Review Article

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# A Review of Genetic Alteration Correlated with Primary and Acquired Resistance to EGFR-TKIs in EGFR-Mutant Non-Small Cell Lung Cancer

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## Abstract:

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) significantly enhance both survival and quality of life for non-small cell lung cancer (NSCLC) patients with EGFR mutations. Nevertheless, an increasing number of patients exhibit primary or acquired resistance. Currently, discoveries of concurrent genetic alterations in EGFR and other genes besides EGFR have been made. Several studies have demonstrated an association between these alterations and resistance to EGFR-TKI. Therefore, it is crucial to gain more understanding of gene alterations and their impact on the effectiveness of EGFR-TKI treatment. In this review, concurrent gene alterations that can predict primary and acquired resistance to EGFR-TKIs in advanced NSCLC are discussed. This may assist clinicians in selecting the appropriate treatment for patients who have failed to respond to EGFR-TKIs.

**Keywords:** acquired resistance, EGFR-TKIs, genetic alteration, non-small cell lung cancer, primary resistance

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## Introduction

Lung cancer is the second most frequently diagnosed cancer globally, with an estimated age-standardized rate (ASR) of 22.4. It is also the primary cause of cancer-related deaths, with an ASR of 18.0[1]. In Thailand, lung cancer is the third most common type of cancer, having an ASR of 18.9, and it is the second leading cause of cancer-related deaths, with an ASR of 15.9[2]. Non-small-cell lung carcinoma (NSCLC) is the predominant histologic type, accounting for 85% of lung cancer cases[3]. Over time, driver mutations in NSCLC have been discovered, enabling the development of personalized targeted treatments that enhance response rates, minimize toxicity, and improve the quality of life for patients[4]. Epidermal growth factor receptors (EGFR) are prevalent driver genes in NSCLC, and are found in 40–60% of Asian populations and 10% of Western populations[4].Overexpression or mutation of the EGFR gene results in aberrant cell growth, differentiation, survival, enhanced formation of blood vessels, and the migration of cancer cells. EGFR tyrosine kinase inhibitors (EGFR-TKIs) can substantially extend the median survival of patients with advanced lung cancer who have EGFR-sensitive mutations[5]. Additionally, it is associated with a high response rate, ranging from 60% to 80%, and results in fewer side effects compared to platinum-based chemotherapy[6,7].

Recently, different EGFR-TKI types, including first-generation EGFR-TKIs (gefitinib, erlotinib, and icotinib) and second-generation EGFR-TKIs (afatinib and dacomitinib) have been authorized for treating patients with EGFR-mutant NSCLC[8]; however, a large number of patients have disease relapse due to treatment resistance[9,10]. Some patients treated with gefitinib, erlotinib, or afatinib are susceptible to primary resistance, but most eventually will acquire complete resistance[11]. Several studies have shown that more than 20% of patients contend with early relapse within 6 months[12–15]. Herein, we reviewed the clinical impact of treatment, focusing on overall response rate (ORR), overall survival (OS), and progression-free survival (PFS). We also reviewed the studies that evaluated concurrent genomic alterations related to resistance in EGFR-mutated NSCLC after EGFR-TKI treatment.

### EGFR mutation

The human EGFR gene is located on the short arm of chromosome 7 (7p11.2). It spans about 200 kb, which contains 28 exons and encodes a protein of 1,210 amino acids[16]. EGFR mutations are classified, according to the American College of Medical Genetics and Genomics (ACMG), into 3 categories: pathogenic, likely pathogenic, and benign, as shown in Figure 1[17]. Exon 19 has the highest mutation frequency at 48.8%, with the majority being pathogenic mutations. Of all the mutations, 18 were deletions, which may be either pathogenic or benign. Exon 21 has the second highest number of pathogenic mutations, totaling 30.95%. Exon 20 has a mutation rate of 14.28%, including 6 pathogenic mutations and 3 benign mutations. Exon 18 has a mutation rate of 5.9%, with 3 mutations classified as pathogenic, and one as likely pathogenic[18]. Drug-sensitive mutations include point mutations in exon 18 (G719X), point mutations in exon 21 (L858R), and in-frame deletions in exon 19 (Del19). Drug-resistant mutations are found in exon 20 (T790M)[19].

### Frequency of EGFR mutations in patients with NSCLC

EGFR is the most prevalent driver mutation in NSCLC, frequently found in ADC (40-60%)[20]. EGFR mutations are rare in SCC, having a prevalence of less than 3%[21]. The lack of reported mutations may restrict the beneficial effects of EGFR-TKIs in treating lung cancer patients with SCC. By geographic region, the prevalence of EGFR mutation is highest in Asia-Pacific, ranging from 20% to 76%, with an average frequency of 47%. Vietnam

has the greatest EGFR mutation frequency in the Asia-Pacific region at 64%, while the Republic of Korea stands at 43%. The frequency of EGFR mutation in Thailand is 54%. In Europe, the overall frequency of EGFR mutation is 15%, the lowest among other geographic regions. Within this region, the EGFR mutation frequency was highest in Turkey at 37%, and lowest in Austria at 7%, as shown in Table 1[20].

### EGFR Tyrosine Kinase Inhibitors (EGFR-TKIs)

EGFR-TKIs are small molecules that prevent the binding of adenosine triphosphates (ATP) to the tyrosine kinase domain of the EGFR within cells. This inhibition affects intracellular signaling, thereby reducing cell division and growth[22]. The EGFR-TKIs are categorized into 3 generations, based on the order of their development.

### First-generation EGFR-TKIs

First-generation EGFR-TKIs, such as erlotinib, gefitinib and icotinib, are quinazoline-derived drugs that bind to EGFR as a reversible inhibitor. It competes with ATP for binding at the active site of the tyrosine kinase domain through non-covalent interactions[23]. These drugs have strong inhibitory effects on activating EGFR mutations,

**Table 1** Frequency of EGFR mutations in patients with NSCLC of ADC (Modified from Midha et al. 2015)[20]

| Country | EGFR mutation frequency | |
|---|---|---|
| | Overall EGFR mutation frequency (%) | EGFR mutation frequency range (%) |
| Asia-Pacific: Overall | 47 | 20-76 |
| China | 48 | 27-66 |
| Japan | 45 | 21-68 |
| Malaysia | 45 | 39-47 |
| Philippines | 52 | N/A |
| Korea | 43 | 20-56 |
| Thailand | 54 | N/A |
| Vietnam | 64 | N/A |
| Europe: Overall | 15 | 6-41 |
| Greece | 22 | 20-22 |
| Italy | 14 | 10-33 |
| The Netherlands | 15 | 11-20 |
| Poland | 13 | 11-14 |
| Russia | 22 | 20-31 |
| Slovakia | 20 | N/A |
| Turkey | 37 | 22-41 |
| United Kingdom | 12 | N/A |
| North America: Overall | 22 | 3-42 |
| Canada | 14 | 14 |
| United States of America | 23 | 3-42 |
| Indian subcontinent: Overall | 26 | 22-27 |
| India | 26 | 22-26 |
| Pakistan | 27 | N/A |
| South America: Overall | 36 | 9-67 |
| Argentina | 19 | N/A |
| Brazil | 28 | 9-34 |
| Peru | 67 | N/A |

ADC=denocarcinoma, EGFR=epidermal growth factor receptor, NSCLC=non-small cell lung cancer

either exon 19 deletions or exon 21 L858R[24]. However, they are only effective in the short term due to the occurrence of acquired resistance caused by mutations within the drug-binding site[24].

### Second-generation EGFR-TKIs

Second-generation EGFR-TKIs, including afatinib, dacomitinib and neratinib, are quinazoline-based drugs that act as irreversible inhibitors by binding to EGFR. The development of these drugs is intended to overcome the acquired resistance resulting from the failure of first-generation EGFR-TKIs. The mechanisms of action of second-generation EGFR-TKIs differ from those of first-generation EGFR-TKIs. The drugs exhibit an affinity via covalent bonding to cysteine residues within the tyrosine kinase domain[23].

### Third-generation EGFR-TKIs

First and second-generation EGFR-TKIs have high efficacy, although resistance often develops within 10–14 months after treatment. EGFR T790M is the most common mutation associated with acquired resistance, which is present in 50–70% of patients[6]. Third-generation EGFR-TKIs are aminopyrimidine-based drugs that act as irreversible inhibitors by binding to EGFR. Osimertinib is currently recommended as a treatment option for NSCLC patients who have developed resistance to first- or second-generation EGFR-TKIs, as a second- or third-line therapy[23].

### Clinical studies regarding EGFR-TKIs treatments in advanced NSCLC patients

Herein, we have retrieved the study demonstrating objective response rate (ORR), overall survival (OS), and progression-free survival time (PFS) in various populations of advanced NSCLC patients following EGFR-TKI. As shown in Table 2, there was a total of 1,881 cases of patients harboring EGFR-activating mutations (deletion 19, G719X, L858R, and L861Q). All patients received either first-generation EGFR-TKIs (gefitinib and erlotinib) or second- generation (afatinib) EGFR-TKIs, and adenocarcinoma (ADC) was diagnosed in almost all cases (99.57%; 1,873/1,881 cases).

The ORR, which is the proportion of patients that achieved a response, was comparable for 3 EGFR-TKIs: erlotinib (53.8–78.0%), gefitinib (56.0–62.5%), and afatinib (50.0–70.0%). The median OS was 20.4 months for erlotinib, ranging from 17.0 to 25.0 months for gefitinib, and from 23.1 to 27.9 months for afatinib. The PFS, which is calculated as the interval between the initiation of treatment and progression or death, was found to be 11.2–12.1 months for erlotinib, 8.0–12.1 months for gefitinib, and 10.3–16.4 months for afatinib.

Furthermore, we have shown in this review a subset of patient recurrence of various durations. PFS was categorized into 3 time periods: less than 3 months, 3 to 6 months, and more than 6 months (Table 2). Most patients usually show relapse after treatment over 6 months: ranging from 53.6% to 80.3% of the total patients. Nevertheless, a subset of patients developed the disease within 6 months of undergoing treatment. This varied among patients from 19.7% to 46.4%. These findings suggest a pattern of patients having developed acquired, early resistance to treatment or not responding to it (referred to as primary resistance).

### Resistance to EGFR-TKIs

Although EGFR-TKIs are presented as a preferred method of lung cancer treatment compared to traditional chemotherapy, some patients develop resistance after exposure to the drugs. Resistance to EGFR-TKI is clinically complex and can present at various times during treatment, including immediately after treatment initiation (primary resistance), weeks or months after evidence of the initial clinical benefit (secondary resistance), or after treatment has been halted for a variety of reasons[9,25].

**Table 2** Prospective clinical trials of first-and second-generation EGFR-TKIs in patients with advanced NSCLC

| Studies | Population | Histology | EGFR-TKIs | ORR, % | Median OS, months | Median PFS, months | Progression-free survival | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 3 months, n (%) | 3-6 months, n (%) | 6 months, n (%) |
| Goto, 2013[35] | Japanese | ADC (n=102) | Erlotinib (n=102) | 78.0 | NA | 11.8 | 13 (12.7) | 17 (16.7) | 72 (70.6) |
| Noronha, 2020[13] | Indian | ADC (n=170)<br>ADS (n=4)<br>SCC (n=2) | Gefitinib (n=176) | 62.5 | 17.0 | 8.0 | 20 (11.4) | 23 (13.1) | 33 (75.5) |
| Wu, 2018[14] | Chinese | ADC (n=217) | Afatinib (n=217) | 66.8 | 23.1 | 11.0 | 32 (14.7) | 39 (18.0) | 146 (67.3) |
| Park, 2016[36] | Korean | ADC (n=317)<br>ADS (n=2) | Afatinib (n=160)<br>Gefitinib (n=159) | 70.0<br>56.0 | 27.9<br>25.0 | 11.0<br>10.9 | 18 (11.2)<br>27 (17.0) | 30 (18.8)<br>26 (16.3) | 112 (70.0)<br>106 (66.7) |
| Pluzanski, 2020[15] | Polish | ADC (n=619) | Erlotinib (n=252)<br>Gefitinib (n=255)<br>Afatinib (n=112) | 53.8<br>56.5<br>50.0 | 20.4<br>17.5<br>NA | 12.1<br>10.3<br>16.4 | 36 (14.3)<br>38 (14.9)<br>14 (12.5) | 55 (21.8)<br>57 (22.4)<br>38 (33.9) | 161 (63.9)<br>160 (62.7)<br>60 (53.6) |
| Kuan, 2016[37] | Taiwanese | ADC (n=448) | Erlotinib (n=63)<br>Gefitinib (n=304)<br>Afatinib (n=81) | NA | NA | 11.2<br>12.1<br>10.3 | 10 (15.9)<br>34 (11.2)<br>4 (4.9) | 9 (14.3)<br>51 (16.8)<br>12 (14.8) | 44 (69.8)<br>219 (72.0)<br>65 (80.3) |

EGFR-TKIs=epidermal growth factor receptor tyrosine kinase inhibitors, NSCLC=non-small cell lung cancer, ADC=adenocarcinoma, ADS=adenosquamous carcinoma, SCC=squamous cell carcinoma, PFS=progression free survival, ORR=objective response rate, OS=overall survival, NA=not available

### Primary resistance

Primary resistance is defined as a failure to respond to treatment for the first time after receiving EGFR-TKIs, without any evidence of an objective response. Primary resistance in NSCLC patients can be divided into 4 classes: 1) harboring drug-resistant EGFR mutations; 2) genomic alterations along with drug-sensitizing EGFR mutations; 3) EGFR wild-type; and 4) unknown mechanisms[26]. The most prevalent mutations that result in primary resistance to EGFR-TKIs in drug-resistant EGFR mutation are the T790M mutation and insertions in exon 20[27].In addition, many studies have shown that genomic alterations, along with drug-sensitizing EGFR mutations, may be linked to primary resistance. These details are summarized in Table 3. Clinical samples, including tissue and blood, were obtained prior to treatment in patients treated with either of the EGFR first- or second-generation TKIs (gefitinib, erlotinib, afatinib, and icotinib).

The clinical definitions of primary resistance provided are diverse and non-uniform. Two studies define primary resistance to EGFR-TKIs as a combination of no evidence of objective response rate (ORR) and progression-free survival (PFS) for less than 3[11] and 4 months[28], respectively. In contrast, the other 2 studies only considered PFS of less than 6 months as the criterion for primary resistance[29,30]. The genomic alteration of all clinical samples was determined using target sequencing, which involved analyzing a set of 22-458 genes. The TP53 mutation was the most common genetic alteration reported in all of the studies. It was higher in cases conferring primary resistance than responders: 100.0% vs. 45.7%[28], 80.0% vs. 52.9%, and 87.5% vs. 16.7%[30]. Four genes were found in 3 studies exhibiting different mutation frequencies. CTNNB was found in 11.1% of patients with primary resistance[11], 5.0% in responders vs. 5.2% in non-responders[28], and 13.3% in short PFS vs. 17.6% in long PFS. SMAD4 was found in 11.1% of patients with primary resistance[11], 10% of non-responders vs 23.3% of non-responders[28], and 33.3% in short PFS[29]. PTEN was found in 5% of non-responders vs 1.7% of non-responders[28], 5.9% in long PFS[29], and 6.3% in short-term responders[30]. ERBB2-4 was found in 5-15% of non-responders vs 6.0-8.6% in responders[28], 6.7% in short PFS vs. 5.9% in long PFS[29], and 12.5% in short-term responders[30]. In addition, a study conducted by Jin et al. (2018) has shown that RB1, which is only found in the short PFS, has a high mutation frequency, accounting for 26.7% of cases. In contrast, another study by Xu *et.al.* (2018) observed that the frequency of RB1 mutation was comparable in both short-term responders (31.3%) and long-term responders (33.3%) (Table 3).

### Acquired resistance

EGFR-TKIs have been shown to have good response rates and prolong PFS in NSCLC patients with sensitive activating EGFR mutations. Despite the initial benefits, almost all patients will experience significant tumor progression or acquire resistance to EGFR-TKI. The clinical definition of acquired resistance is non-uniform. Jackman et al. (2010) have proposed a criteria for defining more precisely acquired resistance to EGFR-TKIs. This criteria consists of: 1) previously received treatment with a single-agent EGFR-TKI (first- or second-generation); 2) either or both of the following: a tumor that harbors an EGFR mutation known to be associated with drug sensitivity or objective clinical benefit from treatment with an EGFR-TKI (either partial/complete response or stable disease longer than 6 months. evaluated by RECIST or WHO); 3) systemic progression of disease while on continuous treatment with EGFR-TKI within the last 30 days; and 4) no intervening systemic therapy between cessations of EGFR-TKI and the initiation of a new therapy[10].

It is well known that a secondary point mutation that substitutes methionine for threonine at amino acid position 790 (T790M) is mainly the molecular mechanism

**Table 3** Genomic alterations in EGFR-mutant NSCLC with primary resistance to EGFR-TKI

| Study | Population | Sample/Drug | Sample collection/Number | Genomic alteration |
|---|---|---|---|---|
| Lee, 2013[11] | Korean | - FFPE<br>- Gef (n=179)<br>- Erl (n=18) | Definition of primary resistance:<br>- PFS <3 months<br>- no evidence of ORR<br>- 11 cases (11/197, 5.6%) | TP53 (3/9, 33.3%)/NOTCH1 (1/9, 11.1%)/CTNNB1 (1/9, 11.1%)/SMAD4 (1/9, 11.1%)<br>**Note:** Sequence only in cases of primary resistance |
| Lim, 2016[28] | Korean | - FFPE<br>- Gef (n=136) | Definition of primary resistance:<br>- PFS <4 months<br>- no evidence of ORR<br>- 20 cases (20/136, 14.7%) | **Non-responders:** TP53 (20/20, 100%)/DDR2, MET, PIK3CA, STK11 (4/20, 20%)/ERBB4, FGFR1-3, KRAS (3/20, 15%)/AKT1, ALK, MAP2K1, SMAD4 (2/20, 10%)/BRAF, CTNNB1, ERBB2, FBXW7, PTEN (1/20, 5%)<br>**Responders:** TP53 (53/116, 45.7%)/ SMAD4 (27/116, 23.3%)/ DDR2 (23/116, 19.8%)/ STK11 (18/116, 15.5%)/ MET (14/116, 12.1%)/ FGFR1-3 (11/116, 9.5%)/ ERBB4 (10/116, 8.6%)/BRAF (9/116, 7.8%)/ ERBB2 (8/116, 6.9%)/AKT1, PIK3CA (7/116, 6.0%)/ MAP2K1, NRAS, CTNNB1 (6/116, 5.2%)/ FBXW7 (5/116, 4.3%)/ NOTCH1, ALK (3/116, 2.6%)/ PTEN (2/116, 1.7%) |
| Jin, 2018[29] | Chinese | - FFPE/plasma<br>- Gef (n=17)<br>- Ico (n=51)<br>- Afa (n=1) | Definition of primary resistance:<br>- PFS <6 months (Short PFS; n=15)<br>- PFS >12 months (Long PFS; n=17) | **Short PFS:** TP53 (12/15, 80.0%)/SMAD4 (5/15, 33.3%)/RB1 (4/15, 26.7%)/ARID1A, PIK3CA (3/15, 20.0%)/CDC73, EP300, MCL1, AKT1, CTNNB1, STK11 (2/15, 13.3%)/TSHR, CDKN2B, CREBBP, JAK2, MLH1, MAP2K4, FGFR3, HGF, NRAS, PRKCI, CCNE1, PRF1, NKX2-1, ARAF, JUN, NBN, RICTOR, ARID2, FLT1, POT1, NOTCH2, DNMT3A, APC, EPHA3, ERBB3, FAT1, BLM, PDE11A, ROS1, PDK1, PTK2, ATR, PMS2, NFKBIA (1/15, 6.7%)<br>**Long PFS:** TP53 (9/17, 52.9%)/CTNNB1, PDK1 (3/17, 17.6%)/AKT1, FLT4, THADA (2/17, 11.8%)/AKT2, CDK6, TSHR, SMAD2, CTCF, PTEN, EP300, PIK3CA, MLH1, ERCC4, CHD4, CSF1R, GRIN2A, NF1, PPP2R1A, GATA1, PGR, NKX2-1, MYC, ARAF, JUN, NSD1, KDM5A, KMT2A, RAD51D, POT1, NOTCH2NL, CYP3A4, APC, EPHA2, ERBB3, FGFR4, SOX2, BUB1B, PDE11A,RAD50, PDCD1, ATRX, POLE, MDM2, RECQL4, SMARCA4, TNFSF11, TOP2A, YAP1 (1/17, 5.9%)<br>**Note:** Only found in Short PFS and confer primary resistance.<br>SMAD4, RB1, ARID1A, CDC73, STK1, MCL1, ARID2, DNMT3A, ATR, ARAF, HGF, PRKCI, CNE1, ARAF |
| Xu, 2018[30] | Chinese | - FFPE<br>- Gef (n=11)<br>- Ico (n=16)<br>- Erl (n=1) | Definition of primary resistance:<br>- PFS <6 months (Short-term responders; n=16)<br>- PFS >24 months (Long-term responders; n=12) | **Short-term responders:** TP53 (14/16, 87.5%)/EGFR amp. (6/16, 37.5%)/RB1 (5/16, 31.3%)/ ERBB2 (2/16, 12.5%)/PTEN, MET, AKT2, HRAS (1/16, 6.3%)/No mutation in AKT2<br>**Long-term responders:** RB1 (4/12, 33.3%)/TP53, AKT1 (2/12, 16.7%)/EGFR amp. (1/12, 8.3%)/ No mutation in PTEN, ERBB2, MET, AKT2, HRAS |

EGFR-mutant NSCLC=non-small cell lung cancer with EGFR mutation, EGFR-TKI=epidermal growth factor receptor tyrosine kinase inhibitor FFPE=formalin-fixed paraffin-embedded tissue, Gef=gefitinib, Erl=erlotinib, Ico=icotinib, Afa=afatinib

**Table 4** Genomic alterations in EGFR-mutant NSCLC with acquired resistance to EGFR-TKI

| Study | Population | Sample/Drug | Sample collection/Number | Genomic alteration |
|---|---|---|---|---|
| Jin, 2016[34] | Chinese | - FFPE/pleural effusions<br>- Gef (n=26)<br>- Erl (n=15)<br>- Ico (n=42) | - Post-treatment<br>- T790M+; 30 cases<br>- T790M-; 53 cases | **T790M+:** multiple EGFR (30/30, 100.0%)/TP53 (21/30, 70.0%)/TET1 (4/30, 13.3%)/APC, RB1 (2/30, 6.7%)/ERBB2, SOX2 (1/30, 3.3%)/No mutation in KRAS, MET, ALK, FGFR1<br>**T790M-:** multiple EGFR (26/53, 49.1%)/TP53 (27/53, 50.9%)/ TET1 (6/53, 11.3%)/ ERBB2 (5/53, 9.4%)/ KRAS, MET, SOX2 (4/53, 7.5%)/APC, RB1, ALK, FGFR1 (3/53, 5.7%) |
| Lee, 2017[32] | Korean | - FFPE/pleural effusions<br>- Gef (n=17)<br>- Erl (n=2) | - Pre-treatment and post-treatment<br>- T790M+; 30 cases<br>- T790M-; 12 cases | **T790M+:** SYNE1 (2/12, 16.7%)/ITGA10, BCL2L2, TGM7, MSH2, RET, MAGEA1, PIK3CG, LPHN3, CREBBP, CMPK1, NTRK3, GRM8, NOTCH4, PLCG1, AMER1, MAGEA1, MN1, DST*, TAF1*, FANCA*, KAT6B (1/12, 8.3%)<br>**T790M-:** **TP53*** (3/5, 60.0%)/ CSMD3*, AKAP9 (2/5, 40.0%)/SEPT9, EML4, MUTYH, AKT3, ATR, LIFR, ATRX, SOX2, CD79B, AXL, COL1A1, MAML2, CYP2D6, KMT2D, XPC, JAK2, MAF, AR*, CASC5, FGFR4, ERCC2, RAF1, PBX1, CTNNB1, NIN, NUP214, MYH9, NUP98, PDE4DIP, KDM5C, RNF213, RNASEL, TOP1, TGFBR2, TRIM24, IGF1R (1/5, 20.0%)<br>**Note:** * is significantly mutated genes in post-treatment compared to pre-treatment |
| Otsubo, 2019[31] | Japanese | - Plasma<br>- Gef (n=5)<br>- Erl (n=7)<br>- Afa (n=12) | - Post-treatment<br>- T790M+; 17 cases<br>- T790M-; 7 cases | **T790M+:** TP53 (4/7, 57.1%)/MET (3/7, 42.9%)/ERBB2 (1/7, 14.3%)<br>**T790M-:** MET (6/17, 35.3%)/TP53, PIK3CA, APC (5/17, 29.4%), multiple EGFR (4/17, 23.5%) |
| Chua, 2021[33] | Singaporean | - Fresh-frozen tissue<br>- 1st and 2nd EGFR TKI (n=59) | - Post-treatment<br>- T790M+; 38 cases<br>- T790M-; 21 cases | **T790M+:** TP53 (19/38, 50%)/FOXA1 (9/38, 24%)/amplification EGFR, NKX2-1, YEATS4, CDH10 (8/38, 21.0%)/MDM2, PIK3CA (7/38, 18%)/Arm Chr3q, FGFR4 (5/38, 13%)/RB1, CTNNB1 (3/38, 8%)/ERBB2, PTEN, HGF (2/38, 5%)/MET, AXIN1 (1/38, 3%)/ No mutation in PIK3CB<br>**T790M-:** TP53 (18/21, 86%)/ Arm Chr3q (12/21, 57%)/MET, HGF (4/21, 19%)/ amplification EGFR, PIK3CA (3/21, 14%)/ ERBB2, RB1, PIK3CB (2/21, 10%)/ MDM2, PTEN, FOXA1, NKX2-1, YEATS4 (1/21, 5%)/No mutation in FGFR4, APC, CTNNB1, AXIN1, CDH10 |

EGFR-mutant NSCLC=non-small cell lung cancer with EGFR mutation, Gef=gefitinib, Erl=erlotinib, Ico=icotinib, Afa=afatinib, FFPE=formalin-fixed paraffin-embedded, T790M+=T790M mutation, T790M-=non-T790M mutation

that produces acquired resistance after drug treatment. In addition, concurrent genomic alterations in genes other than EGFR may be responsible for acquired resistance (Table 4). Clinical samples, including tissues, pleural effusions and blood, were obtained at the time of acquired resistance to EGFR-TKI (gefitinib, erlotinib, afatinib, and icotinib). All studies have shown that patients having received EGFR-TKI develop the T790M mutation (T790M+) at: 70.8% by Otsubo et al. (2019)[31], 71.4% by Lee et al. (2017)[32], 64.4% by Chua et al. (2021)[33], and 36.1% by Jin et al. (2016)[34]. In addition, genetic alterations coexisting with T790M, as well as concurrent genomic alterations in genes other than EGFR, may be responsible for acquired resistance. TP53 was the most frequently mutated gene across all the studies, 2 of which showed both T790M+ *and* non-T790M- mutation (T790M-)[33,34], whereas in the other 2 studies, it was only shown to be present in T790M+[31] or T790M-[32]. In the T790M+, the mutation of multiple EGFR positions (100.0%)[34] and MET (42.9%)[31] was found in high frequencies. Similarly, high mutation frequencies have been identified at multiple EGFR positions (49.1%)[34], CSMD3 (40.0%)[32], AKAP9 (40.0%)[32], MET (35.3%)[31] and Arm Chr3q (57%%)[33].

## Conclusion

First or second-generation EGFR-TKIs are the approved first-line therapy for NSCLC with EGFR-activating mutations. This report provides a comprehensive overview of many studies evaluating the effectiveness of EGFR-TKI treatments in various populations of advanced NSCLC patients. The studies evaluated various outcomes, including ORR, OS and PFS. We additionally reviewed concurrent gene alterations related to both primary and acquired resistance. Our findings could aid in the identification of patients that may derive more benefits from EGFR-TKI treatment, even though possessing sensitive EGFR mutations.

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## Conflict of interest

The authors declare no conflict of interest.

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