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High PD-L1 expression correlates with primary resistance to EGFR-TKIs in treatment naïve advanced *EGFR*-mutant lung adenocarcinoma patients

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ABSTRACT

Objectives: The main objective was to investigate the relationship between Programmed cell Death-ligand 1 (PD-L1) expression levels and the frequency of primary resistance to Epidermal Growth Factor Receptor (EGFR)-Tyrosine Kinase Inhibitor (TKI) in treatment naïve advanced *EGFR*-mutant lung adenocarcinoma patients.
Materials and methods: From 2012–2017, we enrolled advanced *EGFR*-mutant lung adenocarcinoma patients who displayed primary resistance to EGFR-TKI therapy, along with patients with disease control, and patients experiencing either stable disease or partial response to EGFR-TKI treatment.
Results: Sixty-six patients were enrolled as the primary resistance group, while 57 patients were included as the disease control group. Fifteen-five (22.7%) patients had a PD-L1 Tumor Proportion Score (TPS) ≧50% in the primary resistance group, with only one patient (1.8%) having that score in the disease control group ($P < 0.001$). Twenty (30.3%) patients had a PD-L1 ≧25% in the primary resistance group, with 2 (3.5%) patients having that level in the disease control group ($P < 0.001$). Thirty (45.5%) patients had a PD-L1 ≧1% in the primary resistance group, with 7 (12.3%) patients at that level in the disease control group ($P = 0.001$). Patients with a PD-L1≧1% displayed a higher incidence of primary resistance to EGFR-TKIs than those with a PD-L1 < 1% (Odds Ratio (OR), 5.95; 95% Confidence Interval (CI), 2.35–15.05; $P < 0.001$). The phenomenon existed still when the cutoff value was changed to both 25% (OR, 11.96; 95% CI, 2.65–53.87; $P = 0.001$) and 50% (OR, 16.47; 95% CI, 2.10–129.16; $P = 0.008$). The estimated median Progression-free Survival (PFS) rate was 7.3 months in patients with a PD-L1 < 1%, 2.1 months in patients with a PD-L1≧1%, 1.8 months in patients with a PD-L1≧25%, and 1.6 months in patients with a PD-L1≧50%.

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Conclusions: Treatment for advanced *EGFR*-mutant lung adenocarcinoma patients displaying a higher PD-L1 expression level experienced a higher frequency of primary resistance to EGFR-TKIs.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. The treatment of lung cancer is individualized, and is thus determined by the results of molecular biology tests and each patient's histology [1]. *Epidermal Growth Factor Receptor (EGFR)* mutation is the most common driver mutation gene amongst lung cancer patients in East Asian, including Taiwan [2,3]. In Non-small Cell Lung Cancer (NSCLC) patients, activating *EGFR* mutation could be found in approximately 10% of Caucasian patients, and more than 50% of Asian patients [3,4]. Previous clinical trials had shown that EGFR-tyrosine Kinase Inhibitors (TKI) offered a better response rate and displayed fewer adverse effects than platinum-based chemotherapy, in *EGFR*-mutant NSCLC patients with a median Progression-free Survival (PFS) of 9.2–13.1 months [5–9]. A current study has revealed that third-generation EGFR-TKI had a longer PFS than first-generation EGFR-TKI (18.9 months vs. 10.2 months; $P < 0.001$) [10].

Although most *EGFR*-mutant NSCLC patients have shown a positive response to EGFR-TKIs, approximately 5–10% of sensitizing *EGFR*-mutant patients did not achieve disease control with EGFR-TKIs [5,7–9,11,12], with their condition thus being called primary resistance. There is little known about the primary resistance to EGFR-TKIs in NSCLC patients harboring *EGFR* mutation.

Current studies have demonstrated that *EGFR*-mutated NSCLC patients exhibited a low response to Programmed cell Death-1 (PD-1) and Programmed cell Death-ligand 1 (PD-L1) checkpoint blockade, and that immune checkpoint inhibitors do not improve overall survival [13,14]. Additionally, the PD-L1 expression level was one of the predictive biomarkers regarding immune checkpoints treatment effects [15,16]. The pooled analysis from 18 studies performed by Soo, et al. presented that patients with *EGFR*-mutant NSCLC had a lower PD-L1-positive rate than those with *EGFR*-wild type tumors [17]. Their results were consistent with our previous research [18].

We conducted this study in order to investigate the distribution of the PD-L1 expression level in treatment for naïve advanced *EGFR* mutant lung adenocarcinoma with primary resistance to EGFR-TKIs. We attempted to compare the non-responsive patients with the disease control patients regarding the differences in PD-L1 expression and their clinical characteristics.

2. Material and methods

2.1. Study design

This study was a retrospective, single-center, observational study at Taichung Veterans General Hospital (TCVGH) in Taiwan. The study was approved by the Institutional Review Board (IRB) of TCVGH, Taiwan.

2.2. Patients

We enrolled lung cancer patients between 2012 and 2017. To be eligible for the study, patients had to fulfill the following inclusion criteria: a diagnosis of histologically and cytologically confirmed lung adenocarcinoma, stage IIIB-IV according to the 7th edition of the American Joint Committee for Cancer (AJCC) staging system [19], sensitizing *EGFR* mutation, treatment naïve to EGFR-TKIs, and possess sufficient specimens to assess their PD-L1 expression level. Patients were excluded if they had *EGFR* mutations with T790 M and exon 20 insertion, or if they had been diagnosed with another active malignancy. We evaluated each patient's treatment response to EGFR-TKIs through use of the Response Evaluation Criteria in Solid Tumors (Version 1.1) [20]. The definition of primary resistance was when patients experiencing progressive disease after EGFR-TKIs treatment did not meet the Jackman's criteria of acquired resistance [21]. We enrolled all patients with primary resistance to EGFR-TKIs as the primary resistance group, and selected patients with stable disease and partial response to EGFR-TKIs as the disease control group. Demographic characteristics and clinical data, including age, gender, smoking status, baseline *EGFR* mutation status, the type of EGFR-TKIs treatment, Progression-free Survival (PFS) of EGFR-TKIs, and Overall Survival (OS) was collected for analysis. The definition of PFS was the time from the first dose of EGFR-TKI to progression or death, and the definition of OS was the time from the first dose of EGFR-TKI to death. A non-smoker was defined as someone who had never smoked or smoked less than 100 cigarettes in his or her lifetime. Written informed consent for genetic testing and clinical data records was obtained from all patients.

2.3. EGFR mutation and PD-L1 expression test

EGFR mutations were assessed through use of a Matrix-assisted Laser Desorption Ionization-time Of Flight Mass Spectrometry (MALDI-TOF MS). Each patient's PD-L1 expression level was evaluated by the Ventana PD-L1 SP263 assay conducted on the Ventana BanchMark platform. All tests were performed at the ISO15189-certified TR6 Pharmacogenomics Lab (PGL), as part of the National Research Program for Biopharmaceuticals (NRPB), in the National Center of Excellence for Clinical Trial and Research of NTUH.

2.4. Statistical analyses

Regarding the differences between the patient's characteristic and demographic data in the primary resistance group and control group, we used the Fisher's exact test for assessing age, gender, smoking status, baseline *EGFR* mutation status, and the type of EGFR-TKI treatment. A Cox proportional hazard model was performed to evaluate both PFS and OS. The association between the PD-L1 expression level and frequency of primary resistance to EGFR-TKIs was analyzed through the use of logistic regression models. Survival curves were estimated with the Kaplan-Meier method. All statistical tests were done with a SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Two-tailed tests and P values < 0.05 for determining significance were used.

3. Results

3.1. Patients characteristics

In total, 123 patients diagnosed with advanced *EGFR*-mutant lung adenocarcinoma were enrolled for analysis. Sixty-six patients were included in the primary resistance group, while 57 patients were included in the disease control group. Among the disease control group, 9 patients had stable disease to EGFR-TKIs, while 48 patients had partial response to EGFR-TKIs. The baseline characteristics are shown in Table 1. Thirty-four (51.5%) patients were older than 65 years in the primary resistance group, with 28 (49.1%) patients in the disease control group also being older than 65. Thirty-two (48.5%) patients were male in the primary resistance group, and 26 (45.6%) patients were male in the disease control group. Most of the patients were non-smokers in both groups (46 (69.7%) in the primary resistance group, 35 (61.4%) in the disease control group).

Regarding baseline *EGFR* mutation status, 23 (34.8%) patients

Table 1
Patients' characteristics and demographic data.

Characteristics	Primary resistance	Disease control	P value*
Age (years), median (range)			0.857
≦65	32 (48.5)	29 (50.9)	
> 65	34 (51.5)	28 (49.1)	
Gender, n (%)			0.857
Male	32 (48.5)	26 (45.6)	
Female	34 (51.5)	31 (54.4)	
Smoking status, n (%)			0.348
NS	46 (69.7)	35 (61.4)	
C/FS	20 (30.3)	22 (38.6)	
Baseline *EGFR* mutation status, n (%)			0.545
Exon 19 deletions	23 (34.8)	24 (42.1)	
Exon 21 L858R	38 (57.6)	27 (47.4)	
Other mutations	5 (7.6)#	6 (10.5)&	
TKI, n (%)			0.482
Gefitinib	32 (48.5)	24 (42.1)	
Erlotinib	29 (43.9)	25 (43.9)	
Afatinib	5 (7.6)	8 (14.0)	
PD-L1 status (1%), n (%)			< 0.001
< 1%	36 (54.5)	50 (87.7)	
≧1%	30 (45.5)	7 (12.3)	
PD-L1 status (25%), n (%)			< 0.001
< 25%	46 (69.7)	55 (96.5)	
≧25%	20 (30.3)	2 (3.5)	
PD-L1 status (50%), n (%)			0.001
< 50%	51 (77.3)	56 (98.2)	
≧50%	15 (22.7)	1 (1.8)	
PFS of TKI (months), median (range)	1.9 (0.2-6.0)	15.9 (4.2-33.9)	
OS of TKI (months), median (range)	7.2 (0.3-47.2)	21.6 (4.2-59.3)	

NS, never-smokers; C/FS, current/former-smokers; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-L1, Programmed death-ligand 1; PFS, progression-free survival; OS, overall survival.

* By Fisher's exact test.

\# G719 A, G719S, Exon 19 deletions + L858R, 2 L861Q.

& L861Q, G719S + L861Q, S768I, 3 Exon 19 deletions + G719C.

harbored the exon 19 deletion mutation in the primary resistance group, while there were 24 (42.1%) similar patients in the disease control group. Thirty-eight (57.6%) patients possessed the exon 21 L858R point mutation in the primary resistance group, with 27 (47.4%) in the disease control group. Thirty-two (48.5%) patients used EGFR-TKI with gefitinib in the primary resistance group, and 24 (42.1%) patients in the disease control group. In the primary resistance group, 29 (43.9%) patients had been treated with erlotinib, while 25 (43.9%) patients had been treated in the disease control group. Five (7.6%) patients took afatinib in the primary resistance group, with 8 (14.0%) patients being prescribed in the disease control group.

Concerning the PD-L1 expression level, 15 (22.7%) patients registered a Tumor Proportion Score (TPS) ≧50% in the primary resistance group, with only one (1.8%) in the disease control group. Twenty (30.3%) patients had a TPS ≧25% in the primary resistance group, with 2 (3.5%) patients at that level in the disease control group. Thirty (45.5%) patients had a TPS ≧1% in the primary resistance group, while 7 (12.3%) patients were at that level in the disease control group (Fig. 1).

Through the use of univariate analysis, there were no significant differences in age, gender, smoking status, baseline *EGFR* mutation status, or the type of EGFR-TKI treatment between the primary resistance and disease control groups. Most importantly is that patients in the primary resistance group exhibited a significantly higher PD-L1 expression level than those in the disease control group, no matter whether the cutoff value was 1% (P < 0.001), 25% (P < 0.001) or 50% (P = 0.001) (Table 1).

3.2. The association between PD-L1 expression level and the frequency of primary resistance

Patients with a PD-L1≧1% had a higher incidence of primary resistance to EGFR-TKIs than patients with a PD-L1 < 1% (Odds Ratio (OR), 5.95; 95% Confidence Interval (CI), 2.35–15.05; P < 0.001) (Table 2). There was a higher frequency of primary resistance to EGFR-TKIs in patients with a PD-L1≧25%, than there was with patients having a PD-L1 < 25% (OR, 11.96; 95% CI, 2.65–53.87; P = 0.001). Patients with a PD-L1≧50% had a greater chance of primary resistance to EGFR-TKIs than patients with a PD-L1 < 50% (OR, 16.47; 95% CI, 2.10–129.16; P = 0.008). Through multivariate analysis, the above results remained highly statistically significant.

3.3. The difference of PFS and OS in the primary resistance and disease control groups

The estimated median PFS time was 1.9 months (95% CI, 1.7–2.1) in the primary resistance group, and 16.7 months (95% CI, 13.8–19.6) in the disease control group (Fig. 2A). The estimated median OS time was 10.1 months (95% CI, 8.3–11.9) in the primary resistance group, and 47.0 months (95% CI, 32.5–61.7) in the disease control group (Fig. 2B). When comparing PFS between the primary resistance and disease control groups, the Hazard Ratio (HR) was 51.27 (95% CI, 19.55–134.47; P < 0.001) in univariate analysis, and 51.44 (95% CI 19.48–135.83; P < 0.001) in multivariate analysis (Table 3). Concerning OS, HR was 6.84 (95% CI, 3.44–13.57; P < 0.001) in univariate analysis, and 7.75 (95% CI 3.73–16.09; P < 0.001) in multivariate analysis.

3.4. The association between PD-L1 expression level and PFS, OS

Fig. 3A demonstrates the relationship between different PD-L1 expression levels and OS. The estimated median OS time was 38.2 months (95% CI, 26.1–50.3) in patients with a PD-L1 < 1%, and 11.2 months (95% CI, 4.8–17.6) in patients with a PD-L1≧1% (Log Rank P = 0.002). In patients with a PD-L1 < 25%, the estimated median OS time was 38.2 months (95% CI, 26.3–50.1), and 10.1 months (95% CI, 8.6–11.6) in patients with a PD-L1≧25% (Log Rank P = 0.003). Additionally, the estimated median OS time was 38.2 months (95% CI, 28.9–47.5) in patients with a PD-L1 < 50%, and 10.1 months (95% CI, 6.4–13.8) in patients with a PD-L1≧50% (Log Rank P = 0.001).

Fig. 3B shows the association of different PD-L1 expression levels and PFS. The estimated median PFS period was 7.3 months (95% CI, 2.7–12.0) in patients with a PD-L1 < 1%, and 2.1 months (95% CI, 1.6–2.6) in patients with a PD-L1≧1% (Log Rank P < 0.001). In patients with a PD-L1 < 25%, the estimated median PFS period was 6.6 months (95% CI, 3.4–9.8), and 1.8 months (95% CI, 1.5–2.1) in patients with a PD-L1≧25% (Log Rank P < 0.001). Finally, the estimated median PFS period was 4.9 months (95% CI, 1.8–8.1) in patients with a PD-L1 < 50%, and 1.6 months (95% CI, 1.1–2.0) in patients with a PD-L1≧50% (Log Rank P < 0.001).

4. Discussion

This is the first study which discusses the association between PD-L1 expression levels and primary resistance to EGFR-TKIs. Our research has demonstrated that advanced sensitizing *EGFR*-mutant lung adenocarcinoma patients in the primary resistance group exhibited a higher positive rate (TPS ≧1%) of PD-L1 expression than patients in the disease control group (45.5% vs. 12.3%, P < 0.001). In the primary resistance group, 22.7% patients displayed a PD-L1 expression with a strong positive (TPS ≧50%), while this occurred in only one patient in the disease control group. Therefore, a higher level of PD-L1 expression was found to be associated with a higher incidence of primary resistance to EGFR-TKIs in treatment naïve sensitizing *EGFR* mutant lung adenocarcinoma patients.

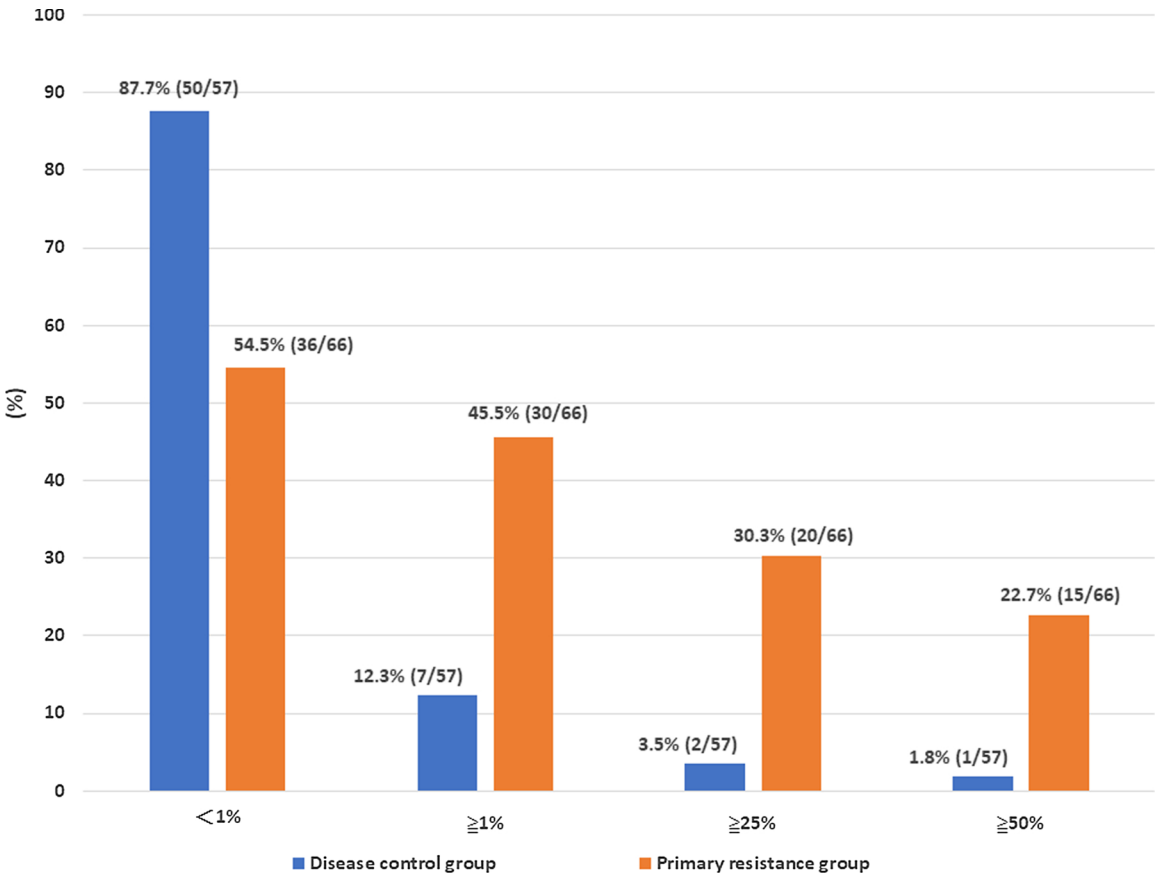

Fig. 1. The distribution of Programmed cell Death-ligand 1 (PD-L1) expression levels in the primary resistance group and disease control group.

Table 2
The association between PD-L1 expression level and primary resistance.

	Univariate analysis*		Multivariate analysis*	
	OR (95% CI)	P value	OR (95% CI)	P value#
PD-L1 ≧1% versus < 1%	5.95 (2.35–15.05)	< 0.001	7.48 (2.70–20.77)	< 0.001
PD-L1 ≧25% versus < 25%	11.96 (2.65–53.87)	0.001	16.29 (3.16–83.96)	0.001
PD-L1 ≧50% versus < 50%	16.47 (2.10–129.16)	0.008	20.67 (2.33–183.46)	0.007

PD-L1, Programmed death-ligand 1; OR, odds ratio; CI, confidence interval.
* By logistic regression model.
# Adjusted by age, gender, smoking, epidermal growth factor receptor, tyrosine kinase inhibitor.

In this study, there were 16 patients with a PD-L1 ≧50%. Amongst them, 15 patients were primary resistance to EGFR-TKIs (93.8%). The one patient with a PD-L1 ≧50% had partial response to EGFR-TKI, with their PFS only 4.4 months.

A higher PD-L1 expression level was related to the treatment efficacy of immune checkpoint inhibitors in NSCLC in several studies [16,22,23]. A previous study had stated that patients with *EGFR*-mutant NSCLC were less likely to be PD-L1-positive when compared with *EGFR*-wild type patients with an OR of 0.59 (95% CI 0.39–0.92; P < 0.02) [17]. Furthermore, a meta-analysis presented that immune checkpoint inhibitors significantly prolonged OS over those prescribed with docetaxel in the *EGFR* wild-type patients (HR, 0.66; 95% CI 0.58–0.76; p < 0.0001), but not in the *EGFR*-mutant subgroup (HR, 1.05; 95% CI 0.70–1.55; p < 0.81) [14]. Lower PD-L1 expression levels in *EGFR*-mutated patients could explain the poor treatment efficacy when treating with immune checkpoint inhibitors. However, a recent phase II trial has demonstrated that pembrolizumab lacked efficacy in advanced *EGFR*-mutant NSCLC patients with TKI naïve and a PD-L1 expression ≧1%, including those with a PD-L1 expression ≧50% [24]. This represents that PD-L1 expression levels may not be a suitable predictor of efficacy for immune checkpoint inhibitors in TKI naïve *EGFR*-mutant lung cancer patients.

The above studies focused on the association between PD-L1 expression levels and immune checkpoint inhibitors. In the present research, we limited the scope of our investigation to the relationship between PD-L1 expression levels and treatment response to EGFR-TKIs. Although 45% of patients in the primary resistance group were PD-L1 positive, there were still more than 50% of patients who were PD-L1 negative. This implies that not only is PD-L1 expression a predictor for primary resistance to EGFR-TKI treatment, but also that other mechanisms could influence the treatment results. In addition to PD-L1 expression, current studies have demonstrated that a higher Tumor Mutational Burden (TMB) was associated with an improved objective response and improved PFS of immune checkpoint inhibitors [25,26]. Therefore, if our findings in this study are considered reasonable, TMB may possibly prove to be another factor which affects the treatment response of EGFR-TKIs.

Regarding OS and PFS of EGFR-TKIs, this study has revealed that a higher PD-L1 expression level was related to lower OS and PFS through use of the Kaplan-Meier method (Fig. 3). In patients with a PD-L1≧50%, the estimated median OS time was 10.1 months (95% CI, 6.4–13.8) and estimated median PFS was 1.6 months (95% CI, 1.1–2.0). In patients with a PD-L1 < 1%, the estimated median OS time was 38.2 months (95% CI, 26.1–50.3) and estimated median PFS was 7.3 months (95% CI, 2.7–12.0). The analysis has supported our hypothesis that *EGFR*-

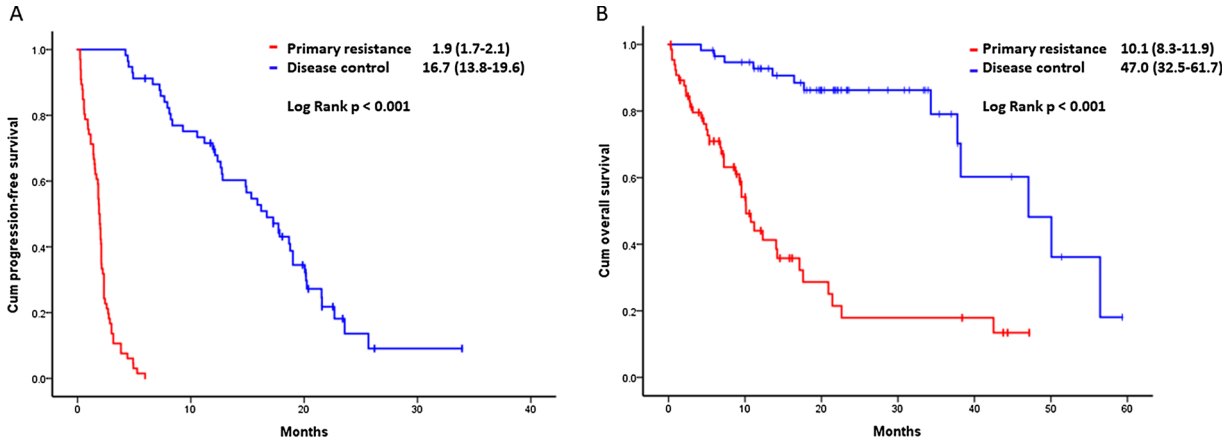

Fig. 2. (A) The Kaplan-Meier survival curve analysis of progression-free survival in the primary resistance group and disease control group. (B) The Kaplan-Meier survival curve analysis of overall survival in the primary resistance group and disease control group.

Table 3
Comparison PFS and OS between primary resistance and disease control group.

	Univariate analysis*		Multivariate analysis*	
Characteristics	HR (95% CI)	P value	HR (95% CI)	P value#
PFS				
Primary resistance versus disease control group	51.27 (19.55–134.47)	< 0.001	51.44 (19.48–135.83)	< 0.001
OS				
Primary resistance versus disease control group	6.84 (3.44–13.57)	< 0.001	7.75 (3.73–16.09)	< 0.001

PFS, progression free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.
* By Cox proportional hazard model.
# Adjusted by age, gender, smoking, epidermal growth factor receptor, tyrosine kinase inhibitor.

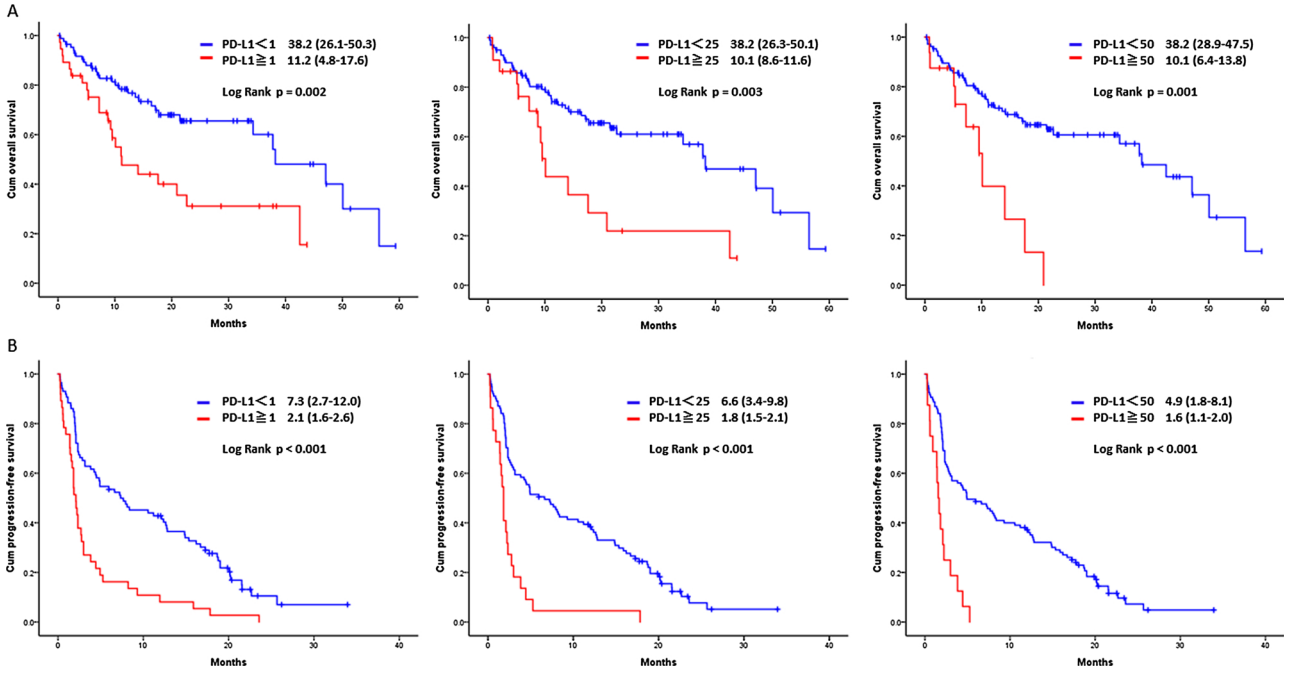

Fig. 3. (A) The relationship between Programmed cell Death-ligand 1 (PD-L1) expression levels and overall survival through Kaplan-Meier survival curve analysis. (B) The relationship between Programmed cell Death-ligand 1 (PD-L1) expression levels and progression-free survival through Kaplan-Meier survival curve analysis.

mutated patients with a higher PD-L1 expression level would display a tendency towards resistance to EGFR-TKI within short periods. Several other possible mechanisms regarding primary resistance to EGFR TKI in *EGFR* mutant lung cancer patients include *BIM* deletion polymorphism, overexpression of *CRIPTO1*, co-existence of *MET* amplification, Phosphatase and Tensin homolog (*PTEN*) loss, *ERBB2* amplification, and v-Ki-ras2 Kirsten Rat Sarcoma viral oncogene homolog (*KRAS*) mutations [27–30].

Our study did have some limitations. First, it was a retrospective study, and thus more bias may have been present compared with other studies that had been prospectively designed. Second, the research was undertaken at a single medical center, and we did not enroll all patients who had been treated with EGFR-TKIs due to insufficient specimen numbers available for assessment. Thus, there may have been selection bias. Third, all patients in this study were Taiwanese. Therefore, our findings may be too generalized, and not suitable for other ethnic populations. Although this study is the first to discuss the relationship between PD-L1 expression level and the frequency of primary resistance to EGFR-TKIs treatment, further research is still required in order to confirm our results.

5. Conclusions

Our study demonstrated that treatment naïve advanced *EGFR*-mutant lung adenocarcinoma patients with higher PD-L1 expression levels experienced a higher frequency of primary resistance to EGFR-TKIs treatment. Clinically, while we treat advanced *EGFR*-mutant and PD-L1 positive lung adenocarcinoma patients with the use of EGFR-TKIs, other treatment regimens should be considered without hesitation if the efficacy rates were not as expected.

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