Targeted Therapy and Drug Resistance of EGFR Mutations in Non-Small Cell Lung Cancer

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Abstract. Lung cancer remains one of the most lethal malignancies, with non-small cell lung cancer (NSCLS) being the predominant subtype. Activating mutations in the epidermal growth factor receptor (EGFR) increase the receptor's affinity for ATP, leading to sustained downstream signaling and uninhibited tumor proliferation. Small-molecule EGFR inhibitors have transformed treatment, with Osimertinib significantly prolonging progression-free and overall survival while improving control of brain metastases. Long-term treatment inevitably leads to drug resistance, often through targeted secondary or tertiary EGFR mutations (e.g., T790M and C797S), activation of bypass pathways involving MET, HER2, and FGFR, and histologic or phenotypic transformation. These challenges highlight the need for personalized treatment strategies guided by molecular profiling and active monitoring. In recent years, treatment strategies have expanded to include fourth-generation EGFR allosteric inhibitors, antibody-drug conjugates, rational combinations targeting alternative signaling pathways, and immunotherapy. This review will summarize the current evidence for EGFR-targeted therapies, highlight established and emerging mechanisms of resistance, and discuss emerging approaches that may help optimize clinical outcomes.

Keywords: Tyrosine kinase inhibitors, EGFR, T790M, C797S, NSCLC.

1. Introduction

Lung cancer is the leading cause of cancer incidence and death in China and approximately 85 percent of these is NSCLC [1]. Approximately 10 to 15 percent of NSCLC patients of Caucasian ancestry and about 30 to 35 percent of Asian patients harbor EGFR activating mutation. This discovery not only reveals the genetic differences between populations, but also provides a basis for precise targeted therapies. As a transmembrane receptor tyrosine kinase, EGFR comprises an extracellular ligand-binding domain, a transmembrane helix, a juxta membrane region, a catalytic kinase domain, and a C-terminal signaling region [2]. EGFR is a tyrosine kinase composed of an extracellular ligand-binding domain, a transmembrane domain, a juxta-membrane domain, a tyrosine kinase domain, and a C-terminal signaling domain [3]. Upon ligand binding, EGFR dimerizes and auto phosphorylates, engaging multiple downstream effectors that collectively enhance proliferation and resistance to cell death.

Tyrosine kinase inhibitors (TKIs) competitively bind to the ATP-binding site of EGFR, thereby blocking downstream signaling and inhibiting or even preventing cancer cell proliferation. The identification of EGFR mutations and the development of TKIs have significantly improved the prognosis for patients with advanced EGFR-mutant NSCLC, representing a major milestone in precision oncology. First-generation EGFR inhibitors such as gefitinib and erlotinib block signaling by reversibly occupying the kinase pocket, but they are easily overcome by secondary resistance and have limited activity against brain metastases. Second and third generation TKIs achieve enhanced on target inhibition by forming irreversible covalent adducts at nucleophilic residues in the ATP binding pocket, thereby reducing the impact of elevated ATP affinity produced by resistance mutations such as T790M.

However, as the duration of TKI treatment increases, more and more cases will inevitably develop drug resistance. Resistance to first- and second-generation EGFR inhibitors primarily stems from the T790M mutation, which increases ATP affinity and impairs drug binding. Resistance to third-generation drugs exhibits a broader, structurally diverse landscape, categorized as EGFR target-site

mutations and EGFR-independent resistance [3]. Target-site resistance is often driven by tertiary mutations, such as C797S, that disrupt the covalent binding site used by third-generation drugs. EGFR-independent resistance often involves focal amplification of bypassing kinases, particularly MET or HER2, as well as examples of lineage plasticity, including small cell or epithelial-mesenchymal transition. To address C797S-mediated resistance failure, three conceptually distinct strategies have been investigated: fourth-generation allosteric inhibitors, which bind outside the ATP pocket and retain activity against triple mutants; antibody-based approaches and antibody-drug conjugates, which target the extracellular domain or recruit immune effector mechanisms; and combination regimens that pair immune checkpoint inhibitors with antiangiogenic agents or chemotherapy to reshape the tumor microenvironment. This article aims to explore the targeted therapy, resistance mechanisms and latest treatment strategies for advanced NSCLC.

2. Molecular Features of EGFR-Driven NSCLC

EGFR mutations are predominantly located in exons 18–21 of the tyrosine kinase domain. Approximately 90% of sensitizing alterations consist of exon 19 deletions and the L858R missense substitution. More than 20 distinct deletion variants have been described, including large deletions, deletions accompanied by point mutations, and deletions with insertions; G719X accounts for ~3%, and S768I, exon 20 insertions and L861Q are also relatively common. More than 20 distinct deletion variants have been described, including large deletions, deletions accompanied by point mutations, and deletions with insertions; G719X accounts for ~3%, and S768I, exon 20 insertions and L861Q are also relatively common [4]. Among patients treated with EGFR-TKIs, roughly half harbor an acquired T790M mutation at disease progression; T790M increases the kinase's affinity for ATP and thereby reduces inhibitor binding. The third-generation irreversible inhibitor osimertinib was developed to selectively inhibit sensitizing mutations and T790M while sparing wild-type EGFR. These molecular profiles are critical for therapeutic selection: molecular testing is routinely used to guide first-line therapy and to characterize resistance at progression. Moreover, uncommon EGFR variants differ in sensitivity across TKI generations, which supports individualized, mutation-guided treatment combined with serial molecular monitoring.

3. TKIs Targeting Common EGFR Mutations

Table 1. Effects of TKIs and Associated Resistance Mechanisms

Category	Typical Drugs	Mechanism of Action	Resistance Mechanisms
First-generation TKIs	Gefitinib; Erlotinib	Reversibly binds to the ATP binding site of sensitive mutations	T790M mutation
Second-generation TKIs	Afatinib	Irreversibly binds to EGFR	MET amplification
Third-generation TKIs	Dacomitinib	Irreversibly binds to EGFR	T790M mutationn and histological transformation

As shown in Table 1, the mechanisms of action and resistance of the three generations of EGFR-TKIs are summarized. Both the first-generation reversible TKIs (gefitinib, erlotinib) and the second-generation irreversible inhibitors (afatinib, dacomitinib) block signal transduction by competitively binding to the ATP-binding site. However, there are notable differences between the two. Gefitinib (Fig. 1A) uses its quinazoline ring to form van der Waals interactions and hydrogen bonds with the hydrophobic pocket of the EGFR kinase domain, thereby stabilizing the inactive conformation of the kinase. In contrast, afatinib (Fig. 1B) covalently binds to a cysteine residue in the kinase domain through its acrylamide group, leading to sustained pathway inhibition and tumor growth suppression. Due to this irreversible binding property, afatinib shows stronger activity than first-generation agents against certain uncommon mutations such as G719X and L861Q. Nevertheless, these drugs demonstrate limited efficacy against exon 20 insertions (ex20ins), acquired T790M mutations, and

are associated with dose-limiting toxicities. The T790M substitution is among the most frequent resistance mechanisms following treatment with first- or second-generation EGFR-TKIs. Osimertinib (Fig. 1C), a representative third-generation EGFR-TKI, was specifically designed to overcome T790M-mediated resistance while retaining potent activity against sensitizing mutations [5]. For patients with advanced EGFR-mutated NSCLC (ex19del or L858R), the phase III FLAURA trial showed that osimertinib delayed the emergence of acquired resistance compared with standard EGFR-TKI, with a median PFS of 18.9 months and a median OS of 38.6 months. In addition, osimertinib also showed fewer grade ≥3 adverse events in first-line treatment [6]. The third-generation EGFR-TKI osimertinib is highly selective for both EGFR-sensitive mutations and T790M-resistant mutations through its acrylamide moiety covalently attached to the C797 residue. Importantly, its reduced activity against wild-type EGFR means fewer skin-related adverse events compared with previous-generation inhibitors. Beyond systemic disease control, osimertinib demonstrated greater efficacy in the central nervous system (CNS) compared with platinum-based chemotherapy. Due to its robust blood-brain barrier penetration, clinical studies have reported an objective response rate (ORR) of 54% and a median progression-free survival (PFS) of 8.5 months in patients with brain metastases. However, in recent years, Osimertinib resistance has become an increasingly recognized challenge. Among various mechanisms, the C797S triple mutation has become a key mechanism underlying acquired resistance to third-generation EGFR-TKIs. To this end, work is currently in progress to develop fourth-generation inhibitors, such as JBJ-04-125-02, designed to overcome resistance after Osimertinib treatment and currently undergoing preclinical evaluation.

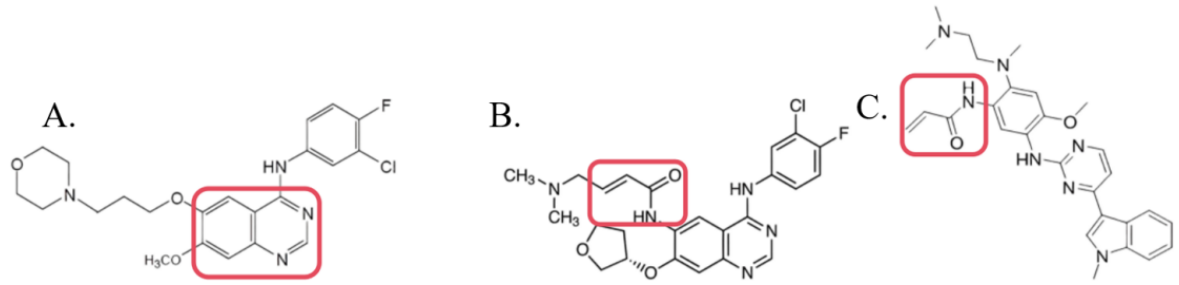

Figure 1. Representative targets of EGFR-TKIs. (A) Gefitinib. (B) Afatinib. (C) Osimertinib. Picture credit : Original

4. EGFR-TKI Resistance Mechanisms and Countermeasures

4.1. Resistance Mechanisms

With the continuous accumulation of secondary EGFR mutations in clinical practice, there are increasing reports of resistance to third-generation EGFR-TKIs. The mechanisms of acquired resistance are diverse and include not only mutations in EGFR itself, but also alterations in non-EGFR genes, aberrant activation of downstream signaling pathways, and in some cases, histological transformation of the tumor phenotype [7]. First-generation EGFR-TKIs (erlotinib, gefitinib, and icotinib) and second-generation drugs (afatinib and dacomitinib) have been extensively evaluated in phase III clinical trials. These drugs consistently outperform platinum-based chemotherapy in the first-line treatment of patients with advanced NSCLC harboring activating EGFR mutations. Although initial EGFR-targeted therapy often elicits rapid tumor responses, these are usually transient. Disease progression associated with acquired resistance typically emerges at a median of 10 to 14 months. The biological underpinnings of resistance fall into three broad categories. The first comprises on-target alterations, most notably the T790M substitution in exon 20, which increases the kinase's affinity for ATP and thereby reduces binding of EGFR tyrosine kinase inhibitors, leading to clinical failure. The second involves compensatory activation of bypass pathways, commonly through focal amplification of receptor kinases such as MET or HER2, which sustain proliferation via

alternative signaling cascades. The third encompasses histologic or phenotypic change, for example small-cell transformation or epithelial–mesenchymal transition, both of which can shift oncogenic dependency and reshape the profile of drug sensitivity [8, 9].

When third-generation EGFR-TKIs such as Osimertinib are used in the second-line setting, the spectrum of resistance mechanisms becomes even more complex. In fact, a single T790M mutation seldom leads to resistance against Osimertinib. Broadly speaking, resistance mechanisms to third-generation EGFR-TKIs can be categorized as either EGFR-dependent or EGFR-independent. Within the EGFR-dependent mechanism, tertiary EGFR mutations are associated with acquired resistance to Osimertinib in the second line. In the first-line setting, C797S/G and L718Q are the most common EGFR-dependent mutations, with the C797S mutation occurring in approximately 7% of patients. This tertiary mutation occurs in exon 20 of EGFR and ranks second in prevalence only to MET amplification. EGFR-independent resistance mechanisms include MET or HER2 amplification and reactivation of downstream signaling pathways such as MAPK and PI3K. Among them, MET amplification is the most common, reported in up to 35% of patients [10]. The incidence of HER2 amplification [11, 12]. Studies have demonstrated that combining Osimertinib with trastuzumab can effectively overcome resistance mediated by HER2 amplification in EGFR T790M-positive NSCLC cell lines.

4.2. Overcoming Acquired Resistance to EGFR Inhibitors

In recent years, strategies to overcome Osimertinib resistance have become increasingly diverse. The first approach is the development of fourth-generation EGFR-TKIs. Novel molecules such as BLU-945 and EAI-045 utilize allosteric binding to circumvent resistance mutations in the canonical ATP binding site [13, 14]. As a result, they can inhibit triple-mutated EGFR (L858R/T790M/C797S) and other complex mutations. These drugs have demonstrated significant inhibitory activity in preclinical cell lines, human xenograft (PDX) models, and intracranial metastasis models. When used alone, they can overcome resistance to Osimertinib, and when used in combination with third-generation EGFR-TKIs, they often exhibit significant synergistic effects. Among them, BLU-945 has entered Phase I/II clinical trials, and initial reports indicate a reasonable safety profile and encouraging signs of activity in patients harboring drug-resistant EGFR mutations. This makes it an attractive strategy for directly addressing targetable resistance.

The second approach that has attracted attention is antibody-based combination therapy [15]. The traditional EGFR-targeted monoclonal antibody cetuximab has shown activity in vitro and in animal models when combined with the ALK/EGFR dual inhibitor brigatinib. This combination can achieve dual-site blockade, reduce EGFR signaling, and overcome certain T790M or C797S resistance. In addition, antibody-based strategies aimed at activating bypass pathways have also made progress. The EGFR-MET bispecific antibody amivantazumab is an example. In the MARIPOSA-2 trial, when combined with chemotherapy, it significantly improved PFS and ORR in patients with EGFR-mutated NSCLC who had progressed during Osimertinib treatment. It also showed advantages on multiple post-progression endpoints. Together, these findings suggest that antibody combination therapy can not only block the main driver pathway but also widely inhibit resistance-associated bypass signaling.

A third strategy involves immunotherapy-based combination regimens. As immune checkpoint inhibitors gain wider use in non-small-cell lung cancer, their role after progression on Osimertinib has become an intense focus of research. Clinical data suggest that combining PD-1 inhibitors with anti-VEGF agents (such as bevacizumab) and chemotherapy can prolong PFS and OS after resistance, and in some patients, this approach has achieved durable efficacy. The benefits appear to derive from two pathways: inhibiting angiogenesis to improve the tumor microenvironment and alleviating T cell suppression, which together enhance anti-tumor activity. The PD-1/VEGF-A bispecific antibody ivonescimab is a newer option. It has been approved in China for the treatment of EGFR-mutant NSCLC after failure of EGFR-TKI therapy and is currently being evaluated in a global Phase III clinical trial. By combining immune activation and anti-angiogenesis in a single agent, these

bifunctional antibodies can simplify treatment regimens while reducing the risk of additional toxicities in the management of resistance.

In addition, inhibiting bypass signaling pathways is also an important strategy. Among them, MET amplification or activation is the most common, accounting for about 15% to 25% of Osimertinib resistance cases [16]. Targeting this mechanism, the combination of MET inhibitors (such as tepotinib and savolitinib) with Osimertinib has been used in clinical practice. Early results from platform studies including ORCHARD and SAVANNAH have shown encouraging efficacy. In summary, these advances indicate that a personalized combination therapy model guided by resistance classification is beginning to take shape. Targeted resistance can be treated with fourth-generation TKIs. Bypass-driven resistance can usually be treated with antibodies or small molecule inhibitors. At the same time, people are studying resistance related to immunity and the microenvironment through combinations of immunotherapy and anti-angiogenic drugs. Looking ahead, routine molecular testing to identify resistance mechanisms and select the most appropriate combination is expected to help delay disease progression and ultimately improve patient survival.

5. Conclusion

The use of EGFR-TKIs has greatly improved the prognosis of patients with EGFR-mutant NSCLC. In particular, the third-generation inhibitor Osimertinib has achieved milestone progress by prolonging PFS and OS while effectively controlling central nervous system (CNS) metastases. Despite these advances, drug resistance has rapidly become a major obstacle to long-term benefits. Current evidence generally divides resistance mechanisms into two categories: EGFR-dependent resistance mechanisms and EGFR-independent resistance mechanisms. These mechanisms include secondary or tertiary EGFR mutations, activation of bypass signaling pathways, and the development of new drug resistance mechanisms. and histological or phenotypic transformation. The biological heterogeneity of these mechanisms emphasizes the need for more precise and stratified therapeutic approaches in clinical practice.

In recent years, strategies for overcoming Osimertinib resistance have expanded rapidly. For targeted resistance, fourth-generation EGFR-TKIs (such as BLU-945 and EAI-045) are able to address triple mutations (L858R/T790M/C797S) and other complex mutations through allosteric binding. These drugs have demonstrated encouraging anti-tumor activity and a favorable safety profile in preclinical studies and early clinical trials. Furthermore, they appear to have synergistic effects when used in combination with third-generation TKIs. For off-target resistance, antibody-based therapies have rapidly developed. For example, combining the EGFR-targeting monoclonal antibody cetuximab with brigatinib synergistically blocks EGFR signaling. Another approach is the EGFR-MET bispecific antibody amivantamab, which significantly prolonged progression-free survival (PFS) and improved objective response rate when combined with chemotherapy in the MARIPOSA-2 study. Combination therapy strategies based on immunotherapy have also attracted widespread attention. Triple therapy combining a PD-1 inhibitor with an anti-VEGF agent and chemotherapy has achieved lasting clinical benefits in some patients with Osimertinib-resistant non-small cell lung cancer. Furthermore, the novel PD-1/VEGF-A bispecific antibody ivonescimab has been approved in China for the treatment of EGFR-mutant non-small cell lung cancer after progression on TKI therapy. Drug resistance driven by bypass pathways such as MET, HER2, and FGFR can be addressed through targeted combination therapies. For example, combination therapy with MET inhibitors (such as tepotinib or savolitinib) and Osimertinib has shown encouraging results in multiple clinical trials.

Looking ahead, the management of Osimertinib resistance will largely depend on precision therapy guided by molecular typing. First, highly sensitive molecular detection technologies (such as next-generation sequencing and circulating tumor DNA analysis) should be used to identify drivers early so that treatment regimens can be adjusted quickly and dynamically. Second, more multicenter, prospective, randomized controlled trials are needed to verify the efficacy and safety of new-

generation drugs and combination regimens in real-world populations. Third, the optimization of combination therapy should balance efficacy and toxicity management, while taking into account drug accessibility and economic burden. With the continuous development of fourth-generation EGFR-TKIs, bispecific antibodies and new immunomodulators, combined with AI-assisted prediction of resistance mechanisms and treatment guidance, the treatment of EGFR-mutant NSCLC is expected to enter a new era of more precise, efficient and lasting disease control.

References

[1] Wang R., Luo Z., Zhang H., et al. Tanshinone IIA reverses gefitinib-resistance in human non-small-cell lung cancer via regulation of VEGFR/Akt pathway. OncoTargets and Therapy, 2019, 12: 9355 - 9365.

[2] Wu L., Ke L., Zhang Z., et al. Development of EGFR TKIs and options to manage resistance of third-generation EGFR TKI Osimertinib: conventional ways and immune checkpoint inhibitors. Frontiers in Oncology, 2020, 10: 602762.

[3] Kitadai R., Okuma Y. Treatment strategies for non-small cell lung cancer harboring common and uncommon EGFR mutations: drug sensitivity based on exon classification, and structure-function analysis. Cancers, 2022, 14 (10): 2519.

[4] Yang J.C.H., Sequist L.V., Geater S.L., et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. The Lancet Oncology, 2015, 16 (7): 830 - 838.

[5] Ponnusamy M.P., Singh A.P., Jain M., et al. MUC4 activates HER2 signalling and enhances the motility of human ovarian cancer cells. British Journal of Cancer, 2008, 99 (3): 520 - 526.

[6] Lin D.C., Xu L., Chen Y., et al. Genomic and functional analysis of the E3 ligase PARK2 in glioma. Cancer Research, 2015, 75 (9): 1815 - 1827.

[7] Halder S., Basu S., Lall S.P., et al. Targeting the EGFR signaling pathway in cancer therapy: what's new in 2023? Expert Opinion on Therapeutic Targets, 2023, 27 (4 - 5): 305 - 324.

[8] Bhatia R., Siddiqui J.A., Ganguly K., et al. Muc4 loss mitigates epidermal growth factor receptor activity essential for PDAC tumorigenesis. Oncogene, 2023, 42 (10): 759 - 770.

[9] Chaturvedi P., Singh A.P., Chakraborty S., et al. MUC4 mucin interacts with and stabilizes the HER2 oncoprotein in human pancreatic cancer cells. Cancer Research, 2008, 68 (7): 2065 - 2070.

[10] Papadimitrakopoulou V.A., Wu Y.L., Han J.Y., et al. Analysis of resistance mechanisms to Osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Annals of Oncology, 2018, 29: 486 - 495.

[11] Cho J.H., Lim S.H., An H.J., et al. Osimertinib for patients with non-small-cell lung cancer harboring uncommon EGFR mutations: a multicenter, open-label, phase II trial (KCSG-LU15-09). Journal of Clinical Oncology, 2020, 38 (5): 488 - 495.

[12] Oxnard G.R., Hu Y.B., Mileham K.F., et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. JAMA Oncology, 2018, 4 (11): 1527 - 1534.

[13] Xu H.Q., Zhang H.J., Jia S.Y., et al. Diethenyl sulfoximine (DESI) as an irreversible lysine-targeting warhead enables the design of covalent allosteric EGFR inhibitor. Chemistry–A European Journal, 2025, 31 (41): e202500009.

[14] Eno M.S., Brubaker J.D., Campbell J.E., et al. Discovery of BLU-945, a reversible, potent, and wild-type-sparing next-generation EGFR mutant inhibitor for treatment-resistant non-small-cell lung cancer. Journal of Medicinal Chemistry, 2022, 65 (14): 9662 - 9677.

[15] Passaro A., Wang J., Wang Y., et al. Amivantamab plus chemotherapy with and without lazertinib in EGFR-mutant advanced NSCLC after disease progression on Osimertinib: primary results from the phase III MARIPOSA-2 study. Annals of Oncology, 2024, 35 (1): 77 - 90.

[16] Hartmaier R.J., Markovets A.A., Ahn M.J., et al. Osimertinib plus savolitinib to overcome acquired MET-mediated resistance in epidermal growth factor receptor-mutated, MET-amplified non-small cell lung cancer: TATTON. Cancer Discovery, 2023, 13 (1): 98 - 113.