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EGRF-T790M mutation associated with acquired resistance to first-generation EGFR tyrosine kinase inhibitors in Vietnamese non-small-cell lung carcinoma patients

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Abstract: This study investigated the incidence of EGFR T790 mutation and progression-free survival (PFS) in 66 non-small cell lung cancer (NSCLC) patients with acquired resistance to first-generation EGFR tyrosine kinase inhibitors (TKIs). The results revealed that 54.5 % of patients developed the EGFR T790M mutation during progression, with a median PFS of 14.48 ± 3.9 months. Older patients and those with comorbidities experienced significantly shorter PFS. However, factors such as age, gender, smoking status, comorbidities, and pathological features were not significantly correlated with EGFR-T790M development. The study concluded that age and comorbidities were associated with PFS, while EGFR-T790M mutation did not show a significant correlation with PFS in this cohort.

Keywords: EGFR-T790M mutation, acquired resistance EGFR-TKIs, targeted therapy, NSCLC.

Classification numbers: 1.3.4, 1.3.2

1. INTRODUCTION

Lung cancer remains the most common etiology of cancer death in the world [1]. The majority of lung cancer is non-small cell lung cancer (NSCLC), most NSCLC patients are diagnosed at the late stage. Patients who had actionable mutations could benefit from targeted therapies [2]. Epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK) and Kirsten ras (KRAS) mutations are identified as the most frequent mutations in lung cancer [3]. EGFR mutations are present in about 13 - 22 % of NSCLC patients, 80 % of them response to

EGFR tyrosine kinase inhibitors (TKIs) [4]. Therefore, targeted therapy with EGFR-TKIs was proven to be an effective choice for NSCLC patients with EGFR mutations [5, 6]. However, most NSCLC patients become resistant after treating EGFR TKIs. Resistance to first-generation EGFR-TKIs occurs in most patients after 10 - 16 months of treatment [7]. There are multiple mechanisms of acquired resistance to first- and second-generation TKIs, in which, EGFR-T790M mutation is the most common one, accounting for about 60 % of cases with acquired resistance to gefitinib or erlotinib [2, 8, 9].

This study describes the incidence of EGFR-T790M mutation associated with acquired resistance to first-generation EGFR-TKIs, and the association of EGFR-T790M mutation with clinical, subclinical features, progression-free survival (PFS) of NSCLC Vietnamese patients.

2. MATERIALS AND METHODS

2.1. Study population

This prospective cohort study described 66 patients from 3 hospitals in Ha Noi, Viet Nam (Bach Mai Hospital, Vietnam National Cancer Hospital and 108 Central Military Hospital) from January 2015 to December 2019. The study included any patients who satisfy the following criteria: histological confirmation of NSCLC; the presence of EGFR mutations associated with drug sensitivity such as G719X, LREA, L858R; the treatment with a single-agent first-generation EGFR-TKI (erlotinib or gefitinib), acquired resistance to first-generation EGFR-TKIs according to the criteria of Jackman *et al*. [10]. The exclusion criteria were: treated with first-generation EGFR-TKIs but no TKIs sensitive EGFR mutations confirmed, not responding or stable state after at least 6 months of first-generation EGFR-TKIs treatment, refused to participate in the research.

Participants were volunteers and had the right to withdraw from the research. Patients' personal data were secured. Techniques, procedures performed on patients were warranted to be right according to the Ministry of Health. The research was conducted for scientific purposes but not any other one. All patients in this study agreed to participate. The research was approved by the Ethics Committee of Hanoi Medical University.

2.2. Data management

The data collection was obtained at the time of starting EGFR-TKIs therapy and during the progressive period. After starting treatment, imaging tests such as neck ultrasound, chest computed tomography (CT), abdomen CT, brain magnetic resonance imaging were conducted every 3 months, and bone scan was performed when suspected of bone metastasis. Response evaluation criteria in solid tumor (RECIST) version 1.1 was used to determine systemic progression of the disease [11]. PFS was defined as the first day of starting EGFR-TKI treatment to disease progression. The tissue sample was collected from all patients during the progressive period by CT-guided lung biopsy, bronchoscopic biopsy, cell block of pleural fluid, cell block of pericardial fluid or biopsy of metastatic site. The EGFR gene mutations of treatment-resistance tissue were analyzed using the Scorpion amplification refractory mutation system (Scorpion-ARMS) and real-time Polymerase chain reaction (PCR) techniques. The Cobas® EGFR Mutation Test v2 kit was employed for the detection of sensitive EGFR gene mutations such as G719X (exon 18), LREA (exon 19), L858R (exon 21), and secondary resistance EGFR mutation T790M (exon 20) at the Center for Gene and Protein Research, Hanoi Medical University, Ha Noi, Viet Nam.

2.3. Statistical analysis

Statistical analysis was performed using SPSS, version 20 (IBM Corp, New York, USA). Patient characteristics were described using descriptive statistics. The PFS was analyzed by the Kaplan-Meier method. The log-rank test was used to compare the PFS between subgroups of patient characteristics. Cox proportional hazard analysis was performed to explore the effect of each variable on PFS. Chi-square and Fisher's exact tests were used to analyze correlations between the clinical, subclinical features and EGFR-T790M mutation status. All statistical tests were two-sided and P values < 0.05 were considered significantly different.

3. RESULTS

3.1. Baseline characteristics

From January 2015 to December 2019, 66 NSCLC patients were evaluated in this study. The basic characteristics of patients are summarized in Table 1. The mean age was 60.6 ± 10.7 years (range: 26 - 80 years old), thirty-four (51.5 %) of the 66 patients were male. In our study, all patients were diagnosed with stages IV. The history of tobacco smoking was found in 45.5 % of patients. The majority of histology was adenocarcinoma, all patients had EGFR gene mutations including LREA, L858R and G719S. They did have KRAS mutation. All patients were treated with first-generation EGFR-TKIs, as follows: 53 % with Erlotinib and 47 % with Gefitinib. The mean PFS was 14.48 ± 3.9 months (range: 8 - 26 months).

After 6 months of treating with first-generation EGFR-TKIs, all patients had an incomplete response to first-generation EGFR-TKIs, in which 87.9 % and 12.1 % had a partial response and stable disease, respectively (Table 2). EGFR-T790M mutation was found in 54.5 % of NSCLC patients who had acquired resistance to first-generation EGFR-TKI treatment (Figure 1).

3.2. The association of clinical features with PFS

The correlation between the clinical features and PFS is presented in Figure 2.

The average PFS was not significantly different between males (13.8 months; 95 % CI 12.6 - 14.9 months) and females (15 months 95 % CI 13.5 - 16.5 months) (p = 0.18). No significant differences were found in average PFS characteristics including smoking (smoker and non-smoker groups), EGFR-mutation (LREA group, L858R group and G719S group), EGFR-TKIs treatment (erlotinib group and gefitinib group) (P > 0.05).

Patients who were younger than or equal to 60 years old tended to have significantly longer PFS than the older group (15.7 months vs. 13.3 months, p = 0.028. The average PFS in patients without comorbidities was 15.6 months (95 % CI, 14.2 - 17.0 months), which was significantly longer than in the patients with comorbidities (13.4 months; 95 % CI 12.3 - 14.6 months) (p = 0.039).

3.3. The association between clinical, subclinical features, PFS and EGFR-T790M mutation

The age, gender, smoking status, comorbidities, pathological features were not significantly correlated with EGFR-T790M mutation (P > 0.05) (Table 3).

The average PFS for patients in the EGFR-T790M group was 14.5 months (95 % CI, 13.3 - 15.8 months) and in the non-EGFR-T790M group was 14.1 months (95 % CI, 12.7 - 15.6

months). The average PFS was not significantly different between the EGFR-T790M group and the non-EGFR-T790M group (P = 0.642) (Figure 3).

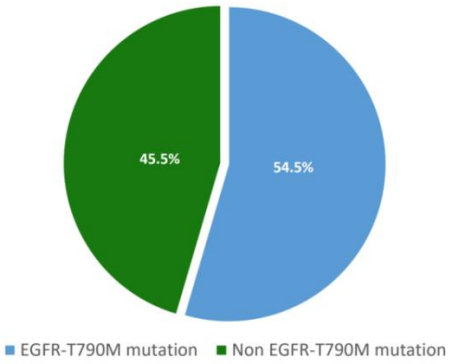

Figure 1. Incidence of EGFR-T790M mutation in NSCLC patients.

Table 1. Baseline characteristics of lung cancer patients (n = 66).

Characteristics		n	%
Mean age: 60.6 ± 10.7 years (range: 26-80 years old)			
Gender	Male	34	51.5
	Female	32	48.5
Smoking	Non-smoker	36	54.5
	Ex-smoker	10	15.2
	Current smoker	20	30.3
Comorbidities	Cardiovascular diseases[a]	16	18.2
	Pulmonary diseases[b]	12	24.2
	Metabolic disorders[c]	10	15.2
Histopathologic characteristics	Adenocarcinoma	65	98.5
	Non-adenocarcinoma	1	1.5
EGFR mutations	LREA (exon 19)	36	54.5
	L858R (exon 21)	29	44.0
	G719S	1	1.5
EGFR-TKIs	Erlotinib	35	53.0
	Gefitinib	31	47.0
Mean PFS: 14.48 ± 3.9 months (range: 8 - 26 months)			

[a]Cardiovascular diseases: hypertension, coronary artery disease, heart failure.

[b]Pulmonary diseases: COPD, asthma, bronchiectasis.

[c]Metabolic disorders: diabetes, gout.

Table 2. Patients' response after 6 months of treating with EGFR-TKIs (n = 66).

Patients' response	n	%
Complete response	0	0
Partial response	58	87.9
Stable disease	8	12.1
Progressive disease	0	0
Total	66	100

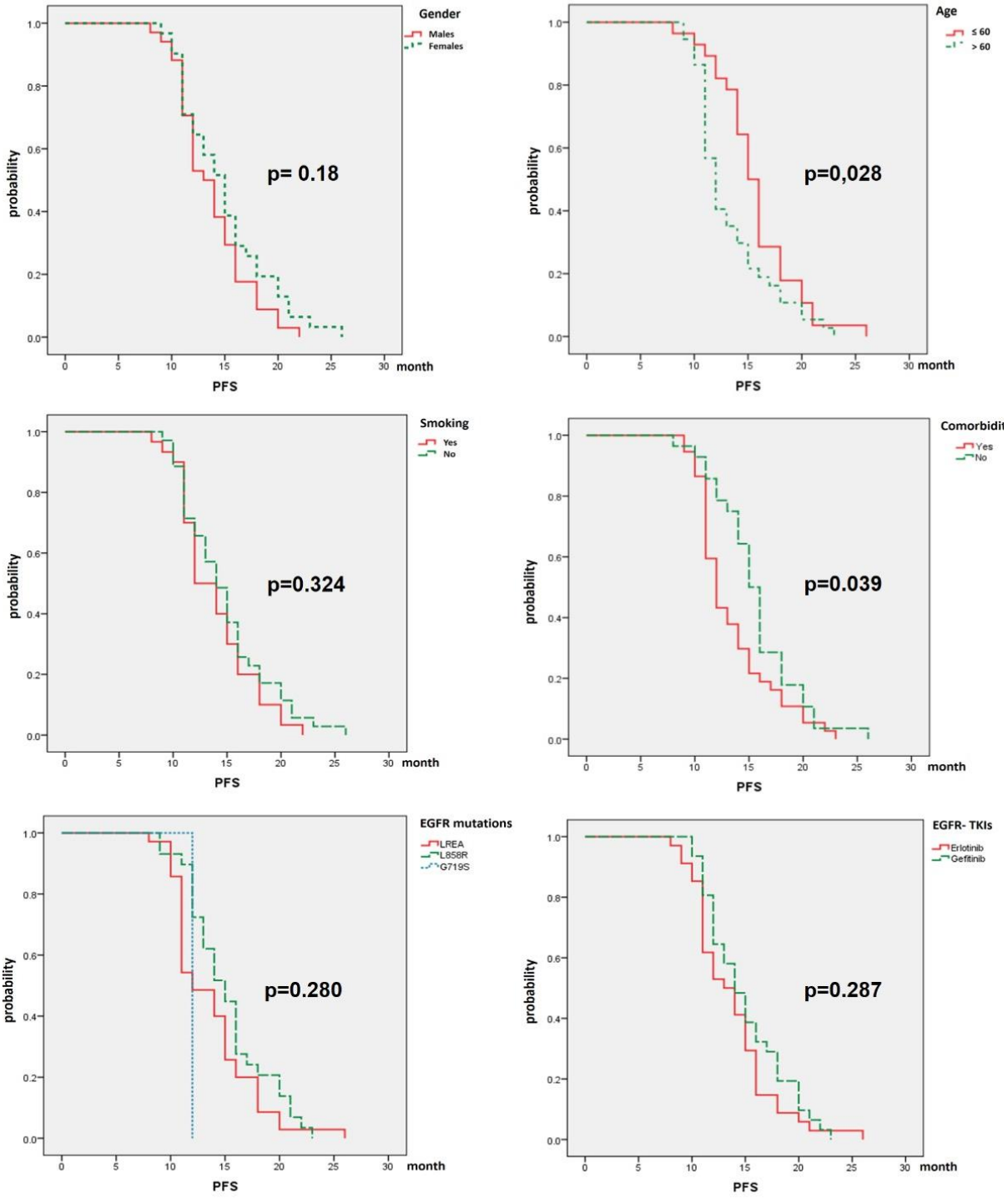

Figure 2. The association of clinical features with PFS (n = 66). Comparing the difference in PFS with several characteristics: gender, age, smoking status, comorbidities, EGFR mutation subtype, and type of first-generation EGFR-TKIs.

Table 3. The association of clinical and subclinical features with EGFR-T790M mutation (n = 66).

Features		EGFR-T790M mutation		Non EGFR-T790M mutation		*p*
		n	%	n	%	
Gender	Male	22	33.3	12	18.3	0.169
	Female	16	24.2	16	24.2	
Age	≤ 60	17	25.7	11	16.7	0.425
	> 60	21	31.9	17	25.7	
Smoking	Yes	21	31.9	9	13.6	0.053
	No	17	25.7	19	28.8	
Cormorbidities	Yes	20	30.3	18	27.3	0.244
	No	18	27.3	10	15.1	
Adenocarcinoma	Yes	38	57.6	27	40.9	0.424
	No	0	0	1	1.5	

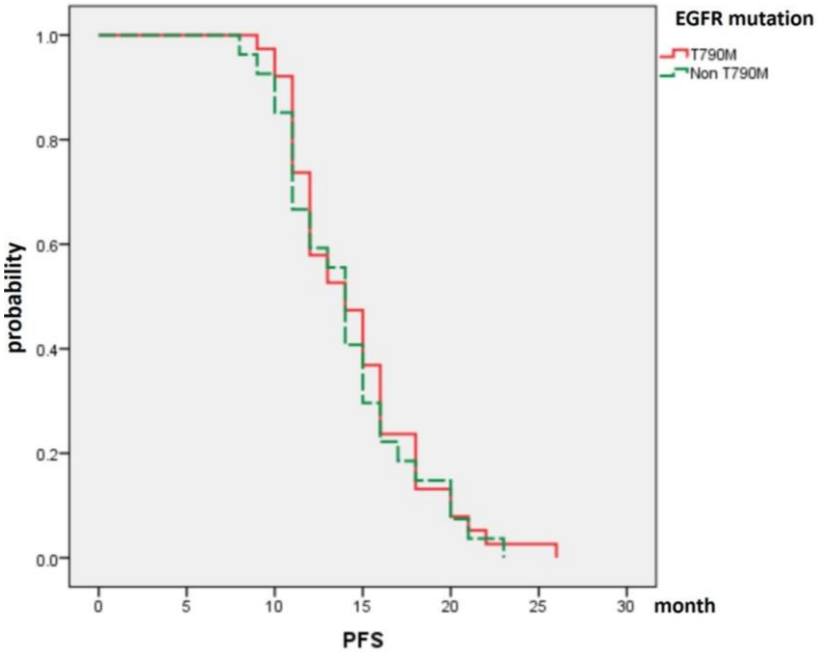

Figure 3. The association of EGFR-T790M mutation with PFS. The average PFS shows no statistically significant difference between the group carrying the EGFR-T790M mutation and the group without EGFR-T790M mutation.

4. DISCUSSION

The majority of patients treated with first-generation EGFR-TKIs developed resistance after an initial response period. The mechanisms of resistance were investigated in re-biopsy or fluid samples after acquired resistance of EGFR-TKIs. Understanding more clearly about this mechanism may be useful in developing new therapeutic strategies. In this prospective cohort of 66 patients, we analyzed the incidence of T790M mutation, the PFS, the association between PFS, T790M mutation and the baseline characteristics. Our study indicated that 54.5 % of

patients had EGFR-T790M mutations after receiving first-generation EGFR-TKIs. This result was in accordance with the previous study [12 - 14].

In terms of the PFS, our study demonstrated that the average PFS was 14.48 ± 3.9 months. This result was consistent with other international publications with a PFS range of 7.7-16.8 months [15, 16].

This study demonstrated that the PFS was significantly longer in younger patients (≤ 60 years old) or non-comorbidities group than older patients or patients with comorbidities ($p < 0.05$), however, no association between gender, smoking status, EGFR mutations, EGFR-TKIs and PFS was observed. In a study by Chantharasame *et al*. [17], genders, smoking status, mutation subtype, and line of TKI therapy were not associated with PFS. According to Jaiswal *et al*. [14], there was no significant association between PFS and age, genders, smoking status, type of baseline EGFR mutation. These results were similar to two previous studies conducted by Zhang and Li *et al*. [18,19], *Kim et al*. [20], where it was reported that the median TTP for all patients was 10.2 months, and confirmed that females, non-smokers, and patients with adenocarcinoma had longer PFS than males, smokers and patients with non-adenocarcinoma histology ($P < 0.05$). The association between gender, histological, smoking status, EGFR mutation, or response to first-line EGFR TKIs was analyzed in a study by Xu *et al*. [21], but the study indicated that no significant difference between the < 65 years old group and ≥ 65 years old group.

Our study revealed that there was no association between factors such as age, gender, smoking status, co-morbidity and histopathology with the presence of EGFR-T790M mutation ($p > 0.05$). A study by Wang *et al*. [22] demonstrated that the T790M mutation did not correlate with age, gender, smoking status, and initial EGFR mutation. These results were also consistent with the studies by Li *et al*. [19] and Zhang *et al*. [18]. Other reports also revealed that there was no significant difference in gender, age, or smoking status between T790M-positive and T790M-negative patients [13]. Jaiswal *et al*. [14] confirmed that the EGFR-T790M mutation correlated with younger age, smoking status, nonerlotinib TKI treatment, and adenocarcinoma histology ($p < 0.05$).

In the total of 66 patients, the mean PFS was not statistically different between groups with and without EGFR-T790M mutations ($p = 0.642$). This result is similar to the study results of Li *et al*. [19] which demonstrated that PFS was not statistically significantly different between patients with and without T790M mutation (13.0 vs. 10.5 months, $p = 0.894$). It is also consistent with a study by Zhang *et al*. [18]. Another study revealed that patients with T790M mutation had significantly longer PFS than those without [12, 22].

This study has some limitations. First, the small sample size may affect the results. Second, our study does not mention any resistance mechanisms other than T790M. Several other resistant mechanisms may be present and associated with PFS. Finally, the presence of the EGFR-T790M mutation was not demonstrated on the same lesions throughout the course of the disease, and tissue samples for T790M detection were also inconsistent, however, the heterogeneity of tumors genetics is an important characteristic of lung cancer.

5. CONCLUSIONS

The EGFR-T790M mutation is a common underlying cause of secondary resistance to first-generation EGFR-TKIs in patients with non-small cell lung cancer in Viet Nam. Patients older than 60 years old or with comorbidities had significantly shorter PFS than the subgroups

without. The average PFS was not significantly different between the EGFR-T790M group and the non-EGFR-T790M group.

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CRediT authorship contribution statement. Hoan Le, Chi Khanh Tran designed the present study and anylyzed the data. Van Khanh Tran and Thinh Huy Tran performed the data collection. Hoan Le and Tra My Thieu wrote the manuscript. Ngoc Cuong Nguyen checked the data and revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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