Prognostic impact of programmed death-ligand 1 expression in correlation with human leukocyte antigen class I expression status in stage I adenocarcinoma of the lung

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ABSTRACT

Objective: The study objective was to investigate the prognostic impact of programmed death-ligand 1 expression in correlation with human leukocyte antigen class I expression on tumor cells in early-stage adenocarcinoma of the lung, because both programmed death-ligand 1 and human leukocyte antigen class I molecules play important roles in cancer immunity.

Methods: Ninety-four patients with completely resected pathologic stage I lung adenocarcinoma were retrospectively reviewed. Programmed death-ligand 1 expression on tumor cells was evaluated with immunohistochemistry in correlation with several clinicopathologic and molecular features, including human leukocyte antigen class I expression on tumor cells.

Results: Fifteen patients (16.0%) had tumor with positive programmed death-ligand 1 expression (percentage of tumor cells expressing programmed death-ligand 1, $\geq 5\%$), and the incidence was significantly higher in poorly differentiated tumors. There was no significant correlation between human leukocyte antigen class I expression and programmed death-ligand 1 expression. Programmed death-ligand 1 positivity was a significant factor to predict a poor survival (5-year survival, 66.7% vs 85.9%; $P = .049$), which was enhanced in tumors with normal human leukocyte antigen class I expression ($P = .029$) but was not evident in tumors with reduced human leukocyte antigen class I expression ($P = .552$).

Conclusions: The prognostic impact of programmed death-ligand 1 expression on tumor cells in early-stage lung adenocarcinoma may be distinct according to concurrent human leukocyte antigen class I expression. (J Thorac Cardiovasc Surg 2018;155:382-92)

Survival curves according to PD-L1 status in patients with HLA class I expression ($\geq 80\%$).

Central Message

The prognostic impact of PD-L1 expression on tumor cells in lung adenocarcinoma was distinct according to concurrent HLA class I expression status.

Perspective

The prognostic impact of PD-L1 expression on tumor cells was distinct according to HLA class I expression status in pathologic stage I adenocarcinoma of the lung. Tumor cells with reduced HLA class I expression may evade immune attack to survive regardless of PD-L1 status. In contrast, tumor cells with normal HLA class I expression may evade immune attack when they express PD-L1.

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Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer, which is the leading cause of cancer-related deaths worldwide.¹ For patients with early-stage NSCLC, surgery is the optimal treatment for cure.² However, the

Scanning this QR code will take you to supplemental table and video for this article.

Abbreviations and Acronyms

CI	= confidence interval
CTL	= cytotoxic T cell
HLA	= human leukocyte antigen
HR	= hazard ratio
IHC	= immunohistochemistry
NSCLC	= non-small cell lung cancer
OS	= overall survival
PD-1	= programmed-cell death 1
PD-L1	= programmed death-ligand 1

postoperative prognosis remains poor, and current adjuvant chemotherapy provides only a modest survival benefit.^{3,4} To improve the prognosis, it is important to establish novel biomarkers to predict prognosis and response to adjuvant chemotherapy.⁴

The programmed-cell death 1 (PD-1) and its ligand (PD-L1) are emerging therapeutic targets in a variety of malignant tumors, including NSCLC. Tumor cells that express PD-L1 may survive immune regulation by binding to PD-1 on cytotoxic T cells (CTLs) and thereby downregulating CTL function, which otherwise would have attacked and killed the tumor cells.⁵ Accordingly, blockade of the PD-1/PD-L1 axis may lead to killing tumor cells by restoring cancer immunity, and several antibodies against PD-1 or PD-L1 have achieved promising anti-tumor activity for advanced NSCLC.⁶⁻¹⁰ More important, PD-L1 expression status on tumor cells has been established as a useful biomarker to predict anti-tumor activity of these therapeutic agents.⁶⁻⁹

In contrast, the prognostic impact of PD-L1 expression status in early-stage NSCLC remains unclear, partly because of heterogeneity in the patient population (eg, heterogeneous stage and histology) included in each study.¹¹ In addition, its prognostic impact may be affected by the status of other biological factors involved in cancer immunity, such as human leukocyte antigen (HLA) class I molecules that play critical roles in an earlier phase for recognition of tumor cells by CTLs.¹²⁻¹⁴ Accordingly, tumor cells with reduced HLA class I molecules may evade immune attack to survive regardless of PD-L1 expression status, which can be associated with a poor prognosis. In contrast, tumor cells with normal HLA class I expression may be recognized and killed by CTLs without PD-L1 expression, and may evade immune attack only when they express PD-L1. However, only a few clinical studies, 1 on cholangiocarcinoma¹⁵ and 1 on hepatocellular carcinoma,¹⁶ have assessed the prognostic impact of PD-L1 expression status in combination with HLA class I status. To assess the prognostic impact of PD-L1 expression status in correlation with HLA class I expression status in NSCLC, we conducted a retrospective study in a

homogenous patient population with completely resected pathologic (p-) stage I lung adenocarcinoma.

PATIENTS AND METHODS**Patients**

Patients with p-stage I lung adenocarcinoma who received complete resection at our institute (Second Department of Surgery [Chest Surgery], University of Occupational and Environmental Health, Japan) from January 2003 to December 2006 without induction therapy before surgery were retrospectively reviewed. All patients received lung resection through minimally invasive thoracotomy. Systemic nodal dissection was principally performed, but was actually performed in 83 patients. Nodal sampling was performed in 8 patients (3 aged ≥ 80 years, 3 with pulmonary comorbidities, and 2 with cardiovascular comorbidities). No nodal sampling was performed in 3 patients (2 patients with pulmonary comorbidities and 1 patient with immunosuppressive treatment for rheumatoid arthritis). In 5 patients who were not fit for lobectomy, limited surgery was performed as follows: segmentectomy in 2 patients (1 aged ≥ 80 years, 1 with severe chronic obstructive pulmonary disease) and wedge resection in 3 patients (2 with cardiovascular comorbidities and 1 with idiopathic pulmonary fibrosis) (Table 1).

Patients who did not provide written informed consent for this study were excluded. Patients were ineligible if adequate primary tumor samples for immunohistochemistry (IHC) were not available, and a total of 94 patients were finally included in the study (Figure 1). Whole-body computed tomography, brain magnetic resonance imaging, and bone scan were performed preoperatively. P-stage was determined according to the TNM classification (Union for International Cancer Control TNM staging system, 7th edition). Patients with p-stage IB disease who were fit for adjuvant chemotherapy were encouraged to participate in clinical trials, and 10 patients actually received adjuvant chemotherapy (Table 1). The institutional review board of the University of Occupational and Environmental Health, Japan, approved the present study.

Follow-up Protocol

For each patient, a routine follow-up was performed at the outpatient clinic as follows: chest roentgenography every 3 months, as well as chest computed tomography, brain magnetic resonance imaging, and bone scan every 6 months for the first 3 years after surgery; all examinations were performed annually thereafter. Additional examinations were performed when any symptoms or signs of recurrence were detected.

The overall survival (OS) was estimated from the date of surgery to death of any cause or the date of the last follow-up. Patients who were not confirmed dead were censored on the date of the last follow-up. A telephone follow-up would be made if the patient did not come to our clinic for a routine follow-up.

Immunohistochemistry

Serial 4- μ m sections were cut from each formalin-fixed and paraffin-embedded primary tumor specimen, and received hematoxylin-eosin staining and IHC staining as described previously.¹⁶ Sections were heated in 1 mmol/L ethylenediaminetetraacetic acid (pH 8.0) at 98°C for 15 minutes for antigen retrieval and incubated in 3% hydrogen peroxide for 10 minutes to inactivate endogenous peroxidase. After blocking with Protein Block Serum-Free (Agilent Technologies, Carpinteria, Calif) for 30 minutes, sections were incubated with rabbit anti-PD-L1 monoclonal antibody (clone E1L3N, Cell Signaling Technology Japan, Tokyo, Japan) diluted at 1:200 for 1 hour at room temperature. Sections were then washed and incubated with SignalStain Boost IHC Detection Reagent HRP Rabbit (Cell Signaling Technology Japan) for 30 minutes. Thereafter, they were visualized with DAB+ Liquid (Agilent Technologies) and counterstained with hematoxylin.

TABLE 1. Patient characteristics

Variables	PD-L1 expression									HLA class I expression		
	≥1%	<1%	<i>P</i>	≥5%	<5%	<i>P</i>	≥50%	<50%	<i>P</i>	Normal	Reduced	<i>P</i>
Age, median (range, y)	75.5 (57-85)	71.8 (40-88)	.150	75.0 (58-85)	71.3 (40-88)	.354	75.0 (62-85)	71.5 (40-88)	.263	72.4 (40-85)	69.5 (45-88)	.738
Sex												
Male (n = 53)	15 (71.4)	38 (52.1)	.139	12 (80.0)	41 (51.9)	.051	4 (80.0)	49 (55.1)	.382	30 (55.6)	23 (57.5)	1.000
Female (n = 41)	6 (28.6)	35 (47.9)		3 (20.0)	38 (48.1)		1 (20.0)	40 (44.9)		24 (44.4)	17 (42.5)	
Smoking status												
Former or current (n = 56)	16 (76.2)	40 (54.8)	.129	12 (80.0)	44 (55.7)	.093	4 (80.0)	52 (58.4)	.645	30 (55.6)	26 (65.0)	.400
Never (n = 38)	5 (23.8)	33 (45.2)		3 (20.0)	35 (44.3)		1 (20.0)	37 (41.6)		24 (44.4)	14 (35.0)	
Brinkman Index, median (pack-y)	40	7.5	.064	45	10	.059	45	15	.497	3.8	25	.264
Comorbidities, combined												
Pulmonary (n = 26)	10 (47.6)	16 (21.9)	.028	6 (40.0)	20 (25.3)	.344	2 (40.0)	24 (27.0)	.614	17 (31.5)	9 (22.5)	.362
Cardiovascular (n = 23)	8 (38.1)	15 (20.5)	.148	4 (26.7)	19 (24.1)	1.000	2 (40.0)	21 (23.6)	.593	15 (27.8)	8 (20.0)	.470
Others (n = 9)	0 (0)	9 (12.3)	.200	0 (0)	9 (11.4)	.346	0 (0)	9 (10.1)	1.000	5 (9.3)	4 (10.0)	1.000
Cell differentiation												
Well (n = 47)	8 (42.1)	39 (62.9)	<.001	6 (46.2)	41 (60.3)	<.001	1 (25.0)	46 (59.7)	<.001	27 (60.0)	20 (55.6)	.757
Moderate (n = 29)	6 (31.6)	23 (37.1)		2 (15.4)	27 (39.7)		0 (0)	29 (37.7)		16 (35.6)	13 (36.1)	
Poorly (n = 5)	5 (26.3)	0 (0)		5 (38.5)	0 (0)		3 (75.0)	2 (2.6)		2 (4.4)	3 (8.3)	
EGFR mutation (del 19 or L858R)												
Mutated (n = 32)	3 (14.3)	29 (39.7)	.037	2 (13.3)	30 (38.0)	.079	0 (0)	32 (36.0)	.162	18 (33.3)	14 (35.0)	1.000
Wild-type (n = 62)	18 (85.7)	44 (60.3)		13 (86.7)	49 (62.0)		5 (100)	57 (64.0)		36 (66.7)	26 (65.0)	
HLA class I expression												
Reduced (n = 40)	8 (38.1)	32 (43.8)	.803	7 (46.7)	33 (41.8)	.780	1 (20.0)	39 (43.8)	.390			
Normal (n = 54)	13 (61.9)	41 (56.2)		8 (53.3)	46 (58.2)		4 (80.0)	50 (56.2)				
Surgery type												
Wedge or segmentectomy (n = 5)	0 (0)	5 (6.8)	.584	0 (0)	5 (6.3)	1.000	0 (0)	5 (5.6)	1.000	2 (3.7)	3 (7.5)	.648
Lobectomy or pneumonectomy (n = 89)	21 (100)	68 (93.2)		15 (100)	74 (93.7)		5 (100)	84 (94.4)		52 (96.3)	37 (92.5)	
No. of nodes dissected, median	22.0	22.0	.958	21.0	22.1	.838	21.0	21.7	.844	22.5	19.5	.452
Pathologic stage												
IA (n = 67)	13 (61.9)	54 (74.0)	.288	9 (60.0)	58 (73.4)	.353	1 (20.0)	66 (74.2)	.023	40 (74.1)	27 (67.5)	.499
IB (n = 27)	8 (38.1)	19 (26.0)		6 (40.0)	21 (26.6)		4 (80.0)	23 (25.8)		14 (25.9)	13 (32.5)	
Adjuvant chemotherapy, performed (n = 10)	4 (19.0)	6 (8.2)	.223	2 (13.3)	8 (10.1)	.659	1 (20.0)	9 (10.1)	.438	3 (7.5)	7 (13.0)	.509

Data represented as absolute counts (%) or median (range). *PD-L1*, Programmed death-ligand 1; *HLA*, human leukocyte antigen; *EGFR*, epidermal growth factor receptor.

Each slide was independently examined by 2 of the investigators (A.H. and S.S.) without any knowledge of the clinical data. When a discrepancy was found between the 2 investigators, a consensus was reached via their simultaneous examination using a double-headed microscope. Each tumor cell was judged as positive staining for PD-L1 when the membrane was stained at any intensity. Each patient was classified into “negative” PD-L1 or “positive” PD-L1 according to the percentage of PD-L1-positive tumor cells; 3 exploratory cutoff

values (1%, 5%, 50%), which were identified in previous clinical studies,⁶⁻¹¹ were used and analyzed.

HLA class I expression had been evaluated in a previous study with IHC using an anti-HLA class I antibody (EMR8-5, Cosmo Bio, Tokyo, Japan).¹⁷ According to the percentage of HLA class I-positive tumor cells (HLA+ tumor cells), each patient had been classified as “normal” expression (HLA+ tumor cells, ≥80%) or “reduced” expression (HLA+ tumor cells, <80%).¹⁷

FIGURE 1. CONSORT diagram of the study.

Statistical Analyses

The proportions of categorical data were compared by the chi-square test. Continuous data were compared using a nonparametric test (Mann–Whitney *U* test).

The Kaplan–Meier method was used to estimate the probability of survival, and survival differences were analyzed by the log-rank test. To identify independent prognostic factors, univariable and multivariable analyses using Cox proportional hazards regression models were performed. Variables with a *P* value less than .1 in the first univariable analyses were included in multivariable models. The hazard ratio (HR) and 95% confidence interval (CI) were calculated for each variable.

All statistical analyses were performed with EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a modified version of R (The R Foundation for Statistical Computing, Vienna, Austria).¹⁷

RESULTS

Programmed Death-Ligand 1 Expression in Lung Adenocarcinoma

PD-L1 expression was positive in 21 patients (22.3%) at the cutoff value of 1%, 15 patients (16.0%) at the cutoff value of 5%, and 5 patients (5.3%) at the cutoff value of 50%.

Positive PD-L1 expression was more frequently seen in poorly differentiated tumors than in well-differentiated tumors. In addition, positive PD-L1 expression seemed to be less frequent among epidermal growth factor receptor–mutated tumors. No significant correlation between PD-L1 expression status at any cutoff value and smoking status was observed (Table 1).

In this cohort, HLA class I expression was “normal” in 54 patients (57%) and “reduced” in 40 patients (43%).

No significant correlation was observed between HLA class I expression and PD-L1 expression, and no other patient characteristic was observed (Table 1).

Programmed Death-Ligand 1 Expression and Survival

The median follow-up period for all patients was 61.2 months. The 5-year OS of PD-L1–positive and PD-L1–negative patients using each cutoff value was 76.2% and 85.1% (cutoff, 1%; *P* = .249) (Figure 2, A), 66.7% and 85.9% (cutoff, 5%; *P* = .049) (Figure 2, B), and 40.0% and 85.3% (cutoff, 50%; *P* = .005) (Figure 2, C), respectively, showing that positive PD-L1 expression with higher cutoff values was associated with a poor prognosis.

There was no significant difference in OS according to HLA class I status (5-year survival, 79.1% in “reduced” patients vs 85.3% in “normal” patients; *P* = .378). An additional exploratory analysis in which the percentage of tumor cells expressing HLA class I was used as a continuous variable also revealed no significant correlation between HLA class I expression status and OS (HR, 0.999; 95% CI, 0.981–1.017; *P* = .891).

When analyzed by PD-L1 expression status with stratification by HLA class I status, a significantly poor prognosis in PD-L1–positive patients (cutoff value, 5% or 50%) was documented only when HLA class I expression was “normal” (Video 1). The 5-year survivals of PD-L1–negative and positive patients were 88.6% versus 76.9% at the cutoff value of 1% (*P* = .186); 89.4% versus 62.5% at the cutoff value of 5% (*P* = .029) (Figure 3, A); and

FIGURE 2. OS curves according to PD-L1 expression status classified by the percentage of tumor cells expressing PD-L1 at the cutoff value of 1% (A), 5% (B), or 50% (C). *CI*, Confidence interval; *PD-L1*, programmed death-ligand 1.

88.3% versus 50.0% at the cutoff value of 50% ($P = .022$) (Figure 3, C), respectively. PD-L1 expression (cutoff value, 5%) along with sex and p-stage were included in a multivariable analysis (model 1), and PD-L1 positivity was a marginal factor to predict a poor prognosis (HR, 3.099; 95% CI, 0.956-9.261; $P = .058$) (Table 2). In a multivariable analysis in which PD-L1 expression (cutoff value, 50%) was included

(Model 2), the prognostic impact of PD-L1 positivity seemed less significant (HR, 4.372; 95% CI, 0.804-13.771; $P = .088$) (Table 2).

Among patients with “reduced” HLA class I expression, PD-L1 expression status provided no prognostic impact: 5-year survival, 80.4% versus 75.0% at the cutoff value of 1%; 80.9% versus 71.4% at the cutoff value of 5%

VIDEO 1. This retrospective study indicated that the prognostic impact of PD-L1 expression on tumor cells might be distinct according to HLA class I expression status in pathologic stage I lung adenocarcinoma. When HLA class I expression was reduced, PD-L1 expression status was not a significant prognostic factor. By contrast, when HLA class I expression was sufficient as represented by “normal,” positivity of PD-L1 expression was associated with a poor prognosis. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(17\)31190-X/fulltext](http://www.jtcvsonline.org/article/S0022-5223(17)31190-X/fulltext).

(Figure 3, B); 81.4% versus 0.0% at the cutoff value of 50% (Figure 3, D). Analyses among patients who did not receive adjuvant chemotherapy provided similar results (Table E1).

DISCUSSION

In the present study, we first showed that positive PD-L1 expression status on tumor cells was a potential factor to predict a poor prognosis, which may depend on concurrent HLA class I expression status on tumor cells in lung adenocarcinoma. PD-L1 positivity was found to be correlated with a poor prognosis when concurrent HLA class I was adequately expressed on tumor cells, but PD-L1 expression status provided no prognostic impact when concurrent HLA class I expression was reduced.

The clinical implications of PD-L1 expression status in NSCLC have been reported in previous studies,¹¹ in correlation with tumor characteristics including mutational burden, prognosis, and response to immune-checkpoint inhibitors, such as anti-PD-1 antibodies (ie, nivolumab^{6,7} and pembrolizumab⁸) and anti-PD-L1 antibodies (ie, atezolizumab⁹ and durvalumab¹⁰). In many studies conducted for advanced NSCLC, positive or stronger PD-L1 expression was correlated with favorable clinical benefits achieved with anti-PD-1/PD-L1 antibodies.⁷⁻⁹ As for the prognostic impact of PD-L1, however, many conflicting results have been reported, partly because of the vast heterogeneity of patient characteristics, including histologic subtypes and tumor stage, indicating the need for studies with homogenous patient populations. Accordingly, in the current study, we assessed the prognostic impact exclusively among patients with completely resected p-stage I lung adenocarcinoma. When we systematically searched for similar articles in PubMed as of December 31, 2016,

using the terms “lung” and “adenocarcinoma” and “PD-L1” and “survival OR prognosis” only in the English literature, 13 eligible articles were identified¹⁸⁻³⁰ (Table 3). Ten of 23 searched articles were ineligible, because no prognostic impact of PD-L1 expression status was examined. Most studies (9 of 13 searched studies, along with the present study) indicated a significantly poor prognosis in positive PD-L1 patients, whereas 3 studies indicated a favorable prognosis in positive PD-L1 patients.^{18,25,27} Concerning clinicopathologic and molecular features, most studies showed that positive PD-L1 expression was associated with increased smoking history and poor differentiation, and the significant association between PD-L1 expression and poor differentiation also was documented in the current study. Five studies showed a positive association between the PD-L1 expression and the absence of epidermal growth factor receptor mutations, as a similar trend shown in the present study. In contrast, 1 study showed a positive association between the PD-L1 expression and the presence of epidermal growth factor receptor mutations.²⁰ Overall, positive PD-L1 expression was associated with more aggressive tumor behavior such as poor differentiation in adenocarcinoma of the lung, but the prognostic impact may remain controversial in such a homogenous population. Therefore, the prognostic impact of PD-L1 expression may be influenced by other factors associated with cancer immunity, such as HLA class I.

Cancer immunity comprises a series of steps as follows: (1) release of neoantigens from tumor cells; (2) capture and processing of neoantigens by antigen presenting cells (APCs); (3) activation of cytotoxic T-cells (CTLs) through presentation of processed neoantigens by APCs; (4) recognition and binding to tumor cells by CTLs through interaction between T-cell receptor on CTLs and its cognate neoantigen on HLA class I molecules on T cells; and (5) killing of target T cells by CTLs.³¹⁻³³ HLA class I molecules expressing on tumor cells play critical roles in the step of recognition and binding by CTLs, and tumor cells with reduced expression of HLA class I molecules may evade recognition by CTLs to survive.¹²⁻¹⁴ Accordingly, expression status of HLA class I molecules on tumor cells may influence prognosis, and several clinical studies showed that downregulation or low expression of HLA class I antigens was associated with a poor prognosis in a variety of malignant tumors, including NSCLC.^{17,34-36} PD-L1, expressing on tumor cells, also plays an important role in immune evasion at the final step by inhibiting activated CTLs. Accordingly, tumor cells with reduced HLA class I expression may evade immune attack regardless of PD-L1 expression on tumor cells, which may account for the lack of prognostic impact of PD-L1 status in tumors with reduced HLA class I expression found in the present study. Nevertheless,

FIGURE 3. OS according to PD-L1 expression status after stratification by HLA class I expression status. HLA class I status was classified on the basis of the percentage of tumor cells expressing HLA class I molecules into “normal” ($\geq 80\%$) or “reduced” ($< 80\%$). A, OS curves of PD-L1 5% or greater and less than 5% cases among “normal” HLA class I cases. B, OS curves of PD-L1 5% or greater and less than 5% cases among “reduced” HLA class I cases. C, OS curves of PD-L1 50% or greater and less than 50% cases among “normal” HLA class I cases. D, OS curves of PD-L1 50% or greater and less than 50% cases among “reduced” HLA class I cases. *CI*, Confidence interval; *PD-L1*, programmed death-ligand 1.

tumor cells with HLA class I expression can be recognized by CTLs and may evade immune attack by expressing PD-L1, which also may account for the result that PD-

L1 positivity was associated with a poor prognosis in tumors with normal HLA class I expression. In the present study, a multivariate analysis failed to show a significant

TABLE 2. Univariable and Multivariable Cox model of prognostic factors for overall survival

Variables	Univariable analysis			Multivariable analysis (Model 1)								
	HR	95% CI	P	All patients			HLA normal only			HLA reduced only		
				HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (per 1-y increase)	1.016	0.964-1.069	.558									
Sex, female (vs male)	0.165	0.037-0.726	.017	0.214	0.048-0.968	.045	0.190	0.023-1.578	.124	0.189	0.022-1.637	.130
Smoker (vs never smoker)	2.273	0.733-7.052	.155									
Comorbidities, present (vs absent)												
Pulmonary	0.665	0.188-2.357	.527									
Cardiovascular	0.764	0.215-2.706	.676									
Others	0.787	0.099-5.751	.756									
Good differentiation (vs moderate to poor)	0.372	0.109-1.273	.115									
EGFR mutation (del 19 or L858R)	0.674	0.215-2.118	.500									
Normal HLA class I expression (vs reduced HLA class I expression)	0.636	0.231-1.755	.382									
PD-L1 expression, positive (vs negative)												
≥1% (vs <1%)	1.864	0.636-5.465	.257									
≥5% (vs <5%)	2.811	1.060-8.227	.046	1.848	0.625-5.466	.267	3.099	0.956-9.261	.058	0.463	0.053-4.071	.488
≥50% (vs <50%)	5.136	1.342-18.279	.022									
No. of nodes dissected, median or greater (vs less than median)	0.803	0.291-2.215	.672									
Pathologic stage IB (vs IA)	3.101	1.123-8.565	.029	2.236	0.800-6.246	.125	1.822	0.398-8.340	.440	2.922	0.620-13.775	.175
Adjuvant chemotherapy, performed (vs not performed)	0.532	0.070-4.048	.542									

Variables	Multivariable analysis (Model 2)								
	All patients			HLA normal only			HLA reduced only		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (per 1-y increase)									
Sex, female (vs male)	0.192	0.043-0.854	.030	0.188	0.023-1.539	.119	0.212	0.025-1.816	.157
Smoker (vs never smoker)									
Comorbidities, present (vs absent)									
Pulmonary									
Cardiovascular									
Others									
Good differentiation (vs moderate to poor)									
EGFR mutation (del 19 or L858R)									
Normal HLA class I expression (vs reduced HLA class I expression)									
PD-L1 expression, positive (vs negative)									
≥1% (vs <1%)									
≥5% (vs <5%)									
≥50% (vs <50%)	3.195	0.854-11.957	.084	4.372	0.804-13.771	.088	2.624	0.279-24.683	.399
No. of nodes dissected, median or greater (vs less than median)									
Pathologic stage IB (vs IA)	2.023	0.705-5.800	.190	1.349	0.293-6.212	.701	2.824	0.611-13.066	.184
Adjuvant chemotherapy, performed (vs not performed)									

HLA, Human leukocyte antigen; HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; PD-L1, programmed death-ligand 1.

TABLE 3. Summary of reported studies on programmed death-ligand 1 expression in adenocarcinoma of the lung

Author (y) [reference]	N	Stage (n)	Distribution of PD-L1 expression	Cutoff for PD-L1 positivity	Antibody used in IHC	No. of positive PD-L1 patients (%)	Prognosis of positive PD-L1 patients	Correlation between PD-L1 positivity and other factors
Yang CY 2014 ¹⁸	163	I (163)	Membrane	≥5%, any intensity	Proteintech (Proteintech, Rosemont, Ill)	65 (40%)	Better RFS (<i>P</i> = .027) No OS difference (<i>P</i> = .933)	Higher-grade differentiation Vascular invasion; not correlated with EGFR
Zhang Y 2014 ¹⁹	143	I (66) II-III (77)	Membrane and cytoplasm	Intensity and percentage (score)	SAB2900365 (Sigma-Aldrich, St Louis, Mo)	70 (49%)	Poor RFS (<i>P</i> < .001) Poor OS (<i>P</i> = .002)	Higher p-stage (T-/N-factor) Solid-predominant type Not correlated with EGFR
Song Z 2016 ²⁰	385	I (121) II (79), III (185)	Membrane	≥5%, any intensity	Proteintech	186 (48%)	Poor DFS (38.0 mo vs 50.4 mo; <i>P</i> = .014) Poor OS (trend, 52.9 mo vs 68.2 mo; <i>P</i> = .069)	Coexisting mutations including EGFR- mutations
Ji M 2016 ²¹	100	I (42) II (27), III (31)	Membrane and cytoplasm	Intensity and percentage (score)	Abcam (Abcam, Cambridge, UK)	40 (40%)	Poor 3-y OS (62.5% vs 83.3%; <i>P</i> = .034)	EGFR wild-type
Cha YJ 2016 ²²	323	I (177) II (58) III (81), IV (7)	Membrane		SP142 (Spring Bioscience, Pleasanton, Calif)	60 (19%)	Poor RFS (<i>P</i> < .001) Poor OS (<i>P</i> < .001)	Solid-predominant type Higher stage EGFR wild-type
Shimoji M 2016 ²³	165	I (113) II-IV (52)	Membrane	Intensity and percentage (score)	E1L3N (Cell Signaling, Danvers, Mass)	37 (22%)	Poor OS (<i>P</i> = .0051)	Smoking solid- predominant subtype
Inamura K 2016 ²⁴	268		Membrane	≥5%, any intensity	E1L3N	43 (16%)	Poor OS (<i>P</i> = .0014) Poor lung cancer- specific survival (<i>P</i> = .019)	Smoking (trend) Less differentiation (moderate to poorly) EGFR wild-type
Huang JJ 2016 ²⁵	112	I (94) II (4) III (14)	Membrane and cytoplasm	Intensity and percentage (score)	Proteintech	99 (88%)	Better OS (<i>P</i> = .023)	Acinar- predominant Less papillary- predominant not correlated with EGFR
Takada K 2016 ²⁶	417	I (305) II (63) III (49)	Membrane	≥5% or ≥1%, any intensity	SP142	144 (35%, ≥1%) 85 (20%, ≥5%)	Poor OS (<i>P</i> < .001 ≥1%; .0010 ≥5%) Poor DFS (<i>P</i> < .0001/1%; 0.0049/5%)	Smoking Advanced p-stage micropapillary, solid- predominant pleural, vessel invasion EGFR wild-type
Huynh TG 2016 ²⁷	261	I (201) II (34) III (22), IV (4)	Membrane	≥1%, 5%, or 50%, any intensity	E1L3N	129 (49% ≥1%) 95 (36% ≥5%) 62 (24% ≥50%)	Poor 5-y PFS (63% vs 79%, <i>P</i> < .001/1%; 66% vs 75%,	Smoking; solid- predominant higher nuclear gradelymphatic,

(Continued)

TABLE 3. Continued

Author (y) [reference]	N	Stage (n)	Distribution of PD-L1 expression	Cutoff for PD-L1 positivity	Antibody used in IHC	No. of positive PD-L1 patients (%)	Prognosis of positive PD-L1 patients	Correlation between PD-L1 positivity and other factors
							$P = .007/5\%$; 66% vs 73%, $P = .032/50\%$ Poor 5-y OS (70% vs 89%, $P = .002/1\%$; 72% vs 85%, $P = .011/5\%$; 79% vs 81%, $P = .118/50\%$)	venous invasion EGFR wild-type
Ohue T 2016 ²⁸	120	I-III A	Membrane and cytoplasm	Intensity and percentage (score)	ProSci (ProSci, Poway, Calif)	53 (44%)	Better OS (trend, $P = .09$)	
Uruga H 2016 ²⁹	109	II-III	Membrane	$\geq 1\%$, 5% or 50%, any intensity	E1L3N	56 (51% $\geq 1\%$) 43 (39% $\geq 5\%$) 19 (17% $\geq 50\%$)	No 5-y PFS (57% vs 45%, $P = .283/1\%$; 55% vs 48%, $P = .373/5\%$; 55% vs 50%, $P = .888/50\%$)	Solid-dominant presence of tumor island, necrosis, vascular invasion; higher nuclear grade nodal involvement
Mori S 2017 ³⁰	296	I-III		Intensity and percentage (score)	EPR1611 (Abcam)	107 (36%)	Poor DFS (69% vs 81% at 3 y; $P = .021$) Poor OS (85% vs 94% at 3 y; $P = .005$)	Smoking; poor differentiation Not correlated with EGFR
Current study	94	I	Membrane	$\geq 1\%$, 5%, or 50%, any intensity	E1L3N	21 (22% $\geq 1\%$) 15 (16% $\geq 5\%$) 5 (5% $\geq 50\%$)	Poor 5-y OS (trend, 76% vs 85%, $P = .249/1\%$; 67% vs 86%, $P = .048/5\%$; 40% vs 85%, $P = .0049/50\%$)	Less differentiation EGFR wild-type (trend)

PD-L1, Programmed death-ligand 1; IHC, immunohistochemistry; RFS, recurrence-free survival; OS, overall survival; EGFR, epidermal growth factor receptor; DFS, disease-free survival; PFS, progression-free survival.

prognostic value of PD-L1 expression in tumor with normal HLA class I expression ($P = .058$), most likely because of a relatively small number of patients.

Study Limitations

Although this study suggested that the prognostic values of PD-L1 expression were distinct according to HLA class I expression, this study had several limitations due to a variety of weaknesses other than its small number of patients. First, this study was a retrospective single-institutional study. Second, a certain percentage of patients were lost to follow-up in this study (approximately one fourth of patients at 5 years after surgery). In addition, the impact of PD-L1 status

on postoperative recurrence and postoperative death is important, which was not examined in this study because adequate recurrence data were not available. Finally, the number of events for OS analysis was relatively small (15 deaths in the entire study cohort). To draw more conclusive results, we are currently conducting a larger-scale retrospective study. In addition, the prognostic impact of PD-L1 may be influenced not only by HLA class I but also by other factors associated with immune response (eg, major histocompatibility complex class II molecules and PD-1) and other pathologic factors (eg, specific subtypes of adenocarcinoma), and these factors should be included and analyzed in future studies.

CONCLUSIONS

The prognostic impact of PD-L1 expression was distinct according to HLA class I expression status in resectable adenocarcinoma of the lung. PD-L1 positivity on tumor cells was associated with a poor prognosis when HLA class I expression was normal, but was not associated with prognosis when HLA class I expression was reduced.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: PD-L1, HLA, lung, adenocarcinoma, prognosis

TABLE E1. Univariable and multivariable Cox model of prognostic factors for overall survival

Variables	Univariable analysis			Multivariable analysis (Model 1)								
	HR	95% CI	P	All patients			HLA normal only			HLA reduced only		
				HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (per 1-y increase)	1.011	0.960-1.065	.676									
Sex (female)	0.088	0.011-0.671	.019	0.128	0.016-1.022	.052	0.265	0.030-2.344	.232	0.249	0.027-2.267	.217
Smoker (vs never smoker)	2.977	0.830-10.677	.194									
Comorbidities												
Pulmonary	0.747	0.208-2.678	.654									
Cardiovascular	0.930	0.259-3.334	.911									
Others	0.711	0.093-5.440	.743									
Good differentiation (vs moderate to poor)	0.391	0.175-1.127	.117									
EGFR mutation (del 19 or L858R)	0.687	0.215-2.191	.526									
Normal HLA class I expression	0.790	0.277-2.253	.659									
PD-L1 expression												
≥1% (vs <1%)	2.435	0.813-7.293	.112									
≥5% (vs <5%)	3.316	1.110-9.904	.032	1.889	0.617-5.780	.265	3.541	0.976-12.441	.054	1.228	0.223-6.747	.813
≥50% (vs <50%)	6.789	1.867-24.696	.014									
No. of nodes dissected, median or greater (vs less than median)	0.828	0.290-2.362	.724									
Pathologic stage IB (vs IA)	4.782	1.669-13.704	.004	3.162	1.075-9.303	.036	2.851	0.640-12.694	.169	3.201	0.665-15.407	.147
Multivariable analysis (Model 2)												
Variables	All patients			HLA normal only			HLA reduced only					
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P			
Age (per 1-y increase)												
Sex (female)	0.124	0.016-0.985	.048	0.238	0.027-2.073	.194	0.252	0.028-2.245	.217			
Smoker (vs never smoker)												
Comorbidities												
Pulmonary												
Cardiovascular												
Others												
Good differentiation (vs moderate to poor)												
EGFR mutation (del 19 or L858R)												
Reduced HLA class I expression												
PD-L1 expression												
≥1% (vs <1%)												
≥5% (vs <5%)												
≥50% (vs <50%)	2.554	0.642-10.162	.183	3.415	0.856-15.985	.085	2.210	0.211-22.140	.500			
No. of nodes dissected, median or greater (vs less than median)												
Pathologic stage IB (vs IA)	2.849	0.922-8.807	.069	2.317	0.490-10.964	.289	2.859	0.547-14.950	.213			

HLA, Human leukocyte antigen; HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; PD-L1, programmed death-ligand 1.