



Original Research

PD-L1 polymorphisms predict survival outcomes in advanced non-small-cell lung cancer patients treated with PD-1 blockade



Hironori Yoshida ^{a,1}, Takashi Nomizo ^{a,1}, Hiroaki Ozasa ^{a,*},
 Takahiro Tsuji ^a, Tomoko Funazo ^a, Yuto Yasuda ^a, Hitomi Ajimizu ^a,
 Masatoshi Yamazoe ^a, Kiyomitsu Kuninaga ^a, Tatsuya Ogimoto ^a,
 Kazutaka Hosoya ^a, Ryo Itotani ^a, Yuichi Sakamori ^b, Young Hak Kim ^a,
 Toyohiro Hirai ^a

^a Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Japan

^b Department of Therapeutic Oncology, Graduate School of Medicine, Kyoto University, Japan

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Abstract Background: We previously reported that PD-L1 polymorphisms are associated with the efficacy and immune-related adverse events of PD-1 blockade with nivolumab. However, the association between PD-L1 polymorphisms and survival outcomes under PD-1/PD-L1 blockade is still uncertain. Here, we aimed to investigate whether PD-L1 polymorphisms are associated with survival outcomes in advanced non-small-cell lung cancer (NSCLC) patients treated with nivolumab.

Methods: PD-1/PD-L1 polymorphisms and survival outcomes were retrospectively analysed in two independent cohorts (133 patients treated with nivolumab and 96 patients with no treatment history of an immune checkpoint inhibitor (ICI) (the non-ICI cohort)) with advanced NSCLC.

Results: Among the 7 studied single-nucleotide polymorphisms, PD-L1 rs822339 and rs1411262 were associated with overall survival (OS) in patients treated with nivolumab. Patients with the A/A genotype of rs822339 had a significantly longer OS than those with A/G or G/G genotypes (not reached versus 12.0 months; hazard ratio (HR), 0.35; 95% confidence interval (CI), 0.18–0.64; $p = 0.0008$). A similar survival benefit with the A/A genotype was observed regardless of driver mutation status. In multivariate analysis, performance status

* Corresponding author: Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, 54, Shogoin-kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan. Fax: +81 75 751 4643.

E-mail address: ozahiro@kuhp.kyoto-u.ac.jp (H. Ozasa).

¹ Hironori Yoshida and Takashi Nomizo equally contributed to this work.

(PS) and PD-L1 rs822339 genotype were independent prognostic factors for OS. In the non-ICI cohort, the PD-L1 rs822339 genotype did not correlate with OS (HR, 0.77; 95% CI, 0.31–1.70; $p = 0.55$). The T/T genotype of rs1411262 also showed a significant prolongation of OS compared to that with the C/T or C/C genotypes in patients treated with nivolumab.

Conclusions: PD-L1 polymorphisms are associated with favourable OS in nivolumab-treated NSCLC patients and may be useful predictive biomarkers, regardless of driver mutation status.

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1. Introduction

The advent of an immune checkpoint inhibitor (ICI) dramatically changed the treatment of various tumours. In advanced non-small-cell lung cancer (NSCLC), programmed death-ligand 1 (PD-L1) or programmed death 1 (PD-1) blockade therapy significantly prolongs overall survival (OS) compared with no blockade therapy and has become a standard treatment [1–4]. To date, pre-treatment tumour PD-L1 expression assessed by immunohistochemistry is the only approved predictive biomarker for PD-1/PD-L1 blockade response in clinical practice [5,6]; however, it is not a sufficiently satisfactory biomarker because of the heterogeneity of PD-L1 expression within tumours, the possibility of temporal changes, and the indeterminate staining depending on tissue specimen status [7–9]. Alternative assessments, such as tumour mutational burden (TMB), have also been vigorously investigated; however, conclusive evidence is still lacking, and there are several challenges to overcome for clinical application [10–12].

In addition to the features of the tumour itself, the components of host immunity are considered important factors contributing to the response to ICI treatment [13]. One such factor is the role of host immune gene polymorphisms. Generally, patients differ in susceptibility to autoimmune disease, partly because of host immune gene polymorphisms [14,15]. Similarly, immune surveillance mechanisms, including the PD-1/PD-L1 axis, have the potential to vary in their effects against tumour cells according to host immune gene polymorphisms, resulting in individual differences in tumour shape and tumour prevalence. Indeed, single-nucleotide polymorphism (SNP) of PD-1/PD-L1 has previously been implicated in the development and prevalence of various diseases, such as Addison disease, Grave disease, rheumatoid arthritis, gastric cancer, bladder cancer, and hepatocellular carcinoma [16–20].

We thus assumed that host immune gene polymorphisms also have the potential to impact the response to ICI treatment, and we subsequently showed that the genotypes of PD-L1 SNPs were associated with progression-free survival (PFS) and immune-related

adverse events in advanced NSCLC patients treated with PD-1 blockade therapy (nivolumab) [21,22]. However, the association between PD-L1 polymorphisms and survival outcomes under PD-1/PD-L1 blockade has not yet been discussed. Here, we aimed to investigate whether PD-L1 polymorphisms are associated with survival outcomes in advanced NSCLC patients treated with nivolumab.

2. Patients and methods

2.1. Study patients

Between January 2016 and March 2018, a total of 162 consecutive NSCLC patients were treated with nivolumab at Kyoto University Hospital. Of these, 133 patients were analysed for an association between the studied SNPs and survival outcomes. Twenty-three patients with a lack of DNA samples, 5 with another active concomitant malignancy, and 1 who died within one day after nivolumab administration were excluded from the analysis. All patients were treated with nivolumab as a second or later line treatment. Nivolumab was administered at a dose of 3 mg/kg.

To determine whether the role of the studied SNPs was PD-1/PD-L1 treatment specific, 283 metastatic NSCLC patients diagnosed between January 2010 and December 2015 were identified; of these, patients with available genomic DNA and exclusively treated with cytotoxic chemotherapy or tyrosine kinase inhibitor (TKI) were extracted. A total of 96 patients were analysed for an association with the studied SNPs and survival outcomes (non-ICI cohort).

Medical records were retrospectively reviewed, and clinical data, such as age, sex, Eastern Cooperative Oncology Group (ECOG) performance status (PS), tumour histology, status of driver mutation: epidermal growth factor receptor (EGFR) mutation and anaplastic lymphoma kinase (ALK) rearrangement, treatment histories, PD-L1 expression status, and survival outcomes, were extracted. Participants in this study provided written informed consent. All experimental methods were in accordance with the 1975 Declaration

of Helsinki. The study was approved by the Review Board of Kyoto University Hospital.

2.2. Genotyping and SNP selection

The methods of SNP genotyping have already been described in detail in our previous reports [21,22]. We selected specific PD-L1 SNPs that previously described the association with the prevalence of autoimmune disease and cancer [16–22]. SNPs with a reported minor allele frequency above 5% were included.

2.3. Efficacy and survival outcomes

PFS was measured from the date of the initiation of nivolumab to the date of disease progression or death. The responses were evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 [23]. OS was measured from the start of nivolumab administration to death or the last follow-up date. The cutoff date for data collection was 30 September 2019. In the non-ICI cohort, PFS was measured from the date of the initiation of cytotoxic chemotherapy or TKI to the date of disease progression or death. OS was measured from the first date of administration of cytotoxic chemotherapy or TKI to the date of death or the last follow-up. The cutoff date for data collection was 31 December 2016.

2.4. Statistical analysis

Differences in characteristics among the genotypes of SNPs were compared using the Chi-square test for categorical data. PFS and OS were estimated using the Kaplan–Meier method. Patients who were lost to follow-up without events were censored at the last known date of follow-up. Statistical significance of the relationship between PFS or OS and genotypes of SNPs was assessed using the log-rank test. Univariate and multivariate Cox proportional hazards models were used to estimate HRs with 95% confidence intervals (CIs). Bonferroni's correction was applied to Fig. 1, Supplementary Fig. S4 and Table S1 (raw p value $\times 7$), and a p value < 0.05 was considered statistically significant. All analyses were performed with JMP 14 software (SAS Institute, Cary, NC, USA). HaploView 4.2 software was used to calculate the linkage disequilibrium (LD), expressed as D' and r^2 .

3. Results

3.1. Patient characteristics according to PD-L1 SNPs

The baseline characteristics of the 133 patients are summarised in Table 1. The majority of patients (91.0%) had an ECOG PS of 0–1. Adenocarcinoma was the most common histology (72.2%). Thirty-one (23.3%)

patients had EGFR mutations, and 5 (3.8%) had ALK rearrangements. PD-L1 expression status was available for only 63 (47.4%) patients. Eighty-five (63.9%) patients were administered nivolumab at the second or third treatment line. Regarding the frequency of PD-L1 rs822339 genotypes, 34 (25.6%) were A/A, 66 (49.6%) were A/G, and 33 (24.8%) were G/G. The genotypes of rs822339 were not associated with clinical factors. The frequencies of the other PD-L1 and PD-1 SNP genotypes are shown in Supplementary Table S1.

3.2. Associations between PD-L1 SNPs and survival outcomes

As of 30 September 2019, the median follow-up was 12.9 (95% CI, 9.6–18.5) months. At the time of analysis, 81 (60.9%) patients had died before the data cutoff date, and 14 (10.5%) patients were lost to follow-up. The median PFS was 2.1 (95% CI, 1.9–2.8) months, and the median OS was 16.2 (95% CI, 10.3–23.0) months for the overall population. The relationships of the studied SNP genotypes and survival are listed in Supplementary Table S1. Among the 7 studied SNPs, the PD-L1 rs822339 and rs1411262 genotypes were associated with survival. Regarding PD-L1 rs822339, although it was not significant after Bonferroni's correction, the median PFS was longer in the A/A genotype group than in the A/G or G/G genotype group (Fig. 1A). The PFS rate at 6 and 12 months was higher in the A/A genotype group than in the A/G or G/G genotype group. The median OS was not reached (NR) (95% CI, 16.2–NR) in the A/A genotype group, while it was 12.0 (95% CI, 9.1–19.1) months in the A/G or G/G genotype groups (HR, 0.35; 95% CI, 0.18–0.64; $p = 0.0008$) (Fig. 1B). This p value remained significant after Bonferroni's correction. The survival rate at 1 and 2 years in the A/A genotype group was higher than that in the A/G or G/G genotype group. Multivariable analysis revealed that the ECOG PS and PD-L1 rs822339 genotypes were independent prognostic factors for OS (Table 2). The T/T genotype of rs1411262 was also significantly associated with prolongation of OS compared to that with the C/T or C/C genotype (HR, 0.40; 95% CI, 0.21–0.70; $p = 0.0017$). The G/G genotype of rs2282055 tended to indicate favourable OS, but this association was not significant after Bonferroni's correction. In this study, rs822339 and rs1411262 were in LD ($|D'| = 1.0$ and $r^2 = 0.97$) (Supplementary Fig. S1).

3.3. Association between PD-L1 SNPs and OS according to driver mutation status

All patients with driver mutations were treated with nivolumab after disease progression on EGFR or ALK TKIs. In the subgroup analysis of the relationship between PD-L1 rs822339 genotypes and OS according to driver mutation status, the median OS was 32.4 (95%

Table 1

Patient characteristics according to PD-L1 rs822339 genotypes.

	Overall (n = 133)	PD-L1 rs822339			p value*
		A/A (n = 34)	A/G (n = 66)	G/G (n = 33)	
Median age (range), years	69 (30–85)	68.5 (41–85)	69 (30–83)	69 (41–84)	0.89
Sex					
Female	48 (36.1%)	11 (32.4%)	20 (30.3%)	17 (51.5%)	0.60
Male	85 (63.9%)	23 (67.6%)	46 (69.7%)	16 (48.5%)	
Smoking status					
Current/former	90 (67.7%)	22 (64.8%)	48 (72.7%)	20 (60.6%)	0.67
Never	43 (32.3%)	12 (35.2%)	18 (27.3%)	13 (39.4%)	
ECOG PS					
0–1	121 (91.0%)	33 (97.1%)	58 (87.9%)	30 (90.9%)	0.15
2–3	12 (9.0%)	1 (2.9%)	8 (12.1%)	3 (9.1%)	
Histology					
Adenocarcinoma	96 (72.2%)	24 (70.6%)	49 (74.2%)	23 (69.7%)	0.53
Squamous	27 (20.3%)	6 (17.6%)	14 (21.2%)	7 (21.2%)	
Others	10 (7.5%)	4 (11.8%)	3 (4.6%)	3 (9.1%)	
Driver mutation					
EGFR mutation	31 (23.3%)	9 (26.5%)	13 (19.7%)	9 (27.3%)	0.86
ALK rearrangement	5 (3.8%)	1 (2.9%)	4 (6.1%)	0 (0%)	
Negative or unknown	97 (72.9%)	24 (70.6%)	49 (74.2%)	24 (72.7%)	
PD-L1 status					
Positive	28 (21.1%)	6 (17.6%)	14 (21.2%)	8 (24.2%)	0.24
Negative	35 (26.3%)	6 (17.6%)	19 (28.8%)	10 (30.3%)	
Unknown	70 (52.6%)	22 (64.8%)	33 (50.0%)	15 (45.5%)	
Treatment line					
2nd or 3rd	85 (63.9%)	23 (67.6%)	42 (63.6%)	20 (60.6%)	0.60
≥4th	48 (36.1%)	11 (32.4%)	24 (36.4%)	13 (39.4%)	

ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase.

*p value was compared between patients with the A/A genotype and those with the A/G or G/G genotype of rs822339.

CI, 14.8–NR) months in the A/A genotype group and 15.7 (95% CI, 9.4–23.0) months in the A/G or G/G genotype group among patients without driver mutations (HR, 0.45; 95% CI, 0.21–0.88; $p = 0.025$) (Fig. 2A). The survival rates at 1 and 2 years in the A/A versus the A/G or G/G genotype group were 76.5% versus 55.9% and 67.0% versus 33.9%, respectively. Additionally, among patients with driver mutations, the median OS was not reached (95% CI, 1.5–NR) in the A/A genotype group and was 8.9 (95% CI, 3.6–12.9)

months in the A/G or G/G genotype groups (HR, 0.18; 95% CI, 0.029–0.61; $p = 0.0093$) (Fig. 2B). The survival rates at 1 and 2 years in the A/A versus the A/G or G/G genotype group were 77.1% versus 33.3% and 77.1% versus 24.3%, respectively. These results showed that the survival benefit with the A/A genotype of PD-L1 rs822339 was observed regardless of the driver mutation status. The subgroup analysis according to other clinical factors is listed in Fig. 3. The survival benefit with the A/A genotype was observed across most

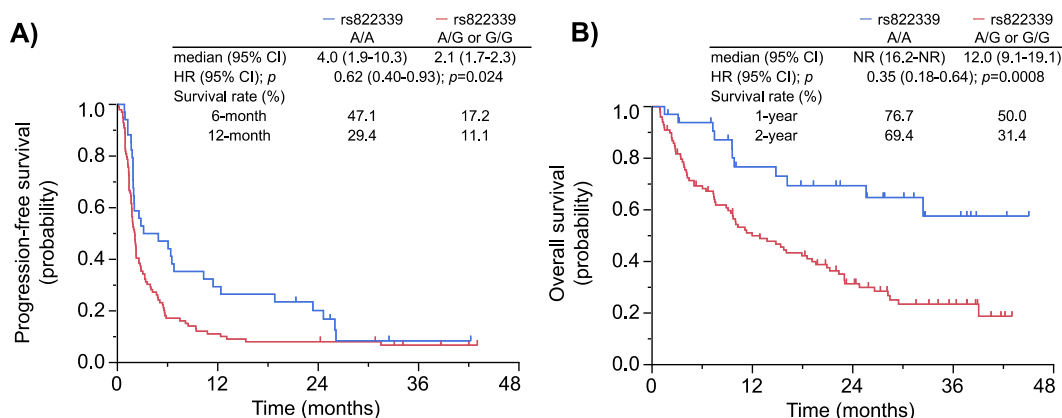


Fig. 1. Kaplan–Meier curves for progression-free survival (PFS) (A) and overall survival (OS) (B) after nivolumab administration according to PD-L1 rs822339 genotypes. CI, confidence interval; NR, not reached; HR, hazard ratio.

Table 2
Univariate and multivariate analysis of OS.

Variable	Univariate			Multivariate		
	HR	95% CI	p value	HR	95% CI	p value
Age (≥ 75 versus < 75 years)	0.95	0.52–1.62	0.86	0.91	0.49–1.59	0.75
Smoking status (current/former versus never)	0.69	0.44–1.10	0.12	0.70	0.38–1.30	0.26
ECOG PS (≥ 2 versus 0–1)	3.32	1.65–6.07	0.002	2.60	1.26–4.91	0.011
Histology (non-Sq versus Sq)	1.17	0.69–2.09	0.58	1.01	0.56–1.88	0.98
Driver mutation (positive versus negative or unknown)	1.29	0.78–2.05	0.31	0.87	0.51–1.75	0.87
Treatment line (2nd or 3rd versus ≥ 4 th)	0.57	0.37–0.88	0.013	0.70	0.42–1.16	0.16
PD-L1 rs822339 (A/A versus A/G or G/G)	0.36	0.18–0.64	< 0.001	0.38	0.19–0.69	< 0.001

OS, overall survival; ECOG, Eastern Cooperative Oncology Group; PS, performance status; Sq, squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

subgroups of the patients. In terms of PFS, the median PFS was longer in the A/A genotype group than that in the A/G or G/G genotype group among patients without driver mutations (Supplementary Fig. S2A). The PFS rate at 6 and 12 months was higher in the A/A genotype group than in the A/G or G/G genotype group. In addition, among patients with driver mutations, the median PFS was slightly longer in the A/A genotype group than in the A/G or G/G genotype group (Supplementary Fig. S2B). The PFS rate at 6 and 12 months in the A/A genotype group was higher than that in the A/G or G/G genotype group (30.0% versus 3.9% and 20.0% versus 3.9%, respectively). These results showed that the A/A genotype tended to indicate favourable PFS regardless of the driver mutation status. The T/T genotype of rs1411262 also showed similar results (data not shown).

3.4. Associations between PD-L1 expression and survival outcomes

PD-L1 expression status was positive in 28 (21.1%) patients, negative in 35 (26.3%) patients, and unknown in 70 (52.6%) patients. Among the 63 patients with PD-L1 test results, both the median PFS and OS were longer in

patients with PD-L1 positive than those with PD-L1 negative ($p = 0.0075$ and 0.049 , respectively) (Supplementary Fig. S3). As reported in previous studies, PD-L1 expression was associated with improved survival outcomes. The genotypes of rs822339 were not associated with PD-L1 expression status (Table 1).

3.5. Association between PD-L1 SNPs and OS in the non-ICI cohort

The baseline characteristics of the 96 patients are summarised in Supplementary Table S2. Seventy-two (75.0%) had driver mutations (EGFR mutations or ALK rearrangements). Regarding the frequency of PD-L1 rs822339 genotypes, twenty-three (23.9%) were A/A, 54 (56.3%) were A/G, and 19 (19.8%) were G/G. The genotypes of rs822339 were not associated with clinical factors. At the time of analysis, OS data were slightly immature because only 32 (33.3%) patients had died before the data cutoff date. Four (4.2%) patients were lost to follow-up. The median follow-up was 24.4 (95% CI, 21.6–28.8) months, and the median OS was 61.5 (95% CI, 47.9–NR) in the overall population. Based on the driver mutation status, patients with driver mutations had significantly longer survival than those

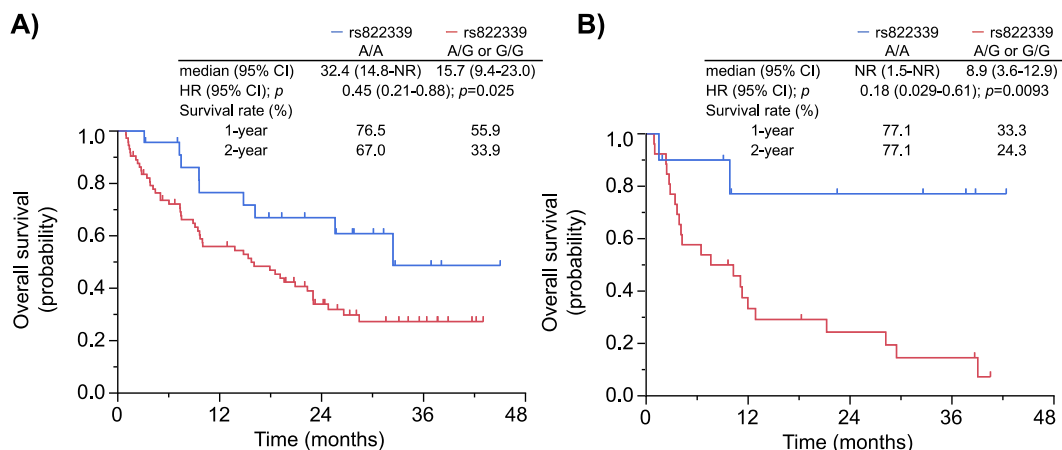


Fig. 2. The association between OS and PD-L1 rs822339 according to driver mutation status. Kaplan–Meier curves for OS in patients without driver mutations (A) and with driver mutations (B). CI, confidence interval; NR, not reached; HR, hazard ratio.

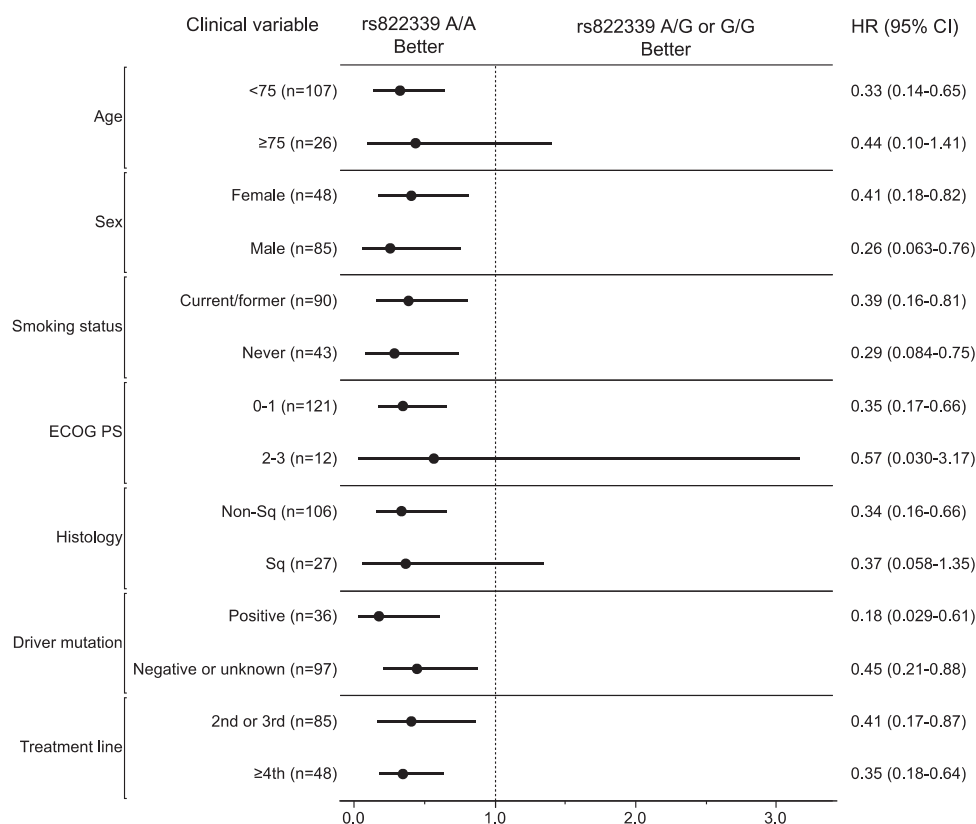


Fig. 3. Subgroup analysis of the association between OS and PD-L1 rs822339. ECOG, Eastern Cooperative Oncology Groups; PS, performance status; Sq, squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

without driver mutations (NR versus 25.7 months; HR, 0.20; 95% CI, 0.094–0.41; $p < 0.0001$). Regarding PD-L1 rs822339, the median PFS was 12.0 (95% CI, 8.2–13.4) months in the A/A genotype group and 12.2 (95% CI, 10.8–15.8) months in the A/G or G/G genotype group (no significant differences) (Supplementary Fig. S4A). In the subgroup analysis of the relationship between PD-L1 rs822339 genotypes and PFS according to first-line treatment, the A/A genotype did not correlate with favourable PFS in both cytotoxic chemotherapy and TKI treatments (Supplementary Fig. S5). Similar trends were observed in the PFS for second-line treatment (data not shown). In terms of OS, there was no significant difference in OS between the A/A genotype and the A/G or G/G genotype of PD-L1 rs822339 (NR versus 61.5 months; HR, 0.77; 95% CI, 0.31–1.70; $p = 0.55$) (Supplementary Fig. S4B), and similar trends were observed across all studied SNPs (data not shown).

4. Discussion

This study showed that the PD-L1 rs822339 and rs1411262 genotypes are associated with prolonged survival in advanced NSCLC patients treated with nivolumab. This survival benefit was also observed regardless of driver mutation status. In contrast, there was no survival difference according to these SNPs in

the non-ICI cohort. Our results suggest that PD-L1 polymorphisms may be useful predictive biomarkers for advanced NSCLC patients treated with nivolumab.

PD-L1 expression is observed on both tumour cells and host immune cells in the tumour microenvironment. In previous studies, good responses to PD-1/PD-L1 blockade were shown in tumours expressing high levels of PD-L1, especially when PD-L1 was expressed by immune cells [24,25]. Other studies demonstrated that PD-L1 expression on immune cells, probably host myeloid cells, may be the relevant target for PD-1/PD-L1 blockade [26,27]. In addition, it has been reported that PD-L1 polymorphisms are associated with PD-L1 expression levels in resected NSCLC and gastric cancer [28,29]. Given these results, the PD-L1 polymorphisms influencing PD-L1 expression on host immune cells may modify the function of the PD-1/PD-L1 axis, leading to different responses to PD-1/PD-L1 blockade. Although the direct contribution of PD-L1 rs822339 and rs1411262 to PD-L1 expression had not been investigated in our study, this scenario would support our study's results.

In contrast, Refae et al. reported that PD-L1 polymorphisms are associated with populations who develop hyperprogressive disease (HPD), an unexpected acceleration of tumour growth kinetics [30]. They demonstrated that the PD-L1 rs2282055 G/G or G/T genotype

is associated with the risk of developing HPD in patients treated with PD-1/PD-L1 blockade. However, the PD-L1 rs2282055 G/G genotype was related to favourable OS in our study, but the association was not significant. In general, the frequency of the rs2282055 G-allele was relatively higher in the Asian population than in the European population in the 1000 Genomes Project and HapMap project. Notably, the frequency of G/G genotypes was 33.8% in our study, but it was only 3% in their study. Thus, the different trends between the two studies may be explained by the different frequencies of the PD-L1 rs2282055 genotype.

In addition, we also assessed the association with PD-L1 polymorphisms and survival in the non-ICI cohort to determine whether the role of PD-L1 polymorphisms was ICI treatment specific. To date, several studies have shown that the efficacy of anticancer drugs depends not only on direct cytotoxic effects but also on the activation of host immune responses against tumour cells [31,32]. Lee et al. reported that the PD-L1 rs4143815 C/C genotype is a poor prognostic factor in advanced NSCLC patients treated with cytotoxic chemotherapy [33]. These results suggested that the PD-1/PD-L1 axis plays a critical role in patients treated with chemotherapy and that PD-L1 polymorphisms may have the potential to exhibit different clinical outcomes. However, our study showed no survival difference according to the studied PD-L1 polymorphisms, including rs4143815, in the non-ICI cohort. A possible explanation is that our cohort had a large number of patients with driver mutations. TKI treatment is greatly beneficial for patients with driver mutations and is the most influential prognostic factor. Therefore, the association of PD-L1 polymorphisms with survival may be obscured in a cohort with a high proportion of driver mutations, such as that in our study.

Interestingly, our study showed that PD-L1 polymorphisms may be useful in predicting survival outcomes after nivolumab treatment, even in patients with driver mutations. In general, the meta-analysis of a large number of retrospective studies revealed that ICI treatment had lower efficacy and did not contribute to OS prolongation in patients with driver mutations [34,35]. Indeed, a prospective phase II study for EGFR-mutant patients with PD-L1-positive and EGFR-TKI naive status was discontinued due to lack of efficacy of PD-1 blockade with pembrolizumab, suggesting that PD-L1 expression was an unreliable marker [36]. However, as a phase I study showed that some patients with driver mutations exhibited long-term survival after nivolumab treatment [37], identifying patients who derive meaningful responses from ICI treatment is of clinical importance. Recently, it has been reported that EGFR-TKIs alter the tumour microenvironment and make it more responsive to ICI treatment [38,39]. As the impact of PD-L1 polymorphism on the tumour

microenvironment after EGFR-TKI treatment is still uncertain, further investigations are warranted.

Our study has several limitations. First, this was a retrospective and small sample-size study conducted at a single institution with only east Asian patients. We have been conducting a prospective multicentre study to confirm our observational results (UMIN000033839); however, this study has a slow patient accrual as the combination with PD-1/PD-L1 blockade and chemotherapy has become a standard first-line treatment for advanced NSCLC. Second, the Common Fund's Genotype-Tissue Expression Program reported that the A/A genotype of rs822339 and the T/T genotype of rs1411262 groups had higher PD-L1 mRNA expression levels in the specific tissue samples; however, we could not show the association between PD-L1 polymorphisms and PD-L1 expression levels because available data for tumour PD-L1 expression status were limited in our study. Despite these limitations, this is the first study suggesting the predictive impact of PD-L1 polymorphisms in the context of ICI treatment and may provide some advantages for future studies in the era of immunotherapy.

In conclusion, we showed that PD-L1 polymorphisms are associated with survival outcomes in NSCLC treated with PD-1 blockade therapy (nivolumab), regardless of driver mutation status. PD-L1 polymorphisms may be useful predictive biomarkers for NSCLC patients treated with nivolumab.

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Author contributions section

H. Yoshida, T. Nomizo and H. Ozasa contributed to the concept and design of the study. H. Yoshida, H. Ozasa, Y. Sakamori and Y.H. Kim recruited and cared for the patients. H. Yoshida, T. Nomizo and T. Funazo performed the experiments. H. Yoshida and T. Nomizo analysed the data. H. Yoshida drafted the manuscript and all authors contributed to data interpretation, discussion and final approval of the manuscript.

Conflict of interest statement

H.Y. reports personal fees from Chugai Pharmaceutical Co., Ltd., and Ono Pharmaceutical Co., Ltd., outside the submitted work. K.H. reports personal fees from Ono Pharmaceutical Co., Ltd., outside the submitted work. Y.S. reports personal fees from Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Astra-Zeneca K.K., and Boehringer Ingelheim Co., Ltd., outside the submitted work. Y.H.K. reports personal

fees from Chugai Pharmaceutical Co., Ltd., and Astra-Zeneca K.K. outside the submitted work. All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2020.11.035>.

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