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RESEARCH

Comparison of programmed death ligand-1 expression in preoperative transbronchial lung biopsy and resected specimens in non-small cell lung cancer

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Abstract

Purpose Programmed death-ligand 1 expression is heterogeneous in non-small cell lung cancer, and small specimens may not accurately represent the entire tumor. The current study investigated the discordance in programmed death-ligand 1 expression between preoperative biopsy samples and resected specimens.

Methods We retrospectively collected data of patients with non-small cell lung cancer who underwent surgical resection from May 2022 to June 2024. The programmed death-ligand 1-positive tumor proportion score was evaluated for each case.

Results In total, 118 patients were included in this study. Programmed death-ligand 1 expression was discordant between the biopsy and resected specimens in 34 cases (28.8%), and it was underestimated in 25 (21.2%) biopsy specimens. The concordance according to Cohen's kappa was $\kappa = 0.410$ (95% confidence interval: 0.243–0.577). The number of discordant cases decreased as the number of tumor cells in biopsy specimens increased. In the group with >400 tumor cells, agreement rate was 100%. The least absolute shrinkage and selection operator model identified never smoker, small tumor size, clinical stage II-IV and ≤200 tumor cells in biopsy specimens as predictors of underestimation. The area under the receiver operating characteristic curve using those four factors was 0.773 (0.663–0.884).

Conclusions Programmed death-ligand 1 expression in biopsy and resected specimens is often discordant, often being underestimated in biopsy specimens. Discordance is more likely when tumor cell counts are low in the biopsy samples. Therefore, caution is advised when treatment decisions are made based on programmed death-ligand 1 assessments of small specimens.

Keywords Biopsy · Immunohistochemistry · Non-small cell lung cancer · Programmed cell death 1 ligand 1 protein · Thoracic surgery

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Abbreviations

ICI	Immune checkpoint inhibitors
PD-1	Programmed death-1
PD-L1	Programmed death ligand-1
NSCLC	Non-small cell lung cancer
IHC	Immunohistochemistry
TC	Tumor cell
TPS	Tumor proportion score
LASSO	Least absolute shrinkage and selection operator
CT	Computed tomography
CTR	Consolidation to tumor ratio
SUV	Standard uptake value
PET	Positron emission tomography
ROC	Receiver operating characteristic
EGFR	Epidermal growth factor receptor

Introduction

Immune checkpoint inhibitors (ICIs) targeting the programmed cell death 1/programmed death-ligand 1 (PD-1/PD-L1) pathway have shown remarkable clinical benefits in the treatment of metastatic and locally advanced non-small cell lung cancer (NSCLC) (Herbst et al. 2016; Brahmer et al. 2015; Borghaei et al. 2015; Garon et al. 2015; Fehrenbacher et al. 2016). In addition, the clinical benefits of neoadjuvant and adjuvant ICIs have been demonstrated in recent years (Forde et al. 2022; Felip et al. 2021). PD-L1 expression in tumor cells (TCs), determined using an immunohistochemistry assay, is a biomarker that can predict the efficacy of PD-1/PD-L1 inhibitor (Abdel-Rahman 2016). Therefore, accurate PD-L1 analysis is indispensable for appropriate treatment.

As the indications for ICIs expand, PD-L1 expression is being evaluated in many situations; however, most of these evaluations are performed on small tissue samples. More than 65% of patients with NSCLC have locally advanced or metastatic disease upon initial presentation (Morgensztern et al. 2010), and PD-L1 testing of such patients are mainly performed on biopsy samples. Moreover, clinical trials on neoadjuvant or perioperative ICIs for patients with resectable NSCLC have been conducted or are ongoing (Peng et al. 2023), and the opportunity to evaluate PD-L1 expression in preoperative biopsy samples is increasing. In CheckMate 816, an open-label phase 3 trial, neoadjuvant nivolumab plus chemotherapy resulted in longer event-free survival and a higher percentage of patients with a pathological complete response (pCR). In that trial, patients with a tumor PD-L1 expression level of 1% or more had a longer event-free survival (hazard ratio: 0.41, 95% confidence interval [CI]: 0.24–0.70) than those with a level of less than 1% (hazard ratio: 0.85, 95% CI: 0.54–1.32) (Forde et al. 2022). Those results indicated that PD-L1 expression according to preoperative samples are predictive of the response to preoperative ICI therapy. However, in the CheckMate 816 study the pCR rate in PD-L1-negative patients was 16.7% for PD-1 inhibitors plus chemotherapy and 2.6% for chemotherapy alone, supporting the efficacy of ICIs for PD-L1-negative patients and indicating that the evaluation of PD-L1 expression is uncertain. In the Keynote 671 study, a randomized, double-blind, phase 3 trial evaluating perioperative pembrolizumab, the relative benefit in the pembrolizumab group increased with increasing PD-L1 expression, but even in cases with PD-L1 TPS < 1% the hazard ratio favored the pembrolizumab group (0.77) (Spicer et al. 2024).

Of note, PD-L1 expression is reportedly heterogeneous in NSCLC (Ilie et al. 2016; Hendry et al. 2018). If the biopsy sample is not representative of the entire tumor, the results of PD-L1 expression will be inaccurate. Knowing the frequency of discordance in PD-L1 expression between biopsy and resected specimens would help to interpret the results of clinical trials for application in clinical practice. In addition, the clinical and pathological factors leading to the discordance between biopsy and resected specimens have not been examined in detail.

To evaluate PD-L1 expression, specimens must contain a minimum of 100 viable TCs (Tsao et al. 2017). However, the number of viable TCs required in a small sample to represent the PD-L1 expression of the entire tumor has not been reported.

In this study, we compared PD-L1 expression in preoperative biopsy samples and resected specimens of the same tumors with the aim to examine the frequency of discordance between them, factors leading to underestimation of PD-L1 expression in biopsy samples, and the association between such discordance and the TC count.

Materials and methods

Study cohort

Patients diagnosed with NSCLC upon preoperative transbronchial biopsy who underwent surgical resection at the Kanagawa Cancer Center (Japan) were included in this study. We retrospectively collected data from 225 patients with a histological diagnosis based on preoperative transbronchial biopsies who underwent surgery from May 2022 to June 2024. PD-L1 expression was routinely evaluated using both biopsy and resected specimens for all patients, regardless of the pathological stage, as long as the sample was large enough to test PD-L1 expression, and patient consent for PD-L1 testing was obtained. Patients who received any type of preoperative therapy and those for whom

biopsies were performed not on the main tumor but on the metastatic lymph nodes were excluded. Patient data were obtained from electronic medical records. Ethical approval for this study was obtained from the Kanagawa Cancer Center (no. 2023 − 149).

Immunohistochemical analysis

PD-L1 immunostaining was performed on only one section selected by pathologists. For biopsy specimens, sections with high TC counts were selected, except when genetic testing was prioritized. For resected specimens, the section with the largest diameter of the invasive part of the tumor was selected. If that diameter spanned more than one section, the section with the largest area of invasive part was selected. Immunohistochemistry was performed using formalin-fixed, paraffin-embedded tissue blocks that were cut into serial 4-µm-thick sections and deparaffinized. Immunostaining with 22C3 pharmDx (Dako; Agilent Technologies, Santa Clara, CA, USA) was performed using the Dako Autostainer Link 48 platform. Each sample was stained according to the manufacturer's instructions. At the same time as the specimens, lung tissues that were confirmed positive or negative for PD-L1 were stained as controls. A trained pathologist evaluated the TPS for each patient. Cutoff values of 1% and 50%, commonly used in clinical practice and previous clinical trials, were used. We defined a PD-L1 TPS of less than 1% as negative and that of 1% or more as positive. Cases negative for PD-L1 in biopsy specimens and positive for PD-L1 in resected specimens were defined as "underestimated cases," and those positive in biopsy specimens and negative in resected specimens as "overestimated cases." For biopsy specimens, the number of TCs in the sections selected for PD-L1 staining was counted.

Statistical analysis

To evaluate PD-L1 expression in biopsy and resected specimens, Cohen's κ coefficient of agreement was calculated. κ values less than 0.60 was defined as weak agreement, 0.60 to 0.79 as moderate, 0.80 to 0.90 as strong, and above 0.90 as almost perfect agreement (McHugh 2012). Fisher's exact test was used to examine whether each clinical or pathological factor was associated with underestimation of PD-L1 expression. To identify clinical and pathological factors that predict such underestimation while controlling for model overfitting, we used a least absolute shrinkage and selection operator (LASSO) logistic model, adjusting for smoking status (current or former/never), tumor size on a computed tomography (CT) scan, the consolidation-to-tumor ratio (CTR), the tumor diameter at the mediastinal/lung window (< 1/≥1), clinical stage (I/II/III-IV), the maximum standard uptake value upon positron emission tomography-CT (PET-CT), the histological diagnosis of biopsy (adenocarcinoma or "NSCLC favor adenocarcinoma"/others), and the TC count in biopsy specimens (≤200/>200). The cutoff value of 200 for the number of TCs was determined based via a receiver operating characteristic (ROC) curve. A ROC curve was generated based on whether TC counts were used to predict underestimated cases. The cut-off value was 200 TCs and the area under the curve was 0.601 with a 95% confidence interval of 0.485–0.717. Age, tumor size on CT scan, CT, and the maximum standard uptake value upon PET-CT were included in the LASSO analysis as a continuous variables. To evaluate the predictive accuracy of the factors selected via the LASSO model, a ROC curve was generated. A p value less than 0.05 was considered statistically significant. All statistical analyses were performed using EZR software, version 1.61 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (Kanda 2013) and R version 4.4.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient and tumor characteristics

A total of 118 patients were included in this analysis. Their clinicopathological features are summarized in Table 1. The median patient age was 72 years, and 77 males and 41 females were included. Ninety current or former smokers and 28 never-smokers were included. The median tumor size on CT scans was 34 mm, with a median CTR of 1. The clinical stages were as follows: 60 cases were stage I, 30 were stage II, 27 were stage III, and 1 was stage IV. The number of patients with TC counts of less than 100, 100–200, 201–300, 301–400, and more than 400 were 3, 52, 24, 9, and 16, respectively. In terms of histological subtypes, 75 cases were adenocarcinomas, 40 were squamous-cell carcinomas, 2 were large-cell carcinomas, and 1 was pleomorphic carcinoma. The pathological stages were as follows: 47 cases were stage I, 37 were stage II, 30 were stage III, and 4 were stage IV.

PD-L1 immunohistochemical staining

Immunohistochemical PD-L1 expression in the biopsy and resected specimens is presented in Table 2. PD-L1-positivity was identified in 63 (53.4%) biopsy specimens and 79 (66.9%) resection specimens. The prevalence of PD-L1 positivity in resected specimens was higher in squamous-cell carcinomas (72.5%) than that in adenocarcinomas (64.0%). No significant difference was observed between adenocarcinomas with

Table 1 Characteristics of 118 patients with NSCLC included in the study

Variables	*n* (%)
Age, median (range)	72 (42–92)
Sex, male/female	77 (65.3)/41 (34.7)
Smoking status, current or former/never	90 (76.3)/28 (23.7)
Tumor size on CT (mm), median (range)	34 (12–110)
CTR, median (range)	1 (0.43–1.00)
Tumor diameter at the mediastinal/lung window, < 1/≥1	85 (72.0)/33 (28.0)
cStage, I/II/III/IV	60 (50.8)/30 (25.4)/27 (22.9)/1 (0.8)
SUVmax with PET-CT (*n* = 110), median (range)	11 (0–29)
Histological diagnosis of biopsy, adenocarcinoma or "NSCLC favor adenocarcinoma"/ others	70 (59.3)/48 (40.7)
TC count of biopsy specimens (*n* = 104), < 100/100–200/201–300/301–400/>400	3 (2.9)/52 (50.0)/24 (23.1)/9 (8.7)/16 (15.4)
Histology of resected specimens, adenocarcinoma/squamous-cell carcinoma/large-cell carcinoma/pleomorphic carcinoma	75 (63.6)/40 (33.9)/2 (1.7)/1 (0.8)
pStage, I/II/III/IV	47 (39.8)/37 (31.4)/30 (25.4)/4 (3.4)

CT, computed tomography; CTR, consolidation-to-tumor ratio; NSCLC, non-small cell lung cancer; PET, positron emission tomography; SUVmax, maximum standardized uptake value; TC, tumor cell

Table 2 Overall PD-L1 positivity according to histology using a cut-off value of 1%

Histology (*n*)	Biopsied specimens		Resected specimens	
	Positive *n* (%)	Negative *n* (%)	Positive *n* (%)	Negative *n* (%)
All (118)	63 (53.4)	55 (46.6)	79 (66.9)	39 (33.1)
Adenocarcinoma (75)	34 (45.3)	41 (54.7)	48 (64.0)	27 (36.0)
Lepidic component (41)	16 (39.0)	25 (61.0)	25 (61.0)	16 (39.0)
EGFR mutation+ (22)	5 (22.7)	17 (77.3)	14 (63.6)	8 (36.4)
EGFR wild-type (53)	29 (54.7)	24 (45.3)	34 (64.2)	19 (35.8)
Squamous-cell carcinoma (40)	27 (67.5)	13 (32.5)	29 (72.5)	11 (27.5)
Large-cell carcinoma (2)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
Pleomorphic carcinoma (1)	1 (100)	0	1 (100)	0

EGFR, epidermal growth factor receptor; PD-L1, programmed death-ligand 1

epidermal growth factor receptor (*EGFR*) mutations (63.6%) and those without (64.2%) in resected specimens. In both adenocarcinomas and squamous-cell carcinomas, the prevalence of PD-L1 positivity was higher in the resected specimens than that in the biopsy specimens. Among adenocarcinomas, with biopsy specimens, 34 (45.3%) cases were positive, and with resected specimens 48 (64.0%) cases were positive. Among squamous-cell carcinomas, with biopsy specimens, 27 (67.5%) cases were positive and with resected specimens 29 (72.5%) cases were positive. In cases of adenocarcinoma with *EGFR* mutations, there was a discrepancy in the percentage of PD-L1-positive observations between biopsy (22.7%) and resected (63.6%) specimens. In adenocarcinoma cases with a lepidic component, PD-L1 positivity was 39% in biopsy specimens and 61% in resected specimens. Percentages of underestimation in cases with and without a lepidic component are shown in Supplemental Data 1. More cases with lepidic components were underestimated compared to those without a lepidic component, although the association was not significant (31.7% vs. 15.6%; $p = 0.058$). Overall PD-L1 positivity using cut off values of ≥50%, 1–49%, and <1% are shown in Supplemental Data 2. As with the 1% cutoff, with the 50% cutoff there were more positive resected specimens than positive biopsy specimens. However, the difference in positivity rates was not as large as at the 1% cutoff.

Discordant PD-L1 expression between biopsy and resected specimens

Figure 1 is a Sankey diagram showing the PD-L1 TPS movement in resected specimens in each case where TPS was <1%, 1–49%, and ≥50% in biopsy specimens. Of the 55 patients with TPS of <1% in biopsy specimens 21 had a TPS of 1-49% in resected specimens, and 4 had a TPS>50%. Of the 31 patients with TPS of 1–49% in biopsy specimens 6 had a TPS>50% in resected specimens. Figure 2 shows PD-L1 TPSs of biopsy and resected specimens of the same tumor. Figure 2A presents the 55 PD-L1-negative cases with biopsy specimens, and TPSs in resected specimens varied from 0 to 95%. Of these 55 cases, 25 cases had a PD-L1 TPS of 1% or more in resected specimens, indicating these cases were underestimated in the biopsy specimens. Figure 2B presents the 31 cases with a PD-L1 TPS of 1–49% in the biopsy specimens. The TPS of these cases in resected specimens varied between 0% and 85%. Seventeen cases exhibited a lower TPS and 13 a higher TPS in the surgical specimens than those in the biopsy specimens. Figure 2C presents the 32 cases with a PD-L1 TPS of 50% or more. The TPS of these cases in resected specimens varied between 5% and 95%. Sixteen cases exhibited a lower TPS and 10 a higher TPS in the surgical specimens than those in the biopsy specimens. Differences in PD-L1 positivity between the biopsy and resected specimens are summarized in Table 3. A total of 34 cases (28.8%) had discordant results, with 25 (21.2%)

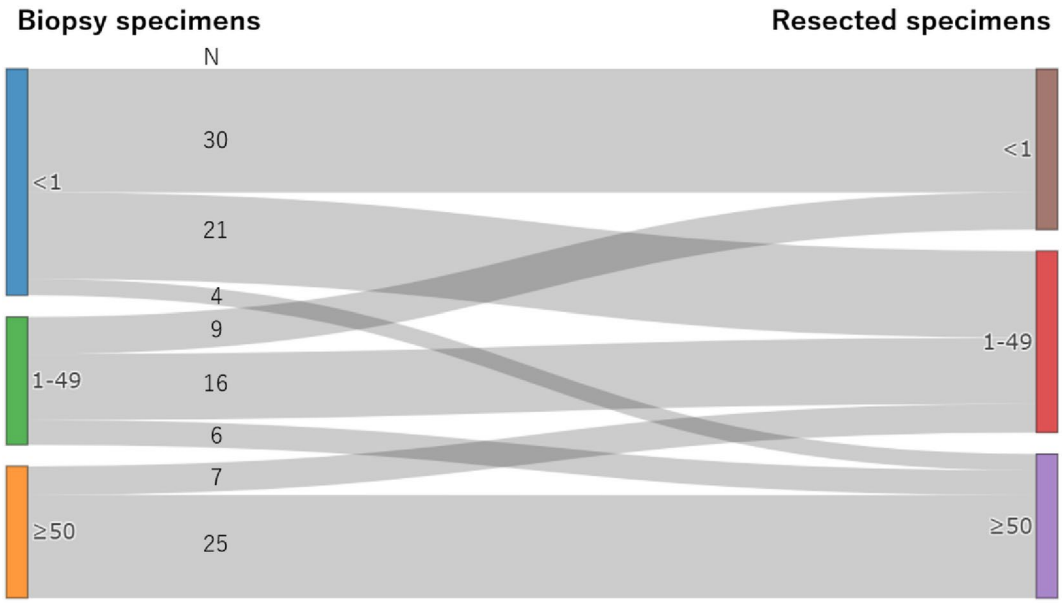

Fig. 1 Sanky diagram representing PD-L1 TPS movement. The numbers of cases in the PD-L1 TPS < 1%, 1–49%, and > 50% in biopsy and resected specimens are shown, as are changes in TPS categories

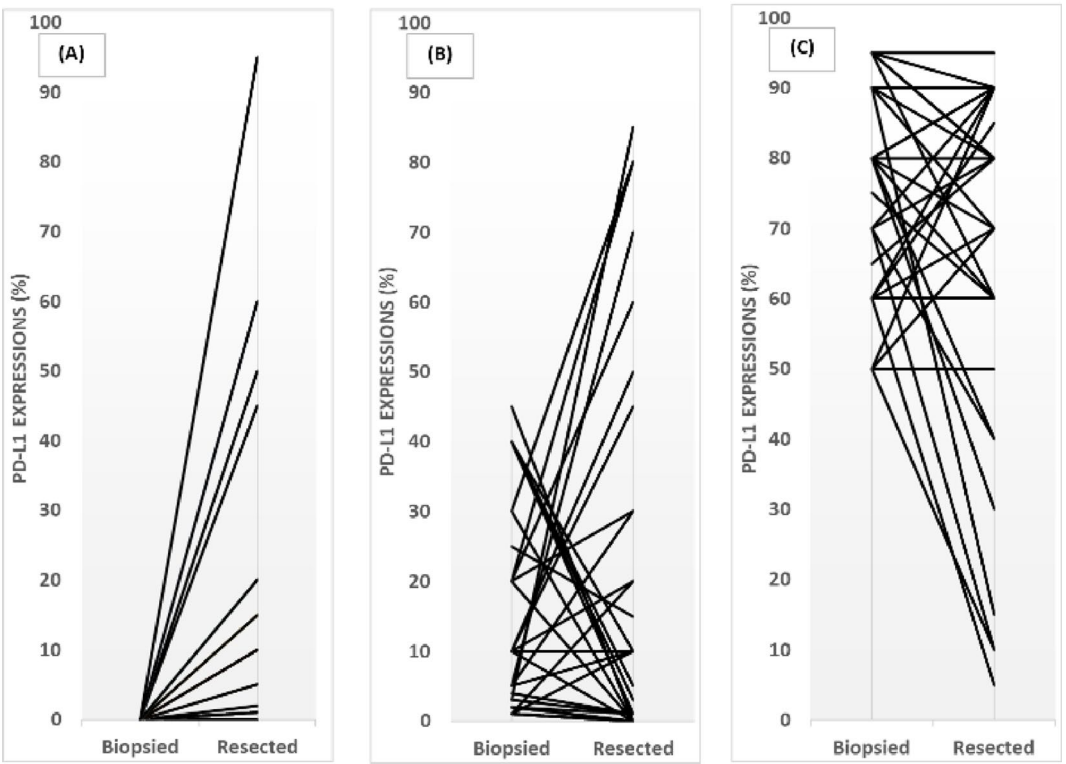

Fig. 2 PD-L1 TPSs of biopsy and resected specimens. The PD-L1 TPS of the biopsy and resected specimens in each case are indicated by connecting lines. The cases in which biopsy specimens were PD-L1-negative are shown in (**A**), those with a TPS of 1-49% are shown in (**B**), and those with a TPS of 50% or more are shown in (**C**). PD-L1, programmed death-ligand 1; TPS, tumor proportion score

underestimated cases and 9 (7.6%) overestimated cases. The concordance between biopsy and resected specimens according to Cohen's kappa was $\kappa = 0.410$ (95% CI: 0.243–0.577), indicating weak agreement. Among adenocarcinomas, 26 cases (34.7%) were discordant, with 20 (26.7%) underestimated cases. Among squamous-cell carcinomas, eight cases (20.0%) were discordant, with five (12.5%) underestimated cases.

Table 3 Differences in PD-L1 positivity with a 1% cut-off between biopsy samples and matched resected specimens

PD-L1 status	Biopsy		Total ($n=118$)	Kappa value (95% CI)
	≥1%	<1%		
Resection				
≥1%	54	25	79	0.410 (0.243–0.577)
<1%	9	30	39	
Total	63	55	118	

CI, confidence interval; PD-L1, programmed death-ligand 1

Table 4 Underestimated and overestimated cases and agreement rate according to tumor cell count ($n=104$)

Tumor cell count	Positivity rate		Underestimated cases	Overestimated cases	Agreement rate
	Biopsy specimens	Resected specimens			
≤200	21/55 (38.2)	35/55 (63.6)	17/55 (30.9)	3/55 (5.5)	35/55 (63.6)
201–400	17/33 (51.5)	20/33 (60.6)	7/33 (21.2)	4/33 (12.1)	22/33 (66.7)
>400	13/16 (81.3)	13/16 (81.3)	0/16 (0)	0/16 (0)	16/16 (100.0)

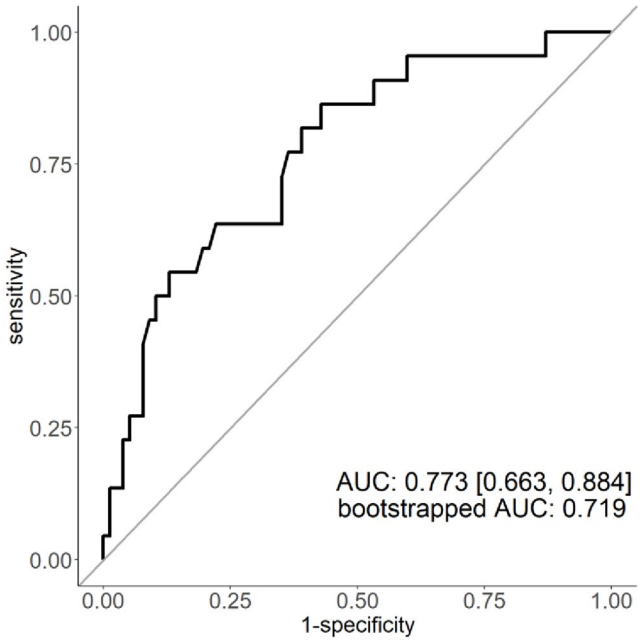

Fig. 3 Receiver operating characteristic analysis to predict underestimated cases. Receiver operating characteristic analysis performed using four factors selected via least absolute shrinkage and selection operator regression (never smoking, tumor size, clinical stage: II-IV, and tumor cell count≤200). The AUC was 0.773 (95% CI 0.663–0.884). AUC, area under the receiver operating characteristic curve

Concordance rate of PD-L1 expression between biopsy and resected specimens according to TC count

The number of underestimated and overestimated cases and agreement rate in each tumor cell count are shown in Table 4. Fourteen cases in which the number of TCs was not evaluated were excluded from the analysis, and 104 cases were included. The median TC count was 200, and the average TC count was 296. The concordance rate was evaluated in three groups according to the number of TCs in the biopsy specimens: ≤200 TCs, 201–400 TCs, and >400 TCs. Both the underestimated and overestimated cases decreased as TC increased. In the group with >400 TCs, the agreement rate was 100%.

Predictive factors for underestimation via biopsy specimens

Clinical and pathological factors predictive of underestimation were examined, limited to factors that could be identified without resection. Four factors (never smoking, small tumor upon CT scan, clinical stage II/III or IV, and a TC count≤200) were selected via the LASSO (Supplementary Data 3.). Using these four factors, ROC analysis was conducted to predict underestimated cases (Fig. 3). The area under the ROC curve was 0.773 (95% CI: 0.663–0.884).

Discussion

The aim of this study was to compare PD-L1 expression in biopsy and resected specimens of the same primary tumor and to examine the frequency of discordance. PD-L1-positivity was more common in resected specimens than that in biopsy specimens; this was also true in the subgroups of adenocarcinomas and squamous-cell carcinomas. Underestimated cases were more common ($n=25$, 21.2%) than overestimated cases ($n=9$, 7.6%) in biopsy specimens. Among the PD-L1-negative cases in biopsy specimens, 45.5% (25/55 cases) were PD-L1-positive in the surgical specimens, an underestimation. Llie et al. revealed a discordance between biopsy and resected specimens ($\kappa=0.218$) and reported that all cases of discordance were underestimations (Ilie et al. 2016). In clinical practice, underestimations may result in missed opportunities for effective treatment (Felip et al. 2021; Mok et al. 2019). Previous studies were inconsistent as to whether biopsy and resected specimens were concordant or discordant in terms of PD-L1 expression. Several studies revealed concordance, with $\kappa\geq0.7$ (Kitazono et al. 2015; Elfving et al. 2019; Gradecki et al. 2018). Those studies had relatively small samples ($n=30$–80). Other studies revealed discordance, with discordance rates of 19-48% ($\kappa=0.218$–0.630) (Ilie et al. 2016; Shen et al. 2021; Li et al. 2017). In the present study, the discordance rate was 28.8% ($\kappa=0.410$), consistent with those of previous studies that revealed a discordance between specimen types.

Discordant cases are reportedly more common in squamous-cell carcinomas than that in adenocarcinomas, although no statistically significant differences have been demonstrated (Spicer et al. 2024; Shen et al. 2021). In this study the rate of discordant cases was higher in adenocarcinomas (34.7%) than that in squamous-cell carcinomas (20.0%), though the association between underestimation and adenocarcinomas compared with other histological types was not significant (26.7% versus 11.6%; $p=0.064$). Adenocarcinomas reportedly differ in PD-L1 expression according to histological subtype (Ng Kee Kwong et al. 2018). In the present study, more cases with lepidic components were underestimated compared to those without a lepidic component, although the association was not significant (31.7% vs. 15.6%; $p=0.058$). PD-L1 expression is reportedly low or undetectable in lepidic components (Shen et al. 2021). The lepidic component of biopsy specimens might have caused the apparent underestimation of PD-L1 expression in adenocarcinomas in this study.

In this study, we also examined the association between the number of TCs in biopsy specimens and the discordance in PD-L1 expression. Gradecki et al. reported that PD-L1 false-negative results for needle biopsies had a specimen length of only 0.2 cm compared with an average of 1.14 cm (Gradecki et al. 2018), and Llie et al. reported that the mean number of biopsy specimens differed between concordant and discordant cases of PD-L1 expression in biopsy and resection specimens (3.4 versus 6.8; $p=0.07$) (Ilie et al. 2016). In the present study, both underestimated and overestimated cases decreased with increasing TC count, and PD-L1-positivity was concordant in all cases with >400 TCs. This result indicates that higher TC counts may decrease the effect of heterogeneity.

Although several reports of discrepancies in PD-L1 expression between biopsy and resected specimens have been published, the factors associated with such discrepancies have not yet been revealed. Li et al. reported that the discordance in PD-L1 expression was not significantly associated with age, sex, smoking status, histological subtype, or tumor stage (Li et al. 2017). In the present study, we examined the factors associated with underestimation of PD-L1 expression, limiting factors to those that can be identified without resection, and four clinical and pathological factors were selected using LASSO regression. The first was smoking status (never-smoker). Adenocarcinoma is the most common histology among never-smokers (Sun et al. 2007), and in the present study, histological analysis revealed that tumors of all never-smokers were adenocarcinomas. In addition, adenocarcinomas in never-smokers are often associated with *EGFR* mutations (Pham et al. 2006). In the present study, 14 of 28 never-smokers (50%) had *EGFR*-mutant adenocarcinomas, and *EGFR*-mutant adenocarcinomas were significantly associated with underestimations compared with adenocarcinomas without *EGFR* mutations (54.5% vs. 18.9%; $p=0.024$). Mutant *EGFR* expression in bronchial epithelial cells reportedly induces the PD-L1 pathway (Akbay et al. 2013). The process by which *EGFR*-mutant tumors activate the PD-L1 pathway may be related to PD-L1 heterogeneity, leading to underestimation.

The second factor included after LASSO regression was small tumor size. In the diagnosis of lung cancer via transbronchial biopsy, a target-lesion diameter≥20 mm is an important factor, according to one report (Kurihara et al. 2022). Biopsies of small tumors may not be representative of the whole tumor, because of its difficulty, which may influence the evaluation of PD-L1 expression.

The third factor was clinical stage II or III or IV tumor. Nagasaki et al. reported that high-grade adenocarcinomas had a significantly higher PD-L1-heterogeneity index than low- and moderate-grade adenocarcinomas. They explained that cancer-cell plasticity, which enables cancer cells to enhance their proliferation, invasion, and survival, contributes to intratumoral heterogeneity and promotes cancer progression and metastasis (Nagasaki et al. 2024). More advanced tumors may be more heterogeneous, which may influence underestimation.

The fourth factor associated with underestimation of PD-L1 expression was a TC count of 200 cells or less in biopsy specimens. In this study the cutoff value of 200 for the number of TCs was determined via a ROC curve. Currently, a TC count of 100 or more cells is considered sufficient for PD-L1 testing; however, the number of TCs needed to avoid underestimation of PD-L1 expression in small specimens is unclear. Although further research is required to this effect, this study was able to provide an indicator as a first step.

In clinical practice it is sometimes difficult to decide whether to administer preoperative ICIs to patients with resectable NSCLCs when preoperative biopsy samples test negative for PD-L1 expression. Effects of preoperative ICIs have been reported even in PD-L1 negative cases (Forde et al. 2022), suggesting that PD-L1 expression is underestimated in preoperative specimens, as supported by the present study. In addition, even if PD-L1 expression was negative in preoperative biopsy specimens and no preoperative ICIs were administered, if PD-L1 expression is positive in the resected specimens, adjuvant ICI would remain a treatment option (Felip et al. 2021). However, the question of when and in which samples PD-L1 expression should be evaluated is controversial, and the re-evaluating of PD-L1 expression in surgical specimens is not specifically recommended. In recurrent cases, whether treatment should be based on PD-L1 expression in preoperative biopsy or resected specimens has not been determined. This study lays the groundwork for such a discussion, including

interpretation of the results of PD-L1 expression in a small sample.

This study had several limitations. First, PD-L1 expression was evaluated in only one section of each tumor; therefore, PD-L1 expression in the resected specimens did not necessarily reflect that in the entire tumor. Second, we included patients with early stage tumors that did not require perioperative ICIs and were unlikely to recur. Third, this was a retrospective, single-center study. Larger studies limited to patients with advanced tumors are needed in the future. Fourth, the study did not investigate responses to immunotherapy. Responses to immunotherapy in underestimated cases and other cases should be further investigated.

In conclusion, this study revealed a significant discordance in PD-L1 expression between biopsy and resected specimens of NSCLC, with an apparent tendency towards underestimation in biopsies. To improve the accuracy, adequate TC counts (>400 cells) must be obtained in biopsies. The observed discordance and potential underestimation of PD-L1 expression in biopsy samples should be considered when deciding whether a patient should receive immunotherapy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00432-025-06189-8.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Approval of the research protocol by an Institutional Reviewer Board: Ethical approval for this study was obtained from the Kanagawa Cancer Center (No. 2023−149). The study was conducted in accordance with the Declaration of Helsinki.

Informed consent Informed consent was obtained from all patients.

Registry and the registration no. of the study/trial N/A.

Animal studies N/A.

Competing interests Financial interests: Shuji Murakami reports personal fees from Ono Pharmaceutical, Bristol Myers Squibb and MSD. Haruhiro Saito reports grants from Ono Pharmaceutical and personal fees from Ono Pharmaceutical, Bristol Myers Squibb, MSD. Hiroyuki Ito reports personal fees from Chugai Pharmaceutical, Ono Pharmaceutical and Bristol Myers Squibb. The other authors have no relevant financial or non-financial interests to disclose.

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