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*Original Article*

# Survival and prognosis analyses of concurrent PIK3CA mutations in EGFR mutant non-small cell lung cancer treated with EGFR tyrosine kinase inhibitors

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**Abstract:** In non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutation, the prognostic impact of a concurrent Phosphoinositide-3-kinase catalytic alpha polypeptide (PIK3CA) mutation was still unknown. Some studies have shown that EGFR mutant NSCLC patients treated with EGFR tyrosine kinase inhibitors (TKIs) when concurrent PIK3CA mutation have a worse prognosis and shorter survival time. This study conducted a retrospective analysis of NSCLC patients with EGFR mutant or concurrent PIK3CA mutations from January 2015 to October 2019 in the First Affiliated Hospital of Nanchang University. Relative to EGFR alone mutations (Single-Mt), we found that NSCLC patients with EGFR mutations coexisting with PIK3CA mutations (Double-Mt) treated with EGFR-TKIs had a shorter median time to progression (TTP): 7.8 months versus 10.9 months (Double-Mt versus Single-Mt, $P = 0.001$), and decrease in median overall survival (OS): 20.6 months versus 32.4 months ($P < 0.001$). The objective response rate (ORR) between Double-Mt and Single-Mt was 36.7% versus 61.9% ($P = 0.044$), disease control rates (DCR) was 80.1% versus 91.7% ($P = 0.179$). Obviously, EGFR-TKIs for EGFR mutate NSCLC patients when concurrent PIK3CA mutations have a worse prognosis and shorter survival time.

**Keywords:** EGFR mutation, PIK3CA mutation, non-small cell lung cancer, survival and prognosis analyses

## Introduction

Lung cancer remains a significant global health issue and the leading cause of death from various types of malignancies worldwide or in China [1, 2]. Non-small cell lung cancer (NSCLC) accounts for the majority, reaching 80%-85%, of newly diagnosed carcinoma of the lung each year [3]. Although a deeper understanding of NCSLC has been gained after particular development, many patients with NSCLC have poor efficacy and overall survival prognosis due to the absence of apparent symptoms at an early stage and most advanced stage at diagnosis, with less than 18% of patients surviving more than five years [4].

Targeted therapy has gradually developed into a highly significant one in lung cancer in recent years. As the primary mutant gene in NSCLC, the Epidermal growth factor receptor (EGFR), with a mutation rate of up to 46% [5]. Even in Asian NSCLC patients, the positive rate of EGFR gene mutation sensitive population is as high as 50%, which is much higher than that of white people [6]. The targeted therapy has a significant effect on EGFR mutant patients, and the targeted therapy drugs epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have also appeared in the situation of three generations [7, 8].

Phosphatidylinositol 3-kinase/protein kinase B/the mammalian target of Rapamycin (PI3K/Akt/mTOR) signaling pathway, as a downstream pathway of EGFR, has received sufficient attention in recent years on NSCLC, whose activation can regulate cell proliferation, inhibit apoptosis,

promote tumor angiogenesis and tumor invasion [9]. PI3K family was derived from the intracellular phosphoinositide kinase family and can be divided into three types, among which the most studied is class I PI3K, which can catalyze the conversion of phosphatidylinositol 4,5-diphosphate to phosphatidylinositol 3,4,5-triphosphate (PIP3) in vivo. It plays an important role in cell multiplication, growth, movement, metabolism, survival, and inflammatory response [10, 11]. Phosphatidylinositol-3-kinase catalytic alpha polypeptide (PIK3CA) mutation was the only tumor-specific mutation in the PI3K family and can activate the PI3K/Akt/mTOR signaling pathway by encoding type IA PI3K, thereby promoting the growth of cancer cells [12, 13]. In NSCLC, PIK3CA gene mutation frequency is 2%-7% [14, 15]. Compared with other oncogene mutations, PIK3CA mutation may have weak independent carcinogenicity and mostly co-exist with other oncogene mutations. Whereas the most commonly co-mutated gene with PIK3CA mutations in lung cancer is EGFR, in which EGFR exon 21 L858R is more common than EGFR exon 19 deletions [16]. Mutations of PIK3CA occur mainly in exon 9 and exon 20, which encode the helical binding domain and catalytic subunit of PIK3CA, respectively [17]. Many literature data showed that EGFR-mutant NSCLC patients combined with PIK3CA exon 9 or exon 20 mutations were generally believed to have a poor prognosis and a shorter survival period [15, 18, 19]. However, due to heterogeneity and lack of data from previous studies, prognostic data of PIK3CA gene mutation in NSCLC are still inconclusive.

This retrospective study explored the clinical characteristics and the impact of combined PIK3CA mutation on survival and prognosis in EGFR mutant NSCLC patients in response to EGFR-TKIs.

## Materials and methods

### *Patients*

This retrospective study was approved by the Research Ethics Committee of the First Affiliated Hospital of Nanchang University. Thirty NSCLC patients treated in our hospital from January 2015 to October 2019 with histologically or cytologically confirmed the presence of EGFR mutation combined with PIK3CA mutation by genetic testing were included in this study. Further inclusion criteria were an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-2. While the exclusion criteria for this study were as follows: primary organs function failure; autoimmune disease; having two or more primary malignancies; unable to follow-up.

The pathology of NSCLC histology was confirmed by the pathology department. Gene detection is performed by Polymerase Chain Reaction (PCR) or Next-Generation Sequencing (NSG) techniques to detect tumor tissue samples or peripheral blood cell-free tumor DNA (ctDNA) [20]. A review of the type and site of gene mutation features was conducted.

The clinical characteristics of each patient will be extracted from their previous inpatient medical records. Smoking status was defined according to their smoking history as: never, former (< 5 pack-year and quit > 10 years ago), and current (within the most recent year). The pathological types were classified according to WHO (2015 edition) pulmonary tumor tissue type [21]. The clinical-stage at diagnosis was performed in accordance with the American Joint Commission on Cancer (AJCC), 8th Edition tumor-node-metastasis staging system [22].

### *Response evaluation*

The treatment outcomes were evaluated and determined in all patients undergoing EGFR-TKI monotherapy. Therapy outcomes were assessed every 6-12 weeks from the start of treatment with TKI therapy according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) by computed tomography (CT) scan. Including the objective response rate (ORR), disease control rate (DCR), time to progression (TPP), and overall survival (OS). TTP was the time from initiation of EGFR-TKI monotherapy to disease progression. Moreover, OS was defined as the time from receiving EGFR-TKI monotherapy to death due to cancer-related causes or the last available follow-up. Patients' adverse events were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute (version 4.03). Patients were followed from the date of diagnosis until death or the last available follow-up. The follow-up of all included patients will be from diagnosis until death due to various causes or last available

**Table 1.** Patient characteristics

| Feature | Number of patients | (%) |
|---|---|---|
| All patient | 30 | 100.0 |
| Gender | | |
| Male | 16 | 53.3 |
| Female | 14 | 46.7 |
| Age (years) | | |
| < 60 | 14 | 46.7 |
| ≥ 60 | 16 | 53.3 |
| ECOG PS | | |
| 0-1 | 23 | 76.7 |
| 2 | 7 | 23.3 |
| Smoking status | | |
| Never | 12 | 40.0 |
| Former | 5 | 16.7 |
| Current | 13 | 43.3 |
| Histology | | |
| Adenocarcinoma | 27 | 90.0 |
| Squamous carcinoma | 2 | 6.7 |
| Sarcomatoid carcinoma | 1 | 3.3 |
| Clinical stage | | |
| I-IIIA | 7 | 23.3 |
| IIIB-IV | 23 | 76.7 |
| Therapy | | |
| Total patients treated with EGFR-TKI monotherapy | 21 | 70.0 |
| Patients treated with gefitinib | 16 | 53.3 |
| Patients treated with erlotinib | 1 | 3.3 |
| Patients treated with icotinib | 2 | 6.7 |
| Patients treated with afatinib | 1 | 3.3 |
| Patients treated with osimertinib | 1 | 3.3 |
| Total patients treated with chemotherapy | 3 | 10.0 |
| Total patients treated with Surgery | 6 | 20.0 |

ECOG PS: Eastern Cooperative Oncology Group performance status.

follow-up. Furthermore, the data deadline was September 2020.

*Statistics*

The statistical analyses of categorical variables were performed using Pearson's chi-square test or Fisher's exact test. TTP and OS were calculated using the Kaplan-Meier method, and comparisons between groups were performed with the log-rank test. Independent indicators associated with TTP and OS were looked for by applying univariate and multivariate cox regression models. All statistical analysis tests were work by SPSS 25.0 software, and the level of significance was 5% ($P < 0.05$).

## Results

*Patient characteristics*

A total of 30 patients with coexistence of EGFR and PIK3CA mutations were enrolled in this study. The clinical features and general conditions of these patients are detailed in **Table 1**. Most patients were male (16/30, 53.3%), older than or equal to 60 years (16/30, 53.3%), had a history of previous smoking (18/30, 60.0%), were stage IIIB-IV at diagnosis (23/30, 76.7%), and most ECOG PS was 0-1 (23/30, 76.7%).

PIK3CA mutations were mainly occurred in the exon 9 helical domain (19/30, 63.3%; including 7 E542K, 10 E545K, and 2 E545D), and exon 20 catalytic domain (10/30, 33.3%; including 7 H1047R and 3 H1047L). And remarkably, we found a rare case in exon 2 K111N (**Figure 1A**). EGFR mutations were located in exon 19 deletions (43.3%, 13/30), exon 21 (43.3%, 13/30; 12 L858R, 1 L861Q), and exon 20 (13.3%, 4/30; T790M). Among them, there were 4 cases of double EGFR mutations (1 exon 19 L747-T751del + exon 20 T790M, 3 exon 21 L858R + exon 20 T790M) (**Figure 1B**).

The results of the assessment of the PIK3CA mutation in kinase versus helical domain alterations and the correlations between clinicopathological characteristics and EGFR exon 19 deletions or exon 21 mutations are summarized in **Table 2**. No statistically evident correlation was identified between PIK3CA mutation in kinase versus helical domain alterations and clinicopathological parameters.

*Response to EGFR-TKIs of concurrent PIK3CA mutations*

Among the 30 patients with EGFR concurrent PIK3CA mutations, 21 received EGFR-TKI monotherapy, 3 received chemotherapy, and 6

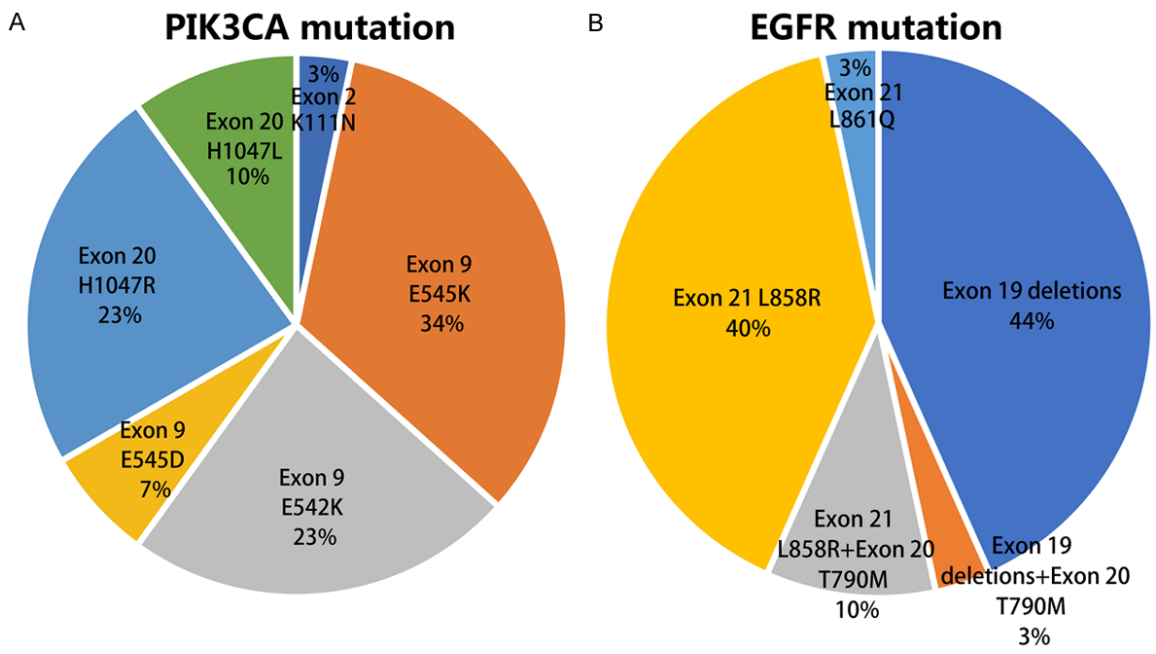


**Figure 1.** Distribution of PIK3CA mutation (A) and EGFR mutation (B) sites in patients with co-mutation.

**Table 2.** Correlations between PIK3CA mutations

| Patients' characteristics | PIK3CA mutations | | |
|---|---|---|---|
| | Exon 9 | Exon 20 | *P* |
| Gender | | | |
| Male | 10 | 6 | 0.705 |
| Female | 9 | 4 | |
| Age (years) | | | |
| <60 | 11 | 3 | 0.153 |
| ≥60 | 8 | 7 | |
| Smoking status | | | |
| Never | 8 | 3 | 0.523 |
| Former/current | 11 | 7 | |
| Clinical stage | | | |
| I-IIIA | 4 | 2 | 0.947 |
| IIIB-IV | 15 | 8 | |
| EGFR mutations | | | |
| Exon 19 | 8 | 6 | 0.359 |
| Exon 21 | 11 | 4 | |

were treated early with surgery. Of the 21 patients who received EGFR-TKIs, 16 received gefitinib, 2 icotinib, 1 erlotinib, 1 afatinib, and 1 osimertinib (**Table 1**).

As summarized in **Table 3**, neither the EGFR exon 19 deletions or exon 21 mutations nor PIK3CA mutations in kinase versus helical

**Table 3.** Response to EGFR-TKIs according to mutation sites

| | Response to EGFR-TKIs | | | |
|---|---|---|---|---|
| | Total | CR+PR n (%) | SD+PD n (%) | *P* |
| EGFR mutations | | | | |
| Exon 19 | 10 | 3 (30.0) | 7 (70.0) | 0.466 |
| Exon 21 | 11 | 5 (45.5) | 6 (54.5) | |
| PIK3CA mutations | | | | |
| Exon 9 | 14 | 6 (42.9) | 8 (57.1) | 0.525 |
| Exon 20 | 7 | 2 (28.6) | 5 (66.7) | |

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

domain alterations were statistically significantly associated with response ($P$ = 0.466 and $P$ = 0.525, respectively). The ORR to EGFR-TKIs of the 21 EGFR concurrent PIK3CA mutations patients was 36.7%, DCR was 80.1%, median TTP was 7.8 months, median OS was 20.6 months, and 1-year OS was 78.0% (**Table 4**).

*Survival and prognostic impact of concurrent PIK3CA mutations*

For the patients who received EGFR-TKI monotherapy overall, 21 patients in the EGFR/PIK3CA Double-Mt group and 60 cases in the

**Table 4.** Impact of concurrent PIK3CA mutations on EGFR-TKIs

| | EGFR/PIK3CA Double-Mt | EGFR Single-Mt | *P* |
|---|---|---|---|
| TKI analysis | 21 | 60 | |
| Local therapy during therapy | 7 (33.3%) | 24 (40.0%) | 0.589 |
| Response | | | |
| ORR | 36.7% | 61.9% | 0.044 |
| DCR | 80.1% | 91.7% | 0.179 |
| TTP | | | |
| Median TTP, 95% CI (months) | 7.8 (6.6-9.0) | 10.9 (10.1-11.7) | 0.001 |
| 6 months progression free | 61.9% | 86.7% | |
| 12 months progression free | 4.8% | 38.3% | |
| 18 months progression free | 0% | 1.7% | |
| OS | | | |
| Median OS, 95% CI (months) | 20.6 (15.1-26.1) | 32.4 (28.6-36.2) | < 0.001 |
| 1-year OS | 78.0% | 90.0% | |
| 2-year OS | 31.7% | 73.3% | |
| 3-year OS | NA | 31.6% | |

Mt: mutation; NA: unable to perform check calculation.

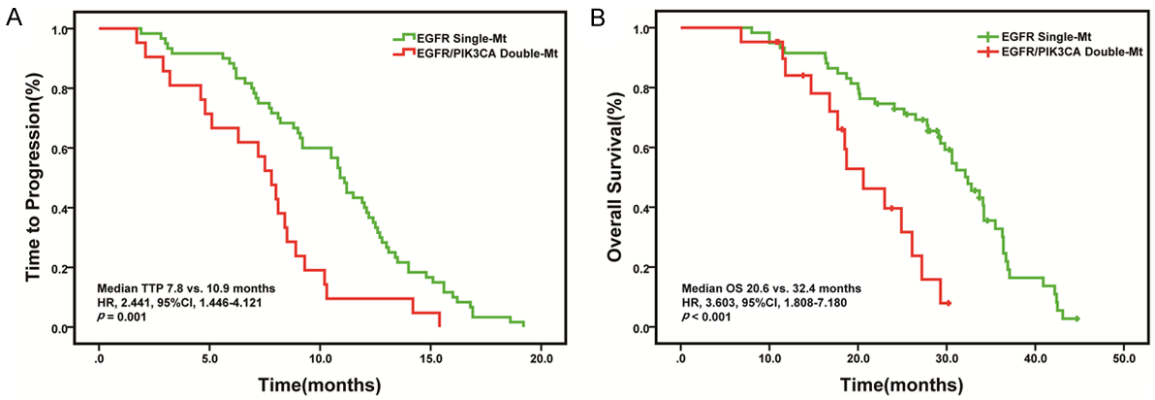


**Figure 2.** TTP (A) and OS (B) curves of patients with EGFR Single-Mt group and EGFR/PIK3CA Double-Mt group.

EGFR Single-Mt group. The ORR between Double-Mt and Single-Mt was 36.7% versus 61.9% ($P$ = 0.044), DCR was 80.1% versus 91.7% ($P$ = 0.179). There was no difference between the two groups in the receipt of local therapy (pleural infusion, radiotherapy, and surgery) (7 of 21, 33.3% versus 24 of 60, 40.0%; $P$ = 0.589). These results are shown in **Table 4**.

There were statistically significant differences in TTP and OS between the Double-Mt group and Single-Mt group. The median TTP was 7.8 months (95% CI, 6.6-9.0 months) versus 10.9 months (95% CI, 10.1-11.7 months, $P$ = 0.001) (**Table 4**, **Figure 2A**). And the median OS was 20.6 months (95% CI, 15.1-26.1 months) versus 32.4 months (95% CI, 28.6-36.2 months, $P$ < 0.001) (**Table 4**; **Figure 2B**).

Furthermore, the two subgroups of EGFR/PIK3CA Double-Mt group stratified according to PIK3CA mutations in exon 9 and exon 20 showed no obvious statistical differences in TTP ($P$ = 0.743, **Figure 3A**) or OS ($P$ = 0.660, **Figure 3B**). Similarly, subgroups stratified according to EGFR exon 19 deletions or exon 21 mutations of Single-Mt and Double-Mt patients were tested separately, and there were also no significant statistical differences in TTP ($P$ = 0.376 and $P$ = 0.532, respectively, **Figure 3C**) or OS ($p$ = 0.175 and $P$ = 0.334, respectively, **Figure 3D**).

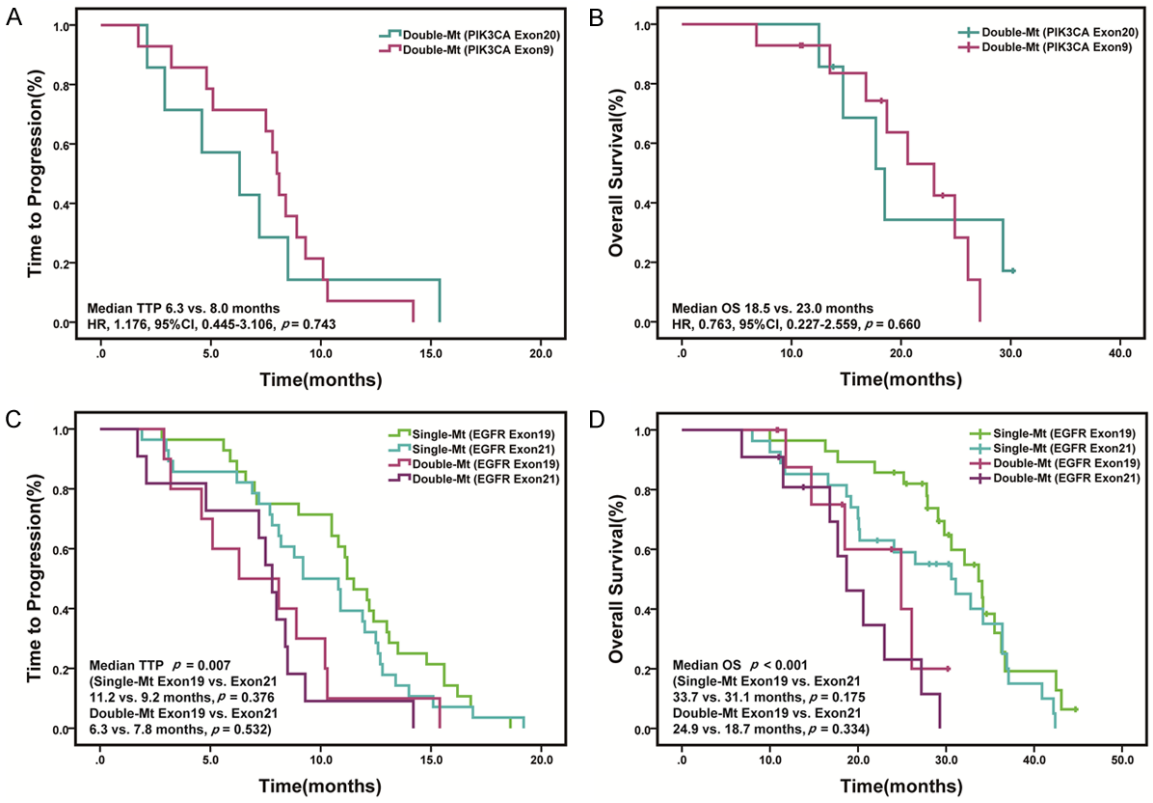


**Figure 3.** (A, B) TTP (A) and OS (B) curves of patients with two Double-Mt subgroups stratified according to PIK3CA mutation in exon 9 and exon 20; (C, D) TTP (C) and OS (D) curves of Single-Mt and Double-Mt patients with subgroups stratified according to EGFR exon 19 deletions and exon 21 mutations.

*Univariate and multivariate cox regression analyses of molecular and clinical parameters for TTP and OS of concurrent PIK3CA mutant patients*

Univariate and multivariate Cox regression models for TTP and OS were built using molecular and clinical parameters in patients with concurrent PIK3CA mutations. According to the univariate analysis, smoking status at Former/Current and ECOG PS = 2 were statistically valuable predictors of poor TTP ($P = 0.005$ and $P = 0.001$, respectively) and OS ($P = 0.044$ and $P = 0.017$, respectively). The multivariate analysis revealed the following factors related to a shorter TTP: smoking status at Former/Current and ECOG PS = 2, and correlated with worse OS was ECOG PS = 2 (**Table 5**).

*EGFR-TKIs related adverse events*

Of all patients receiving EGFR-TKIs, 62 of the 81 (76.5%) patients reported a total of 109 AEs. Skin toxicity (39/81, 48.1%) and gastrointestinal symptoms (33/81, 40.7%) were the most common AEs; the following was abnormal liver function (15/81, 18.5%), anemia (9/81, 11.1%), leukopenia (7/81, 8.6%), thrombocytopenia (5/81, 4.9%), oral ulcer (1/81, 1.2%). Among them, 5 patients (2 rash, 2 diarrhea and 1 anemia) reported grade 3 or 4 AEs. There was no statistically significant difference between the Double-Mt group and the Single-Mt group ($P > 0.05$).

## Discussion

PIK3CA is a common oncogene in human malignancies and is expressed in several malignancies such as lung, breast, and colorectal cancer. The present study showed that the frequency of PIK3CA mutation in NSCLC accounts for about 3% in adenocarcinoma and 5%-10% in squamous cell carcinoma (SCC) [14, 15, 23]. As mean that PIK3CA mutations are more common in lung SCC relative to lung adenocarcinoma. However, in this study, squamous cell carcinoma accounted for 6.7% (2/30) and adeno-

**Table 5.** Univariate and multivariate cox regression analyses

| Variables | TTP | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Gender | | 0.839 | | | | 0.383 | | |
| Male vs. Female | 0.880 (0.258-3.004) | | | | 2.116 (0.393-11.389) | | | |
| Age (years) | | 0.753 | | | | 0.628 | | |
| < 60 vs. ≥60 | 1.279 (0.277-5.911) | | | | 1.491 (0.205-5.922) | | | |
| Smoking status | | 0.005 | | 0.003 | | 0.044 | | 0.203 |
| Former/current vs. Never | 16.889 (2.300-123.988) | | 11.445 (2.328-56.260) | | 5.587 (1.049-29.769) | | 2.361 (0.629-8.871) | |
| Clinical stage | | 0.523 | | | | 0.126 | | |
| IIIB vs. IV | 2.145 (0.206-22.328) | | | | 0.053 (0.001-2.287) | | | |
| ECOG PS | | 0.001 | | < 0.001 | | 0.017 | | 0.025 |
| 0-1 vs. 2 | 0.041 (0.006-0.270) | | 0.035 (0.005-0.222) | | 0.054 (0.005-0.597) | | 0.138 (0.025-0.777) | |
| PIK3CA mutations | | 0.922 | | | | 0.132 | | |
| Exon 20 vs. Exon 9 | 0.943 (0.289-3.079) | | | | 0.218 (0.030-1.584) | | | |
| EGFR mutations | | 0.811 | | | | 0.132 | | |
| Exon 19 vs. Exon 21 | 1.168 (0.327-4.174) | | | | 0.220 (0.031-1.576) | | | |

carcinoma accounted for 90.0% (27/30), which were not consistent with domestic and foreign studies. The reason was that NSCLC patients with EGFR mutations were included at the beginning of this research, and EGFR mutations were significantly higher in lung adenocarcinoma than in lung SCC, leading to fewer cases of lung SCC enrolled in our study, which results in inconformity with relevant research. PIK3CA mutations may have weak independent carcinogenicity and mostly co-exist with other oncogene mutations. The most common co-mutation gene is EGFR mutation in lung cancer [16].

The human EGFR family belongs to the tyrosine kinase receptor family, also known as the HER or ErbB family. EGFR family members are similar in structure and consist of an extracellular domain, a transmembrane domain, and an intracellular domain. The intracellular domain contains the tyrosine kinase (TK) domain, and its TK activity plays an essential regulatory role for cell proliferation and differentiation. EGFR mutations mainly occur in the first four exons (18~21) of the intracellular TK region, leading to ligand-independent activation of EGFR TK, thereby activating the downstream EGFR pathway and promoting tumor growth, called activation mutations [24]. The patients with EGFR mutations in this study all fell into this category. For advanced EGFR mutant NSCLC patients, EGFR-TKI treatment has made a qualitative leap compared with chemotherapy. The phase III randomized clinical trials IPASS study confirmed the EGFR mutation is a significant prognostic factor of EGFR-TKIs curative effect [25]. In this study, among the 261 EGFR mutant patients, progression-free survival (PFS) was significantly longer in patients who received gefitinib than in those who received carboplatin plus paclitaxel. OPTIMAL [7] and EURTAC [26] study comparing erlotinib with gemcitabine plus carboplatin solution, CONVINCE study [27] comparing icotinib with pemetrexed plus carboplatin subsequent pemetrexed maintain a plan, as well as several III randomized controlled studies confirmed that first-generation EGFR-TKIs was superior to standard platinum-based chemotherapy in patients with EGFR mutations, with ORR of 58%-83%, and the median PFS could reach 8 to 13 months, with significantly fewer adverse effects than chemotherapy. In this study, the ORR of EGFR single-mutant patients was 61.9%, TTP (equivalent to PFS) was 10.9 months, which were consistent with these studies.

For most EGFR mutant NSCLC patients, drug resistance occurs about 10 months after treatment with EGFR-TKIs and resulting in disease progression. Numerous studies have shown that PIK3CA was significantly correlated with EGFR-TKIs resistance. Song et al. reported that 810 patients with lung adenocarcinoma after surgery showed that PIK3CA was correlated with reduced PFS and OS in patients who received EGFR-TKIs [23]. Ludovini et al. showed that PIK3CA mutation was significantly associated with inferior PFS and OS and indicated developing drug resistance and worse survival during EGFR-TKIs therapy [28]. Eng et al. explored the NSCLC patients of EGFR-mutant and co-exist with PIK3CA mutation were related to poor median OS: 18 versus 33 months (Double-Mt versus Single-Mt), and median TTP was 7.8 versus 11.1 months [19]. All these studies suggested that PIK3CA mutation is associated with drug resistance and shorter survival after EGFR-TKIs therapy. The conclusion of our study is in line with these studies.

In addition, PIK3CA mutations mainly exist in exon 9 and exon 20, and EGFR mutations are most commonly found in exon 19 deletions or exon 21 mutations. When subgroup analysis was performed, we found that the prognosis of patients with PIK3CA co-mutation was not affected by the mutation in kinase versus helical domain alterations. However, although subgroups stratified according to EGFR exon 19 or exon 21 of Single-Mt or Double-Mt patients were tested respectively and made no apparent statistical difference, the median TTP (11.2 versus 9.2 months, $P = 0.376$) and median OS (33.7 versus 31.1 months, $P = 0.175$) in EGFR single-mutant patients with exon 19 deletions were longer than those with exon 21 mutations. A meta-analysis of 8 phase III randomized controlled clinical trials published at the 2014 ASCO annual meeting included 1489 cases of first-line therapy by EGFR-TKIs. The results showed that patients with exon 19 deletion mutations had better PFS outcomes than those with exon 21 L858R point mutations (HR = 0.587, $P = 0.02$) [29]. Nevertheless, some early phase III randomized trials, for instance, the NEJ002 [30] and WJTOG3405 [31] study, reported EGFR mutations in exon 19 or 21 we-

re not significantly associated with PFS in advanced patients with TKI first-line therapy. Those need to be verified and supported by more evidence-based medicine and basic research.

Li et al. carried out genetic testing on 5125 Chinese NSCLC specimens and found that PIK3CA was prone to co-mutation with EGFR exon 19 and 21, among which EGFR exon 19 was more prone to co-mutation with PIK3CA [15]. Moreover, PIK3CA exon 9 E545K has been implicated as a biomarker of poor clinical outcome, which may be due to acquired drug-resistant mutations to TKI therapy. EK45K accounts for a large proportion of exon 9, which is confirmed in this study (E545K accounts for 52.6% of exon 9), so the prognosis of exon 9 should theoretically be worse than that of exon 20. However, no statistical difference was shown between PIK3CA and EGFR exon 19 and exon 21 in our study. Furthermore, there was also no significant difference in the outcome of PIK3CA mutations in exon 9 or 20 according to univariate and multivariate analysis. We probed into the reasons for this presumably related to the small sample size included, which may lead to more accurate conclusions when the sample size is available. Furthermore, studies reported that ECOG PS and smoking status as independent prognostic factors in NSCLC, which our research confirms [32, 33].

Studies have confirmed that PIK3CA mutation promotes tumor growth by activating the PI3K/Akt/mTOR signaling pathway, and this pathway was closely related to EGFR-TKIs resistance [34, 35]. Currently known PIK3CA gene abnormalities include mutation and amplification, while both gene abnormalities can lead to the activation of PI3K to promote tumor growth, and found that mutation and amplification may be mutually exclusive in lung cancer, meaning that these two alterations of PIK3CA gene may have equal potential to promote lung carcinogenesis [36, 37]. The current study shows that PIK3CA mutations occur predominantly in exon 9 and exon 20, whereas mutations occurring in these regions promote tumor growth by activating the PI3K/Akt/mTOR signaling pathway, and the TKIs treated co-exist PIK3CA mutated cases in this study all belong to this category of mutations. PIK3CA mutation was the only tumor-specific mutation in the PI3K family and can activate the PI3K-Akt-mTOR signaling pathway by encoding type IA PI3K. Moreover, PIK3CA mutation encodes the p110a catalytic subunit of class IA PI3K, which has been identified in approximately 5% of NSCLC [38]. Prior studies suggested that these mutations lead to sustained Akt activation under serum-deprived conditions and were not controlled by the upstream signal pathway of EGFR, resulting in resistance to EGFR-TKIs [34, 38, 39]. Phosphorylated activated Akt phosphorylates many downstream proteins, and mTOR was a critical downstream signaling branch of Akt, which plays a significant role in cell growth, proliferation, survival, movement, protein synthesis, and transcription, and also plays a considerable role in tumorigenesis [40, 41]. In addition, the PI3K/Akt/mTOR pathway can also be activated by deletion or mutation of PTEN, which also needs our attention for the PTEN gene [41].

Currently, multiple PI3K/Akt/mTOR signaling pathway targeted inhibitors are being investigated and used in preclinical researches and cancer-related tests, which include targeted agents acting on PI3K, Akt, mTOR signaling proteins alone (simple inhibitors), or targeted agents acting on PI3K and mTOR dual signaling proteins (dual inhibitors) [42-44]. BEZ235, a PI3K/mTOR dual inhibitor, was the first agent to get into clinical tests [43, 44]. The current clinical trial results indicate that the drug has vigorous antitumor activity and well-tolerated, with important implications for tumor treatment. Isoyama et al. showed that PI3K and mTOR inhibitors have a synergistic effect on the inhibition of all NSCLC, even low concentrations of mTOR can increase PI3K inhibitors' sensitivity, and the combination of EGFR-TKIs can further inhibit tumor growth [45]. Targeted therapy of EGFR-TKIs combined with PI3K/Akt/mTOR inhibitors will be a prospective therapeutic schedule for NSCLC patients with EGFR and PIK3CA co-mutations. This is of great significance for precise individualized therapy of NSCLC patients.

There are limitations to this study, such as its being a retrospective study, the variable therapeutic agents, and the comparatively small sample capacity. Above all, the quality of samples sent for genetic testing and how the gene was tested may lead to the false detection of gene mutations, which will be included in our study, affecting the accuracy of the results.

Additionally, although most patients received gefitinib, some received other first-generation EGFR-TKIs, such as erlotinib and icotinib, or even used the second or third generation directly as first-line treatment. Therefore, our results may also be affected by different TKI drugs. Finally, because the frequency of PIK3CA mutation is only about 2%-7% in NSCLC, fewer cases have EGFR mutation, resulting in a relatively small sample size included in this study. Although our findings are interesting, the impact of PIK3CA co-mutation on the outcome of EGFR mutant NSCLC patients and its predictive value needs to be further validated by randomized studies. Despite these limitations, this study provides a reference value, both therapeutically and prognostically, for NSCLC patients with EGFR and PIK3CA co-mutations.

In conclusion, we explored that concurrent PIK3CA mutations may be an adverse prognostic factor in EGFR mutant NSCLC patients. With increasing attention to precision medicine, the role of PIK3CA mutations will become increasingly prominent. Moreover, EGFR-TKIs combined with PI3K/Akt/mTOR signaling pathway inhibitors will be an individualized and precise treatment strategy for NSCLC patients with EGFR and PIK3CA co-mutations. Of course, these preliminary results warrant further research.

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## Disclosure of conflict of interest

None.

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