



Significance of PD-L1 expression in the cytological samples of non-small cell lung cancer patients treated with immune checkpoint inhibitors

Naofumi Hara¹ · Eiki Ichihara² · Daijiro Harada³ · Koji Inoue⁴ · Keiichi Fujiwara⁵ · Shinobu Hosokawa⁶ · Daizo Kishino⁷ · Kawai Haruyuki⁸ · Nobuaki Ochi⁹ · Naohiro Oda¹⁰ · Katsuyuki Hotta¹¹ · Yoshinobu Maeda¹ · Katsuyuki Kiura²

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Abstract

Objectives The programmed death-ligand 1 (PD-L1) tumor proportion score (TPS) in tumor tissue samples is an established clinical biomarker for non-small cell lung cancer (NSCLC). However, the significance of PD-L1 expression in other types of samples has not been fully investigated.

Patients and methods We conducted a multicenter retrospective cohort study of advanced NSCLC patients who received ICI treatment during the clinical course and investigated the effects of ICIs according to PD-L1 expression in cytology samples, including cell block and endobronchial ultrasound-guided (EBUS) transbronchial needle aspiration (TBNA) samples.

Results A total of 264 patients were included in this study: PD-L1 expression was determined in cell block or TBNA specimens in 55 patients, and in tissue samples in 209 patients. Among the former patients, the median progression-free survival (PFS) of those with a TPS for PD-L1 $\geq 50\%$ was significantly longer compared to that of those with a TPS $< 50\%$ (6.5 vs. 1.9 months, respectively, $p = 0.008$). When the cutoff value was set at 1%, the median PFS was 4.2 months in patients with a TPS $\geq 1\%$ and 1.5 months in patients with a TPS $< 1\%$ ($p < 0.001$).

Conclusion PD-L1 expression determined using cytology specimens predicts the efficacy of ICIs.

Keywords Non-small cell lung cancer · Immune checkpoint inhibitor · PD-L1 expression · EBUS-TBNA · Cytology

✉ Eiki Ichihara
ichiha-e@md.okayama-u.ac.jp

¹ Department of Hematology, Oncology and Respiratory Medicine, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

² Department of Allergy and Respiratory Medicine, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama, Okayama 700-8558, Japan

³ Department of Thoracic Oncology, National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan

⁴ Department of Respiratory Medicine, Ehime Prefectural Central Hospital, Matsuyama, Japan

⁵ Department of Respiratory Medicine, National Hospital Organization Okayama Medical Center, Okayama, Japan

⁶ Department of Respiratory Medicine, Japanese Red Cross Okayama Hospital, Okayama, Japan

⁷ Department of Respiratory Medicine, Himeji Red Cross Hospital, Himeji, Japan

⁸ Department of Internal Medicine, Okayama Saiseikai General Hospital, Okayama, Japan

⁹ Department of General Internal Medicine 4, Kawasaki Medical School, Okayama, Japan

¹⁰ Department of Internal Medicine, Fukuyama City Hospital, Fukuyama, Japan

¹¹ Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan

Introduction

Immune checkpoint inhibitors (ICIs), such as programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) antibodies, have markedly changed the treatment strategies for advanced non-small cell lung cancer (NSCLC) (Borghaei et al. 2015; Brahmer et al. 2015) and various other cancers. Among the most important benefits of ICIs are their long-lasting tumor-inhibiting effects and potentially curative action (Reck et al. 2019). However, the rate of long-term inhibition is only ~ 10% (Borghaei et al. 2015), and the establishment of predictive biomarkers is important for ICI therapy. Only the PD-L1 tumor proportion score (TPS) in tumor tissue samples is an established clinical biomarker for NSCLC (Reck et al. 2016), although there are some conflicting data (Carbone et al. 2017; Zhang et al. 2020). Pembrolizumab has become a standard therapy in previously untreated NSCLC patients with PD-L1 \geq 50%, where more than half of the patients were still alive after 30 months of treatment with pembrolizumab in the KEYNOTE-024 trial (Reck et al. 2019). Combination treatment with ICI and platinum doublet chemotherapy was shown to confer a significant survival benefit compared to chemotherapy alone in multiple recent phase III clinical trials and has become a standard therapy in advanced NSCLC regardless of PD-L1 expression (Gandhi et al. 2018; Socinski et al. 2018). However, it remains unclear whether pembrolizumab alone or ICI in combination with chemotherapy is better for NSCLC patients with PD-L1 \geq 50%. PD-L1 is also an important biomarker when determining the treatment for patients intolerant to platinum doublet chemotherapy.

PD-L1 expression was determined in tumor tissue samples, and the use of cytology samples was not permitted in the KEYNOTE-024 trial (Reck et al. 2016). Therefore, the clinical significance of PD-L1 expression in cytology samples remains unclear. However, tissue samples appropriate for determining PD-L1 levels are not always available in clinical practice, whereas the acquisition of cytology samples is minimally invasive and could be available even in patients whose histology sample is unavailable. Therefore, determining the significance of PD-L1 expression in the cytological samples is very important for clinical practice.

This study was performed to investigate the clinical significance of PD-L1 expression in cytological samples, including cell block (coelomic fluid) and endobronchial ultrasound-guided (EBUS) transbronchial needle aspiration (TBNA) samples, in advanced NSCLC.

Materials and methods

Patients

This branch study analyzed our data from the Okayama Lung Cancer Study Group-Immunotherapy Database (OLCSG-ID), which was established to allow various analyses of ICI efficacy for NSCLC (Ichihara et al. 2020a, b). OLCSG-ID includes the medical records for NSCLC patients who received PD-1/PD-L1 antibody monotherapy at nine institutions between December 2015 and May 2018.

Response assessment

Responses were reevaluated by each investigator according to the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1; the iRECIST criteria, which are often used in ICI studies, were not employed.

Statistical analysis

Overall survival (OS) was evaluated from the beginning of ICI therapy to the day of death from any cause, and progression-free survival (PFS) was evaluated from the beginning of ICI therapy to the day of disease progression or death from any cause using the Kaplan–Meier method. Multivariate analysis was performed using the Cox proportional hazards model. Statistical analyses were performed using STATA software (version 11.0; StataCorp, College Station, TX, USA). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

Results

Patient characteristics

We retrospectively investigated 265 NSCLC patients in whom PD-L1 expression had been determined. We excluded one patient whose PD-L1 expression was determined from an unknown sample, and therefore finally analyzed the data of 264 patients. The patients were divided into two groups: those whose PD-L1 TPS was determined in cell block using coelomic fluid or EBUS-TBNA samples (cytology group), and those in whom tissue samples were analyzed (histology group). The patient characteristics are listed in Table 1. The cytology and histology groups consisted of 55 and 209 patients, respectively. There were no significant differences in demographic characteristics between the two groups, except for the disease stage. The rate of postoperative recurrence was significantly higher in the histology group,

Table 1 Patients characteristics

	Cytology <i>n</i> = 55	Histology <i>n</i> = 209	Fisher's test
Age, years			
Median (range)	71 (52–87)	70 (28–93)	
≥ 65	42	159	<i>p</i> = 1.000
< 65	13	50	
Gender			
Male	45	166	<i>p</i> = 0.850
Female	10	43	
Performance status			
> 1	7	31	<i>p</i> = 0.831
0/1	48	178	
Smoking history			
Yes	46	172	<i>p</i> = 1.000
No	9	37	
Histology			
Squamous	11	63	<i>p</i> = 0.177
Non-squamous	44	146	
PD-L1 expression			
≥ 1%	48	163	<i>p</i> = 0.184
< 1%	7	46	
PD-L1 expression			
≥ 50%	34	100	<i>p</i> = 0.071
< 50%	21	109	
EGFR or ALK alteration			
Yes	1	8	<i>p</i> = 0.690
No/not determined	54	201	
Disease stage			
Advanced	53	163	<i>p</i> = 0.001
Rec	2	46	

rec postoperative recurrence, *EGFR* epidermal growth factor receptor, *ALK* anaplastic lymphoma kinase

suggesting that resected tumor samples were likely used for the PD-L1 testing in this group. The majority of the patients were male, had a good performance status (PS), were smokers, and had adenocarcinoma histology.

The proportions of patients with a PD-L1 TPS of 0%, 1–49%, and ≥ 50% were 13, 26, and 61%, respectively, in the cytology group, and 22, 30, and 48%, respectively, in the histology group (Fig. 1).

Response to ICIs according to PD-L1 expression

The objective response rate (ORR) was determined according to PD-L1 expression in both the cytology and histology groups (Fig. 2). PD-L1 expression predicted response to ICI treatment in both the cytology and histology groups (cytology group, TPS < 50 vs. ≥ 50%: 17.6 vs. 48.4%; histology group: 18.1 vs. 42.6%).

Higher PD-L1 expression in cytological specimens predicted PFS

We determined the PFS according to PD-L1 expression in both groups. In the cytology group, a higher PD-L1 expression level predicted significantly better PFS, with cutoff values of both 50 and 1% (PD-L1 ≥ 50 vs. < 50%: median PFS, 6.5 vs. 1.9 months, hazard ratio [HR], 0.43, log-rank test *p* = 0.008, Fig. 3a; PD-L1 ≥ 1 vs. < 1%: median PFS, 4.2 vs. 1.5 months, HR, 0.18, log-rank test *p* < 0.001, Fig. 3b). Similar results were obtained in the histology group (PD-L1 ≥ 50 vs. < 50%: median PFS, 8.1 vs. 3.2 months, HR, 0.49, log-rank test *p* < 0.001, Fig. 3c; PD-L1 ≥ 1 vs. < 1%, median PFS, 6.5 vs. 2.5 months, HR, 0.50, log-rank test *p* < 0.001, Fig. 3d). We also determined OS. In the cytology group, PD-L1 expression with a cutoff value of 50% was not significantly associated with OS (median OS, not reached vs. 12.4 months, HR, 0.40, log-rank test *p* = 0.393, Fig. 4a), while that with a cutoff value of 1% was significantly associated with OS (median OS, not reached vs. 3.9 months, HR, 0.27, log-rank test *p* = 0.023, Fig. 4b). In the histology group, higher PD-L1 expression predicted better OS with cutoff values of both 50 and 1%, although the difference with a cutoff value of 1% was not significant (PD-L1 ≥ 50 vs. < 50%: 28.4 vs. 16.1 months, HR, 0.59, log-rank test *p* = 0.018, Fig. 4c; PD-L1 ≥ 1 vs. < 1%: 20.4 vs. 16.1 months, HR, 0.67, log-rank test *p* = 0.102, Fig. 4d).

Discussion

Some previous studies have reported a correlation of PD-L1 expression between tissue and cytology samples (Capizzi et al. 2018; Wang et al. 2018; Gagné et al. 2019). Therefore, it has been assumed that using cytology to determine PD-L1 can predict the efficacy of ICIs. However, there have been no studies on the direct correlation between PD-L1 expression in cytology samples and the efficacy of ICIs. In this study, we demonstrated that the PD-L1 expression level in cytological samples predicted the efficacy of ICIs.

Consistent with a previous study (Herbst et al. 2016), the proportions of patients with a PD-L1 TPS < 1%, 1–49%, and ≥ 50% were roughly equivalent in our histology group. In our cytology group, the proportion of NSCLC patients with a PD-L1 TPS ≥ 50% was high, accounting for more than 60% of the patients (Fig. 1). One plausible reason for this is that cytology samples may be more amenable to PD-L1 staining. However, this seems unlikely, because PD-L1 expression in cytology samples was reported to be equivalent to that in histology samples (Capizzi et al. 2018; Wang et al. 2018; Gagné et al. 2019). Another possible reason is that, in the group with cytology samples (which require the presence of coelomic fluid or lymph node swelling), inflammation

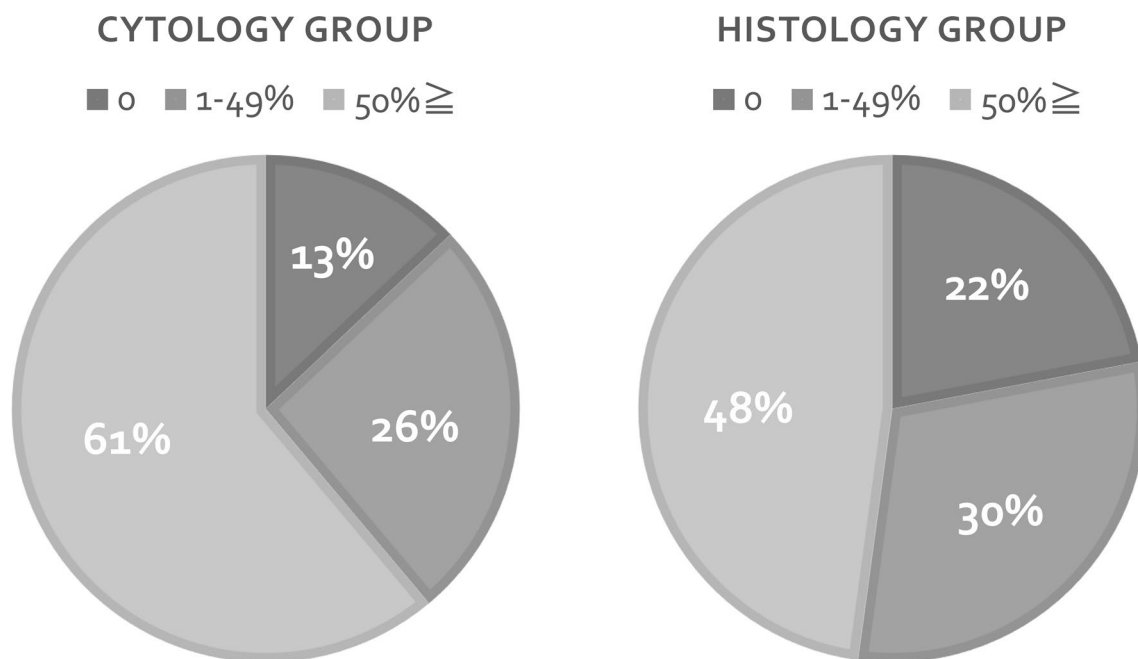


Fig. 1 Patient rates according to programmed cell death ligand 1 (PD-L1) expression. **a** Proportions of patients with a PD-L1 tumor proportion score (TPS) of 0%, 1–49%, and $\geq 50\%$ in cytology samples and **b** histology samples

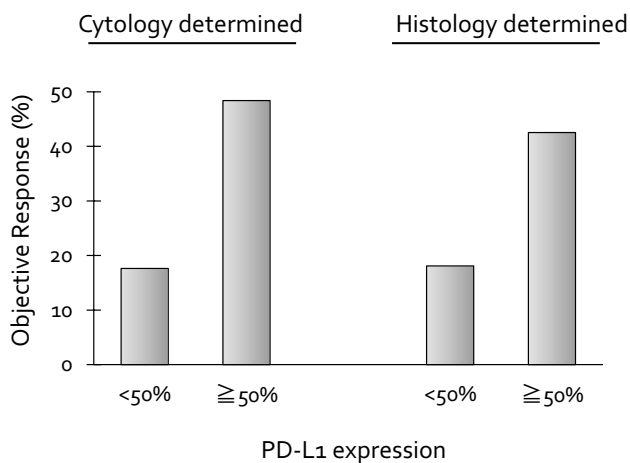


Fig. 2 Objective response rate (ORR) to immune checkpoint inhibitors (ICIs). The ORR to ICIs in the cytology and histology groups are shown

may have been more severe with higher levels of PD-L1 expression.

With regard to the efficacy of ICIs, patients with a PD-L1 TPS < 1% in our cytology group showed an especially short response (all within 3 months of starting ICI therapy; Fig. 3). This is an important finding; although

further studies are required for confirmation, PD-L1 < 1% in cytology samples may be a good marker to exclude ICI therapy, while PD-L1 < 1% in histology samples cannot fully exclude ICI (because some of the NSCLC cases still showed a long-term response to ICI therapy in a previous study) (Borghaei et al. 2015). Malignant pleural effusion is known to be a poor prognostic factor (Morgensztern et al. 2012), which may partly explain why such patients in our cytology group, with a TPS < 1%, showed poor outcomes.

In contrast to previous clinical trials of ICI (Herbst et al. 2016), some of the analyses conducted in the present study, such as the cytology sample analyses, indicated that, when the cutoff value was 50%, PD-L1 did not predict OS (Fig. 4a). In the present study, there was large variation among the patients in terms of regimen numbers prior to ICI therapy and OS was calculated from the start date of ICI therapy in all cases. Therefore, the OS data were heterogeneous, which probably reduced the differences in survival according to PD-L1 expression level.

This study had some limitations. First, the cell block preparation process was not standardized among institutions, which may have led to bias in the PD-L1 staining results. Second, we did not investigate the concordance between PD-L1 expression in cytology and that in histology using

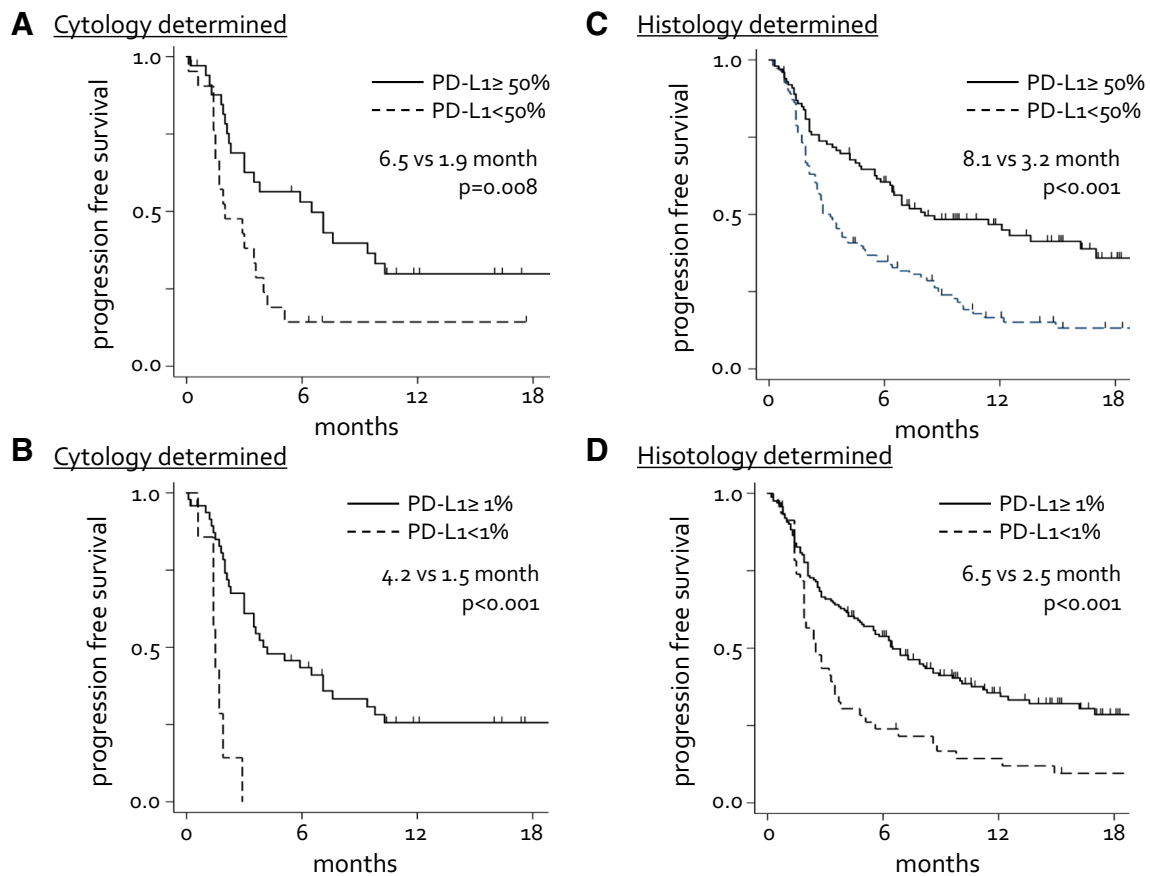


Fig. 3 Progression-free survival (PFS) according to PD-L1 expression. **a** PFS in the cytology group using a PD-L1 expression cutoff value of 50%. **b** PFS in the cytology group using a PD-L1 expres-

sion cutoff value of 1%. **c** PFS in the histology group using a PD-L1 expression cutoff value of 50%. **d** PFS in the histology group using a PD-L1 expression cutoff value of 1%

paired samples. Paired samples were not available in most patients, because cytology samples were used for PD-L1 examination only when histology samples were unavailable. Third, different from previous studies (Mok et al. 2019), tissue-determined PD-L1 with a 1% cut-off value did not predict OS (Fig. 4d), which is most likely to be due to many censored cases with an immature follow-up period, which would obscure the difference. Finally, this

was a retrospective analysis of data that were heterogeneous in terms of patient cohorts and follow-ups, such that the results cannot be considered definitive and should therefore be interpreted cautiously.

In conclusion, PD-L1 expression in cytological samples, including cell block (coelomic fluid) samples, can predict the efficacy of ICI for NSCLC.

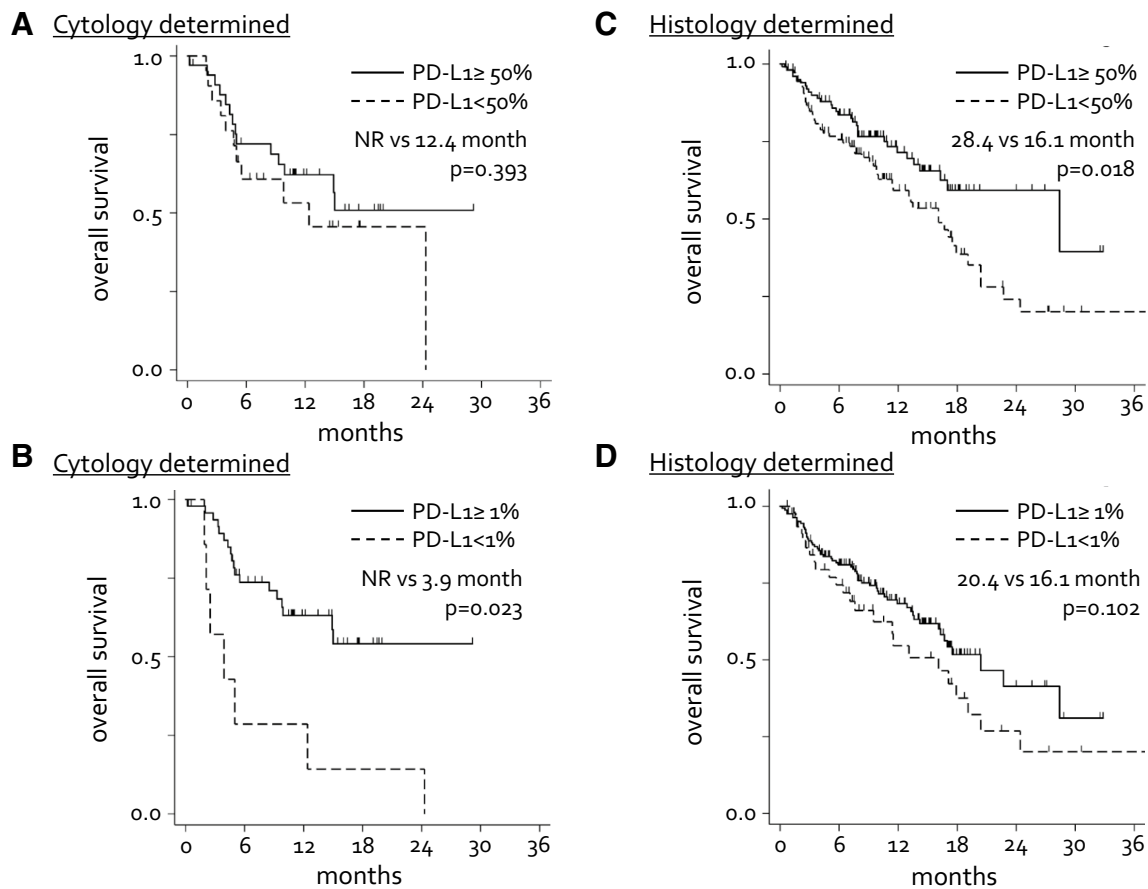


Fig. 4 Overall survival (OS) according to PD-L1 expression. **a** OS in the cytology group using a PD-L1 expression cutoff value of 50%. **b** OS in the cytology group using a PD-L1 expression cutoff value

of 1%. **c** OS in the histology group using a PD-L1 expression cutoff value of 50%. **d** OS in the histology group using a PD-L1 expression cutoff value of 1%

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Author contributions Conception and design: NH and EI; Data acquisition: NH, EI, DH, KI, KF, SH, DK, HK, NO, and NO; Data analysis and interpretation: NH, EI, KH, YM and KK; Writing and review of the manuscript: NH, EI, DH, KI, KF, SH, DK, HK, NO, NO, KH, YM and KK.

Declarations

Conflict of interest EI received honoraria from Chugai Pharmaceutical. EI received additional research funding from MSD. KH received honoraria from Taiho Pharmaceutical, and Chugai Pharmaceutical. KH received additional research funding from MSD, and Chugai Pharmaceutical. TM received honoraria from Chugai Pharmaceutical, and Bristol–Myers Squibb. KK received honoraria from Chugai Pharmaceuticals. All other authors declare no conflicts of interest regarding this study.

References

- Borghaei H, Paz-Ares L, Horn L et al (2015) Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa1507643>
- Brahmer J, Reckamp KL, Baas P et al (2015) Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa1504627>
- Capizzi E, Ricci C, Giunchi F et al (2018) Validation of the immunohistochemical expression of programmed death ligand 1 (PD-L1) on cytological smears in advanced non-small cell lung cancer. *Lung Cancer*. <https://doi.org/10.1016/j.lungcan.2018.10.017>
- Carbone DP, Reck M, Paz-Ares L et al (2017) First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med*. <https://doi.org/10.1056/nejmoa1613493>
- Gagné A, Wang E, Bastien N et al (2019) Impact of specimen characteristics on PD-L1 testing in non-small cell lung cancer: validation of the IASLC PD-L1 testing recommendations. *J Thorac Oncol*. <https://doi.org/10.1016/j.jtho.2019.08.2503>
- Gandhi L, Rodríguez-Abreu D, Gadgeel S et al (2018) Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa1801005>
- Herbst RS, Baas P, Kim DW et al (2016) Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced

- non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet*. [https://doi.org/10.1016/S0140-6736\(15\)01281-7](https://doi.org/10.1016/S0140-6736(15)01281-7)
- Ichihara E, Harada D, Inoue K et al (2020a) The impact of body mass index on the efficacy of anti-PD-1/PD-L1 antibodies in patients with non-small cell lung cancer. *Lung Cancer*. <https://doi.org/10.1016/j.lungcan.2019.11.011>
- Ichihara E, Harada D, Inoue K et al (2020b) Characteristics of patients with EGFR-mutant non-small-cell lung cancer who benefited from immune checkpoint inhibitors. *Cancer Immunol Immunother*. <https://doi.org/10.1007/s00262-020-02662-0>
- Mok TSK, Wu YL, Kudaba I et al (2019) Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet*. [https://doi.org/10.1016/S0140-6736\(18\)32409-7](https://doi.org/10.1016/S0140-6736(18)32409-7)
- Morgensztern D, Waqar S, Subramanian J et al (2012) Prognostic impact of malignant pleural effusion at presentation in patients with metastatic non-small-cell lung cancer. *J Thorac Oncol*. <https://doi.org/10.1097/JTO.0b013e318267223a>
- Reck M, Rodríguez-Abreu D, Robinson AG et al (2016) Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa1606774>
- Reck M, Rodríguez-Abreu D, Robinson AG et al (2019) Updated analysis of KEYNOTE-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol*. <https://doi.org/10.1200/JCO.18.00149>
- Socinski MA, Jotte RM, Cappuzzo F et al (2018) Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa1716948>
- Wang H, Agulnik J, Kasymjanova G et al (2018) Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. *Ann Oncol*. <https://doi.org/10.1093/annonc/mdy126>
- Zhang B, Liu Y, Zhou S et al (2020) Predictive effect of PD-L1 expression for immune checkpoint inhibitor (PD-1/PD-L1 inhibitors) treatment for non-small cell lung cancer: a meta-analysis. *Int Immunopharmacol*. <https://doi.org/10.1016/j.intimp.2020.106214>

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