Original Article

Prognostic and predictive significance of soluble programmed death ligand 1 in bronchoalveolar lavage fluid in stage IV non-small cell lung cancer

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Background: Patients with non-small cell lung cancer (NSCLC) have been shown to exhibit elevated levels of soluble programmed death-ligand 1 (sPD-L1) in the blood, associated with poor survival in NSCLC. The bronchoalveolar lavage fluid (BALF) composition reflects the tumor microenvironment of lung cancer. In this study, we investigated sPD-L1 levels in BALF and its role as a prognostic and predictive marker in patients with stage IV NSCLC.

Methods: We prospectively obtained BALF from lung cancer patients who underwent bronchoscopy between January 2020 and September 2022 at Chungnam National University Hospital (CNUH). Finally, 94 NSCLC stage IV patients were included in this study. Soluble PD-L1 levels in BALF were measured using a human PD-L1 Quantikine ELISA kit.

Results: The correlation between PD-L1 expression in tumor cells and sPD-L1 in BALF was weakly positive (rho =0.314, P=0.002). The median overall survival (OS) of the low sPD-L1 in BALF group was 16.47 months [95% confidence interval (CI): 11.15–21.79 months], which is significantly longer than 8.87 months (95% CI: 0.0–19.88 months, P=0.001) in the high sPD-L1 in BALF group. In 64 patients treated with or without immune checkpoint inhibitors (ICIs), sPD-L1 in BALF was significantly associated with progression-free survival (PFS) and OS. In the subgroup analysis of 31 patients treated with ICI, the objective response rate (ORR) in the low sPD-L1 BALF group was significantly higher than in high sPD-L1 in BALF group (ORR: 60.9% *vs.* 12.5%, P=0.02).

Conclusions: Soluble PD-L1 in BALF is a potential prognostic indicator for patients with stage IV NSCLC and a predictive marker for ICI treatment response.

Keywords: Non-small cell lung cancer (NSCLC); soluble programmed death-ligand 1 (sPD-L1); biomarker; bronchoalveolar lavage fluid (BALF)

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Introduction

Although the development of various drugs, such as targeted agents and immune checkpoint inhibitors (ICIs), have contributed to the survival rate improvement of patients with advanced-stage cancer, lung cancer remains the leading cause of cancer-related mortality worldwide (1). The evolution of various anticancer agents has resulted in the development of more diverse treatment options for patients with lung cancer; however, treatment responses vary widely. Numerous studies on the prognosis of lung cancer and predicting the efficacy of existing treatments have been conducted. In patients receiving ICIs, which have recently become the standard treatment for advanced lung cancer, programmed death ligand 1 (PD-L1) expression in tumor cells is currently the only predictive marker used in clinical practice (2-4).

PD-L1 is a transmembrane protein that binds to its receptor programmed death-1 (PD-1) expressed by T cells and other immune cells (5,6). The interaction between PD-L1 and PD-1 suppresses T cell function and blocks antitumor immune responses, leading to tumor progression (7). ICIs block the interaction between PD-L1 and PD-1, enhancing anti-tumor immunity (8-11). However, PD-L1 expression in tumor cells has not been standardized as a prognostic marker for lung cancer. Some studies have shown that high PD-L1 expression in tumor cells is associated with poor prognosis in non-small cell lung cancer (NSCLC) (12,13). Other studies have suggested that the relationship between PD-L1 expression in tumor cells and prognosis differs depending on the cancer stage (14). PD-L1 expression in tumor cells is determined using tissue biopsy, which is an invasive procedure. Moreover, it is sometimes difficult to obtain sufficient tissue samples to detect PD-L1 expression in tumor cells, depending on the patient's condition, tumor location, and tumor size (15). Additionally, PD-L1 expression in tumor cells differs depending on tumor size and heterogeneity (16,17).

Due to the high variability and difficulty in obtaining sufficient tissue samples, circulating blood-based biomarkers are being investigated to predict the response to ICIs and prognosis in lung cancer patients. Unlike PD-L1 expression in tumor cells, blood-based biomarkers offer advantages in terms of ease of sampling, repeatability, and reduced invasiveness (18). Circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and blood tumor mutational burden (bTMB) are some common blood-based biomarkers (19). Some studies have shown that soluble PD-L1 (sPD-L1) levels in the blood are elevated in patients with cancer compared with those in healthy controls (20-22). Other studies have reported that high levels of sPD-L1 in the blood are associated with poor survival and response to ICI treatment in lung cancer (23,24). However, blood-based biomarkers have some limitations in patients with early-stage cancer that exhibit a low tumor burden (25-27).

Compared to tissue or blood, bronchoalveolar lavage fluid (BALF) demonstrates an enhanced ability for the preservation of tumor-related DNA, improved sensitivity for detecting tumor-derived mutations, and a high potential to reflect intra-tumoral heterogeneity in lung cancer (27,28). BALF samples can reflect tumor microenvironments and are more sensitive than blood or other fluid samples as bronchoscopy operators proximally target pulmonary lesions to obtain BALF samples (29). Using saline, large amounts of tumor-related BALF samples can be obtained (27). In addition, BALF samples could enable the analysis of the cellular and non-cellular contents of the bronchial and alveolar spaces, serving as excellent markers of the tumor microenvironment (30). Therefore, BALF may be more optimal for assessing immune profiles in the lung tumor microenvironment and predicting the efficacy of ICIs (31).

Accordingly, in the present study, we investigated sPD-L1 levels in BALF and their role as a prognostic and

Highlight box

Key findings

- Soluble programmed death-ligand 1 (sPD-L1) in bronchoalveolar lavage fluid (BALF) was a significant prognostic factor affecting both progression-free survival (PFS) and overall survival (OS) in patients with non-small cell lung cancer (NSCLC) who received treatment. sPD-L1 in BALF can serve as a predictive and prognostic marker for the efficacy and outcomes of immune checkpoint inhibitor (ICI) treatment.

What is known and what is new?

- High levels of sPD-L1 in the blood are associated with poor survival and response to ICI treatment in lung cancer.
- We demonstrated a positive correlation between programmed death-ligand 1 (PD-L1) expression in tumor cells and sPD-L1 levels in BALF. Patients with high sPD-L1 in BALF showed a significantly lower response to ICI treatment.

What is the implication, and what should change now?

- We demonstrated the potential of sPD-L1 in BALF as a promising biomarker in NSCLC prognosis and ICI efficacy. Our results suggest that ICI combination therapy using cytotoxic agents may be better than ICI monotherapy when the levels of both PD-L1 in tumor cells and sPD-L1 in BALF are high.

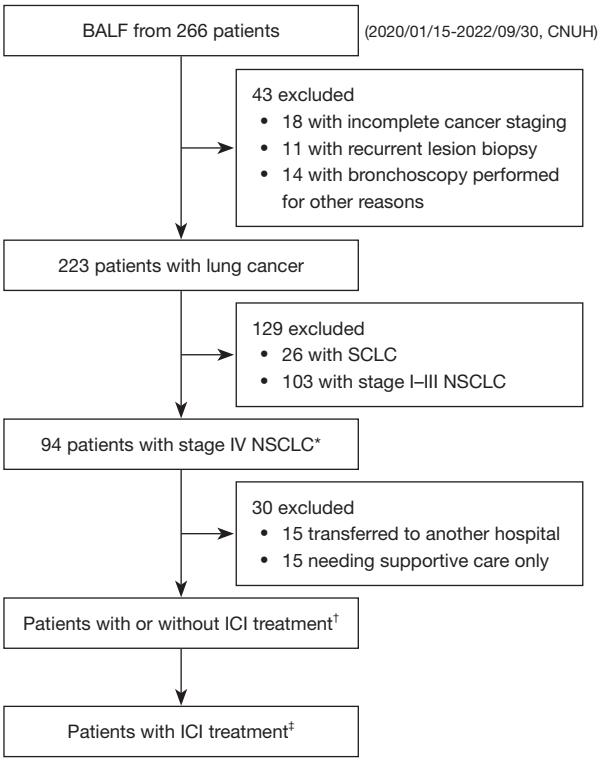

Figure 1 Flow diagram of the patients included in the study. *, we analyzed the overall survival of these patients (N=94); †, subgroup analysis was performed for patients with or without ICI treatment (N=64); ‡, subgroup analysis was performed for patients who received ICI treatment (N=31). BALF, bronchoalveolar lavage fluid; CNUH, Chungnam National University Hospital; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; ICI, immune checkpoint inhibitor.

predictive marker of ICI treatment response in patients with advanced lung cancer. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-392/rc).

Methods

Study design and participants

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Institutional Review Board of Chungnam National University Hospital (IRB No. 2018-01-059). All study samples were obtained after acquiring the study participants' written informed consent. We prospectively obtained BALF from lung cancer patients who underwent bronchoscopy between clinical need from January 2020 and September 2022 at Chungnam National University Hospital (CNUH). A flowchart depicting the study population is shown in *Figure 1*. A total of 266 BALF samples were collected. Of these, 43 samples were excluded, including 11 BALF samples obtained from recurrent lesions, 14 samples collected for other reasons (e.g., pneumonia), and 18 BALF samples with incomplete lung cancer staging. A total of 223 samples from patients with lung cancer with complete cancer staging were included. These samples were obtained during the initial bronchoscopy conducted for the diagnosis of lung cancer without prior treatment for lung cancer. The stage and histology of lung cancer are powerful factors affecting overall survival (OS); therefore, in the present study, we included 94 patients initially diagnosed with stage IV NSCLC. We performed a subgroup analysis on 64 patients treated with or without ICIs at our hospital, and among them, we also performed a subgroup analysis on 31 patients who received ICI treatment.

Soluble PD-L1 in BALF

Bronchoscopy was performed using a flexible bronchoscope (Olympus, Tokyo, Japan). BALF was obtained in accordance with official recommendations (32) by the instillation of isotonic saline solution in a total of 100 mL into the wedged pulmonary segment that showed the most prominent finding on chest computed tomography (CT). Through gentle suction of the injected solution, the BALF was collected and pooled in a collection tube. The BALF was deemed to be of sufficient quality when the volume, and the number of cells per mL, of BALF were >30 mL and >60,000, respectively, and there was no increase in the number of epithelial cells. For processing the samples, BALF was filter through a 100 μm cell strainer (SPL) to remove clumps and debris and centrifuged at 2,000 rpm for 10 min at room temperature. Separated cell-pellets were treated with ammonium-chloride-potassium lysis buffer (BioLegend) to lyse the red blood cells (RBCs). After separating cell-pellets, the supernatant was aliquoted and stored at −80 ℃ until analysis. Thawed samples were used for measuring sPD-L1 levels in BALF using a human PD-L1 Quantikine ELISA kit (DB7H10; R&D Systems, Minneapolis, MN, USA).

PD-L1 expression in tumor tissue

PD-L1 expression in tumor cells was evaluated using the

PD-L1 IHC 22C3 pharmDx test (Agilent Technologies, Santa Clara, CA, USA) on the Dako Autostainer and the PD-L1 IHC SP263 test on the Ventana BenchMark platform. The percentage of tumor cells showing immunoreactivity was quantified according to the respective manufacturer's guidelines. Positive expression was defined by membrane staining of cancer cells, with cytoplasmic reactions disregarded. PD-L1 protein expression was assessed based on the proportion of viable tumor cells displaying partial or complete membrane staining [tumor proportion score (TPS)] (33). The study categorized PD-L1 expression into three groups based on TPS cut-offs: no expression (<1%), low expression (1–49%), and high expression (≥50%). Classification of subgroups according to PD-L1 expression relied on the 22C3 pharmDx assay results, with patients lacking 22C3 pharmDx results categorized based on SP263 assay results, as both assays are interchangeable (33).

Treatment response and survival analysis

Treatment response was assessed using CT. Treatment response was assessed based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) was defined as the time from the date of the first drug administration to the date of documented progression or death from any cause. OS was defined as the period from the date of first drug administration to the date of death or the last day of follow-up.

Statistical analysis

The demographic and clinical parameters were analyzed using descriptive statistics. The correlation between PD-L1 expression in tumor cells and sPD-L1 in BALF was analyzed using the Student's *t*-test, analysis of variance (ANOVA), and Spearman's rho method because the data did not follow a normal distribution. Conventional receiver operating characteristic curves were generated to calculate the sensitivity and specificity of biomarkers, conventional receiver operating characteristic (ROC) curves were generated. The optimal cut-off value was determined as the point at which the Youden index was maximized by the ROC curve. The relationships between sPD-L1 in BALF and clinical variables were analyzed using Pearson's chi-square test. Kaplan-Meier survival analyses and Cox proportional hazard models were used to analyze PFS and OS. Statistical significance was set at P<0.05. SPSS version 26 (IBM Corp., Armonk, NY, USA), Excel 2016 (Microsoft, Redmond, WA, USA), and PRISM version 9 (GraphPad Software Inc., Boston, MA, USA) were used for all statistical analyses and graphics generation.

Results

Soluble PD-L1 levels in BALF in patients with stage IV NSCLC

The trends of the levels of sPD-L1 in BALF are shown in *Figure 2A*. The median value of sPD-L1 in BALF was 1.45 pg/mL and the mean value was 10.12 pg/mL. We investigated the correlation between PD-L1 expression in tumor cells and sPD-L1 levels in BALF. A Spearman's rho value of 0.314 suggested that the correlation between these two factors was weakly positive (P=0.002; *Figure 2B*). ANOVA revealed that the mean value of sPD-L1 in BALF was significantly different (P=0.02) in the no PD-L1 expression group (2.5 pg/mL), low PD-L1 expression group (8.78 pg/mL), and high PD-L1 expression group (24.31 pg/mL) (*Figure 2C*). In addition, the mean value of PD-L1 expression in tumor cells was significantly different (P=0.01) in patients in the low sPD-L1 in BALF (18.2%) and high sPD-L1 in BALF (44.86%) groups, based on the cutoff value determined by ROC curve analysis (7.35 pg/mL) (*Figure 2D*).

Patient baseline characteristics according to sPD-L1 in BALF

Based on the cutoff value for sPD-L1 in BALF determined by ROC curve analysis (7.35 pg/mL), all patients were classified into the low sPD-L1 in BALF group (71 patients) and high sPD-L1 in BALF group (23 patients). The clinical parameters of the two groups are summarized in *Table 1*. There were no significant differences between the two groups in terms of sex, smoking status, Eastern Cooperative Oncology Group Performance Score (ECOG PS), histology, comorbidities, ICI use, targeted mutations, brain metastasis, or NLR. Mean age and PD-L1 expression in tumor cells were significantly different between the two groups.

OS according to sPD-L1 in BALF

To investigate the prognostic role of sPD-L1 in BALF in patients with NSCLC, we used an ROC curve to determine

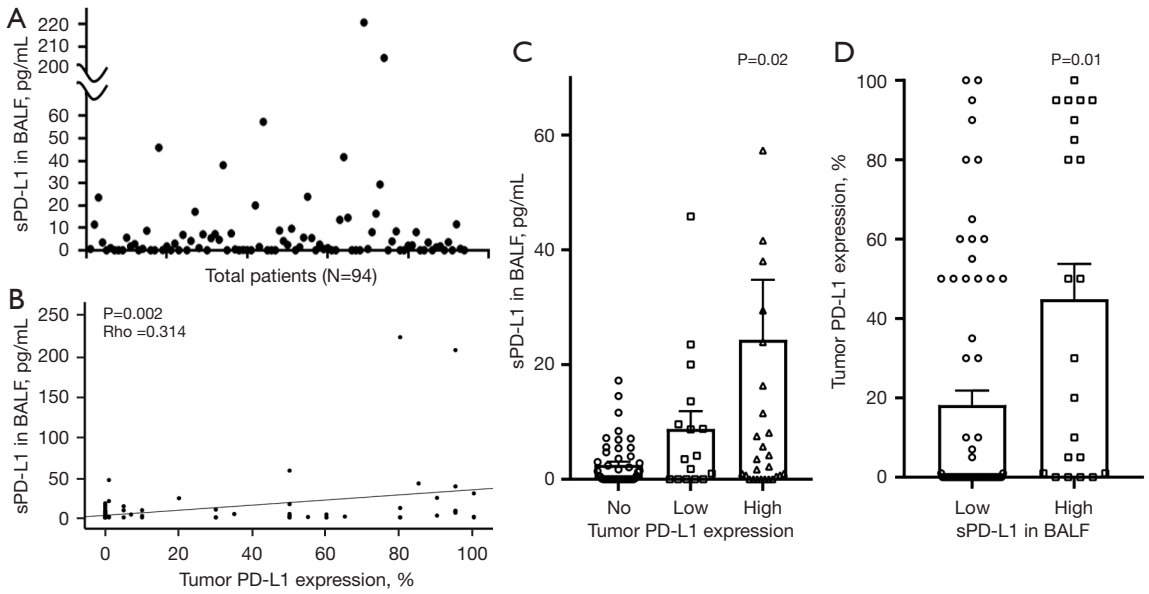

Figure 2 Distribution and correlation analysis of PD-L1 and sPD-L1 expression in BALF. (A) Scatterplot showing sPD-L1 levels in BALF in all patients (N=94). (B) Graph showing weakly positive correlation between PD-L1 expression in tumor cells and sPD-L1 levels in BALF (P=0.002, Spearman's rho value =0.314). (C) Mean sPD-L1 in BALF based on tumor PD-L1 expression. (D) Mean tumor PD-L1 expression based on sPD-L1 in BALF. BALF, bronchoalveolar lavage fluid; PD-L1, programmed death-ligand 1; sPD-L1, soluble programmed death-ligand 1.

Table 1 Baseline characteristics of total patients (N=94)

Variables	Whole group	Low BALF sPDL1 (N=71)	High BALF sPDL1 (N=23)	P value
Age (years)	72.11±9.4	70.99±9.7	75.77±7.22	0.04*
<65	18 (19.1)	17 (23.9)	1 (4.3)	
≥65	76 (80.9)	54 (76.1)	22 (95.7)	
Sex				0.95
Male	69 (73.4)	52 (73.2)	17 (73.9)	
Female	25 (26.6)	19 (26.8)	6 (26.1)	
Smoking status				0.54
Never	25 (26.6)	20 (28.2)	5 (21.7)	
Former	37 (39.4)	29 (40.8)	8 (34.8)	
Current	32 (34.0)	22 (31.0)	10 (43.5)	
Smoking history (packs × years)	31.54±25.97	31.12±26.54	24.55±7.22	0.86
ECOG PS				0.66
0	18 (19.1)	14 (19.7)	4 (17.4)	
1	56 (59.6)	44 (62.0)	12 (52.2)	
2	15 (16.0)	10 (14.1)	5 (21.7)	
3	5 (5.3)	3 (4.2)	2 (8.7)	

Table 1 *(continued)*

Table 1 *(continued)*

Variables	Whole group	Low BALF sPDL1 (N=71)	High BALF sPDL1 (N=23)	P value
Histology				0.32
AD	61 (64.9)	49 (69.0)	12 (52.2)	
SCC	28 (29.8)	19 (26.8)	9 (39.1)	
Other	5 (5.3)	3 (4.2)	2 (8.7)	
Comorbidity (COPD)	41 (43.6)	31 (43.7)	10 (43.5)	0.75
ICI use				0.83
Yes	31 (33.0)	23 (32.4)	8 (34.8)	
No	63 (67.0)	48 (67.6)	15 (65.2)	
PD-L1 expression in tumor cell				0.001*
No	48 (51.1)	44 (62.0)	4 (17.4)	
Low	16 (17.0)	9 (12.7)	7 (30.4)	
High	28 (29.8)	17 (23.9)	11 (47.8)	
N/A	2 (2.1)	1 (1.4)	1 (4.3)	
EGFR				0.14
Wild-type	67 (71.3)	48 (67.6)	19 (82.6)	
Mutant	23 (24.5)	20 (28.2)	3 (13.0)	
N/A	4 (4.3)	3 (4.2)	1 (4.3)	
ALK rearrangement				0.91
Negative	77 (81.9)	60 (84.5)	17 (73.9)	
Positive	5 (5.3)	4 (5.6)	1 (4.3)	
N/A	12 (12.8)	7 (9.9)	5 (21.7)	
BRAF				0.34
Wild-type	41 (43.6)	28 (39.4)	13 (56.5)	
Mutant	2 (2.1)	2 (2.8)	0 (0.0)	
N/A	51 (54.3)	41 (57.7)	10 (43.5)	
Brain metastasis				0.07
Yes	26 (27.7)	23 (32.4)	3 (13.0)	
No	68 (72.3)	48 (67.6)	20 (87.0)	
NLR				0.51
<2.3	26 (27.7)	21 (29.6)	5 (21.7)	
≥2.3	66 (70.2)	49 (69.0)	17 (73.9)	
N/A	2 (2.1)	1 (1.4)	1 (4.3)	
BAL cells (%)				
Neutrophil	54.0±28.3	51.5±30.0	67.3±11.3	0.32
Lymphocyte	9.6±9.3	9.5±9.8	10.5±6.8	0.85
Eosinophil	0.84±1.1	0.7±0.9	1.5±1.9	0.48
Macrophage	34.7±26.3	37.5±27.6	20.0±10.6	0.23

Data are presented as mean ± standard deviation or n (%). *, P<0.05. ECOG PS, Eastern Cooperative Oncology Group performance status; AD, adenocarcinoma; SCC, squamous cell carcinoma; COPD, chronic obstructive pulmonary disease; ICI, immune checkpoint inhibitor; PD-L1, programmed death ligand 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; BRAF, v-Raf, murine sarcoma viral oncogene homolog B; NLR, neutrophil-to-lymphocyte ratio; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; sPDL1, soluble programmed death ligand 1; N/A, not available.

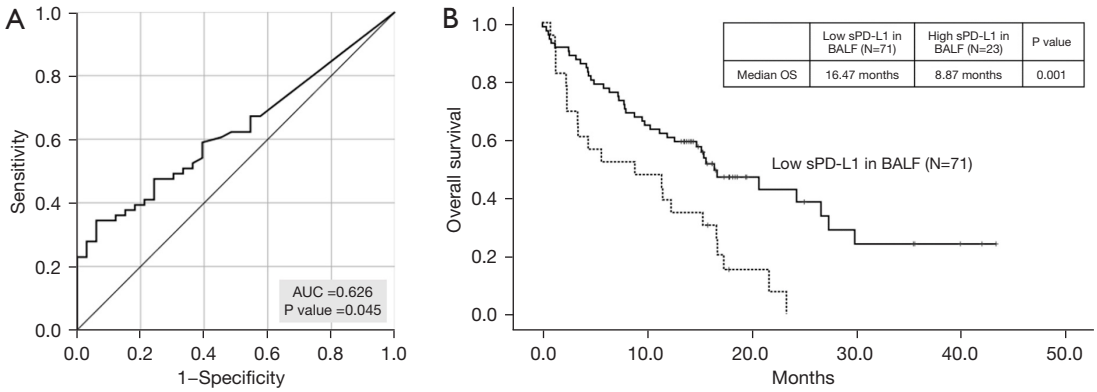

Figure 3 OS in all patients based on soluble PD-L1 levels in BALF (N=94). (A) ROC curve of sPD-L1 levels for survival rate in total patients (N=94) showing an AUC value of 0.626 (P=0.045) based on the 7.35 pg/mL cutoff. (B) Graph showing significantly longer median OS of the low sPD-L1 in BALF group (16.47 months; 95% CI: 11.15–21.79) than in the high sPD-L1 in BALF group (8.87 months; 95% CI: 0.0–19.88). AUC, area under the curve; OS, overall survival; sPD-L1, soluble programmed death-ligand 1; PD-L1, programmed death-ligand 1; BALF, bronchoalveolar lavage fluid; ROC, receiver operating characteristic; CI, confidence interval.

the optimal cut-off level of sPD-L1 in BALF for the prediction of survival (*Figure 3A*). We found that a cutoff value of 7.35 pg/mL distinguished best survival and yielded an AUC of 0.626 (P=0.045). Univariate and multivariate analyses were performed for prognostic factors, including sPD-L1 in BALF, age, sex, smoking status, ECOG PS, comorbidity, histology, PD-L1 expression in tumor cells, and brain metastasis (Table S1). Univariate analysis revealed that a longer OS was associated with never smoking, better performance status, no PD-L1 expression in tumor cells, and low sPD-L1 in BALF. Multivariate logistic regression analyses showed that only sPD-L1 in BALF was significantly associated with OS. The median OS of the low sPD-L1 in BALF group was 16.47 months [95% confidence interval (CI): 11.15–21.79 months], which is significantly longer than 8.87 months (95% CI: 0.0–19.88 months) in the high sPD-L1 in BALF group. The hazard ratio (HR) for OS was 2.349 in the high sPD-L1 in BALF group than in the low sPD-L1 in BALF group. In the survival graph, patients with high sPD-L1 in BALF exhibited poorer OS outcomes than patients with low sPD-L1 in BALF (P=0.001, *Figure 3B*).

Survival analysis in patients who received first-line treatment

Of the 94 patients finally enrolled in the study, we separately analyzed 64 patients who received first-line treatment in our hospital. Of the 64 patients, 49 were classified into the low sPD-L1 in BALF group and 15 in the high sPD-L1. Except for PD-L1 expression in tumor cells (P=0.02), none of the other variables differed significantly between the two groups (*Table 2*).

We performed univariate and multivariate analyses for prognostic factors, including sPD-L1 levels in BALF, age, sex, smoking status, ECOG PS, comorbidity, histology, ICI use for chemotherapy, ICI and chemotherapy combination therapy, PD-L1 expression in tumor cells, molecular expression (EGFR, ALK, and BRAF), and brain metastasis (*Table 3*). Univariate and multivariate analyses revealed that both histology and sPD-L1 in BALF were significantly associated with PFS. Univariate analysis for OS showed that sPD-L1 in BALF was the only significant factor associated with OS. In the survival graph of 64 patients who received first-line treatment at our hospital, the high sPD-L1 in BALF group exhibited poorer PFS and OS than the low sPD-L1 in BALF group. The median PFS of the low sPD-L1 in BALF group was 10.2 months (95% CI: 6.45–13.95 months), which was significantly longer than 3.9 months (95% CI: 1.38–6.43 months) in the high sPD-L1 in BALF group (P=0.001, *Figure 4A*). The median OS of the low sPD-L1 in BALF group was 20.57 months (95% CI: 13.86–27.27 months), which was significantly longer than 10.60 months (95% CI: 5.87–15.33 months) in the high sPD-L1 in BALF group (P=0.003, *Figure 4B*).

Survival analysis in patients treated with immunotherapy

We analyzed 31 patients treated with ICI separately. The 31 patients were classified into the low sPD-L1 in BALF

Table 2 Baseline characteristics of patients treated with or without ICIs (N=64)

Variables	Low BALF sPDL1 (N=49)	High BALF sPDL1 (N=15)	P value
Age (years)	71.38±9.311	75.27±6.923	0.17
<65	11 (22.4)	1 (6.7)	
≥65	38 (77.6)	14 (93.3)	
Sex			0.36
Male	37 (75.5)	13 (86.7)	
Female	12 (24.5)	2 (13.3)	
Smoking status			0.12
Never	14 (28.6)	2 (13.3)	
Former	20 (40.8)	4 (26.7)	
Current	15 (30.6)	9 (60.0)	
Smoking history (pack × year)	33.827±28.596	37.980±21.186	0.61
ECOG PS			0.79
0	7 (14.3)	2 (13.3)	
1	33 (67.3)	9 (60.0)	
2	8 (16.3)	4 (26.7)	
3	1 (2.0)	0 (0.0)	
Comorbidity (COPD)	23 (46.9)	7 (46.7)	0.71
Histology			0.15
AD	36 (73.5)	7 (46.7)	
SCC	11 (22.4)	7 (46.7)	
Other	2 (4.1)	1 (6.7)	
ICI use			0.60
Yes	20 (40.8)	5 (33.3)	
No	29 (59.2)	10 (66.7)	
Chemotherapy combined			0.31
ICI only	7 (14.3)	3 (20.0)	
Combined	13 (26.5)	2 (13.3)	
Best response to treatment			0.50
CR	2 (4.1)	0 (0.0)	
PR	28 (57.1)	7 (46.7)	
SD	13 (26.5)	4 (26.7)	
PD	6 (12.2)	4 (26.7)	
First response to treatment			0.47
CR	2 (4.1)	0 (0.0)	
PR	19 (38.8)	6 (40.0)	
SD	22 (44.9)	5 (33.3)	
PD	6 (12.2)	4 (26.7)	

Table 2 *(continued)*

Table 2 *(continued)*

Variables	Low BALF sPDL1 (N=49)	High BALF sPDL1 (N=15)	P value
ORR	61.2%	46.7%	0.32
PD-L1 expression in tumor cell			0.02*
No	29 (59.2)	3 (20.0)	
Low	8 (16.3)	6 (40.0)	
High	12 (24.5)	6 (40.0)	
EGFR			0.13
Wild-type	32 (65.3)	13 (86.7)	
Mutant	16 (32.7)	2 (13.3)	
N/A	1 (2.0)	0 (0.0)	
ALK rearrangement			0.38
Negative	42 (85.7)	11 (73.3)	
Positive	3 (6.1)	0 (0.0)	
N/A	4 (8.2)	4 (26.7)	
BRAF			0.38
Wild-type	23 (46.9)	9 (60.0)	
Mutant	2 (4.1)	0 (0.0)	
N/A	24 (49.0)	6 (40.0)	
Brain metastasis			0.11
Yes	17 (34.7)	2 (13.3)	
No	32 (65.3)	13 (86.7)	
NLR			0.96
<2.3	16 (32.7)	5 (33.3)	
≥2.3	33 (67.3)	10 (66.7)	

Data are presented as mean ± standard deviation or n (%). *, P<0.05. ICIs, immune checkpoint inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status; COPD, chronic obstructive pulmonary disease; AD, adenocarcinoma; SCC, squamous cell carcinoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; PD-L1, programmed death ligand 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homolog B; NLR, neutrophil-to-lymphocyte ratio; BALF, bronchoalveolar lavage fluid; sPDL1, soluble programmed death ligand 1; N/A, not available.

(23 patients) and high sPD-L1 in BALF group (8 patients). There were no significant differences in baseline characteristics between the two groups (*Table 4*). The objective response rate (ORR) was significantly higher in the low sPD-L1 in BALF group than in the high sPD-L1 in BALF group (ORR: 60.9% *vs.* 12.5%, P=0.02, *Figure 5A*).

We performed univariate analysis for prognostic factors, including sPD-L1 levels in BALF, age, sex, smoking status, ECOG PS, comorbidity, histology, ICI and chemotherapy combined therapy, PD-L1 expression in tumor cells, and brain metastasis (*Table 5*). Univariate analysis revealed no significant factors associated with PFS. Although there was not significantly, the high sPD-L1 in BALF group showed poor PFS than the low sPD-L1 in BALF group. The median PFS of the low sPD-L1 in BALF group was 7.57 months (95% CI: 3.65–11.48 months), which was longer than 2.0 months (95% CI: 0.0–5.74 months) in the high sPD-L1 in BALF group (*Figure 5B*). Univariate analysis for OS revealed sPD-L1 in BALF as the only significant factor associated with OS (P=0.01). In the Kaplan-Meier survival curve for OS, the high sPD-L1

Table 3 Univariate and multivariate analyses of PFS and OS in patients treated with or without ICIs (N=64)

Variables	N=64	PFS					OS		
		Univariate analysis			Multivariate analysis		Univariate analysis		
		Median (95% CI) (months)	Exp(B) (95% CI)	P value	Exp(B) (95% CI)	P value	Median (95% CI) (months)	Exp(B) (95% CI)	P value
Age (years)				0.96					0.58
<65	12	10.17 (2.97–12.70)	1				14.90 (10.25–17.72)	1	
≥65	52	7.75 (5.30–10.58)	1.021 (0.493–2.113)				9.00 (5.98–11.31)	1.279 (0.530–3.087)	
Sex				0.17					0.39
Male	50	6.28 (4.37–10.08)	1				12.62 (8.97–14.20)	1	
Female	14	12.30 (7.47–14.00)	0.614 (0.305–1.235)				15.10 (12.90–17.93)	0.696 (0.304–1.593)	
Smoking status				0.11					0.33
Never	16	12.70 (7.93–14.00)	1				15.60 (13.77–17.93)	1	
Former	24	5.12 (3.10–10.20)	2.167 (1.031–4.557)				11.82 (6.69–14.20)	1.896 (0.797–4.510)	
Current	24	8.32 (3.83–10.67)	1.728 (0.810–3.684)				12.75 (6.52–16.37)	1.387 (0.573–3.360)	
ECOG PS				0.12					0.10
0	9	12.97 (9.93–16.60)	1				16.30 (9.97–18.10)	1	
1	42	6.78 (3.92–10.50)	2.321 (0.901–5.980)				12.93 (9.73–14.40)	3.226 (0.757–13.757)	
2	12	6.25 (2.37–10.93)	3.037 (1.047–8.807)				10.73 (4.08–15.93)	5.366 (1.172–24.574)	
3	1	12.97 (12.97–12.97)	0 (0)				12.90 (12.90–12.90)	0 (0)	
Comorbidity				0.33					0.08
COPD	30	5.75 (3.83–10.50)	1.352 (0.740–2.470)				12.38 (7.47–12.20)	1.931 (0.914–4.080)	
Histology				0.01*		0.02*			0.60
AD	43	10.67 (4.72–12.40)	1		1		14.00 (12.13–16.18)	1	
SCC	18	7.02 (4.57–10.20)	1.502 (0.796–2.834)		0.994 (0.481–2.051)		11.18 (8.72–13.60)	1.439 (0.688–3.007)	
Other	3	2.97 (0.23–5.30)	5.177 (1.500–17.874)		5.718 (1.643–19.905)		13.43 (7.60–15.80)	1.402 (0.326–6.023)	
ICI use for 1st treatment				0.82					0.72
Yes	25	7.93 (3.88–10.67)	0.932 (0.517–1.681)				12.63 (9.97–14.97)	0.882 (0.439–1.773)	
No	39	9.07 (4.93–12.20)	1				13.76 (9.42–15.60)	1	

Table 3 *(continued)*

Table 3 *(continued)*

Variables	N=64	PFS					OS		
		Univariate analysis			Multivariate analysis		Univariate analysis		
		Median (95% CI) (months)	Exp(B) (95% CI)	P value	Exp(B) (95% CI)	P value	Median (95% CI) (months)	Exp(B) (95% CI)	P value
ICI + chemotherapy combined for 1st treatment				0.34					0.34
ICI only	10	4.53 (0.62–12.27)	1				11.70 (2.77–16.28)	1	
Combined	15	9.53 (4.38–12.98)	0.627 (0.238–1.657)				12.63 (11.05–14.97)	0.580 (0.186–1.814)	
PD-L1 expression in tumor cell				0.77					0.26
No	32	9.33 (4.93–12.40)	1				13.90 (10.87–16.37)	1	
Low	14	5.67 (3.90–10.50)	1.286 (0.629–2.628)				11.18 (7.97–14.80)	1.955 (0.867–4.409)	
High	18	8.50 (2.42–12.97)	1.025 (0.519–1.986)				13.37 (5.43–15.52)	1.362 (0.618–3.006)	
EGFR				0.11					0.49
Wild-type	45	7.47 (4.38–9.93)	1				12.63 (10.30–13.77)	1	
Mutant	18	12.60 (9.03–14.57)	0.593 (0.309–1.137)				15.50 (10.58–17.92)	0.767 (0.363–1.620)	
ALK rearrangement				0.18					0.19
Negative	53	7.93 (4.93–10.50)	1				13.60 (10.60–15.50)	1	
Positive	3	12.97 (12.40–12.97)	0.281 (0.038–2.053)				12.90 (12.90–14.97)	0.045 (0.000–57.157)	
BRAF				0.38					0.19
Wild-type	32	4.90 (3.77–9.97)	1				11.50 (5.43–14.00)	1	
Mutant	2	10.62 (7.47–13.77)	0.419 (0.057–3.097)				16.30 (13.77–18.83)	0.043 (0.000–57.999)	
Brain metastasis				0.55					0.17
Yes	19	6.03 (3.10–13.00)	1.201 (0.659–2.189)				14.40 (4.07–16.07)	1	
No	45	9.53 (5.40–11.07)	1				12.97 (11.18–14.28)	1.601 (0.814–3.151)	
sPD-L1 in BALF (pg/mL)				0.003*		0.006*			0.003*
<7.35	49	10.20 (6.45–13.95)	1		1		20.57 (13.86–27.27)	1	
≥7.35	15	3.90 (1.38–6.43)	2.637 (1.381–5.038)		2.872 (1.362–6.055)		10.60 (5.87–15.33)	2.771 (1.389–5.530)	

*, P<0.05. PFS, progression-free survival; CI, confidence interval; OS, overall survival; ICIs, immune checkpoint inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status; COPD, chronic obstructive pulmonary disease; AD, adenocarcinoma; SCC, squamous cell carcinoma; PD-L1, programmed death ligand 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homolog B; BALF, bronchoalveolar lavage fluid; sPD-L1, soluble programmed death ligand 1.

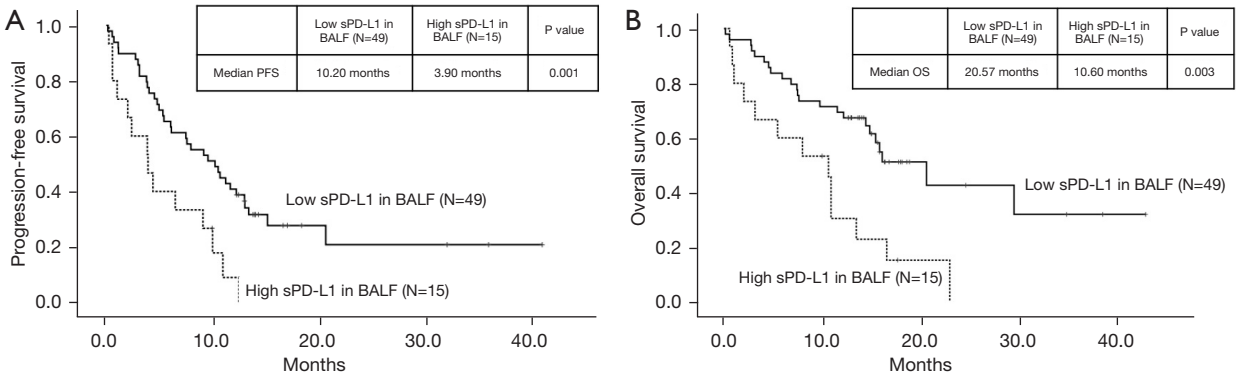

Figure 4 PFS and OS in patients with or without ICI treatment (N=64). (A) The median PFS of the low sPD-L1 in BALF group was 10.20 months (95% CI: 6.45–13.95 months), which was significantly longer than 3.90 months (95% CI: 1.38–6.43 months) in the high sPD-L1 in BALF group (P=0.001). (B) The median OS of the low sPD-L1 in BALF group was 20.57 months (95% CI: 13.86–27.27 months), which was significantly longer than 10.60 months (95% CI: 5.87–15.33 months) in the high sPD-L1 in BALF group (P=0.003). PFS, progression-free survival; sPD-L1, soluble programmed death-ligand 1; BALF, bronchoalveolar lavage fluid; OS, overall survival; ICI, immune checkpoint inhibitor; CI, confidence interval.

Table 4 Baseline characteristics of patients who received ICI treatment (N=31)

Variables	Low BALF sPD-L1 (N=23)	High BALF sPD-L1 (N=8)	P value
Age (years)	71.30±7.82	75.38±2.07	0.21
<65	4 (17.4)	0 (0.0)	
≥65	19 (82.6)	8 (100.0)	
Sex			0.55
Male	22 (95.7)	8 (100.0)	
Female	1 (4.3)	0 (0.0)	
Smoking status			0.50
Never	1 (4.3)	0 (0.0)	
Former	10 (43.5)	2 (25.0)	
Current	12 (52.2)	6 (75.0)	
Smoking history (pack × year)	51.44±23.76	43.09±13.86	0.36
ECOG PS			0.18
0	1 (4.3)	2 (25.0)	
1	18 (78.3)	4 (50.0)	
2	4 (17.4)	2 (25.0)	
3	0 (0.0)	0 (0.0)	
Comorbidity (COPD)	15 (65.2)	4 (50.0)	0.28
Histology			0.06
AD	14 (60.9)	1 (12.5)	
SCC	7 (30.4)	6 (75)	
Other	2 (8.7)	1 (12.5)	

Table 4 *(continued)*

Table 4 *(continued)*

Variables	Low BALF sPD-L1 (N=23)	High BALF sPD-L1 (N=8)	P value
Chemotherapy combined			0.12
ICI only	10 (43.5)	6 (75.0)	
Combined	13 (56.5)	2 (25.0)	
Agent (ICI)			0.30
Pembrolizumab	20 (87.0)	5 (62.5)	
Atezolizumab	3 (13.0)	3 (37.5)	
ICI treatment line			0.30
1st	20 (87.0)	5 (62.5)	
≥2nd	3 (13.0)	3 (37.5)	
First response to treatment			0.19
PR	11 (47.8)	1 (12.5)	
SD	8 (34.8)	4 (50.0)	
PD	4 (17.4)	3 (37.5)	
BR to treatment			0.06
PR	14 (60.9)	1 (12.5)	
SD	5 (21.7)	4 (50.0)	
PD	4 (17.4)	3 (37.5)	
ORR	60.9%	12.5%	0.02*
PD-L1 expression in tumor cell			0.09
No	10 (43.5)	1 (12.5)	
Low	5 (21.7)	5 (62.5)	
High	8 (34.8)	2 (25.0)	
IRAE			0.47
Yes	9 (39.1)	2 (25.0)	
No	14 (60.9)	6 (75.0)	
Type of IRAE			0.23
Pneumonitis	4 (17.4)	0 (0.0)	
Thyroid disease	0 (0.0)	1 (12.5)	
Dermatitis	4 (17.4)	1 (12.5)	
Pleural effusion	1 (4.3)	0 (0.0)	
Brain metastasis			0.11
Yes	6 (26.1)	0 (0.0)	
No	17 (73.9)	8 (100.0)	
NLR			0.11
<2.3	7 (30.4)	5 (62.5)	
≥2.3	16 (69.6)	3 (37.5)	

Data are presented as mean ± standard deviation or n (%). *, P<0.05. ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; COPD, chronic obstructive pulmonary disease; AD, adenocarcinoma; SCC, squamous cell carcinoma; PR, partial response; SD, stable disease; PD, progressive disease; BR, best response; ORR, objective response rate; PD-L1, programmed death ligand 1; IRAE, immune-related adverse events; NLR, neutrophil-to-lymphocyte ratio; BALF, bronchoalveolar lavage fluid; sPD-L1, soluble programmed death ligand 1.

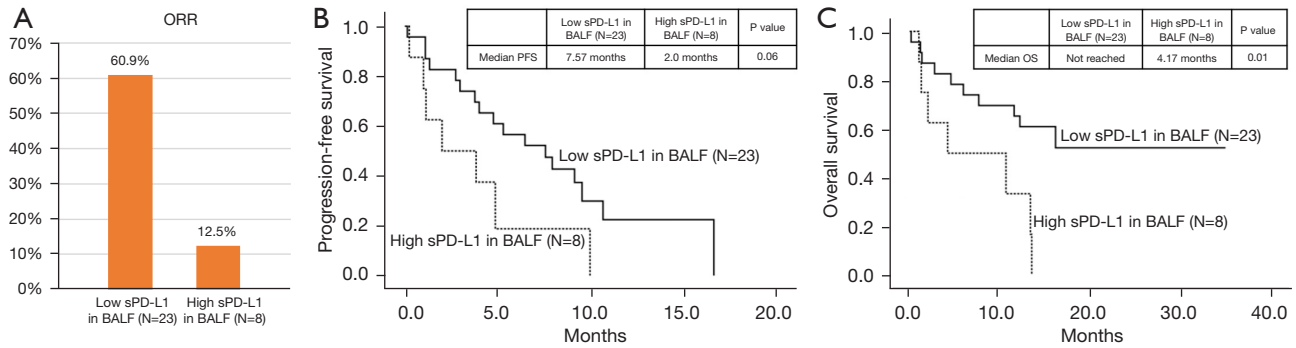

Figure 5 ORR, PFS, and OS in patients who received immunotherapy (N=31). (A) Bar graph showing significantly higher ORR in patients with low sPD-L1 in BALF (60.9%) than in those with high sPD-L1 in BALF (12.5%) (P=0.02). (B) Graph showing significantly longer median PFS in the low sPD-L1 in BALF (7.57 months; 95% CI: 3.65–11.48) than in the high sPD-L1 in BALF group (2.0 months; 95% CI: 0.0–5.74). (C) Graph showing significantly longer median OS in the low sPD-L1 in BALF (not reached) than in the high sPD-L1 in BALF group (4.17 months; 95% CI: 0.0–14.38). ORR, objective response rate; sPD-L1, soluble programmed death-ligand 1; BALF, bronchoalveolar lavage fluid; PFS, progression-free survival; OS, overall survival; CI, confidence interval.

in BALF group exhibited poorer outcomes than the low sPD-L1 in BALF group. The median OS of the low sPD-L1 in BALF group was not reached, which was significantly longer than 4.17 months (95% CI: 0.0–14.38 months) in the high sPD-L1 in BALF group (P=0.01, *Figure 5C*).

Discussion

To our knowledge, this is the first study to analyze the predictive and prognostic significance of sPD-L1 in the BALF of patients with stage IV NSCLC (*Figure 6*), and to discover the correlation between sPD-L1 in BALF and PD-L1 expression in tumor cells. We found that sPD-L1 in BALF was a significant prognostic factor affecting both PFS and OS in patients with NSCLC who received treatment, regardless of the type of treatment agent. Among patients receiving ICI treatment, those with high sPD-L1 in BALF showed a significantly lower response to ICI treatment, confirming that sPD-L1 in BALF can serve as a predictive and prognostic marker for the efficacy and outcomes of ICI therapy.

We enrolled 94 NSCLC patients who underwent bronchoscopy for the diagnosis of lung cancer. These patients had not received any anticancer treatment for lung cancer, minimizing the potential confounding effects associated with changes in PD-L1 expression in tumor cells after chemotherapy, as observed in previous studies (34-36). In addition, we examined the influence of factors affecting sPD-L1 in BALF. sPD-L1 in BALF is elevated in patients with COPD or a history of smoking (37-39). We categorized patients into several groups based on COPD and smoking status, conducted chi-square tests, and demonstrated that these factors were not associated with sPD-L1 levels in the BALF of NSCLC patients.

As immunotherapy has become the standard treatment for lung cancer, it is crucial to identify potential biomarkers suitable for screening patients who may benefit from immunotherapy (40). PD-L1 expression in tumor cells, a suboptimal marker for predicting the therapeutic efficacy of NSCLC immunotherapy, is currently the most established and widely used biomarker for immunotherapy for NSCLC (4,41,42). High PD-L1 expression in tumor cell is associated with a favorable response to ICIs (2,43). Furthermore, PD-L1 expression is associated with increased tumor proliferation and aggressiveness in lung cancer (44). However, small biopsies can misclassify PD-L1 expression in tumor cells by up to 35% and PD-L1 expression of tumor cells can vary depending on sample size of biopsy (16,17). The site of biopsy and primary and metastatic lesions may also affect changes in PD-L1 expression (40). Therefore, the prognostic and predictive performance of PD-L1 expression in tumor cells remains suboptimal.

sPD-L1 is usually detected in the blood samples of patients with cancer. Soluble PD-1 (sPD-1) and sPD-L1, both soluble forms of checkpoints, are elevated in patients with advanced cancer, and high levels of sPD-L1 in the blood indicate a poorer prognosis in patients with lung cancer (20,22,45,46). However, sPD-L1 levels in the blood are also elevated in other diseases such as sepsis, acute

Table 5 Univariate analysis of PFS and OS in patients who received ICI treatment (N=31)

Variables	N=31	PFS			OS		
		Univariate analysis			Univariate analysis		
		Median (95% CI) (months)	Exp(B) (95% CI)	P value	Median (95% CI) (months)	Exp(B) (95% CI)	P value
Age (years)				0.90			0.20
<65	4	7.63 (2.97–9.13)	1		15.75 (7.60–19.50)	1	
≥65	27	4.77 (2.73–7.93)	1.084 (0.317–3.709)		12.13 (4.53–13.43)	3.443 (0.454–26.093)	
Sex				0.97			0.91
Male	30	4.82 (3.37–8.17)	1		12.37 (5.90–13.76)	1	
Female	1	7.93 (7.93–7.93)	1.039 (0.138–7.832)		16.07 (16.07–16.07)	1.120 (0.146–8.582)	
Smoking status				0.65			0.48
Never	1	7.93 (7.93–7.93)	1		16.07 (16.07–16.07)	1	
Former	12	3.05 (1.10–8.53)	1.239 (0.155–9.932)		11.82 (1.30–13.82)	1.277 (0.156–10.480)	
Current	18	5.67 (3.83–9.13)	0.831 (0.107–6.462)		12.78 (6.07–15.67)	0.700 (0.086–5.674)	
ECOG PS				0.56			
0	3	4.20 (1.13–11.33)	1		9.97 (1.27–16.77)	1	0.92
1	22	4.81 (2.73–8.80)	2.797 (0.367–21.285)		12.62 (4.55–13.60)	1.490 (0.193–11.513)	
2	6	6.61 (2.47–11.63)	2.249 (0.261–19.349)		13.20 (5.03–17.32)	1.580 (0.176–14.170)	
3	0	–	–		–	–	
Comorbidity (COPD)	19	7.53 (2.00–9.43)	0.460 (0.182–1.166)	0.09	12.63 (2.73–14.20)	0.850 (0.307–2.356)	0.76
Histology				0.11			0.54
AD	15	7.93 (1.92–9.90)	1		13.77 (4.32–17.25)	1	
SCC	13	4.20 (3.77–7.57)	1.726 (0.683–4.365)		10.60 (4.17–13.13)	1.831 (0.626–5.359)	
Other	3	2.97 (0.23–5.30)	3.950 (0.998–15.634)		13.43 (7.60–15.80)	1.387 (0.280–6.879)	
ICI + chemotherapy combined				0.08			0.11
ICI only	16	3.80 (1.10–5.30)	1		7.93 (1.33–15.80)	1	
Combined	15	8.80 (4.00–9.43)	0.480 (0.208–1.108)		12.63 (11.05–14.58)	0.451 (0.166–1.226)	
PD-L1 expression in tumor cell				0.96			0.82
No	11	6.47 (2.97–9.13)	1		12.60 (4.17–16.37)	1	
Low	10	4.48 (2.00–9.43)	0.948 (0.352–2.555)		11.05 (2.38–14.97)	1.438 (0.437–4.736)	
High	10	6.22 (1.10–9.45)	0.869 (0.321–2.351)		13.13 (2.77–16.77)	1.352 (0.412–4.435)	
Brain metastasis				0.27			0.16
Yes	6	8.53 (3.20–13.98)	0.547 (0.184–1.630)		16.42 (8.48–26.27)	1	
No	25	4.20 (2.73–7.57)	1		11.50 (4.55–13.13)	0.358 (0.079–1.610)	
sPD-L1 in BALF (pg/mL)				0.06			0.01*
<7.35	23	7.57 (3.65–11.48)	1		Not reached	1	
≥7.35	8	2.0 (0.0–5.74)	2.319 (0.928–5.796)		4.17 (0.0–14.38)	3.414 (1.250–9.322)	

*, P<0.05. PFS, progression-free survival; OS, overall survival; ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; COPD, chronic obstructive pulmonary disease; AD, adenocarcinoma; SCC, squamous cell carcinoma; PD-L1, programmed death ligand 1; BALF, bronchoalveolar lavage fluid; sPDL1, soluble programmed death ligand 1.

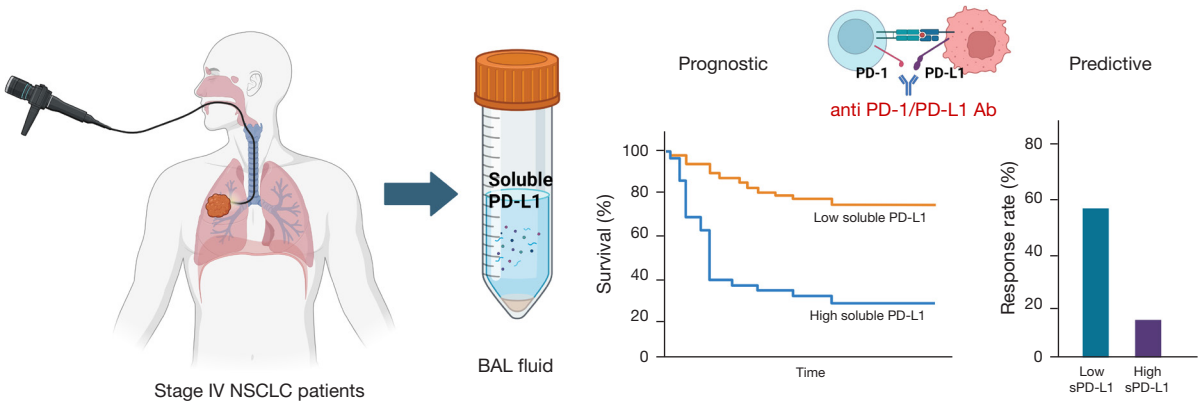

Figure 6 Graphical summary of the study. NSCLC, non-small cell lung cancer; BAL, bronchoalveolar lavage; PD-L1, programmed death-ligand 1; sPD-L1, soluble programmed death-ligand 1.

respiratory distress syndrome (ARDS), acute pancreatitis, and auto-inflammatory diseases (5). Moreover, as a circulating protein, it reflects conditions not specifically implicated in tumors. However, sPD-L1 in BALF is typically collected in close proximity to tumor lesions, making it more specific to the tumor microenvironment (27,28,30). In addition, obtaining sPD-L1 in BALF is less invasive than biopsy and repeated examinations, and obtaining a substantial sample quantity is easier than biopsy. In a previous study, it was reported that increased sPD-L1 levels in the BALF of ARDS patients was associated with increased lung inflammation and ARDS severity (47). Another study reported that sPD-L1 level in the BALF was increased in patients with neutrophilic asthma and that sPD-L1 was associated with Th17/IL-17 immune responses (48). However, thus far, no study has investigated the sPD-L1 expression in the BALF of lung cancer patients.

In this study, we demonstrated a positive correlation between PD-L1 expression in tumor cells and sPD-L1 levels in BALF. However, in contrast to the results of previous studies that showed that patients with high PD-L1 expression in tumor cells showed a better response to ICI treatment, we found that patients with higher soluble PD-L1 in BALF showed a poorer response to ICI treatment. We classified patients into four groups based on PD-L1 expression in tumor cells and sPD-L1 levels in BALF (Table S2). Even in patients with no or low tumor PD-L1 expression, the ORR was approximately 60% when BALF sPD-L1 levels were low. The ORR in patients with high tumor PD-L1 expression and high sPD-L1 in BALF was 0%, whereas that in patients with high tumor PD-L1 expression and low sPD-L1 in BALF was 62.5%. The efficacy of ICI treatment may decrease when both PD-L1 expression in tumors and sPD-L1 levels in BALF are high, although it has been reported that there is no significant difference in efficacy between ICI monotherapy and ICI combination therapy when PD-L1 expression in tumor cells is high (49,50). Although it was difficult to determine this because the number of patients in each group was very small, our results suggest that ICI combination therapy using cytotoxic agents may be better than ICI monotherapy when the levels of both PD-L1 in tumor cells and sPD-L1 in BALF are high. Moreover, among patients with similar PD-L1 expression in tumor cells, sPD-L1 in BALF could be a valuable indicator for predicting the response to ICI.

This study had several limitations. First, it was conducted at a single center, resulting in a relatively small number of participants. In particular, the number of patients who received ICI treatment was small; therefore, validation of our results in a larger cohort is required. Second, the mechanism by which sPD-L1 in BALF contributes to poor prognosis in patients with lung cancer and is associated with reduced efficacy of ICI treatment remains to be elucidated.

Conclusions

We demonstrated the potential of sPD-L1 in BALF as a promising biomarker in NSCLC prognosis and ICI efficacy, offering advantages such as ease of sampling, less invasive procedure compared to traditional biopsy, and increased sensitivity for reflecting the tumor microenvironment compared to other established blood biomarkers.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-392/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-392/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted per the Declaration of Helsinki (as revised in 2013) and approved by the Institutional Review Board of Chungnam National University Hospital (IRB No. 2018-01-059). Informed consent was obtained from all participants involved in the study.

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