CLINICAL FACTORS PREDECTING T790M MUTATION IN NON-SMALL CELL LUNG CANCER PROGRESSED AFTER FIRST- AND SECOND-GENERATION TKI TREATMENT

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ABSTRACT

Objective: We aimed to identify the T790M mutation rate and clinical factors predicting the occurrence of acquired T790M mutations in advanced non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutations after first- and second-generation tyrosine kinase inhibitor (TKI) treatment. ***Method:*** The EGFR-mutant NSCLC patients who experienced progression after first- and second-generation TKI treatment and underwent testing for EGFR using plasma samples between January 2021 and April 2023 were enrolled. The T90M mutation rate and related clinical factors were evaluated. We utilized the Chi-square test or Fisher's exact test for univariate analysis and multivariable logistic binary regression for multivariate analysis. ***Results:*** A total of 247 patients were eligible for the study. Patients harbored the exon 19 deletion mutation (Del19), exon 21 L858R point mutation, and uncommon mutations in 63.2%, 34.4%, and 2.4% of cases, respectively. The overall T790M mutation rate was 33.6%. In multivariate analyses, the Del19 mutation versus L858R mutation, duration of first-line TKI exceeding 12 months versus below 12 months, and solitary lesion progression versus multiple lesion progression were identified as favorable prognostic factors for the occurrence of T790M mutation. ***Conclusion:*** Patients with EGFR exon 19 deletion, a prolonged treatment duration of first-line EGFR-TKI, and solitary lesion progression pattern are more likely to acquire the T790M mutation, as detected by the PCR method in plasma samples.

Keywords: *lung cancer, T790M acquired, progression pattern, treatment duration, baseline EGFR mutation*

I. INTRODUCTION

Lung cancer is one of the leading types of cancer with a high incidence and mortality rate worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 80-85% of all lung cancer cases, and several new treatment modalities have helped improve overall survival, including targeted therapy for EGFR mutations. Epidermal growth factor receptor (EGFR) mutations are the most common driver mutations among patients with lung cancer in Southeast Asians. Multiple phase III trials have shown higher response rates and significantly improved PFS in EGFR-mutant tumors treated with first- and second-generation EGFR tyrosine kinase inhibitors (TKIs) compared with platinum-based first-line chemotherapy; consequently, erlotinib, gefitinib, and afatinib are the first-line standard of care in EGFR-mutant NSCLC.

Although most EGFR-mutant NSCLC patients initially have a good response to EGFR-TKIs, acquired resistance is

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Date of receipt: 12/2/2024
Date of scientific judgment: 11/3/2024
Reviewed date: 18/3/2024

commonly observed between 8 to 13 months after the initiation of first-line therapy with either first- or second-generation EGFR-TKI. Some mechanisms have been identified, including the development of the EGFR T790M mutation, MET amplification, transformation to small cell lung cancer, and the development of PIK3CA and KRAS mutations. Among the above mechanisms, the secondary T790M mutation accounts for the largest proportion, approximately 50-60% of acquired resistance. The T790M mutation causes steric hindrance that affects the ability of EGFR TKIs to bind to the ATP-kinase pocket. In addition, the T790M mutation restores the balance of affinity of the mutant receptor in favor of ATP, thus reducing the potency of competitive inhibitors.

The AURA 2 and AURA 3 studies demonstrated the efficacy of the third-generation EGFR-TKI osimertinib in the treatment of in advanced NSCLC patients who had progressive disease after first- or second-generation EGFR-TKI therapy. At the time of development of resistance to the initial EGFR-TKIs, identification of the EGFR T790M mutation after EGFR-TKI failure has been an important requirement for subsequent treatment. However, rebiopsy performed to examine acquired T790M mutations are associated with both risks and discomfort and may not provide enough tumor tissue for genetic analyses in some patients. To overcome this problem, plasma biopsy, which detects cancer cell DNA in the blood, has been developed to detect the T790M mutation. Plasma biopsy is a noninvasive, repeatable, quicker, and less expensive technique; however, it is associated with lower sensitivity compared to tissue biopsy [1].

The aim of this study was to provide information about the frequency of T790M mutation detected by the PCR method in plasma samples and to identify the clinical factors predicting the occurrence of acquired T790M mutation after first- and second-generation TKI treatment.

II. MATERIALS AND METHODS

This retrospective, single-center, observational study was conducted at the National Cancer Hospital in Vietnam. EGFR-mutated NSCLC patients who experienced progression after first- and second-generation TKI treatment and underwent testing for EGFR using plasma samples between January 2021 and April 2023 were enrolled, and patient medical records were reviewed. The ethics committee of the National Cancer Hospital approved the study and protocol.

Patient Population: Patients were required to meet the following criteria: 1) histologically confirmed EGFR-mutant NSCLC, 2) stage IIIB, IIIC, and IV according to the eighth edition of the American Joint Committee for Cancer staging system, 3) first-line EGFR-TKI treatment by first- or second-generation, and 4) retesting EGFR by plasma sample after disease progression confirmed by the Response Evaluation Criteria in Solid Tumors Ver. 1.1. We excluded patients who had met one of the following criteria: 1) a history of primary treatment failure with EGFR-TKI, 2) a lack of prior EGFR mutation examination, 3) the presence of an EGFR exon 20 insertion mutation prior to initiating EGFR-TKI treatment, 4) concurrent diagnosis of another active malignancy, and 5) receipt of other anticancer medications simultaneously or

first-line EGFR TKI treatment for a duration of less than 90 days.

EGFR T790M mutation test: Plasma samples for the analysis of circulating cell-free tumor DNA (cfDNA) were collected at disease progression and analyzed using the QUIamp Circulating Nucleic Acid kit was by the real-time PCR EGFR mutation assay.

Progression pattern: This study evaluated the disease progression patterns when patients were tested for the T790M mutation. All progressive lesions were assessed, including progression in the lungs, bones, liver, adrenal glands, brain, pleura, peritoneum, lymph node metastasis, and soft tissue. We divided the patients into two groups: those with solitary lesion progression and those with multiple lesion progression.

Statistical analyses: Univariate analysis using the Chi-square test or Fisher's exact test was conducted to assess the associations between the frequency of T790M mutation and patient characteristics, as well as progression patterns. Multivariate analysis was performed using multivariable logistic binary regression (backward elimination approach) to identify clinical factors related to the occurrence of the T790M mutation. All statistical tests were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). All reported p values were two-sided, and $p < 0.05$ was considered to indicate statistical significance.

III. RESULTS

A total of 247 patients who met the study criteria were included. The patient characteristics are shown in Table 1. After the development of resistance to EGFR-TKIs, the overall T790M mutation was identified in 83 (33.6%) of 247 patients.

Table 1. ***Patient characteristics***

Characteristic		**Number (n = 247)**	**Percentage**
Age: Median (range, yr)		61 (29-79)	
Sex	Male	127	51.4%
	Female	120	48.6%
Smoking status	Yes	91	36.8%
	No	156	63.2%
Baseline EGFR mutation status	Exon 19 del	156	63.2%
	L858R	85	34.4%
	Rare mutations	6	2.4%
First-line TKI treatment	Erlotinib	92	37.2%
	Gefitinib	121	49%
	Afatinib	34	13.8%
Treatment duration of first-line TKI	≥ 12 months	123	49.8%
	< 12 months	124	50.2%
Progression patterns	Solitary lesion	108	43.7%
	Multiple lesion	139	56.3%

3.1. Correlation between the acquired T790M mutation rate and patient characteristics

The associations between the T790M mutation rate and patient characteristics are summarized in Table 2. According to the baseline EGFR mutation status, 61 of 156 patients with the Del19 mutation had the T790M mutation (39.1%), 22 of 85 patients with the L858R mutation harbored the

T790M mutation (25.9%), and 0 of 6 patients with rare mutations tested positive for the T790M mutation (0%). Patients with a baseline Del19 mutation had a higher T790M mutation rate than those with L858R mutations (p=0.039). Multivariate analysis confirmed the correlation of exon 19 deletion with the presence of the T790M mutation (adjusted odds ratio, 0.53; 95% CI, 0.29 to 0.96; p=0.035).

There were no significant differences in the frequency of the T790M mutation in univariate analysis between the two groups in terms of patient characteristics, including age, sex, and smoking status.

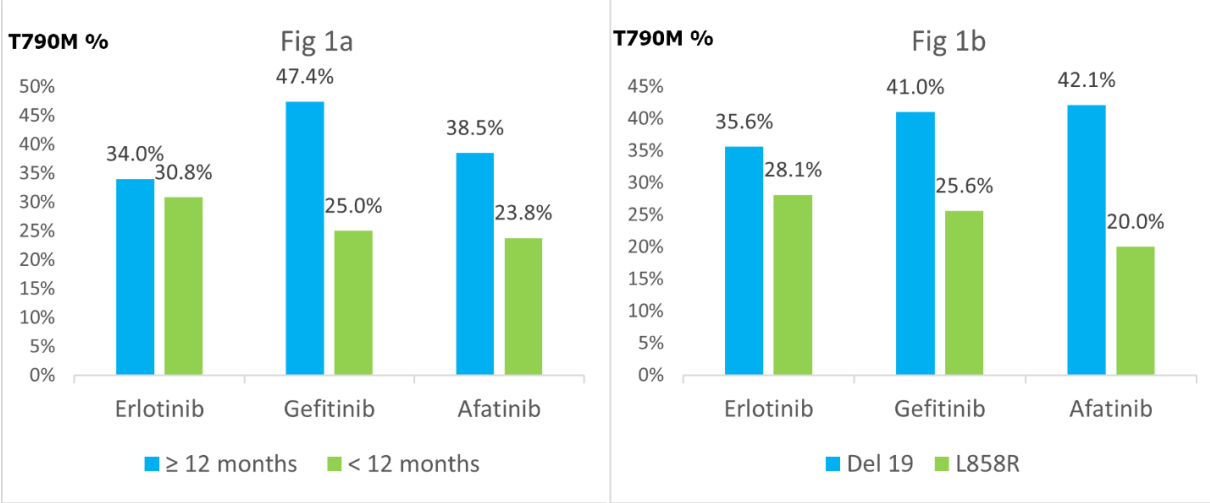

Figure 1. ***Comparison of positive T790M mutation rates among three EGFR TKIs based on treatment duration (1a) and EGFR mutation baseline (1b).***

3.2. The correlation between the T790M mutation rate and the type of first-line EGFR-TKI

Assessing the relationship between T790M acquired and the type of first-line EGFR-TKI, our study noted that the group of patients using gefitinib had the highest rate of T790M acquired mutation at 35.5%, followed by the group of patients using erlotinib (32.6%), and the lowest was the group of patients using afatinib (29.4%), the difference was not statistically significant. Two factors that showed a significant correlation with the rate of acquired T790M mutation were baseline EGFR mutation and treatment duration of first-line EGFR TKI. We studied these two factors categorized into three groups based on the three types of EGFR-TKIs used. The results are shown in Figure 1. Accordingly, each group of patients using one of the three TKIs showed similar results to the general group: the frequency of the T790M mutation was higher in the EGFR del exon 19 group, and the treatment duration of first-line EGFR-TKI was ≥ 12 months.

3.3. The correlation between the T790M mutation rate and the treatment duration of first-line EGFR-TKIs

Patients with a treatment duration of first-line EGFR-TKI ≥ 12 months harbored a higher frequency of the T790M mutation than those with a first-line EGFR-TKI treatment duration < 12 months (p=0.02). This conclusion was confirmed using multivariate analysis by logistic regression models with

an adjusted odds ratio of 0.51 (95% CI, 0.29 of 0.89, p=0.017).

We divided the patients into three groups (treatment duration: ≤ 10 months, 11–19 months, ≥ 20 months), and it appeared that the frequency of the T790M mutation and the treatment duration of first-line EGFR-TKI treatment were directly correlated (Figure 2).

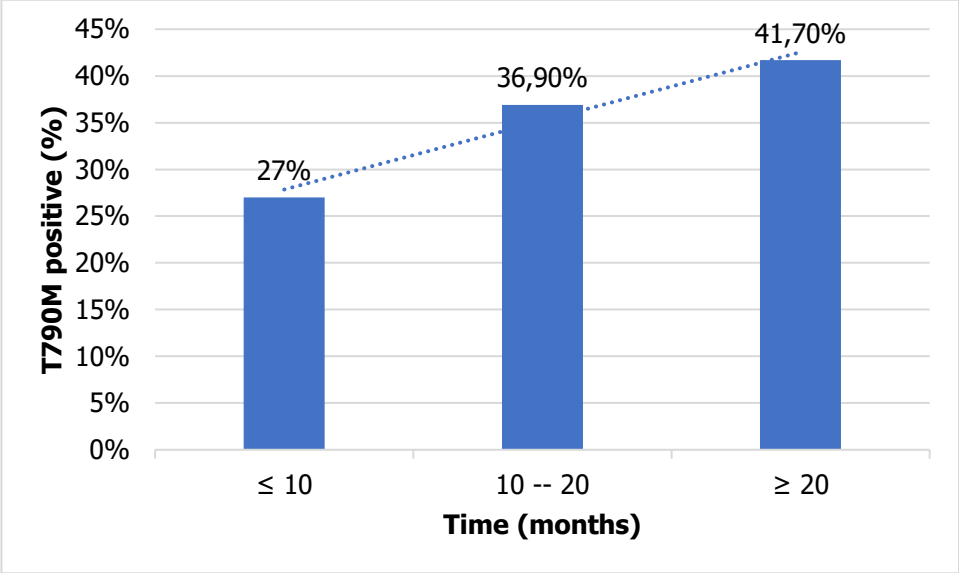

Figure 2. ***The correlation between treatment duration of EGFR-TKI first-line and T790M mutation status.***

3.4. The correlation between the T790M mutation rate and the progression patterns at failure of first-line EGFR-TKI.

We analyzed the progression patterns of all patients at the time of disease progression. In univariate analysis, the presence of the acquired T790M mutation was significantly correlated with solitary lesion progression in 45 (41.7%) patients compared with 38 (27.3%) patients in the multiple lesion progression subgroup (p=0.018). This difference was confirmed by the multivariate analysis (adjusted odds ratio, 0.48; 95% CI, 0.27 to 0.83; p=0.009).

Table 2. ***The T790M Mutation Rate and Related Factors***

Characteristic	No.	T790M positive n (%)	Univariate analysis (*)		Multivariate analysis (**)	
			p	OR (95% CI)	p	OR (95% CI)
Age						
•≥ 65	82	28 (34.1)	0.803	1.00 (ref)	-	-
•< 65	165	59 (35.8)		1.14 (0.65-2.00)		
Sex						
•Male	127	39 (30.7)	0.322	1.00 (ref)	-	-
•Female	120	44 (36.7)		1.31 (0.77-2.22)		
Smoking status						
•Yes	91	35 (38.5)	0.416	1.00 (ref)	-	-
•No	156	52 (33.3)		1.02 (0.59-1.77)		
Baseline EGFR mutation status						
•Exon 19 del	156	61 (39.1)	**0.039[a]**	1.00 (ref)	**0.035[a]**	1.00 (ref)
•L858R	85	22 (25.9)	0.084[b+]	0.54 (0.30-0.97)	0.999[b]	0.53 (0.29-0.96)
•Rare mutations	6	0 (0)	0.152[c+]	-		-

Characteristic	No.	T790M positive n (%)	Univariate analysis (*)		Multivariate analysis (**)	
			p	OR (95% CI)	p	OR (95% CI)
First-line TKI						
•Erlotinib	92	30 (32.6)	0.656[d]	1.00 (ref)	-	-
•Gefitinib	121	43 (35.5)	0.732[e]	1.14 (0.64-2.02)		
•Afatinib	34	10 (29.4)	0.506[f]	0.86 (0.37-2.03)		
Treatment duration of first-line TKI						
•≥ 12 months	123	50 (40.7)	**0.02**	1.00 (ref)	**0.017**	1.00 (ref)
•< 12 months	124	33 (26.6)		0.53 (0.31-0.91)		0.51 (0.29-0.89)
Progression pattern						
•Solitary organ	108	45 (41.7)	**0.018**	1.00 (ref)	**0.009**	1.00 (ref)
•Multiple organ	139	38 (27.3)		0.53 (0.31-0.90)		0.48 (0.27-0.83)

(*): chi-square test *(**): Multivariable logistic binary regression*

p^a: Exon 21 vs. *exon 19; p^b: Rare mutations* vs. *exon 19; p^c: Rare mutations* vs. *exon 21*

p^d: gefitinib vs. *erlotinib; p^e: afatinib* vs. *erlotinib; p^f: afatinib* vs. *gefitinib;*

+: Fisher's exact test

IV. DISCUSSION

The acquired T790M mutation plays a decisive role in treatment selection after the failure of first- and second-generation EGFR-TKIs. Consequently, numerous studies have assessed the factors influencing the occurrence of acquired T790M mutations. There is a difference in the rate of T790M mutations between previous studies, partly due to the type of samples used for repeat biopsies and the ability to repeat biopsies after obtaining negative results. Jenkins et al published the results of a study using data compiled from two randomized trials: AURA and AURA 2 and found that the rate of acquired T790M mutation was 63% [2]. Isabella et al., when evaluating the rate of acquired T790M mutations, found a mutation rate of up to 71%. This result was achieved by using repeat biopsies after a negative result that allowed for the detection of positive mutations in an additional 17% of the cases. This study showed that the detection rate of the resistance mutation T790M in liquid biopsies was only 33%, lower than that in tissue biopsies (57%) [3]. This conclusion is consistent with previous studies indicating that liquid biopsies have a rate of false negatives. Thus, the literature suggests that when liquid biopsies are negative, patients should undergo a tissue biopsy to increase the likelihood of detecting resistance gene mutations.

In our study, the rate of acquired T790M mutation was 33.6%, lower than in previous real-world data studies. There are two main reasons for this outcome. First, the rate of detecting T790M mutations in liquid biopsy is generally lower than that in tissue biopsy. Second, it is related to the testing technique. Del Re et al. evaluated the acquired T790M mutation using plasma samples for testing with the ddPCR (droplet digital) method and found that the T790M mutation rate was 56.6%. The authors noted that the high T790M mutation rate was due to the technique used for EGFR analysis being

ddPCR, a highly sensitive approach capable of detecting mutations at levels as low as 0.0001%, a sensitivity higher than ARMS real-time PCR [4].

Most studies on acquired T790M mutations share a common observation, which is that the baseline EGFR mutation is related to the rate of T790M mutations at the time of disease progression. Jenkins et al. stated that the frequency of the T790M mutation was higher in patients with the EGFR exon 19 deletion than in those with the L858R mutation (73% vs. 58%, p=0.0002), the lowest rate was observed in the rare mutation group at only 32% [2]. Huang et al. also reported that patients with EGFR mutation 19 deletion had a higher frequency of the T790M mutation than patients with the L858R mutation and other EGFR mutations (55% vs. 37.3% and 27.3%, p=0.022) [5]. Our study also reached a similar conclusion, where patients with mutations in exon 19 had a T790M acquired rate of 39.1%, which was significantly higher than the rate for those with L858R mutations at 25.9% in both the univariate and multivariate analysis (p=0.039). There were only six patients with rare mutations, and no cases were observed with T790M acquired mutations.

Other factors such as age, sex, performance status, and histopathological characteristics, have been evaluated for their association with the occurrence rate of acquired T790M mutations. However, only a few studies have found a significant correlation between one of these factors and the T790M mutation. Kaburagi's study with a median age of 70 years concluded that the group of patients over 75 years old had a significantly higher rate of acquired T790M mutations than the group of patients under 75 years old (55.6% vs. 36.6%, p=0.0096) [6]. However, studies by Maso (2019) and Isabella (2020) used different age cutoffs and reached the opposite conclusion, stating that the younger patient group (under 65 years old) had a significantly higher rate of acquired T790M mutations than the older patient group (over 65 years old) [3] [7]. Regarding sex, the study by Oya et al., observed that the prevalence of the T790M mutation was significantly higher in men than in women (63% vs. 38%, $p < 0.05$) [8]. In our study, when assessing the relationship of age, sex, smoking status with the incidence of acquired T790M mutations, we did not find statistically significant differences with p-values of 0.803, 0.322 and 0.416, respectively.

About the treatment duration of first-line EGFR-TKI treatment, Isabella et al. concluded that the group of patients with treatment duration ≥ 12 months had a significantly higher rate of T790M mutations (p=0.005). Huang et al. found that patients with a first-line EGFR-TKI treatment duration > 11 months had a higher likelihood of developing T790M mutation (p=0.044) [5]. The results of our study also demonstrate that patients with a longer duration of first-line EGFR-TKI treatment have a higher rate of acquired T790M mutations, the patients with a first-line EGFR-TKI treatment duration ≥ 12 months had a higher rate of T790M mutations compared to the group with treatment duration < 12 months (40.7% vs. 26.6%, p value=0.02).

The type of first-generation (1st or 2nd) EGFR TKI is a major concern in studies assessing the correlation with the emergence of acquired T790M mutations. Our study

results align with the majority of real-world data studies, showing no statistically significant differences in the incidence of acquired T790M mutation between erlotinib, gefitinib, and afatinib. However, some recent studies have presented different conclusions. Wagener et al. found that 45% of afatinib-treated patients developed a T790M mutation, compared to 65% of reversible EGFR TKI-treated patients (p=0.02) [9]. The analysis of data from 29 studies involving 3385 patients showed that the T790M mutation rate was significantly lower with afatinib (33%) than with gefitinib (49%) and erlotinib treatments (47%) (p<0.001). The mechanisms underlying the lower T790M mutation rate after afatinib treatment are unclear. Several hypotheses have been proposed based on the resistance mechanisms of cancer cells to targeted therapies, the formation of T790M mutation acquired, or the clonal selection during different EGFR-TKI treatments [10].

The study by Oya et al. is the first study to investigate the association between progression patterns at the time of resistance to initial EGFR-TKIs and T790M mutation status in patients resistant to EGFR-TKIs. The progression patterns are classified as either solitary lesion progression or multiple lesion progression. The conclusion of their study is that the prevalence of the T790M mutation was significantly greater in patients with solitary lesion progression than in those with multiple lesion progression (58% vs 24%, p<0.001) [8]. Maso et al. evaluated the type of progression, which includes the categories of isolated, oligo-, or systemic progression. The results of their study showed that the isolated group had a slightly higher emergence of the T790M mutation than the systemic + oligo group (59% vs. 50.6%), and the isolated + oligo group also had a slightly higher T790M mutation rate than the systemic group (53.5% vs. 50.5%) [7]. Chai et al. focused on the number of new progression sites in the pattern of disease progression in patients with acquired T790M mutation and found that the group of patients progressing at a single site had a higher rate of T790M mutation compared to the group of patients progressing at two or more sites, with corresponding rates of 67.4% vs. 58.5% [11]. However, this difference was not statistically significant.

When evaluating the relationship between progression patterns and T790M mutations, our study categorizes patients similarly to Oya's study [8]. We found that the group of patients with solitary lesion progression had a T790M acquired mutation rate of 41.7%, which was higher than that of the group of patients with multiple lesion progression, which was only 27.3%. This difference was statistically significant by both univariate and multivariate analysis (adjusted odds ratio, 0.48; 95% CI, 0.27 to 0.83; p =0.009). This result is consistent with the findings of previous studies, suggesting that tumors that progress more slowly with fewer progressing sites are more likely to develop T790M acquired mutations.

V. CONCLUSION

The treatment duration of first-line EGFR-TKI, type of EGFR mutation at diagnosis, and progression pattern (solitary lesion vs multiple lesion progression) were significantly associated with the occurrence of acquired T790M mutation. The present study also indicates a trend favoring acquired T790M mutations in the group of patients

treated with first-generation EGFR-TKIs compared to those treated with second-generation EGFR-TKIs.

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