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

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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. The PD-L1 testing with 3 commercial PD-L1 IHC assays using small biopsy samples is reliable in patients with nonsmall cell 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.[5,11] New therapeutic strategies, including target agents or immunotherapy, are improving overall and progression-free survival in patients with advanced NSCLC.[5] 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.[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.
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 k statistic.[33,34] Specitivity, 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 1

| 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.

### Table 2

| Scoring target | Interpretation of positivity | Minimum requirement |
|----------------|------------------------------|---------------------|
| 22C3           | Viable tumor cells           | 100 viable tumor cells |
| SP263          | Membranous staining of any intensity | 100 viable tumor cells |
| SP142          | Membranous staining of any intensity | 50 tumor cells with associated stroma |

HCC = immunohistochemistry, PD-L1 = programmed death-ligand 1.
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 0.813).
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, 0.498–0.813), 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...
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,[17,33–38] 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[33] 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[33] 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 ≥

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

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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**

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