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ORIGINAL RESEARCH

High PD-L1 Expression is Associated with Unfavorable Clinical Outcome in *EGFR*-Mutated Lung Adenocarcinomas Treated with Targeted Therapy

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Byung Woo Yoon [1,2]
Boksoon Chang [3]
Seung Hyeun Lee [3]

[1]Department of Internal Medicine, Seoul Paik Hospital, Seoul, South Korea; [2]Department of Internal Medicine, Inje University College of Medicine, Gimhae, South Korea; [3]Department of Internal Medicine, Kyung Hee University School of Medicine, Seoul, South Korea

Purpose: Although programmed death-ligand 1 (PD-L1) expression is widely accepted as a predictive and prognostic biomarker in immunotherapy, its implications in lung cancer patients with driving mutations are still unclear. The objective of this study is to determine the association between PD-L1 expression and treatment outcome in epidermal growth factor receptor (*EGFR*)-mutated lung cancer treated with tyrosine kinase inhibitors (TKIs).

Methods: We retrospectively enrolled *EGFR*-mutant, advanced lung adenocarcinoma patients who received first-line *EGFR*-TKIs and evaluated the PD-L1 tumor proportion score (TPS) using the 22C3 pharmDx assay. We investigated the distribution of patients with different PD-L1 TPS values, followed by the analysis of response rate (RR), survival rate, and incidence of secondary T790M mutation according to the PD-L1 TPS group.

Results: Among the 131 patients analyzed, the proportion of patients with PD-L1 TPS ≥ 50%, 1–49%, and <1%, was 17.6%, 32.8%, and 49.6%, respectively. The RR was significantly lower in the group with PD-L1 TPS ≥ 50% than in the other groups (43.5% vs 72.1% vs 78.5%, all $p = 0.001$). In multivariate analysis, PD-L1 TPS ≥ 50% was independently associated with a significantly shorter PFS in the overall population (hazard ratio [HR] = 2.64, $p = 0.004$) and associated with shorter OS in patients with exon 19 deletion (HR = 2.55, $p = 0.041$) compared with PD-L1 TPS < 50%. In addition, the frequency of secondary T790M mutation after TKI failure was significantly lower in the group with PD-L1 TPS ≥ 50% than in the other groups (13.3% vs 40.0% vs 53.3%, all $p = 0.001$). PD-L1 TPS ≥ 50% was an independent predictor of a lower frequency of this mutation (HR = 0.63, $p = 0.043$).

Conclusion: High PD-L1 expression was associated with unfavorable clinical outcome and less development of secondary T790M mutation, suggesting a distinct subgroup warranting active surveillance and tailored therapeutic approach.

Keywords: lung cancer, programmed death-ligand 1, epidermal growth factor receptor mutation, tyrosine kinase inhibitor, clinical outcome, prognosis, T790M

Correspondence: Seung Hyeun Lee
Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Internal Medicine, Kyung Hee University School of Medicine, Kyungheedae-Ro 23, Dongdaemun-Gu, Seoul 02447, South Korea
Tel +82 2 958 8511
Fax +82 2 968 1848
Email v3mann@naver.com

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. In Korea, approximately 27,000 new cases and 18,000 lung cancer-related deaths were reported in 2017.[1,2] Adenocarcinoma is the major histologic subtype constituting 60% of all cases of non-small cell lung cancers (NSCLC).[3] Although new treatment modalities, including molecular-targeted therapy and immune checkpoint inhibitors, have demonstrated remarkable survival benefits in patients with advanced lung

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adenocarcinoma,[3,4] the prognosis is still poor suggesting the need for individualized therapeutic strategies to improve the clinical outcomes.

Immunotherapy based on immune checkpoint inhibitors represents one of the most important breakthroughs in the management of solid tumors, including lung cancers with promising results in numerous clinical trials.[5] Programmed death-ligand 1 (PD-L1) expression is consistently associated with clinical efficacy in anti-PD-1/PD-L1 treatment.[6–8] The US Food and Drug Administration approved PD-L1 22C3 and 28–8 clones as a companion diagnostic for pembrolizumab and as a complementary diagnostic method for nivolumab, respectively, in 2015. However, the clinical implications of this biomarker in different clinical settings including chemotherapy or targeted therapy are largely unknown. Given that PD-1/PD-L1 interaction is a major immune checkpoint involved in immune escape during cancer development and progression, the upregulated PD-L1 expression may be associated with dismal clinical outcome in lung cancer patients undergoing treatment other than immunotherapy. This concept was partially evident in a previous study demonstrating the poor prognosis of patients with positive PD-L1 expression who received surgical resection for lung cancer.[9]

Epidermal growth factor receptor (*EGFR)* mutations are major driver genetic alterations, which are detected in approximately 50% of the population in the Far East diagnosed with lung adenocarcinoma.[10,11] *EGFR*-tyrosine kinase inhibitors (TKIs) have doubled the progression-free survival (PFS) compared with chemotherapy and increased the overall survival (OS) to more than 2 years, and thus recommended as a frontline treatment in patients harboring this mutation.[12] However, the response to TKIs is not similar in all *EGFR*-mutant tumors and resistance inevitably occur after approximately 11 months of treatment in most patients.[13–15] The emergence of resistance is the major cause of treatment failure, which is a challenge for the management of those patients.[16] The mechanism of primary resistance to *EGFR*-TKIs is not fully understood, although it is partly explained by *De novo* T790M mutation, the presence of concurrent genetic alterations, or current smoking.[17,18] Thus, identification of mechanisms leading to TKI resistance or determination of predictive and prognostic factors before TKI use is an important step to improve clinical outcomes in this patient population.

To address this issue, we conducted the present study to determine the clinical impact of PD-L1 expression in patients with *EGFR*-mutant advanced lung adenocarcinoma treated with first-line TKIs. First, we evaluated whether PD-L1 expression was associated with treatment response or survival. We then evaluated its association with the emergence of secondary T790M mutation after TKI failure.

Materials and Methods

Study Subjects and Data Collection

We retrospectively recruited *EGFR*-mutant patients treated with first-line *EGFR*-TKIs for histologically confirmed, locally advanced or metastatic lung adenocarcinoma at three referral hospitals in South Korea (Kyung Hee University Medical Center, Kyung Hee University Hospital at Gangdong, and Dongnam Institution of Radiological & Medical Sciences) from January 2014 to November 2019. Patients without follow-up data, a history of other cancers, or other driving genetic alterations including ROS proto-oncogene 1 (*ROS1)* and anaplastic lymphoma kinase (*ALK)* fusions, and those with a previous history of chemotherapy or radiotherapy were excluded.

All patients underwent staging workup, including chest computed tomography (CT), brain magnetic resonance imaging, and ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography. TNM staging was performed according to the 8th edition of the International Association for the Study of Lung Cancer TNM staging system.[19] Tumor response was assessed with CT after every two cycles of systemic treatment and evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.[20] We collected demographic information, past medical or social history, and clinical outcome of all participants by reviewing electronic medical records. This study protocol was approved by the Clinical Research Ethics Committee of the Kyung Hee University Medical Center (KHMC 2019–06-030). Written informed consent was obtained from all patients who were alive. All research was carried out in compliance with the Declaration of Helsinki.

PD-L1 Immunohistochemical Staining and Scoring

Immunohistochemical staining for PD-L1 expression was performed using 22C3 pharmDx assay (Agilent, Santa Clara, CA, US) and the Automated Link 48 Platform (Dako, Carpinteria, CA, US) in formalin-fixed

tumor samples obtained by surgical resection or small biopsy (percutaneous needle biopsy, bronchoscopic mucosal biopsy, or endobronchial ultrasound-guided transbronchial biopsy) before commencement of first-line TKI treatment. Neoplastic cells were considered positive in the presence of cell membrane staining. PD-L1 expression was determined using the tumor proportion score (TPS), which is defined as the percentage of viable tumor cells showing partial or complete membrane staining. Based on PD-L1 expression, the tumors were categorized into three groups (< 1%, 1–49%, and ≥50%), according to the TPS by counting at least 100 viable cells.[21]

EGFR Mutation Testing

All the *EGFR* tests were performed using the tumor tissues. Genomic DNA was extracted from formalin-fixed, paraffin-embedded, 5-μm-thick tissue sections using the High Pure Template Preparation Kit (Roche Applied Science, Mannheim, Germany). The extracted DNA was stored at −20°C until analysis. EGFR Pyro Kit (QIAGEN Korea Ltd., Seoul, Korea) and PyroMark Q24 System (QIAGEN Korea Ltd., Seoul, Korea) were used to detect *EGFR* mutations via real-time polymerization chain reaction (PCR). The primer sets covered mutations or deletions spanning exons 18 to 21 of the genes encoding the tyrosine kinase domain of *EGFR*. The results were interpreted according to the manufacturer's instructions.

Statistical Analyses

Baseline characteristics of different groups were compared using Chi-square test or Fisher's exact test as appropriate. Clinical outcomes were assessed using response rate (RR), PFS and OS. RR was defined as the percentage of patients who showed complete or partial remission. PFS and OS were defined as the periods from the first day of treatment to disease progression/death and death from any cause, respectively. Data of patients without tumor recurrence or death were censored at the last follow-up. Associations between clinical/pathologic parameters and survival were evaluated by univariate analysis using the Log rank test. Subsequently, the multivariate Cox's proportional hazard regression analysis was conducted by adjusting parameters with p values < 0.3 from the univariate analysis. Survival curves were generated using the Kaplan–Meier method. P values < 0.05 were considered significant. All analyses were performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

Results

Patient Characteristics

During the study period, 684 patients were newly diagnosed with NSCLC in three institutes and 412 patients were diagnosed with advanced disease. Of these patients, 160 underwent first-line treatment with *EGFR*-TKIs for *EGFR*-mutant lung adenocarcinoma. Fifteen patients without survival data available, 11 with a history of other cancers, and 3 who received other cancer treatments before the initiation of TKIs were excluded. Finally, a total of 131 patients were eligible for the analysis.

The clinical characteristics of the study population are summarized in Table 1. All subjects were Korean and their median age was 70 years (range, 42–86 years). Sixty-seven (51.1%) patients were aged ≥70 years. Seventy-one (54.2%) patients were female. Forty-seven (35.9%) patients were current or former smokers. One hundred and three (78.6%) patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Eight (6.1%) patients had stage III and 123 (93.9%) patients had stage IV disease. Twenty-one (16.0%) patients had metastases involving more than three organs. Thirty-three (25.2%) and 15 (11.5%) patients had brain or liver metastasis, respectively. Seventy-four (56.5%) patients received gefitinib or erlotinib, while 57 (43.5%) received afatinib as a first-line TKI. Sixty-four (48.9%) patients had exon 19 deletion (19del), 52 (39.7%) had L858R point mutations, and 15 (11.5%) had uncommon or compound mutations. The list of uncommon or compound mutations was provided in Table S1. Clinicopathological characteristics did not differ according to *EGFR* mutational subtypes (Table 1).

Distribution of PD-L1 TPS Expression

The prevalence of different PD-L1 TPS is resented in Table 1. Number of patients with PD-L1 TPS ≥ 50%, 1–49%, and <1% was 23 (17.6%), 43 (32.8%), and 65 (49.6%), respectively. The proportion of patients with PD-L1 TPS ≥ 50% was lower compared with historical NSCLC population without driver mutations[22] and was in accordance with previous data evaluating PD-L1 expression in *EGFR*-mutant

Table 1 Characteristics of 131 Study Patients Stratified by *EGFR* Mutational Subtypes

	No. of Patients (%)	*EGFR* Mutational Subtypes			*p*-value
		19del (n=64)	L858R (n=52)	Others (n=15)	
Age (years)					0.936
Median, range	70 (42–86)	70 (42–86)	69 (47–83)	72 (53–76)	
Age					0.433
<70	64 (48.9)	32 (50.0)	27 (51.9)	5 (33.3)	
≥70	67 (51.1)	32 (50.0)	25 (48.1)	10 (66.7)	
Sex					0.583
Male	60 (45.8)	30 (46.9)	25 (48.1)	5 (33.3)	
Female	71 (54.2)	34 (53.1)	27 (51.9)	10 (66.7)	
Smoking history					0.261
Never	84 (64.1)	45 (70.3)	29 (55.8)	10 (66.7)	
Ever	47 (35.9)	19 (29.7)	23 (44.2)	5 (33.3)	
ECOG PS					0.713
0,1	103 (78.6)	50 (78.1)	40 (76.9)	13 (86.7)	
≥2	28 (21.4)	14 (21.9)	12 (23.1)	2 (13.3)	
Stage					0.152
III	8 (6.1)	2 (3.1)	3 (5.8)	2 (13.3)	
IV	123 (93.9)	62 (96.9)	49 (94.2)	13 (86.7)	
Organs involved					
≤3	110 (84.0)	53 (82.8)	45 (86.5)	12 (80.0)	0.781
>3	21 (16.0)	11 (17.2)	7 (13.5)	3 (20.0)	
Brain metastasis					
No	98 (74.8)	44 (68.8)	41 (78.8)	13 (86.7)	0.245
Yes	33 (25.2)	20 (31.2)	11 (21.2)	2 (13.3)	
Liver metastasis					
No	116 (88.5)	58 (90.6)	43 (82.7)	15 (100.0)	0.137
Yes	15 (11.5)	6 (9.4)	9 (17.3)	0 (0.0)	
First-line TKI					0.578
Gefitinib	35 (26.7)	18 (28.1)	12 (23.1)	3 (20.0)	
Erlotinib	39 (29.8)	20 (31.2)	17 (32.7)	2 (13.3)	
Afatinib	57 (43.5)	26 (40.7)	21 (44.2)	10 (66.7)	
PD-L1 TPS					0.379
<1%	65 (49.6)	37 (57.8)	23 (44.2)	5 (33.3)	
1–49%	43 (32.8)	18 (28.1)	18 (34.6)	7 (46.7)	
≥50%	23 (17.6)	9 (14.1)	11 (21.2)	3 (20.0)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-L1 TPS, programmed death-ligand 1 tumor proportional score.

lung cancer.[23,24] The reduced incidence of high PD-L1 (PD-L1 TPS ≥ 50%) expression was consistent regardless of *EGFR* mutational subtypes (Table 1).

Response Rate and Progression-Free Survival According to PD-L1 TPS

The median follow-up time for this cohort was 32.8 months (range, 4.2–57.7 months). The RRs according to the PD-L1 TPS and *EGFR* subtypes are summarized in Table S2. The RRs of patients with PD-L1 TPS ≥ 50%, 1–49%, and <1% were 43.5%, 72.1%, and 78.5%, respectively. The RRs of patients with PD-L1 TPS ≥ 50% were significantly lower compared with those with PD-L1 TPS 1–49% and <1% (both $p = 0.001$, Figure 1). The lower RR of high PD-L1 TPS group was consistent throughout different *EGFR* mutational subtypes (Table S2).

The results of PFS analysis according to clinicopathological parameters are summarized in Table 2. The median PFS of all study subjects was 15.1 months (range, 2.2–30.8 months). Based on univariate analysis, metastases involving more than three organs, presence of liver metastasis, and PD-L1 TPS ≥ 50% were significantly associated with shorter PFS (all $p < 0.05$). Multivariate analysis showed that metastases involving than three organs (HR = 3.20, 95% confidence interval [CI]: 1.01–10.8) and PD-L1 TPS ≥ 50% (HR = 2.64, 95% CI: 1.43–4.80) were independently associated with shorter PFS. Kaplan–Meier survival curves showed that patients with PD-L1 TPS ≥ 50% were likely to have poor PFS compared with other TPS groups (Figure 2A).

To evaluate whether the PD-L1 expression has any different impact on the PFS according to different *EGFR* mutational subtypes, we performed subgroup analysis. Survival analysis results of patients with 19del and

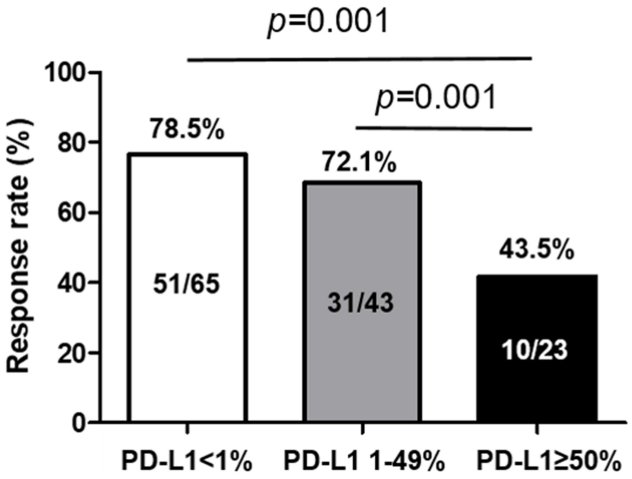


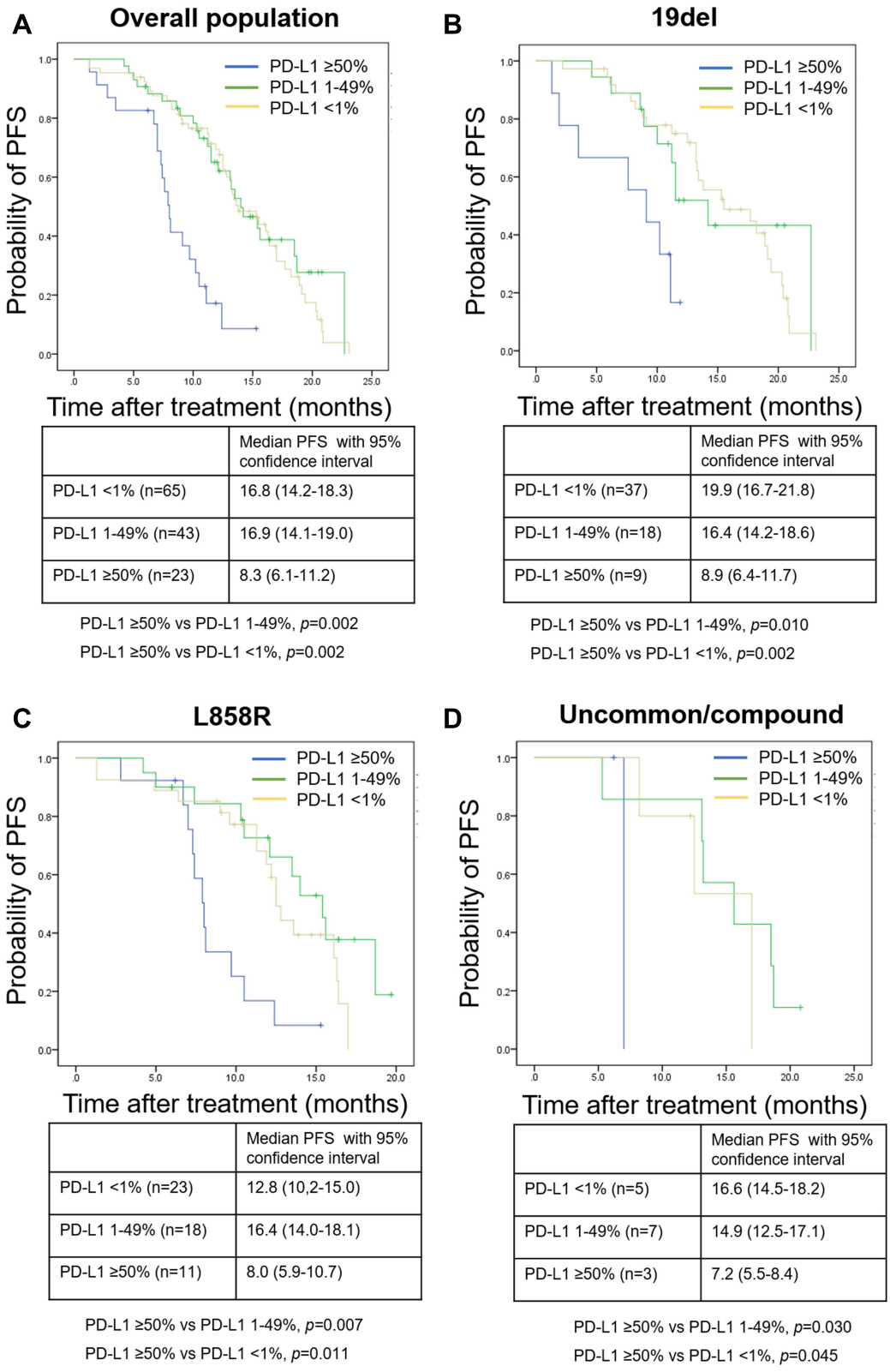
Figure 1 Response rate (RR) according to different PD-L1 TPS in patients with *EGFR*-mutations treated with first-line TKIs. The RRs of patients with PD-L1 TPS ≥ 50% were significantly lower compared with patient groups of PD-L1 TPS 1–49% and <1% (both $p = 0.001$).

Table 2 Progression-Free Survival Analyses Results According to Clinicopathological Parameters of All Study Subjects (n=131)

	Median PFS (months)	Univariate		Multivariate	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
All	15.1				
Age (years)			0.294		0.198
<70	17.1	reference		reference	
≥70	12.9	1.26 (0.82–1.94)		1.66 (0.78–2.35)	
Sex			0.122		0.100
Female	17.1	reference		reference	
Male	12.6	1.43 (0.91–2.24)		1.50 (0.93–2.40)	
Smoking history			0.354	NA	
Never	14.5	reference			
Ever	11.6	1.24 (0.79–1.95)			
ECOG PS			0.537	NA	
0.1	15.7	reference			
≥2	11.6	1.18 (0.70–1.97)			
Stage			0.104		0.247
III	16.5	reference		reference	
IV	13.3	1.54 (0.74–2.26)		1.12 (0.56–2.10)	
Organ involved			0.043		0.046
≤3	16.7	reference		reference	
>3	11.6	3.03 (0.93–9.69)		3.20 (1.01–10.8)	
Brain metastasis			0.214		0.741
No	15.5	reference		reference	
Yes	11.6	1.36 (0.84–2.19)		1.09 (0.65–1.84)	
Liver metastasis			0.039		0.183
No	16.5	reference		reference	
Yes	12.6	1.89 (1.03–3.45)		1.50 (0.81–3.08)	
EGFR mutation subtypes			0.213		0.610
19del	17.7	reference		reference	
L858R	12.2	1.43 (0.98–4.15)		1.27 (0.68–3.11)	
Others	16.5	1.01 (0.48–3.43)		1.12 (0.85–2.62)	
First-line TKI			0.106		0.211
Gefitinib/Erlotinib	11.5	1.25 (0.84–2.39)		1.86 (0.91–3.95)	
Afatinib	13.7	reference		reference	
PD-L1 TPS			0.002		0.004
<50%	16.9	reference		reference	
≥50%	8.3	2.48 (1.39–4.41)		2.64 (1.43–4.80)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-L1 TPS, programmed death-ligand 1 tumor proportional score; HR, hazard ratio; CI, confidence interval; NA, not analyzed.

L858R are summarized in Tables S3 and S4, respectively. The univariate analysis of 19del-positive population revealed that male sex, metastases involving more than three organs, and PD-L1 TPS ≥ 50% were significantly associated with shorter PFS (all $p < 0.05$). Multivariate analysis showed that male sex (HR = 1.91, 95% CI: 1.02–3.71) and PD-L1 TPS ≥ 50% (HR = 3.85, 95% CI: 1.23–11.92) were independently associated with shorter PFS. In case of L858R-positive population, metastases involving more than three organs, and PD-L1 TPS ≥ 50% were significantly associated with shorter PFS (all $p < 0.05$) in univariate analysis, and only PD-L1



	Median PFS with 95% confidence interval
PD-L1 <1% (n=65)	16.8 (14.2-18.3)
PD-L1 1-49% (n=43)	16.9 (14.1-19.0)
PD-L1 ≥50% (n=23)	8.3 (6.1-11.2)

PD-L1 ≥50% vs PD-L1 1-49%, p=0.002

PD-L1 ≥50% vs PD-L1 <1%, p=0.002

	Median PFS with 95% confidence interval
PD-L1 <1% (n=37)	19.9 (16.7-21.8)
PD-L1 1-49% (n=18)	16.4 (14.2-18.6)
PD-L1 ≥50% (n=9)	8.9 (6.4-11.7)

PD-L1 ≥50% vs PD-L1 1-49%, p=0.010

PD-L1 ≥50% vs PD-L1 <1%, p=0.002

	Median PFS with 95% confidence interval
PD-L1 <1% (n=23)	12.8 (10,2-15.0)
PD-L1 1-49% (n=18)	16.4 (14.0-18.1)
PD-L1 ≥50% (n=11)	8.0 (5.9-10.7)

PD-L1 ≥50% vs PD-L1 1-49%, p=0.007

PD-L1 ≥50% vs PD-L1 <1%, p=0.011

	Median PFS with 95% confidence interval
PD-L1 <1% (n=5)	16.6 (14.5-18.2)
PD-L1 1-49% (n=7)	14.9 (12.5-17.1)
PD-L1 ≥50% (n=3)	7.2 (5.5-8.4)

PD-L1 ≥50% vs PD-L1 1-49%, p=0.030

PD-L1 ≥50% vs PD-L1 <1%, p=0.045

Figure 2 Kaplan–Meier curves of progression-free survival (PFS) according to different PD-L1 TPS. Curves of (**A**) overall population, (**B**) patients with exon 19 deletion, (**C**) patients with L858R mutation, and (**D**) patients with uncommon or compound mutations. *P*-values were determined using the Log rank test.

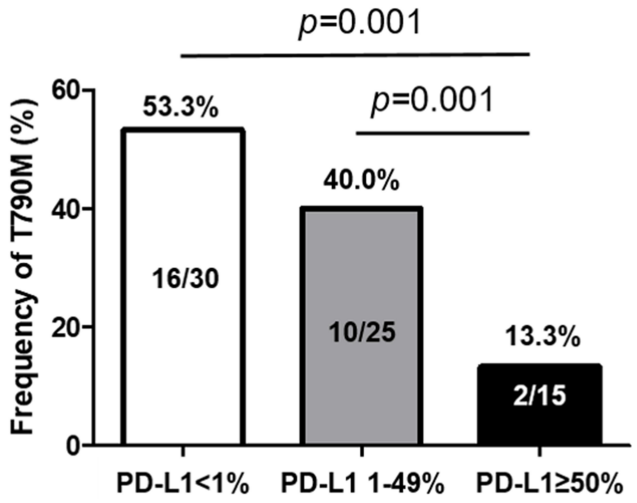


Figure 3 Frequency of secondary T790M mutation after TKI failure according to different PD-L1 TPS. PD-L1 TPS ≥ 50% showed significantly low frequency of T790M mutation compared with the other two groups (all $p = 0.001$).

TPS ≥ 50% was an independent predictor of shorter PFS (HR=2.15, 95% CI: 1.12–6.98). Kaplan–Meier survival curves of patients with different mutational subtypes showed that patients with PD-L1 TPS ≥50% were likely to have poor PFS compared with other TPS groups (Figure 2BD).

Overall Survival According to PD-L1 TPS

The results of OS analysis of the overall population are summarized in Table S5. The median OS of all study subjects was 36.8 months (range, 4.2–54.6 months). Univariate analysis showed that male sex, current or former smoking, metastases involving more than three organs, and presence of liver metastasis were significantly associated with shorter OS (all $p < 0.05$). Multivariate analysis showed that metastases involving more than three organs (HR = 3.05, 95% CI: 1.36–6.72) and presence of liver metastasis (HR = 2.46, 95% CI: 1.10–5.76) were independently associated with shorter OS. PD-L1 TPS ≥ 50% was not associated with shorter OS.

In the subgroup analysis according to different *EGFR* mutational subtypes, metastases involving more than three organs (HR = 2.90, 95% CI: 1.07–9.23), presence of liver metastasis (HR= 3.70, 95% CI: 1.01–10.86), and PD-L1 TPS ≥ 50% (HR = 2.55, 95% CI: 1.17–6.40) were independently associated with shorter OS in patients with 19del (Table S6). However, PD-L1 TPS ≥ 50% was not associated with shorter OS in those with L858R mutations (Table S7). Kaplan–Meier survival curves for OS of the population and each mutational subtypes are presented in Figure S1.

Frequency of Acquired T790M Mutation According to PD-L1 TPS

To identify the possible association between the emergence of secondary T790M mutation and PD-L1 expression, we evaluated the frequency of the mutation according to different PD-L1 TPS. Among 70 patients who underwent rebiopsy, 28 (40.0%) patients were found to carry T790M mutation. The mutation frequency was 13.3% (2/15), 40.0% (10/25), and 53.3% (16/30) in PD-L1 TPS ≥ 50%, 1–49%, and <1% groups, respectively. Patients with PD-L1 TPS ≥ 50% showed significantly low frequency of T790M mutation compared with the other two groups (all $p = 0.001$, Figure 3). The analyses results for the factors associated with the emergence of secondary T790M mutation are presented in Table 3. Univariate analysis showed that L858R mutation, TKI use <12 months, and PD-L1 TPS ≥ 50% were significantly associated with lower incidence of T790M (all $p < 0.05$). Multivariate analysis showed that duration of TKI use <12 months was independently associated with a low emergence of acquired T790M (OR = 0.54, 95% CI: 0.-11–0.89). In addition, PD-L1 TPS > 50% was significantly associated with lower frequency of secondary T790M mutation (OR = 0.63, 95% CI: 0.12–0.95).

Discussion

The present study demonstrated that high PD-L1 expression was significantly associated with poor treatment response and shorter PFS regardless of *EGFR* mutational subtypes in patients who were treated with first-line *EGFR*-TKIs. In addition, it was associated with less frequent development of secondary T790M mutation after TKI failure. To the best of our knowledge, this is the second study to suggest the possible association between pre-TKI PD-L1 expression and the emergence of T790M mutation. Our data suggest that high PD-L1 expression might confer an aggressive phenotype requiring different therapeutic approaches among *EGFR*-mutant tumors.

Although components of tumor immune microenvironment (TME) including tumor PD-L1 expression, tumor nonsynonymous mutation burden (TMB), and tumor-infiltrating lymphocytes (TILs) are emerging as predictors of response to immune checkpoint blockade in NSCLC,[25,26] their clinical implications in patients with

Table 3 Analysis of the Factors Associated with Emergence of Secondary T790M Mutation

	Univariate		Multivariate	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
Age (years)		0.228		0.632
<70	reference		reference	
≥70	0.55 (0.21–1.46)		0.76 (0.24–2.40)	
Sex		0.912	NA	
Female	reference			
Male	1.06 (0.41–2.72)			
Smoking history		0.568	NA	
Never	reference			
Ever	0.74 (0.26–2.08)			
ECOG PS		0.276		0.362
0.1	reference		reference	
≥2	1.51 (0.91–4.94)		1.77 (0.52–6.10)	
Stage		0.391		
III	reference		NA	
IV	0.41 (0.05–3.15)			
Organ involved		0.924		
≤3	reference		NA	
>3	1.06 (0.30–3.75)			
Brain metastasis		0.241		0.741
No	reference		reference	
Yes	1.90 (0.65–5.57)		1.12 (0.35–3.70)	
Liver metastasis		0.856		
No	reference		NA	
Yes	1.12 (0.32–3.96)			
EGFR mutation subtypes				
19del	reference		reference	
L858R	0.82 (0.21–1.94)	0.047	0.71 (0.15–1.86)	0.061
Others	0.20 (0.02–1.65)	0.134	0.29 (0.03–1.45)	0.156
First-line TKI		0.106		0.278
Gefitinib/Erlotinib	1.25 (0.74–2.45)		1.16 (0.51–3.41)	
Afatinib	reference		reference	
Duration of TKI use		0.035		0.040
<12 months	0.33 (0.08–0.74)		0.54 (0.11–0.89)	
≥12months	reference		reference	
PD-L1 TPS		0.047		0.043
<50%	reference		Reference	
≥50%	0.54 (0.07–0.89)		0.63 (0.12–0.95)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-L1 TPS, programmed death-ligand 1 tumor proportional score; OR, odds ratio; CI, confidence interval; NA, not analyzed.

EGFR-mutations remain unclear. Earlier studies demonstrated that *EGFR* mutations induced PD-L1 expression, suggesting the role of *EGFR* signaling in remodeling the TME to increase sensitivity to anti-PD-1/PD-L1 treatment.[27,28] However, subsequent studies identified that *EGFR*-mutant lung adenocarcinoma was correlated with an uninflamed phenotype with a high frequency of inactive TIL and low TMB.[29,30] Several studies on PD-L1

Table 4 Summary of Published and Present Data on the Association Between PD-L1 Expression and Clinical Outcomes of EGFR-TKIs

Author, Year	No. of Patients	PD-L1 IHC	Scoring Method and Cutoff	Outcome Parameters Associated with High PD-L1 Expression
D'Incecco et al, 2015[32]	95	Ab58810	5% staining	Better RR and longer TTP
Lin et al, 2015[33]	56	Ab58810	Mean H score	Better DCR and longer PFS
Tang et al, 2015[39]	64	E1L3N	H score 5	No association
Soo et al, 2017[34]	90	SP142	Median H score	Shorter PFS
Yoneshima et al, 2018[31]	71	Dako 22C3	1% of TPS	Shorter PFS
Su et al, 2018[35]	101	SP142	TC3/IC3, TC1-2/IC1-2, TC0/IC0	Poor RR, shorter PFS, higher primary resistance rate
Hsu et al, 2019[36]	123	SP263	1% of TPS	Shorter PFS, higher primary resistance rate
Yang et al, 2020[24]	153	Dako 22C3	501% of TPS	Poor RR, shorter PFS, higher primary resistance rate Less acquired T790M
Present study	131	Dako 22C3	501% of TPS	Poor RR and shorter PFS Less acquired T790M Shorter OS in exon19del

Abbreviations: IHC, immunohistochemical staining; PD-L1, programmed death ligand-1; H score, histological score; RR, response rate; TTP, time to progression; DCR, disease-control rate; PFS, progression-free survival; TPS, tumor proportional score; OS, overall survival.

expression demonstrated a lower proportion of elevated PD-L1 expression among patients with *EGFR*-mutations (11.8% to 17.5%) compared with *EGFR* wild-type patients, which are consistent with our results (17.6%).[23,24,31] Taken together, our study confirmed the previous findings suggesting that *EGFR*-mutant NSCLC is characterized by less immunogenic TME in terms of tumor PD-L1 expression, suggesting that immune checkpoint blockade might be of limited benefit in such tumors compared with *EGFR*-wild type NSCLC.

PD-L1 expression is widely accepted as predictive and prognostic in immunotherapy, however, the implications of this biomarker in *EGFR*-mutant lung cancer treated with TKIs are still inconclusive. A summary of the published studies and our data is presented in Table 4. Earlier studies showed that positive PD-L1 expression was associated with better RR and longer time to progression or PFS.[32,33] However, five recent studies consistently demonstrated that PD-L1 expression was associated with poor response and unfavorable clinical outcomes.[24,31,34-36] Yang et al reported that RR and PFS were significantly poor in patients with PD-L1 TPS ≥50%, and PD-L1 TPS<50% was an independent prognostic factor for longer PFS (HR=0.433, 95% CI: 0.250–0.751).[24] Our results are in accordance with those recent studies and suggest the predictive and prognostic value of baseline PD-L1 expression in *EGFR*-mutant tumors. The reason for the inconsistent results is not clear but it can be partly explained by differences in sample size, patients' ethnicities, antibody clones, and scoring cutoffs among different studies.

In addition, male sex was independently associated with shorter PFS in our study. Although the clinical significance of sex in *EGFR*-mutant NSCLC treated with *EGFR*-TKIs as first-line treatment is still disputed, many studies have suggested that male sex is an unfavorable predictive factor.[31,37,38] Our results are consistent with previous findings. However, further large-scaled studies are needed to validate the impact of sex on survival in this clinical setting.

In the present study, PD-L1 expression was not associated with OS in overall population. To date, three studies have investigated the prognostic value of PD-L1 in *EGFR*-mutant NSCLC treated with EGFR-TKIs.[33,34,39] Although the cutoffs and the antibodies used differed among the studies, no association was observed between PD-L1 positivity and OS, which was in accordance with our finding. However, in the subgroup analysis, we identified that high PD-L1 expression was independently associated with shorter OS among patients with 19del but not among those with L858R. A preclinical study has demonstrated that these two *EGFR*-mutations show distinct biological properties that may affect the efficacy of EGFR-TKIs.[40] In addition, our data showed that compared to other mutational subtypes, patients with 19del are more likely to carry secondary T790M mutation which is associated with longer survival. Although the reason why PD-L1 expression was significant only in 19del-positive patients is not clear, our findings suggest that this immune checkpoint protein may have prognostic value in a certain

group of *EGFR*-mutant patients. This hypothesis should be validated by further studies with long-term follow-up data.

Studies have demonstrated that PD-L1 expression is inducible and can be upregulated by various genomic alterations such as *EGFR, ALK*, and mitogen-activated protein kinase (MAPK).[41–43] Thus high PD-L1 expression may confer to presence of concurrent genetic alterations, resulting in TKI resistance as reported in previous studies.[30,44] Recent studies suggest that TME interacts with *EGFR*-mutant tumors, which affects the efficacy of TKI treatment as shown in the field of immunotherapy.[24,45] Matsumoto et al divided the TME into four types based on PD-L1 expression and CD8+ T cells, and demonstrated that PD-L1+/CD8+ tumors exhibited the lowest RR (14.3%) and the shortest median PFS (2.4 months), while PD-L1-/CD8+ patients showed best RR (78.6%) and the longest PFS (17.5 months) in *EGFR*-mutant patients treated with TKIs.[45] In addition, Yang et al evaluated the impact of various immune cells in TME including regulatory T cells and macrophages, and demonstrated the possible association between CD20+ B cell and tumor response in the same clinical setting.[24] More interestingly, a recent study suggested that TKI treatment may alter TME in *EGFR*-mutant NSCLC.[46] Using rebiopsy samples after TKI failure, Isomoto et al reported that the proportion of patient with high PD-L1 expression was significantly increased (14% to 28%, $p = 0.001$) and TMB tended to increase after TKI (3.3 to 4.1 mutations/Mbp, $p = 0.0508$) and suggested the possible benefit of subsequent anti-PD-1/PD-L1 treatment in those patients.[46] Previous studies have demonstrated that patients with driving genetic alterations are characterized by impaired response to immune checkpoint inhibitors.[47] However, Sun et al reported that the clinical response to PD-1/PD-L1 blockade reached almost 30% even in *EGFR*-mutant patients, especially in males or smokers with high PD-L1 expression suggesting the possible clinical benefit of immunotherapy in selected patients harboring the mutation.[48] Taken previous and our data together, subsequent immunotherapy after TKI failure in patients with high PD-L1 expression might be a feasible treatment option although further prospective investigations are essential.

In this study, we found that high PD-L1 expression was associated with a low frequency of acquired T790M mutation as a resistance mechanism. The T790M gatekeeper mutation, localized to exon 20 of *EGFR* has been identified in about half of patients who progressed after first-line *EGFR*-TKI, which is a major resistance mechanism.[16] Because T790M-positive tumors are susceptible to osimertinib, the detection of this mutation is critical for the management of *EGFR*-mutant patients. Although mutational analysis using tumor tissue or body fluid is the standard method, it is often limited by issues such as invasiveness of the tissue acquisition procedures, inaccessibility or insufficiency of sample tissues. Previous studies demonstrated that 19del and long-term exposure to *EGFR*-TKIs were associated with more frequent T790M mutation.[49–51] Our present data identified that low PD-L1 expression (PD-L1 TPS <50%) can be a predictor for T790M positivity after TKI failure, which is consistent with a recent study.[24] The reason why low PD-L1 is associated with frequent T790M emergence is unclear; however, it may be attributed to the longer duration of treatment with *EGFR*-TKI in patients with low PD-L1 expression as suggested by previous studies.[52–54] Chmielecki et al demonstrated that T790M-positive cells were selected after long-term exposure to first-line *EGFR*-TKIs.[52] Other studies have shown that T790M-positive cells undergo selection and enrichment during *EGFR*-TKI treatment.[53,54] T790M-positive tumor is slow-growing with a longer survival compared with T790M-negative counterparts.[55] Interestingly, Hata et al reported higher PD-L1 expression in T790M-negative patients compared with T790M-positive patients after *EGFR*-TKI failure, suggesting a potential benefit of anti-PD-1/PD-L1 treatment in T790M-negative population.[56] This hypothesis was partly supported by a subsequent study demonstrating favorable response to nivolumab in such patients.[57]

Based on our findings, we cautiously suggest that *EGFR*-mutant tumors should be managed with different treatment strategies according to baseline PD-L1 status. TKI monotherapy would be the standard-of-care for patients with low PD-L1 expression, as indicated by the current guidelines. In contrast, for patients with high PD-L1 expression who show poor response to TKIs, the combination with other agents including chemotherapy or immunotherapy may be a feasible option. Indeed, a very recent NEJ009 trial demonstrated that gefitinib plus carboplatin/pemetrexed combination showed better RR (84% vs 67%, $p < 0.001$), longer PFS (20.9 vs 11.9 months, HR = 0.49; $p < 0.001$), and OS (50.9 vs 38.,8 months, HR = 0.722; $p = 0.021$) in *EGFR*-mutant NSCLC compared with gefitinib monotherapy.[58] Because the elevated PD-L1 expression confers a distinct aggressive phenotype among *EGFR*-mutant tumors, large-scaled prospective investigations are urgently required to determine the optimal treatment strategy for those patients population.

This study has several limitations. First, it is a retrospective study and selection bias is inevitable. However, we strived to enhance the validity of our data by using a relatively large cohort with long-term follow-up. Second, we did not include clinical characteristics such as underlying medical diseases or nutritional status, which may affect the clinical outcome in our study population. Third, we only used a single antibody (22C3) and did not evaluate other PD-L1 antibodies simultaneously. The recent Blueprint Project has shown that the results of various PD-L1 antibody clones were concordant except SP142.[59] Fourth, patients with other genetic alterations such as *ROS1* and *ALK* fusions were excluded because of their rarity among lung adenocarcinoma patients. Fifth, the dynamic changes of PD-L1 expression in patients with progressive disease after *EGFR*-TKI were not evaluated. As mentioned above, TKI therapy can affect the level of PD-L1 expression and the impact of upregulated or downregulated PD-L1 expression on the subsequent treatment is an interesting topic for future studies. Finally, we did not assess pre-TKI genetic alterations or other immune phenotypes. To address this issue, we are currently working on a comprehensive study investigating the impact of concurrent genetic alterations and immunologic signatures on the clinical course of *EGFR*-mutant patients.

Conclusion

Our data demonstrate that a high PD-L1 expression is associated with unfavorable clinical outcome not only due to the poor response to frontline TKI treatment but also because of the diminished likelihood of secondary T790M mutation after TKI failure. Although further studies are needed to verify our results, our findings suggest that elevated expression of PD-L1 might confer a distinct aggressive phenotype among EGFR-mutant tumors requiring a tailored therapeutic approach. In addition, future studies should focus on the optimal treatment strategy for populations with high PD-L1 expression to facilitate personalized medicine for patients with driving mutations.

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Disclosure

The authors declare that they have no competing interests for this work.

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