

EGFR mutations in non-small cell lung cancer: Classification, characteristics and resistance to third-generation EGFR-tyrosine kinase inhibitors (Review)

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Abstract. Mutations in EGFR (mEGFRs) in non-small cell lung cancer (NSCLC) are key factors driving tumor development and treatment response. The present article aimed to review the classification, characteristics and molecular mechanisms of resistance to third-generation EGFR-tyrosine kinase inhibitors (EGFR-TKIs) associated with mEGFRs. Activating mutations (such as L858R and exon 19 deletions) are the primary markers of sensitivity to EGFR-TKIs, while rare mutations (such as G719X and S768I) require individualized treatment strategies. Resistance mechanisms are categorized into EGFR-dependent (such as T790M and C797S mutations) and -independent (bypassing signaling activation, epithelial-mesenchymal transition, tumor microenvironment remodeling and epigenetic regulation). Third-generation EGFR-TKIs (such as osimertinib) markedly improve patient survival by selectively targeting the T790M mutation, but novel resistance mutations, such as C797S, limit their long-term efficacy. Combination therapies (such as MET proto-oncogene, receptor tyrosine kinase/EGFR dual-target inhibitors) and fourth-generation TKIs (such as BLU-945) offer novel directions to overcome resistance. Future research should focus on precise subtyping, dynamic monitoring of resistance

mechanisms and regulation of the immune microenvironment to advance personalized treatment for NSCLC.

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1. Introduction

Non-small cell lung cancer (NSCLC) represents 80-85% of all lung cancer cases, making it a notable contributor to cancer morbidity and mortality worldwide (1). The incidence of NSCLC is increasing, particularly in countries such as China, where it is the leading cause of cancer-associated mortality (2). Risk factors for NSCLC include tobacco use, family history and environmental exposure, alongside chronic lung diseases such as chronic obstructive pulmonary disease, which further exacerbate the risk (3). The clinical importance of NSCLC lies not only in its high prevalence but also in its poor prognosis, with numerous patients diagnosed at advanced stages when treatment options are limited, and survival rates are low (4).

Mutations in the EGFR gene (mEGFRs) are prevalent in NSCLC, occurring in ~40% of patients in Asian populations and ~20% in non-Asian populations (5). These mutations serve a key role in the pathogenesis of NSCLC, leading to uncontrolled cell proliferation and survival (4). Among mutations, the EGFR T790M mutation is a key driver of resistance to first- and second-generation EGFR-tyrosine kinase inhibitors (TKIs) (6). Understanding the biological role of mEGFRs is crucial, as they not only dictate the therapeutic approach but also influence patient outcomes and survival rates (7).

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Third-generation EGFR-TKIs (such as osimertinib) are designed to selectively target both activating mutations [such as L858R and exon 19 deletions (19del)] and the T790M mutation via irreversible binding to the EGFR kinase domain. Their enhanced selectivity for mutant over wild-type EGFR decreases off-target toxicity, making them preferable first-line options for advanced NSCLC. However, the emergence of resistance mutations (such as C797S) is a challenge.

The present review aimed to summarize mEGFRs in NSCLC, the role of third-generation EGFR-TKIs and the challenges associated with resistance mechanisms to highlight the importance of targeted therapies in improving patient outcomes and elucidate potential pathways for overcoming resistance to EGFR-TKIs. Understanding these dynamics is key for optimizing treatment strategies and enhancing the clinical management of patients with NSCLC.

2. Classification of mEGFRs

Activating mutations (such as L858R and 19del). Activating mutations in the EGFR gene are key in the pathogenesis of NSCLC (8). The most common of these mutations include the L858R point mutation and 19del (Fig. 1). These mutations lead to constitutive activation of the EGFR signaling pathway, promoting tumor cell proliferation and survival. Studies have demonstrated that patients harboring these mutations respond favorably to first-line EGFR-TKIs, such as gefitinib and erlotinib (9,10). For example, in a retrospective study involving 532 patients, the median PFS for patients with the L858R mutation receiving first-line TKI treatment was 11.4 months, while wild-type patients had a poorer response to TKI (median PFS typically below 3 months) (11).

The presence of 19del is associated with a higher sensitivity to EGFR-TKIs. This association has been reported in several previous studies (12,13), highlighting that patients with NSCLC harboring these specific mutations exhibit improved clinical outcomes when treated with EGFR-TKIs compared with those with other mutations, such as the L858R mutation (14). For example, patients with 19del had markedly longer PFS compared with patients with the L858R mutation, indicating a more favorable response to treatment (14).

Moreover, the coexistence of the T790M mutation, which is known to confer resistance to first-generation TKIs, has been reported to be more prevalent in patients with the L858R mutation compared with patients with 19del (15). This suggests that 19del not only predicts an improved response to initial treatment but may also be less likely to be associated with resistance mutations that complicate therapy (16). In addition, previous research indicates that certain exon 19 variants exhibit decreased ATP-binding affinity, which is associated with increased sensitivity to specific TKIs, such as afatinib (17). Thus, understanding the specific types of activating mutation is essential for tailoring personalized therapeutic strategies in NSCLC.

Rare mutations (such as G719X and S768I). Rare mEGFRs, such as G719X and S768I, represent a unique subset of mEGFR alterations that influence treatment outcomes. Although less frequent than L858R and 19del, these mutations have been shown to confer sensitivity to EGFR-TKIs, albeit with variable response rates (18-20).

Previous studies have highlighted that patients with NSCLC exhibiting G719X and S768I mutations may experience favorable outcomes when treated with second-generation TKIs, suggesting these agents could be optimal choices for this patient population (21,22). For example, the objective response rate (ORR) for patients with G719X mutations treated with afatinib reached 77.8%, with a median progression-free survival (PFS) of 13.8 months, and similar efficacy was observed for the S768I mutation (11). By contrast, the ORR for G719X patients using first-generation TKIs (such as gefitinib or erlotinib) was only 42.9%, with a median PFS of 5.98 months (18). A retrospective study further confirmed that the median PFS for patients treated with afatinib who carry the S768I compound mutation (such as S768I+L858R) reached 9.79 months, significantly better than gefitinib's 4.6 months ($p=0.049$) (19). Furthermore, the presence of these mutations complicates treatment decisions, as they may coexist with other mutations, leading to variability in response to therapy (23). For example, a previous study demonstrated that patients with lung adenocarcinoma harboring the G719X mutation often present compound mutations, such as G719X combined with S768I or L861Q, which can alter the efficacy of TKIs such as gefitinib and erlotinib (23). A retrospective analysis reported that patients with the G719X mutation alone have a different response rate compared with those with compound mutations involving S768I: The objective response rate for patients with G719X-only mutations is reported to be higher compared with patients with G719X combined with other mutations (24).

A previous study reported a patient with both G719X and S768I mutations who responded well to afatinib, maintaining disease control for an extended period. For example, a case study reported a 67-year-old male patient with G719X and S768I compound mutations. After failing initial chemoradiotherapy, he received afatinib treatment (40 mg/day), achieving partial remission and maintaining disease control for up to 17 months (25). This result is consistent with the conclusions of several multicenter studies: the G719X mutation (especially when present in combination with S768I) is associated with a longer PFS (26-28). Specifically, a real-world study in Vietnam showed that patients with the G719X-S768I compound mutation had a median time to treatment failure (TTF) of 23.2 months, significantly better than other mutation types (12.3 months) (26). A multicenter study in Taiwan also confirmed that the G719X mutation (whether as a single mutation or in combination) is an independent favorable predictor of PFS ($HR=0.578$), with an overall median PFS of 17.3 months (27). Furthermore, a retrospective analysis in Spain further indicated that the objective response rate (ORR) in the compound mutation group (including G719X+S768I) reached 70%, significantly higher than in other mutation subgroups (28). The clinical importance of these rare mutations underscores the necessity for comprehensive genetic testing in patients with lung cancer, as they may benefit from targeted therapies that are typically reserved for more common mutations. Further research is needed to elucidate the mechanisms underlying the differential responses observed in patients with these rare mutations, which may inform future therapeutic development and clinical guidelines (8).

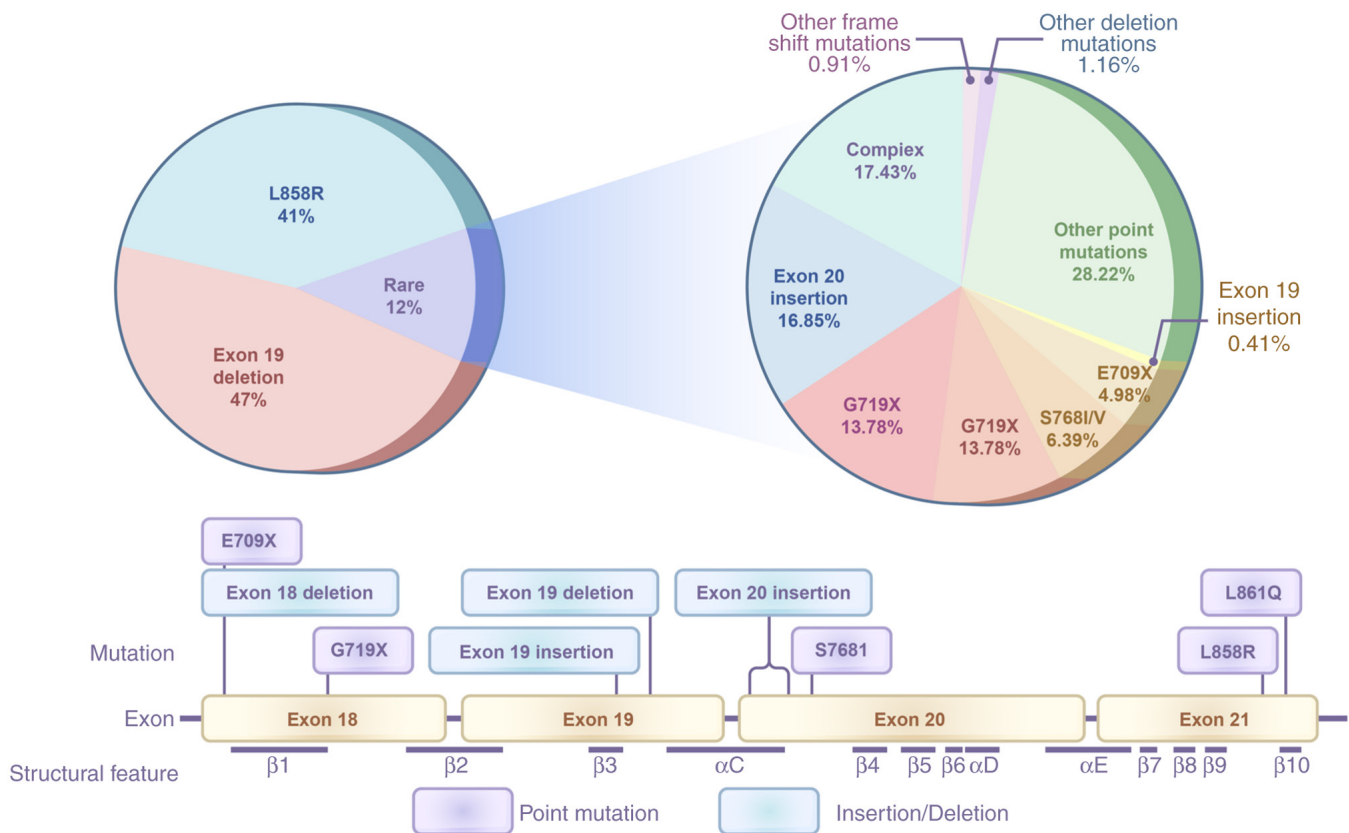


Figure 1. Mutation types of EGFR and EGFR kinase domain structure, marking high-frequency mutation sites and drug binding areas. Reproduced from a previous study (14) Figure created using Figdraw (figdraw.com) and Home for Researchers (www.home-for-researchers.com).

Other mutations (such as T790M and C797S). The T790M mutation is an acquired alteration in EGFR and occurs in 50-60% of NSCLC cases (29). By contrast, the C797S mutation represents a tertiary resistance mechanism specific to third-generation EGFR-TKIs (such as osimertinib). Both mutations are clinically notable markers of therapeutic resistance, and their detection necessitates tailored treatment strategies (30).

3. Characteristics of mEGFRs

Association between mutation frequency and clinical features. The association between mEGFR frequency and clinical features in NSCLC has been a subject of extensive research. Several studies have demonstrated that the prevalence of mEGFRs is notably influenced by demographic and clinical characteristics such as age, sex, smoking status and histological subtype (11,15,18). For example, a study involving 388 patients with NSCLC demonstrated that mEGFRs are more prevalent in younger patients, particularly those aged <80 years, with a mutation rate of 49.6% compared with 24.1% in older patients (31). This suggests that age may serve a key role in the mutation landscape of NSCLC. Additionally, female patients had a higher incidence of mEGFRs, aligning with findings from other cohorts that report a strong association between female sex and mEGFR status (32).

Smoking status is an important factor influencing mEGFR frequency. Research has consistently reported that mEGFRs are more common in never-smokers compared

with smokers (26,33). For example, a cohort study from India reported a notable association between mEGFRs and non-smoking status, with EGFR mutations being statistically significant among non-smokers compared to smokers (58.7% vs. 18.4%) (32). This trend is echoed in other studies, reinforcing the hypothesis that smoking may be negatively associated with the likelihood of mEGFRs in patients with NSCLC (34,35).

Histological subtype also affects mEGFR frequency. Adenocarcinoma, the most common subtype of NSCLC, is associated with a higher rate of EGFR mutations compared with squamous cell carcinoma. In 697 Chinese patients, mEGFRs were detected in 52.9% of adenocarcinomas, while 14.5% of squamous cell carcinomas exhibited similar mutations (36). This highlights the value of histological classification in understanding the mutation profile of NSCLC.

Association between mutation type and prognosis. The types of mEGFR impact the prognosis of patients with lung adenocarcinoma. For example, a meta-analysis indicated that patients with the 19del mutation exhibit a higher objective response rate and longer PFS compared with those with the L858R mutation in exon 21 (37). This suggests the 19del mutation may serve as a more effective clinical marker for predicting responses to EGFR-TKIs in patients with NSCLC (37). Furthermore, the presence of concomitant mutations alongside the primary mEGFRs also impacts prognosis. Patients with both 19del and L858R mutations had different sensitivity to EGFR-TKIs, with 19del showing a more favorable response (38).

Moreover, the prognostic value of TP53 mutations in conjunction with mEGFRs has been explored: TP53 mutations are associated with worse overall survival in advanced NSCLC, particularly when coupled with specific mEGFR types (39). This highlights the complexity of the molecular landscape in NSCLC and the need for comprehensive genetic profiling to improve prediction of patient outcomes.

Mechanisms influencing the tumor microenvironment (TME). The role of mEGFR in NSCLC extends beyond its function as a driver of tumor growth; it notably influences the TME. The TME is a complex ecosystem composed of several cell types, including cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and immune cells, which interact dynamically with cancer cells (40,41). A key mechanism by which mEGFR affects the TME is the modulation of CAFs. CAFs promote tumor progression and drug resistance by creating an immunosuppressive environment. Studies have demonstrated that mEGFR signaling enhances the recruitment and activation of CAFs, leading to increased secretion of pro-tumorigenic factors that further support cancer cell survival and proliferation (42,43). This not only facilitates tumor growth but also contributes to the remodeling of the extracellular matrix (ECM), which can hinder the efficacy of therapeutic agents. The aberrant deposition of ECM components (such as collagen and laminin) establishes a physical barrier that impedes drug penetration, limiting the efficacy of EGFR-TKIs such as osimertinib in tumor cores (44). Concurrently, EGFR activation upregulates MMP2/MMP9, which not only degrade the basement membrane to facilitate metastasis but also liberate ECM-sequestered growth factors (e.g., TGF- β and VEGF) to reactivate PI3K/AKT and MAPK signaling pathways, thereby bypassing targeted therapy (45,46). Integrin-mediated interactions with remodeled ECM components (e.g., fibronectin) further activate FAK/SRC/STAT3 survival cascades, fostering cell adhesion-mediated drug resistance (44).

Moreover, mEGFR influences the polarization of TAMs within the TME. Elevated levels of mEGFRs are associated with an increase in M2-polarized macrophages, which are known to support tumor growth and metastasis (47). This shift in macrophage polarization is mediated by several signaling pathways, including the STAT5A-indoleamine 2,3-dioxygenase 1 axis, which promotes an immunosuppressive environment in NSCLC (48). By enhancing the presence of M2 macrophages, mEGFR contributes to immune evasion, allowing tumor cells to escape detection and destruction by the immune system.

Additionally, the interaction between mEGFR and immune checkpoint molecules, such as programmed death-ligand 1 (PD-L1), is key in shaping the TME. mEGFR signaling can upregulate PD-L1 expression on tumor cells, which inhibits T cell activation and proliferation, further exacerbating the immunosuppressive landscape of the TME (49). This dynamic interaction highlights the potential for combining mEGFR inhibitors with immune checkpoint inhibitors to enhance anti-tumor immunity and improve patient outcomes.

4. Mechanism of action of third-generation EGFR-TKIs

Characteristics of drugs targeting mEGFRs. Drugs targeting mEGFRs are a cornerstone in the treatment of NSCLC (Fig. 2).

These targeted therapies, primarily TKIs, have shown notable efficacy in patients harboring specific mEGFRs, such as 19del and the L858R point mutation. A notable TKI is gefitinib, which was one of the first agents approved for mEGFR-positive NSCLC by the US FDA. It works by competitively inhibiting the ATP-binding site of the EGFR TK domain, thereby blocking downstream signaling pathways that promote tumor growth and survival (50). Gefitinib can significantly inhibit the phosphorylation of ERK1/2 in the MAPK/ERK pathway, leading to downregulation of Cyclin D1 expression and cell cycle arrest (51). At the same time, gefitinib promotes the upregulation of the pro-apoptotic protein Bim and activates Caspase-3/7 by inhibiting the phosphorylation of AKT at the Ser473 site in the PI3K/AKT/mTOR pathway, inducing apoptosis in tumor cells (52). In addition, gefitinib can block the abnormal activation of the JAK2/STAT3 pathway, reducing STAT3 nuclear translocation, thereby inhibiting the secretion of the angiogenic factor VEGF and the expression of the tumor stem cell marker CD133 (53). Erlotinib is another TKI used in combination with chemotherapy to enhance treatment efficacy (54). Afatinib, a second-generation TKI, has been developed to irreversibly bind to EGFR, providing a broader spectrum of activity against mEGFRs (50).

Despite the initial success of these therapies, resistance to mEGFR-targeted treatments remains a challenge. Mechanisms of resistance can include secondary mEGFRs, such as T790M, which can diminish the effectiveness of first-generation TKIs (55). This has led to the development of third-generation inhibitors, such as osimertinib, which are designed to target both the primary mEGFRs and the T790M mutation (55). In addition to TKIs, combination therapies that may enhance the efficacy of mEGFR-targeted treatments. For example, combining TKIs with immune checkpoint inhibitors or other targeted agents may provide synergistic effects and overcome resistance mechanisms (56). Furthermore, the identification of predictive biomarkers, such as specific serum metabolites, may aid in stratifying patients who are most likely to benefit from mEGFR-targeted therapy (54).

Comparison of third- and second-generation EGFR-TKIs. Second-generation EGFR-TKIs, such as afatinib and dacomitinib, have demonstrated improved efficacy compared with first-generation agents such as gefitinib and erlotinib (57). However, the introduction of third-generation EGFR-TKIs, particularly osimertinib, has advanced treatment options, especially for patients who develop resistance due to the T790M mutation (58,59). Third-generation EGFR-TKIs are designed to selectively target both the activating mutations and the T790M mutation, providing a clinical advantage over second-generation agents (60). For example, osimertinib has shown a median overall survival of 38.6 months in patients with NSCLC, which was significantly better than the control group (31.8 months) (58). Furthermore, third-generation inhibitors have a more favorable safety profile, with fewer adverse effects compared with their predecessors (61).

Despite these advancements, resistance to third-generation TKIs is a challenge, with the emergence of tertiary mutations such as C797S (62). This has prompted research into fourth-generation EGFR-TKIs aimed at overcoming these novel resistance mechanisms (63). By contrast, second-generation

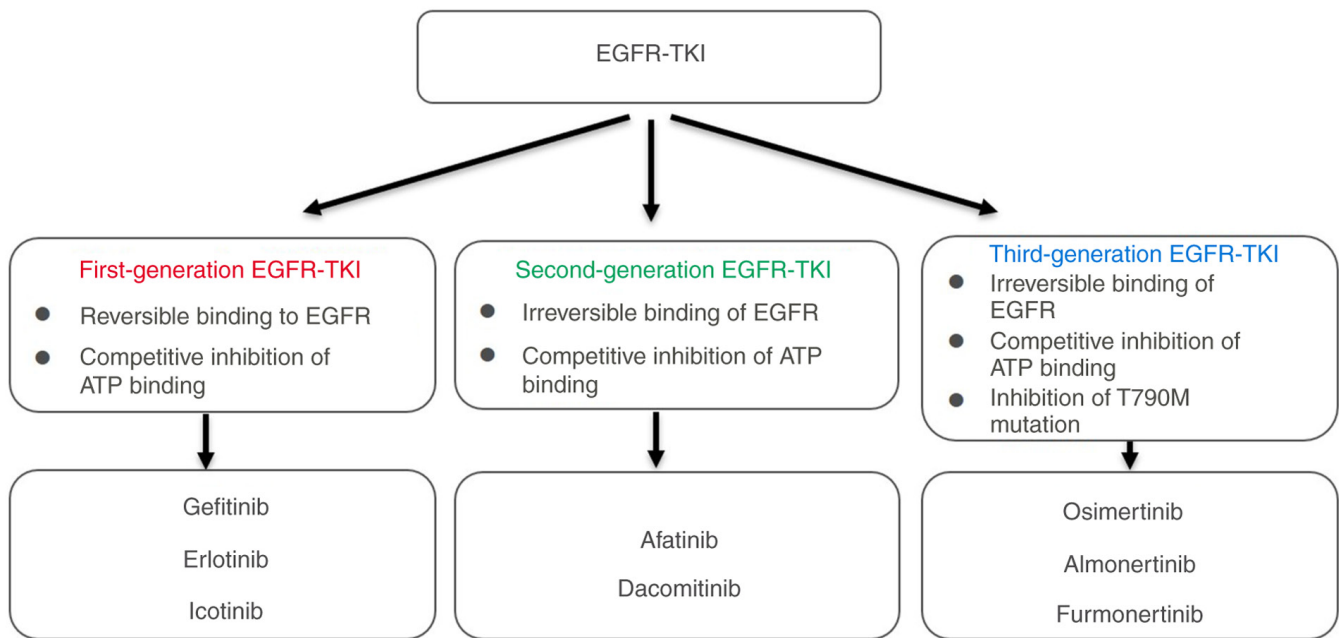


Figure 2. Types of EGFR-TKI. First generation EGFR-TKIs (gefitinib, erlotinib, icotinib) reversibly bind to EGFR, competitively inhibiting ATP. Second generation EGFR-TKIs (afatinib, dacomitinib) irreversibly bind EGFR, competitively inhibiting ATP. Third generation EGFR-TKIs (osimertinib, amivantamab, furmonertinib) irreversibly bind and inhibit the T790M mutation. TKI, tyrosine kinase inhibitor.

TKIs, while effective, often lead to resistance through several pathways, including the activation of alternative signaling routes (64).

Association between drug selectivity and resistance. The relationship between drug selectivity and resistance in the context of EGFR-TKIs is complex and multifaceted. Third-generation TKIs, such as osimertinib, are designed with enhanced selectivity for mutant over wild-type EGFR, which decreases the likelihood of adverse effects associated with off-target inhibition (65). However, the emergence of resistance is a notable clinical challenge. Studies indicate that while the selective pressure exerted by these drugs can initially lead to substantial tumor regression, it can also promote the selection of resistant clones that harbor secondary mutations, such as C797S or alterations in the MET proto-oncogene, receptor tyrosine kinase (MET) pathway (66,67). This underscores the importance of understanding the genetic landscape of tumors when administering third-generation TKIs. Additionally, combination therapies that target multiple pathways may be necessary to mitigate resistance and prolong the effectiveness of treatment. Ongoing research aims to identify biomarkers that can predict resistance and tailor treatment strategies, thereby improving outcomes for patients with mEGFR-NSCLC (68,69).

5. Mechanisms of resistance

EGFR-dependent resistance. The secondary mEGFR T790M is caused by the substitution of threonine with methionine in the tyrosine kinase domain of EGFR. This mutation increases the affinity of the ATP binding site, leading to spatial hindrance that interferes with the binding of TKIs, resulting in resistance (70). The C797S mutation is caused by the substitution of cysteine with serine at the C797 site of EGFR. This

mutation eliminates the covalent binding site between TKIs and EGFR, rendering third-generation TKIs such as osimertinib ineffective (71). In addition to the T790M and C797S mutations, the L718Q mutation is also noteworthy. Studies have demonstrated that the L718Q mutation can coexist with other mutations such as C797S, further enhancing resistance to osimertinib (72,73). To address these challenges, researchers are developing fourth-generation EGFR-TKIs, such as BLU-945, specifically targeting T790M/C797S mutations. These novel inhibitors overcome resistance issues through higher selectivity and specificity (74). Additionally, dual-target inhibitors such as aurora kinase B (AURKB)/EGFR inhibitors enhance therapeutic effects and delay the onset of resistance by simultaneously targeting multiple signaling pathways (MAPK and PI3K pathways) (8). Highly selective allosteric inhibitors represent another promising strategy. These inhibitors suppress EGFR activity by altering its conformation without directly competing with the ATP binding site, potentially decreasing the impact on wild-type EGFR and lowering toxicity. This strategy shows promise in overcoming resistance caused by EGFR T790M and C797S mutations (75).

EGFR amplification compensates for the inhibitory effects of EGFR-TKIs by upregulating EGFR levels, leading to resistance to EGFR-TKIs. This resistance mechanism has been reported in multiple studies (76,77). EGFR amplification can counteract the inhibitory effects of TKIs by enhancing the activity of the EGFR signaling pathway. For example, amplification of the wild-type EGFR allele is sufficient to confer acquired resistance to selective mutant EGFR-TKIs (78). This indicates that even in the context of mEGFRs, signaling from wild-type EGFR serves a notable role in resistance. Secondly, EGFR amplification is not limited to the EGFR signaling pathway but may also involve the activation of other bypass signaling pathways. For example, MET amplification and

MET receptor activation are also mechanisms of EGFR-TKI resistance (79). The activation of these bypass signaling pathways maintains cell proliferation and survival through different mechanisms, such as cross-activation of receptor TKs. Additionally, EGFR amplification may interact with other genetic alterations to enhance resistance. For example, in patients with advanced NSCLC, EGFR amplification coexists with the T790M mutation, and when treated with third-generation EGFR-TKIs (such as Osimertinib), patients experience improved PFS compared to those without acquired EGFR amplification (80). This suggests that EGFR amplification may synergize with other resistance mechanisms, affecting treatment outcomes.

Non-EGFR-dependent resistance. A mechanism involved in non-EGFR-dependent resistance is bypass signal activation. In addition to the T790M mEGFR, MET gene amplification is an important mechanism of resistance to first- or second-generation EGFR-TKIs (81). In certain studies (81,82), MET amplification has been identified as a key mechanism of acquired resistance to third-generation EGFR-TKIs (such as osimertinib), particularly when used as first-line treatment. In NSCLC with mEGFRs, MET amplification or protein over-activation may lead to escape from EGFR inhibition via the PI3K/AKT/mTOR pathway (83). To overcome this resistance, previous research suggests combining osimertinib with MET or MEK inhibitors (81). Furthermore, HER2 amplification is also regarded as an acquired resistance mechanism to EGFR inhibition (84). First, HER2 amplification can mediate resistance by enhancing the activity of the EGFR signaling pathway. Both EGFR and HER2 belong to the ErbB receptor family, and the overexpression or amplification of HER2 can activate downstream signaling pathways by forming heterodimers, thereby counteracting the inhibitory effects of EGFR-TKIs (84). Secondly, HER2 amplification may also promote resistance by affecting other signaling pathways. For example, studies have found that HER2 amplification can activate the PI3K/Akt signaling pathway, which plays a key role in cell proliferation and survival (85). Previous studies have reported that HER2 amplification and mEGFR (T790M) are mutually exclusive, revealing a mechanism of resistance to EGFR-TKIs and providing a rationale for assessing HER2 status and potentially targeting HER2 (76,84). Although there are currently limited standard treatment options for HER2 abnormalities, previous studies have demonstrated that the third-generation TKI osimertinib exhibits strong antitumor efficacy in lung cancer with HER2 abnormalities, providing a strong basis for future clinical trials (86,87).

Another mechanism involved in non-EGFR-dependent resistance is epithelial-mesenchymal transition (EMT). In NSCLC, zinc finger E-box binding homeobox 1 (ZEB1) and Twist family BHLH transcription factor 1 (TWIST1) are key transcription factors that serve important roles in the EMT process. ZEB1 is a notable regulator of EMT, promoting cell mesenchymalization by inhibiting the expression of E-cadherin, thereby enhancing the invasive and migratory ability of cells (88). Studies have demonstrated that the upregulation of ZEB1 is associated with the development of TKI resistance, especially in mEGFR NSCLC cells (89,90). Additionally, ZEB1 promotes the occurrence of EMT through

interactions with other signaling pathways, such as the TGF- β signaling pathway (91). TWIST1 is also an important regulator of EMT, facilitating EMT by regulating genes (such as E-cadherin) associated with cytoskeletal reorganization and cell adhesion (92). Furthermore, TWIST1 enhances the effects of EMT through interactions with other signaling molecules, such as STAT3 (93).

In response to EMT-mediated resistance, researchers have proposed potential therapeutic strategies. For example, inhibiting the EMT-associated transcription factor TWIST1 enhances the efficacy of EGFR-TKIs (92). Studies have demonstrated that genetic silencing of TWIST1 or the use of TWIST1 inhibitors can suppress the growth of mEGFR NSCLC and induce apoptosis, thereby overcoming resistance (92,94). Additionally, glycogen synthase kinase-3 (GSK-3) inhibitors reverse EMT-associated resistance, particularly in resistant cells with a mesenchymal phenotype, where GSK-3 inhibitors markedly inhibit cell proliferation and induce apoptosis (95). Another strategy is the combined use of EGFR and fibroblast growth factor receptor (FGFR) inhibitors to suppress the survival and proliferation of resistant cells in mEGFR NSCLC. Dual inhibition of EGFR and FGFR can suppress proliferation of resistant cells over an extended period, preventing the development of fully resistant cancer (96). Dual inhibition of EGFR and FGFR can suppress cancer cell proliferation by blocking the cross-activation of these two signaling pathways. For example, in NSCLC, resistance to EGFR inhibitors is often associated with the activation of the FGFR signaling pathway. By simultaneously inhibiting EGFR and FGFR, cancer cell proliferation and survival is reduced (97). Secondly, dual inhibition of EGFR and FGFR can enhance apoptosis by inhibiting downstream signaling pathways such as the MEK/ERK and mTOR pathways. Studies have shown that feedback activation of EGFR and FGFR limits the efficacy of single FGFR inhibitors, while inhibition of EGFR can enhance the effects of FGFR inhibitors, leading to more significant apoptosis and tumor shrinkage (97). Additionally, dual inhibition of EGFR and FGFR can limit the development of resistance by reducing the frequency of tumor stem cells. Dual inhibition of EGFR and FGFR can decrease the frequency of tumor-initiating cells, thereby enhancing the response to radiotherapy and other treatments (98). These findings provide a theoretical basis for developing novel treatment regimens and offer new insight for overcoming EMT-mediated resistance.

TME remodeling is also involved in non-EGFR-dependent resistance. TAMs serve an important role in the TME, as they weaken the therapeutic effect of EGFR-TKIs by inhibiting the activation of CD8⁺ T cells (99). In addition, the ECM in the TME is an important factor in EGFR-TKI resistance. Even in the absence of genetic changes, ECM can immediately confer resistance to EGFR-TKIs in tumor cells through its interaction with integrin β 1. This effect is dose-dependent and reversible, suggesting that targeting the ECM and integrin β 1 may be a potential strategy for treating resistance (100). Moreover, immune evasion mechanisms in the TME may lead to EGFR-TKI resistance. EGFR-TKI resistance may promote immune evasion by upregulating the expression of PD-L1. Studies have found that the expression of PD-L1 is significantly increased in NSCLC cells resistant to EGFR-TKIs, which may facilitate tumor immune evasion by inhibiting

lymphocyte activation and cytotoxicity (101,102). EGFR-TKI resistance may enhance the immunosuppressive microenvironment by inhibiting the anti-tumor function of CD8⁺ T cells. Furthermore, RNA methylation modifications may also play a role in the immune evasion associated with EGFR-TKI resistance. Research indicates that in EGFR-TKI resistant NSCLC, the inhibition of the m6A RNA demethylase ALKBH5 leads to the upregulation of CD47, thereby triggering immunosuppression (103). This mechanism may promote tumor immune evasion by inhibiting the phagocytic activity of dendritic cells and the cytotoxicity of CD8⁺ T cells. Finally, EGFR-TKI resistance may also reshape the tumor microenvironment from a non-inflammatory state to an inflammatory state, thereby affecting the infiltration and function of immune cells. Studies have found that after EGFR-TKI treatment, pro-inflammatory signals such as interferon- γ and inflammatory responses are significantly enriched in tumor samples, which may be associated with upregulation of effector cells after resistance (104).

Epigenetic regulation also serves a key role in EGFR-TKI resistance, including changes in histone modification, DNA methylation and non-coding RNA (ncRNA), which provide potential therapeutic targets and biomarkers for overcoming drug resistance (105). In the mechanisms of EGFR-independent resistance, epigenetic regulation affects the survival and proliferation of cancer cells through several pathways. For example, the loss of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) is associated with EGFR-TKI resistance, as it influences cell proliferation and resistance by regulating the expression and phosphorylation of MET (106). Additionally, changes in the expression of ncRNAs, such as nc886. In the study of the HCC827 NSCLC cell line, it was found that the expression of nc886 is not related to EMT (epithelial-mesenchymal transition), but the CRISPR/Cas9-mediated disruption of its sequence can increase the sensitivity of cells to TKIs. Furthermore, the disruption of the nc886 sequence hinders the activation of MET RTK, which is a mechanism of TKI resistance with EMT as the endpoint (107). To address the resistance induced by these epigenetic regulations, researchers have proposed various therapeutic strategies: Combination therapy with PI3K/AKT inhibitors and EGFR-TKIs breaks the MET-AKT-EZH2 feedback loop, thereby enhancing tumor suppression effects (106). Furthermore, targeted therapies against ncRNAs are potential strategies to overcome resistance by modulating their expression or function to restore drug sensitivity (107).

6. Clinical applications and future research directions

Personalized treatment strategies for mEGFRs. The advent of targeted therapies has revolutionized the management of NSCLC, particularly in patients harboring mEGFRs. These mutations are key in dictating the responsiveness to TKIs, such as gefitinib and erlotinib. Personalized treatment strategies that consider the specific type of mEGFR are key for optimizing therapeutic outcomes. For example, 19del and the L858R point mutation exhibit distinct responses to TKIs, necessitating tailored approaches (108). Recent studies have underscored the importance of comprehensive genomic profiling prior to treatment initiation, which can guide clinicians in selecting the most effective agent and dosage for individual patients, thereby

enhancing efficacy and minimizing adverse effects (109,110). Furthermore, Combination therapy, where TKIs are paired with immunotherapies or chemotherapy, aims to overcome resistance mechanisms that often develop during treatment (111). The integration of artificial intelligence in analyzing patient data and predicting treatment responses presents a promising avenue for future research, potentially leading to more refined and effective personalized treatment protocols (112).

Advances in the development of novel targeted drugs. Previous studies have highlighted the importance of understanding the molecular mechanisms of NSCLC to identify potential therapeutic targets (113,114). For example, the discovery of mEGFRs and mutations in genes such as anaplastic lymphoma kinase (115) and KRAS (116) has led to the development of targeted agents that specifically inhibit these oncogenic drivers. The implementation of these therapies has shown promising results in clinical trials, demonstrating improved response rate and survival outcomes compared with conventional treatment (117,118). Furthermore, the integration of immunotherapy into the treatment paradigm harnesses the immune system to combat cancer cells, offering hope for patients with advanced disease (119).

Despite these advancements, challenges remain in the clinical application of targeted therapies for NSCLC. A notable hurdle is the development of resistance to these agents, which can occur through various mechanisms, including secondary mutations and activation of alternative signaling pathways. Understanding these resistance mechanisms is key for developing strategies to overcome them, such as combination therapies that target multiple pathways simultaneously (120,121). Additionally, the identification of robust predictive biomarkers is key for patient stratification, ensuring that individuals most likely to benefit from specific therapies are selected for treatment (121,122).

There is a need for clinical trials to evaluate the efficacy of new targeted agents and combination therapy, particularly in biomarker-selected populations. Secondly, the exploration of novel molecular targets and therapeutic strategies, including the potential repurposing of existing drugs, may provide additional avenues for treatment (123,124). Lastly, enhancing the understanding of the TME and its role in cancer progression is key for developing innovative therapies that effectively combat NSCLC (125).

7. Conclusion

mEGFRs serve a key role in the molecular classification and treatment of NSCLC. Activating mutations (L858R/19del) are sensitive to EGFR-TKIs but prone to secondary T790M/C797S resistance; rare mutations (G719X/S768I) require second-generation TKIs or combination therapy. mEGFRs are also involved in complex resistance mechanisms. EGFR-dependent resistance (such as T790M/C797S) diminishes TKI efficacy through steric hindrance or covalent binding site disruption, whereas EGFR-independent resistance (MET/HER2 amplification, EMT, TME remodeling) relies on bypass activation or immune evasion, necessitating multi-target intervention (104). The present review highlighted the innovative treatment strategies for NSCLC. Fourth-generation TKIs

(BLU-945) and dual-target inhibitors (AURKB/EGFR) target resistant mutations and combining immune checkpoint inhibitors or epigenetic modulators (such as histone deacetylase inhibitors) can reverse resistance (126). Future studies should integrate liquid biopsy, artificial intelligence classification and multi-omics data to achieve dynamic monitoring of resistance mechanisms. In addition, evaluating the immune interactions of TME and developing new targeted drugs (such as CD47 inhibitors) may be key to precision treatment for NSCLC.

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Authors' contributions

ZT, LC and FW conceived the study, performed the literature review and wrote the manuscript. JueD, YuH and YiH performed the literature review and revised the manuscript. ZW and JunD wrote the manuscript. YJ reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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