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Reliability of PD-L1 assays using small tissue samples compared with surgical specimens

Insu Kim, MD[a], Ahrong Kim, MD[b], Chang Hun Lee, MD[b], Geewon Lee, MD[c], Ahreum Kim, BS[d], Eun Jung Jo, MD[a], Mi-Hyun Kim, MD[a], Jeongha Mok, MD[a], Kwangha Lee, MD[a], Ki Uk Kim, MD[a], Hye-Kyung Park, MD[a], Min Ki Lee, MD[a], Jung Seop Eom, MD[a,e,*]

Abstract

Programmed death ligand 1 (PD-L1) immunohistochemistry (IHC) assays are widely used for complementary or companion diagnostic purposes during treatment with immune checkpoint inhibitors. However, limited information is available on the clinical reliability of the PD-L1 IHC assay using small biopsy samples.

Participants included 46 patients with nonsmall cell lung cancer who underwent PD-L1 testing using 3 PD-L1 IHC assays (22C3, SP142, and SP263) for both small biopsy samples and surgical specimens from November 2017 to June 2018. The PD-L1 IHC assay results were analyzed with cut-off values of 1%, 5%, 10%, and 50%. The PD-L1 IHC results obtained from the surgical specimens were regarded as the reference values.

The 22C3, SP142, and SP263 PD-L1 IHC assays were performed in 26 (57%), 20 (43%), and 46 (100%) patients, respectively. Biopsy methods included radial probe endobronchial ultrasound using a guide sheath, endobronchial ultrasound-guided transbronchial needle aspiration, bronchoscopic biopsy, and percutaneous needle aspiration in 26 (57%), 4 (9%), 12 (25%), and 4 (9%) patients, respectively. The 22C3, SP142, and SP263 PD-L1 assays had concordance rates of 73–96, 65–80, and 72%–91%, respectively, compared with the reference values.

PD-L1 testing with 3 commercial PD-L1 IHC assays using small biopsy samples is reliable in patients with nonsmall cell lung cancer.

Abbreviations: ADC = adenocarcinoma, CI = confidence interval, CPS = combined positive score, CT = computed tomography, EBUS-GS = radial probe endobronchial ultrasound using a guide sheath, EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration, F = female, FDA = US Food and Drug Administration, HCC = hepatocellular carcinoma, IC = tumor infiltrating immune cell, IHC = immunohistochemistry, IQR = interquartile range, M = male, Neo-adj. CTx. = neo-adjuvant chemotherapy, NPV = negative predictive value, NR = not reported, NSCLC = nonsmall cell lung cancer, PCNA = percutaneous needle aspiration, PD-L1 = programmed death 1 and its ligand-programmed death ligand 1, PPV = positive predictive value, SqCC = squamous cell carcinoma, TC = tumor cells, TPS = tumor proportion score, VATS = video-assisted thoracoscopic surgery.

Keywords: biologic assay, biopsy, bronchoscopy, immunologic diagnosis, lung cancer

1. Introduction

Lung cancer is one of leading causes of cancer-related mortality worldwide,[1] and nonsmall cell lung cancer (NSCLC) accounts for the majority of lung cancers.[2,3] New therapeutic strategies, including target agents or immunotherapy, are improving overall and progression-free survival in patients with advanced NSCLC.[4] In particular, immune checkpoint inhibitors provide an additional treatment option for patients with the wild-type epidermal growth factor receptor mutation or the anaplastic lymphoma kinase rearrangement.[5–8]

Immune checkpoint inhibitors hinder tumor proliferation by blocking inhibitory pathways, such as programmed death 1 and its ligand-programmed death ligand 1 (PD-L1).[9–11] PD-L1 immunohistochemistry (IHC) is used as a biomarker to predict the feasibility and response to immune checkpoint inhibitors in various diseases (Table 1).[12–15] Therefore, accurate PD-L1 IHC analyses are necessary for appropriate treatment and accurate prognostic prediction.

PD-L1 IHC assays are usually performed after routine hematoxylin and eosin staining and epidermal growth factor receptor mutation or anaplastic lymphoma kinase rearrangement analyses.[16–18] Therefore, a large amount of tissue is required for an accurate PD-L1 IHC assay. However, the majority of patients with advanced NSCLC are diagnosed using a small biopsy sample, which is generally collected by radial probe endobronchial ultrasound using a guide sheath (EBUS-GS), endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), bronchoscopic biopsy, or percutaneous needle aspiration (PCNA).[19–21] No data are available regarding the reliability of the PD-L1 IHC assay using small biopsy specimens. Thus, we performed this retrospective study to identify the reliability of PD-L1 IHC assays using a small biopsy sample.

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[a] Department of Internal Medicine, [b] Department of Pathology, [c] Department of Radiology, Pusan National University School of Medicine, [d] Biostatistics Team of Regional Center for Respiratory Diseases, [e] Biomedical Research Institute, Pusan National University Hospital, Busan, Korea.

[*] Correspondence: Jung Seop Eom, Department of Internal Medicine, Pusan National University School of Medicine, 179 Gudeok-ro, Seo-gu, Busan 49241, Korea (e-mail: ejspulm@gmail.com).

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Table 1

Comparison of PD-L1 study criteria by disease as a single agent usage.

Disease	Application	Immunotherapy agent	Indications	Companion diagnostic test (Developer)
NSCLC	1st. line	Pembrolizumab	TPS ≥50% in FDA approval test	PD-L1 IHC 22C3 pharmDx (Dako)
	2nd. line	Pembrolizumab	TPS ≥1% in FDA approval test	PD-L1 IHC 22C3 pharmDx (Dako)
	2nd line	Nivolumab	All-comers	PD-L1 IHC 28–8 pharmDx (Dako)
	2nd line	Atezolizumab	All-comers	PD-L1 IHC SP142 assay (Ventana)
Gastroesophageal cancer	1st line	Pembrolizumab	CPS ≥1 in FDA approval test	PD-L1 IHC 22C3 pharmDx (Dako)
HCC	2nd line	Pembrolizumab	All-comers	PD-L1 IHC 22C3 pharmDx (Dako)
	2nd line	Nivolumab	All-comers	PD-L1 IHC 28–8 pharmDx (Dako)
Head and neck squamous cell cancer	2nd line	Pembrolizumab	All-comers	PD-L1 IHC 22C3 pharmDx (Dako)
Melanoma	2nd line	Nivolumab	All-comers	PD-L1 IHC 28–8 pharmDx (Dako)

CPS = combined positive score, FDA = US Food and Drug Administration, HCC = hepatocellular carcinoma, IHC = immunohistochemistry, NSCLC = nonsmall cell lung cancer, TPS = tumor proportion score.

2. Material and methods

2.1. Study population

We performed a retrospective study from November 2017 to June 2018 using the NSCLC database at Pusan National University Hospital (a university-affiliated tertiary referral hospital in Busan, Republic of Korea). During the study period, 59 patients underwent surgery following a histopathological diagnosis of lung cancer. Of these, 46 subjects with PD-L1 IHC staining of small biopsy and surgical samples were included in this study. Eligible patients for this study were those who underwent surgery and biopsy in our institution and were able to undergo PD-L1 immunohistochemical staining using both specimens. However, patients who were unsuitable for PD-L1 immunohistochemical staining due to a small amount of tissue, or who did not have surgical or biopsy tissue available, were excluded from the study. Considering the retrospective nature of the study, the Institutional Review Board of Pusan National University Hospital approved this study with no additional patient consent required.

2.2. Biopsy methods

EBUS-GS, EBUS-TBNA, bronchoscopic biopsy, and PCNA were performed on patients suspected of having lung cancer. The biopsy method was selected based on the location of the lesion, ease of the procedure, and systemic condition of the patient. Endobronchial lesions were generally approached by flexible bronchoscopy, and EBUS-TBNA was performed for central tumors or lymph nodes adjacent to the bronchial tree.[22] EBUS-GS was performed for peripheral lung lesions with the bronchus sign, which was defined as identification of a peripheral bronchus leading to a lung lesion on an axial computed tomography (CT) scan, and PCNA was performed if there was no bronchus sign.[23,24]

2.3. PD-L1 IHC assay

Immunostaining was conducted on tumor specimens using the 22C3 PharmDx kit (Agilent Technologies Carpentaria, CA) or the Ventana SP142 or SP263 antibody clones (Ventana Medical Systems Inc., Tucson, AZ) according to the manufacturer's manuals. Paraffin blocks with more than 100 tumor cells were selected on hematoxylin and eosin-stained slides, and tumor cell areas were counted under different magnifications for the PD-L1 test. The results were interpreted under low- and high-power fields, as staining of weak intensity was considered to be positive for all 3 antibodies; the overall percentage was used in the analyses. The cut-off values of the 3 PD-L1 IHC results were 1%, 5%, 10%, and 50%, based on previous studies (Supplement table 1, http://links.lww.com/MD/C903).[5–7,12,25–30] And, features of the 3 PD-L1 assays were described in Table 2. The tumor proportion score was used for the 22C3 PD-L1 assay,[7,12] and the tumor expression score was used to interpret the SP142 and SP263 assays.[8,31,32] Representative figures for each cut-off value using SP263 PD-L1 assay were shown in Figure 1.

2.4. Statistical analysis

Variables are reported as numbers (%) or medians (interquartile range [IQR]), as appropriate. Agreement analyses between small biopsy samples and surgical specimens were conducted using Cohen's κ statistic.[33,34] Specificity, sensitivity, negative predictive value, and positive predictive value were analyzed using exact binomial confidence intervals. All statistical analyses were conducted using SPSS version 22.0 for Windows software (SPSS Inc., Chicago, IL). A P-value $< .05$ was considered significant.

3. Results

3.1. Patients

Table 3 lists the baseline characteristics of the 46 study subjects: 35 patients were male (76%) and the median age was 69 years (IQR, 64–73 years). Pathological diagnoses were as follows: adenocarcinoma in 20 patients (44%), squamous cell carcinoma in 24 (52%), large cell carcinoma in 1 (2%), and pleomorphic carcinoma in 1 (2%). The surgical resection methods were as

Table 2

Features of the 3 PD-L1 IHC assays.

	22C3	SP263	SP142
Scoring target	Viable tumor cells	Viable tumor cells	Viable tumor cells and immune cells
Interpretation of positivity	Membranous staining of any intensity	Membrane and/or cytoplasmic staining of any intensity	Membranous staining of any intensity
Minimum requirement	100 viable tumor cells	100 viable tumor cells	50 tumor cells with associated stroma

IHC = immunohistochemistry, PD-L1 = programmed death-ligand 1.

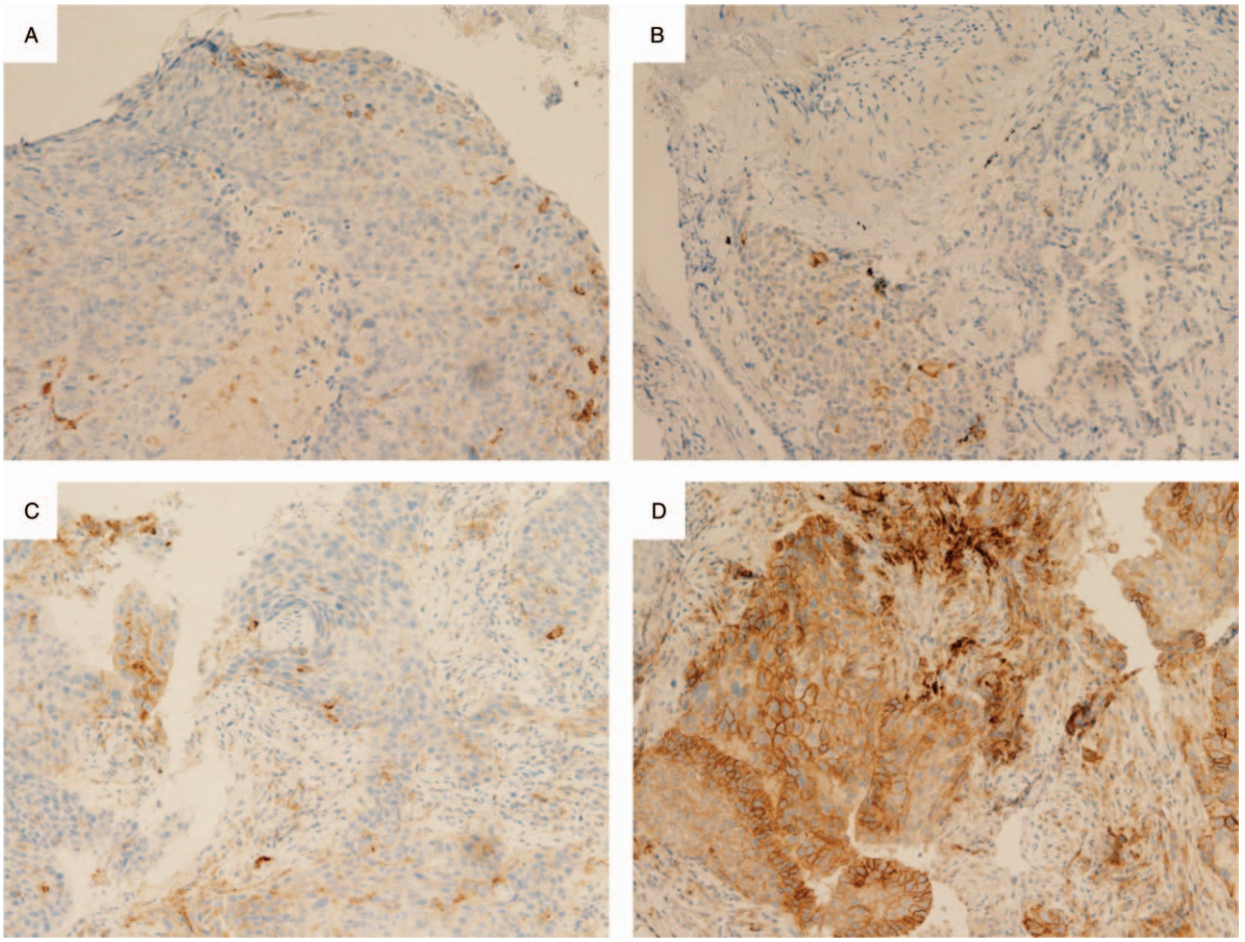

Figure 1. Representative figures by cut-off value from SP263 assay. (A) Positive at 1% cut-off value (×200). (B) Positive at 5% cut-off value in (×200). (C) Positive at 10% cut-off value (×200). (D) Positive at 50% cut-off value (×200). IHC = immunohistochemistry, PD-L1 = programmed death-ligand 1.

Table 3

Baseline characteristics of the 46 study subjects.

Characteristics	No. (%) or median (IQR)
Age, years	69 (64–73)
Male gender	35 (76)
Pathological diagnosis	
Adenocarcinoma	20 (44)
Squamous cell carcinoma	24 (52)
Large cell carcinoma	1 (2)
Pleomorphic carcinoma	1 (2)
Biopsy method	
EBUS-GS	26 (57)
EBUS-TBNA	4 (9)
Bronchoscopic biopsy	12 (25)
PCNA	4 (9)
Surgical resection method	
Lobectomy	34
Sleeve lobectomy	7
Pneumonectomy	1
Segmentectomy	2
Bilobectomy	2
Pathological stage*	
I	18 (39)
II	18 (39)
III	10 (22)
Biopsy site	
Primary site	44 (96)
Lymph node	2 (4)

EBUS-GS = endobronchial ultrasound using a guide sheath, EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration, IQR = interquartile range, PCNA = percutaneous needle lung aspiration.
* Based on the eighth edition of the American Joint Commission on Cancer TNM staging system.

follows: lobectomy in 34 patients (75%), sleeve lobectomy in 7 (15%), pneumonectomy in 1 (2%), segmentectomy in 2 (4%), and bilobectomy in 2 (4%). Twenty-six (57%), 4 (9%), 12 (25%), and 4 (9%) patients underwent EBUS-GS, EBUS-TBNA, bronchoscopic biopsy, and PCNA, respectively (Fig. 2). Forty-four patients (96%) received a biopsy on the primary tumor site. The numbers of patients with stage I, II, and III NSCLC were 18 (39%), 18 (39%), and 10 (22%), respectively, and no patient had stage IV NSCLC. Of the 46 subjects, the 22C3, SP142, and SP263 PD-L1 IHC assays were performed in 26 (57%), 20 (43%), and 46 (100%) patients, respectively (Fig. 3). The median interval between biopsy and operation was 29 days (IQR: 21–35 days). Two patients (4%) received neo-adjuvant therapy.

3.2. 22C3 PD-L1 IHC assay

Among the 26 patients who had the 22C3 PD-L1 IHC assay, the positive rates of the small biopsy samples were 96%, 76%, 69%, and 42% when the cut-off value was 1%, 5%, 10%, or 50%, respectively (Table 4). About 92%, 88%, 85%, and 46% of surgical specimens were positive on the 22C3 PD-L1 IHC assay according to the cut-off values of 1%, 5%, 10%, and 50%, respectively. The agreement rates of the PD-L1 results between the small biopsy samples and the surgical specimens were 96% (kappa coefficient, 0.649; 95% confidence interval [CI], 0.016–1.282), 81% (kappa coefficient, 0.343; 95% CI, −0.090 to

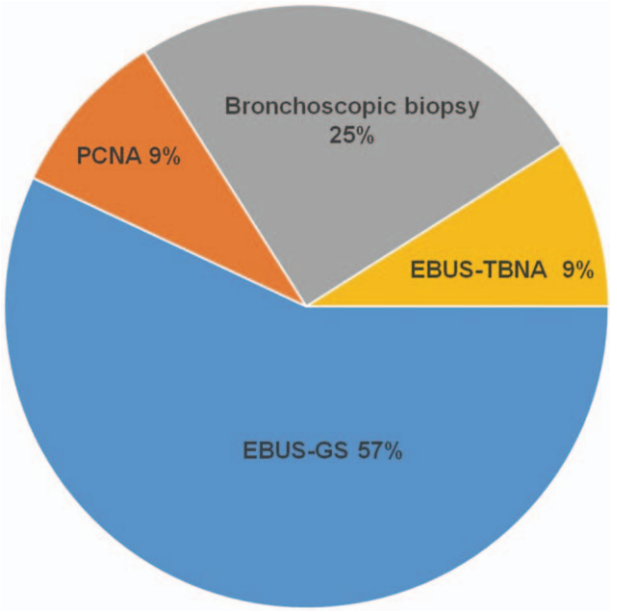

Figure 2. Biopsy methods used for the 46 study subjects. EBUS-GS = endobronchial ultrasound using a guide sheath, EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration, PCNA = percutaneous needle lung aspiration.

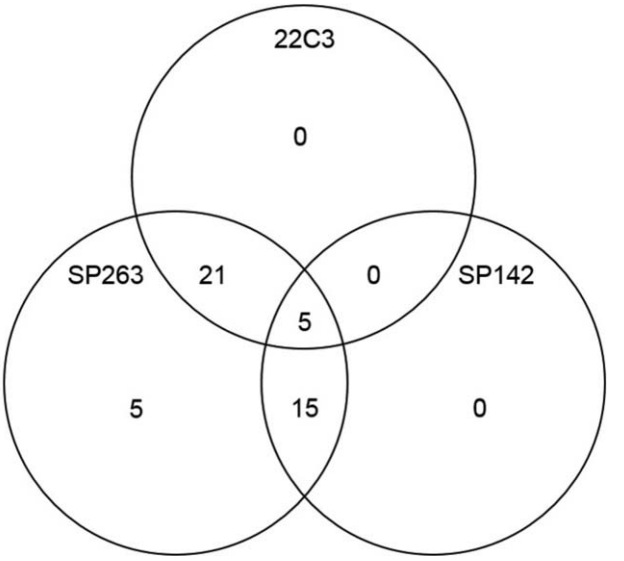

Figure 3. Types of PD-L1 IHC assays performed on the 46 samples. IHC = immunohistochemistry, PD-L1 = programmed death-ligand 1.

0.776), 85% (kappa coefficient, 0.581; 95% CI, 0.238–0.924), and 73% (kappa coefficient, 0.455; 95% CI, 0.112–0.798) when the cut-off value was 1%, 5%, 10%, and 50%, respectively. The sensitivity of the PD-L1 results using small biopsy samples was 100% (95% CI, 0.961–1.000), 83% (95% CI, 0.756–0.867), 82% (95% CI, 0.715–0.818), and 67% (95% CI, 0.420–0.834) when the cut-off value was 1%, 5%, 10%, and 50%, respectively. The specificity of the PD-L1 results using small biopsy samples was 50% (95% CI, 0.028–0.500), 67% (95% CI, 0.132–0.982), 100% (95% CI, 0.435–1.000), and 79% (95% CI, 0.574–0.929) when the cut-off value was 1%, 5%, 10%, and 50%, respectively (Supplement table 2, http://links.lww.com/MD/C903).

3.3. SP142 PD-L1 IHC assay

The positive rates of the SP142 PD-L1 IHC assay using small biopsy samples and surgical specimens were 45% and 35% at a cut-off value of 1%; 40% and 35% at a cut-off value of 5%; 20% and 35% at the cut-off value of 10%; and 10% and 20% at the cut-off value of 50%, respectively (Table 5). The numbers of patients with consistent PD-L1 results between small biopsy samples and surgical specimens were 14 (70%), 15 (75%), 13 (65%), and 16 (80%), and the kappa coefficients were 0.381 (95% CI, −0.021 to 0.783), 0.468 (95% CI, 0.070–0.866), 0.146 (95% CI, −0.281 to 0.573), and 0.231 (95% CI, −0.284 to 0.746) when the cut-off values were 1, 5, 10, and 50%, respectively. The sensitivity of the PD-L1 results using small biopsy samples was 71% (95% CI, 0.353–0.943), 71% (95% CI, 0.354–0.940), 29% (95% CI, 0.055–0.515), and 25% (95% CI, 0.014–0.486) when the cut-off value was 1%, 5%, 10%, and 50%, respectively. The specificity of the PD-L1 results using small biopsy samples was 69% (95% CI, 0.498–0.815), 77% (95% CI, 0.575–0.891), 85% (95% CI, 0.722–0.969), and 94% (95% CI, 0.878–0.997) when the cut-off value was 1%, 5%, 10%, and 50%, respectively (Supplement table 2, http://links.lww.com/MD/C903).

3.4. SP263 PD-L1 IHC assay

Of the 46 patients who participated the SP263 PD-L1 IHC assay, 44 (96%), 38 (83%), 31 (67%), and 16 (35%) patients had

Table 4

Agreement analysis of the 22C3 PD-L1 assay between the small biopsy and surgical specimens.

Cut-off value*	Positive rate†		Agreement rate†	Kappa coefficient (95% CI)
	Small biopsy	Surgery		
≥ 1%	25/26 (96)	24/26 (92)	25/26 (96)	0.649 (0.016–1.282)
< 1%	1/26 (4)	2/26 (8)		
≥ 5%	20/26 (76)	23/26 (88)	21/26 (81)	0.343 (−0.090 to 0.776)
< 5%	6/26 (24)	3/26 (12)		
≥ 10%	18/26 (69)	22/26 (85)	22/26 (85)	0.581 (0.238–0.924)
< 10%	8/26 (31)	4/26 (15)		
≥ 50%	11/26 (42)	12/26 (46)	19/26 (73)	0.455 (0.112–0.798)
< 50%	15/26 (58)	14/26 (54)		

CI = confidence interval, PD-L1 = programmed death-ligand 1.

* Cut-off values are presented with tumor expression score.

† Values are expressed as numbers/total (%).

Table 5

Agreement analysis of the SP142 PD-L1 assay between the small biopsy and surgical specimens.

Cut-off value*	Positive rate†		Agreement rate†	Kappa coefficient (95% CI)
	Small biopsy	Surgery		
≥ 1%	9/20 (45)	7/20 (35)	14/20 (70)	0.381 (−0.021 to 0.783)
< 1%	11/20 (55)	13/20 (65)		
≥ 5%	8/20 (40)	7/20 (35)	15/20 (75)	0.468 (0.070–0.866)
< 5%	12/20 (60)	13/20 (65)		
≥ 10%	4/20 (20)	7/20 (35)	13/20 (65)	0.146 (−0.281 to 0.573)
< 10%	16/20 (80)	13/20 (65)		
≥ 50%	2/20 (10)	4/20 (20)	16/20 (80)	0.231 (−0.284 to 0.746)
< 50%	18/20 (90)	16/20 (80)		

CI = confidence interval, PD-L1 = programmed death-ligand 1.

* Cut-off values are presented with tumor expression score.

† Values are expressed as numbers/total (%).

positive small biopsy specimens at cut-off values of 1%, 5%, 10%, and 50%, respectively (Table 6). The surgical specimens were positive in 42 (91%), 35 (76%), 34 (74%), and 19 patients (41%), respectively. When the cut-off values were 1, 5, 10, and 50%, 42 (91%), 33 (72%), 35 (76%), and 37 (80%) patients had consistent results, and their kappa values were 0.292 (95% CI, −0.206 to 0.790), 0.143 (95% CI, −0.170 to 0.457), 0.426 (95% CI, 0.144–0.708), and 0.587 (95% CI, 0.348–0.826), respectively. The sensitivity of the PD-L1 results using small biopsy samples was 98% (95% CI, 0.954–0.999), 86% (95% CI, 0.796–0.933), 79% (95% CI, 0.697–0.866), and 68% (95% CI, 0.497–0.795) when the cut-off value was 1%, 5%, 10%, and 50%, respectively. The specificity of the PD-L1 results using small biopsy samples was 25% (95% CI, 0.013–0.486), 27% (95% CI, 0.078–0.515), 67% (95% CI, 0.391–0.872), and 89% (95% CI, 0.757–0.967) when the cut-off value was 1%, 5%, 10%, and 50%, respectively (Supplement table 2, http://links.lww.com/MD/C903). A representative figure of the discordant results according to the cut-off values for the SP263 PD-L1 IHC assay is shown in Figure 4.

4. Discussion and conclusions

In this study, we found relatively good agreement between small biopsy samples and surgical specimens in the 3 types of PD-L1 tests. Some previous studies have evaluated the performance of PD-L1 biomarkers and related specimens,[17,35–38] but to date no study has compared the results of 3 commercially available PD-L1 assays (22C3, SP263, and SP142 PD-L1 IHC assays) on surgical specimens and small biopsy samples. Our results suggest that the 3 commercially available PD-L1 assays are relatively accurate even when performed using small biopsy samples, compared with surgical specimens as the reference.

Kitazono et al[19] reported that PD-L1 IHC with the polyclonal clone (catalog no. 4059, dilution 1:1600; ProSci, Inc., Poway, CA) using a hybrid score had a good concordance rate of 92% between 79 paired surgically resected specimens and small biopsy samples collected by EBUS-TBNA, bronchoscopic biopsy, or PCNA. Sakakibara et al[35] found that the concordance rate of PD-L1 expression using a PD-L1 rabbit monoclonal antibody [clone EPR1161(2), dilution 1:200; Abcam PLC, Cambridge, UK] between EBUS-TBNA and matched surgical specimens was 75% in a study population of six patients. However, the antibodies used in those studies were developed for research purposes and were not approved for diagnostic and therapeutic purposes.

Heymann et al[36] reported that the concordance rate of the 22C3 PD-L1 assay between surgical specimens and paired small biopsy samples using EBUS-TBNA, bronchoscopic biopsy, or PCNA was 100% in a study population of six patients with NSCLC. Although small biopsy samples and surgical specimens were completely correlated in the 22C3 PD-L1 assay, the number of study subjects was too small to deduce conclusive results. Sakata et al[37] reported that the concordance rates of the 22C3 PD-L1 IHC assay between EBUS-TBNA samples and surgical specimens were 87% and 82% according to cut-off values ≥ 1% and ≥ 50%, respectively, which did not differ significantly from our results (96% and 73% concordance using cut-off values of ≥

Table 6

Agreement analysis of the SP263 PD-L1 assay between the small biopsy and surgical specimens.

Cut-off value*	Positive rate†		Agreement rate†	Kappa coefficient (95% CI)
	Small biopsy	Surgery		
≥ 1%	44/46 (96)	42/46 (91)	42/46 (91)	0.292 (−0.206 to 0.790)
< 1%	2/46 (4)	4/46 (9)		
≥ 5%	38/46 (83)	35/46 (76)	33/46 (72)	0.143 (−0.170 to 0.457)
< 5%	8/46 (17)	11/46 (24)		
≥ 10%	31/46 (67)	34/46 (74)	35/46 (76)	0.426 (0.144–0.708)
< 10%	15/46 (33)	12/46 (26)		
≥ 50%	16/46 (35)	19/46 (41)	37/46 (80)	0.587 (0.348–0.826)
< 50%	30/46 (65)	27/46 (59)		

CI = confidence interval, PD-L1 = programmed death-ligand 1.

* Cut-off values are presented with tumor expression score.

† Values are expressed as numbers/total (%).

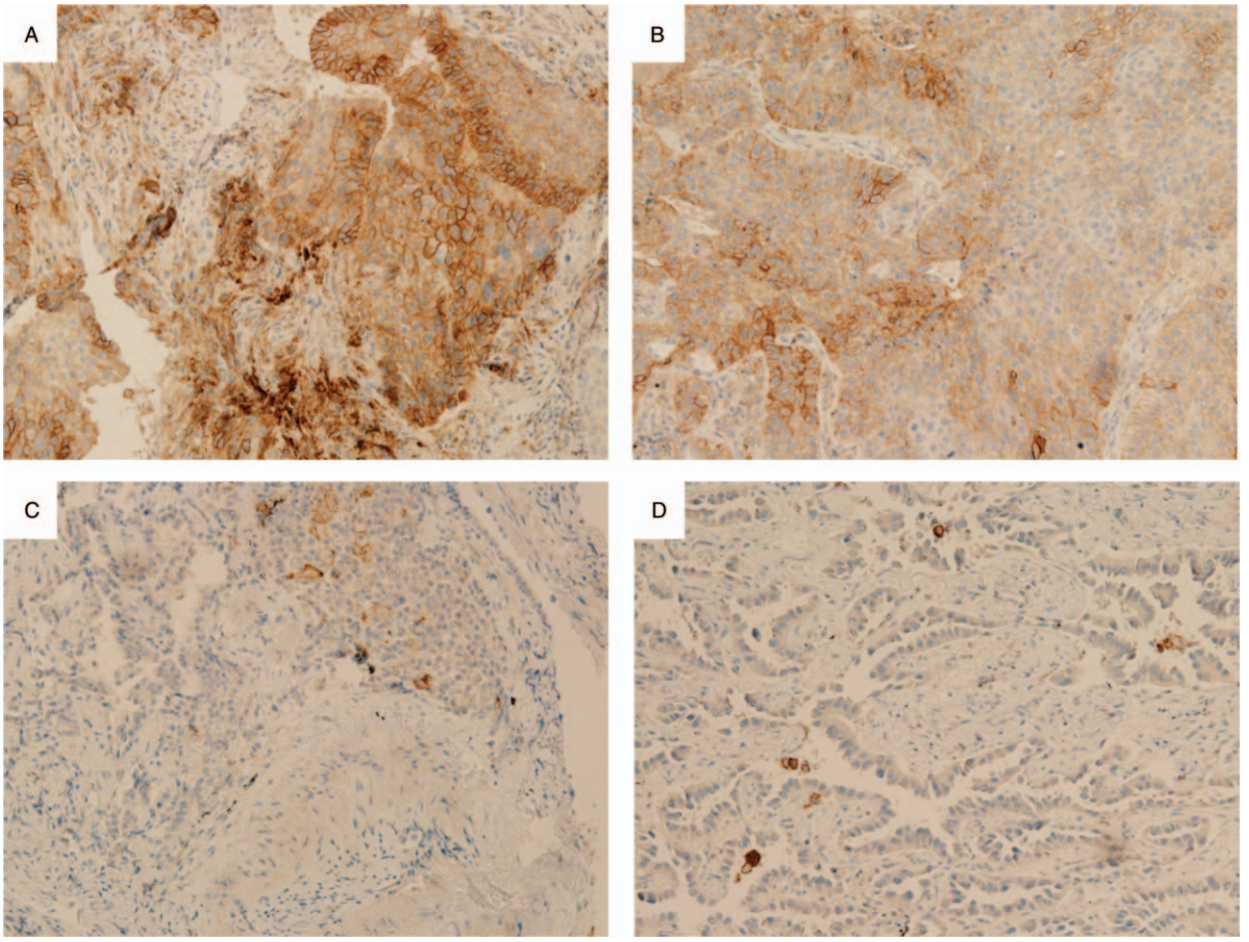

Figure 4. Representative figures of the surgical and biopsy specimens were compared by categorized PD-L1 IHC study result using SP263 assay. (A, B) Concordance cases between 2 samples (A: Biopsy sample, B: Surgical specimen, ×200). (C, D) Discordance cases between 2 samples (C: Biopsy sample, D: Surgical specimen, ×200). IHC = immunohistochemistry, PD-L1 = programmed death-ligand 1.

1% and ≥ 50%, respectively). However, their study provided limited information about the single 22C3 PD-L1 IHC assay and only used 2 cut-off values of ≥ 1% and ≥ 50%. Ilie et al[38] compared the results of a PD-L1 study between surgically resected samples and corresponding small biopsy (EBUS-TBNA, bronchoscopic biopsy, and PCNA) specimens using tumor cells or tumor-infiltrating immune cell scores of the SP142 PD-L1 IHC assay, and the concordance rate was 52% in 160 patients. However, they estimated the agreement of the immunostaining scores without a cut-off value; therefore, interpretation of their results may be ambiguous.

The present study had several limitations. First, it was a single-center study with a small number of patients. Second, because it used surgical specimens as a reference, only patients with operable early lung cancer were included. Immune checkpoint inhibitors are generally used as palliative treatment in patients with advanced NSCLC, so the results of this study may differ from real-world data. However, patients with advanced stage disease have an increased tumor burden and size compared to patients with early stage disease.[39] Therefore, although the amount of tissue obtained from a small biopsy is limited, the accuracy of the PD-L1 study is expected to improve in patients with advanced NSCLC. Third, because this study was conducted retrospectively, the relationship between the volume of the sample and the quantitative PD-L1 results, including the number of tumor cells, could not be determined. Fourth, this study was retrospective, and it was not possible to propose a cut-off value because the study population was small and data on survival and disease progression were not collected. Fifth, this study was retrospective in nature and the design was inadequate for further analysis of tumor heterogeneity, as tissue samples were not obtained from various sites during the histological examination.[40,41] These limitations will need to be verified in future multicenter prospective studies with a larger number of subjects.

In conclusion, the results of 3 commercially available PD-L1 assays (22C3, SP142, and SP263) using small biopsy samples obtained by minimally invasive methods were reliable compared with those using surgical specimens in patients with NSCLC.

Author contributions

Conceptualization: Insu Kim, Ahrong Kim, Hye-Kyung Park, Min Ki Lee, Jung Seop Eom.
Data curation: Insu Kim, Ahreum Kim.
Investigation: Insu Kim, Ahrong Kim, Geewon Lee, Mi-Hyun Kim, Ki Uk Kim, Jung Seop Eom.
Methodology: Ahrong Kim, Geewon Lee, Jeongha Mok.
Project administration: Chang Hun Lee, Jung Seop Eom.
Resources: Eun Jung Jo.
Supervision: Mi-Hyun Kim, Jung Seop Eom.

Validation: Jeongha Mok, Kwangha Lee.
Writing – original draft: Insu Kim.
Writing – review & editing: Ahrong Kim, Jung Seop Eom.

References

[1] Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Adv Exp Med Biol 2016;893:1–9.
[2] Reck M, Rabe KF. Precision diagnosis and treatment for advanced non-small-cell lung cancer. N Engl J Med 2017;377:849–61.
[3] Davies J, Patel M, Gridelli C, et al. Real-world treatment patterns for patients receiving second-line and third-line treatment for advanced non-small cell lung cancer: a systematic review of recently published studies. PLoS One 2017;12:e0175679.
[4] Economopoulou P, Mountzios G. The emerging treatment landscape of advanced non-small cell lung cancer. Ann Transl Med 2018;6:138.
[5] Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627–39.
[6] Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015;373:123–35.
[7] Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540–50.
[8] Fehrenbacher L, Spira A, Ballinger M, 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 2016;387:1837–46.
[9] Shih K, Arkenau HT, Infante JR. Clinical impact of checkpoint inhibitors as novel cancer therapies. Drugs 2014;74:1993–2013.
[10] Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy-inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res 2012;18:6580–7.
[11] Villaruz LC, Kalyan A, Zarour H, et al. Immunotherapy in lung cancer. Transl Lung Cancer Res 2014;3:2–14.
[12] Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018–28.
[13] Teixido C, Vilarino N, Reyes R, et al. PD-L1 expression testing in non-small cell lung cancer. Ther Adv Med Oncol 2018;10:1758835918763493.
[14] Fuchs CS, Doi T, Jang RW, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. JAMA Oncol 2018;10:4.
[15] El-Khoueiry AB, Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 2017;24:2492–502.
[16] Cree IA, Booton R, Cane P, et al. PD-L1 testing for lung cancer in the UK: recognizing the challenges for implementation. Histopathology 2016;69:177–86.
[17] Gridelli C, Ardizzoni A, Barberis M, et al. Predictive biomarkers of immunotherapy for non-small cell lung cancer: results from an Experts Panel Meeting of the Italian Association of Thoracic Oncology. Transl Lung Cancer Res 2017;6:373–86.
[18] Sholl LM, Aisner DL, Allen TC, et al. Programmed death ligand-1 immunohistochemistry—a new challenge for pathologists: a perspective from members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2016;140:341–4.
[19] Kitazono S, Fujiwara Y, Tsuta K, et al. Reliability of small biopsy samples compared with resected specimens for the determination of programmed death-ligand 1 expression in non-small-cell lung cancer. Clin Lung Cancer 2015;16:385–90.
[20] Li C, Huang C, Mok TS, et al. Comparison of 22C3 PD-L1 expression between surgically resected specimens and paired tissue microarrays in non-small cell lung cancer. J Thorac Oncol 2017;12:1536–43.
[21] Hiley CT, Le Quesne J, Santis G, et al. Challenges in molecular testing in non-small-cell lung cancer patients with advanced disease. Lancet 2016;388:1002–11.
[22] Wahidi MM, Herth F, Yasufuku K, et al. Technical aspects of endobronchial ultrasound-guided transbronchial needle aspiration: CHEST guideline and expert panel report. Chest 2016;149:816–35.
[23] Gaeta M, Pandolfo I, Volta S, et al. Bronchus sign on CT in peripheral carcinoma of the lung: value in predicting results of transbronchial biopsy. AJR Am J Roentgenol 1991;157:1181–5.
[24] Ali MS, Sethi J, Taneja A, et al. Computed tomography bronchus sign and the diagnostic yield of guided bronchoscopy for peripheral pulmonary lesions: a systematic review and meta-analysis. Ann Am Thorac Soc 2018;15:978–87.
[25] Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563–7.
[26] Carbone DP, Reck M, Paz-Ares L, et al. First-Line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–26.
[27] Hendry S, Byrne DJ, Wright GM, et al. Comparison of four PD-L1 immunohistochemical assays in lung cancer. J Thorac Oncol 2018;13:367–76.
[28] Marchetti A, Barberis M, Franco R, et al. Multicenter comparison of 22C3 PharmDx (Agilent) and SP263 (Ventana) assays to test PD-L1 expression for NSCLC patients to be treated with immune checkpoint inhibitors. J Thorac Oncol 2017;12:1654–63.
[29] Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627–39. 22.
[30] Fehrenbacher L, Spira A, Ballinger M, 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 2016;387:1837–46. 30.
[31] Hersom M, Jorgensen JT. Companion and complementary diagnostics-focus on PD-L1 expression assays for PD-1/PD-L1 checkpoint inhibitors in non-small cell lung xancer. Ther Drug Monit 2018;40:9–16.
[32] Ratcliffe MJ, Sharpe A, Midha A, et al. Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cut-offs in non-small cell lung cancer. Clin Cancer Res 2017;23:3585–91.
[33] McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22:276–82.
[34] Park CU, Kim HJ. Measurement of inter-rater reliability in systematic review. Hanyang Med Rev 2015;35:44–9.
[35] Sakakibara R, Inamura K, Tambo Y, et al. EBUS-TBNA as a promising method for the evaluation of tumor PD-L1 expression in lung cancer. Clin Lung Cancer 2017;18:527–34. e1.
[36] Heymann JJ, Bulman WA, Swinarski D, et al. PD-L1 expression in non-small cell lung carcinoma: comparison among cytology, small biopsy, and surgical resection specimens. Cancer Cytopathol 2017;125:896–907.
[37] Sakata KK, Midthun DE, Mullon JJ, et al. Comparison of programmed death ligand-1 immunohistochemical staining between endobronchial ultrasound transbronchial needle aspiration and resected lung cancer specimens. Chest 2018;154:827–37.
[38] Ilie M, Long-Mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. Ann Oncol 2016;27:147–53.
[39] Detterbeck FC, Boffa DJ, Kim AW, et al. The eighth edition lung cancer stage classification. Chest 2017;151:193–203.
[40] Casadevall D, Clavé S, Taus Á, et al. Heterogeneity of tumor and immune cell PD-L1 expression and lymphocyte counts in surgical NSCLC samples. Clin Lung Cancer 2017;18:682–91.
[41] Nakamura S, Hayashi K, Imaoka Y, et al. Intratumoral heterogeneity of programmed cell death ligand-1 expression is common in lung cancer. PLoS One 2017;12: 19.