The Role of De Novo T790M Mutation in The Origin of T790M Resistance Clone and in The Clinical Outcomes For Advanced EGFR-Mutant NSCLC Patients Receiving EGFR-TKIs

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Research

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Abstract

**Background:** Increasing evidence suggests that de novo T790M mutation occurs at a low frequency in patients with epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC). However, the effects of this mutation on the formation of T790M resistant clones and efficacy of EGFR tyrosine kinase inhibitors (TKIs) remain unclear.

**Methods:** Fifty-nine treatment-naïve in-patients with advanced EGFR-mutant NSCLC were enrolled in this study between 2017 and 2018. We dynamically monitored T790M mutation in ctDNA of patients before and during treatment with first-generation EGFR-TKIs, which were administered every 2 to 3 months until disease progression.

**Results:** Among the patients, 28.81% (17/59) had a low-frequency de novo T790M mutation, 66.67% (10/15) of them retained T790M mutation and resistance in this group was defined as “selection” resistance. T790M mutation was detected after treatment in 42.3% (11/26) of patients without de novo T790M mutation who experienced disease progression and resistance in this group was defined as “acquisition” resistance. After treatment with third-generation EGFR-TKI, patients with the “selection” T790M resistance mutation had significantly better objective response rate (ORR) and longer progression-free survival (PFS) than those with the “acquisition” T790M resistance mutation.

**Conclusion:** Our study provides evidence that low-frequency de novo T790M mutation is not rare in patients with advanced EGFR-mutant NSCLC. T790M resistance mutations can have two origins: the selection of low-frequency de novo T790M clones or the acquisition of the mutation in initially T790M-negative cells clinically. Since the origin of T790M resistance mutations can affect the efficacy of third-generation EGFR-TKIs, these EGFR-TKIs may be more effective for the treatment of NSCLC patients with “selection” T790M resistance mutations.

**Background**

Over the past decade, tremendous progress has been made in the treatment of advanced non-small cell lung cancer (NSCLC), such as the use of tyrosine kinase inhibitors (TKIs) and molecularly targeted therapy for NSCLC patients with epidermal growth factor receptor (EGFR) mutations [1-5]. However, most patients that initially respond to EGFR-TKIs will eventually become drug resistant. The development of drug resistance to EGFR-TKIs has been attributed to EGFR T790M mutation (p. Thr790Met) in approximately 50~60% of cases [5-9].

A two-model hypothesis has been proposed for the origin of EGFR T790M resistance clones from an evolutionary perspective, namely “selection” and “acquisition” models [10]. Under EGFR-TKI treatment pressure, a clone with a low mutant allele frequency (MAF) for de novo T790M can develop into a tumor with a dominant drug-resistant mutation. This is known as the “selection” resistance model. An EGFR-activating mutant clone that undergoes various processes such as the activation of the insulin-like growth factor 1 receptor (IGF-1R) signaling pathway, an altered chromatin state, or nuclear factor kappa B
NF-kB) activation [11, 12] and finally evolves into a T790M resistance clone is known as an “acquisition” resistance clone. Basic research and retrospective data have revealed differences in the effects of first-generation EGFR-TKIs and the sensitivity of T790M resistance clones to third-generation EGFR-TKIs between patients with “selection” and “acquisition” resistance mutations [10, 13, 14].

At present, the probability of detecting de novo T790M mutation varies according to the detection samples and methods used. More than a decade ago, Michio et al [15] used direct sequencing and mutant-enriched polymerase chain reaction (PCR) to identify T790M mutations in small fractions of tumor cells collected from NSCLC patients before treatment. De novo T790M mutation, which may be one of the main origins of the “selection resistance clone,” was found in only 0.3%-3% of NSCLC patients. However, the prevalence of de novo T790M mutation (ranges from 20% to 80%) has been clearly underestimated due to the sensitivity of the detection methods available. These techniques include matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), TaqMan assay, colony hybridization, peptide nucleic acid (PNA)-clamping PCR, and scorpion amplified refractory mutation system (ARMS). Droplet digital PCR (ddPCR) is a novel method that can detect rare mutations with ultra-sensitive quantification [13, 14, 16-24]. De novo T790M has low frequency and the highest reported MAF for the mutation is between 0.01% and 0.1% [14, 21-23]. However, the effect of low-frequency de novo T790M mutation on the biological behavior of NSCLC patients with EGFR-activating mutations remains unclear. Therefore, detecting de novo T790M mutation and understanding the differences between the two resistance models are of great clinical significance for the first-line treatment of EGFR-activating mutant NSCLC and overcoming drug resistance.

To this end, we used ddPCR to monitor the EGFR T790M mutation in circulating tumor DNA (ctDNA) using serial plasma samples from advanced EGFR-mutant NSCLC patients before and during treatment with first-generation EGFR-TKIs. Using this approach, we evaluated the prevalence of de novo T790M mutation, the influence of the de novo T790M clone on “selection” and “acquisition” resistance models, and the differences in the effects of third-generation EGFR-TKIs on T790M resistance clones with different origins.

**Methods**

**Patients and samples**

A total of 59 treatment-naïve, advanced NSCLC patients with EGFR-activating mutation were enrolled in this study. The patients were admitted to the Fujian Provincial Cancer Hospital (Fuzhou, China) and The First Hospital of Nanping City, Fujian Province (China) during the period from March 10, 2017 to November 28, 2018. Analysis of EGFR-activating mutations in biopsy specimens from the patients was performed using ARMS-PCR or next generation sequencing (NGS). Furthermore, EGFR T790M mutation in the ctDNA of the patient plasma samples was assessed every 2 to 3 months using ddPCR before and during first-generation EGFR-TKI treatment. However, serial plasma ctDNA samples were only collected from a limited number of patients. Based on the results, the eligible patients were stratified into two
groups: EGFR-activating mutation with and without the low-frequency de novo T790M mutation at the baseline.

**Treatment regimens and response evaluation**

All participants received 250 mg Gefitinib (AstraZeneca Pharmaceuticals; Waltham, MA, USA) once a day (QD) or 150 mg Icotinib (Betta Pharmaceuticals Co., Ltd.; Hangzhou, China) three times a day (TID). Some of the patients also received 80 mg of the third-generation EGFR-TKI Osimertinib (AstraZeneca Pharmaceuticals; Waltham, MA, USA) QD when EGFR T790M mutation was detected in their plasma ctDNA or biopsy specimens after disease progression. The Response Evaluation Criteria in Solid Tumor (RECIST version 1.1) was used to evaluate the treatment responses of the patients. Progression-free survival 1 (PFS1) was recorded after treatment with first-generation EGFR-TKIs in patients with T790M resistance mutation if the disease progressed. PFS2 represented the length of survival for all patients after treatment with first-generation EGFR-TKIs. PFS3 represented the length of survival after treatment with the third-generation EGFR-TKI until disease progression.

**Droplet Digital PCR (ddPCR)**

ddPCR was performed following the manufacturer's protocols; therefore, a minimum of 8 ml of peripheral blood was required. The presence of the EGFR T790M mutation in the samples was determined using the QX200 ddPCR System (Bio-Rad, Marnes-la-Coquette, France). Qiamp Circulating Nucleic Acid Kits were obtained from Qiagen (Cat NO. 55114) and used for the purification of free-circulating DNA in the plasma or serum. The quality of the extracted nucleic acid was determined using a Qubit Fluorometer. The samples were first emulsified using a QX200 droplet generator, then transferred to 96-well plates and amplified in a thermal cycler with the following thermal profile: 42°C for 5 min; 94°C for 5 min; 8 cycles of 94°C for 15 s, 60°C for 25 s, and 72°C for 40 s; and 72°C for 5 min. The fluorescence signals from individual sample droplets in each well were measured with a QX200 droplet reader. The MAF relative to the frequency of the wild-type allele was analyzed using QuantaSoft V.1.7.4.0917 software. The MAF was determined according to Poisson's distribution principle and the number and proportion of positive droplets. Positive and negative cut-off points were established for the controls. The cut-off point for positive droplets was set at three droplets based on preliminary assays and the manufacturer's protocols. This kit is very sensitive and can detect 0.33% of EGFR gene mutant DNA in patient samples.

**Statistical analysis**

The clinicopathologic variables were compared using Fisher's exact test or the Chi-squared test. PFS was analyzed using the Kaplan-Meier method and the differences in PFS between groups were compared using the log-rank test. In the tests, a two-sided P value (less than 0.05) was considered statistically significant. The statistical software SPSS version 23.0 (SPSS, Inc.; Chicago, IL, USA) was used for all statistical analyses in this study.

**Informed consent and ethical approval**
The protocol for this study was reviewed and approved by the Ethical Review Committee of Fujian Provincial Cancer Hospital (approval no.2017-069-01). Written informed consent with a detailed description of the study was obtained from all participants.

**Results**

**Clinicopathological characteristics of patients with EGFR mutation**

Fifty-nine advanced EGFR-mutant NSCLC patients were enrolled in this study, comprising 28 men and 31 women with a median age of 56 years (range, 38-79 years) at diagnosis. Among these patients, 98.3% were diagnosed with adenocarcinoma and 94.9% had an Eastern Cooperative Oncology Performance Status (ECOG-PS) score of 0 or 1. Nineteen of the patients had brain metastases and 94.9% were at stage IV at baseline. Thirty-four patients harbored an EGFR exon 19 deletion mutation and the remaining 25 patients harbored an EGFR exon 21 L858R mutation. Using ddPCR, T790M mutation in the ctDNA samples from all 59 patients was assessed before treatment. Based on the ddPCR results, the patients were stratified into two groups: 17 patients had an EGFR-activating mutation with low-frequency de novo T790M mutation and the other 42 patients were T790M-negative pretreatment. Most of the baseline clinicopathological features such as gender, age, ECOG-PS score, smoking status, stage, and brain metastases were similar between the two groups. However, the composition of the EGFR-activating mutation differed between the groups. As shown in Table 1, the low-frequency de novo T790M mutation may coexist with EGFR 19del mutation (p=0.020).

**Low-frequency de novo T790M mutation is not rare in NSCLC patients with EGFR-activating mutation**

T790M mutation in the ctDNA samples from all 59 patients was evaluated with ddPCR before treatment. The results showed that the overall incidence of low-frequency de novo T790M mutation was 28.81% (17/59). The MAF for T790M mutation ranged from 0.01% to 0.28%, with an ultra-low allele frequency between 0.01% and 0.11% in approximately 88.24% (15/17) of patients (Figure 1).

**MAF of plasma T790M mutation showed an upward trend during treatment with first-generation EGFR-TKIs**

Serial plasma samples were collected every 2 or 3 months during treatment with first-generation EGFR-TKIs, although plasma samples could not be collected at every visit for all patients. A total of 214 plasma samples were collected for T790M detection using ddPCR, among which 59 plasma samples were obtained pretreatment and 155 plasma samples were obtained during the first-generation EGFR-TKI treatment. A downward trend in the MAF of T790M was found in most patients with a low-frequency de novo T790M mutation at the early stage of EGFR-TKI treatment, but an upward trend was observed when the tumor progressed. After disease progression, nine patients retained T790M mutation in their ctDNA, which was detected by ddPCR. As shown in Figure 2, the MAF of T790M resistance mutation ranged from 0.04% to 1.75%. Furthermore, in the group without de novo T790M mutation pretreatment, the MAF of T790M was negative at the early stage of EGFR-TKI treatment. However, the MAF of T790M in most patients without de novo T790M mutation pretreatment also showed an upward trend over the course of
tumor development. ddPCR results showed T790M mutation in nine patients after disease progression and the MAF for T790M resistance mutation ranged from 0.034% to 12.9% (Figure 3).

**Most cases of T790M resistance mutation emerge due to the selection of the low-frequency de novo T790M clone**

In this study, EGFR T790M mutation in the plasma ctDNA of the patients was monitored with ddPCR every 2 to 3 months until disease progression during treatment with first-generation EGFR-TKIs. In the group with the low-frequency de novo T790M mutation, 15 patients exhibited disease progression and 66.67% (10/15) of them retained the T790M mutation, which was detected with NGS in biopsy specimens from one patient and with ddPCR in ctDNA samples from nine patients. The resistance model for the above-mentioned cases was defined as “selection” resistance. In the group without T790M pretreatment, 26 patients exhibited disease progression and 42.3% (11/26) of them were found to have developed T790M resistance mutation. T790M mutation was detected in ctDNA samples from nine of these patients using ddPCR and in biopsy specimens from the other two patients using ARMS-PCR. The resistance model for these patients was defined as “acquisition” resistance. Taken together, the probability of T790M mutation was 51.22% (21/41) in all patients after disease progression (Figure 4).

**There may be no difference in the occurrence time of T790M resistance mutation with “selection” and “acquisition” origins in clinical practice**

As shown in Figure 5, among the patients with the T790M resistance mutation after disease progression during the first-generation EGFR-TKI treatment, those with low-frequency de novo T790M mutation had a median PFS1 of 11.0 months (95% confidence interval [CI], 9.141-12.859 months). Patients without the T790M mutation before treatment had a median PFS1 of 11.5 months (95% CI, 9.989-13.011 months; p = 0.649). The median PSF1 for both groups was similar, suggesting there may be no difference in the occurrence time of T790M resistance mutation between patients with “selection” and “acquisition” resistance mutations in clinical practice.

**Low-frequency de novo T790M mutation may not affect the efficacy of first-generation EGFR-TKIs**

The median follow-up period for the patients in this study was 21.3 months (range, 1.2-36.2 months). At the end of the follow-up period (Mar 23, 2020), 88.2% (15/17) of patients with a low-frequency de novo T790M mutation had a median PFS2 of 12.0 months (95% CI, 9.781-14.219 months), which was comparable to that of patients without pretreatment T790M (median PFS2, 11.4 months; 95% CI, 9.342-13.458 months; p = 0.451).

**The “selection” model of T790M resistance mutation may be treated more effectively with third-generation EGFR-TKIs**
In the group of patients with the low-frequency de novo T790M mutation, 10 patients retained the T790M mutation after disease progression. Seven of these patients were further treated with Osimertinib. The optimal efficacy in this group was estimated and indicated a partial response (PR) in six patients (85.7%) and stable disease (SD) in one patient (14.3%), with an ORR of 85.7%. In this group, four patients experienced disease progression at the end of the follow-up period and the PFS3 for five patients was more than 8 months. In the group of patients without T790M mutation before treatment, nine patients further received Osimertinib when T790M mutation was discovered after disease progression. The optimal efficacy in this group was estimated as PR in two (25.0%) patients and SD in six (75.0%) patients, with an ORR of 25.0%. Six patients experienced disease progression at the end of the follow-up period and the PFS3 for five patients was less than 6 months in this group (Figure 7,8).

**Discussion**

EGFR T790M mutation has been widely studied and is commonly recognized as a drug resistance mutation after treatment with first or second-generation EGFR-TKIs [25-28]. However, the understanding of de novo T790M mutation in patients with advanced NSCLC before EGFR-TKI treatment is insufficient. Increasing evidence suggests that de novo T790M mutation exists in NSCLC patients with EGFR-activating mutations. Due to the differences in detection methods and samples, the occurrence probability of de novo T790M mutation was variable in previous reports [13, 14, 16-22]. ddPCR is the most sensitive molecular detection method for de novo T790M mutation at present and its analytical sensitivity is close to 0.001% [14, 29]. Masaru et al [21] used ddPCR to assess T790M mutation and found de novo T790M mutation in 79.9% (298/373 cases) of surgically resected tumor specimens from patients with EGFR-activating mutations. The MAF of de novo T790M mutation in these patients ranged from 0.009% to 26.9%, with an ultra-low allele frequency between 0.01% and 0.1% in approximately 80% of cases. However, there are few reports on the detection rate for de novo T790M mutation in plasma samples from patients using ddPCR. Hence, we evaluated the occurrence of de novo T790M mutation in the plasma ctDNA of advanced NSCLC patients with EGFR-activating mutations using ddPCR. The results showed that the overall incidence of de novo T790M mutation was 28.81%, with a MAF ranging from 0.01% to 0.28% and an ultra-low allele frequency between 0.01% and 0.11% in approximately 88.24% of cases. The difference in the prevalence of de novo T790M mutation between our results and other studies can be attributed to differences in the samples and the limited size of the tested populations. However, the MAF of de novo T790M mutation in our study was similar to those in previous reports.

The most common EGFR-activating mutations are EGFR 19del and 21L858R [5], but the probability of pretreatment de novo T790M that coexists with 19del or 21L858R remains controversial. Several reports showed that the de novo T790M mutation likely coexists with EGFR 21L858R mutation [24, 30-34]. However, in our study, we found that de novo T790M was more likely to coexist with 19del mutation, which was consistent with the report by Julie A et al [14]. Potential explanations for the differences between our results and other reports include our small sample size and inconsistent detection methods for T790M mutation. Therefore, further studies are needed to understand the differences in the de novo T790M mutation between EGFR 19del and 21L858R mutations in NSCLC.
Although the existence of de novo T790M has been recognized, the effect of the mutation on the efficacy of first-generation EGFR-TKIs was inconsistent in previous reports. In our study, patients with and without de novo T790M mutation had a PFS of 12.0 vs 11.4 months after treatment with first-generation EGFR-TKIs, respectively (p = 0.451). Linda Ye et al [23] reported similar results. In previous reports [13, 16, 20, 35], EGFR-activating mutant NSCLC patients harboring the de novo T790M mutation who received first-generation EGFR-TKI treatment had a shorter PFS than those without de novo T790M. Two meta-analysis studies also support these observations [36, 37]. On the contrary, several reports suggested that patients with de novo T790M mutation had a longer median PFS than T790M-negative patients [38-40]. Yoshihiko et al [18] evaluated patients with strongly positive T790M mutation signals and those with modestly positive signals and found that the former had a longer PFS (p=0.0019), suggesting that the MAF of de novo T790M mutation may impact the effect of EGFR-TKIs. Julie A et al [14] detected de novo T790M using ddPCR and defined a specific threshold of 0.1% for the MAF, which separated patients with longer PFS from those with EGFR-activating mutations (p=0.029). Therefore, it is necessary to establish a threshold for low-frequency de novo T790M mutation in future clinical practice in order to determine the prognostic value of de novo T790M for NSCLC patients with EGFR-activating mutation.

There is a two-model hypothesis (i.e. the “selection” and “acquisition” resistance models) for the origin of T790M resistance clones during disease progression after first-generation EGFR-TKIs treatment. However, there is little direct clinical evidence to support the existence of tumor sub-clones before treatment as the main mechanism for the eventual evolution of drug-resistant clones. In our study, 66.67% (10/15) of patients in the group with low-frequency de novo T790M retained the T790M mutation after disease progression, while 42.31% (11/26) of patients without the T790M mutation before treatment developed the T790M resistance mutation after disease progression. In other words, most of the T790M resistance mutations developed due to the evolution of a T790M sub-clone that existed pretreatment and the low-frequency de novo T790M mutation may be primarily responsible for the subsequent development of the T790M resistance clone.

As described above, the T790M resistance clone can develop from two pathways, namely the selection of a pre-existing EGFR T790M-positive clone or via the evolution of drug-tolerant cells (DTCs) that are initially EGFR T790M-negative [10, 41]. Previous studies showed that the time of development for drug-induced T790M resistance clones was different in the two models. The “acquisition” of T790M resistance clone requires a longer drug-induced period because the tumor progresses through a drug-tolerant phase and later evolves into a drug-resistant state [10, 12, 41, 42]. However, the clinical evidence is insufficient to show the difference in the occurrence time of T790M resistance mutation. In all of the patients with the T790M resistance mutation when the disease progressed, we found no differences in the PFS after treatment with first-generation EGFR-TKIs between the patients with and without the de novo T790M mutation (median PFS, 11.0 vs. 11.5 months, respectively; p = 0.649). Therefore, our clinical data did not confirm the existence of a difference in the emergence of the T790M drug-resistant clone in the two models and further investigations with laboratory models and clinical data are required.
The “acquisition” model of T790M resistance evolves from T790M-negative DTCs after the drug-tolerant stage [10, 12, 41, 42]. This evolutionary process makes the tumor cells more heterogeneous, resulting in less sensitivity in the “acquisition” T790M resistance clone to the follow-up drugs. In-vitro studies showed that the acquisition of T790M resistance from DTCs leads to a low level of apoptosis, which can be increased by the over-expression of Bcl-2-like protein 11 (BIM) [10, 43, 44]. Our study showed similar results, as patients with the “selection” T790M resistance mutation had better ORR to Osimertinib than those with the “acquisition” T790M resistance mutation (ORR, 85.7% vs 25.0%). Although we found no statistically significant difference between the PFS in the two groups due to the small sample size in our study, the PFS of most patients with the “acquisition” T790M resistance mutation was less than 6 months after Osimertinib treatment. This suggests that the origin of the T790M resistance mutation may affect the efficacy of third-generation EGFR-TKIs and that the low-frequency de novo T790M mutation is a good prognostic marker for treatment with third-generation EGFR-TKIs among patients with T790M resistance mutation.

Although EGFR-TKIs benefit the survival of patients with EGFR-activating mutations, more individual and detailed management of patients is still required. It remains unclear whether dynamic detection of ctDNA T790M is necessary before and during treatment with first-generation EGFR-TKIs. In our study, most of the MAF for T790M mutation in patients with and without de novo T790M mutation showed an upward trend during tumor progression but had no regularity. D. Zheng et al [45] reported that T790M ctDNA-positivity was confirmed in some patients at a median of 2.2 months prior to clinical disease progression following treatment with first-generation EGFR-TKIs. However, there is no clinical evidence to support the survival benefits of intervention with third-generation EGFR-TKIs in advance for patients with ctDNA T790M positivity or those with an increasing MAF. The AZD9291 (Osimertinib) Treatment on Positive Plasma T790M in EGFR-mutant NSCLC Patients (APPLE) trial assessed the dynamic predictive value of liquid biopsies to confirm the appropriate timing for Osimertinib administration to patients with either positive ctDNA T790M or disease progression according to RECIST [46]. However, the results of this trial have not been announced. The detection of T790M in serial plasma samples may be used to predict the progression of tumors and reverse resistance to first-generation EGFR-TKIs ahead of time. Osimertinib has been recommended as one of the first-line treatment options for NSCLC patients with EGFR-activating mutations. However, due to the potency ratio and other factors, only some patients are eligible for third-generation EGFR-TKIs as the first-line treatment. In NSCLC patients with both EGFR-activating mutation and de novo T790M, better curative effects can be achieved by eliminating the secondary mutation and reducing tumor heterogeneity at the beginning of tumor treatment if the third generation of EGFR-TKIs can be used as the first-line treatment. For example, the AZENT trial (ClinicalTrials.gov, NCT02841579) was performed to evaluate the efficacy and safety of Osimertinib as a first-line treatment for advanced NSCLC patients with de novo T790M mutation, indicating the importance of de novo T790M in tumor therapy.

Conclusion
Low-frequency de novo T790M mutation is common in patients with EGFR-activating mutant NSCLC. The use of ultra-sensitive detection methods such as ddPCR to evaluate de novo T790M mutation is of clinical value for the individualized management of advanced NSCLC patients with EGFR-activating mutation because of the role of this mutation in not only predicting the prognosis of patients but also in the choice of treatment strategies. As previously reported in the literature, de novo T790M mutation may have an effect on the treatment efficacy of first-generation EGFR-TKIs. In order to achieve better efficacy, third-generation EGFR-TKIs may be selected as the first-line treatment for advanced NSCLC patients with both EGFR-activating mutations and de novo T790M mutation. Furthermore, the origin of the T790M resistance mutation might affect the efficacy of third-generation EGFR-TKIs in patients with “acquisition” T790M resistance mutation because of a reduced apoptotic response in the tumor cells to third-generation EGFR-TKIs. Therefore, the combination of third-generation EGFR-TKIs with anti-apoptosis inhibitors may be a good approach in clinical practice.

Abbreviations

epidermal growth factor receptor (EGFR), non-small cell lung cancer (NSCLC), tyrosine kinase inhibitors (TKIs), objective response rate (ORR), progression-free survival (PFS), mutant allele frequency (MAF), insulin-like growth factor 1 receptor (IGF-1R), nuclear factor kappa B (NF-kB), polymerase chain reaction (PCR), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), peptide nucleic acid (PNA), amplified refractory mutation system (ARMS), droplet digital PCR (ddPCR), circulating tumor DNA (ctDNA), next generation sequencing (NGS), once a day (QD), three times a day (TID), Eastern Cooperative Oncology Performance Status (ECOG-PS), partial response (PR), stable disease (SD), drug-tolerant cells (DTCs).

Declarations

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Ethical Review Committee of Fujian Provincial Cancer Hospital (approval no.2017-069-01).

Consent for publication

With the consent for publication for all authors.

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.
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Authors' contributions

Li Meifang carried out data curation, formal analysis, investigation, methodology, software, visualization and writing-original draft. Lin Jinghui participated in data curation, methodology and resources. Zhang Jing participated in methodology and resources. Huang Yunjian participated in methodology and resources. Chen Shengchi participated in methodology and resources. Wang Yan participated in methodology and resources. He Zhiyong carried out conceptualization, data curation, funding acquisition, project administration, supervision and writing-review & editing.

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Tables

**Table 1.** Clinicopathological characteristics of the patients with EGFR mutation
| Characteristics       | Total subjects (n=59) | EGFR+/T790M+ (n=17) | EGFR+/T790M- (n=42) | P-value |
|-----------------------|-----------------------|---------------------|---------------------|---------|
|                       | No. of patients | %    | No. of patients | %    |         |
| Gender                |                       |                    |                     |         |
| Male                  | 28                    | 8                  | 28.6                | 20      | 71.4    | 1.000   |
| Female                | 31                    | 9                  | 29.0                | 22      | 71.0    |         |
| Age (years)           |                       |                    |                     |         |
| >60                   | 25                    | 9                  | 36.0                | 16      | 64.0    | 0.386   |
| ≤60                   | 34                    | 8                  | 23.5                | 26      | 76.5    |         |
| ECOG-PS score         |                       |                    |                     |         |
| 0-1                   | 56                    | 15                 | 26.8                | 41      | 73.2    | 0.197   |
| ≥ 2                   | 3                     | 2                  | 66.7                | 1       | 33.3    |         |
| Smoking status        |                       |                    |                     |         |
| Yes                   | 10                    | 2                  | 20.0                | 8       | 80.0    | 0.708   |
| No                    | 49                    | 15                 | 30.6                | 34      | 69.4    |         |
| Brain metastases      |                       |                    |                     |         |
| Yes                   | 19                    | 6                  | 31.6                | 13      | 68.4    | 0.766   |
| No                    | 40                    | 11                 | 27.5                | 29      | 72.5    |         |
| Stage                 |                       |                    |                     |         |
| A                     | 19                    | 8                  | 42.1                | 11      | 57.9    | 0.781   |
| B                     | 40                    | 9                  | 22.5                | 31      | 77.5    |         |
| Histology             |                       |                    |                     |         |
| Adenocarcinoma        | 58                    | 17                 | 29.3                | 41      | 70.7    | 1.000   |
| Adenosquamous         | 1                     | 0                  | 0                   | 1       | 100     |         |
| EGFR mutations        |                       |                    |                     |         |
| 19del                 | 34                    | 14                 | 41.2                | 20      | 58.8    | 0.020   |
| 21L858R               | 25                    | 3                  | 12.0                | 22      | 88.0    |         |

**Figures**
Detection of EGFR T790M mutation alleles in ctDNA samples from pretreatment NSCLC patients with EGFR-activating mutation. Analysis of the frequency of alleles in 17 NSCLC patients with de novo T790M mutation showed the MAF ranged from 0.01% to 0.28%.

Monitoring plasma T790M mutation in patients with low-frequency de novo T790M mutation during treatment with first-generation EGFR-TKIs. An upward trend in the MAF of T790M mutation was observed in nine patients after tumor progression.
Figure 3

Monitoring plasma T790M mutation during first-generation EGFR-TKI treatment in pre-treatment T790M-negative patients. The MAF of T790M mutation in nine patients over the course of tumor development showed an upward trend.

Figure 4

Process flowchart for monitoring the EGFR T790M mutation in patients during EGFR-TKI treatment.
Figure 5

Kaplan-Meier curves for PFS1 in patients with T790M resistance mutation after disease progression during first-generation EGFR-TKI treatment. Comparison of the median PFS1 between the patients with low-frequency de novo T790M mutation and those without pretreatment T790M mutation did not show a significant difference between the two groups (11.0 months VS. 11.5 months; p = 0.649).

Figure 6
Kaplan-Meier curves for PFS2 for advanced EGFR-activating NSCLC patients with and without de novo T790M mutation during first-generation EGFR-TKI treatment. The median PFS2 was comparable between the patients with and without low-frequency de novo T790M mutation before treatment (12.0 months VS. 11.4 months, respectively; \( p = 0.451 \)).

Figure 7

The efficacy of the third-generation EGFR-TKI for patients with “selection” and “acquisition” T790M resistance mutations. The red histogram represents the patients with the “selection” T790M resistance mutation, among which the ORR was 85.7%. The yellow histogram represents the patients with the “acquisition” T790M resistance mutation and their ORR was 25.0%. (partial response=PR, stable disease=SD, objective response rate=ORR)

Figure 8

PFS3 after third-generation EGFR-TKI treatment for patients with “selection” and “acquisition” T790M resistance mutations. The red histogram represents the patients with the “selection” T790M resistance
mutation; the PFS3 for five patients was more than 8 months in this group. The yellow histogram represents the patients with the “acquisition” T790M resistance mutation; the PFS3 for five patients was less than 6 months in this group.