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Comparing three different anti-PD-L1 antibodies for immunohistochemical evaluation of small cell lung cancer

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ABSTRACT

Objective: Small cell lung cancer (SCLC), which accounts for approximately 15% of all lung cancer cases, has high initial sensitivity to chemotherapy. However, clinical outcomes have not improved in the past two decades. Therefore, novel biomarkers are needed to prolong survival in patients with advanced SCLC.

Material and methods: In this retrospective study, we assessed 44 patients with SCLC who underwent first-line or adjuvant chemotherapy. We analyzed PD-L1 expression in SCLC tumors using three specific anti-PD-L1 antibody clones (28-8, 22C3, and SP263) and assessed their correlation with clinical profiles.

Results: Each clone yielded PD-L1 positivity as follows: 10 cases with 28-8, eight cases with 22C3, and six cases with SP263. Eleven patients tested positive with at least one of the three anti-PD-L1 antibodies, and 33 patients tested negative with all anti-PD-L1 antibodies. Serum neuron-specific enolase levels at diagnosis were significantly higher in negative tumors than in positive tumors with the 28-8 clone ($p = 0.036$) and, similarly, tended to be higher in negative tumors with the 22C3 and SP263 clones.

Conclusion: These observations suggest that PD-L1 is detected in SCLC tumors at a similar rate and with similar clinical correlates when detected using any of these three anti-PD-L1 clones. Further large-scale investigations are warranted to reveal the roles of PD-L1 expression in patients with SCLC.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancer cases [1,2]. Disappointingly, the response rate to first-line chemotherapy and the overall survival (OS) have not improved in the last two decades [3].

Cancer immunotherapy using antibodies such as anti-programmed death 1 (PD-1) and anti-programmed death ligand 1 (PD-L1) has dramatically improved survival as compared to the use of standard cytotoxic agents for locally advanced and metastatic non-small cell lung cancer (NSCLC) [4–12]. Recently, several clinical studies reported good outcomes for immunotherapy in patients with advanced SCLC [13,14]. The IMpower 133 clinical phase 3 trial showed that combination therapy with the anti-PD-L1 antibody atezolizumab and platinum-based chemotherapy extended progression-free survival (PFS) and OS more than when platinum-based chemotherapy alone was used as the first-line treatment of advanced SCLC [14]. However, as the response rate of immune checkpoint inhibitors (ICIs) against several types of solid tumors, including SCLC, was only approximately 20% [5,6,13,15–17], predictive biomarkers related to ICI efficacy in SCLC are needed.

Tumor mutation burden (TMB) is a potential biomarker for predicting ICI efficacy in patients with NSCLC. However, there was no significant difference between high- and low-TMB SCLC in OS after

Abbreviations: SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; OS, overall survival; ICIs, immune checkpoint inhibitors; TMB, tumor mutation burden; CT, computed tomography; RECIST, Response Evaluation Criteria in Solid Tumors; IHC, immunohistochemical; TPS, tumor proportion score; TC, tumor cell; NSE, neuron-specific enolase

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immunotherapy [18]. PD-L1 expression in tumors has been developed as a predictive biomarker for ICI treatment in patients with NSCLC. We therefore evaluated PD-L1 expression in SCLC tumors using multiple anti-PD-L1 antibody clones and assessed the correlation between PD-L1 expression in tumors and the clinical profiles of patients with SCLC.

2. Materials and methods

2.1. Patients

We retrospectively assessed 44 patients with SCLC who received first-line or adjuvant chemotherapy at two institutions in Japan between January 2015 and March 2018. All patient responses were evaluated using conventional computed tomography (CT) or magnetic resonance imaging according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. This study protocol was approved by the Ethics Committees of Kyoto Prefectural University of Medicine (approval no. ERB-C-1320-1) and another hospital.

2.2. Immunohistochemical (IHC) staining and evaluation

Formalin-fixed, paraffin-embedded tissue blocks were cut into serial 4-μm-thick sections and deparaffinized. Immunostaining for anti-PD-L1 clone 28-8 and clone 22C3 antibodies (Agilent Technologies, Santa Clara, CA, USA) was carried out with a Dako autostainer Link48 system and a PD-L1 PharmDx kit (Dako, Carpinteria, CA, USA), respectively. Immunostaining using clone SP263 (Roche Diagnostics, Indianapolis, IN, USA) was carried out with a Ventana BenchMark platform (Ventana Medical Systems, Tucson, AZ, USA). In addition to PD-L1 staining, hematoxylin and eosin staining was performed to identify tumor cells. The PD-L1 tumor proportion score (TPS) was calculated as the percentage of PD-L1-positive tumor cells (TCs) by two experienced pathologists (A. M−H. and Y. S.) who had no prior information on the clinicopathological features of the patient samples. Samples with < 100 viable TCs were excluded from this study. The cutoff for defining a case as positive was based on previous reports: 1% TC staining for clones 28-8 and 22C3 and 25% TC staining for clone SP263 [4–6,19].

2.3. Statistical analysis

Correlations between the PD-L1 TPS and patient characteristics were evaluated using the chi-square test or Student's t-test. Cox proportional hazards models considering several patient factors were used. To analyze PFS, times to events were estimated using the Kaplan-Meier method and compared using the log-rank test. PFS was censored at the date of disease progression. All statistical analyses were performed using Prism (version 8.01; GraphPad Software Inc., La Jolla, CA, USA). All p values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

In 44 patients with SCLC who underwent first-line or adjuvant chemotherapy in the present study, we obtained 43 samples (97.7%) from primary lung lesions and one sample (2.3%) from a bone metastatic lesion using bone biopsy. Among the specimens from lung lesions, nine (20.5%) were obtained via video-assisted thoracic surgery (VATS), 33 (75.0%) via bronchoscopy, and one (2.3%) via CT-guided biopsy. Thirty-five patients (79.5%) were male, the median age was 72.5 years (range: 56–84 years), and all patients had a history of smoking. Thirty-seven patients (84.1%) had advanced SCLC. The median serum pro-gastrin-releasing peptide and neuron-specific enolase (NSE) levels were 137.0 pg/mL (range: 20.0–574.0 pg/mL; normal range: < 81.0 pg/mL) and 51.85 ng/mL (range: 8.8–278.0 ng/mL; normal range: < 16.3 pg/mL; **Supplementary Table 1**), respectively.

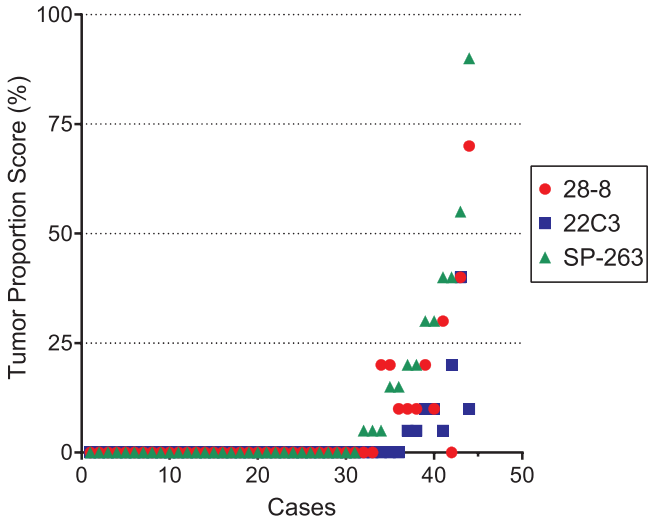


Fig. 1. Immunohistochemical Staining Using Three Anti-PD-L1 Antibody Clones in 44 Patients with SCLC.
PD-L1 antibody clones yielded positive staining in 10 cases using 28-8, eight cases using 22C3, and six cases with SP263. Five patients (11.4%) were positive using three clones, and 33 patients (75.0%) were negative using all three clones.

3.2. Comparing tumor PD-L1 expression with three Anti-PD-L1 antibody clones

We evaluated tumor PD-L1 expression in 44 SCLC specimens obtained from 44 patients using three anti-PD-L1 antibody clones: 28-8, 22C3, and SP263. In most cases, SP263 and 22C3 showed a stronger intensity with full circumferential membrane staining compared to 28-8, which showed weaker and vague staining with partial circumferential membrane staining **(SupplementaryFig. 1A-1C)**. PD-L1 expression was positive in 10 cases with 28-8, eight cases with 22C3, and six cases with SP263. Eleven patients with SCLC (25.0%) were positive for at least one of the three PD-L1 antibodies. Five patients (11.4%) were positive for all three anti-PD-L1 antibody clones, and 33 patients (75.0%) were negative for all three anti-PD-L1 clones (Fig. 1). Of all patients, two (4.5%) were positive for both 28-8 and 22C3 clones, and one (2.3%) was positive for both 22C3 and SP263 clones (Fig. 2A). The results of PD-L1 expression were concordant for 40 patients between clones 28-8 and 22C3, 38 patients between clones 28-8 and SP263, and 42 patients between clones 22C3 and SP263 (Fig. 2D). The concordance in all patients was similar to that in patients with limited and extensive SCLC (Fig. 2B, C, E, and F).

3.3. Relationships between PD-L1 positivity and clinicopathological features for each PD-L1 antibody

We next assessed correlations with clinicopathological features for 44 patients comparing patients who were PD-L1-positive and -negative for each anti-PD-L1 clone. The median serum neuron-specific enolase (NSE) level was significantly higher in patients with PD-L1-negative tumors detected with the 28-8 clone than in those with PD-L1-positive tumors (31.0 ng/mL [range: 14.4–56.1 ng/mL] versus 69.4 ng/mL [range: 8.8–278.0 ng/mL], p = 0.036). Patients with PD-L1-negative tumors also tended to have higher median serum NSE levels than those with PD-L1-positive tumors when detected with the 22C3 and SP263 clones. Apart from serum NSE levels, there were no other significant differences between the two groups with respect to any of the anti-PD-L1 clones **(Supplementary Table 2)**. These observations suggest that PD-L1 expression in tumors detected by any anti-PD-L1 clone may have similar incidence for patients with SCLC.

A) All patients

22C3 \ 28–8	<1	1≤
<1	**33**	3
1≤	1	**7**

Concordant pair: 0.91 (40 of 44)
28–8 higher: 0.07 (3 of 44)
22C3 higher: 0.02 (1 of 44)

SP263 \ 28–8	<1	1≤
<25	**33**	5
25≤	1	**5**

Concordant pair: 0.86 (38 of 44)
28–8 higher: 0.11 (5 of 44)
SP263 higher: 0.02 (1 of 44)

22C3 \ SP263	<25	25≤
<1	**36**	0
1≤	2	**6**

Concordant pair: 0.95 (42 of 44)
22C3 higher: 0.05 (2 of 44)
SP263 higher: 0 (0 of 44)

B) Limited disease

22C3 \ 28–8	<1	1≤
<1	**5**	0
1≤	1	**1**

Concordant pair: 0.86 (6 of 7)
28–8 higher: 0 (0 of 7)
22C3 higher: 0.14 (1 of 7)

SP263 \ 28–8	<1	1≤
<25	**5**	0
25≤	1	**1**

Concordant pair: 0.86 (6 of 7)
28–8 higher: 0 (0 of 7)
SP263 higher: 0.14 (1 of 7)

22C3 \ SP263	<25	25≤
<1	**5**	0
1≤	0	**2**

Concordant pair: 1.00 (7 of 7)
22C3 higher: 0 (0 of 7)
SP263 higher: 0 (0 of 7)

C) Extensive disease

22C3 \ 28–8	<1	1≤
<1	**28**	3
1≤	0	**6**

Concordant pair: 0.92 (34 of 37)
28–8 higher: 0.08 (3 of 37)
22C3 higher: 0 (0 of 37)

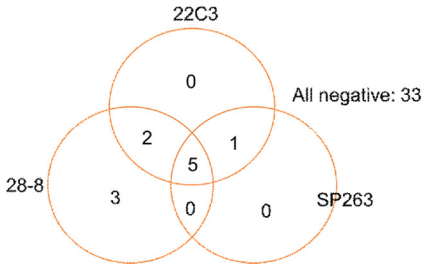
SP263 \ 28–8	<1	1≤
<25	**28**	5
25≤	0	**4**

Concordant pair: 0.86 (32 of 37)
28–8 higher: 0.14 (5 of 37)
SP263 higher: 0 (0 of 44)

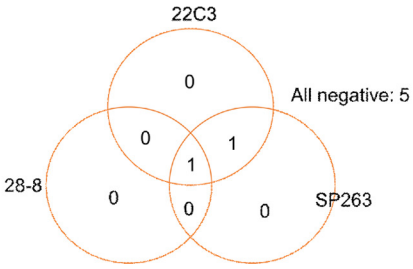
22C3 \ SP263	<25	25≤
<1	**31**	0
1≤	2	**4**

Concordant pair: 0.95 (35 of 37)
22C3 higher: 0.05 (2 of 37)
SP263 higher: 0 (0 of 37)

D) All patients



E) Limited disease



F) Extensive disease

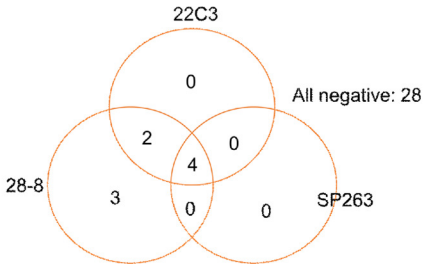


Fig. 2. Comparing PD-L1 TPS Using the Cutoff Indicated by Each Clinical Threshold.
(A) All patients, (B) those with limited disease, and (C) those with extensive disease were compared using the PD-L1 TPS cutoff for each clinical threshold. (D–F) Venn diagram showing PD-L1 immunohistochemical analysis for 44 patients with SCLC using each clone for (D) all patients, (E) those with limited disease, and (F) those with extensive disease. TPS: tumor proportion score

3.4. Relationship between PD-L1 positivity and clinical outcomes in patients with advanced SCLC

The rate of response to platinum-based chemotherapy for patients with SCLC was 77.8%, 83.3%, and 75.0% in patients with positive tumors detected by 28-8, 22C3, and SP263 anti-PD-L1 clones, respectively, while the response rate was 64.3%, 64.5%, and 66.7% in patients with PD-L1-negative tumors detected with each clone,

respectively. Although patients with PD-L1-expressing SCLC tumors showed a relatively better response to platinum-based chemotherapy than those with negative tumors, there was no significant difference in the response rate to platinum-based chemotherapy for any of the anti-PD-L1 antibodies **(SupplementaryFig. 2A-2C)**. In addition, there was no significant difference in PFS between the PD-L1-positive and -negative groups for any of the PD-L1 antibodies **(Supplementary Fig. 3A-3 F)**. These observations suggest that PD-L1-expressing tumors detected by any anti-PD-L1 antibodies may not predict a good response to first-line platinum-based chemotherapy in patients with SCLC.

4. Discussion

PD-L1 expression in tumors has been used clinically as a positive predictive biomarker for effective ICI treatment in NSCLC [7]; in contrast, it has been reported as a negative predictive factor for the response to docetaxel in NSCLC [5]. However, clinically useful biomarkers have not yet been identified to predict the efficacy of systemic chemotherapy for patients with SCLC. This is the first report to evaluate the clinical incidence of PD-L1 expression in SCLC tumors using multiple anti-PD-L1 clones. Previous studies using the anti-PD-L1 antibody clones 28-8 and E1L3N showed that SCLC tumors are relatively less likely to express PD-L1, with approximately 5%–16.1% of patients with SCLC positive for PD-L1, and this expression did not correlate with any clinical features [20–22]. Previous reports showed a lower frequency of PD-L1 positivity in SCLC tumors compared with other respiratory malignancies, such as NSCLC, thymic cancer, and malignant mesothelioma [23–25]. However, we found that, using any of the three PD-L1 clones, 25% of patients with SCLC were positive, a much higher percentage than that of previous reports [20–22]. The frequency of PD-L1 expression significantly correlates with clinical staging and smoking status in several cancers, including SCLC [26–29]. In the present study, the higher rate of PD-L1 positivity might be due to the high number of advanced SCLC tumors and patients with a heavy smoking history. Therefore, our observations might provide useful knowledge to assess the role of PD-L1 expression in patients with advanced SCLC who are treated with systemic chemotherapy and immunotherapy.

The anti-PD-L1 antibody clone SP142 was relatively less concordant in PD-L1 expression than the other antibodies, whereas the clones used in our study (28-8, 22C3, and SP263) gave similar clinical outcomes for ICI treatment in patients with NSCLC [23,30]. Our retrospective findings also showed that PD-L1 expression analysis measured with the anti-PD-L1 clones 28-8, 22C3, and SP263 yielded similar clinical outcomes for patients with SCLC. Therefore, any of these clones could be used to measure PD-L1 expression in SCLC tumors, consistent with NSCLC.

Previous studies reported that serum lactate dehydrogenase and NSE levels in patients with SCLC were correlated with PD-L1 expression in tumors [31]. Although we did not examine serum lactate dehydrogenase, serum NSE was significantly correlated with PD-L1 expression in SCLC tumors using only 28-8; however, the other antibodies, 22C3 and SP263, also showed a nonsignificant correlation. As serum NSE is a prognostic factor for patients with SCLC, PD-L1 expression may also become a potent prognostic factor for patients with SCLC. However, we did not observe a significant correlation between treatment outcome and PD-L1 expression in tumors from patients with SCLC. Recent clinical trials demonstrated that combination therapy with platinum-based chemotherapy and atezolizumab shows promising results as a first-line treatment of patients with advanced SCLC [14]. Tumor PD-L1 expression might be a potent predictor for combination therapy, although the rate of PD-L1 positivity is relatively low. Further, large-scale investigations are needed to reveal the usefulness of PD-L1 expression as a marker for clinical outcomes of chemotherapy and/or immunotherapy for patients with SCLC.

This study has several limitations. First, it comprised a small, retrospective sample. However, previous retrospective observations in SCLC tumors have used similar sample sizes [20–22]. Second, most of the specimens in this study were obtained from biopsy samples, potentially producing a discrepancy in PD-L1 expression regarding the tumor volume of SCLC; however, previous reports have shown that PD-L1 expression in small biopsy samples was similar to that of surgically resected specimens in NSCLC [32,33]. Third, there may have been bias considering, even though treatment was administered in multiple centers, and in the timing of evaluation using CT scanning, even though it was performed every 1–3 months after treatment.

In summary, our observations indicate that PD-L1-expressing tumors, detected using multiple anti-PD-L1 antibody clones, occur at a similar incidence and with similar clinical outcomes for SCLC. Therefore, any of the three PD-L1 antibody clones evaluated in this study can be used to detect the PD-L1 expression levels in SCLC tumors. Further investigations are warranted to reveal the roles of PD-L1 expression on the clinical significance of SCLC, including immunotherapy.

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Disclosure

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.09.012.

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