Original Article

Correlation between PD-L1 expression and clinicopathological characteristics of non-small cell lung cancer: A real-world study of a large Chinese cohort

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Background: Programmed death ligand-1 (PD-L1) is a predictive marker of anti-programmed death protein 1 (PD-1)/PD-L1 therapies for non-small cell lung cancer (NSCLC). However, little is known between PD-L1 expression and the clinicopathological characteristics of NSCLC in the Chinese population in a real-world setting.

Methods: We analyzed PD-L1 expression by immunohistochemistry (IHC) in NSCLC patients using the 22C3 clone on the Dako Autostainer Link 48 platform. We then examined the associations of PD-L1 expression with clinicopathological characteristics, stromal tumor-infiltrating lymphocytes (TILs) and major molecular features.

Results: A total of 1,156 recently NSCLC specimens including 827 sequentially resected specimens and 293 biopsy specimens were enrolled in our study. PD-L1 high expression was observed in 9.7% of 827 NSCLC patients, including 6.5% with adenocarcinoma (ADC, n=690), and 27.4% with squamous cell carcinoma (SqCC, n=117). These results showed higher expression rates than those in archived samples (>5 years old, n=329), that were previously reported by our group (4.9%, 0.5%, and 13.9% in NSCLC, ADC, and SqCC, respectively). The prevalence of PD-L1 expression was lower in surgical resection samples than in small biopsy samples. PD-L1 high expression in the lung biopsy was less likely present in the primary cancer than in metastases, and was also associated with a high level of stromal TILs (P=0.029) and PD-L1-positive immune cells (IC) (P<0.001). Both PD-L1 high and low expressions were more frequent in EGFR-wild type than in mutant type (P<0.001).

Conclusions: This study demonstrates that expression of PD-L1 is linked to the type of tumor specimens, resection versus biopsy specimens, and biopsies of primary versus metastatic cancers. These findings have substantial implications for clinical practice.

Keywords: Programmed death-ligand 1 (PD-L1); non-small cell lung cancer (NSCLC); immunohistochemistry (IHC)

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Introduction

The approach to the diagnosis and molecular analysis of non-small cell lung cancer (NSCLC) has advanced significantly in the last decade. There is a revolution driven by the development of drugs against particular sub-groups of tumor defined by their genetic pathology or protein expression (1,2). The remarkable success of therapies with antibodies that block the interaction of programmed death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) introduced the era of immunotherapy for advanced NSCLC (3-5).

The KEYNOTE trials reported that pembrolizumab prolonged the overall survival (OS) rates of NSCLC patients with PD-L1 immunohistochemistry (IHC) positive expression (at least 1% of tumor cells, TCs) (5-7). Pembrolizumab was approved for the first line treatment of patients with metastatic NSCLC whose tumors demonstrate PD-L1 (22C3, Dako platform) staining in ≥50% of TCs, or patients with locally advanced and/or metastatic NSCLC with prior chemotherapy and a ≥1% of TCs. Hence, the association between PD-L1 expression and clinicopathological characteristics has become an important consideration. A number of investigators have already presented that PD-L1 positive rates range from 13%-35% in archived NSCLC samples (8-11). However, little is known between PD-L1 expression and the clinicopathological characteristics of NSCLC in the Chinese population in a real-world setting.

Immune cells (ICs) in the lung cancer microenvironment primarily comprise T cells, macrophages, and mast cells. They are generally found at the invasive tumor margin (12). Recently, several studies reported that a high number of tumor-infiltrating lymphocytes (TILs) were generally associated with a good outcome in NSCLC patients (13,14). Donnem *et al.* focused on the prognostic impact of TILs on immunoscore assessment and TNM-immunity. Although the implication of TILs has been understood to some extent, these studies neither assessed PD-L1 expression on ICs nor described the relationship between stromal TILs and PD-L1 expression.

The aim of this study was to provide real-world data on PD-L1 protein expression in surgically resected and biopsied NSCLC samples and to compare recently resected with archived tumor samples. We also analyzed the correlation between PD-L1 expression and clinicopathological characteristics, stromal TILs, and pulmonary major driver genes alterations in Chinese NSCLC patients.

Methods

Patients and samples

Tumor samples were collected from 1,156 NSCLC patients who underwent sequentially surgical resection and 293 biopsy specimens in our institution between September 2017 and June 2018. Clinical data, PD-L1 expression data, and molecular alteration data were retrieved from the patients' medical records. Patients who received neoadjuvant chemotherapy and had a history of other malignant tumors were excluded. Patients were classified according to the smoking status as never-smokers (<100 lifetime cigarettes) and smokers. The histology of the lung cancers was classified according to the 2015 WHO classification (15). The tumor pathologic stage was characterized using the AJCC staging system (8th Edition) (16). The Institutional Review Board (IRB) of Fudan University Shanghai Cancer Center had approved this study.

Analysis of PD-L1 protein expression by IHC

PD-L1 protein staining was performed on formalin-fixed paraffin-embedded (FFPE) sections (4 μm thick) by IHC immediately after the operation. In specimens whose the maximum diameter was greater than 1cm, a representative slide was selected for staining. The representative slide was defined as a section contained the most diverse histological subtypes. This assay was performed on the Dako Autostainer Link 48 platform with an automated staining protocol using a mouse monoclonal anti-PD-L1 antibody (22C3). PD-L1 expression was evaluated by the tumor proportion score (TPS), which is defined as the percentage of PD-L1-positive TCs over total TCs. The evaluation of the score included partial or complete membranous staining (at least 1+ intensity). All other cells, such as tumor-associated ICs, normal/non-neoplastic cells, and necrotic cells, were excluded from the evaluation. PD-L1 expression in TCs was classified into three levels: negative expression (TPS <1%), low expression (TPS 1–49%), and high expression (TPS ≥50%). Furthermore, we evaluated PD-L1 expression in ICs. PD-L1 ICs were defined as positive when there were more than 1% positive mononuclear cells (including lymphocytes and plasma cells) in tumor stroma, according to the atlas of the PD-L1 SP142 assay by IHC testing in lung cancer (17). The results were interpreted by using light microscope (Olympus BX43, Japan) by two pathologists who were blinded to clinical data and patient outcomes (Y. L. and Y. J).

Evaluation of stromal TILs

The scoring of stromal TILs was performed on hematoxylin & eosin (H&E)-stained FFPE tissue sections, as previously described (18). We evaluated stromal TILs which often localize to surgical margins, including lymphocytes and plasma cells. The stromal TILs were assessed in multiple stromal regions and not only in hot spots. The percentage of tumor stromal containing ICs was classified as low stromal TILs (1–49%), and high stromal TILs (≥50%).

Status of other driver genes

EGFR mutations within exons 18 to 21 in FFPE tissues were examined using the ADx-ARMS (amplification refractory mutation system) kit (Amoy Diagnostics, Xiamen, China), as described previously (8). The *ALK* rearrangement was analyzed by fluorescence *in situ* hybridization (FISH) using a Vysis break-apart rearrangement probe (Abbott Diagnostics, Abbott Park, IL, USA) or IHC (clone D5F3, VMSI) on FFPE tumors.

Statistical analysis

Statistical analyses were performed using the software package Statistical Package for Social Sciences, version 20.0, for Windows (SPSS, Chicago, IL, USA). The chi-square test or Fisher's exact test was used to determine potential associations. All statistical values were determined using two-tailed statistical analyses, and P values <0.05 were considered to indicate a statistically significant difference.

Results

Patient and tumor characteristics

The surgical resection group consisted of 827 recently resected and 329 archived (>5 years old) NSCLC samples (*Table 1*). The archived tissues have been previously described (8). The current acquired resection tumor

Table 1 Clinicopathological features of patients with non-small cell lung cancer (NSCLC)

Variables	Archival samples	Recent samples		P value
		Resection	Small biopsy	
Total	329	827	293	
Sex				0.002
Male	225	421	216	
Female	104	406	77	
Age (years)				0.201
Median	61	61	64	
Range	27–83	17–83	27–75	
Histology				<0.001
Adenocarcinoma (ADC)	221	690	211	
Squamous cell carcinoma (SQCC)	108	117	63	
Large cell neuroendocrine carcinoma (LCNEC)	0	9	13	<0.001
Adenosquamous carcinoma (ASC)	0	6	2	
Large cell carcinoma (LCC)	0	2	2	
Pleomorphic carcinoma (PC)	0	2	2	
Atypical carcinoid tumor (AC)	0	1	0	
PD-L1 expression				0.001
Negative	283 (86.0%)	572 (69.2%)	94 (32.1%)	
Low expression	30 (9.1%)	175 (21.2%)	74 (25.3%)	
High expression	16 (4.9%)	80 (9.7%)	125 (42.7%)	<0.001
Collection time	Sep 2009–Mar 2013	Sep 2017–Jun 2018	Sep 2017–Jun 2018	0.101

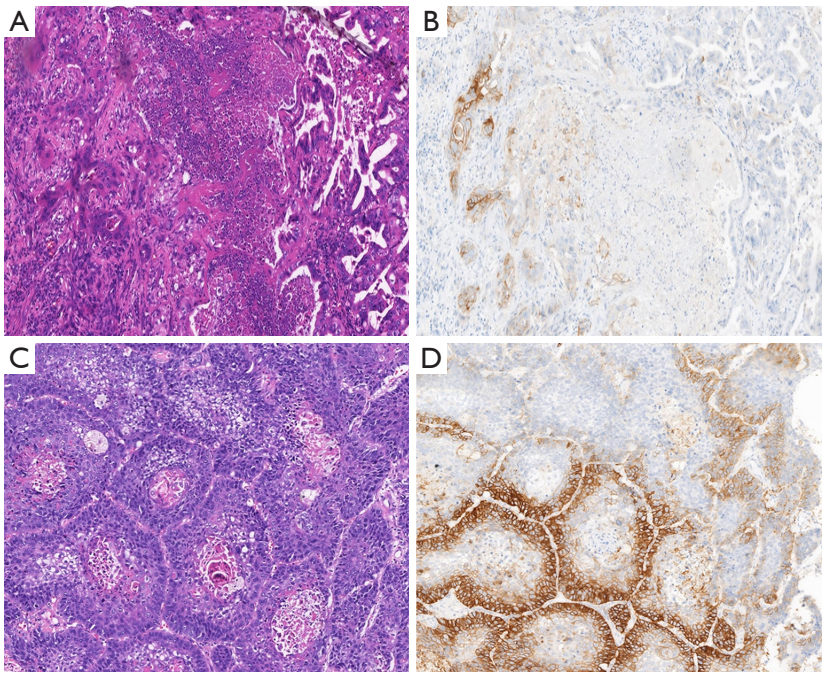

Figure 1 Representative images of programmed death ligand-1 (PD-L1) immunostaining in adenocarcinoma and squamous cell carcinoma (×200). (A) and (B) show hematoxylin and eosin (H&E) staining and the membranous staining of PD-L1 expression in adenocarcinoma; (C) and (D) show H&E staining and PD-L1 expression in squamous cell carcinoma. PD-L1 expression in tumor cells showed different staining patterns based on tumor differentiation. In the same specimens, a well-differentiated area shows low PD-L1 expression, whereas a poorly differentiated area shows strong PD-L1 expression.

samples included those from 406 women (49.1%) and 421 men (50.9%), with a median age of 61 years (range, 17–83 years). Of the 827 patients, 520 (62.9%) were non-smokers, and 307 were smokers. The histological characterization of tumors revealed that 690 samples were adenocarcinoma (ADC, 83.4%), 117 were squamous cell carcinoma (SqCC, 14.1%), and 20 were other histologic type NSCLC (2.4%; 9 large cell neuroendocrine carcinomas, 6 adenosquamous carcinomas, 2 large cell carcinomas, 2 pleomorphic carcinomas, and 1 atypical carcinoid tumor). Of the 827 patients, characterization of the pathological stage of each tumor revealed 606 patients in stage I, 100 patients in II, 114 patients in III, and 7 patients in IV.

A total of 293 small biopsy cases included 244 primary pulmonary specimens and 49 metastatic specimens that involved lymphocytes, the brain, bone, soft tissue, adrenal gland, liver, bronchus, ovary, and pleura. Of these patients, 216 (73.7%) were male and 77 (26.31%) were female. The median age was 64 years (range, 27–75 years) (*Table 1*).

PD-L1 protein expression in NSCLC

PD-L1 protein expression in TCs was defined as complete circumferential or partial cell membrane staining (*Figure 1*). Among the NSCLC patients, 80 (9.7%) had high PD-L1 expression, and 175 (21.1%) had low PD-L1 expression. High PD-L1 expression was significantly more common in SqCC than in non-squamous NSCLC (ADC and other histologic type NSCLC) (SqCC 27.4% *vs.* ADC 6.5% *vs.* other histologic type NSCLC 15%, P<0.001) (*Figure 2*).

Regarding ADC, PD-L1 high expression and low expression were observed in 6.5% and 17.7% of patients, respectively (*Figure 2*). PD-L1 high expression was significantly associated with female sex (P=0.002), a large tumor size (P<0.001), pleural invasion (P=0.001), venous and lymphatic invasion (P<0.001), smoking history (P<0.001), and advanced pathological stage (P<0.001) (*Table 2*). The ADC subtype groups with less frequent PD-L1 expression included the papillary (6/163, 3.7%), and acinar (13/352, 3.7%). The subtype groups with the more frequent

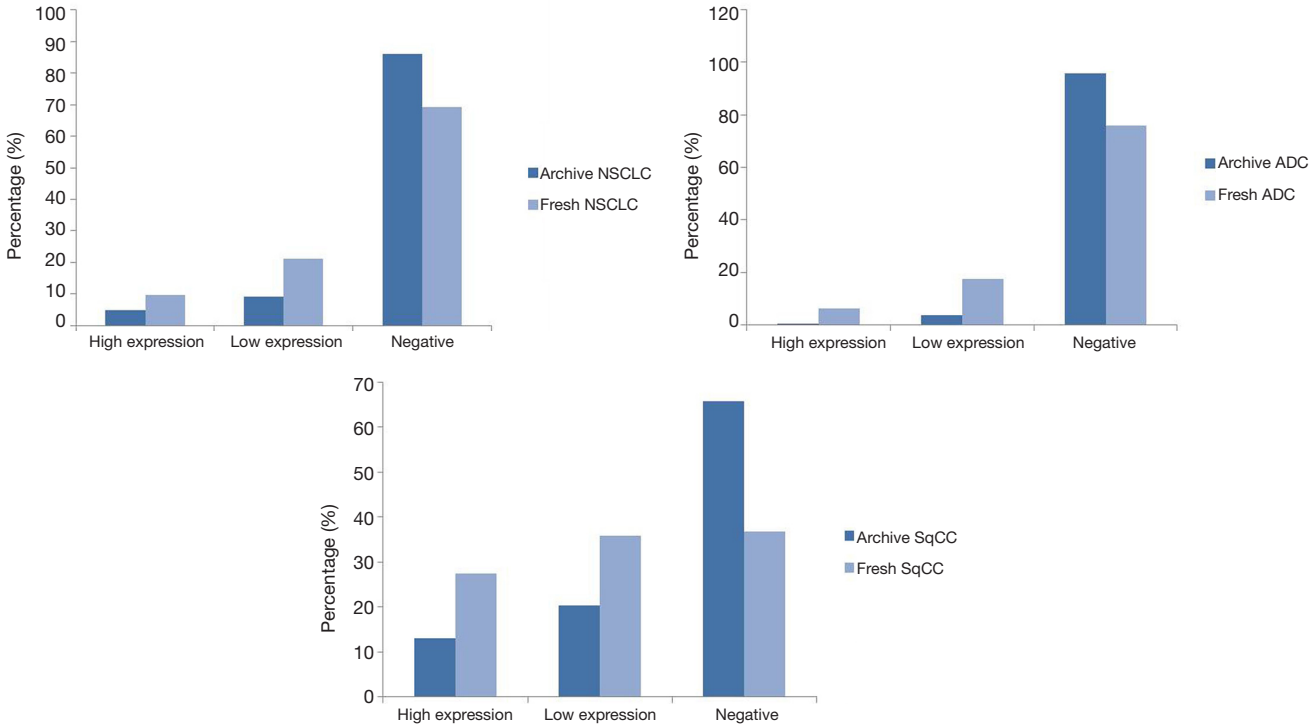

Figure 2 Comparison of PD-L1 expression in recently acquired samples and archived NSCLC samples. PD-L1, programmed death ligand-1; NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; SqCC, squamous cell carcinoma.

PD-L1 expression included the solid (22/63, 34.9%) and micropapillary (4/20, 20.0%) subtypes (P<0.001).

In the SqCC subgroup, the PD-L1 high expression, low expression and negative expression were observed in 27.4%, 35.8%, and 36.8% of patients, respectively. PD-L1 high expression was significantly associated with the non-keratinizing type (P=0.012) and the absence of perineural invasion (P=0.001) (*Table 3*).

PD-L1 high expression was observed in 15% of the remaining patients (3/20, 2 adenosquamous carcinomas, 1 large cell carcinoma). The PD-L1 low expression was observed in 55% of the remaining patients (11/20, 5 large cell neuroendocrine carcinomas, 3 adenosquamous carcinomas, 2 pleomorphic carcinomas, and 1 atypical carcinoid tumor). Four large cell neuroendocrine carcinomas, 1 adenosquamous carcinoma, and 1 large cell carcinoma showed negative PD-L1 expression.

We subsequently evaluated the prevalence of PD-L1 expression in small biopsy specimens. Among the 244 primary pulmonary small biopsy specimens, PD-L1 high and low expression were found in 76 (31.1%) and 63 (25.8%) patients. In the 49 metastasis specimens, PD-L1 high expression and low expression were observed in 18 (36.7%) and 11 (22.4%) patients, respectively.

Correlation of PD-L1 expression and the tumor microenvironment in NSCLC

A total of 368 NSCLC patients were examined to define whether PD-L1 expression correlated with the tumor stromal immune microenvironment (*Figure 3*). High and low stromal TILs were observed in 44 (12.0%) and 324 (88.0%) tumors, respectively. Patients with high stromal TILs were found to exhibit higher PD-L1 protein expression than those with low stromal TILs (P=0.029, *Table 4*). A high stromal TIL density was correlated with clinicopathological parameters, although the difference was not significant. On the other hand, PD-L1 high expression was significantly associated with the PD-L1 IC-positive status (P<0.001, *Table 4*).

Association between PD-L1 expression and the status of major driver genes

A total of 542 NSCLC patients were available to study *EGFR* mutations: 156 patients had wild-type *EGFR*,

Table 2 Clinicopathological features of patients with adenocarcinoma

Variables	Total (n=690)	PD-L1 expression, n (%)			P value
		Negative	Low expression	High expression	
Sex					0.002
Male	393	315 (80.2)	62 (15.8)	16 (4.1)	
Female	297	208 (70.0)	60 (20.2)	29 (9.8)	
Age					0.201
<61 years	339	267 (78.8)	53 (15.6)	19 (5.6)	
≥61 years	351	256 (72.9)	69 (19.7)	26 (7.4)	
Tumor size					<0.001
<2 cm	341	286 (83.9)	44 (12.9)	11 (3.2)	
≥2 cm	349	237 (67.9)	78 (22.3)	34 (9.7)	
Subtype predominant[#]					<0.001
Lepidic	56	52 (92.2)	4 (7.1)	0 (0.0)	
Acinar	352	278 (79.0)	61 (17.3)	13 (3.7)	
Papillary	163	128 (78.5)	29 (17.8)	6 (3.7)	
Solid	63	20 (31.7)	21 (33.3)	22 (34.9)	
Micropapillary	20	9 (45.0)	7 (35.0)	4 (20.0)	
Pleural invasion					0.001
Absent	587	460 (78.4)	92 (15.7)	35 (6.0)	
Visceral invasion	103	63 (61.2)	30 (29.1)	10 (9.7)	
Venous and lymphatic invasion					<0.001
Absent	529	434 (82.0)	70 (13.2)	25 (4.7)	
Present	161	89 (55.3)	52 (32.3)	20 (12.4)	
Perineural invasion					0.101
Absent	680	518 (76.2)	119 (17.5)	43 (6.3)	
Present	10	5 (50.0)	3 (30.0)	2 (20.0)	
Smoking					<0.001
No	483	385 (79.7)	76 (15.7)	22 (4.6)	
Yes	207	138 (66.7)	46 (22.2)	23 (11.1)	
Stage					<0.001
Early stage (I and II)	602	478 (79.4)	91 (15.1)	33 (5.5)	
Advanced stage (III and IV)	88	45 (51.1)	31 (35.2)	12 (13.6)	

[#], invasive adenocarcinoma.

384 had mutant *EGFR*, and 1 had a gene amplification. Histologically, the majority of tumor specimens were ADC (94.6%). PD-L1 high and low expression was more frequent in the *EGFR* wild-type group than in the mutant group (P<0.001) (*Table 5*). There was a significant association between the *EGFR* mutation types and the PD-L1 expression level. Rare *EGFR* mutations (exon 18 and 20 mutations, double mutations) demonstrated higher

Table 3 Clinicopathological features of patients with squamous cell carcinoma

Variables	Total (n=117)	PD-L1 expression, n (%)			P value
		Negative	Low expression	High expression	
Sex					0.110
Male	108	42 (38.9)	36 (33.3)	30 (27.8)	
Female	9	1 (11.1)	6 (66.7)	2 (22.2)	
Age					0.488
<65 years	56	18 (32.1)	23 (41.1)	15 (26.8)	
≥65 years	61	25 (41.0)	19 (31.1)	17 (27.9)	
Tumor size					0.273
<3.5 cm	10	6 (60.0)	2 (20.0)	2 (20.0)	
≥3.5 cm	107	37 (34.6)	40 (37.4)	30 (28.0)	
Keratinizing status					0.012
Keratinizing	16	11 (68.8)	4 (25.0)	1 (6.3)	
Non-keratinizing	101	32 (31.7)	38 (37.6)	31 (30.7)	
Pleural invasion					0.124
Absent	100	33 (33.0)	38 (38.0)	29 (29.0)	
Visceral invasion	17	10 (58.8)	4 (23.5)	3 (17.6)	
Venous and lymphatic invasion					0.859
Absent	84	32 (38.1)	29 (34.5)	23 (27.4)	
Present	33	11 (33.3)	13 (39.4)	9 (27.3)	
Perineural invasion					0.001
Absent	103	40 (38.8)	31 (30.1)	32 (31.1)	
Present	14	3 (21.4)	11 (78.6)	0 (0.00)	
Smoking					0.772
No	32	12 (37.5)	10 (31.3)	10 (31.3)	
Yes	85	31 (36.5)	32 (37.6)	22 (25.9)	
Stage					0.315
Early stage (I and II)	88	32 (36.4)	29 (33.0)	27 (30.7)	
Advanced stage (III and IV)	29	11 (37.9)	13 (44.8)	5 (17.2)	

expression rates than classical mutations (exon 19 and 21 mutations).

In addition, we examined the relationship between the *ALK* gene rearrangement and PD-L1 expression in 320 resected NSCLCs. PD-L1 high expression and low expression were observed in 6.9% (20/289) and 19.0% (55/289) of *ALK*-negative patients, respectively. For the *ALK* gene rearrangement group, PD-L1 high expression and low expression were observed in 6.5% (2/31) and 32.3% (10/31) patients, respectively. PD-L1 expression was not associated with the *ALK* gene rearrangement (P=0.218).

Discussion

To our knowledge, this study represents the largest comprehensive study of PD-L1 expression with clinicopathological characteristics in Chinese patients with NSCLC. Here, we show the following: (I) the prevalence of PD-L1 high expression (27.4%) in the surgically resected SqCC population was higher than that in the ADC population (6.5%), which is in accordance with previous reports (8,11); (II) surgically resected tumor samples showed lower rates of PD-L1 expression

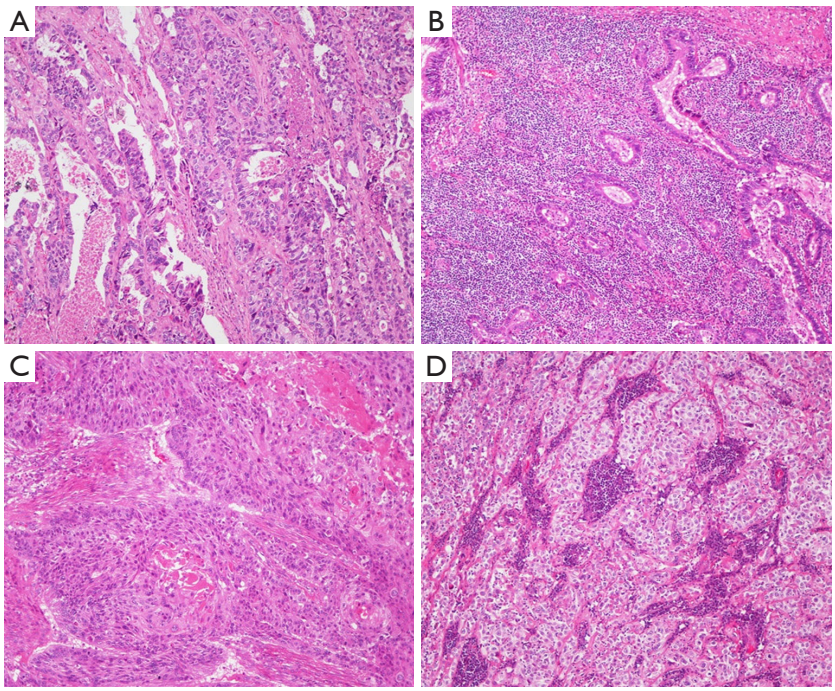

Figure 3 Representative H&E staining images of stromal tumor-infiltrating lymphocytes (TILs) (×200). (A) and (B) show a negative (<50%), and positive (≥50%) stromal TILs status in adenocarcinoma, respectively; (C) and (D) show a negative (<50%), and positive (≥50%) stromal TILs status in squamous cell carcinoma, respectively.

Table 4 Correlation of PD-L1 expression and tumor microenvironment status

Variables	Total (n=368)	PD-L1 expression, n (%)			P value
		Negative	Low expression	High expression	
sTILs status					0.029
High (<50%)	324	215 (66.4)	73 (22.5)	36 (11.1)	
Low (≥50%)	44	21 (47.7)	13 (29.5)	10 (22.7)	
ICs status					<0.001
Positive (<1%)	260	190 (73.1)	55 (21.2)	15 (5.8)	
Negative (≥1%)	108	46 (42.6)	31 (28.7)	31 (28.7)	

PD-L1, programmed death ligand-1; sTILs, stromal tumor-infiltrating lymphocytes; IC, immune cells.

than small biopsy samples; high PD-L1 expression of the lung biopsy was less likely present in the primary cancer than in metastases; (III) in ADC, PD-L1 high expression was more likely to appear among the solid and micropapillary subtypes; in SqCC, PD-L1 high expression was significantly associated with the non-keratinizing type; (IV) PD-L1 TCs high expression was significantly associated with high stromal TILs (≥50%) and the PD-L1 IC-positive status; (V) PD-L1 high expression was significantly associated with no *EGFR* mutation.

Previously reported tumor PD-L1 positivity detected by the 22C3 assay varied widely (5-7,10,11). Several studies consistently reported that high PD-L1 expression was more frequent in SqCC than in ADC. One key finding of our study was that PD-L1 expression in recently resected tumor samples was higher than that in archived samples. In this study, the PD-L1 positive rates were similar to those reported in previous studies. However, the prevalence of PD-L1 in

Table 5 Correlation of programmed death ligand-1 (PD-L1) expression and *EGFR* mutation

EGFR mutation	Total (n=541)	PD-L1 expression, n (%)			P value
		Negative	Low expression	High expression	
Wild type	156	96 (61.5)	41 (26.3)	19 (12.2)	
Mutations					<0.001
Rare mutation	45	34 (75.6)	6 (13.3)	5 (11.1)	
Exon 19 mutation	145	109 (75.2)	29 (20.0)	7 (4.8)	
Exon 21 mutation	195	165 (84.6)	25 (12.8)	5 (2.6)	

fresh tumor samples was conspicuously higher than that in archived samples (8). Our previous study assessed archived (>5 years old) tumor samples (*Figure 2A*). Recently, one study reported that the concordance of archived samples <3 years old was the highest, among recently acquired samples (19). To our knowledge, protein expression can be influenced by the ageing of FFPE tissue blocks (20). It appears that the PD-L1 protein may decay under different storage conditions. Our data suggest that PD-L1 protein expression should ideally be determined immediately after the excision of primary lesions.

Another key finding of our study is the heterogeneity of PD-L1 expression in NSCLC. We identified a highly significant relationship between the histologic patterns of ADC and PD-L1 expression. Poorly differentiated histologic patterns, such as solid and micropapillary patterns were much more likely to express PD-L1, and well or moderately differentiated lepidic, acinar, and papillary patterns were much less likely to express PD-L1. Interestingly, in the same specimens, the well-differentiated area exhibited low PD-L1 expression intensity, whereas the poorly differentiated area exhibited strong PD-L1 expression intensity (*Figure 1*). Similar to ADC, PD-L1 expression was also prevalent in poorly differentiated SqCC (*Figure 1*). Recently, several studies have also reported that the poor differentiation histology was associated with high PD-L1 expression (8,21,22). Considering the potential difference in PD-L1 expression, we should choose a representative section that contained the most diverse histological subtypes and then take an average value of PD-L1 expression. These findings highlight the importance of histologic growth patterns when comparing the prevalence of PD-L1 positivity across studies.

In our study, PD-L1 high expression was observed in 29.3% of NSCLC biopsy specimens, which was higher than in surgically resected specimens (9.7%). Moreover, interestingly, we found that high PD-L1 expression in the lung biopsy showed lower rates than those in the metastatic sites. One prior study reported that prevalence of PD-L1 TPS ≥50% and PD-L1 TPS ≥1% was similar across geographic regions, between surgical specimens and biopsies, and irrespective of whether tissue was from the primary tumor or from metastases (23). Such a discrepancy could be the result of tumor heterogeneity and different clinical stages. It is evident that PD-L1 expression in small biopsy samples might not represent that of the entire tumor specimen because of cancer heterogeneity. The mechanism of intra-tumoral PD-L1 expression heterogeneity is well understood (24-26). Furthermore, most biopsy specimens were obtained from patients with advanced lung cancer. In contrast, the majority of excision specimens were operable in early-stage tumors. These results demonstrate that PD-L1 expression may reveal heterogeneity, and it is important to understand the effect of different sample sites on PD-L1 expression levels to assess their suitability for testing (27). Further work is needed to understand the mechanisms of intra- and inter-tumoral heterogeneity for clinical treatment guidance of patients with advanced unresectable lung cancers.

Recently, studies have indicated a trend that patients suitable for immunotherapy often lack known driver mutations (11,28,29). We also showed that PD-L1 positive expression was more frequent in the *EGFR* wild type group than in the mutation group. However, some (4.4%) patients with the *EGFR* mutation still expressed PD-L1; thus, more attention needs to be paid to these patients.

Some studies have revealed that TILs, especially T cells, have a major impact on the clinical course of several cancers, including melanoma and lung, melanoma, head and neck, breast, bladder, urothelial, ovarian, colorectal, renal, and prostate cancers (12,13). Approximately 80%

of lymphocytes are T cells expressing various activation antigens. Most prior studies evaluating TILs in lung cancer applied IHC (CD4, CD8, and CD3) to differentiate IC subsets and to assess their density, distribution and localization (21,30). Here, we evaluated stromal TILs localized only in the invasive margin by commonly used H&E staining methods. We found a significant correlation between PD-L1 expression and stromal TIL levels. Furthermore, our results showed that high PD-L1 expression was significantly associated with the presence of PD-L1-positive ICs. Further study should be carried out to accurately determine the relationship between PD-L1 expression and ICs by IHC or other new technologies. The limitations of this study include the lack of an accurate evaluation of stromal TILs and a short follow-up time, which was not sufficient to compare differences in OS and disease-free survival (DFS). Nevertheless, this is the first correlation analysis of PD-L1 expression and stromal ICs.

In conclusion, our results revealed that PD-L1 higher expressing patterns of ADC and non-keratinizing SqCC as well as comparison with different metastatic expression. For patients with recurrence and metastasis, a re-biopsy is strongly suggested to evaluate the status of PD-L1. Only when sufficient fresh tissue cannot be obtained by a biopsy for various clinical conditions should archived blocks be considered as a complementary method. Our data also indicate that the PD-L1 protein may decay under poor storage conditions. To ensure the reliability and authenticity of the expression level of PD-L1, IHC should be performed as soon as possible on postoperative specimens.

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Footnote

Conflicts of Interest: 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 approved by the institutional review board of the Shanghai Cancer Center, Fudan University, Shanghai, China (IRB, No. IRB# 090977-1). All procedures in studies that involved human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or with comparable ethical standards.

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