Survival-associated factors of first-line EGFR-tyrosine kinase inhibitor responders and non-responders in lung adenocarcinoma patients with common *EGFR* mutations

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Abstract. The aim of the present retrospective cohort study was to elucidate the clinical presentation of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) responders and non-responders in lung adenocarcinoma patients with common *EGFR* mutations. The cohort included 131 lung adenocarcinoma patients with common exon 19 or exon 21 *EGFR* mutations, who were receiving first-line EGFR-TKI therapy. The patient characteristics, treatment regimen and outcomes were recorded and analyzed. Of the 131 patients, 104 (79.3%) responded to treatment, while 27 (20.7%) did not. A significantly longer median progression-free survival (PFS) [14.3, 95% confidence interval (CI): 12.2-18.4 vs. 5.7, 95% CI: 2.7-9.9 months; P<0.001] and overall survival (OS) (42.2, 95% CI: 28.1-58.1 vs. 11.5, 95% CI: 8.3-19.7 months; P<0.001) were observed in responders compared with non-responders. In responders, bone [hazard ratio (HR)=1.87, 95% CI: 1.11-3.20, P=0.021] and pleural (HR=2.40, 95% CI: 1.37-4.22, P=0.002) metastasis were independent factors of PFS. Exon 19 mutations (HR=0.38, 95% CI: 0.19-0.76, P=0.006), Eastern Cooperative Oncology Group performance status score ≥2 (HR=3.53, 95% CI: 1.42-8.75, P=0.007) and bone metastasis (HR=2.01, 95% CI: 1.05-3.85, P=0.034), were independent factors of OS. In non-responders, smoking (HR=3.97, 95% CI: 1.13-13.91, P=0.031) was an independent factor of PFS. Different survival-associated factors were observed between EGFR-TKI responders and non-responders. The development of new treatment strategies should be advocated in EGFR-TKI non-responders.

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, and the majority of the patients are at the advanced stages of the disease at the time of diagnosis (1). Traditional chemotherapy regimens for advanced-stage lung cancer have exhibited modest efficacy in prolonging survival, and are associated with undesirable side effects (2,3). Previously, therapy targeted towards the epidermal growth factor receptor (EGFR) pathway has achieved great success in the treatment of lung cancer. The EGFR pathway is an attractive target for therapy, as EGFR signaling plays an important role in the growth, proliferation and survival of several solid tumors, including non-small-cell lung cancer (NSCLC) (4).

A subgroup of patients with NSCLC harbor specific mutations in the tyrosine kinase domain of the *EGFR* gene, which are correlated with favorable clinical responsiveness to EGFR tyrosine kinase inhibitor (TKI) therapy (5). All mutations appear to be limited to exons 18, 19, 20 and 21 of the *EGFR* gene (6), and are most frequently observed in lung adenocarcinoma patients (7,8). Missense mutations in exon 21 (L858R) and in-frame deletions in exon 19 are the most frequent EGFR-TKI-sensitive mutations (80%) in NSCLC patients (9). Both the exon 19 deletion and the exon 21 missense mutation are common *EGFR* mutations that are associated with a favorable response to first-line treatment with gefitinib (10,11), as well as other EGFR-TKIs, including erlotinib (12) and afatinib (13),

compared with standard chemotherapy in NSCLC patients. In NSCLC patients with *EGFR* mutations, the overall response rate (ORR) to first-line EGFR-TKI therapy is 66.9-83%, with a progression-free survival (PFS) of 9.2-13.1 months (10-13). Despite the favorable response to EGFR-TKIs in NSCLC patients with EGFR mutations, ~20-30% of patients do not respond to EGFR-TKIs, and the clinical phenotypes and survival-associated factors of these EGFR-TKI responders and non-responders have not been previously described.

The aim of the present study was to elucidate the clinical presentation and significance of EGFR-TKI responders and non-responders in lung adenocarcinoma patients with common exon 21 and 19 *EGFR* activating mutations.

Patients and methods

Patients and study design. The present retrospective cohort study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital. The cohort comprised 131 lung adenocarcinoma patients from the Chang Gung Memorial Hospital, Chiayi Branch (Puzi, Taiwan) (IRB No. 201600601B0), who had been diagnosed between December 2010 and January 2015. All participants were previously treatment-naive advanced-stage (stage IIIB or IV) lung adenocarcinoma patients. The *EGFR* mutation status at exons 18, 19, 20 and 21 of the *EGFR* gene was determined by Sanger sequencing (8) or by using the Therascreen® EGFR RGQ PCR kit (Qiagen, Manchester, UK) (14). All the patients received first-line EGFR-TKI therapy (gefitinib, erlotinib or afatinib) and had *EGFR* mutations at exon 19 or 21. Patients with combined exon 18 or 20 mutations were excluded from the study. Follow-up was extended from the first diagnosis of advanced-stage lung cancer to October 2016. The clinical phenotypes of these patients were recorded and analyzed. The response of the lesions was evaluated by chest computed tomography, brain magnetic resonance or bone scan, according to the Response Evaluation Criteria in Solid Tumors 1.1 (15) at 3 months after the initiation of treatment. EGFR-TKI responders were defined as complete responders (CR) or partial responders (PR), while non-responders were defined as those having stable disease (SD) or progressive disease (PD) at 3 months after the initiation of EGFR-TKI therapy. PFS is defined as the time from the first treatment to PD or death. Overall survival (OS) is defined as the time from diagnosis to death from any cause, or until the patients were censored at the last follow-up.

Statistical analysis. The Pearson's χ^2 test was used to determine the correlations between the categorical variables in the different groups. Survival analysis was performed using a Kaplan-Meier analysis and log-rank test. Multivariate analysis was performed by Cox proportional-hazards regression, and factors that were determined as significant by the log-rank test were included in the analysis. A P-value of <0.05 was considered as statistically significant. All statistical tests were performed using MedCalc software, version 15 (MedCalc Software, Ostend, Belgium).

Results

Clinical characteristics common to all first-line EGFR-TKI patients. In total, 131 patients were enrolled in the present study (Table I). The median age was 70.0 years. The majority of the patients were female (n=72, 55%), non-smokers (n=115, 87.8%), and had stage IV disease (n=121, 92.4%). Of the 131 patients, 59 (45%) had exon 19 deletions and 72 (55%) had exon 21 missense *EGFR* mutations. The EGFR-TKIs gefitinib (n=99, 75.6%), erlotinib (n=27, 20.6%) or afatinib (n=5, 3.8%) were used as the first-line therapy in these patients. Three months after EGFR-TKI treatment, the tumor response to treatment was evaluated. PR was observed in 104 (79.3%), SD in 12 (9.2%), and PD in 15 (11.5%) patients. There were no CR patients. The ORR to EGFR-TKIs was 79.3%, and the disease control rate (DCR) was 88.5%. The median PFS for all first-line EGFR-TKI patients was 12.7 months (95% CI: 12.0-16.70 months), and the median OS was 32.7 months (95% CI: 24.7-57.1 months).

Survival of first-line EGFR-TKI responders and non-responders. EGFR-TKI responders and non-responders were identified based on their response to treatment. A total of 104 (79.3%) EGFR-TKI responders (CR + PR) and 27 (20.7%) non-responders (SD + PD) were identified. No significant differences were observed between EGFR-TKI responders and non-responders in terms of sex, smoking history, age, EGFR-TKI use, EGFR mutation status, carcinoembryonic antigen, Eastern Cooperative Oncology Group (ECOG) performance status (PS), and cancer stage (Table I). A significantly higher proportion of brain metastasis was observed in EGFR-TKI responders compared with non-responders (31.7 vs. 7.4%, respectively; P=0.011; Table I). A significantly longer median PFS was observed in EGFR-TKI responders (14.3 months, 95% CI: 12.2-18.4 months) compared with that in non-responders (5.7 months, 95% CI: 2.7-9.9 months; P<0.001; Fig. 1A). We also observed a significantly longer median OS in responders (42.2 months, 95% CI: 28.1-58.1 months) compared with that in non-responders (11.5 months, 95% CI: 8.3-19.7 months; P<0.001; Fig. 1B).

Characteristics of survival in patients treated with first-line EGFR-TKIs. The associations between measured clinical variables and survival were evaluated. According to the univariate analysis, EGFR-TKI responder status (HR=0.33, 95% CI: 0.16-0.68, P<0.001) and old age (>65 years) (HR=0.64, 95% CI: 0.41-1.00, P=0.038) were significantly associated with a favorable PFS (Table II). Conversely, male sex (HR=1.66, 95% CI: 1.07-2.58, P=0.018), bone metastasis (HR=1.66, 95% CI: 1.07-2.58, P=0.024) and pleural metastasis (HR=1.62, 95% CI: 1.00-2.77, P=0.022) were significantly associated with an unfavorable PFS (Table II). According to the multivariate analysis, EGFR-TKI responder status (HR=0.25, 95% CI: 0.15-0.42, P<0.001), old age (HR=0.58, 95% CI: 0.36-0.92, P=0.020) and male sex (HR=1.70, 95% CI: 1.07-2.67, P=0.024) remained independent factors for PFS (Table II).

According to the univariate analysis, exon 19 mutations (HR=0.55, 95% CI: 0.33-0.92, P=0.027) and EGFR-TKI responder status (HR=0.30, 95% CI: 0.14-0.67, P<0.001) were significantly associated with a favorable OS (Table III). By contrast, ECOG PS ≥2 (HR=2.21, 95% CI: 0.79-6.18, P=0.031) and bone metastasis (HR=1.79, 95% CI: 1.04-3.10, P=0.020) were significantly associated with an unfavorable OS (Table III). According to the multivariate analysis, EGFR-TKI responder status (HR=0.30, 95% CI: 0.17-0.54, P<0.001),

Table I. Clinical characteristics of patients treated with first-line EGFR-TKIs.

	Total	Responders	Non-responders	P-value
Patients	131	104	27	
Sex				0.426
Male	59 (45.0)	45 (43.2)	14 (51.9)	
Female	72 (55.0)	59 (56.8)	13 (48.1)	
Smoking				
Yes	16 (12.2)	12 (11.5)	4 (14.8)	0.645
No	115 (87.8)	92 (88.5)	23 (85.2)	
Age (years)	70	70	74	0.219
Age				0.068
≥65	82 (62.6)	61 (58.6)	21 (77.8)	
<65	49 (37.4)	43 (41.4)	6 (22.2)	
TKI				0.157
Erlotinib	27 (20.6)	24 (23.0)	3 (11.1)	0.059
Gefitinib	99 (75.6)	75 (72.1)	24 (88.9)	
Afatinib	5 (3.8)	5 (4.8)		
Mutations				0.350
Exon 19	59 (45.0)	49 (47.1)	10 (37.0)	
Exon 21	72 (55.0)	55 (52.9)	17 (63.0)	
Stage				0.961
IIIb	10 (7.6)	8 (7.7)	2 (7.4)	
IV	121 (92.4)	96 (92.3)	25 (92.6)	
CEA (ng/ml)	95	79	156	0.325
ECOG PS				0.724
≤1	119 (90.8)	94 (90.4)	25 (92.6)	
≥2	12 (9.2)	10 (9.6)	2 (7.4)	
Metastatic sites				
Lung	42 (32.1)	36 (34.6)	6 (22.2)	0.221
Brain	35 (26.7)	33 (31.7)	2 (7.4)	0.011[a]
Liver	15 (11.4)	12 (11.5)	3 (11.1)	0.951
Bone	51 (38.9)	37 (35.6)	14 (51.9)	0.124
Adrenal	9 (6.9)	8 (7.7)	1 (3.7)	0.467

[a]P<0.05. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; CEA, carcinoembryonic antigen; ECOG PS, Eastern Cooperative Oncology Group performance status.

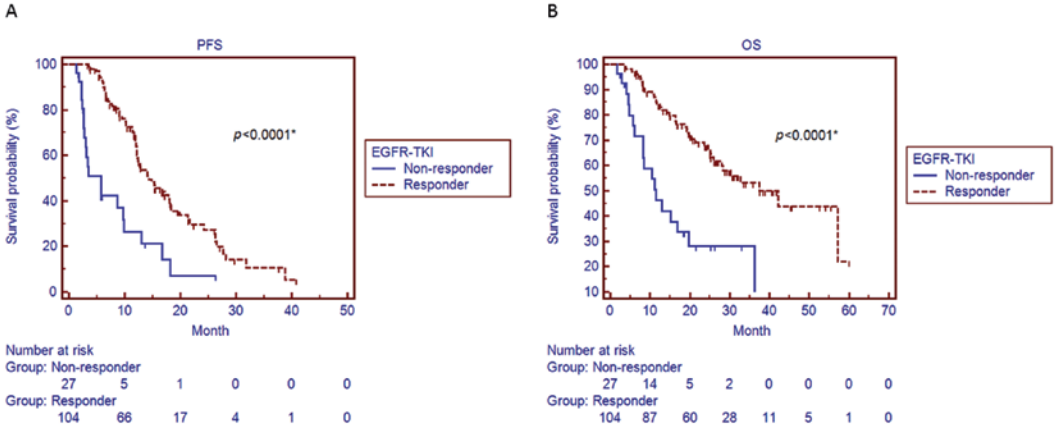

Figure 1. (A) PFS and (B) OS of EGFR-TKI responders and non-responders in first-line EGFR-TKI lung adenocarcinoma patients with common EGFR mutations. PFS, progression-free survival; OS, overall survival; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Table II. Clinical variables associated with PFS in patients treated with first-line EGFR-TKIs.

	Univariate analysis			Multivariate analysis		
Covariates	P-value	HR	95% CI	P-value	HR	95% CI
Male	0.018[a]	1.66	1.07-2.58	0.024[a]	1.70	1.07-2.67
Exon 19 mutation[b]	0.090	0.69	0.45-1.06			
Smoking	0.197	1.49	0.73-3.03			
Responder status	<0.001[a]	0.33	0.16-0.68	<0.001[a]	0.25	0.15-0.42
Old age (≥65 years)	0.038[a]	0.64	0.41-1.00	0.020[a]	0.58	0.36-0.92
ECOG PS ≥2	0.502	1.30	0.55-3.09			
Gefitinib	0.241	1.38	0.84-2.26			
Metastasis						
Lung	0.515	1.16	0.73-1.86			
Brain	0.185	0.71	0.44-1.14			
Liver	0.074	1.70	0.83-3.49			
Bone	0.024[a]	1.62	1.02-2.56			
Adrenals	0.540	1.27	0.54-2.99			
Pleura	0.022[a]	1.67	1.00-2.77			

[a]P<0.05. [b]Reference group is exon 21. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table III. Clinical variables associated with OS in patients treated with first-line EGFR-TKIs.

	Univariate analysis			Multivariate analysis		
Covariates	P-value	HR	95% CI	P-value	HR	95% CI
Male sex	0.303	1.30	0.77-2.21			
Exon 19 mutation[a]	0.027[b]	0.55	0.33-0.92			
Smoking	0.915	0.96	0.42-2.19			
Responder status	<0.001[b]	0.30	0.14-0.67	<0.001[b]	0.30	0.17-0.54
Old age (≥65 years)	0.300	1.33	0.79-2.25			
ECOG PS ≥2	0.031[b]	2.21	0.79-6.18	0.012[b]	2.70	1.25-5.86
Gefitinib	0.019[b]	2.61	1.42-4.80			
Metastasis						
Lung	0.798	1.07	0.61-1.87			
Brain	0.917	0.97	0.54-1.73			
Liver	0.314	1.49	0.59-3.76			
Bone	0.020[b]	1.79	1.04-3.10	0.030[b]	1.82	1.06-3.14
Adrenals	0.080	0.20	0.08-0.53			
Pleura	0.434	1.24	0.70-2.20			

[a]Reference group is exon 21. [b]P<0.05. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

ECOG PS ≥2 (HR=2.70, 95% CI: 1.25-5.86, P=0.012) and bone metastasis (HR=1.82, 95% CI: 1.06-3.14, P=0.030) remained independent factors for OS (Table III).

Survival-associated factors in first-line EGFR-TKI responders and non-responders. Since the initial response to EGFR-TKI treatment was significantly associated with PFS and OS according to both univariate and multivariate analysis, the characteristics of EGFR-TKI responders and non-responders were then analyzed separately.

According to the univariate analysis for EGFR-TKI responders, old age (HR=0.56, 95% CI: 0.34-0.93, P=0.018) was significantly associated with a favorable PFS, while

Table IV. Clinical variables associated with PFS in first-line EGFR-TKI responders.

	Univariate analysis			Multivariate analysis		
Covariates	P-value	HR	95% CI	P-value	HR	95% CI
Male sex	0.150	1.42	0.86-2.36			
Exon 19 mutation[a]	0.189	0.72	0.44-1.19			
Smoking	0.614	1.21	0.54-2.68			
Old age (≥65 years)	0.018[b]	0.56	0.34-0.93			
ECOG PS ≥2	0.657	1.23	0.45-3.33			
Gefitinib	0.474	1.24	0.71-2.21			
Metastasis						
Lung	0.395	1.25	0.73-2.13			
Brain	0.524	0.84	0.49-1.43			
Liver	0.081	1.80	0.78-4.18			
Bone	0.063	1.58	0.92-2.72	0.021[b]	1.87	1.11-3.20
Adrenals	0.512	1.32	0.51-3.39			
Pleura	0.006[b]	2.01	1.08-3.73	0.002[b]	2.40	1.37-4.22

[a]Reference group is exon 21. [b]P<0.05. PFS, progression-free survival; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table V. Clinical variables associated with OS in first-line EGFR-TKI responders.

	Univariate analysis			Multivariate analysis		
Covariates	P-value	HR	95% CI	P-value	HR	95% CI
Male sex	0.456	1.26	0.67-2.39			
Exon 19 mutation[a]	0.006[b]	0.40	0.22-0.74	0.006[b]	0.38	0.19-0.76
Smoking	0.897	0.93	0.34-2.55			
Old age (≥65 years)	0.844	1.06	0.57-1.99			
ECOG PS ≥2	0.023[b]	2.61	0.73-9.30	0.007[b]	3.53	1.42-8.75
Gefitinib	0.158	1.83	0.89-3.76			
Metastasis						
Lung	0.206	1.49	0.76-2.90			
Brain	0.376	1.33	0.67-2.64			
Liver	0.081	2.11	0.66-6.72			
Bone	0.030[b]	1.92	0.98-3.77	0.034[b]	2.01	1.05-3.85
Adrenal	0.235	0.32	0.10-1.05			
Pleura	0.875	1.06	0.53-2.08			

[a]Reference group is exon 21. [b]P<0.05. OS, overall survival; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

pleural metastasis (HR=2.01, 95% CI: 1.08-3.73, P=0.006) was significantly associated with an unfavorable PFS (Table IV). According to the multivariate analysis, bone metastasis (HR=1.87, 95% CI: 1.11-3.20, P=0.021) and pleural metastasis (HR=2.40, 95% CI: 1.37-4.22, P=0.002) were independent factors for PFS (Table IV).

Exon 19 mutations (HR=0.40, 95% CI: 0.22-0.74, P=0.006) were significantly associated with a favorable OS, while ECOG PS ≥2 (HR=2.61, 95% CI: 0.73-9.30, P=0.023) and bone metastasis (HR=1.92, 95% CI: 0.98-3.77, P=0.030) were significantly associated with an unfavorable OS (Table V). Exon 19 mutations (HR=0.38, 95% CI: 0.19-0.76, P=0.006), ECOG PS ≥2 (HR=3.53, 95% CI: 1.42-8.75, P=0.007) and bone metastasis (HR=2.01, 95% CI: 1.05-3.85, P=0.034) remained independent factors of OS according to the multivariate analysis (Table V).

Factors associated with EGFR-TKI non-responders were also analyzed. According to the univariate analysis, male

Table VI. Clinical variables associated with PFS in first-line EGFR-TKI non-responders.

	Univariate analysis			Multivariate analysis		
Covariates	P-value	HR	95% CI	P-value	HR	95% CI
Male sex	0.045[a]	2.24	0.91-5.47			
Exon 19 mutation[b]	0.610	0.79	0.31-1.97			
Smoking	0.020[a]	3.24	0.59-17.70	0.031[a]	3.97	1.13-13.91
Old age (≥65 years)	0.053	0.41	0.12-1.40			
ECOG PS ≥2	0.277	2.17	0.28-16.56			
Gefitinib	0.455	1.56	0.54-4.54			
Metastasis						
Lung	0.717	1.20	0.41-3.47			
Brain	0.642	0.72	0.20-2.62			
Liver	0.718	1.25	0.33-4.72			
Bone	0.951	1.03	0.43-2.48			
Adrenal	0.077	4.92	0.07-356.78			
Pleura	0.552	0.77	0.32-1.87			

[a]P<0.05. [b]Reference group is exon 21. PFS, progression-free survival; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

sex (HR=2.24, 95% CI: 0.91-5.47, P=0.045) and smoking (HR=3.24, 95% CI: 0.59-17.70, P=0.020) were significantly associated with an unfavorable PFS, and smoking (HR=3.97, 95% CI: 1.13-13.91, P=0.031) remained an independent factor of PFS according to the multivariate analysis (Table VI). No factor analyzed in the present study was associated with OS in EGFR-TKI non-responders (data not shown).

Discussion

In the present study, 20.3% of lung adenocarcinoma patients with common sensitizing exon 21 and exon 19 *EGFR* mutations were EGFR-TKI non-responders. Our results are similar to those of previous studies on first-line TKI treatment, in which EGFR-TKI non-responders accounted for 20-30% of the study group (10-13). In the present study, EGFR-TKI non-responders had a poor prognosis. The clinical factors associated with PFS and OS were also assessed and it was observed that, in EGFR-TKI responders, bone and pleural metastasis were independent factors for unfavorable PFS. Poor ECOG PS (≥2) and bone metastasis were independent factors for unfavorable OS, and exon 19 deletions were an independent factor for favorable OS. In EGFR-TKI non-responders, smoking was an independent factor for unfavorable PFS.

PFS and OS were reduced in EGFR-TKI non-responders, confirming the results of an earlier study, in which the median OS was 21 months (95% CI: 26.1-30.4) in responders compared with 8 months (95% CI: 8.7-15.8) in non-responders (16). Based on the multivariate analysis, EGFR-TKI non-responding status was found to be a strongly unfavorable factor for both PFS and OS in patients receiving first-line EGFR-TKI therapy. Rapid progression of lung cancer after the initiation of EGFR-TKI therapy has been reported to be a poor prognostic factor for survival outcomes (17). Our results further suggest that EGFR-TKI non-responders are distinctly different from EGFR-TKI responders. Since this group of patients had a worse prognosis, a treatment strategy that overcomes primary resistance to EGFR-TKI is urgently needed. Close monitoring of EGFR-TKI treatment response is also mandatory for early detection of EGFR-TKI non-responders, so that treatment may be adjusted accordingly.

Exon 19 deletions have been associated with better outcomes compared with L858R mutations in EGFR-TKI patients as, reported in several studies (18-20). In the present study, exon 19 deletions were found to be an independent predictor of outcome in first-line EGFR-TKI responders. Exon 19 deletions have been previously associated with better survival rates compared with exon 21 mutations in gefitinib-treated NSCLC patients, due to the differential inhibition of downstream signaling (21). Recently, exon 19 deletions were reported to be associated with a better outcome after afatinib therapy, compared with that of the exon 21 L858R mutation. Altogether, exon 19 deletions and L858R mutations characterize two distinct groups of patients and, therefore, different clinical treatment strategies for these patients should be considered in the future. A reduced frequency of exon 19 deletions has also recently been reported in EGFR-TKI non-responders (16). However, no significant differences in the frequency of exon 19 deletions and L858R mutations were observed, which may be due to the limited number of patients in this cohort.

In the present study, poor baseline ECOG PS (≥2) was associated with an unfavorable OS in EGFR-TKI responders, which is similar to previously reported results (17). However, we did not observe a significant effect of poor ECOG PS on PFS, which may indicate that EGFR-TKIs are effective and well-tolerated in responders. In addition, 35.6% of EGFR-TKI responders had developed bone metastasis at the time of diagnosis of lung cancer, and the overall incidence of bone

metastasis in patients with *EGFR* mutations was not higher compared with that reported previously (22). Bone metastasis was found to be associated with unfavorable PFS and OS. EGFR-TKIs prolong survival in patients with *EGFR* mutations and bone metastasis (23). However, our results highlight that management of bone metastasis should be a priority in EGFR-TKI responders.

Smoking at the time of diagnosis of lung cancer was associated with unfavorable PFS in EGFR-TKI non-responders. Indeed, smoking for ≥30 pack-years is associated with a decreased ORR and DCR in lung adenocarcinoma with activation *EGFR* mutations (24). No clinical variables were associated with OS in EGFR-TKI non-responders, which may be attributed to the relatively short survival and small number of these patients. In addition, non-response to EGFR-TKIs (primary resistance) may be associated with underlying genetics or molecular mechanisms in lung cancer cells. Several mechanisms for primary EGFR-TKI resistance have been proposed, including v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mutations (25), phosphoinositide-3-kinase catalytic subunit α mutation (26), *de novo MET* amplification (27,28), *Bim* deletion polymorphisms (29,30), and phosphatase and tensin homolog loss (31). *De novo* T790M mutations of the *EGFR* gene have also been reported to be associated with poorer response to first-line EGFR-TKI treatment (32). Since patients with *de novo* T790M mutations were excluded from the present study, we hypothesized that other genetic or molecular changes may be implicated in EGFR-TKI resistance in EGFR-TKI non-responders in the present study, and these changes warrant further investigation.

Although this study was limited by the small cohort size and limited number of *EGFR* mutations, the results may help elucidate the clinical presentation of the EGFR-TKI response, and contribute to the development of novel treatment strategies for lung adenocarcinoma patients with common *EGFR* mutations.

In summary, it was demonstrated that different prognosis and survival-associated factors are observed in EGFR-TKI responders and non-responders. These groups of patients should therefore be considered as two distinct groups, and novel treatment strategies should be developed and applied to EGFR-TKI non-responders.

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Competing interests

The authors declare that they have no competing interests.

References

1. Breathnach OS, Freidlin B, Conley B, Green MR, Johnson DH, Gandara DR, O'Connell M, Shepherd FA and Johnson BE: Twenty-two years of phase III trials for patients with advanced non-small-cell lung cancer: Sobering results. J Clin Oncol 19: 1734-1742, 2001.
2. Goulart BH, Martins RG and Lynch TJ: Twenty-two years of phase III trials for patients with advanced non-small-cell lung cancer: Sobering results. J Clin Oncol 19: 4089, 2001.
3. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J and Johnson DH; Eastern Cooperative Oncology Group: Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346: 92-98, 2002.
4. Arteaga CL: Epidermal growth factor receptor dependence in human tumors: More than just expression? Oncologist 7 (Suppl 4): S31-S39, 2002.
5. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350: 2129-2139, 2004.
6. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, *et al*: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
7. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, *et al*: Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 26: 4268-4275, 2008.
8. Huang SF, Liu HP, Li LH, Ku YC, Fu YN, Tsai HY, Chen YT, Lin YF, Chang WC, Kuo HP, *et al*: High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 10: 8195-8203, 2004.
9. Gazdar AF, Shigematsu H, Herz J and Minna JD: Mutations and addiction to EGFR: The Achilles 'heal' of lung cancers? Trends Mol Med 10: 481-486, 2004.
10. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, *et al*: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361: 947-957, 2009.
11. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, *et al*: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 11: 121-128, 2010.
12. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, *et al*: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12: 735-742, 2011.
13. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, Li W, Hou M, Shi JH, Lee KY, *et al*: Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): An open-label, randomised phase 3 trial. Lancet Oncol 15: 213-222, 2014.
14. Vallée A, Le Loupp AG and Denis MG: Efficiency of the Therascreen® RGQ PCR kit for the detection of EGFR mutations in non-small cell lung carcinomas. Clin Chim Acta 429: 8-11, 2014.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
16. Kim GW, Song JS, Choi CM, Rho JK, Kim SY, Jang SJ, Park YS, Chun SM, Kim WS, Lee JS, *et al*: Multiple resistant factors in lung cancer with primary resistance to EGFR-TK inhibitors confer poor survival. Lung cancer 88: 139-146, 2015.
17. Cha YK, Lee HY, Ahn MJ, Choi YL, Lee JH, Park K and Lee KS: Survival outcome assessed according to tumor burden and progression patterns in patients with epidermal growth factor receptor mutant lung adenocarcinoma undergoing epidermal growth factor receptor tyrosine kinase inhibitor therapy. Clin Lung Cancer 16: 228-236, 2015.
18. Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M and Miller VA: Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 12: 839-844, 2006.
19. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, *et al*: Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 361: 958-967, 2009.

20. Jackman DM, Miller VA, Cioffredi LA, Yeap BY, Jänne PA, Riely GJ, Ruiz MG, Giaccone G, Sequist LV and Johnson BE: Impact of epidermal growth factor receptor and KRAS mutations on clinical outcomes in previously untreated non-small cell lung cancer patients: Results of an online tumor registry of clinical trials. Clin Cancer Res 15: 5267-5273, 2009.
21. Zhu JQ, Zhong WZ, Zhang GC, Li R, Zhang XC, Guo AL, Zhang YF, An SJ, Mok TS and Wu YL: Better survival with EGFR exon 19 than exon 21 mutations in gefitinib-treated non-small cell lung cancer patients is due to differential inhibition of downstream signals. Cancer Lett 265: 307-317, 2008.
22. Bury T, Barreto A, Daenen F, Barthelemy N, Ghaye B and Rigo P: Fluorine-18 deoxyglucose positron emission tomography for the detection of bone metastases in patients with non-small cell lung cancer. Eur J Nucl Med 25: 1244-1247, 1998.
23. Hong SH, Kim YS, Lee JE, Kim IH, Kim SJ, Han D, Yoo IeR, Chung YG, Kim YH, Lee KY and Kang JH: Clinical characteristics and continued epidermal growth factor receptor tyrosine kinase inhibitor administration in EGFR-mutated non-small cell lung cancer with skeletal metastasis. Cancer Res Treat 48: 1110-1119, 2016.
24. Kim MH, Kim HR, Cho BC, Bae MK, Kim EY, Lee CY, Lee JS, Kang DR and Kim JH: Impact of cigarette smoking on response to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors in lung adenocarcinoma with activating EGFR mutations. Lung cancer 84: 196-202, 2014.
25. Takeda M, Okamoto I, Fujita Y, Arao T, Ito H, Fukuoka M, Nishio K and Nakagawa K: De novo resistance to epidermal growth factor receptor-tyrosine kinase inhibitors in EGFR mutation-positive patients with non-small cell lung cancer. J Thorac Oncol 5: 399-400, 2010.
26. Ludovini V, Bianconi F, Pistola L, Chiari R, Minotti V, Colella R, Giuffrida D, Tofanetti FR, Siggillino A, Flacco A, *et al*: Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol 6: 707-715, 2011.
27. Cappuzzo F, Jänne PA, Skokan M, Finocchiaro G, Rossi E, Ligorio C, Zucali PA, Terracciano L, Toschi L, Roncalli M, *et al*: MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 20: 298-304, 2009.
28. Tanaka A, Sueoka-Aragane N, Nakamura T, Takeda Y, Mitsuoka M, Yamasaki F, Hayashi S, Sueoka E and Kimura S: Co-existence of positive MET FISH status with EGFR mutations signifies poor prognosis in lung adenocarcinoma patients. Lung cancer 75: 89-94, 2012.
29. Ying HQ, Chen J, He BS, Pan YQ, Wang F, Deng QW, Sun HL, Liu X and Wang SK: The effect of BIM deletion polymorphism on intrinsic resistance and clinical outcome of cancer patient with kinase inhibitor therapy. Sci Rep 5: 11348, 2015.
30. Lee JY, Ku BM, Lim SH, Lee MY, Kim H, Kim M, Kim S, Jung HA, Sun JM, Ahn JS, *et al*: The BIM deletion polymorphism and its clinical implication in patients with EGFR-mutant non-small-cell lung cancer treated with EGFR tyrosine kinase inhibitors. J Thorac Oncol 10: 903-909, 2015.
31. Sos ML, Koker M, Weir BA, Heynck S, Rabinovsky R, Zander T, Seeger JM, Weiss J, Fischer F, Frommolt P, *et al*: PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer Res 69: 3256-3261, 2009.
32. Lee Y, Lee GK, Hwang JA, Yun T, Kim HT and Lee JS: Clinical likelihood of sporadic primary EGFR T790M mutation in EGFR-mutant lung cancer. Clin Lung Cancer 16: 46-50, 2015.