage		.083	1.142	0.768 to 1.658	0.497		.001	1.745	1.191 to 2.510	.005
1/2	583					335				
3	326					50				
PD-L1		.002	1.638	1.138 to 2.362	.008		.014	1.593	1.117 to 2.281	.011
Positive	276					123				
Negative	638					369				
Ki-67		.112					.188			
High	356					200				
Low	583					264				
CD4		.725					.231			
High	448					235				
Low	495					135				
CD8		.135	1.231	0.892 to 1.725	0.208		.049	1.448	1.039 to 2.055	.028
High	482					321				
Low	323					221				

Abbreviations: HR, hazard ratio; MST, median survival time; PD-L1, programmed death ligand-1.

levels was a significant prognostic factor for OS and PFS. The expression of PD-L1 and CD8+ levels was also found to be an independent predictor for worse prognosis, by multivariate analysis. In patients with CA who were classified as having clinical stages II or III disease, no statistically significant differences in the OS ($P = .422$) and PFS ($P = .751$) were observed between patients with positive and negative PD-L1 expression.

Of the 29 patients who initially underwent chemotherapy, 24 were administered docetaxel; five received other regimens. We performed the survival analysis of these 29 patients. No statistically significant difference was found in the OS ($P = .095$) and PFS ($P = .102$) between patients with positive (median OS, 263 days; median PFS, 133 days) and negative (median OS, 676 days; median PFS, 423 days) PD-L1 expression.

DISCUSSION

Immune checkpoint inhibitors targeting PD-1 or PD-L1 have been available to treat human neoplasms, including malignant melanoma and lung cancer, in Japan and other countries. Several studies have focused on the prognostic significance of PD-L1 expression in certain cancers.[13-18] The positive rate of PD-L1 expression was 50% in breast cancer, 39.2% in pancreatic cancer, 42.2% in gastric cancer, 25% in hepatocellular carcinoma, 43.9% in esophageal cancer, and

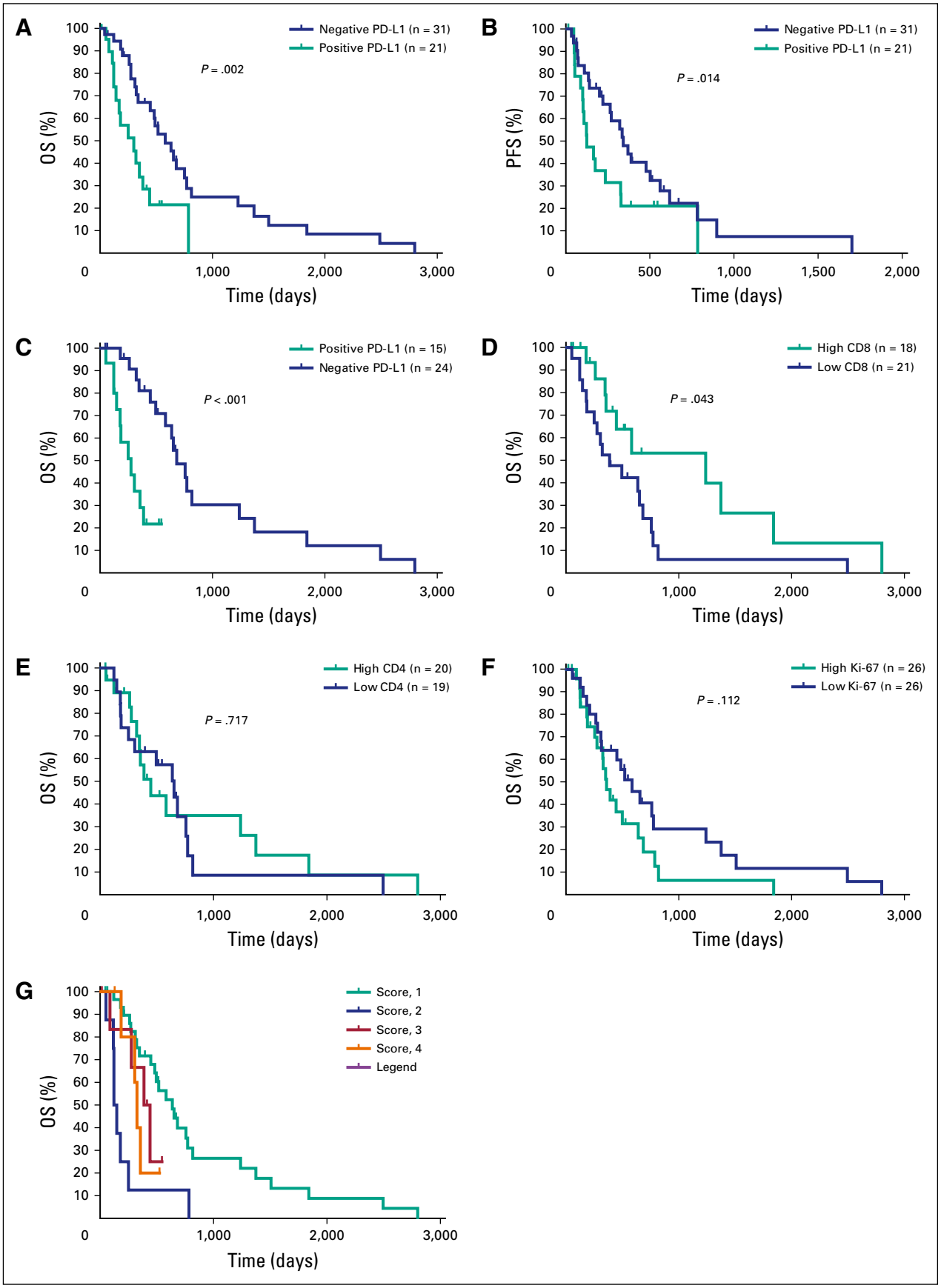
A
Negative PD-L1 (n = 31)
Positive PD-L1 (n = 21)
P = .002
OS (%)
Time (days)
B
Negative PD-L1 (n = 31)
Positive PD-L1 (n = 21)
P = .014
PFS (%)
Time (days)
C
Positive PD-L1 (n = 15)
Negative PD-L1 (n = 24)
P < .001
OS (%)
Time (days)
D
High CD8 (n = 18)
Low CD8 (n = 21)
P = .043
OS (%)
Time (days)
E
High CD4 (n = 20)
Low CD4 (n = 19)
P = .717
OS (%)
Time (days)
F
High Ki-67 (n = 26)
Low Ki-67 (n = 26)
P = .112
OS (%)
Time (days)
G
Score, 1
Score, 2
Score, 3
Score, 4
Legend
OS (%)
Time (days)

Fig 2. Kaplan-Meier curves for the patients with a positive or negative expression of programmed death ligand-1 (PD-L1). A significant difference in the (A) overall survival (OS) and (B) progression-free survival (PFS) was recognized with respect with the expression of PD-L1 in all patients (N = 52). (C) In patients with clinical stage I disease (n = 39), a significant difference in the OS was observed between positive and negative expression of PD-L1 and (D) between high and low numbers of CD8+ TILs, (E) but not between high and low numbers of CD4+ TILs. (F) No statistically significant difference in the OS was observed between high and low expression of Ki-67. (G) OS according to scoring of PD-L1 expression.

66.3% in renal cell carcinoma.[13-18] PD-L1 expression may predict worse outcomes in these carcinomas. Another study also identified PD-L1 expression as an independent indicator of poor prognosis in soft tissue sarcoma.[10] In 105 patients with soft tissue sarcoma, a positive PD-L1 expression was seen in 65%, and PD-L1 expression was significantly associated with advanced clinical stage, distant metastases, and advanced clinicopathological variables.[10] However, that study included only five patients with angiosarcoma, and it was unclear whether the increased expression of PD-L1 could be a significant predictor for poor prognosis in patients with angiosarcoma as well. Recently, D'Angelo et al[19] documented the clinicopathological significance of TILs and PD-L1 expression in 50 soft tissue sarcoma specimens: 14 gastrointestinal stromal tumors, five synovial sarcomas, four leiomyosarcomas, three spindle cell sarcomas, three angiosarcomas, and 21 other cancers. They reported that the expression of PD-L1 was not observed in three angiosarcoma specimens without the description of primary site. They concluded that the expression of PD-L1 was low in sarcoma, and there was no association between PD-L1 expression and survival. In contrast, our study revealed that the positive rate of PD-L1 expression in CA was almost similar to that in human epithelial tumors and was an independent prognostic factor for worse outcome. Because PD-1/PD-L1 therapy has been recognized to be effective in patients with advanced cancers,[20] it is clinically important to examine the PD-L1 expression in tumor tissues before PD-1/PD-L1 immunotherapy. In fact, 36% of patients with positive PD-L1 tumors responded to anti-PD1 immunotherapy, whereas patients with negative PD-L1 tumors did not.[21]

In the current study, we found the expression level of PD-L1 to be closely correlated with tumor cell proliferation. A previous study also reported a strong positive link between PD-L1 expression and the cell proliferative Ki-67 marker in patients with breast cancer.[22] Fujii et al[4] reported that no statistically significant difference was present between the prognosis and Ki-67 labeling index of 30 patients with CA, which corresponds with our results indicating Ki-67 was not identified as a prognostic predictor. Although the Ki-67 index closely correlates with the expression level of PD-L1, it remains unclear why the expression level of Ki-67 was not significantly associated with the prognosis of patients with CA. The small sample size may have biased our results. Further investigation is required to confirm whether the relationship between prognostic significance and Ki-67 labeling index exists in CA.

Angiosarcoma is a rare disease and no effective chemotherapeutic regimen has been established. Surgical resection or radiation therapy has been conventionally recommended. Panel et al[23] have described that paclitaxel is an effective and well-tolerated regimen for patients with unresectable angiosarcoma, yielding an overall response of approximately 18% and median survival time of 8 months. Moreover, Nagano et al[24] reported that six of nine patients treated with docetaxel revealed good response: complete responses in two patients and partial response in four patients. Thus, docetaxel has been accepted as an effective chemotherapeutic agent against CA. In patients with advanced CA, taxane-based agents have been chosen for treatment. In our study, 26 patients were treated with taxane regimens, including docetaxel (n = 25) and paclitaxel (n = 1). However, we found no significant difference in the prognosis after systemic chemotherapy with respect to the expression level of PD-L1. Further investigation is necessary to understand the relationship between the PD-L1 expression and the clinical effectiveness of chemotherapeutic agents.

One limitation of our study was a small sample size. However, CA is an extremely rare sarcoma among the Japanese and its frequency seems to be different among races.[25,26] We could not find a significant difference in OS between patients with positive and negative PD-L1 expression among the 29 patients treated with chemotherapy. Of these 29 patients, 21 had clinical stage I disease and eight had clinical stage III disease. Heterogenous groups of different clinical stages may have biased the results of our survival data. Second, PD-L1 antibodies were not same as those used in previous immunohistochemical studies. We checked several commercially available PD-L1 antibodies to obtain clear staining. Finally, we have not carried out the experimental study using any inhibitors targeting PD-L1. The inhibitors of PD-1/PD-L1 have been widely used for treating lung cancer, malignant melanoma, and other cancers, and these agents show a favorable survival outcome compared with the cytotoxic agents.[27,28] Further studies to investigate the antitumor effect of PD-1/PD-L1 in vivo using the animal model of CA would expand our understanding of PD-L1 expression in angiosarcoma.

In conclusion, high expression levels of PD-L1 in CA were identified as a significant predictor for poor prognosis, especially in patients of the

Table 4. Univariate and Multivariate Survival Analysis in Patients With Clinical Stage I Disease

	Overall Survival					Progression-Free Survival				
	Univariate		Multivariate			Univariate		Multivariate		
Variable	MST (days)	*P*	HR	95% CI	*P*	MST	*P*	HR	95% CI	*P*
Age (years)		.831					.571			
⩽ 75	335					161				
> 75	617					355				
Sex		.315	0.725	0.450 to 1.096	.131		.011	0.457	0.255 to 0.742	< .001
Male	351					187				
Female	618					1,141				
Primary site		.129					.705			
Parietal	367					267				
Nonparietal	610					352				
Tumor size (mm)		.675					.924			
⩽ 45	581					331				
> 45	564					187				
PD-L1		< .001	2.456	1.467 to 4.337	< .001		.013	1.758	1.123 to 2.805	.014
Positive	263					124				
Negative	676					436				
Ki-67		.265					.451			
High	3,712					278				
Low	618					361				
CD4		.717					.395			
High	448					321				
Low	652					335				
CD8		.043	1.511	1.019 to 2.327	.039		.024	1.632	1.064 to 2.607	.024
High	1,239					502				
Low	387					200				

Abbreviation: HR, hazard ratio; MST, median survival time; PD-L1, programmed death ligand-1.

clinical stage I disease, and could be an important clinicopathological marker for choice of treatments. Moreover, we confirmed high levels of CD8+ TILs could also be a significant biomarker for favorable outcome, as previously described.[4] The inhibition of PD-L1 function could be a novel therapeutic target for CA with high PD-L1 expression.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

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REFERENCES

1. Goldblum JR, Folpe AL, Weiss SW: Malignant vascular tumors, in Weiss SW, Goldblum JR (eds), Enzinger and Weiss's Soft Tissue Tumors. Philadelphia, PA, Mosby Elsevier, 2008, pp 703-732.
2. Mendenhall WM, Mendenhall CM, Werning JW, et al: Cutaneous angiosarcoma. Am J Clin Oncol 29:524-528, 2006
3. Guadagnolo BA, Zagars GK, Araujo D, et al: Outcomes after definitive treatment for cutaneous angiosarcoma of the face and scalp. Head Neck 33:661-667, 2011
4. Fujii H, Arakawa A, Utsumi D, et al: $CD8^+$ tumor-infiltrating lymphocytes at primary sites as a possible prognostic factor of cutaneous angiosarcoma. Int J Cancer 134:2393-2402, 2014
5. Maddox JC, Evans HL: Angiosarcoma of skin and soft tissue: A study of forty-four cases. Cancer 48:1907-1921, 1981
6. Zietz C, Rumpler U, Stürzl M, et al: Inverse relation of Fas-ligand and tumor-infiltrating lymphocytes in angiosarcoma: Indications of apoptotic tumor counterattack. Am J Pathol 159:963-970, 2001
7. Carter L, Fouser LA, Jussif J, et al: PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. Eur J Immunol 32:634-643, 2002
8. Liang SC, Latchman YE, Buhlmann JE, et al: Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol 33:2706-2716, 2003
9. Nishimura H, Honjo T: PD-1: An inhibitory immunoreceptor involved in peripheral tolerance. Trends Immunol 22: 265-268, 2001
10. Kim JR, Moon YJ, Kwon KS, et al: Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One 8:e82870, 2013
11. Shen JK, Cote GM, Choy E, et al: Programmed cell death ligand 1 expression in osteosarcoma. Cancer Immunol Res 2:690-698, 2014
12. Kaira K, Endo M, Abe M, et al: Biologic correlation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. J Clin Oncol 28:3746-3753, 2010
13. Ghebeh H, Mohammed S, Al-Omair A, et al: The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: Correlation with important high-risk prognostic factors. Neoplasia 8:190-198, 2006
14. Ohigashi Y, Sho M, Yamada Y, et al: Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 11:2947-2953, 2005
15. Nomi T, Sho M, Akahori T, et al: Clinical significance and therapeutic potential of the programmed death-1 ligand/ programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 13:2151-2157, 2007
16. Wu C, Zhu Y, Jiang J, et al: Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem 108:19-24, 2006
17. Nakanishi J, Wada Y, Matsumoto K, et al: Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. Cancer Immunol Immunother 56:1173-1182, 2007
18. Thompson RH, Gillett MD, Cheville JC, et al: Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. Cancer 104:2084-2091, 2005
19. D'Angelo SP, Shoushtari AN, Agaram NP, et al: Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol 46:357-365, 2015
20. Homet Moreno B, Ribas A: Anti-programmed cell death protein-1/ligand-1 therapy in different cancers. Br J Cancer 112:1421-1427, 2015

21. Sznol M, Chen L: Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res 19:1021-1034, 2013
22. Ghebeh H, Tulbah A, Mohammed S, et al: Expression of B7-H1 in breast cancer patients is strongly associated with high proliferative Ki-67-expressing tumor cells. Int J Cancer 121:751-758, 2007
23. Panel N, Bui BH, Bay JO, et al: Phase II trial of weekly paclitaxel for unresectable angiosarcoma: The ANGIOTAX study. J Clin Oncol 26:269-272, 2008
24. Nagano T, Yamada Y, Ikeda T, et al: Docetaxel: A therapeutic option in the treatment of cutaneous angiosarcoma: Report of 9 patients. Cancer 110:648-651, 2007
25. Luke JJ, Keohan ML: Advances in the systemic treatment of cutaneous sarcomas. Semin Oncol 39:173-183, 2012
26. Sturgis EM, Potter BO: Sarcomas of the head and neck region. Curr Opin Oncol 15:239-252, 2003
27. Brahmer J, Reckamp KL, Baas P, et al: Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373:123-135, 2015
28. Wolchok JD, Kluger H, Callahan MK, et al: Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369: 122-133, 2013