Positive PD-L1 expression is predictive for patients with advanced EGFR wild-type non-small cell lung cancer treated with gemcitabine and cisplatin

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Abstract. This retrospective study aimed to investigate the association between programmed death ligand-1 (PD-L1) expression and the clinicopathological characteristics of patients with advanced epidermal growth factor receptor (EGFR) wild-type non-small cell lung cancer (NSCLC). The predictive role and cut-off value of PD-L1 expression was subsequently investigated. A total of 172 patients with advanced EGFR wild-type NSCLC were enrolled. All patients received platinum-based doublet chemotherapy (gemcitabine plus cisplatin). PD-L1 expression in lung tissues was assessed using immunohistochemical methods. The χ^2 test was used to analyze the association between PD-L1 expression and clinicopathological characteristics. Survival time analysis was performed using the Kaplan-Meier method. The two groups, positive PD-L1 expression and negative PD-L1 expression, were compared using the log-rank test. Multivariate analysis using the Cox proportional hazard regression model was conducted to determine prognostic factors for overall survival (OS) and progression-free survival (PFS) times. Positive PD-L1 expression was observed in 48.3% (84/172), 40.7% (70/172), 21.5% (37/172) and 8.1% (14/172) of patients when using cut-off values of 1, 5, 10 and 50%, respectively. The χ^2 test revealed that elevated pretreatment C-reactive protein (CRP) level and cancer stage IV were significantly associated with positive PD-L1 expression. The OS and PFS of positive PD-L1 (1, 5, 10 and 50% cut-off) expression group were shorter compared with the negative PD-L1 (1, 5, 10 and 50% cut-off) expression group. Multivariate survival analysis revealed that PD-L1 expression ≥50% was significantly associated with decreased OS and PFS [OS time, P=0.001; hazard ratio (HR), 2.768; 95% confidence interval (CI), 1.551-4.940; PFS time, P=0.002; HR, 2.537; 95% CI, 1.423-4.524]. These results indicated that positive PD-L1 (50% cut-off) expression was an independent predictor of poor prognosis for patients with advanced NSCLC treated with gemcitabine plus cisplatin. PD-L1 expression was associated with CRP level and cancer stage. The results obtained in the present study suggest that positive PD-L1 expression serves a prognostic role in advanced NSCLC and that the optimal cut-off value may be 50%.

Introduction

Lung cancer has been the leading cause of cancer-associated mortality worldwide in males (24%) and females (23%) in 2019 (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all types of lung cancer (2). Platinum-based chemotherapy is the standard treatment for patients with advanced epidermal growth factor receptor (EGFR) wild-type NSCLC (3). Gemcitabine was approved as a first-line treatment for advanced NSCLC (4-6). However, the clinical outcome for patients with advanced stage NSCLC remains poor, and novel effective treatment strategies are required (7).

Immune checkpoint inhibitors have yielded promising results in NSCLC. Programmed death ligand-1 (PD-L1) is an important target for immunotherapy. Previous studies have revealed that PD-L1 expression may be a predictor of treatment response (8,9). High expression of PD-L1 was associated with the presence of EGFR mutations (10-12). Activating mutations of EGFR also induced PD-L1 expression in NSCLC, and EGFR tyrosine kinase inhibitors downregulated PD-L1 expression in EGFR mutation-positive NSCLC (13-15). However, the predictive value of PD-L1 expression in patients with EGFR wild-type NSCLC remains unclear. Furthermore, different chemotherapy regimens may affect the clinical outcome (16,17). Therefore, the aim of the current retrospective study was to analyze PD-L1 expression in patients with advanced EGFR wild-type NSCLC treated with gemcitabine plus cisplatin and to potentially determine the cut-off value of PD-L1 expression.

Materials and methods

Patients. A total of 172 eligible patients were enrolled in the current study between August 2011 and December 2017 at The

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First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). The inclusion criteria were as follows: i) Histologically confirmed diagnosis of NSCLC based on the WHO classification (18); ii) newly diagnosed with cancer stage IIIB or IV; iii) ≥18 and ≤80 years of age; iv) European Cooperative Oncology Group (ECOG) performance status (PS) 0-2; v) EGFR wild-type; vi) measurable disease according to revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (19); vii) adequate hematological, hepatic and organ function; and viii) adequate clinicopathological information and follow-up data. The exclusion criteria were as follows: i) Uncontrolled brain metastases; ii) autoimmune disease; iii) previous malignant tumor or second primary tumor; and iv) prior treatment with chemotherapy, radiotherapy or immunotherapy. Clinicopathological characteristics were recorded for each patient, including patient demographics, histology, EGFR status, pretreatment serum C-reactive protein (CRP) level, date of diagnosis, imaging of the involved region, cancer stage, ECOG PS, smoking status, chemotherapy schedule, treatment response, PD-L1 expression, overall survival (OS) and progression-free survival (PFS) times. OS was measured from the date of the initial therapy until the last follow-up. PFS was measured from the date of the initial treatment until the date of disease progression or death from any cause. Patients were followed up at a median duration of 9 months (range, 2-25 months). The current study was approved by The Ethics Committee of The First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from all enrolled patients. All experiments were performed in accordance with approved guidelines and regulations (20,21).

PD-L1 expression. Pretreatment lung cancer tumor tissue was collected for PD-L1 analyses. PD-L1 expression was retrospectively assessed in tumor biopsies using immunohistochemical methods. Sections (4-μm-thick) from each formalin-fixed (10% formaldehyde at 20˚C for 24 h) paraffin-embedded tissue were used, followed by the modified avidin-biotin complex method (Envision method) using an automated immunostainer (model no. 314683; Ventana Medical Systems, Inc., Tucson, AZ, USA) (22). A rabbit antihuman PD-L1 antibody (ready-to-use; cat. no. ZA-0629; OriGene Technologies Inc., Beijing, China) was used to detect PD-L1, and was incubated for 40 min at 37˚C. The tissues were then incubated with the horseradish peroxidase-anti-rat IgG secondary antibody (ready-to-use; cat. no. 760-500; Roche, Basel, Switzerland) for 8 min at 37˚C. The sections were counterstained with hematoxylin at 37˚C for 4 min and then mounted. Images were taken using a light microscope and analyzed using HistoQuest software (version 6.0; TissueGnostics, Vienna, Austria) for an automated measurement. PD-L1 expression was defined by tumor cell membrane expression levels, and classified according to prespecified levels (≥1, ≥5, ≥10 and ≥50%) (23-25).

Treatment and response. Eligible patients received platinum-based doublet chemotherapy (gemcitabine 1,000 mg/m^2 on days 1 and 8; cisplatin 25 mg/m^2 on days 1, 2 and 3, repeated every 3 weeks; both from Hanson Pharma, Lianyungang, China). This treatment is the standard of care for managing patients with advanced NSCLC in China (7). Assessment of treatment response was based on the RECIST version 1.1 guidelines (19).

Statistical analysis. The association between PD-L1 expression and clinicopathological characteristics was analyzed using the χ^2 test. Survival curves and rates were estimated using the Kaplan-Meier method and groups were compared using the log-rank test. Multivariate analysis using Cox proportional hazard regression model was performed to evaluate the prognostic and predictive role of PD-L1 expression. The hazard ratio (HR) and 95% confidence interval (CI) were estimated using a stratified Cox proportional hazards model. Statistical analyses were performed using GraphPad Prism software (version 6; GraphPad Software Inc., La Jolla CA, USA) and SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. The clinicopathological characteristics of the enrolled patients are presented in Table I. Among 172 patients, positive PD-L1 expression was observed in 48.3% (84/172), 40.7% (70/172), 21.5% (37/172) and 8.1% (14/172) of patients when using cut-off values of 1, 5, 10 and 50%, respectively.

Association of PD-L1 expression with clinicopathological characteristics. The χ^2 test revealed that elevated pretreatment serum CRP (≥10 mg/l) was significantly associated with positive PD-L1 expression for 1, 5, 10 and 50% cut-off values (P=0.001, 0.001, 0.001 and 0.008, respectively). Similarly, stage IV cancer was significantly associated with positive PD-L1 expression for 1, 5, 10 and 50% cut-off values (P=0.001, 0.001, 0.001 and 0.018, respectively; Table I). These data suggested that PD-L1 expression was associated with pretreatment serum CRP level and cancer stage.

Survival time. To illustrate the prognostic value of PD-L1, the was association between PD-L1 expression and OS and PFS times was determined. At the median follow-up duration of 9 months, the one-year OS and PFS times of all patients were 43.3 and 22.0% respectively. Positive PD-L1 expression group was significantly associated with shorter OS and PFS times compared with negative PD-L1 expression group (Fig. 1). The one-year OS time for positive PD-L1 expression group were shorter than negative PD-L1 expression group (36.4 vs. 50% at 1% cut-off; 35.4 vs. 49.0% at 5% cut-off; 33.2 vs. 46.5% at 10% cut-off; and 7.1 vs. 46.2% at 50% cut-off, respectively). The one-year PFS time for positive PD-L1 expression group were also shorter than negative PD-L1 expression group (16.4 vs. 27.6% at 1% cut-off; 16.0 vs. 25.8% at 5% cut-off; 11.5 vs. 24.6% at 10% cut-off; and 7.0 vs. 23.3% at 50% cut-off, respectively; Fig. 2). Representative immunohistochemical staining images of tumor biopsies with PD-L1 were shown in Figure 3. Univariate survival analysis revealed that cancer stage IV, positive PD-L1 (1% cut-off) expression, ECOG PS 2 and age ≥60 were significantly associated with shorter OS and PFS times (P<0.0001, 0.0481, 0.0050 and <0.0001 for OS time; P<0.0001, 0.0035, 0.0278 and 0.0010 for PFS time, respectively; Table II).

Table I. Association of PD-L1 expression and clinicopathological characteristics (n=number of patients).

Variables	No. of patients (%)	PD-L1 (1% cut-off) (n)			PD-L1 (5% cut-off) (n)			PD-L1 (10% cut-off) (n)			PD-L1 (50% cut-off) (n)		
		Positive (84)	Negative (88)	P-value	Positive (70)	Negative (102)	P-value	Positive (37)	Negative (135)	P-value	Positive (14)	Negative (158)	P-value
Gender				0.304			0.645			0.947			0.776
Female	55 (32.0)	30	25		21	34		12	43		4	51	
Male	117 (68.0)	54	63		49	68		25	92		10	107	
Age (years)				0.281			0.035			0.071			0.124
<60	83 (48.3)	37	46		27	56		13	70		4	79	
≥60	89 (51.7)	47	42		43	46		24	65		10	79	
Histology				0.634			0.905			0.757			0.568
Adenocarcinoma	122 (70.9)	61	61		50	72		27	95		9	113	
Non-adenocarcinoma	50 (29.1)	23	27		20	30		10	40		5	45	
Stage				0.001			0.001			0.001			0.018
IIIB	89 (51.7)	32	57		22	67		12	77		3	86	
IV	83 (48.3)	52	31		48	35		25	58		11	72	
ECOG PS				0.706			0.291			0.368			0.160
0-1	104 (60.5)	52	52		39	65		20	84		6	198	
2	68 (39.5)	32	36		31	37		17	51		8	60	
Smoking status				0.551			0.908			0.812			0.856
Former or current smoker	82 (47.7)	42	40		33	49		17	65		7	75	
Non-smoker	90 (52.3)	42	48		37	53		20	70		7	83	
CRP				0.001			0.001			0.001			0.008
Elevated	66 (38.4)	43	23		37	29		23	43		10	56	
Normal	106 (61.7)	41	65		33	73		14	92		4	102	

PD-L1, programmed death ligand-1; ECOG, European Cooperative Oncology Group; PS, performance status; CRP, C-reactive protein.
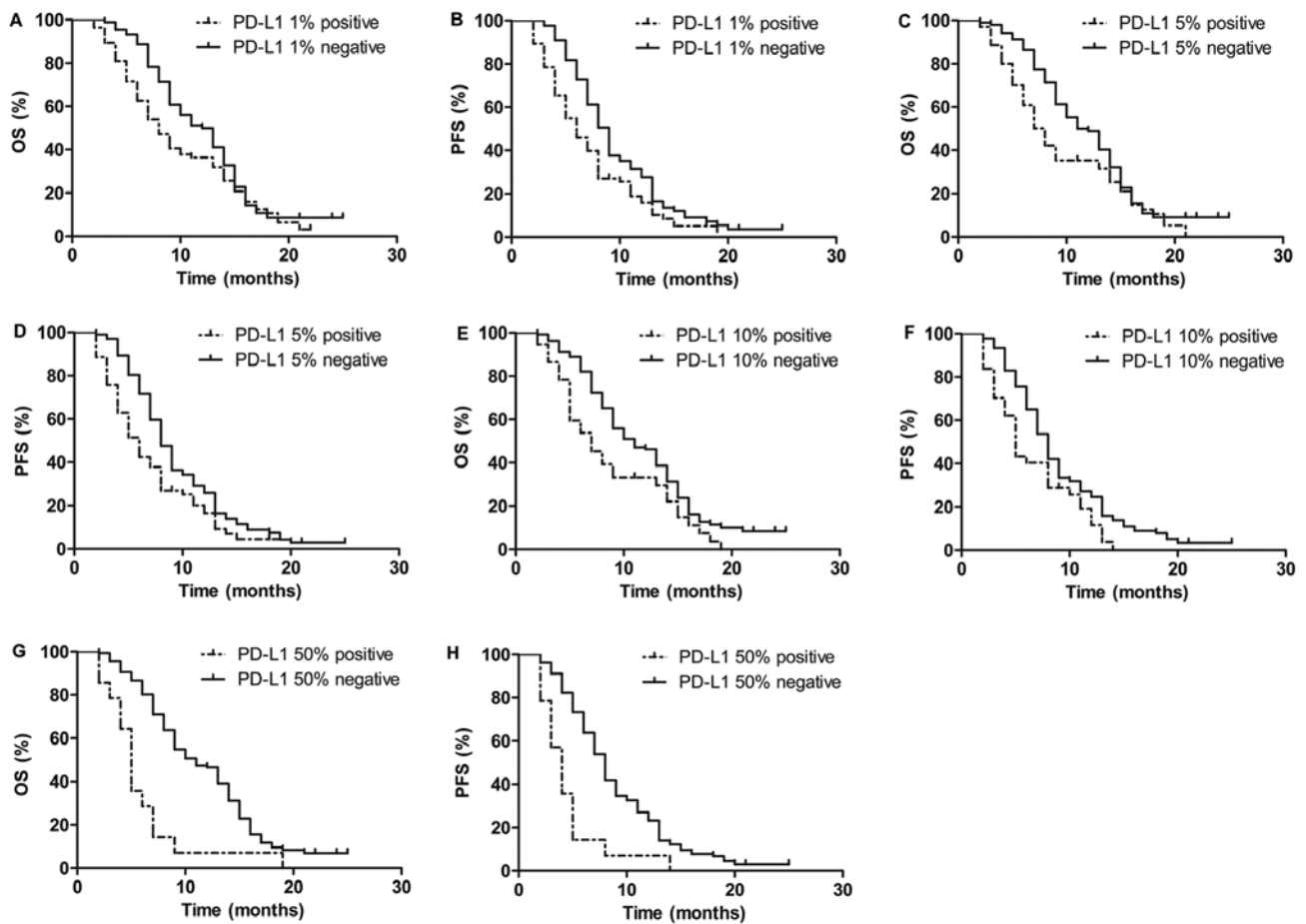
Figure 1. Kaplan-Meier survival curves of OS and PFS times according to PD-L1 expression in patients with advanced non-small cell lung cancer. OS time for the (A) 1%, (C) 5%, (E) 10% and (G) 50% cut-off. PFS time for the (B) 1%, (D) 5%, (F) 10% and (H) 50% cut-off. OS, overall survival; PFS, progression-free survival; PD-L1, programmed death ligand-1.
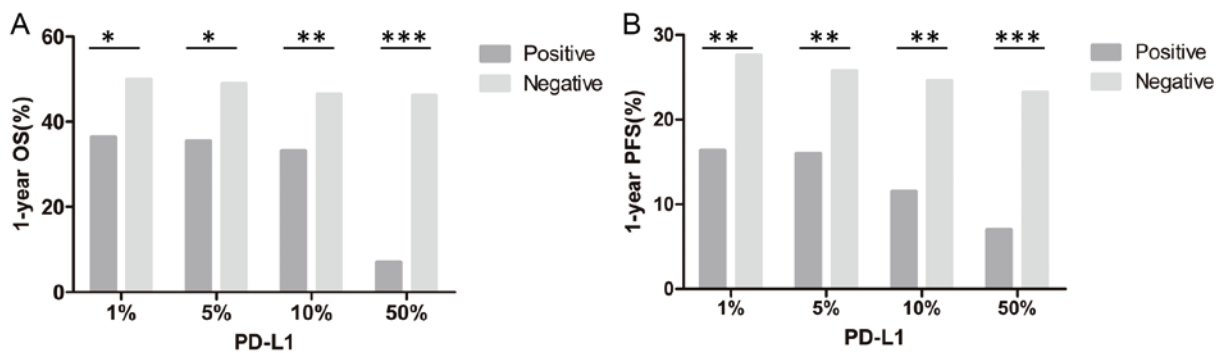

Figure 2. Comparison of one-year OS and PFS times according to PD-L1 expression. (A) Comparison of one-year OS time between patients with positive PD-L1 expression and patients with negative PD-L1 expression (defined as positive, ≥cut-off; and negative, <cut-off). (B) Comparison of one-year PFS time between patients with positive PD-L1 expression and patients with negative PD-L1 expression. *$P<0.05$; **$P<0.01$; ***$P<0.001$. OS, overall survival; PFS, progression-free survival; PD-L1, programmed death ligand-1.

These results indicated that positive PD-L1 (1% cut-off) expression predicted a shorter survival time.

Prognostic significance of PD-L1 expression cut-off values. To investigate the cut-off value of PD-L1 expression, the association between survival and PD-L1 expression at 1, 5, 10 and 50% levels was investigated. Univariate survival analysis revealed that positive PD-L1 expression for 1, 5, 10

and 50% cut-off values was significantly associated with shorter OS and PFS times (OS time: $P=0.0481, 0.0212, 0.0068$ and <0.0001, respectively; PFS time: $P=0.0035, 0.0044,$ 0.0051 and <0.0001, respectively; Fig. 1 and Table III). All parameters that were statistically significant according to the univariate analysis were included in the multivariate analysis, which demonstrated that positive PD-L1 (50% cut-off) expression was significantly associated with shorter survival time

Table II. Univariate and multivariate analysis of the association between clinicopathological characteristics and survival.

| | | OS | | | PFS | | |
| | | Univariate analysis | Multivariate analysis | | Univariate analysis | Multivariate analysis | |
Variable	Category	P-value	HR (95% CI)	P-value	P-value	HR (95% CI)	P-value
Gender	Male	0.094			0.681		
Age	≥60	<0.0001	1.537 (1.067-2.213)	0.021	0.0010	1.298 (0.915-1.840)	0.144
Histology	Non-adenocarcinoma	0.5878			0.5422		
Stage	IV	<0.0001	1.700 (1.187-2.434)	0.004	<0.0001	1.860 (1.299-2.665)	0.001
ECOG PS	2	0.0050	1.346 (0.937-1.935)	0.108	0.0278	1.390 (0.971-1.988)	0.072
Smoking status	Non-smoker	0.8710			0.7849		
CRP	Elevated	0.6622			0.1117		
PD-L1 (1% cut-off)	Positive	0.0481	1.125 (0.783-1.617)	0.524	0.0035	1.266 (0.884-1.813)	0.199

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PD-L1, programmed death ligand-1; ECOG, European Cooperative Oncology Group; PS, performance status; CRP, C-reactive protein.
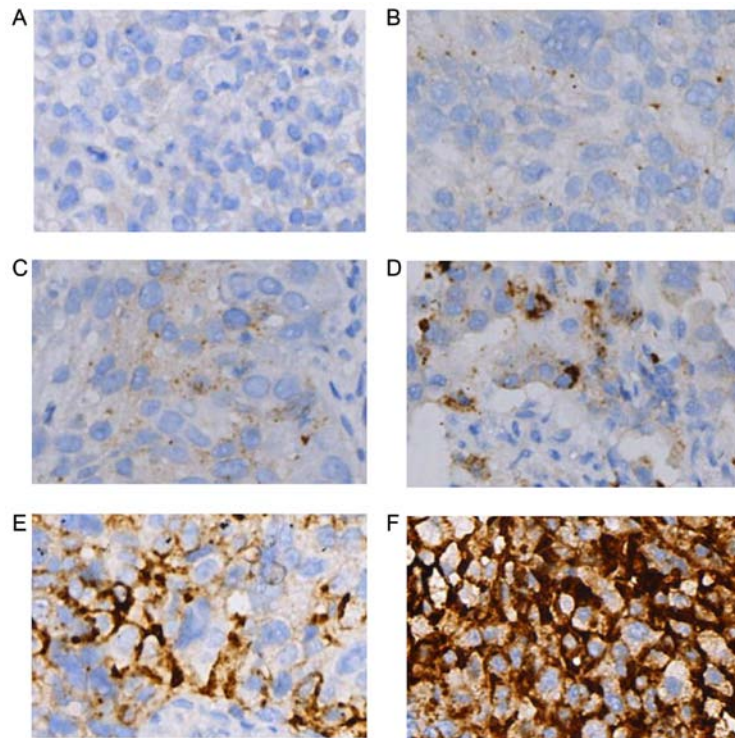

Figure 3. Representative immunohistochemical staining images of tumor biopsies with PD-L1. (A) Negative control. (B) 1% positive. (C) 5% positive. (D) 10% positive. (E) 50% positive. (F) 100% positive. All images were captured at x20 magnification.

(OS time, P=0.001; HR=2.768, 95% CI, 1.551-4.940; PFS, P=0.002; HR=2.537, 95% CI, 1.423-4.524; Table III). These results suggested that positive PD-L1 (50% cut-off) expression was an independent predictor of poor prognosis for patients with advanced NSCLC treated with gemcitabine plus cisplatin.

Discussion

Increased PD-L1 expression was observed in NSCLC and neuroendocrine tumors of the lung (23,26), suggesting that patients with NSCLC may benefit from PD-L1 inhibitors. The results obtained in the current study revealed that high PD-L1 expression was observed in patients with advanced NSCLC, compared with normal lung tissue.

There is no universal method for PD-L1 immunostaining and antibodies used in different studies vary (21). The definition of a positive PD-L1 test result differs depending on which biomarker assay is used. Four immunohisto-chemical assays are approved by the US Food and Drug Administration as diagnostic tests in advanced NSCLC

Table III. Survival and PD-L1 expression level.

PD-L1 cut-off	OS						PFS					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI
≥1%	0.0481	1.434	1.003-2.051	0.524	1.125	0.783-1.617	0.0035	1.691	1.188-2.406	0.199	1.266	0.884-1.813
≥5%	0.0212	1.558	1.069-2.272	0.807	1.049	0.716-1.537	0.0044	1.721	1.185-2.499	0.469	1.147	0.791-1.665
≥10%	0.0068	1.952	1.202-3.171	0.084	1.421	0.954-2.117	0.0051	2.001	1.232-3.249	0.073	1.439	0.937-2.141
≥50%	<0.0001	7.768	3.031-19.91	0.001	2.768	1.551-4.940	<0.0001	8.123	3.137-21.04	0.002	2.537	1.423-4.524

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PD-L1, programmed death ligand-1.

including PD-L1 IHC 22C3 pharmDx (Dako Omnis), PD-L1 IHC 28-8 pharmDx, VENTANA PD-L1 (SP142) assay and VENTANA PD-L1 (SP263) assay (23). The comparative accuracy and utility between the immunohistochemical assay used in the current study and the aforementioned assays have been verified (27).

Previously published studies reported conflicting results on the association between PD-L1 expression and age, gender, histology, ECOG PS, smoking status and cancer stage (28). The present study revealed a significant association between PD-L1 expression and cancer stage and pretreatment serum CRP level. Expression of the PD-L1 gene may be controlled by inflammatory signaling (29). Expression of the PD-L1 was regulated by interferon-γ through the Janus kinase/signal transducer of activation pathway in NSCLC (30). A recently published study demonstrated that the serum CRP level was associated with PD-L1 expression in patients with NSCLC (29). The inflammatory markers, CRP and neutrophil-lymphocyte ratio, were predictive for the efficacy of nivolumab in patients with NSCLC (29). The results obtained in the current study indicated that pretreatment elevated serum CRP level was associated with positive PD-L1 expression. However, future studies are required to verify this association.

PD-L1 is a co-regulatory molecule that can be expressed on tumor cells and its expression suppress T-cell activity (31). Compared with PD-L2, PD-L1 is the dominant inhibitory ligand of PD-1 on T cells (25). Immune checkpoints inhibitors which target the PD-L1/PD-1 pathway (including pembrolizumab and nivolumab which target PD-1, and atezolizumab and duralumab which target PD-L1) are a promising treatment method for patients with advanced NSCLC (8,24,32-36). Thus, the identification of potential biomarkers may guide the choice of inhibitor used.

Currently known biomarkers include the ALK receptor tyrosine kinase fusion oncogene, ROS proto-oncogene 1, receptor tyrosine kinase gene rearrangements, sensitizing EGFR gene mutations and B-Raf proto-oncogene, serine/threonine kinase V600E point mutations (37,38). Activation of the immune checkpoint pathways is one of the main mechanisms underlying tumor development (39). PD-L1 expression on tumor cells negatively regulates the immune response and may lead to cancer progression (31). Although PD-L1 expression may not be an optimal biomarker (40,41), PD-L1 expression is currently used to assess whether patients with NSCLC are candidates for treatment with pembrolizumab (42,43). Identification of PD-L1 expression using immunohistochemical methods may aid in treatment selection (21). Previously published studies have reported conflicting results on whether positive PD-L1 expression may be a predictor of treatment response (44,45). The definition of positive PD-L1 expression is variable in different studies and it may impact the results (21). Future studies are required to define the prognostic role and cut-off value of PD-L1. A previous study reported that a PD-L1 expression level of ≥50% was a positive test result for first-line pembrolizumab therapy (8). In the present study, PD-L1 expression predicted poor clinical outcome at the prespecified PD-L1 expression levels of 1, 5, 10 and 50% and results obtained suggested that the optimal cut-off value may be 50%. The current study was limited by the small sample size and retrospective analysis. Therefore, a future prospective

study with a larger sample is required to validate the results obtained.

In conclusion, the present study demonstrated that positive PD-L1 expression was associated with poor outcomes in patients with advanced NSCLC treated with gemcitabine plus cisplatin.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YJQ analyzed and interpreted the data of the study, and wrote the manuscript. MZ, JJ and YRQ participated in the design of this research. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (Zhengzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin 69: 7-34, 2019.
2. Goldstraw P, Ball D, Jett JR, Le Chevalier T, Lim E, Nicholson AG and Shepherd FA: Non-small-cell lung cancer. Lancet 378: 1727-1740, 2011.
3. Hellmann MD, Li BT, Chaft JE and Kris MG: Chemotherapy remains an essential element of personalized care for persons with lung cancers. Ann Oncol 27: 1829-1835, 2016.
4. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, Serwatowski P, Gatzemeier U, Digumarti R, Zukin M, et al: Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 26: 3543-3551, 2008.
5. Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, Nishiwaki Y, Saijo N, Ariyoshi Y and Fukuoka M: Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol 18: 317-323, 2007.
6. Danson S, Middleton MR, O'Byrne KJ, Clemons M, Ranson M, Hassan J, Anderson H, Burt PA, Fairve-Finn C, Stout R, et al: Phase III trial of gemcitabine and carboplatin versus mitomycin, ifosfamide, and cisplatin or mitomycin, vinblastine, and cisplatin in patients with advanced nonsmall cell lung carcinoma. Cancer 98: 542-553, 2003.
7. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, et al: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12: 735-742, 2011.
8. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, et al: Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 387: 1540-1550, 2016.
9. Cui S, Su X, Dong L, Qian J, Ye L, Zhang T, Fu H, Han H, Huang J, Yao Y, et al: Programmed cell death ligand 1 protein levels predicted survival of non-small cell lung cancer. J Cancer 8: 4075-4082, 2017.
10. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, Matsumoto K, Takayama K, Takamori S, Kage M, et al: Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. Ann Oncol 25: 1935-1940, 2014.
11. Lin C, Chen X, Li M, Liu J, Qi X, Yang W, Zhang H, Cai Z, Dai Y and Ouyang X: Programmed death-ligand 1 expression predicts tyrosine kinase inhibitor response and better prognosis in a cohort of patients with epidermal growth factor receptor mutation-positive lung adenocarcinoma. Clin Lung Cancer 16: e25-e35, 2015.
12. Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z and Huang JA: The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol 49: 1360-1368, 2016.
13. Lin K, Cheng J, Yang T, Li Y and Zhu B: EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-kappaB. Biochem Biophys Res Commun 463: 95-101, 2015.
14. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, Mikse OR, Cherniack AD, Beauchamp EM, Pugh TJ, et al: Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 3: 1355-1363, 2013.
15. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, Zhang Y, He X, Zhou T, Qin T, et al: Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: Implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. J Thorac Oncol 10: 910-923, 2015.
16. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R and Johnson DH: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355: 2542-2550, 2006.
17. Fossella F, Pereira JR, von Pawel J, Pluzanska A, Gorbounova V, Kaukel E, Mattson KV, Ramlau R, Szczesna A, Fidias P, et al: Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: The TAX 326 study group. J Clin Oncol 21: 3016-3024, 2003.
18. Osmani L, Askin F, Gabrielson E and Li QK: Current WHO guidelines and the critical role of immunohistochemical markers in the subclassification of non-small cell lung carcinoma (NSCLC): Moving from targeted therapy to immunotherapy. Semin Cancer Biol 52: 103-109, 2018.
19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
20. Sun C, Mezzadra R and Schumacher TN: Regulation and function of the PD-L1 checkpoint. Immunity 48: 434-452, 2018.
21. Thunnissen E, de Langen AJ and Smit EF: PD-L1 IHC in NSCLC with a global and methodological perspective. Lung Cancer 113: 102-105, 2017.
22. Ramos-Vara JA and Miller MA: Comparison of two polymer-based immunohistochemical detection systems: ENVISION+ and ImmPRESS. J Microsc 224: 135-139, 2006.
23. Brody R, Zhang Y, Ballas M, Siddiqui MK, Gupta P, Barker C, Midha A and Walker J: PD-L1 expression in advanced NSCLC: Insights into risk stratification and treatment selection from a systematic literature review. Lung Cancer 112: 200-215, 2017.

24. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, *et al*: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1627-1639, 2015.
25. Shen X and Zhao B: Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: Meta-analysis. BMJ 362: k3529, 2018.
26. Takada K, Okamoto T, Toyokawa G, Kozuma Y, Matsubara T, Haratake N, Akamine T, Takamori S, Katsura M, Shoji F, *et al*: The expression of PD-L1 protein as a prognostic factor in lung squamous cell carcinoma. Lung Cancer 104: 7-15, 2017.
27. Kerr KM, Tsao MS, Nicholson AG, Yatabe Y, Wistuba II, Hirsch FR and IASLC Pathology Committee: Programmed death-ligand 1 immunohistochemistry in lung cancer: In what state is this art? J Thorac Oncol 10: 985-989, 2015.
28. Chang YL, Yang CY, Huang YL, Wu CT and Yang PC: High PD-L1 expression is associated with stage IV disease and poorer overall survival in 186 cases of small cell lung cancers. Oncotarget 8: 18021-18030, 2017.
29. Akamine T, Takada K, Toyokawa G, Kinoshita F, Matsubara T, Kozuma Y, Haratake N, Takamori S, Hirai F, Tagawa T, *et al*: Association of preoperative serum CRP with PD-L1 expression in 508 patients with non-small cell lung cancer: A comprehensive analysis of systemic inflammatory markers. Surg Oncol 27: 88-94, 2018.
30. Ikeda S, Okamoto T, Okano S, Umemoto Y, Tagawa T, Morodomi Y, Kohno M, Shimamatsu S, Kitahara H, Suzuki Y, *et al*: PD-L1 is upregulated by simultaneous amplification of the PD-L1 and JAK2 genes in non-small cell lung cancer. J Thorac Oncol 11: 62-71, 2016.
31. Boussiotis VA: Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med 375: 1767-1778, 2016.
32. Horn L, Spigel DR, Vokes EE, Holgado E, Ready N, Steins M, Poddubskaya E, Borghaei H, Felip E, Paz-Ares L, *et al*: Nivolumab versus docetaxel in previously treated patients with advanced non-small-cell lung cancer: Two-year outcomes from two randomized, open-label, phase III trials (checkmate 017 and checkmate 057). J Clin Oncol 35: 3924-3933, 2017.
33. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, Gottfried M, Peled N, Tafreshi A, Cuffe S, *et al*: Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375: 1823-1833, 2016.
34. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, *et al*: Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet 387: 1837-1846, 2016.
35. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, *et al*: Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373: 123-135, 2015.
36. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Yokoi T, Chiappori A, Lee KH, de Wit M, *et al*: Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. N Engl J Med 377: 1919-1929, 2017.
37. Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Janne PA, Joshi VA, McCollum D, Evans TL, Muzikansky A, *et al*: First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol 26: 2442-2449, 2008.
38. Miller VA, Riely GJ, Zakowski MF, Li AR, Patel JD, Heelan RT, Kris MG, Sandler AB, Carbone DP, Tsao A, *et al*: Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. J Clin Oncol 26: 1472-1478, 2008.
39. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264, 2012.
40. Petrelli F, Maltese M, Tomasello G, Conti B, Borgonovo K, Cabiddu M, Ghilardi M, Ghidini M, Passalacqua R, Barni S and Brighenti M: Clinical and molecular predictors of PD-L1 expression in non-small-cell lung cancer: Systematic Clin Lung Cancer 19: 315-322, 2018.
41. Martinez P, Peters S, Stammers T and Soria JC: Immunotherapy for the first-line treatment of patients with metastatic non-small cell lung cancer. Clin Cancer Res 2019.
42. Kerr KM and Nicolson MC: Non-small cell lung cancer, PD-L1, and the pathologist. Arch Pathol Lab Med 140: 249-254, 2016.
43. Kerr KM and Hirsch FR: Programmed death ligand-1 immunohistochemistry: Friend or foe? Arch Pathol Lab Med 140: 326-331, 2016.
44. Zhou Y, Chen C, Zhang X, Fu S, Xue C, Ma Y, Fang W, Yang Y, Hou X, Huang Y, *et al*: Immune-checkpoint inhibitor plus chemotherapy versus conventional chemotherapy for first-line treatment in advanced non-small cell lung carcinoma: A systematic review and meta-analysis. J Immunother Cancer 6: 155, 2018.
45. Bethmann D, Feng Z and Fox BA: Immunoprofiling as a predictor of patient's response to cancer therapy-promises and challenges. Curr Opin Immunol 45: 60-72, 2017.