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ORIGINAL RESEARCH

# Differential Responses to Targeted Therapies in Non-Small Cell Lung Cancer: A Comparative Analysis of Outcomes in Patients with Single EGFR Mutation and Concurrent Gene Alterations

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**Background:** Epidermal growth factor receptor tyrosine kinase inhibitors (*EGFR-TKIs*) improve the quality of life in individuals with EGFR mutation-positive non-small cell lung cancer (NSCLC). This study evaluates the treatment outcomes of *EGFR-mutant* NSCLC patients with concurrent gene alterations, aiming to determine their predictive significance concerning responses to *EGFR-TKI* therapy.

**Materials and Methods:** We conducted a retrospective cohort study using next-generation sequencing (NGS) data from January 2019 to June 2023. Patients were categorized into two groups: those with a single *EGFR* mutation (Group 1) and those with concurrent *EGFR* mutations (Group 2).

**Results:** Among 109 patients with EGFR mutations, 72 showed partial responses (66.1%), one had a complete response (0.9%), and 17 had stable disease (15.6%); 19 experienced progressive disease (17.4%). The overall response rate (ORR) was 67%, and the disease control rate (DCR) was 82.6%. Progression-free survival (PFS) was 15.03 months (95% CI: 13.17–16.89) in the single EGFR mutation group and 11.00 months (95% CI: 9.95–12.05) in the concurrent mutations group (P = 0.001). Among 43 patients with concurrent mutations, those with *ALK* mutations had the longest PFS (13.43 months), followed by *PIK3CA* (11.00 months), while *MET* alterations showed the shortest PFS (4.77 months).

**Conclusion:** Concurrent gene alterations in *EGFR-mutant* NSCLC are associated with reduced efficacy of *EGFR-TKIs*. Patients with *KRAS, BRAF, ROS1*, or *MET* mutations have poorer predictive outcomes compared to those without these alterations.

**Keywords:** *EGFR*, NSCLC, concurrent mutations, targeted therapy, treatment outcomes

## Introduction

Epidermal growth factor receptor (*EGFR*) mutations are present in approximately 40% to 50% of East Asian patients with lung adenocarcinoma.[1] *EGFR* tyrosine kinase inhibitors (*EGFR-TKIs*), including erlotinib, gefitinib, and afatinib, have significantly improved survival rates compared to standard chemotherapy in patients with advanced non-small cell lung cancer (NSCLC) harboring *EGFR* mutations.[2,3] Notably, treatment outcomes do not show significant variance among first- and second-generation *EGFR-TKIs*.[4]

Patients with exon 19 deletions or exon 21 *L858R* mutations typically exhibit a median progression-free survival (PFS) ranging from 9 to 13 months,[5–7] accompanied by objective response rates (ORRs) between 60% and 70%.[8] Despite these promising outcomes, primary resistance to *EGFR-TKIs* remains a substantial challenge, particularly in East Asian

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populations. This phenomenon underscores the complexity of oncogenic signaling pathways and highlights the potential influence of concurrent genetic alterations on therapeutic efficacy.

*EGFR-TKI* treatment demonstrates promising efficacy; however, primary resistance remains prevalent, particularly in the East Asian population.[9] This phenomenon presents a complex clinical scenario, necessitating a reevaluation of the conventional understanding of single-gene driver-oncogene relationships.[10] Recent reports underscore the importance of considering concurrent genetic changes that may contribute significantly to resistance mechanisms, thereby elucidating the marked variability observed in individual patient responses.[11] Coexistent genetic alterations, such as *HER2* amplification, *MET* amplification, *PIK3CA* mutation, and *KRAS* mutation, have been identified as potential contributors to primary resistance for *EGFR-TKI* treatment.[11–13]

However, the clinical implications of *EGFR-TKI* treatment in NSCLC patients harboring coexisting genetic alterations remain largely unknown, and current guidelines provide no specific recommendations for their management. This knowledge gap underscores the pressing need for a comprehensive understanding of the relationship between specific gene co-variation and the efficacy of *EGFR-TKI* treatment.[14–16] Addressing this gap not only enhances our ability to predict clinical outcomes but also empowers healthcare professionals to make informed decisions in tailoring optimal treatment strategies for patients with concurrent mutations, paving the way for more personalized and effective treatment approaches.[17–19] The primary objective of this study was to evaluate the treatment outcome of *EGFR-TKIs* in these specific patient cohorts and to determine the predictive significance of these factors concerning the response to *EGFR-TKI* treatment.

Recent studies have investigated the impact of concurrent gene alterations on *EGFR-TKI* treatment outcomes in NSCLC patients with *EGFR* mutations. These alterations, observed in 6.6% to 78.8% of cases, include mutations in key oncogenes such as *PIK3CA, ALK, HER2, FGFR3*, and *CDKN2A*. (Hu et al, 2017;[20] Chang et al, 2019;[21] Chevallier et al, 2020[11]). The presence of these concurrent mutations is generally associated with shorter PFS compared to patients with isolated EGFR mutations, highlighting their prognostic significance. (Hu et al,[20] 2017; Le Tu Linh et al, 2024;[22] Chevallier et al, 2020[11]).

Notably, specific genetic alterations, such as *FGFR3* mutations and *CDKN2A* copy number loss, have been linked to particularly poor clinical outcomes, underscoring the heterogeneity of resistance mechanisms. (Chang et al, 2019). Moreover, the type of concurrent alteration plays a pivotal role in determining treatment efficacy. For instance, *PIK3CA* mutations are often associated with relatively better outcomes compared to other co-alterations. (Hu et al, 2017; Le Tu Linh et al, 2024). These findings emphasize the critical role of comprehensive genomic profiling in *EGFR-mutant* NSCLC, facilitating the identification of resistance mechanisms and guiding the development of personalized therapeutic strategies. (Chevallier et al, 2020; Chang et al, 2019).[11,20,21]

As the landscape of precision oncology continues to evolve, the integration of molecular diagnostics with targeted therapies is paramount in optimizing NSCLC management. Understanding the complex interplay between concurrent genetic alterations and *EGFR-TKI* responsiveness will be instrumental in refining clinical guidelines and improving patient outcomes in this heterogeneous disease.

# Methods

## Study Design and Patient

A multi-center retrospective cohort study was conducted in two large health centers including National Lung Hospital and National Cancer Hospital from January 2019 to June 2023. The patients who were diagnosed with NSCLC carrying *EGFR 19del* mutation or *EGFR 21L858R* mutation were enrolled. The inclusion criteria were as follows: (I) sensitive *EGFR* mutations detected by next-generation sequencing (NGS) from tumor tissue or liquid biopsy; (II) NSCLC patients receiving initial treatment with gefitinib, erlotinib, or afatinib; (III) their response to treatment evaluated after at least 2 months of supervision. We excluded the patients under three months of follow-up and incomplete medical records.

## Genetic Mutation Analysis

Genetic variant detection was performed using next-generation sequencing (NGS) technology on the BGI sequencing platform. This platform enabled the detection of over 2800 hotspot mutations across 50 lung cancer-related genes. The

BGI system ensures high-throughput sequencing with robust sensitivity and specificity for detecting both single EGFR mutations and concurrent genetic alterations.

## Study Procedures

Hospitalized patients were diagnosed with lung cancer under the guidance of a biopsy of the chest wall, diagnosis of pathological tissue, and determination of mutation status with NGS. The resulting single *EGFR* mutation and *EGFR* concurrent mutations will be treated with targeted first- and second-generation such as Gefitinib, Erlotinib, and Afatinib. These are FDA-approved drugs that the Vietnamese Ministry of Health has approved for use. This process is consistent in all the hospitals we have studied. Collection and analyses of single *EGFR* mutation and concurrent genetic alterations.

We used a standardized classification and case record form to collect data on common variables. Data was entered into the database of the study using the password-protected online case report forms.

## Variables

The data for each study patient were recorded from the same unified data collection tool (case record form-CRF). A case record form was adopted across the study sites to collect the common variables. Data were submitted to the study database by Kobotoolbox software, which was used for simple or programmed data entry and data documentation that could prevent data entry errors or mistakes. Patient identifiers were not entered into the database to protect the patients' confidentiality.

We included variables based on CRF, such as information on:

- We collected data on behavioral history (eg, cigarette smoking), medical history (including comorbidities or pre-existing diseases), demographics (ie, sex, age), social status (eg, health insurance, occupations, highest education levels, annual income), ECOG Performance Status (PS).
- Computed tomography (CT) of head, chest, abdomen and whole-body scintigraphy. The disease status was determined according to the Eight edition of the IASlC TNM staging system.
- Histopathological characteristics of ADC after lung biopsy- Classified the subtype according to the dominant histopathological morphology with diagnostic criteria from the classification of WHO 5th edition - 2021.[23]
- *EGFR* gene mutation status: Rate of *EGFR* mutation detection in the total patients and *EGFR* distribution including: *Deletion* in exon 19, *L858R* in exon 21, other mutations in exons 18–21, concurrent mutations.
- Hospital course and outcomes, including length of hospitalization, discharge status (eg, hospital discharge, transfer to another hospital, "discharged to die" decision in which almost all patients were in grave condition or dying) at the time of discharge, and death in hospital); functional outcomes at 30 and 90 days of treatment for targeted therapy, and follow up to the end of study.

## Outcomes

Progression-Free Survival (PFS) of *EGFR-TKIs* was defined as the duration from the initiation of *EGFR-TKI* treatment to disease progression or death from any cause. Overall survival (OS) is the period from the start of the target treatment to the withdrawal from the study (the day of death due to illness, the day of loss of follow-up, the date of the last surviving medical examination, after which no other information is available, or the death date due to other causes). All patients were regularly monitored during the treatment process to assess clinical response and diagnostic imaging every 8 weeks (or earlier for significant progression appeared). The best clinical response to treatment was evaluated based on the RECIST guidelines (version 1.1) by a fully trained clinician or radiologist of the participating. Objective response rate (ORR) included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Disease Control Rate (DCR) was determined by the sum of objective response and stable disease (CR + PR + SD). The predictive outcomes were determined based on progression-free survival (PFS) and overall survival (OS), classifying them into two distinct categories: favorable and unfavorable outcomes.

## Data Analysis

Survival curves for progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method, from the time of advanced NSCLC targeted treatment to death or last follow-up, and statistical comparisons were conducted using the Log rank test. P values were calculated using Fisher's exact test and Pearson's test for categorical and continuous variables, respectively. Continuous variables and binary variables were compared using the Wilcoxon test. All statistical analyses were performed using SPSS 20.0 software (IBM Corporation, NY, USA). A p-value <0.05 was considered as significant.

## Research Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Hanoi Medical University (No. 912/GCN-HĐĐĐNCYSH-ĐHYHN). The study was approved to collect data from the National Lung Hospital, National Cancer Hospital. All data were de-identified to ensure patient confidentiality. The IRB waived the requirement for informed consent due to the retrospective nature of the study and the use of anonymized data.

# Results

## Clinical Characteristics of the Patients

During the observation period from January 2019 to June 2023, a total of 109 patients were enrolled in the study across two groups. The characteristics of study patients are shown in Table 1. Among these patients, 66 patients were diagnosed with single *EGFR* mutation and 43 patients with concurrent gene alterations. Males accounted for 58.1% of the concurrent mutations group, which is higher than that in the single *EGFR* mutation group (34.8%). Additionally,

**Table 1** Comparison of Clinical Profile Between Single EGFR Mutation and Concurrent Gene Alteration Patients

| Characteristics | Single *EGFR* Mutation (N=66) n (%) | Concurrent Alteration (N=43) n (%) | *p*-values |
|---|---|---|---|
| Gender | | | 0.02* |
| Male | 23 (34.8) | 25 (58.1) | |
| Female | 43 (65.2) | 18 (41.9) | |
| Age group | | | 0.70 |
| <60 | 29 (43.9) | 17 (39.5) | |
| ≥60 | 37 (56.1) | 26 (60.5) | |
| Smoking status | | | 0.03* |
| Never | 19 (28.8) | 22 (51.2) | |
| Former/current | 47 (71.2) | 21 (48.8) | |
| Histology | | | 0.40 |
| Adenocarcinoma | 66 (100.0) | 42 (97.7) | |
| No-adenocarcinoma | 0 (0.0) | 1 (2.3) | |
| Stage at *EGFR*-TKI treatment | | | 0.56 |
| IIIB | 5 (7.6) | 4 (9.3) | |
| IV | 61 (92.4) | 39 (90.7) | |
| *EGFR* mutation type | | | 0.03* |
| Exon 19 deletion | 51 (77.3) | 24 (55.8) | |
| L858R | 15 (22.7) | 17 (39.5) | |
| Exon 19 deletion+Exon 21 L858R | 0 (0.00) | 2 (4.7) | |
| Performance score at *EGFR*-TKI treatment | | | 0.56 |
| 0-1 | 65 (98.5) | 41 (95.3) | |
| 2-3 | 1 (1.5) | 2 (4.7) | |

**Note**: *Significant at 0.05.

a higher proportion of patients who reported smoking was found in the concurrent mutations group (71.2%) compared to that on the single *EGFR* mutation group (48.8%). A multivariate Cox regression model was developed, consist of the sex, age, smoking status, type of TKI, *EGFR* status, and mutation types (single vs concurrent), in order to assess the ORR and PFS. Multivariate analysis showed a significant difference in PFS between patients with single *EGFR* mutations and those with concurrent *EGFR* mutations, with mutation type remaining an independent prognostic factor (P = 0.001). (refer to Table 2).

## Gene Results

Among 109 patients with advanced NSCLC, with targeted NGS test, exon 19 deletion (*Ex19del*) mutation was detected in 66 (61.5%) patients, exon 21 *L858R* point mutation was in 41 (38.5%) and both Exon 19 del mutation and exon 21 *L858R* point mutation were in 2 (1.8%) (Table 1). All 109 patients were analyzed for *KRAS, PIK3CA, BRAF, MET* mutations and *ALK, ROS1* fusion genes.

**Table 2** Treatment Outcome for Patients with EGFR-Mutant Treated by EGFR TKIs

| Variable | No. (%) | Objective Response | | PFS (Month) | | Progression-Free Survival | |
|---|---|---|---|---|---|---|---|
| | | No. (%) | P value | Median (95% CI) | P value | Multivarate[a] | |
| | | | | | | HR (95% CI) | P value |
| Sex | | | | | | | |
| Male | 48 (44.1) | 34 (77.3) | 0.183 | 14.43 (10.83–18.02) | 0.996 | 1 [Reference] | 0.996 |
| Female | 65 (55.9) | 46 (70.7) | | 13.53 (12.20–14.87) | | 1.001 (0.620–1.617) | |
| Age | | | | | | | |
| ≥ 60 years | 63 (57.8) | 44 (69.8) | 0.250 | 13.73 (11.97–15.49) | 0.970 | 1 [Reference] | 0.970 |
| < 60 years | 46 (42.2) | 26 (59.1) | | 14.27 (12.79–15.75) | | 1.009 (0.621–1.640) | |
| Smoke status | | | | | | | |
| Never smoking | 41 (37.6) | 26 (63.4) | 0.628 | 13.73 (12.44–15.02) | 0.559 | 1 [Reference] | 0.355 |
| Smoking | 68 (62.4) | 46 (67.6) | | 14.40 (11.40–17.40) | | 1.157 (0.708–1.890) | |
| Type of TKI | | | | | | | |
| Afatinib | 37 (34.6) | 24 (64.9) | 0.995 | 12.57 (11.02–14.12) | 0.453 | 1.478 (0.782–2.795) | 0.229 |
| Gefitinib | 44 (41.1) | 29 (65.9) | | 14.27 (11.83–16.73) | | 1.165 (0.627–2.165) | 0.629 |
| Erlotinib | 26 (24.3) | 17 (65.4) | | 14.67 (13.95–15.39) | | 1 [Reference] | |
| EGFR status | | | | | | | |
| Exon 19 del | 76 (71) | 54 (71.1) | 0.16 | 13.37 (12.36–15.10) | 0.33 | 1 [Reference] | |
| Exon 21 mut | 29 (27.1) | 15 (51.7) | | 14.67 (8.36–20.96) | | 0.258 (0.03–2.00) | 0.19 |
| Exon 19Del and 21 mut | 2 (1.8) | 1 (50%) | | 7.47 (NA)* | | 0.296 (0.04–2.39) | 0.25 |
| Characteristics | | | | | | | |
| Single EGFR mutation | 66 (60.5) | 44 (66.7) | 0.93 | 15.17 (12.84–17.50) | 0.0001* | 1 [Reference] | 0.0001* |
| Concurrent EGFR mutation | 43 (39.6) | 29 (67.4) | | 11.07 (10.67–11.47) | | 3.24 (1.89–5.55) | |

**Notes**: *Significant at 0.05. [a]Sex, age, smoking status, type of TKI, EGFR status, Single EGFR and concurrent mutations were entered into the multivariable Cox proportional hazard regression model.

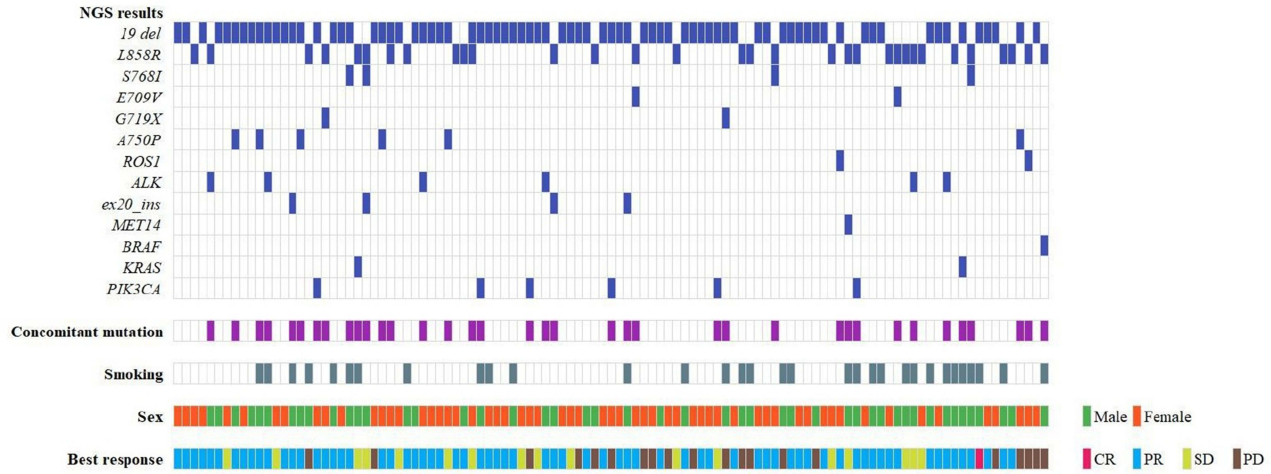


**Figure 1** Gene mutations in NGS analysis of patients in two groups.

Out of the total, concurrent mutations or fusions were found in 43 patients, accounting for 39.4%. *PIK3CA* and *ALK* rearrangement were the main mutations found in these patients, each accounted for 16.2%, following by *KRAS* mutation (5.4%), *ROS1* rearrangement (5.4%). The concurrent mutations were shown in Figure 1. Among the 43 patients, 24 had deletions in exon 19, 17 had the *L858R* mutation in exon 21, and 2 had both the *Del 19* deletion and *L858R* mutation.

## Efficacy Analysis

The Objective Response Rate (ORR) of the total patient was 62.5%, while 84.9% of patient experienced Disease Control Rate (DCR). One hundred and nine patients with *EGFR* mutation showed partial responses [PR] (70.2%), one with complete response [CR] (0.3%) and 67 showed stable disease [SD] (22.4%); 46 patients had progressive disease [PD] (Table 3). We compared the clinical features of 66 patients harboring single *EGFR* mutation with those of the 43 patients harboring co-alterations with an *EGFR* mutation. The analysis of data show that a significant differences in *EGFR* del19 group compared with *EGFR* exon 21 group and those who never smoke compared with those who smoke (Table 1). Furthermore, when we compared the clinical features and treatment effect of the two groups, we found the significant differences in PFS. The median PFS in all the 109 patients was 9.8 months (95% CI, 8.0–12.6). In the concurrent *EGFR* group, the PFS in the group with single *EGFR* mutation and concurrent gene alteration groups were 11.83 months (95% CI,10.16–13.05) and 10.35 months (95% CI, 2.34–18.32), respectively (P = 0.02) (Figure 2). The predictive analysis revealed a significant distinction in outcomes among patients with favorable characteristics marked by *PIK3CA* and *ALK*, reporting a median survival of 11.0 months and 14.3 months. Conversely,

**Table 3** Clinical Efficacy Comparison of EGFR-TKI in Single EGFR Mutation and Concurrent Gene Alterations

| Best Response | Single *EGFG* Mutation (N=66) n (%) | Concurrent Gene Alterations (N=43) n (%) | *p*-values |
|---|---|---|---|
| CR | 1 (1.5) | 0 (0) | |
| PR | 43 (65.2) | 29 (67.4) | |
| SD | 9 (13.6) | 8 (18.6) | |
| PD | 13 (19.7) | 6 (14.0) | |
| ORR | 66.7 | 67.4 | 0.93 |
| DCR | 80.3 | 86.0 | 0.61 |
| Median PFS (month) | 15.03 | 11.00 | 0.0001* |
| Median OS (month) | 35.1 | 30.2 | 0.025* |

**Note**: *Significant at 0.05.

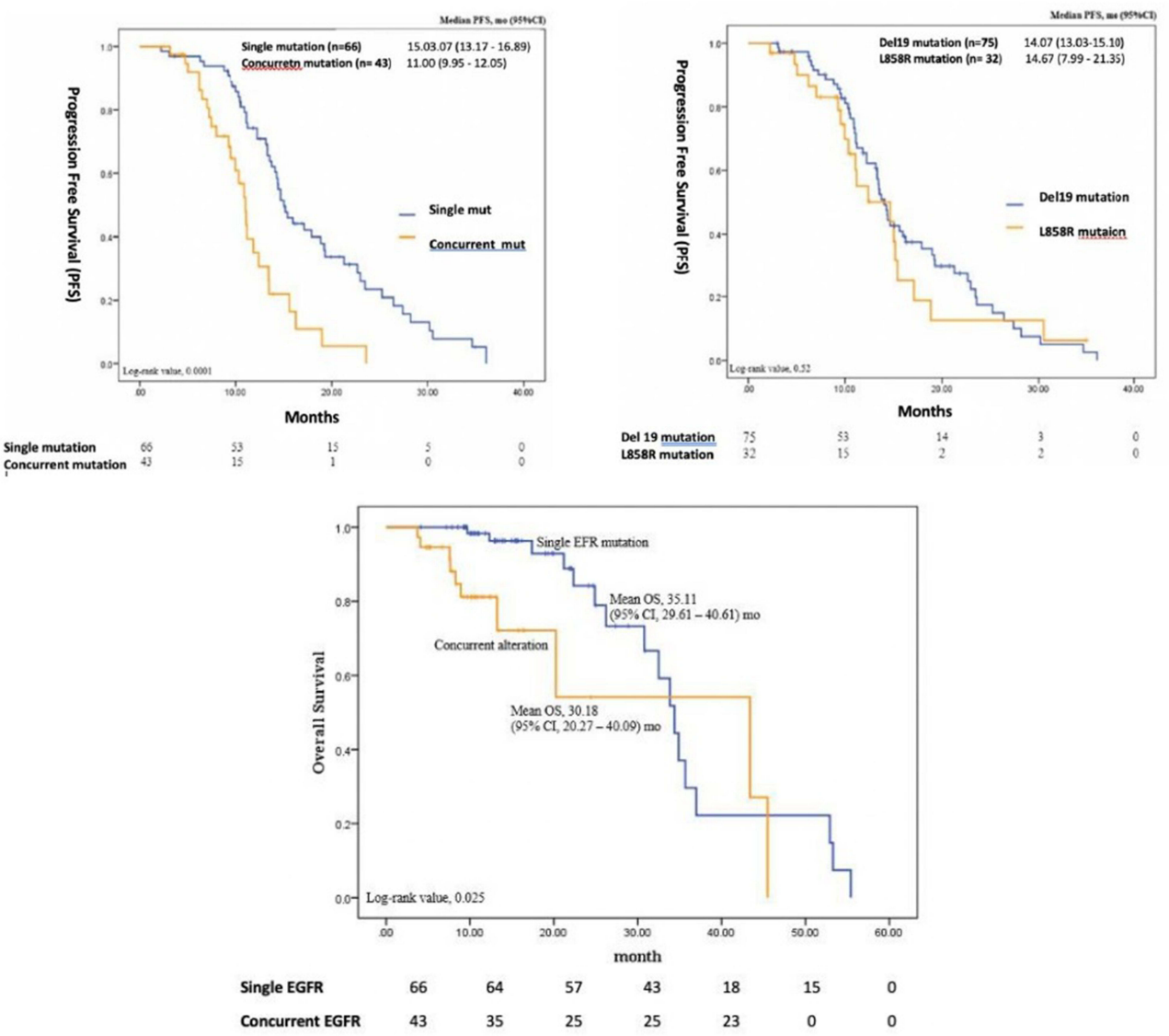


**Figure 2** Comparison of PFS and OS patients with EGFR-TKI treatment between single EGFR mutation and concurrent gene alterations patients.

patients characterized by *KRAS, MET*, and *BRAF* exhibited unfavorable outcomes, displaying median survivals of 4.7 months, 5.03 months and 6.20 months, respectively (P=0.02) (Figure 3).

The PFS in the group with single *EGFR* exon 19 deletion mutation and concurrent gene alterations group were 13.7 months (95% CI,12.1–14.5) and 11.8 months (95% CI,10.16–13.05) (P=0.001) (Figure 2). The PFS in the group with single *EGFR* exon 21 *L858R* mutation and concurrent gene alterations group were 14.6 months (95% CI, 12.5–15.6) and 7.0 months (95% CI, 4.1–8.9) (P=0.009).

The median overall survival time (OS) in the group single *EGFR* mutation and concurrent gen alterations group were 35.11 months (95% CI, 29.61–40.61) and 30.18 months (95% CI, 20.27–40.09), respectively (P=0.025).

## Discussion

The understanding of oncogenic mutations in NSCLC has evolved significantly, challenging the previously held notion that driver mutations occur in isolation. Our study reinforces the concept that *EGFR* mutations frequently co-exist with other oncogenic alterations, profoundly impacting treatment outcomes. The presence of concurrent mutations in genes such as *ALK, ROS1, RET, PIK3CA, BRAF, KRAS*, and *NRAS* was associated with significantly worse PFS compared to

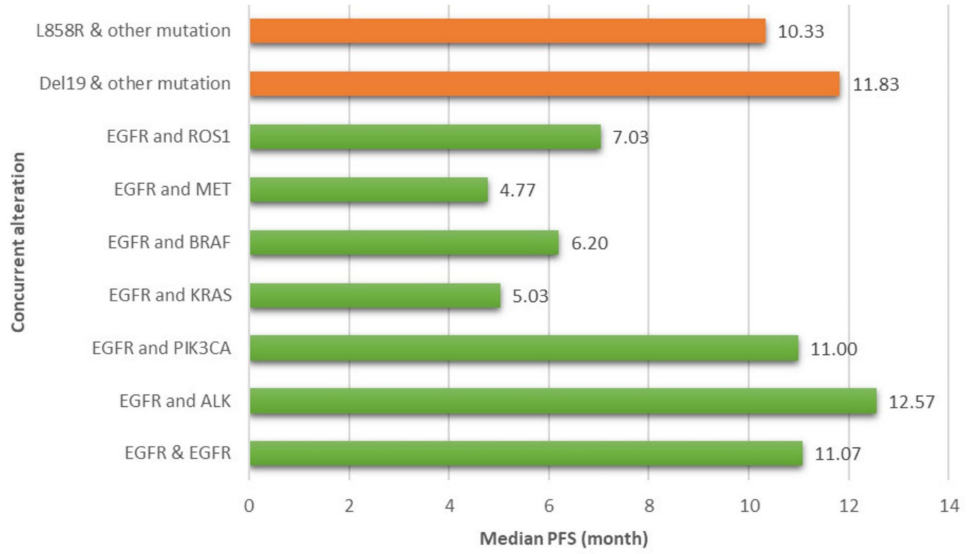


**Figure 3** Outcome treatment in sub-group concurrent mutations.

patients with single *EGFR* mutations, despite a similar ORR between the two groups—66.7% in the single *EGFR* mutation group and 67.4% in the concurrent mutation group—with no statistically significant difference ($P > 0.05$) (Table 3).

Our findings highlight the differential impact of specific concurrent mutations on treatment response. Notably, patients with *EGFR* exon 19 deletions (Del19) showed superior treatment responses compared to those harboring the *L858R* mutation, regardless of the presence of concurrent mutations. These results align with previous studies indicating that the *Del19* mutation is more sensitive to *EGFR-TKIs*, whereas *L858R* mutations are associated with reduced response rates. This suggests that inherent biological differences, rather than concurrent mutations alone, may account for the distinct sensitivities of *Del19* and *L858R* to *EGFR-TKIs*, as proposed by Liang et al.[24]

This finding indicates that the emergence of concurrent mutations can influence the outcome of targeted therapy, thereby establishing crucial predictive indicators for treatment response.[25–27] However, not all patients with co-existing mutations experience unfavorable treatment outcomes. The PFS observed in *EGFR* concurrent mutation patients with *ALK* and *PIK3CA* mutations was comparable to findings from recent studies focusing solely on *EGFR-mutated* patients. Among these, *ALK* and *PIK3CA* were favorable predictive factors. A previous study reported that concurrent mutations were found in about 5% of patients with lung adenocarcinoma.[28] In the present study, *EGFR/ALK* was reported in 1.3%–1.6% of patients with EGFR mutations, compared with 0.2–4% of patients with EGFR mutations combined with other gene mutations.[29–31] First-line *EGFR-TKI* treatment significantly improved PFS compared to *ALK-TKI* therapy.[31] In our study, all six patients with *EGFR/ALK* co-mutations showed clinical benefit from *EGFR-TKI* treatment, as evidenced by a prolonged median PFS.

Aberrant activation of the *PI3K/AKT/mTOR* pathway has been identified as a mechanism of acquired resistance to *EGFR-TKIs*, particularly in lung adenocarcinoma with *EGFR* mutations. Studies by Cheng et al[32] and Fumarola et al[33] have highlighted the pivotal role of this pathway in cancer progression and drug resistance, with targeted agents currently in development. Additionally, Liu et al[34] emphasized that *PIK3CA* mutations can drive acquired resistance to *EGFR-TKIs*, presenting clinical challenges and opportunities for targeted therapy. Alterations in the *PTEN/PI3K/AKT* pathway, mainly *PTEN* inactivation, are also associated with resistance to *EGFR-TKI* therapy and lower survival in NSCLC patients, as reported by Pérez-Ramírez et al.[35] Furthermore, Jeannot et al[36] demonstrated that the *PI3K/AKT* pathway promotes gefitinib resistance in *KRAS* mutant lung adenocarcinoma, suggesting that combining *PI3K/AKT* and *EGFR* inhibitors could be a promising treatment approach.

*PIK3CA* mutations frequently coexist with *EGFR/KRAS* mutations in NSCLC, as shown by Wang et al, and are associated with poor prognosis, especially in the *EGFR/KRAS* wildtype subgroup. Moreover, Gadgeel et al[37] provided

a preclinical rationale for *PI3K/AKT/mTOR* pathway inhibitors as therapy for *EGFR* inhibitor-resistant NSCLC. The *PI3K/AKT* pathway, a downstream signaling cascade of the *HER* family, plays a pivotal role in oncogenesis and lung cancer progression.[38,39] *PIK3CA* encodes the catalytic subunit of *PI3K*, and mutations in this gene can activate the pathway.[34] *PIK3CA* mutations occur in approximately 2–5% of NSCLC cases.[40,41] Predominantly, these mutations manifest in exon 9 (*E545K, E545Q, E545G, E545A, Q546R, E542K* and *T536I*) and exon 20 (*H1047R, H1047L, M1043L, G1007R*, and *Y1021C*), with *E545K* and *H1047R* being the most prevalent.[42]

In this study, patients with *EGFR* and *PIK3CA* concurrent mutations achieved notable treatment efficacy. Within our cohort, five of sixths displayed the *EGFR Del19* mutation. Among these, four patients had a *PIK3CA* exon 9 mutation, while one had an exon 20 mutation. The remaining patients harbored mutations in either exon 4 or exon 8 of the *PIK3CA* gene. According to Naixin Liang, the *PIK3CA* exon 9 mutation had a higher probability of concurrent mutations than the exon 20 mutation,[24] although the number of patients with *EGFR Del19* was higher than those with *EGFR L858R*, though the difference was not statistically significant. Our results support these assertions. Additionally, three of six patients carried the *PIK3CA E545K* mutation on exon 9, though little research has compared the effects of *PIK3CA E545K* to other exon 9 mutations.

On the other hand, patients with other concurrent mutations, such as *MET, KRAS, BRAF*, and *ROS1*, had unfavorable treatment outcomes. *MET* amplification and *KRAS* represent additional recognized mechanisms of resistance to *EGFR-TKI* therapy.[43–45] The prevalence of baseline MET amplification in EGFR-mutant patients appears to be minimal (3.2%).[46,47] Previous reports suggest that these patients might respond to *MET* inhibitors and crizotinib.[43] Concurrent mutation involving *BRAF V600E* is also infrequent.[48] However, defining the optimal targeted therapy for these patients remains uncertain due to limited cases, primarily documented in case reports. In this study, a patient with an *EGFR* mutation alongside concurrent amplification of *MET, KRAS*, and *BRAF* exhibited poor clinical outcomes. Moving forward, devising effective therapeutic strategies for concurrent *KRAS, MET*, and *BRAF* mutations in *EGFR-mutant* NSCLC will necessitate more sophisticated molecular screening approaches and well-designed clinical trials. Therefore, we suggest that chemotherapy remains the mainstay of treatment for patients with *KRAS* and *MET* mutations.

Our findings showed no significant difference in ORR between patients in the single *EGFR* group and those with concurrent genes, differing from Wentao Hu et al.[20] One potential contributing factor could be the limited sample size of patients with concurrent genes. However, the OS in our study had similarities with many other studies worldwide, demonstrating statistically significant differences between the single mutation group and the concurrent group in OS.[49]

A limitation of our study pertains to the small sample size of concurrent mutations. Furthermore, the availability of multiple *EGFR TKIs* for treating advanced NSCLC patients with *EGFR* mutations adds complexity to accurately assessing targeted medication efficacy. The frequency of gene mutations and the presence of missing data may have influenced the results. Subsequent large-scale investigations are essential to establish a correlation between sample size and mutation frequency. Nevertheless, as a pioneering study examining the role of multiple genes in patients with *EGFR* mutations, our findings hold clinical significance. We recommend conducting randomized clinical trials (RCTs) to accurately assess the impact of genetic mutations on TKI therapy effectiveness. Next-generation sequencing (NGS) should be applied to all stage IV lung cancer patients to enhance precision treatment guidance.

In summary, *EGFR* mutations concurrent with *PIK3CA* and *ALK* are recognized as favorable indicators for treatment response, whereas *EGFR* mutations with *KRAS, BRAF*, and *MET* are associated with worse outcomes. Moreover, patients with the Exon 19 deletion mutation exhibited superior treatment responses compared to those with the Exon 21 mutation. Future prospective, large-scale studies are warranted to validate our findings and refine treatment algorithms.

Several studies have further reinforced our findings on the impact of concurrent gene alterations in *EGFR-mutant* NSCLC. Hu et al[20] found that concurrent gene alterations, particularly *PIK3CA* mutation and *EML4-ALK* rearrangement, were associated with reduced efficacy of *EGFR-TKIs* in Chinese patients, leading to significantly shorter PFS compared to those with single *EGFR* mutations. Similarly, Linh et al[22] demonstrated that patients with concurrent *EGFR* mutations exhibited a significantly shorter PFS (11 months) compared to those with single *EGFR* mutations (15.03 months), with *ALK-positive* patients having the longest PFS and *MET-mutant* patients having the shortest.

Chevallier et al[11] reported that resistance mutations in genes like P*IK3CA, PTEN, KRAS*, and *SMAD4* negatively impacted overall survival in *EGFR-mutant* NSCLC patients treated with *EGFR-TKIs*. This aligns with Chang et al,[21] who

found that *TP53, CDK4*, and *CDKN2A* alterations correlated with worse clinical outcomes. Furthermore, Chen et al noted that nearly half of *EGFR-mutant* patients harbored concomitant genetic alterations, most commonly in *TP53, KRAS, and PIK3CA*, resulting in reduced ORR and shorter PFS.

The significance of *EGFR/ALK* concurrent mutations was also highlighted by Sweis et al,[50] who observed distinct clinical characteristics and modest response rates to targeted therapies among these patients. Cheng et al emphasized that *TP53, ERBB2*, and *FGF19* amplifications negatively influenced *EGFR-TKI* efficacy, while Hong et al reinforced the role of concurrent mutations in predicting response to *EGFR* targeted therapy. Additionally, Yang et al[51] and Won et al[31] reported that NSCLC cases with concomitant *EGFR* mutations and *ALK* translocations displayed diverse responses to TKIs, necessitating comprehensive genomic profiling for optimal treatment selection.

Taken together, these studies highlight the critical impact o f concurrent genetic alterations on treatment outcomes in *EGFR-mutant* NSCLC. As Guo et al[52] and Blakely et al[15] emphasized, recognizing these alterations is crucial for optimizing *EGFR-TKI* therapy, suggesting that combination treatments may be necessary for patients with unfavorable genetic profiles.

## Conclusion

Our study underscores the prognostic significance of concurrent gene alterations in *EGFR-mutant* NSCLC. While single *EGFR* mutations predict favorable responses to TKIs, the presence of concurrent mutations, particularly in *KRAS, MET*, and *BRAF*, portends poorer outcomes. Conversely, *ALK* and *PIK3CA* concurrent mutations may not preclude effective TKI therapy. Comprehensive genomic profiling should be integrated into routine clinical practice to guide personalized treatment strategies. Further randomized clinical trials are essential to elucidate the optimal management of this heterogeneous patient population.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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