

Causal Relationship between Postoperative Recurrence and PD-L1 and EGFR Mutations in NSCLC: A Retrospective Cohort Study

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Abstract

Although information on the PD-L1 expression and *EGFR* mutations in non-small cell lung cancer (NSCLC) is important for determining therapeutic strategies, the causality between these two factors and postoperative recurrence and the association between each factor have remained unclear. We retrospectively assessed the PD-L1 expression and *EGFR* mutations in tumors of 280 NSCLC patients. The causality between the PD-L1 expression, *EGFR* mutations and postoperative recurrence were evaluated by a multivariate Cox proportional hazard analysis. The association between the PD-L1 expression and *EGFR* mutations was evaluated by a multinomial logistic regression analysis. The adjusted hazard ratio (HR) in cases with high ($\geq 50\%$) PD-L1 expression was 4.83 (95% confidence interval [CI]: 1.51–15.5). The adjusted HRs in cases with *EGFR* major and minor mutations were 0.42 (95% CI: 0.14–1.25) and 0.63 (95% CI: 0.18–2.15), respectively. The high expression of PD-L1 ($\geq 50\%$) was significantly associated with exon 21 L858R mutation (Ex21) of *EGFR* (adjusted odds ratio, 0.10; 95% CI, 0.01–0.87). The high expression of PD-L1 was an independent risk factor for postoperative recurrence in NSCLC, whereas *EGFR* mutations were not. The high expression of PD-L1 was negatively associated with Ex21. These findings may help identify NSCLC patients with an increased risk of postoperative recurrence.

Introduction

In recent years, the importance of immune checkpoint inhibitors (ICIs) in the treatment of lung cancer has increased [1]. Because the use of ICIs has been suggested to be potentially effective in the pre-surgical setting for surgically resectable non-small cell lung cancer (NSCLC), the benefits of ICIs in the surgical field have also been attracting attention [2]. The programmed death-ligand 1 (PD-L1) expression in tumors has already been shown to be a predictor of the treatment efficacy of ICIs for NSCLC [3]. On the other hand, epidermal growth factor receptor gene (*EGFR*) mutations are important in the pathogenesis of lung adenocarcinoma and EGFR tyrosine kinase inhibitor treatment has been shown to significantly prolong survival of patients with lung adenocarcinoma with *EGFR* mutations [4]. Thus, the information on the PD-L1 expression and *EGFR* mutations in NSCLC are extremely important for therapeutic strategies.

The role of the PD-L1 expression and *EGFR* mutations in the postoperative prognosis of NSCLC and the association of the PD-L1 expression with *EGFR* mutations has been reported in several previous studies [5, 6]. Recently, a large cohort study on postoperative patients with NSCLC suggested that the expression of PD-L1 might be a poor prognostic factor for recurrence-free survival (RFS) [7]. In addition, a large cohort study examining the association between the type of *EGFR* mutations and postoperative recurrence showed that patients with exon 19 deletion (Ex19) had significantly shorter postoperative RFS in comparison to those with exon 21 L858R mutation (Ex21) [8]. Regarding the association between the expression of PD-L1 and *EGFR* mutations in NSCLC, it has been reported that NSCLC with *EGFR* mutation was less likely to express PD-L1 and exhibited a poor response to ICIs in comparison to *EGFR* wild-type [9].

However, the previous studies have not been considered simultaneously analyzing the interaction between different PD-L1 expression levels and various subtypes of *EGFR* mutation. Therefore, the details of the influence of these two factors on the postoperative prognosis and the understanding of the differential expression of PD-L1 depending on *EGFR* mutations remain unknown. By clarifying this association, it may be possible to identify NSCLC patients with a high risk of postoperative recurrence based on the information of the PD-L1 expression and *EGFR* mutations within the resected tumor. Furthermore, the knowledge on the association between PD-L1 expression and *EGFR* mutations may be useful in predicting the effect of ICIs on NSCLC patients with *EGFR* mutations.

The present study aimed to evaluate the effect of PD-L1 expression and *EGFR* mutations on postoperative recurrence in NSCLC, and their associations.

Results

Patient characteristics

The patient characteristics of each PD-L1 expression group are shown in Table 1. Chi-squared tests showed that the high expression of PD-L1 ($\geq 50\%$) was more common in male patients, smokers, the histological type of SCC and other, pathological stage II and IIIA, the presence of adjuvant therapy, and wild-type *EGFR* mutation status. The PD-L1 expressing group tended to include a higher percentage of patients with *EGFR* mutation wild-type. The characteristics of patients categorized according to their *EGFR* mutation status are shown in Supplementary Table S1. The presence of *EGFR* mutations was associated with female sex, never smoker, ADC, earlier pathological stage, and low PD-L1 expression status of $<1\%$ or 1-49%.

Recurrence-free survival

During a median follow-up period of 788 days after surgery, 39 patients developed recurrent disease (13.9%). The Kaplan-Meier curves for RFS according to the PD-L1 expression status are shown in Fig. 1. RFS in the PD-L1-high group was significantly shorter in comparison to the PD-L1-negative and PD-L1-low groups, before adjustment for patient background ($P < 0.001$). The Kaplan-Meier curves for RFS according to the *EGFR* mutation status are shown in Fig. 2. Before adjustment for patient background, RFS did not differ among the *EGFR* mutation status groups ($P = 0.06$).

The multivariate analyses of postoperative RFS according to the expression of PD-L1 and the *EGFR* mutation status

The results of multivariate Cox proportional hazards analysis of factors associated with RFS are shown in Tables 2 and 3. A significant differences in RFS was observed between the PD-L1-high group and the PD-L1-negative group (hazard ratio [HR], 4.83; 95% confidence interval [CI], 1.51-15.5) after adjustment for

patient background factors including the *EGFR* mutation status (Table 2). RFS did not differ to a statistically significant extent in the PD-L1-low expression group (HR, 1.78; 95% CI, 0.59-5.43) (Table 2). In contrast, in the multivariate Cox proportional hazards analysis adjusted for patient background factors including the PD-L1 expression status, RFS did not differ to a statistically significant extent in the major mutation group (HR, 0.42; 95% CI, 0.14-1.25) or the minor mutation group (HR, 0.63; 95% CI, 0.18-2.15) with reference to the wild-type group (Table 3).

The associations between the expression of PD-L1 and *EGFR* mutations

A multinomial logistic regression analysis was performed to examine the link between the expression of PD-L1 and *EGFR* mutations (Table 4). None of the *EGFR* mutations were significantly associated with the low expression of PD-L1: Ex21 (OR, 0.87; 95% CI 0.41-1.85); Ex19 (OR, 0.54; 95% CI 0.23-1.30); minor mutation (OR 1.05; 95% CI 0.35-3.22). The high expression of PD-L1 showed a significant negative association with Ex21 mutation of *EGFR* (OR, 0.10; 95% CI 0.01-0.87). Neither Ex19 nor minor *EGFR* mutations showed a significant association with the high expression of PD-L1: Ex19 (OR, 0.23; 95% CI 0.04-1.22); minor mutation (OR, 0.38; 95% CI 0.06-2.50).

Discussion

The multivariate Cox proportional hazards analysis adjusted for *EGFR* mutation status, pathological stage, histological type, and adjuvant chemotherapy revealed that postoperative recurrence of lung cancer was five times as likely to occur with a PD-L1-high expression status in comparison to PD-L1-negative cases. The Cox proportional hazards analysis with adjustment for the expression of PD-L1, pathological stage, histological type and adjuvant chemotherapy revealed that *EGFR* mutations were not significantly associated with postoperative recurrence. A multinomial logistic regression analysis showed a significant negative association between the high expression of PD-L1 and the presence of the Ex21 mutation.

Previous studies have not shown consistent results regarding the prognosis associated with the expression of PD-L1 and *EGFR* mutations in NSCLC. One study showed that the low expression of PD-L1 was associated with significantly reduced RFS in NSCLC patients with *EGFR* mutations [10]. However, this study did not show a significant association between the high expression of PD-L1 and RFS. In addition, this study focused solely on the influence of the presence or absence of *EGFR* mutations, and the interaction between the PD-L1 expression and various *EGFR* mutations was not considered in the analysis. Furthermore, another study showed that the patients with Ex21 had a significantly longer RFS in comparison to those with WT or Ex19 [8]. However, this study did not evaluate RFS, with the inclusion of the PD-L1 expression as a confounding factor in the multivariate analysis.

In the present study, we performed multivariate analyzes considering the interaction of different PD-L1 expression levels with *EGFR* mutations in NSCLC and revealed the following three novel points. First, patients with the high expression of PD-L1 had significantly shorter postoperative RFS, whereas those with the low expression of PD-L1 showed no significant difference. Second, the presence of *EGFR* mutations did not contribute significantly to postoperative RFS. Third, the high expression of PD-L1 showed a significant negative connection with Ex21.

These three points may be biologically plausible. First, the high expression of PD-L1 in NSCLC may affect the postoperative prognosis. Because cancer cells inactivate T cells via the PD-L1 expression [11], the higher the expression of PD-L1, the more the immune system is suppressed, which may lead to greater cancer progression than expected after surgery. Some studies have reported that the expression of PD-L1 was associated with the AKT-mTOR pathway, which has been shown to be necessary for cell proliferation [12]. Therefore, the expression of PD-L1 may be correlated with oncogenic signal, the high expression of which may be involved in high tumor progression. Second, *EGFR* mutations in NSCLC may not affect postoperative recurrence. An *in vitro* study using cell lines have reported that *EGFR* mutations promote the expression of PD-L1 [13]. However, *in vivo*, it has been reported that various cytokines in the tumor microenvironment affect the PD-L1 expression on cancer cells [14]. An experiment in which cell lines were stimulated with cytokines, such as interferon- γ due to mimicking of the *in vivo* environment demonstrated that the expression of PD-L1 is higher in cells with wild-type *EGFR* in comparison to those with *EGFR* mutations [15]. In the *in vivo* tumor microenvironment, *EGFR* mutation may not be an independent risk factor for recurrence because of attenuated immunosuppression in connection with the tendency for low PD-L1 expression levels in cells with *EGFR* mutations. Third, among the *EGFR* mutation subtypes, Ex21 may be negatively associated with the high expression of PD-L1 in NSCLC. The tumor mutation burden (TMB) has been known to be an effective predictor of an improved response to ICIs in NSCLC [16]. One study suggested that there was no relevant association between the expression of PD-L1 and a high TMB in tumors [17]. In addition, another study examining the association between *EGFR* mutations and the TMB reported that the TMB tended to be higher in cells with Ex21 than in those with Ex19 [18]. Thus, the high TMB in cancer cells with Ex21 may contribute to the suppression of the high expression of PD-L1.

The present study was associated with some limitations. First, because the number of variables that could be included in the multivariable Cox proportional hazards analysis was limited due to the small number of postoperative recurrence cases, it was not possible to incorporate all of other confounding factors into the model at once. A larger sample size using a model that simultaneously includes all variables is needed in order to evaluate the influence of the PD-L1 expression and *EGFR* mutation status on the postoperative recurrence with higher accuracy. Second, our study was limited to surgical cases; we did not examine advanced cases that were managed without surgery. *EGFR* mutation positivity was reportedly associated with the expression of PD-L1 in stage IV [19]. This result is completely opposite to the results of our study. Therefore, the PD-L1 expression in advanced stage may behave differently than other stages. A prognostic analysis that analyzed the influence of the PD-L1 expression and *EGFR* mutation status, including patients with advanced stage disease, may also be needed.

In summary, we performed multivariate analyses using a cohort of patients who had undergone complete surgical resection for lung cancer to compare postoperative RFS between groups categorized according to their PD-L1 expression and *EGFR* mutation status. We showed that $\geq 50\%$ PD-L1 positivity within the resected tumor tissue was an independent risk factor for recurrence after lung cancer surgery, whereas the *EGFR* mutation status was not. We showed that Ex21 may be not a risk factor for postoperative recurrence due to the difficulty in expressing a high level of PD-L1. Our findings may contribute to the selection of patients who are eligible for adjuvant chemotherapy using ICIs, regardless of pathological stage.

Methods

Patients

A total of 280 lung cancer patients who had undergone surgical lung resection at Kinki-Chuo Chest Medical Center (KCMC) from April 2017 to January 2020 were included in our study. We selected the patients who had undergone complete resection. Those who had received limited resection were excluded because a previous report showed that limited resection was associated with a higher rate of locoregional recurrence in comparison to lobectomy [20]. The histopathological diagnosis according to the current 2015 World Health Organization classification was performed by pathologists [21]. Adjuvant chemotherapy with a platinum-based regimen was administered to patients with indications and who had given their informed consent. Patients who received neoadjuvant therapy were excluded because it was reported that the PD-L1 expression status of cancer cells was altered after neoadjuvant chemotherapy [22]. Other clinicopathological features, including age, sex, smoking status, pathologic tumor-node-metastasis (TNM) classification (eighth edition) and the presence of postoperative recurrence were collected from medical records. The present study was approved by the Institutional Review Board of KCMC (Approval number: 725). Informed consent was obtained by an opt-out method using the website of our institution. All methods were performed in accordance with relevant guidelines and regulations.

Tumor PD-L1 immunohistochemistry and the EGFR mutation assay

All viable cancer cells on the entire pathological tissue section of each tumor sample were evaluated. We used the PD-L1 clone 22C3 pharmDx kit and Dako Automated Link 48 platform (Agilent Technologies, Dako, Carpinteria, CA, USA) to measure the PD-L1 expression. The PD-L1 tumor proportion score (TPS) was calculated as the percentage of complete or partial membrane staining in a sample. The cut-off value for the expression of PD-L1 was set at 50% and 1% based on a previous clinical trial [23]. The tumor samples of each patient were separated into 3 groups based on the presence of positivity stained cells in specimen, as follows: $<1\%$ (negative), 1-49% (low expression), and $\geq 50\%$ (high expression). All patients

were subjected to an *EGFR* mutation assay by the testing laboratories (Cobas *EGFR* Mutation Test; Roche Molecular Diagnostics, Pleasanton, CA, USA).

Recurrence-free survival

The primary outcome of this study was recurrence-free survival (RFS), defined as the time from the date of curative resection to the date on which disease relapse was diagnosed. The patients received blood tests and a chest X-ray after surgery every three or six months. Additional screenings were performed in cases where abnormal findings were observed. The diagnosis of relapse was comprehensively determined based on the results of examinations.

Statistical analyses

Chi-squared tests were used to compare the proportions of categorical variables between each of the PD-L1 expression groups and *EGFR* mutation groups. The probability of RFS was assessed using the Kaplan-Meier method and log-rank tests. A multivariate Cox proportional hazards analysis was performed to estimate the hazard ratios (HRs) with adjustment by risk factors for recurrence. A multinomial logistic regression analysis was performed to assess the odds ratios (ORs) for each *EGFR* mutation status in the PD-L1-high and PD-L1-low groups, with the PD-L1-negative group as a reference. A multivariate Cox proportional hazards analysis and a multivariate logistic regression analysis can analyze the covariates of the number of cases with an outcome divided by 10 or 5 [24, 25]. In this study, the number in the Cox proportional hazards analysis was 39 (i.e., the number of cases with relapse) divided by 10 or 5 (result: 4 or 8). And the number of cases included in the logistic regression analysis was 56 (i.e., the number of cases with the high expression of PD-L1) divided by 10 or 5 (coming to 5 or 11). We selected the following 8 factors in a multivariate Cox hazards analysis: the PD-L1 expression level (low and high [reference: negative]); histological type (squamous cell carcinoma (SCC) and other types [reference: adenocarcinoma (ADC)]); pathological stages (stage II-IIIa [reference: stage I]); adjuvant chemotherapy (platinum [reference: none]); the *EGFR* mutation status (major mutation (Ex19 or Ex21) and minor mutation (all mutations except Ex21 and Ex19) [reference: wild-type]). These factors have been reported to evaluate factors associated with postoperative recurrence [26]. In addition, we selected the following 10 factors in the multinomial logistic regression analysis: histological types (SCC and other types [reference: ADC]); pathological stages (stage II and stage IIIa [reference: stage I]); the *EGFR* mutation status (Ex21, Ex19 and minor mutation [reference: wild-type]); age; sex; and smoking status. All statistical analyses were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of R commander with added biostatistical functions [27]. *P* values of < 0.05 were considered to indicate statistical significance.

Abbreviations

ADC Adenocarcinoma

EGFR Epidermal growth factor receptor

Ex19 Exon 19 deletion

Ex21 Exon 21 L858R

NSCLC Non-small cell lung cancer

PD-L1 Programmed cell death-ligand 1

RFS Recurrence free survival

SCC Squamous cell carcinoma

Declarations

Author contributions

K.K. conceived and designed the study. K.K., T.S. and H.Y. collected clinical specimens. T.K. performed pathological diagnosis. K.K., T.S. and H.Y. collected clinical data. K.K., T.K. and S.A. performed the data analysis. K.K. wrote the manuscript. All authors reviewed the manuscript.

Conflicts of interest statement

S.A. has received research funding from AstraZeneca, MSD, Eli-Lilly, Chugai, Ono, Taiho, Boehringer-Ingelheim, Pfizer, Bristol-Myers Squibb, F. Hoffmann-La Roche, Hisamitsu, Kyowa Hakko Kirin, Novartis Pharma and Thermo Fisher Scientific. The remaining authors have no conflicts of interest to disclose.

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Tables

Table 1

Association between the PD-L1 expression and patient clinicopathological variables.

Variables	PD-L1 expression			P
	<1% (N = 78)	1-49% (N = 146)	≥50 (N = 56)	
Age: ≥70 years – no. (%)	39 (50.0)	88 (60.3)	29 (51.8)	0.27
Sex: Male – no. (%)	30 (38.5)	84 (57.5)	43 (76.8)	< 0.001
Current or former smoker – no. (%)	40 (51.3)	87 (59.6)	47 (83.9)	< 0.001
Histological Type – no. (%)				< 0.001
ADC	76 (97.4)	115 (78.7)	25 (44.6)	
SCC	1 (1.3)	22 (15.1)	23 (41.1)	
Other	1 (1.3)	9 (6.2)	8 (14.3)	
Pathological Stage – no. (%)				< 0.001
I	64 (82.1)	107 (73.3)	30 (53.6)	
II	11 (14.1)	24 (16.4)	15 (26.8)	
IIIA	3 (3.8)	15 (10.3)	11 (19.6)	
Surgical procedure – no. (%)				0.74
Lobectomy	76 (97.4)	135 (92.5)	51 (91.1)	
Bilobectomy	1 (1.3)	3 (2.1)	2 (3.6)	
Lobectomy with combined resection	1 (1.3)	8 (5.5)	3 (5.4)	
Lymph node dissection – no. (%)				< 0.001
ND1	17 (21.8)	7 (4.8)	1 (1.8)	
ND2	61 (78.2)	139 (95.2)	55 (98.2)	
Adjuvant therapy – no. (%)				0.018
Platinum	4 (5.1)	13 (8.9)	11 (19.6)	
EGFR mutation – no. (%)				< 0.001
Wild-type	38 (48.7)	92 (63.0)	51 (91.1)	
Ex21	19 (24.4)	28 (19.2)	1 (1.8)	
Ex19	15 (19.2)	14 (9.6)	2 (3.6)	
Minor mutation ^a	6 (7.7)	12 (8.2)	2 (3.6)	

^a Defined as all mutations except Ex21 and Ex19.

PD-L1 programmed cell death-ligand 1, *ADC* adenocarcinoma, *SCC* squamous cell carcinoma, *EGFR* epidermal growth factor receptor gene, *Ex21* exon 21 L858R mutation, *Ex19* exon19 deletion mutation.

Table 2

The Cox proportional hazards analysis of RFS according to the PD-L1 expression.

PD-L1 expression	N	Recurrence	Unadjusted		Adjusted	
		N (%)	HR (95% CI),	<i>P</i>	HR ^a (95% CI),	<i>P</i>
< 1%	78	4 (5.13)	Reference		Reference	
1-49%	146	15 (10.3)	2.12 (0.70-6.39),	0.18	1.78 (0.59-5.43),	0.31
≥50%	56	20 (35.7)	8.96 (3.01-26.3),	< 0.001	4.83 (1.51-15.5),	0.008

^a Adjusted by pathological stage, histological type, adjuvant chemotherapy and *EGFR* mutation status.

RFS recurrence-free survival, *PD-L1* programmed death-ligand 1, *HR* hazard ratio, *CI* confidence interval, *EGFR* epidermal growth factor receptor gene.

Table 3

The Cox proportional hazards analysis of RFS according to the *EGFR* mutation status.

<i>EGFR</i> mutation	N	Recurrence N (%)	Unadjusted HR (95% CI),	<i>P</i>	Adjusted HR ^a (95% CI),	<i>P</i>
Wild-type	181	32 (17.7)	Reference		Reference	
Major mutation ^b	79	4 (5.06)	0.26 (0.09-0.74),	0.01	0.42 (0.14-1.25),	0.12
Minor mutation ^c	20	3 (15.0)	0.77 (0.23-2.51),	0.66	0.63 (0.18-2.15),	0.46

^a Adjusted by PD-L1 expression status, pathological stage, histological type and adjuvant chemotherapy.

^b Ex21 or Ex19.

^c Defined as all mutations except Ex21 and Ex19.

RFS recurrence-free survival, *EGFR* epidermal growth factor receptor gene, *HR* hazard ratio, *CI* confidence interval, *PD-L 1* programmed death-ligand 1, *Ex21* exon 21 L858R mutation, *Ex19* exon19 deletion mutation.

Table 4

The multinomial logistic regression analysis to investigate the association between the expression of PD-L1 and *EGFR* mutations.

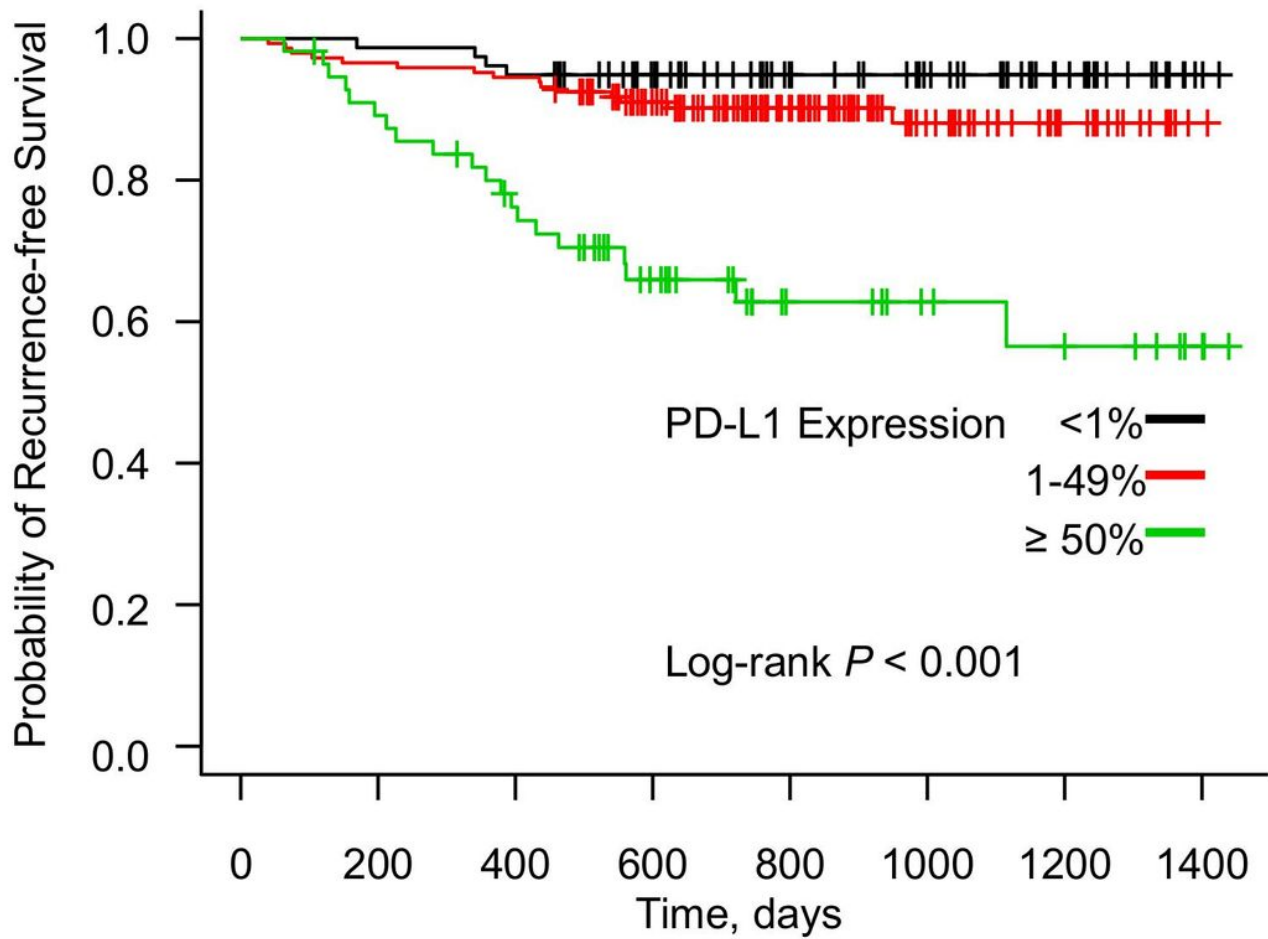
<i>EGFR</i> mutation	PD-L1 expression							
	1-49% vs. <1%				≥50% vs. <1%			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
	OR (95% CI),	<i>P</i>	OR ^a (95% CI),	<i>P</i>	OR (95% CI),	<i>P</i>	OR ^a (95% CI),	<i>P</i>
Wild-type	Reference		Reference		Reference		Reference	
Ex21	0.61 (0.30-1.22),	0.16	0.87 (0.41-1.85),	0.72	0.04 (0.01-0.31),	< 0.001	0.10 (0.01-0.87),	0.03
Ex19		0.02		0.17		<		0.08
Minor mutation ^b	0.39 (0.17-0.88),	0.72	0.54 (0.23-1.30),	0.93	0.09 (0.02-0.46),	0.001	0.23 (0.04-1.22),	0.31
	0.83 (0.29-2.36),		1.05 (0.35-3.22),		0.25 (0.04-1.30),	0.09	0.38 (0.06-2.50),	

^a Adjusted by age, sex, smoking status, pathological stage and histological type.

^b Defined as all mutations except Ex21 and Ex19.

PD-L1 programmed death-ligand 1, *EGFR* epidermal growth factor receptor gene, *OR* odds ratio, *CI* confidence interval, *Ex21* exon 21 L858R mutation, *Ex19* exon19 deletion mutation.

Figures

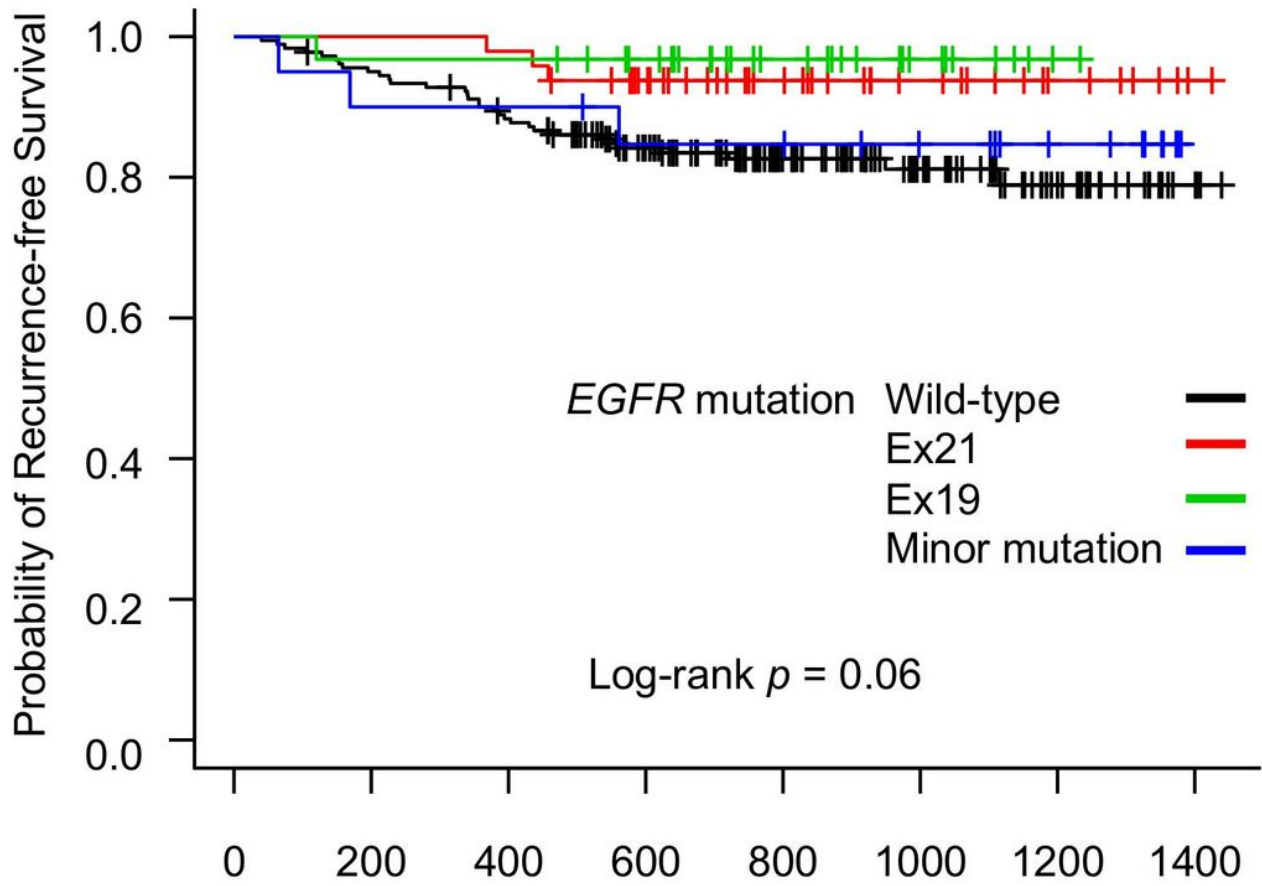


No. at Risk

<1%	78	77	74	62	44	36	18	2
1-49%	146	141	138	112	76	37	16	1
≥ 50%	56	49	40	27	15	11	9	3

Figure 1

Kaplan-Meier curve showing the probability of a recurrence-free survival among patients after lung cancer resection according to the expression of PD-L1.



No. at Risk	Time, days							
	0	200	400	600	800	1000	1200	1400
Wild-type	181	171	157	123	80	49	26	5
Ex21	48	48	47	36	23	14	7	1
Ex19	31	30	30	26	16	8	1	0
Minor mutation	20	18	18	16	16	13	9	0

Figure 2

Kaplan-Meier curve showing the probability of a recurrence-free survival among patients after lung cancer resection according to the EGFR mutation status.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1.pdf](#)