



Association between *EGFR* gene mutant protein expression and T790M mutation after first-generation EGFR-TKI treatment resistance: a retrospective, single-arm clinical study

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Background: Epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) is an important treatment for lung adenocarcinoma patients with *EGFR* gene mutations. The purpose of this study was to review the efficacy of first-generation EGFR-TKIs and the incidence of T790M after first-generation TKI resistance in stage IV lung adenocarcinoma patients with positive *EGFR* gene mutation expression associated with EGFR mutant protein.

Methods: Tumor tissues were collected from stage IV lung adenocarcinoma patients with *EGFR* gene mutation who received first-generation EGFR-TKI targeted therapy. Patients were followed up through outpatient and inpatient systems. Immunohistochemistry was used to detect the expression of corresponding EGFR mutant protein in tumor tissues. The incidence of T790M mutation after first-generation TKI resistance and the correlation between the mutant protein and progression-free survival (PFS) after first-generation TKI treatment were investigated.

Results: T790M mutation rates were 37.93% (11/29) and 42.50% (17/40) in the *EGFR* mutation groups, respectively, after first-generation TKI treatment for drug resistance. In patients with exon 19 deletion, T790M mutations were found in 63.64% (7/11) of patients with positive protein expression and 22.22% (4/18) of patients with negative protein expression ($P=0.026$; $\chi^2=4.974$). The mutation rate of T790M after drug resistance in patients with L858R mutation was 53.57% (15/28) and 16.67% (2/12) in the protein expression-positive and negative groups, respectively ($\chi^2=4.682$, $P=0.030$). The variations were statistically significant.

Conclusions: After resistance to the first-generation EGFR-TKI treatment, the occurrence of T790M mutation may be related to the expression of EGFR mutant protein in patients with *EGFR* gene mutation.

Keywords: Lung adenocarcinoma; epidermal growth factor receptor (*EGFR*); EGFR-tyrosine kinase inhibitor (EGFR-TKI); T790M mutation

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Introduction

With the development of precision medicine, gene detection is more and more widely used. Targeted therapy has become an important treatment method for patients with advanced lung cancer (1). Driver gene variation can be detected in the tumor tissue of most patients with lung adenocarcinoma, including epidermal growth factor receptor (*EGFR*) mutation, anaplastic lymphoma kinase (*ALK*) rearrangement (2), *ROS-1* rearrangement (3), rearranged during transfection (*RET*) rearrangement, mesenchymal-epithelial transition (*MET*) exon 14 deletion (4), *KRAS* mutation, and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) mutation, among others. Targeted therapy based on these driver genes has greatly improved therapeutic efficacy in patients with advanced lung adenocarcinoma (5).

EGFR is a receptor tyrosine kinase that is widely distributed on the surface of mammalian epithelial cells and is involved in cell proliferation and differentiation. *EGFR* binds to its ligand to form a dimer. It regulates the growth and reproduction of tumor cells by activating downstream signaling pathways through autophosphorylation, including the mitogen-activated protein kinase (*MAPK*) pathway and phosphatidylinositol 3-kinase (*PI3K*)/*AKT* pathway, among others (6). Multiple studies have shown that *EGFR* mutations are common in Asian, nonsmoker or mild smoker, female adenocarcinoma patients who may benefit from *EGFR*-tyrosine kinase inhibitor (TKI)-targeted therapy. The 2009 Iressa Pan-Asia Study (IPASS) compared gefitinib with carboplatin/paclitaxel in patients with advanced non-small cell lung cancer. It showed that gefitinib had significantly longer progress-free survival than carboplatin/paclitaxel and confirmed that *EGFR* gene mutations could be used as biomarkers to evaluate gefitinib efficacy (7). *EGFR*-TKI is approved for advanced non-small cell lung cancer with *EGFR* mutations.

EGFR-TKIs are an important treatment option for patients with *EGFR*-mutated lung adenocarcinoma. However, most patients eventually develop acquired resistance after 1 year, including T790M mutation (8), *MET* amplification, human epidermal growth factor receptor 2 (*HER-2*) amplification (9), and *PIK3CA* mutation (10). T790m mutation is currently known to be a common cause of acquired drug resistance following first-generation TKIs and an effective target for third-generation *EGFR*-TKIs. However, it is unknown which patients will develop the T790M mutation following first-generation TKIs. The

IPASS study systematically compared the predictive value of *EGFR* gene mutations and mutant protein expression for the response to first-generation *EGFR*-TKI treatment. However, it failed to observe the association between the T790M mutation and the above markers after first-generation *EGFR*-TKI resistance. Although third-generation *EGFR*-TKIs are becoming the standard of care for *EGFR* mutation-positive NSCLC patients worldwide, first- or second-generation *EGFR*-TKIs followed by third-generation TKIs may offer longer survival, so predicting T790M positivity is highly suggestive. In this study, we used immunohistochemistry (IHC) to detect the expression of *EGFR* protein in tumor tissues of patients with stage IV lung adenocarcinoma who had a known *EGFR* mutation, analyzed the relationship between protein expression and the efficacy of the first-generation TKI, and investigated the incidence of T790M mutation in patients who developed drug resistance. The aim of this research was to explore the population characteristics of patients with T790M mutation and to try to find markers that could be used to predict T790M resistance after first-generation *EGFR*-TKI treatment. The findings are summarized below. We present the following article in accordance with the REMARK reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-3850/rc>).

Methods

Study subjects

The tumor tissue samples and clinical treatment data of stage IV lung adenocarcinoma patients who visited The First Affiliated Hospital of Anhui Medical University and The Second Affiliated Hospital of Anhui Medical University between January 1, 2018 and March 30, 2021 were analyzed retrospectively in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Commission of the First Affiliated Hospital of Anhui Medical University (PJ2022-10-43). Written informed consent to participate in this study was provided by all participants/next of kin.

The criteria for inclusion were: aged ≥ 18 years old; pathologically confirmed adenocarcinoma; stage IV patients with distant organ metastasis at the time of oral *EGFR*-TKI; tumor tissue samples confirmed by sequencing to have *EGFR* mutations, including exon 19 deletion and L858R point mutation; received first-generation *EGFR*-TKI

targeted therapy for at least 2 months, including gefitinib, icotinib, and erlotinib; with RECIST 1.1-compliant target lesions; and Eastern Cooperative Oncology Group (ECOG) score 0–1.

The exclusion criteria were: second and third-generation EGFR-TKIs after first-line EGFR-TKI therapy; patients with other malignancies; patients with *EGFR* non-19 exon deletion or L858R point mutation; and patients whose efficacy could not be determined (for example, due to no measurable lesions, lack of imaging data, and loss to follow-up).

Immunohistochemical assays

The Department of Pathology reviewed all tumor tissue samples, and the proportion of tumor cells met the quality control criteria. After quality control, the content of tumor cells in the submitted samples shall not be lower than the detection standard (detection standard: proportion of tumor cells $\geq 20\%$, number of tumor cells ≥ 100). Protein antibody against *EGFR* 19 exon deletion (E746-A750del) and *EGFR* 21 exon mutation (L858R) were rabbit monoclonal antibodies (clone numbers SP111, SP125; Fuzhou Maixin Biotechnology Development Co., Ltd., Beijing, China), and secondary antibodies were enzyme-labeled goat anti-mouse/rabbit immunoglobulin G (IgG) polymers (Fuzhou Maixin Biotechnology Development Co., Ltd.). The mouse/rabbit MaxVision-HRP kit was used. Paraffin sections (4 μm) were cut, routinely deparaffinized, hydrated, and repaired at high temperature using ethylenediaminetetraacetic acid (EDTA) antigen retrieval solution. Blocking agent was added to block endogenous peroxidase, and primary antibody reagent was incubated overnight at 4 °C. The samples were rinsed, incubated with secondary antibody reagent at 37 °C for 15 minutes, developed by 3,3'-diaminobenzidine (DAB), counterstained with hematoxylin, rinsed, and dehydrated with gradient ethanol.

Evaluation of immunohistochemical results

The protein expressions were evaluated after the NSCLC was diagnosed. The immunohistochemical results were judged independently by two pathologists. The presence of yellow or brownish-yellow staining in the membrane/cytoplasm of the tumor tissue without background staining was considered positive, and the absence of any yellow or brownish-yellow staining in the tumor tissue was considered negative.

Evaluation of efficacy

Patients who met all of the inclusion and exclusion criteria had their medical histories collected retrospectively, including information on the use of first-generation TKIs, progression-free survival (PFS) for first-generation TKIs, and T790M mutation detection results in peripheral blood by digital polymerase chain reaction (PCR) or Super-ARMS after first-generation TKI resistance. The time from the first dose to radiographic progression was defined as PFS. The progression refers to the minimum sum of diameters of all target lesions measured in the whole treatment process as a reference, and the relative increase of diameters and diameters should be at least 20%, and the absolute increase of diameters and diameters should be at least 5 mm. If one or more new lesions appear, the disease progression is also considered.

Statistical analysis

The statistical software packages SPSS for Windows, software version 25.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 8.0 (GraphPad Software, San Diego, CA, USA) were used for analysis. Enumeration data were statistically described by relative number (percentage), and the χ^2 test was used for comparison. The Kaplan-Meier method was used to calculate PFS curves, and differences were compared using the log-rank test, with $P < 0.05$ indicating a statistically significant difference.

Role of the funding source

The funding agencies did not participate in study design, data collection, data analysis, or manuscript writing. The corresponding authors were responsible for all aspects of the study to ensure that issues related to the accuracy or integrity of any part of the work were properly investigated and resolved. The final version was approved by all authors.

Results

Basic characteristics of the study population

This study included 69 stage IV lung adenocarcinoma patients (aged 40–81 years, median age 67 years) who met the inclusion criteria, had complete medical history data, and had peripheral blood T790M detection after drug resistance (Table 1).

Table 1 Baseline characteristics of 69 patients with stage IV lung adenocarcinoma

Clinical data	19 exon deletion	L858R point mutation	χ^2 value	P value
Gender			2.077	0.150
Male	12	10		
Female	17	30		
Age (years)			0.960	0.327
≤ 65	12	12		
> 65	17	28		
Medicine				
Gefitinib	13	24	1.556	0.212
Icotinib	12	11	1.457	0.227
Erlotinib	4	5	0.025	0.875
Protein expression of 19del/L858R when diagnosed as NSCLC			7.035	0.008
Positive	11	28		
Negative	18	12		
T790M expression after TKI resistance			0.146	0.703
Yes	11	17		
No	18	23		

NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor.

Protein expression

There were no statistically significant differences in efficacy between the groups with positive and negative protein expression. The median PFS (mPFS) of all patients with exon 19 deletion was 13.20 months (95% CI: 11.18–15.23 months), including 13.20 months (95% CI: 10.45–15.95 months) in the protein expression-positive group and 13.00 months (95% CI: 2.67–23.33 months) in the protein expression-negative group, with no statistically significant difference, $\chi^2=0.405$, $P=0.817$ (Figure 1). The mPFS of all patients with L858R point mutation was 12.13 months (95% CI: 8.10–16.16 months), including 12.13 months (95% CI: 7.50–16.76 months) in the protein expression-positive group and 11.93 months (95% CI: 9.48–14.39 months) in the protein expression-negative group, with no statistically significant difference, $\chi^2=0.191$, $P=0.909$ (Figure 2).

T790M mutation and protein expression

We analyzed the T790M mutation in peripheral blood after resistance to first-generation EGFR-TKI treatment, and the

results showed that the protein expression-positive group was more likely to produce the T790M mutation after resistance, and the difference was statistically significant. In patients with exon 19 deletion, T790M mutation developed in 7 (63.64%, 7/11) patients in the positive protein expression group and 4 (22.22%, 4/18) in the negative protein expression group ($\chi^2=4.974$, $P=0.026$). In patients with L858R point mutation, there were 15 (53.57%, 15/28) and 2 (16.67%, 2/12) in the positive and negative protein expression groups, respectively ($\chi^2=4.682$, $P=0.030$) (Figure 3).

Discussion

Lung cancer is a deadly malignant tumor that affects millions of people worldwide. A common type of driver gene mutation in lung adenocarcinoma is the *EGFR* gene mutation. Exon 19 deletion and exon 21 L858R point mutation are 2 common *EGFR* effective mutations (11). The IPASS study published in 2009 found that gefitinib treatment for patients with *EGFR* gene mutations resulted in significantly longer PFS time than chemotherapy (7).

EGFR gene mutation, gene copy number, and protein

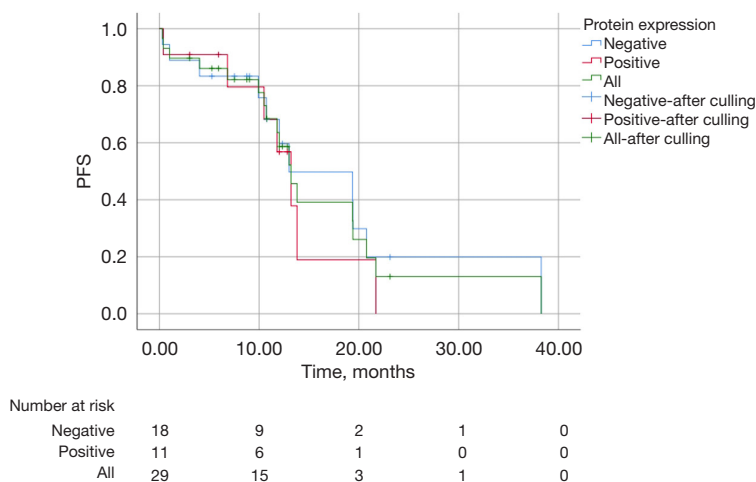


Figure 1 Protein expression and efficacy of exon 19 deletion mutation in stage IV lung adenocarcinoma patients. PFS, progression-free survival.

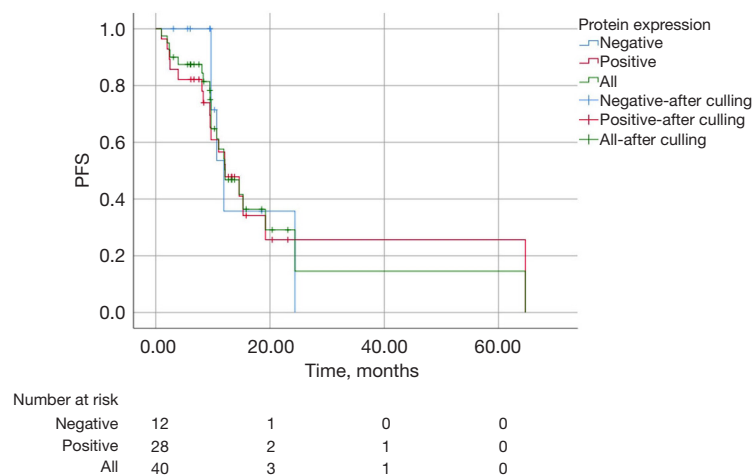


Figure 2 Protein expression and efficacy of L858R point mutation in stage IV lung adenocarcinoma patients. PFS, progression-free survival.

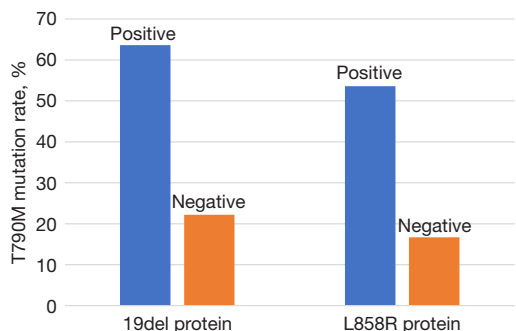


Figure 3 T790M mutation rate after first-generation TKI resistance. TKI, tyrosine kinase inhibitor.

expression are all common biomarkers used to predict the efficacy of EGFR-TKIs, and they frequently overlap (12). The relationship between the 3 biomarkers and the efficacy of targeted therapy was investigated in the IPASS study’s biomarker analysis. The results showed that in patients with a positive *EGFR* gene mutation, gefitinib significantly prolonged PFS and increased overall response rate (ORR) compared to chemotherapy. In the protein expression analysis, the gefitinib group had PFS in patients with positive protein expression, but there was no significant difference in ORR and overall survival (OS) between the two groups (13). As a result, *EGFR* mutations distinguished

the benefit population more effectively than EGFR protein expression, establishing *EGFR* mutations as predictive markers of response to TKI therapy.

This study re-examined the relationship between protein expression and the efficacy of targeted therapy in stage IV lung adenocarcinoma patients with known *EGFR* gene mutations. Among 69 patients with *EGFR* mutations, 39 had corresponding protein expression detected. The mPFS was similar in the protein expression-positive and negative groups, showing no statistically significant difference. This was consistent with the findings of the IPASS study, which found that protein expression had little predictive value for the efficacy of targeted therapy. In a retrospective study of patients with advanced non-small cell lung cancer (NSCLC) treated with EGFR-TKI, patients with exon 19 deletion had better clinical benefit than those with exon 21 mutation, with PFS (13.2 vs. 10.8 months, $\chi^2=4.700$, $P=0.030$) and OS of 30.2 and 25.6 months, respectively, in the two groups (14). It has been proposed that this difference may be related to structural changes caused by different mutations and subsequent T790M mutations (15). In this study, patients with exon 19 deletion had a slightly longer mPFS than patients with the L858R point mutation (13.20 vs. 12.13 months).

The mPFS with targeted first-generation EGFR-TKIs is now estimated to be 10–14 months (16). There are diverse mechanisms of subsequently acquired resistance, including T790M mutation, *c-MET* amplification (17), *HER-2* amplification (18), and *PIK3CA* mutation (19), with the most common being the T790M mutation, which accounts for more than half of acquired resistance (20). In the phase III AURA3 study, PFS was significantly longer in the osimertinib group than in the pemetrexed/platinum group (10.10 vs. 4.40 months, hazard ratio 0.30, 95% CI: 0.23–0.41, $P<0.001$). In addition, the ORR was also significantly better than in the chemotherapy group (71.00% vs. 31.00%, odds ratio 5.39, 95% CI: 3.47–8.48, $P<0.001$) (21), suggesting that osimertinib was more effective than platinum-based combination chemotherapy for patients with NSCLC who have a T790M mutation and have progressed after first-generation EGFR-TKI therapy. For patients with NSCLC who have the T790M resistance mutation, third-generation TKIs are currently recommended (22).

Furthermore, the OS time for patients with the T790M mutation treated with first-generation or second-generation EGFR-TKIs followed by third-generation TKIs can be up to 40 months. The treatment time with EGFR-TKIs was 27.6 months (90% CI: 25.9–31.3 months) in a real-world study (NCT03370770) of afatinib and osimertinib

in the sequential treatment of patients with *EGFR*-mutated lung cancer, and it is worth noting that the treatment time was 30.3 months for patients with exon 19 deletions and 46.7 months for Asian patients (23). This study investigated protein expression and T790M mutations in the peripheral blood of patients with subsequent drug resistance and discovered that patients with *EGFR* mutations and protein expression were more likely to have T790M mutations. A greater proportion of T790M mutations occurred in the positive protein expression group compared to the negative protein expression group (63.64% vs. 22.22%, Figure 2; $\chi^2=4.974$, $P=0.026$). In patients with L858R point mutations, the T790M mutation rates were 53.57% and 16.67% in the positive and negative protein expression groups, respectively ($\chi^2=4.682$, $P=0.030$). Although the IPASS study found that protein expression had little predictive value for the efficacy of targeted therapy, the T790M mutation was not investigated. Suppose patients with an *EGFR* gene mutation and protein expression are more likely to have a T790M mutation. In that case, after patients became resistant to first-generation or second-generation EGFR-TKI, patients with T790M mutation showed significantly better efficacy with third-generation EGFR-TKI than those without T790M mutation. The patients with *EGFR* gene mutations and protein expression were more likely to have T790M mutations after first-generation EGFR-TKI treatment progression. As a result, EGFR protein expression could be used to predict the efficacy of third-generation TKIs and select the candidate to receive afatinib-osimertinib sequential therapy. However, because this was a retrospective study with a small sample size and potentially skewed results, additional prospective and multicenter studies are required for validation.

Conclusions

The efficacy of EGFR-TKI as first-line treatment for lung adenocarcinoma with *EGFR* gene mutation is not related to protein expression, similar to the results from IPASS study. However, this study discovered that patients with *EGFR* gene mutations and protein expression were more likely to have T790M mutations after first-generation EGFR-TKI treatment progression, and these patients benefited from third-generation TKI treatment.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-3850/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Commission of the First Affiliated Hospital of Anhui Medical University (PJ2022-10-43). Written informed consent to participate in this study was provided by all participants/next of kin.

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