Mechanisms and management of 3rd-generation EGFR-TKI resistance in advanced non-small cell lung cancer (Review)

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Abstract. Targeted therapy with epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) is a standard modality of the 1st-line treatments for patients with advanced *EGFR*-mutated non-small cell lung cancer (NSCLC), and substantially improves their prognosis. However, *EGFR* T790M mutation is the primary mechanism of 1st- and 2nd-generation EGFR-TKI resistance. Osimertinib is a representative of the 3rd-generation EGFR-TKIs that target T790M mutation, and has satisfactory efficacy in the treatment of T790M-positive NSCLC with disease progression following use of 1st- or 2nd-generation EGFR-TKIs. Other 3rd-generation EGFR-TKIs, such as abivertinib, rociletinib, nazartinib, olmutinib and alflutinib, are also at various stages of development. However, the occurrence of acquired resistance is inevitable, and the mechanisms of 3rd-generation EGFR-TKI resistance are complex and incompletely understood. Genomic studies in tissue and liquid biopsies of resistant patients reveal multiple candidate pathways. The present review summarizes the recent findings in mechanisms of resistance to 3rd-generation EGFR-TKIs in advanced NSCLC, and provides possible strategies to overcome this resistance. The mechanisms of acquired resistance mainly include an altered EGFR signaling pathway (*EGFR* tertiary mutations and amplification), activation of aberrant bypassing pathways (hepatocyte growth factor receptor amplification, human epidermal growth factor receptor 2 amplification and aberrant insulin-like growth factor 1 receptor activation), downstream pathway activation (RAS/RAF/MEK/ERK and PI3K/AKT/mTOR) and histological/phenotypic transformations (SCLC transformation and epithelial-mesenchymal transition). The combination of targeted therapies is a promising strategy to treat osimertinib-resistant patients, and multiple clinical studies on novel combined therapies are ongoing.

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1. Introduction

Lung cancer is the leading cause of cancer-related death throughout the whole world. More than half (57%) of all patients with lung cancer have metastasis at the time of diagnosis, and the 5-year survival rate is only 5% (1). Non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancer cases (2). Epidermal growth factor receptor (*EGFR*) is one of the most common driver genes in NSCLC. Female patients have higher

mutation rates than male patients. Adenocarcinomas and non-smoking are also associated with *EGFR* mutations (3). The EGFR belongs to the HER/ErbB family; it locates on the cell surface, and binds to its ligands, such as EGF and transforming growth factor. Upon ligand binding, EGFR undergoes dimer formation and auto-phosphorylation at the tyrosine residues, and activates downstream signaling through MAPK and PI3K pathways, leading to cancer cell proliferation, differentiation and migration (4). Approximately 20% of NSCLC patients bear activating mutations in *EGFR*. The deletions in exon 19 (Ex19del) and the L858R point mutation within exon 21 constitute ~90% of *EGFR* activating mutations (5). The activating mutations of *EGFR* are the targets of EGFR tyrosine kinase inhibitors (EGFR-TKIs). Current treatment guidelines recommend EGFR-TKIs as the 1st-line treatment for patients with advanced NSCLC with *EGFR* activating mutations.

The 1st-generation EGFR-TKIs, including gefitinib, erlotinib and icotinib, are reversible inhibitors that can inhibit the EGFR tyrosine kinase domain in an ATP-competitive and -reversible manner (Fig. 1) (6). The Iressa Pan-Asia study is a phase III and open-label study comparing gefitinib with carboplatin-paclitaxel as the 1st-line treatment for patients with advanced NSCLC in East Asia. The results showed that in the patients with *EGFR*-activating mutations, the gefitinib group experienced longer progression-free survival (PFS) than the carboplatin/paclitaxel group, while in patients without *EGFR* mutations, there was no PFS benefit in the gefitinib group (7). In 2015, gefitinib was approved by the U.S. Food and Drug Administration (FDA) for the 1st-line treatment of advanced NSCLC with *EGFR*-activating mutations (8). The European Tarceva versus Chemotherapy study is a multicenter, open-label and randomized phase III trial, which aimed to compare erlotinib with platinum-based chemotherapy as the 1st-line treatment for European patients with advanced NSCLC. In this study, erlotinib significantly improved PFS time compared with platinum-based chemotherapy in the patients with *EGFR*-activating mutations (9.7 vs. 5.2 months; HR, 0.37; 95% CI, 0.25-0.54; P<0.0001) (9). Icotinib is independently developed in China, and the CONVINCE study was designed to compare the efficacy and safety of 1st-line icotinib versus cisplatin/pemetrexed plus pemetrexed maintenance in patients with advanced lung adenocarcinoma. The results have demonstrated superior efficacy in that the PFS time of the icotinib group was significantly longer than that of the pemetrexed plus cisplatin chemotherapy group following the 1st-line treatment of *EGFR*-mutated NSCLC (11.2 vs. 7.9 months; HR, 0.61; 95% CI, 0.43-0.87; P=0.006) (10).

The 2nd-generation EGFR-TKIs, such as afatinib and dacomitinib, were originally designed to overcome the resistance to the 1st-generation EGFR-TKIs (Fig. 1). Afatinib is an irreversible dual specificity EGFR/human epidermal growth factor receptor 2 (HER2) inhibitor that is designed to covalently bind to EGFR and HER2, while dacomitinib is an irreversible pan-HER inhibitor. The broad spectrum of activity enables them to improve inhibition of EGFR-dependent tumor growth compared with the 1st-generation EGFR-TKIs. In the LUX-Lung7 and ARCHER studies, afatinib and dacomitinib significantly prolonged PFS and overall survival (OS) times compared with gefitinib in the 1st-line treatment of patients with NSCLC and *EGFR* activating mutations (11,12). However,

afatinib and dacomitinib exhibited low maximum tolerated doses in the patients. Compared to gefitinib, they were associated with a higher incidence of adverse events, including increased skin and gastrointestinal toxicity. The 2nd-generation EGFR-TKIs therefore have limited roles in patients with developed resistance to the 1st-generation EGFR-TKIs (13-15). Most patients inevitably develop acquired resistance through various mechanisms after 9-13 months of treatment with the 1st- and 2nd-generation EGFR-TKIs. The T790M mutation in *EGFR* exon 20 is the predominant cause, occurring in 50-60% of patients (15-17).

The so-called 'gatekeeper' T790M mutation increases the competition between ATP and the reversible EGFR-TKIs by exerting effects on both steric hindrance and increased ATP affinity to mutant EGFR receptor, thereby decreasing the efficacy of 1st- and 2nd-generation EGFR-TKIs (15). The 3rd-generation EGFR-TKIs, represented by osimertinib, have satisfactory efficacy in overcoming acquired resistance to the 1st- and 2nd-generation EGFR-TKIs mediated by T790M mutation (Fig. 1). Osimertinib selectively targets the *EGFR* T790M mutation, forming covalent bonds with the C797 residue in the ATP-binding site of mutant EGFR. Since their binding is irreversible, this agent overcomes the enhanced ATP affinity conferred by the T790M mutation; it exhibits ~200 times greater potency against L858R/T790M mutant EGFR than wild-type EGFR (18). The 3rd-generation EGFR-TKIs have promising clinical activity and tolerability. For patients who progressed on the 1st-generation EGFR-TKI treatment and have a T790M mutation, osimertinib has significantly improved their PFS and OS times compared with chemotherapy (17). Furthermore, osimertinib has shown better efficacy than gefitinib or erlotinib in untreated NSCLC with *EGFR* mutations (19). Patients receiving osimertinib have a lower incidence of serious adverse events than those treated with the 1st- or 2nd-generation EGFR-TKIs (12,19). However, patients with *EGFR*-activating mutations administered osimertinib as the 1st-line treatment have also been found to inevitably develop resistance after 18.9 months (19). Previous studies on the mechanisms of 3rd-generation EGFR-TKI resistance have revealed both primary and acquired resistance (Fig. 2). For patients with NSCLC progression after the 3rd-generation EGFR-TKI treatments, the use of next-generation sequencing (NGS) of plasma or tissue samples to clarify the resistance mechanism is important to guide further treatment. The molecular targets and resistance mechanisms of different generations of EGFR-TKIs are shown in Table I. Comprehensive and in-depth studies on the resistance to the 3rd-generation EGFR-TKIs are urgently required to provide better therapeutic strategies.

The present review summarizes the emerging evidence on mechanisms of 3rd-generation EGFR-TKI resistance in advanced NSCLC, and outlines the latest clinical strategies used to overcome this problem.

2. Third-generation EGFR-TKIs

In addition to osimertinib, several other 3rd-generation EGFR-TKIs targeting the T790M mutation (Fig. 1), such as rociletinib (CO1686), lazertinib (YH25448), abivertinib (AC0010), nazartinib (EGF816), naquotinib (ASP8273) and
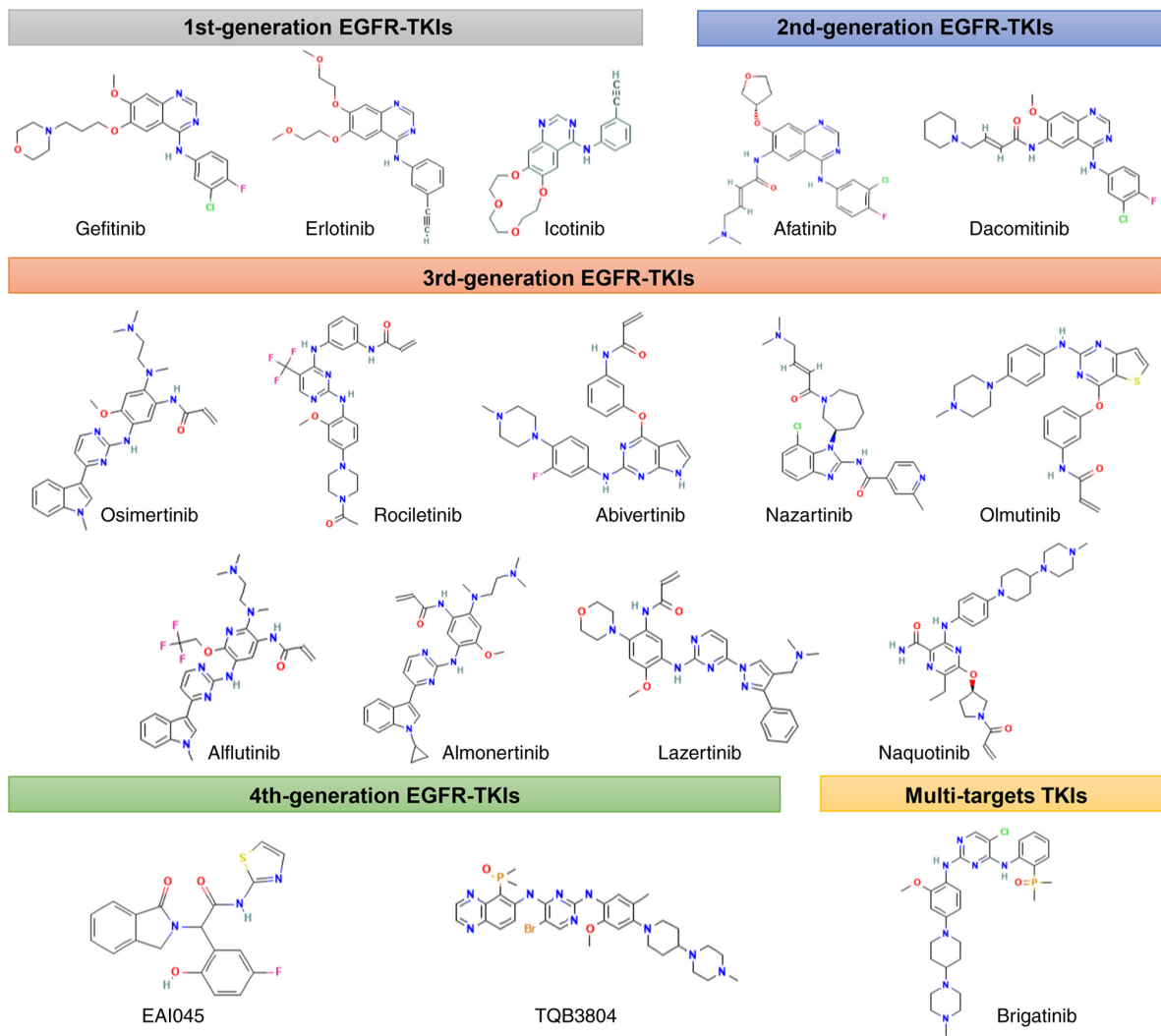
Figure 1. Chemical structures of different generations of EGFR-TKIs. Chemical structures were sourced from https://pubchem.ncbi.nlm.nih.gov/. EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.
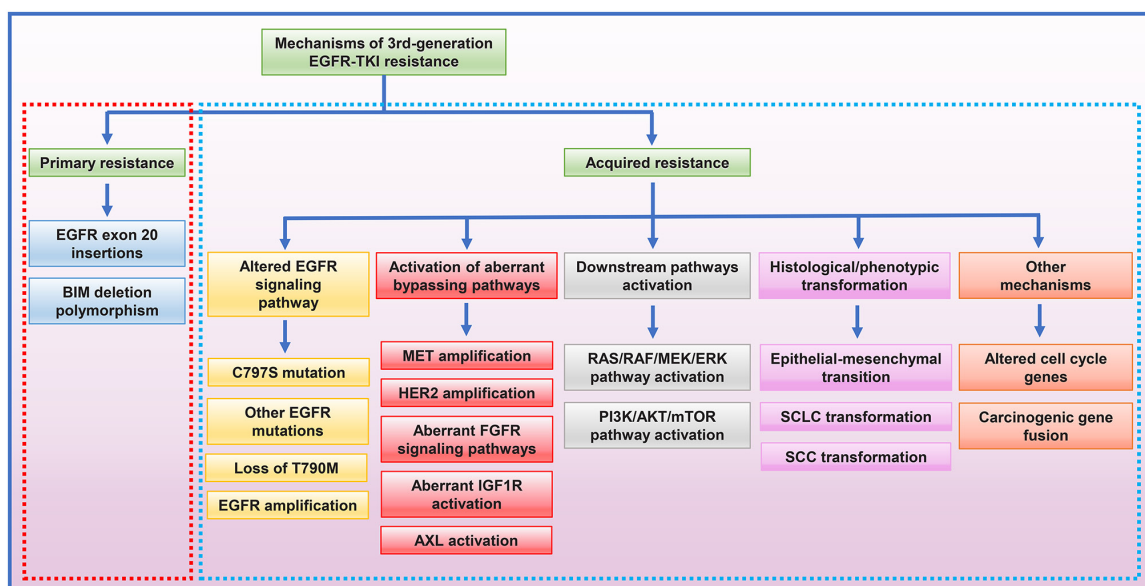
Figure 2. Mechanisms of primary and acquired 3rd-generation EGFR-TKI resistance in advanced *EGFR*-mutated non-SCLC. *BIM*, B-cell lymphoma-2 (BCL-2)-like 11; *MET*, hepatocyte growth factor receptor; *HER2*, human epidermal growth factor receptor 2; FGFR, fibroblast growth factor receptor; IGF1R, insulin-like growth factors 1 receptor; AXL, anexelekto; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

Table I. Different generations of EGFR-TKIs.

A, 1st-generation EGFR-TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
Gefitinib (ZD1839)	EGFR Ex19del, L858R	Reversible; competitive	T790M, EGFR amp, HER2 amp, MET amp	Approved by the FDA in 2005	Skin rash/acne, abnormal LFT, anorexia (7)
Erlotinib (OSI-774)	EGFR Ex19del, L858R	Reversible; competitive	T790M, EGFR amp, HER2 amp, MET amp	Approved by the FDA in 2003	Skin rash, abnormal LFT, diarrhea (9)
Icotinib (BPI-2009H)	EGFR Ex19del, L858R	Reversible; competitive	T790M, EGFR amp, HER2 amp, MET amp	Approved by the SFDA in 2011	Skin rash, cough, diarrhea, abnormal LFT (10)

B, 2nd-generation EGFR-TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
Afatinib (BIBW 2992)	EGFR Ex19del, L858R, HER2, HER4, uncommon mutations	Irreversible; covalent	T790M, EGFR amp, HER2 amp, MET amp	Approved by the FDA in 2013	Diarrhea, paronychia, skin rash (11)
Dacomitinib (PF-00299804)	EGFR Ex19del, L858R, HER2, HER4	Irreversible; covalent	T790M, EGFR amp, HER2 amp, MET amp	Approved by the FDA in 2018	Diarrhea, skin rash/acne, paronychia (12)

C, 3rd-generation EGFR-TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
Osimertinib (AZD9291)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S, MET amp, EGFR amp, HER2 amp/mut, PIK3CA amp/mut, SCLC transformation	Approved by the FDA in 2015	Diarrhea, skin rash, dry skin, paronychia (17-19)
Rociletinib (AVL301/CO1686)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S, EGFR amp, MET amp, HER2 amp, KRAS mut	After phase I/II trial, rejected by the FDA	Hyperglycemia, QTc prolong, cataracts (20,26)
Abivertinib (AC0010)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S, EGFR amp, MET amp, HER2 amp, KRAS mut	Phase II	Diarrhea, skin rash, abnormal LFT (22,27)
Nazartinib (EGF816)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S, MET amp	Phase III	Rash, diarrhea, pruritus (23,28)
Olmutinib (HM61713)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S	Approved by the FDA in 2015	Diarrhea, skin exfoliation, nausea (25,29)
Alflutinib (AST2818)	EGFR G719X, Ex19del, L858R, L861Q, T790M	Irreversible; covalent	N/A	Approved in China in 2021	Diarrhea, skin rash, abnormal LFT (30)
Almonertinib (HS-10296)	EGFR G719X, Ex19del, L858R, L861Q, T790M	Irreversible; covalent	N/A	Approved in China in 2020	Creatine phosphokinase elevation, skin rash, abnormal LFT, leukopenia (31)

Table I. Continued.

C, 3rd-generation EGFR-TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
Lazertinib (YH25448/GNS-1480)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	C797S, EGFR amp, PIK3CA, MET amp, HER2 amp	Approved in South Korea in 2021	Skin rash, pruritus, paresthesia (21,32)
Naquotinib (ASP8273)	EGFR Ex19del, L858R, T790M	Irreversible; covalent	N/A	Phase III discontinued	Diarrhea, hyponatremia, abnormal LFT, nausea (24,33)

D, 4th-generation EGFR-TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
EAI045	EGFR L858R, C797S, T790M	Non-ATP-competitive; reversible	N/A	Preclinical (91)	N/A
BLU-945	EGFR Ex19del, L858R, C797S, T790M	N/A	N/A	Preclinical (93)	N/A
TQB3804	EGFR Ex19del, L858R, C797S, T790M	N/A	N/A	Phase I (94)	N/A

E, Multi-target TKIs

Drug name	Molecular target	Target binding mode	Resistance mechanisms	Status	Adverse events
Brigatinib (AP26113)	EGFR Ex19del, L858R, C797S, T790M	ATP-competitive; reversible	N/A	Approved by the FDA in 2016	Nausea, diarrhea, headache, creatine phosphokinase elevation (92,95)

amp, amplification; Ex19del, exon 19 deletion; FDA, Food and Drug Administration; SFDA, Chinese State Food and Drug Administration; LFT, liver function test; mut, mutation; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; QTc, corrected QT interval; SCLC, small cell lung cancer; MET, hepatocyte growth factor receptor; HER2, human epidermal growth factor receptor 2.

olmutinib (HM61713), are at various stages of development (Table I) (17,20-33). Osimertinib, rociletinib and olmutinib are currently approved for clinical use by the U.S. FDA. Osimertinib has dominated the global landscape of treatment of *EGFR*-positive NSCLC, since it is the current standard of care for the 1st-line treatment of advanced *EGFR*-mutated NSCLC and for *EGFR* T790M-positive NSCLC after 1st- or 2nd-generation EGFR-TKIs (17,19).

Osimertinib. In 2015, the FDA approved osimertinib for the treatment of patients with metastatic *EGFR*-mutant NSCLC who have acquired the *EGFR* T790M resistance mutation. The drug was then approved as the 1st-line therapy for advanced *EGFR*-mutated NSCLC in 2018 (34). Osimertinib is an oral, irreversible EGFR-TKI that is selective for both *EGFR* and T790M mutations and has activity in the central nervous system (CNS). AURA3 is a randomized, open-label, phase 3 trial that has been conducted to show the superiority of osimertinib over platinum therapy plus pemetrexed in patients with T790M-positive advanced NSCLC after 1st-generation EGFR-TKI therapy. The results showed that the median PFS time was significantly longer with osimertinib than that with platinum therapy plus pemetrexed (10.1 vs. 4.4 months; HR, 0.30; 95% CI, 0.23-0.41; P<0.001), which establish a

standard protocol for the treatment of *EGFR* T790M-positive patients after failure of 1st-generation EGFR-TKI therapy, as well as targeted therapy for brain metastasis (17). The double-blind, phase 3 FLAURA trial compared the efficacy and safety of osimertinib with the standard EGFR-TKIs (gefitinib or erlotinib) in patients with untreated *EGFR* mutation-positive advanced NSCLC. It demonstrated that the median PFS time of untreated patients with *EGFR* mutation was significantly longer following osimertinib administration compared with that following gefitinib or erlotinib administration (18.9 vs. 10.2 months; HR, 0.80; 95% CI, 0.37-0.57; P<0.001) (19), as was the median OS time (38.6 vs. 31.8 months; HR, 0.46; 95% CI, 0.64-1.00; P=0.046) (35). In patients with measurable brain metastases, the objective response rate (ORR) was 91% with osimertinib versus 68% with the 1st-generation EGFR-TKIs (odds ratio, 4.6; 95% CI, 0.9-34.9; P=0.066) (36). Several studies have investigated the safety and efficacy of EGFR-TKIs in combination with other targeted therapies (37-39). The phase Ib TATTON study assessed the safety and tolerability of osimertinib in combination with selumetinib (MEK inhibitor), savolitinib [hepatocyte growth factor receptor (MET) inhibitor] or durvalumab, a human anti-programmed cell death-ligand-1 (PD-L1) antibody. The ORR in the selumetinib, savolitinib and durvalumab arms was 42, 44 and 43%, respectively (39).

Overall, osimertinib is a promising 3rd-generation EGFR-TKI in current lung cancer treatment regimens, and osimertinib-based combination therapy deserves broad investigations to improve outcomes in patients with advanced EGFR-mutated NSCLC.

Rociletinib. Rociletinib (CO1686) is a small-molecule, orally administered and irreversibly selective EGFR-TKI that inhibits *EGFR* mutations, including Ex19del, L858R and T790M, but not exon 20 insertion (e20ins) (26). The TIGER-X trial (phase I/II study) and the TIGER-2 trial (phase II, open-label, multicenter study) evaluated the safety and efficacy of rociletinib in previously treated patients with *EGFR*-mutated NSCLC. A total of 130 patients were enrolled in the TIGER-X study. Of the 46 evaluable T790M patients, the ORR was 59% (95% CI, 45-73), and the estimated median PFS time was 13.1 months (95% CI, 5.4-13.1) (26). However, results of the TIGER-X and TIGER-2 trials using a pooled cohort of patients found that the rate of confirmed response was 28-34% for rociletinib, which significantly differed from the result of the TIGER-X trial. After independent analysis, the maturation confirmation response rate was updated to 45% and the median PFS time to 6.1 months (20). Sequist *et al* (40) reported that 9 patients with progression on rociletinib were subsequently treated with osimertinib. Among them, 2 patients exhibited a partial response, 3 presented with stable disease and 4 developed progressive disease. The 3 patients with brain metastasis after rociletinib treatment also showed improvement in CNS disease response to osimertinib. The development of rociletinib was terminated on May 6, 2016, due to a lower efficacy than osimertinib and incidences of adverse events such as high-grade hyperglycemia and corrected QT interval prolongation (41).

Abivertinib. Abivertinib (AC0010) is one of the 3rd-generation EGFR-TKIs developed in China; it is a pyrrolopyrimidine compound, which is highly selective, irreversible and potent (42). A phase I study of abivertinib presented significant clinical benefits and a good safety profile in patients with NSCLC and acquired resistance to the 1st-generation EGFR-TKIs. The ORR in patients with T790M mutation was 42%, and the median PFS time estimated by Kaplan-Meier ranged between 14.0 and 35.6 weeks in those patients receiving a daily dose of 350-600 mg (42). A phase I, open, multicenter study explored the efficacy of abivertinib in patients with NSCLC and CNS metastasis. The median intracranial PFS time was 142 days (95% CI, 31.1-252.9) in 7 patients with brain metastasis, suggesting that abivertinib had good control of asymptomatic brain metastasis (43). A recent study reported that 9 patients received osimertinib treatment after progression on abiverinib, with a median total treatment duration of 15.9 months (95% CI, 12.5-19.3) (27). These results have demonstrated that osimertinib might be an option for subsequent therapy in patients with disease progression after abivertinib treatment. Further studies on abivertinib are required to investigate its interaction with osimertinib.

Other 3rd-generation EGFR-TKIs. Several other 3rd-generation EGFR-TKIs are also under development. Nazartinib (EGF816) is a covalent and irreversible EGFR-TKI. Preliminary results from an open-label, multicenter, phase I/II study of 180 patients at 9 academic medical centers in Europe, Asia and North America demonstrated that nazartinib had an excellent safety profile, with low dermal toxicity and a primary adverse event of maculopapular rash (40%) (28). Olmutinib (HM61713), approved by the FDA in December 2015 for the treatment of NSCLC, has potent inhibitory activity against L858R/T790M mutant NSCLC cells (44). In a recently published single-arm, open-label, phase I/II trial, *EGFR* T790M-positive patients receiving olmutinib (800 mg/day) had an ORR of 55% (38/69 evaluable patients; 95% CI, 42.6-67.1) and an estimated median PFS time of 6.9 months (95% CI, 5.6-9.7). The common adverse events included diarrhea (59%), pruritus (42%), rash (41%) and nausea (40%) (29). On September 30, 2016, two cases of toxic epidermal necrolysis/Stevens-Johnson syndrome, one of which was fatal, were reported in a phase II trial of olmutinib in South Korea. On April 13, 2018, the development of olmutinib was terminated (41). Alflutinib (AST2818) is a newly developed 3rd-generation EGFR-TKI; its ORR was 77% (89 out of 116 patients), and 9% of patients (11 out of 130 patients) experienced grade 3 or higher drug-related adverse events (30). Almonertinib, a 3rd-generation EGFR-TKI developed in China, has also shown good efficacy in the treatment of T790M-positive NSCLC after EGFR-TKI resistance. In a phase II trial, it exhibited a median PFS time of 12.3 months, an ORR of 69% and acceptable toxicity. On March 19, 2020, almonertinib was approved by the Center for Drug Evaluation of the China National Medical Products Administration (31). Phase I/II clinical studies on lazertinib showed that lazertinib had a favorable safety profile and exhibited promising antitumor activity in patients with *EGFR* T790M-positive NSCLC, with a median PFS time of 11.0 months and an ORR of 58%. Lazertinib was approved for *EGFR* T790M-positive NSCLC in South Korea in 2021 (21,32). Naquotinib has antitumor activity in preclinical models of *EGFR*-mutant NSCLC that targets mutant *EGFR*, including *EGFR* T790M. In phase I

trials, it had an ORR of 31% against T790M-positive NSCLC. However, the phase III clinical trial of naquotinib was terminated due to toxicity and limited predicted efficacy (24,33). Further investigations on 3rd-generation EGFR-TKIs are still ongoing.

3. Primary resistance mechanisms

Similar to chemotherapy-associated resistance, resistance to EGFR-TKIs includes both primary (intrinsic) and acquired (secondary) types. The primary type is generally defined as resistance that is present prior to the EGFR-TKI treatment. There are few studies related to primary resistance, and the mechanisms are unclear. Possible causes include *EGFR* e20ins and B-cell lymphoma-2 (BCL-2)-like 11 (*BIM*) deletion polymorphism.

EGFR e20ins. EGFR e20ins occur in ~3% of lung adenocarcinoma patients and 9% of EGFR-mutated tumors. EGFR e20ins block EGFR-TKI binding to EGFR target sites, leading to primary resistance (45). For patients with EGFR e20ins, the overall effectiveness of 1st-line treatment with 1st- and 2nd-generation EGFR-TKIs is 3-8%, with a median PFS time of only 2 months. An *in vitro* study showed that NSCLC cells stably expressing EGFR e20ins were resistant to the 3rd-generation EGFR-TKIs, suggesting that EGFR e20ins might be primary mechanisms of 3rd-generation EGFR-TKI resistance (46). Qin *et al* (47) demonstrated that p.A767_V769dup (25%) and p.S768_D770dup (18%) were the 2 most common EGFR e20ins, accounting for 44% of cases and serving as primary targets for future drug development. In addition, different EGFR e20ins might be associated with different outcomes in patients with advanced NSCLC. p.A763_Y764insFQEA conferred better PFS times than the other e20ins when patients were treated with various EGFR-TKIs, while p.S768_D770dup showed a limited response and p.D770delinsGY patients experienced a short PFS time. Therefore, it is important to select the appropriate EGFR-TKIs according to the specific EGFR e20in type (47). van Veggel *et al* (48) reported that osimertinib (80 or 160 mg/day) treatment had an ORR of 5% and a median PFS time of 3.6 months (95% CI, 2.6-4.5) in 21 patients with advanced NSCLC and EGFR e20ins (48). Phase II clinical studies on osimertinib for EGFR e20ins in NSCLC are currently underway (NCT03414814 and NCT03191149).

BIM deletion polymorphism. BIM is a member of the BCL-2 family and a pro-apoptotic molecule. The *BIM* deletion polymorphism is present in ~21% of East Asian individuals, and is absent in African and European populations (49,50). Tanimoto *et al* (51) demonstrated that *EGFR*-mutant NSCLC cells with *BIM* deletion polymorphism were resistant to 3rd-generation EGFR-TKIs. Li *et al* (49) first reported that a patient with NSCLC plus the *EGFR* L858R/T790M mutation and the *BIM* deletion polymorphism exhibited a poor clinical outcome after treatment with osimertinib (49). In addition, another study indicated that the median PFS time of patients with *BIM* deletion polymorphism was significantly shorter than that of wild-type *BIM* patients (227 vs. 533 days; P<0.001). Multivariate Cox regression analysis showed that the *BIM* deletion polymorphism was an independent indicator of

shorter PFS time (52). However, Liu *et al* (53) concluded that it was the concomitant genetic alterations, rather than *BIM* deletion polymorphism, that influenced the EGFR-TKI response. Cox multivariate regression analysis showed that *TP53* mutations, *NTRK1* mutations and amplifications, *RB1* mutations and *PIK3CA* mutations were associated with poorer PFS. In addition, *TP53* mutations, *KEAP1* mutations and deletions were shown to have an impact on OS in patients treated with EGFR-TKI. By contrast, neither analysis showed an effect of *BIM* deletion polymorphisms on PFS and OS (53). The roles of *BIM* deletion polymorphism in the prediction of EGFR-TKI responses are still controversial. Further studies are required to explore the exact functions of *BIM* deletion polymorphism in the primary resistance of 3rd-generation EGFR-TKIs.

4. Acquired resistance mechanisms

Acquired resistance refers to the evading effects of EGFR-TKIs in tumor cells via alteration of their metabolic pathways after exposure to the drugs. With the application of NGS-based genomic profiling, the mechanisms of 3rd-generation EGFR-TKI resistance have been extensively studied. The acquired resistance of osimertinib as the 2nd- and 1st-line therapy (54-60), as well as rociletinib and abivertinib (42,61-63), is summarized in Fig. 3. The mechanisms of acquired resistance to 3rd-generation EGFR-TKIs include an altered EGFR signaling pathway, aberrant activation of bypass and downstream signaling pathways, and histological transformation. The molecular mechanisms are depicted in Fig. 4.

Altered EGFR signaling pathway
EGFR tertiary mutations. *EGFR* tertiary mutations are common resistance mechanisms against 3rd-generation EGFR-TKIs in lung cancer, and multiple *EGFR* tertiary mutations have been observed. The C797S mutation at *EGFR* exon 20 is the most common tertiary mutation mediating 3rd-generation EGFR-TKI resistance. C797S is a missense mutation in the tyrosine kinase region of EGFR, which prevents the 3rd-generation EGFR-TKIs forming covalent bonds in the ATP-binding domain of EGFR kinase, resulting in EGFR-TKI dysfunction. The C797S mutation has been found to affect the sensitivity of NSCLC to 3rd-generation EGFR-TKIs in both *in vitro* and *in vivo* models (54,56,59). Ercan *et al* (64) evaluated the sensitivity of EGFR inhibitors in models harboring drug-resistant *EGFR* mutations. It was found that NSCLC cell lines harboring C797S mutation were resistant to irreversible EGFR-TKIs such as osimertinib, rociletinib and olmutinib (64). NGS analysis of plasma samples from 73 patients with progression after 2nd-line treatment of osimertinib in the AURA3 study showed that 10 patients (14%) had the C797S mutation, and all retained the T790M mutation (54). Le *et al* (56) found 8 (19%) C797S mutations in 42 patients coinciding with the T790M mutation. In the FLAURA study, the frequency of the C797S mutation was 7% (6/91) (59). In addition to that in osimertinib, the C797S mutation was reported to mediate resistance to other 3rd-generation TKIs, such as olmutinib, larzertinib, rociletinib and abivertinib (61-63,65,66). Zhang *et al* (61) first indicated that *EGFR* tertiary mutations mediated abivertinib resistance. The *EGFR* C797S mutation was detected in 3 patients (10%), all occurred
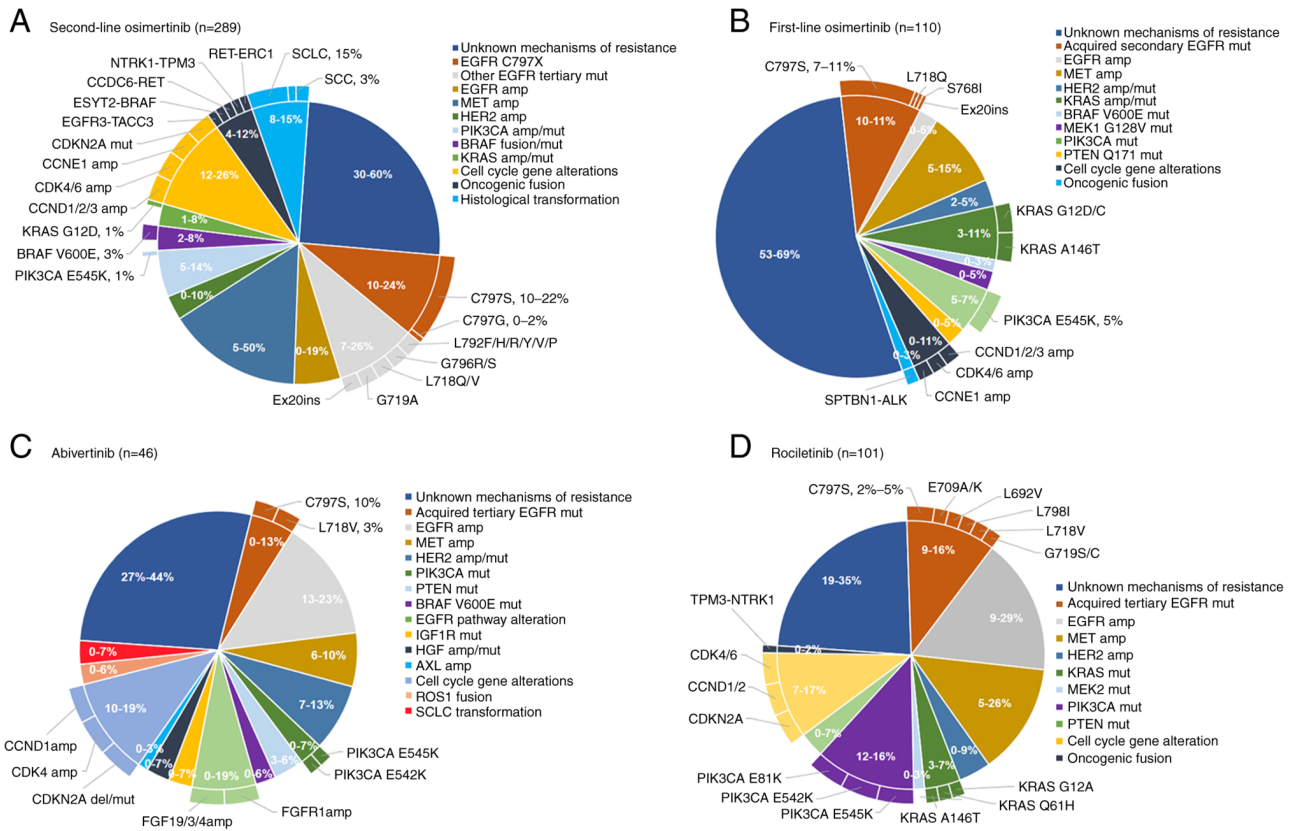
Figure 3. Mechanisms of acquired 3rd-generation EGFR-TKI resistance in advanced *EGFR*-mutated non-SCLC. (A) Osimertinib as the 2nd-line treatment. (B) Osimertinib as the 1st-line treatments. (C) Abivertinib. (D) Rociletinib. These pie-charts summarize data from studies that enrolled >15 patients. Different resistant mechanisms might co-exist in the same patient. mut, mutation; amp, amplification; *PIK3CA*, phosphatidylinositol-3-kinase catalytic α; *PTEN*, phosphatase and tensin homolog; del, deletion; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; *MET*, hepatocyte growth factor receptor; *HER2*, human epidermal growth factor receptor 2; FGFR, fibroblast growth factor receptor; IGF1R, insulin-like growth factors 1 receptor; HGF, hepatocyte growth factor; AXL, anexelekto; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; ROS1, c-ros oncogene 1, receptor tyrosine kinase.

in cis with the *EGFR* T790M mutation. By contrast, the *EGFR* C797S mutation is an infrequent mechanism of rociletinib resistance, only observed in 2.3-4.5% of patients (62,63).

Other rare *EGFR* tertiary mutations associated with drug resistance observed in the AURA3 and FLAURA studies are L792X (3%), G796S (1%), L718Q (1-2%) and S768I (1%), which have the potential to sterically interfere with the osimertinib-EGFR interaction (59). Osimertinib treatment may be ineffective against G724S mutation (67,68). Structural analysis revealed that the G724S mutation might induce a glycine-rich loop conformation, which is incompatible with the binding of 3rd-generation EGFR-TKIs (68). Further studies indicated that the L718Q mutation showed cross-resistance to all the 3rd-generation EGFR-TKIs (69). In addition, the L718V mutation was observed in abivertinib-resistant patients, while the E709K, L692V and L798I mutations were detected in rociletinib-resistant patients (61,62).

Loss of T790M. Patients enrolled in the AURA3 study all developed the T790M mutation after the 1st-line treatment with 1st- or 2nd-generation EGFR-TKIs, and 49% (36/73) of them had loss of T790M after osimertinib resistance (54). The frequency of T790M mutation loss in osimertinib-resistant patients in the studies by Le *et al* (56) and Oxnard *et al* (57) was 53% (21/40) and 68% (28/41), respectively. Oxnard *et al* (57)

conducted tumor biopsies for genomic analysis in 41 patients, and suggested that the loss of T790M was associated with a small decrease in *EGFR*-activating mutations after 1-3 weeks of treatment. Another recent study demonstrated a markedly shorter median PFS time in the T790M-loss group than that in the T790M group (5.93 vs. 11.87 months, P=0.0004). Loss of T790M mutation was correlated with earlier disease progression and worse survival in the osimertinib-resistant patients (70). In addition, the loss of T790M was present in 50% (6/12) of patients resistant to rociletinib (71). In T790M cases, the resistant mechanism is mostly related to C797S mutation or bypass pathway activation, while patients without T790M often show EGFR-independent mechanisms (56,71).

EGFR amplification. Le *et al* (56) demonstrated that *EGFR* amplification occurred in 8 (19%) of 42 patients who developed resistance to osimertinib (56). One patient with progression after osimertinib treatment developed amplified copies of *EGFR* Ex19del, with amplification levels parallel to clinical and radiological progression (72). *EGFR* amplification is more commonly observed in patients treated with rociletinib. Piotrowska *et al* (71) reported that 25% (3/12) of patients resistant to rociletinib showed *EGFR* amplification. This was comparable to 29% (17/58) in the study by Helman *et al* (63). In addition, *EGFR* amplification in 7 (23%) patients was
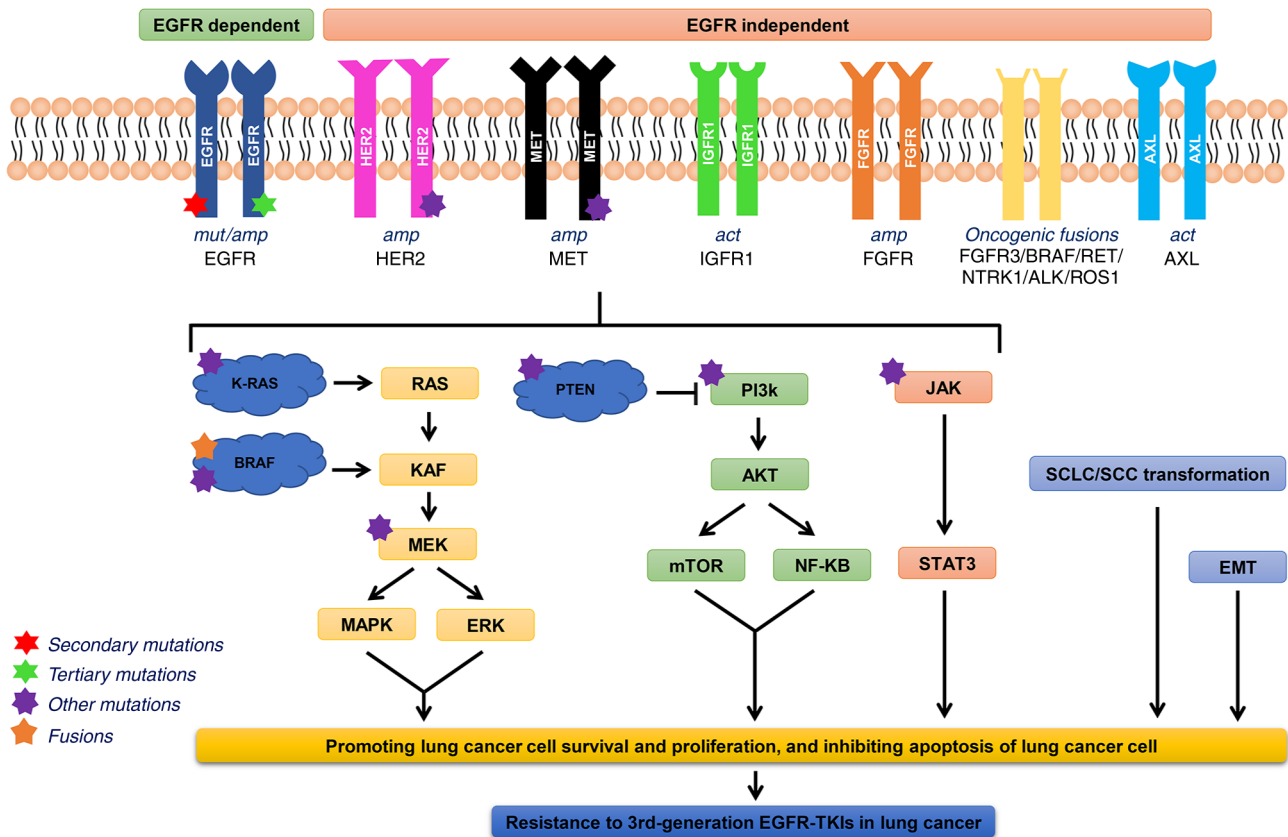
Figure 4. Molecular mechanisms of acquired 3rd-generation EGFR-TKI resistance. act, activation; NTRK1, neurotrophic receptor tyrosine kinase 1; ALK, anaplastic lymphocyte kinase; ROS1, c-ros oncogene 1, receptor tyrosine kinase; STAT3, signal transducer and activator of transcription 3; EMT, epithelial-mesenchymal transition; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; *MET*, hepatocyte growth factor receptor; *HER2*, human epidermal growth factor receptor 2; FGFR, fibroblast growth factor receptor; IGF1R, insulin-like growth factors 1 receptor; AXL, anexelekto.

considered as putative mechanisms of abivertinib resistance. Among them, 4 patients had *EGFR* amplification after abivertinib treatment, and the other 3 had *EGFR* amplification prior to treatment but had elevated *EGFR* copy numbers at progressive disease (61).

Activation of aberrant bypassing pathways
MET amplification. Amplification of c-Met plays an important role in 1st- and 3rd-generation EGFR-TKI resistance; it functions as a transmembrane receptor tyrosine kinase. When hepatocyte growth factor ligands bind to c-Met, c-Met is activated, inducing homodimerization and phosphorylation of intracellular tyrosine residues, which activates the downstream RAS/ERK/MAPK and PI3K-AKT signaling pathways (73,74). These pathways can drive cell survival, proliferation, motility, migration, invasion, angiogenesis and epithelial-mesenchymal transition (EMT), providing a bypass pathway in the presence of an EGFR inhibitor (73,74). The AURA3 study showed that 19% (14/73) of patients developed *MET* gene amplification after the 2nd-line treatment with osimertinib, which is the second most common cause of resistance following the C797S mutation (54). Moreover, the incidence of *MET* amplification was 14% (6/42) in the study by Le *et al* (56). In the FLAURA study, 15% (14/91) of patients resistant to osimertinib developed *MET* amplification, exceeding the 7% (6/91) of the C797S mutation (59). *MET* amplification might be the most common cause of resistance

when osimertinib is used as 1st-line treatment. It is also one of the most common resistance mechanisms to rociletinib, occurring in 8-26% of rociletinib-resistant patients (62,63). In the abivertinib-resistant cohort, *MET* amplification was detected in 3 (10%) patients (61).

HER2 amplification. HER2 belongs to the EGFR family. *HER2* amplification has a sustained effect on the PI3K/AKT and MAPK signaling pathways downstream of EGFR, thereby promoting osimertinib resistance in lung cancer cells (54,56,59). In the AURA3 study, *HER2* amplification occurred in 6% of patients resistant to 2nd-line treatment with osimertinib (4/73) (54), and in 10% (4/42) of patients in the study by Le *et al* (56). In the FLAURA study, for the patients resistant to 1st-line treatment with osimertinib, the incidence of *HER2* amplification was 2% (2/91) (59). Moreover, *HER2* e20ins and E1247K were also identified in osimertinib-resistant patients (60). A recent *in vitro* study found that *HER2* D16 can form a homodimer and activate downstream signaling pathways in NSCLC cells, resulting in acquired resistance to osimertinib. The combination of osimertinib and afatinib could synergistically inhibit H1975-*HER2* D16 cells (75). In addition, the *HER2* amplification also occurred in rociletinib-resistant patients with a frequency of 9% (62).

Aberrant fibroblast growth factor receptor (FGFR) signaling pathway. FGFR signaling pathways regulate cell proliferation,

migration, cycle progression, metabolism and survival (76). Kim *et al* (77) found that *FGFR1* amplification and FGF2 expression were upregulated in patients resistant to osimertinib, suggesting that the FGF2-FGFR1 autocrine loop might be associated with osimertinib resistance (77). The plasma-based circulating tumor DNA (ctDNA) assay revealed that the *FGFR3-TACC3* fusion mutation was present in patients with the T790M mutation, and disease progression occurred upon osimertinib and naquotinib treatment (78). These results suggested that abnormalities of the FGFR signaling pathway might contribute to 3rd-generation EGFR-TKI resistance.

Aberrant insulin-like growth factor 1 receptor (IGF1R) activation. IGF1R is present in numerous cell types in various tissues, such as muscle, cartilage, bone and brain; it is a tyrosine kinases receptor that is involved in the pathogenesis and progression of various malignancies, including lung cancer (79). Abnormalities of IGF1R confer resistance to 3rd-generation EGFR-TKIs by activating the EGFR bypass signaling pathway (79). Manabe *et al* (80) demonstrated for the first time that IGF2 autocrine-mediated activation of the IGF1R pathway in lung cancer cells was involved in osimertinib resistance. Immunohistochemistry confirmed the increased IGF2 expression in lung cancer patients with acquired osimertinib resistance (80). Phosphate-receptor tyrosine kinase array analysis of osimertinib-resistant cells showed that IGF1R was activated in PC9/T790M/AZDR and H1975/AZDR cells. Inhibition of IGF1R activation by small interfering RNA or linstinib (IGF1R inhibitor) significantly restored osimertinib sensitivity, suggesting that aberrant IGF1R activation might be one of the causes of 3rd-generation EGFR-TKI resistance (79).

Anexelekto (AXL) activation. AXL is a member of the receptor tyrosine kinases, which are involved in the regulation of cell survival, proliferation, migration and metabolism. A study by Taniguchi *et al* (81) demonstrated that the overexpression of AXL was associated with a poor response to osimertinib. This study showed that osimertinib stimulated AXL by inhibiting the feedback inhibition of SPRY4. Activated AXL interacted with EGFR and HER3 to maintain cell survival and induced osimertinib resistance, which could be delayed by AXL inhibitors (81). Another study demonstrated that AXL was upregulated in osimertinib-resistant lung adenocarcinoma cells. Osimertinib combined with cabozantinib (AXL inhibitor) inhibited the growth of these cells both *in vitro* and *in vivo* (82). In addition, *AXL* amplification was also detected in abivertinib-resistant patients (61). Therefore, AXL activation and *AXL* amplification might also contribute to the primary and acquired resistance to 3rd-generation EGFR-TKIs.

Downstream pathways activation
RAS/RAF/MEK/ERK pathway activation. Acquired resistance to the 3rd-generation EGFR-TKIs is associated with the activation of the RAS-MAPK pathway. BRAF is a serine/threonine protein kinase, which regulates cell survival, proliferation, differentiation and apoptosis, and plays a crucial role in RAS/RAF/MEK/ERK pathways. Nakatani *et al* (83) observed KRAS overexpression in osimertinib-resistant cells. One case (1%) in the AURA3 study had a *KRAS* (G12D) mutation after resistance and 2 cases (3%) had *BRAF V600E* mutations (54).

KRAS Q61K mutation, *BRAF* mutations and *ESYT2-BRAF* gene fusion have also been detected in patients with acquired osimertinib resistance (57). *KRAS* mutations in the FLAURA study included A146T (1%), G12C (1%) and G12D (1%), and the incidence of the *BRAF V600E* mutation was 3% (3/91) (59).

PI3K/AKT/mTOR pathway activation. Phosphatidylinositol-3-kinase catalytic α (*PIK3CA*) amplification or mutations promote tumor infiltration and activate the PI3K/AKT/mTOR pathway. In the AURA3 study, *PIK3CA* amplification and E545K mutation were detected in patients with osimertinib resistance (54). Oxnard *et al* (57) reported a 10% (4/41) incidence of *PIK3CA* amplification in osimertinib-resistant patients (57). *PIK3CA* mutations in the FLAURA study included E453K (1%), E545K (4%) and H1047R (1%) (59). These results suggested that PI3K/AKT/mTOR pathway activation might be involved in the 3rd-generation EGFR-TKI resistance.

Histological/phenotypic transformation. Marcoux *et al* (84) reported that 19 NSCLC patients developed SCLC transformation after treatment with the 3rd-generation EGFR-TKIs. The median time of transformation was 17.8 months (95% CI, 14.3-26.2) and the median survival time after SCLC transformation was 10.9 months (95% CI, 8.0-13.7). The incidence of squamous cell carcinoma (SCC) transformation after the 1st- and 2nd-line osimertinib treatment was 15 and 14%, respectively (85). SCLC and SCC transformation have not yet been identified *in vitro*, suggesting that the tumor microenvironment might play potential roles in these processes. The transdifferentiation of epithelial cells into mesenchymal cells, a process known as EMT, involves the loss of epithelial cell markers, such as E-cadherin, and the acquisition of mesenchymal markers, such as vimentin, N-cadherin, fibronectin, β-catenin and zeb-1, which confers on cancer cells increased tumor-initiating and metastatic potential (86). Nukaga *et al* (87) demonstrated that osimertinib- and rociletinib-resistant H1975 cells exhibited a shuttle cell-like morphology and acquired a more mesenchymal phenotype *in vitro*. NSCLC cells from patients with acquired osimertinib resistance were reported to exhibit EMT features, including decreased epithelial junction proteins and increased mesenchymal markers, confirming that EMT was closely associated with 3rd-generation EGFR-TKI resistance (88). Therefore, in addition to liquid biopsy to clarify the relevant mutant genes, it is also important to clarify the presence of histological/phenotypic transformation by tissue biopsy in osimertinib-resistant patients.

Altered cell cycle genes. The AURA3 and FLAURA studies demonstrated that the frequency of cell cycle gene alterations was 11 and 12%, respectively, in patients with disease progression after osimertinib treatment as the 1st- or 2nd-line therapy (54,59). Both *CCNE1* and *CDK6* amplification occurred at a frequency of 7% (5/73) as the most common cell cycle gene alterations in the AURA3 study (54). The most common cell cycle gene alteration in the FLAURA study was *CDK6* amplification (3%) (59). In the study by Le *et al* (56), *CCND1*, *CCNE1* and *CDKN2A* deletions were also found. Alterations in cell cycle genes may be involved in the development of osimertinib resistance.
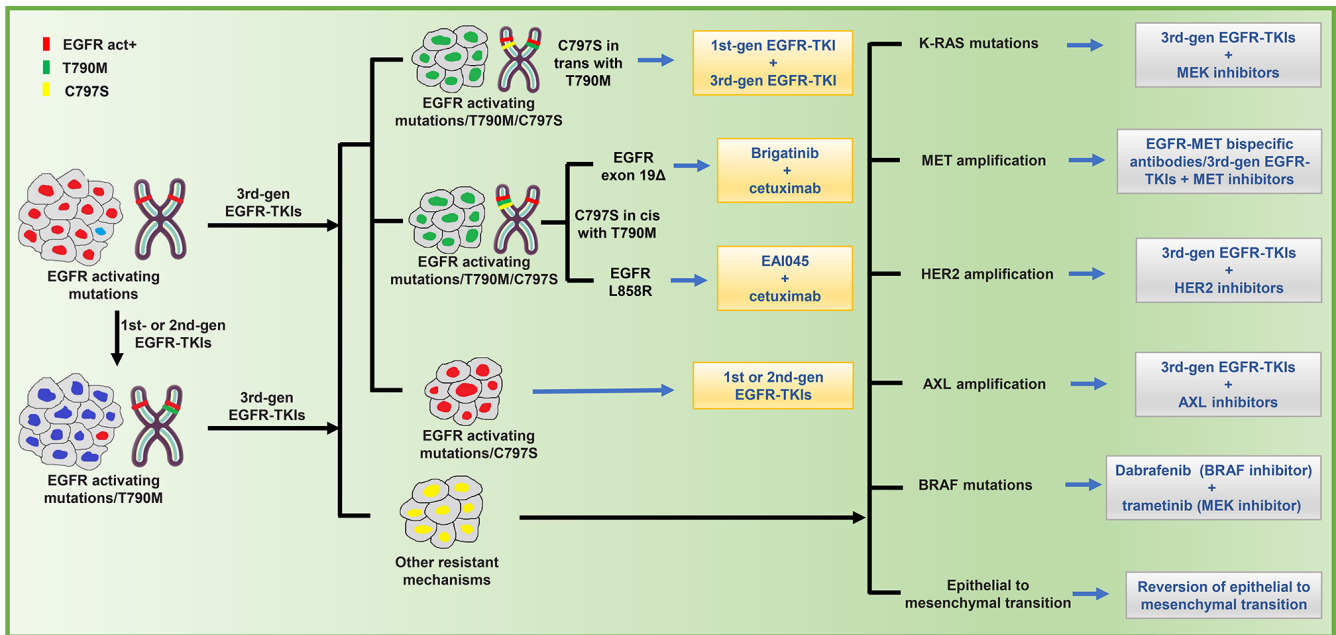
Figure 5. Possible overcome strategies to the 3rd-generation EGFR-TKI resistance in advanced *EGFR*-mutated NSCLC. For patients who developed resistance to the 3rd-generation EGFR-TKI after 1st- or 2nd-line treatment with osimertinib, according to the status of the C797S mutation, different treatment regimens can be adopted. If the C797S and T790M mutations are in the cis structure, a combination of cetuximab with brigatinib or EAI045 is effective, while C797S and T790M mutations in trans are sensitive to a combination of 1st- and 3rd-generation TKIs. For NSCLC with C797S mutation but without T790M mutation, 1st- or 2nd-generation EGFR-TKIs are promising agents. For other heterogeneous resistance mechanisms, combinations of 3rd-generation EGFR-TKIs and other targeted agents, such as MEK inhibitors, MET inhibitors and HER2 inhibitors, are effective treatment options. gen, generation; act, activation; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; *MET*, hepatocyte growth factor receptor; *HER2*, human epidermal growth factor receptor 2; AXL, anexelekto.

Carcinogenic gene fusion. RET-ERC1 and NTRK1-TPM3 gene fusions were found in osimertinib-resistant patients in the AURA3 study (54). *ALK* gene fusion was detected in the FLAURA study (59). Schrock *et al* (89) identified *BRAF*, *ALK*, *RET* and *FGFR3* gene fusions in 31 patients with osimertinib resistance. One of the patients with the T790M mutation had disease progression after osimertinib treatment. Analysis of tumor and blood-based ctDNA samples by NGS revealed a *PLEKHA7-ALK* gene fusion. Combined treatment of osimertinib and the ALK inhibitor alectinib alleviated the disease. A blood-based ctDNA test 4 months later revealed the disappearance of the *PLEKHA7-ALK* gene fusion (89). Batra *et al* (90) reported the case of a patient who developed echinoderm microtubule-associated protein like 4-*ALK* fusion after osimertinib resistance, and revealed that further treatment with the combination of osimertinib and alectinib was effective, suggesting that the oncogenic gene fusion might contribute to EGFR-TKI resistance (90).

5. Strategies to overcome resistance

Scientists are exploring approaches to overcome the different mechanisms of 3rd-generation EGFR-TKI resistance. Combination of targeted therapies is a promising strategy. For the reversion of 3rd-generation EGFR-TKI resistance, possible strategies to overcome different mechanisms are shown in Fig. 5. Some representative 4th-generation EGFR-TKIs are summarized in Table I (91-95). Case reports and ongoing clinical trials exploring the emerging combination are shown in Table II (84,89,96-104).

Targeting EGFR tertiary mutations
Fourth-generation EGFR-TKIs. If the C797S and T790M mutations are cis-structured (located in the same allele; 85%), patients are resistant to 1st-, 2nd- and 3rd-generation EGFR-TKIs (105). The 4th-generation EGFR-TKI, EAI045, is the first selective, non-ATP-competitive allosteric TKI targeting the L858R, T790M and C797S mutations (Fig. 1). However, EGFR dimerization affects the efficacy of allosteric EGFR inhibitors. EAI045 is ineffective when used alone. Cetuximab is a chimeric monoclonal antibody that inhibits EGFR dimerization (91). EAI045 in combination with cetuximab was demonstrated to significantly inhibit the proliferation of lung cancer cells with *EGFR* L858R/T790M/C797S mutations both *in vitro* and *in vivo*. However, it was ineffective against drug resistance caused by *EGFR* Ex19del/C797S/T790M mutation (91). Brigatinib, an ALK and EGFR inhibitor, was effective against both *EGFR* Ex19del/C797S/T790M and L858R/C797S/T790M mutant cells *in vitro* and *in vivo* (96). Uchibori *et al* (92) demonstrated that brigatinib fits into the ATP-binding pocket of triple-mutant EGFR. The structure-activity relationship analysis revealed that the chloro, phosphine oxide group and the methoxy group of brigatinib worked as key components to inhibit the triple-mutant EGFR. The efficacy of brigatinib was significantly enhanced when used in combination with anti-EGFR antibody due to the reduction of surface and total EGFR expression (92). A recent study reported the cases of 15 osimertinib-resistant patients with *EGFR* T790M-cis-C797S mutation, 5 of whom were treated with a combination of brigatinib and cetuximab, and the remainder of whom received cisplatin-based doublet

Table II. Combined therapies to overcome 3rd-generation EGFR-tyrosine kinase inhibitor resistance.

Targeted resistance	Target	Combined strategies	Primary outcomes	Clinical trial identifier	(Refs.)
EGFR-dependent resistance					
EGFR C797S, in cis	C797S	Brigatinib + cetuximab	mPFS time of 14 months and an ORR of 60%	None	(96)
		Osimertinib + bevacizumab + brigatinib	PR after 1 month of treatment and had fewer *EGFR* mutations	None	(97)
EGFR C797S, in trans	C797S	Osimertinib + erlotinib	PR after 1 month of treatment, PD after 3 months	None	(98)
		Osimertinib + geftinib	Clinical improvement within 3 days, PD after 1 month	None	Recruiting
EGFR-independent resistance					
MET amplification	c-Met	Osimertinib + crizotinib	PR after 1 month of treatment	None	(99)
		Osimertinib + savolitinib	Safety (DLTs and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT02143466	(100)
		Nazartinib + INC280	Safety (DLTs and MTD) and efficacy (ORR and PFS)	NCT02335944	None
HER2 amplification	HER2	Osimertinib + trastuzumab	Safety (intensity and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT03784599	None
		Osimertinib + necitumumab + trastuzumab	Safety (intensity and incidence of AEs) and efficacy (ORR, PFS and OS)	NCT04285671	None
AXL amplification	AXL	Osimertinib + DS-1205c	Safety (DLTs and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT03255083	None
RAS/RAF/MEK/ ERK pathway aberrant activation	MEK	Osimertinib + selumetinib	Safety (DLTs and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT02143466	None
BRAF mutation	BRAF	Dabrafenib + trametinib	ORR of 63% and a DCR of 79%	None	(101)
	BRAF	Dabrafenib + trametinib	Safety (intensity and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT04452877/N CT04507919	None
	mTOR	Osimertinib + sapanisertib	Safety (DLTs and incidence of AEs) and efficacy (ORR, PFS, DCR and OS)	NCT02503722	None
JAK/STAT3	JAK	Osimertinib + itacitinib	Safety (intensity and incidence of AEs) and efficacy (ORR, PFS and OS)	NCT02917993	None
		Osimertinib + AZD4205	Safety (incidence of AEs) and efficacy (ORR)	NCT03450330	None
Histological transformation					
SCLC transformation	None	Etoposide + cisplatin	Responded well to chemotherapy	None	(102)
		Etoposide + cisplatin	Clinical response rate of 54% and an estimated mPFS time of 3.4 months	None	(84)

Table II. Continued.

Targeted resistance	Target	Combined strategies	Primary outcomes	Clinical trial identifier	(Refs.)
Others					
CCDC6-RET fusion	RET	Osimertinib + BLU-667	PR after 2 months of treatment with grade 1 toxicities	None	(103)
PLEKHA7-ALK fusion	ALK	Osimertinib + alectinib	PR and a duration of response of 6 months	None	(89)
CDK4, *CDK6* amplification	CDK4/6	Osimertinib + G1T38	Safety (DLTs and incidence of AEs) and efficacy (PFS and OS)	NCT03455829	None
Unknown	VEGFR	Osimertinib + apatinib	Optimal dosage, safety (intensity and incidence of AEs) and efficacy (PFS and OS)	NCT03050411	None
		Osimertinib+ ramucirumab	Safety (intensity and incidence of AEs) and efficacy (CR, PR, SD, PFS and OS)	NCT02411448	None
	VEGF	Osimertinib + bevacizumab	Safety (MTD and intensity and incidence of AEs) and efficacy (ORR, PFS and OS)	NCT02803203/N CT02971501	None
	BCL-2	Osimertinib + navitoclax	Safety (intensity and incidence of AEs) and efficacy (ORR)	NCT02520778	None
	BIM	Osimertinib + aspirin	The mPFS time was 15.3 and 9.3 months for the combination therapy and osimertinib groups, respectively.	None	(104)

mPFS, median progression free survival; ORR, objective response rate; PR, partial response; PD, progressive disease; DLTs, dose limiting toxicities; AEs, adverse events; DCR, disease control rate; OS, overall survival; VEGFR, vascular endothelial growth factor receptor; CR, complete response; SD, stable disease; MTD, maximum tolerated dose; BIM, B-cell lymphoma-2 (BCL-2)-like 11; MET, hepatocyte growth factor receptor; HER2, human epidermal growth factor receptor 2; AXL, anexelekto; EGFR, epidermal growth factor receptor.

chemotherapy. The median PFS time was 14 and 3 months, respectively, with an ORR of 60 and 10%, respectively (96). Blu-945 is a 4th-generation EGFR-TKI designed to target *EGFR* T790M/C797S and T790M-resistant mutations. A recent study reported that BLU-945 alone or in combination with osimertinib significantly inhibited osimertinib-resistant lung cancer cell growth both *in vitro* and *in vivo* (93). TQB3804 is a 4th-generation EGFR-TKI developed in China (Fig. 1). A preclinical study showed that TQB3804 exhibits a prominent inhibitory effect on the Ex19del/T790M/C797S and L858R/ T790M/C797S triple mutations (94). A phase I clinical trial of TQB3804 is currently recruiting (NCT04128085). More clinical trials are required to validate the efficacy of the novel EGFR-TKIs in patients with advanced NSCLC.

Combination of the 1st- and 3rd-generation EGFR-TKIs. When *EGFR* C797S and T790M mutations are in the trans structure (on separate alleles; 10%), the tumor is resistant to 3rd-generation TKIs, but sensitive to combined therapy with 1st- and 3rd-generation TKIs (105). Erlotinib combined with osimertinib was effective for a patient developing T790M and C797S trans mutations after osimertinib treatment. However, resistance reappeared 3 months later and liquid biopsies showed T790M and C797S mutations in cis (106). An ongoing phase I/ II study investigated the safety and efficacy of osimertinib plus

gefitinib as the 1st-line treatment for *EGFR*-mutated NSCLC. The adverse events were tolerable, and the ORR was 85.2% (95% CI, 67.5-94.1), consistent with the 1st-line ORR to osimertinib. The median PFS time was not yet reached (107).

1st- or 2nd-generation EGFR-TKIs. *In vitro* studies have shown that NSCLC cells with *EGFR* Ex19del/C797S mutation and without *EGFR* T790M mutation are resistant to 3rd-generation EGFR-TKIs, but remain sensitive to 1st- or 2nd-generation EGFR-TKIs (105). One patient with *EGFR* Ex19del/C797S mutation had a partial tumor remission after gefitinib treatment (108). Rangachari *et al* (109) demonstrated that osimertinib-resistant NSCLC with *EGFR* C797S mutation responded transiently to the 1st-generation EGFR-TKIs, but acquired *EGFR* T790M/C797S mutation eventually (109). Yang *et al* (110) reported that afatinib was effective against *EGFR* L858R/L718Q mutation with T790M loss after osimertinib resistance, indicating that 2nd-generation EGFR-TKIs might be promising agents for selected osimertinib-resistant patients.

Targeting EGFR-independent resistance
Bypassing pathway inhibitors. Against drug resistance due to *MET* or *HER2* amplification, use of c-Met or HER2 inhibitors alone or in combination with the 3rd-generation EGFR-TKIs

is a potential therapeutic strategy. Romaniello *et al* (111) demonstrated that the combination of cetuximab, trastuzumab and osimertinib inhibited ERK activation and reduced the abundance of c-Met, AXL and HER3 *in vitro* and *in vivo*, suppressing tumor growth and preventing recurrence (111). An ongoing phase I study demonstrated that patritumab deruxtecan (a HER3-directed antibody drug conjugate) had good efficacy in patients with various types of osimertinib resistance, including *EGFR* C797S, *MET* amplification and *HER2* mutations (112). The combination of crizotinib and osimertinib substantially reduced tumor size after a 1-month treatment in a patient with *MET* amplification (99). In a multicenter, open-label, phase Ib TATTON study, osimertinib in combination with the c-Met inhibitor savolitinib was used to treat patients with advanced NSCLC harboring *EGFR* mutations and *MET* amplification after the 3rd-generation EGFR-TKI treatment, and encouraging results were obtained. In 69 patients with disease progression on the 3rd-generation EGFR-TKI treatments, the ORR was 30% (20-43) and the median PFS time was 5.4 months (95% CI, 4.1-8.0) (100). In addition, amivantamab (EGFR-MET bispecific antibody) with lazertinib has demonstrated synergistic inhibition of tumor growth in preclinical studies. Ongoing studies are exploring the safety and preliminary efficacy of this combined therapy in patients with disease progression upon osimertinib treatment (113).

Currently, the studies on AXL inhibitors are mainly at the preclinical stages, and there are few clinical trials. Okura *et al* (114) demonstrated that the novel AXL inhibitor ONO-7475 increased the sensitivity of AXL-overexpressing NSCLC cells to osimertinib and suppressed the emergence of osimertinib-resistant cells *in vitro* (114). Kim *et al* (115) found that promoting AXL degradation in combination with osimertinib delayed or overcame osimertinib resistance in *EGFR*-mutant patient-derived xenograft models (115). In addition, the newly developed drug CB469 inhibited both AXL and c-Met activation and phosphorylation, thereby overcoming acquired resistance to EGFR-TKIs mediated by AXL and c-Met activation both *in vitro* and *in vivo* (116).

Downstream signaling pathway inhibitors. For drug resistance caused by RAS/RAF/MEK/ERK signaling pathway activation, 3rd-generation EGFR-TKIs combined with MEK inhibitors are potential therapeutic strategies. Preclinical studies demonstrated that the combination of osimertinib and selumetinib overcame the resistance caused by *KRAS* amplification and overexpression (83). In another *in vitro* study, MEK inhibitors restored the sensitivity of *EGFR* ex19del/T790M/ C797S-mutated PC9 cells to osimertinib by modulating MEK/ERK-dependent degradation of BIM and MCL-1 (117). Osimertinib and MEK/ERK inhibitors synergistically promoted apoptosis and inhibited survival in osimertinib-resistant cells, and this combination could delay the emergence of osimertinib resistance both *in vitro* and *in vivo* (118). In 2017, the FDA approved the combination of dabrafenib (*BRAF* V600E inhibitor) plus trametinib (MEK inhibitor) for the treatment of patients with *BRAF V600E* mutations, with an ORR of 63 and 61%, respectively, for the 1st- and 2nd-line treatments (101). Xie *et al* (119) demonstrated that osimertinib combined with vemurafenib was effective to overcome *BRAF*

V600E-mediated osimertinib resistance (119). More clinical trials are required to validate the roles of RAS/RAF/MEK/ ERK signaling pathway inhibitors in osimertinib-resistant patients.

Targeting EMT- and BIM-mediated resistance. In a recent study, the 3rd-generation EGFR inhibitor WZ-4002 and the FGFR inhibitor dovitinib synergistically inhibited EMT-mediated resistant cell survival and prevented the generation of resistant clones. It was shown that FGFR signaling is critical for the emergence of mesenchymal-like drug tolerant clones. Dual EGFR plus FGFR inhibition might be a promising strategy to avoid resistance (120). Yochum *et al* (121) reported that the transcription factor TWIST1 inhibitor harmine and the Bcl-2/BclXL inhibitor ABT737 were able to target the EMT transcription factor TWIST1 in *EGFR*-mutant NSCLC. The study demonstrated that downregulated TWIST1 and upregulated BIM could overcome EMT- and BIM-mediated drug resistance (121). Another recent study demonstrated that combined treatment with osimertinib and aspirin promoted BIM-dependent apoptosis in osimertinib-resistant lung cancer cells. A retrospective analysis of 45 patients with NSCLC also confirmed that the median PFS time of the osimertinib plus aspirin group was significantly longer than that of the osimertinib alone group (104).

Targeting EGFR 20 exon insertion mutations. A recent study included 693 patients with *EGFR* e20ins and uncommon mutations (G719X, L861Q and S768). Afatinib was demonstrated to have broad activity against uncommon *EGFR* mutations and several e20ins, with an ORR of 60.0 and 24.3%, respectively (122). *In vitro* studies showed that cetuximab combined with afatinib was specifically sensitive to *EGFR* e20ins tumors, and this was also confirmed in clinical studies (123-125). Fang *et al* (123) reported that afatinib and cetuximab achieved long-term disease control in a patient with NSCLC and a rare *EGFR* e20ins. Poziotinib is a potent inhibitor of *EGFR* and *HER2* e20ins mutations; it can effectively inhibit *EGFR* e20ins cells *in vitro* with 100-fold greater potency than osimertinib. In a phase II clinical study, the ORR of poziotinib in patients with NSCLC and *EGFR* e20ins was 64%, and the median OS was not achieved (46). In addition, the promising clinical activity of amivantamab and mobocertinib (oral EGFR/HER2 inhibitor) in ongoing phase I/II studies also provides hope for the treatment of patients with *EGFR* e20ins in future (126,127).

Other strategies
Vascular endothelial growth factor receptor (VEGFR) inhibitors. VEGFR inhibitors have synergistic antitumor effects with EGFR inhibitors. Phase I/II clinical trials of osimertinib in combination with ramucirumab (128) or bevacizumab (129) in patients with advanced NSCLC and *EGFR* mutations are ongoing. In addition, an open-label, phase II, multicenter, single-arm trial is exploring the efficacy of afatinib plus bevacizumab in patients with *EGFR*-mutant NSCLC and osimertinib resistance (130).

Immunotherapy. A retrospective analysis showed that anti-programmed cell death-1 (PD-1) monotherapy was poorly effective in *EGFR*-mutated patients, even for patients with

PD-L1 expression ≥50% (131). In phase Ib TATTON study, osimertinib in combination with durvalumab had an ORR of 67% in patients with *EGFR* T790M mutation. However, the incidence of interstitial lung disease was 38% (132). The osimertinib plus durvalumab combination arm of the TATTON study was suspended due to severe adverse events. Immunotherapy combined with chemotherapy may provide new hope for patients resistant to the 3rd-generation EGFR-TKIs. A recent study reported that combined therapy of toripalimab (anti-PD-1 antibody) and pemetrexed/carboplatin achieved a partial response for >8 months in a osimertinib-resistant patient. A phase II clinical trial of toripalimab combined with chemotherapy in patients without *EGFR* T790M mutation achieved an ORR of 50% and a median PFS time of 7.0 months (133). Clinical trials of nivolumab (anti-PD-1 antibody) in combination with chemotherapy or ipilimumab (anti-cytotoxic T lymphocyte-associated antigen-4 antibody) (NCT02864251) and pembrolizumab (anti-PD-1 antibody) in combination with chemotherapy (NCT03515837) in patients who were resistant to the 1st- or 2nd-line treatment of osimertinib are currently underway.

Chemotherapy. For patients who developed SCLC transformation, chemotherapy after osimertinib resistance is an option. Marcoux *et al* (84) demonstrated that patients with SCLC transformation had higher response rates to etoposide, cisplatin and paclitaxel. For patients with unknown resistant mechanisms, chemotherapy is still an option. If the patient is asymptomatic or has symptomatic local progression, osimertinib can be combined with local treatment according to National Comprehensive Cancer Network (NCCN) guidelines (134). Furthermore, carboplatin/paclitaxel/bevacizumab/atezolizumab (anti-PD-L1 antibody) are also options for patients who experience systemic progression after osimertinib treatment (135). Whether chemotherapy can delay resistance to the 3rd-generation EGFR-TKIs remains unknown. A study on osimertinib with or without chemotherapy as the 1st-line treatment in patients with *EGFR*-mutated NSCLC is currently recruiting (NCT04035486).

6. Conclusions and perspectives

The resistance mechanism of 3rd-generation EGFR-TKIs for advanced NSCLC is very complex. The mechanism of 3rd-generation EGFR-TKI resistance in *EGFR*-mutated tumors is different between patients, and heterogeneous among different tumor sites. Therefore, NGS of blood-based ctDNA or tissue samples to clarify the resistance mechanism is of importance to guide the next treatment, which will be conducive to the clinical research of novel combined therapy to overcome drug resistance. According to the NCCN guidelines (version 4. 2021) (136), radical local therapy combined with osimertinib continuation should be considered for patients who progressed on osimertinib, except those who developed extensive progression after resistance, for whom platinum-based two-drug chemotherapy with or without bevacizumab is recommended. To improve survival in patients with EGFR mutations, a range of new therapies targeting different resistance mechanisms are being developed. 4th-generation EGFR-TKIs have emerged, such as EAI045, TQB3804 and BLU-945, and the multi-target TKI brigatinib, showing high potential and being worthy of attention with regard to targeting *EGFR*

T790M/C797S mutation. EAI045 is a representative of allosteric *EGFR*-mutant selective compounds. A number of other allosteric EGFR inhibitors, including JBJ-04-125-02 and DDC-01-163, are currently in development. Studies have suggested that EGFR dimerization affects the efficacy of allosteric EGFR inhibitors, so it is more effective when allosteric EGFR inhibitors are combined with osimertinib or EGFR-targeted monoclonal antibodies (cetuximab), than when used as single agents. Furthermore, the allogeneic EGFR inhibitors have potential to improve the clinical efficacy of osimertinib and delay the emergence of mutant *EGFR*-mediated resistance in NSCLC (91,137,138). Receptor structure-based drug design and library screening provides novel allogeneic EGFR inhibitors. Combined therapy of allogeneic EGFR inhibitors with conventional EGFR-TKIs, EGFR-targeted monoclonal antibodies, chemotherapy or immunotherapy offers an effective treatment option for patients with 3rd-generation EGFR-TKI-resistant NSCLC. More preclinical and clinical studies are required to validate the efficacy of combined therapies. The combinations of EGFR-TKIs with agents targeting aberrant EGFR bypassing pathways, as well as downstream pathways, are also promising to overcome EGFR-TKI resistance. Mutations in some EGFR loci are insensitive to conventional EGFR-TKIs, and the emergence of new agents such as mobocertinib, amivantamab and poziotinib have shown promising efficacy against *EGFR* e20ins. Recent studies have shown that chemotherapy plus EGFR-TKIs or bevacizumab is superior to chemotherapy alone in SCLC-transformed EGFR-mutant lung adenocarcinoma. In addition, the rapid development of immunotherapy has brought new hope for EGFR-TKI resistant patients. The combination of immune checkpoint inhibitors, anti-angiogenic agents and chemotherapy may be a promising research direction in future. Although the combination of immunotherapy and EGFR-TKIs has achieved satisfactory efficacy, serious adverse events have hindered further promotion of clinical trials. The manner in which the occurrence of adverse reactions can be reduced in the process of combination therapy is an important concern. Most combined therapies are still under development, several of which have shown satisfactory results at the preclinical stage, but failed in clinical trials. Therefore, more studies should be conducted in the future to improve our understanding of the complex signaling pathways involved in the resistance to 3rd-generation EGFR-TKIs and to find the best combination treatments modality to overcome the 3rd-generation EGFR-TKI resistance under the guidance of NGS.

ncbi.nlm.nih.gov/compound/57335384#section=2D-Structure; Abivertinib: CID 72734520; https://pubchem.ncbi.nlm.nih.gov/compound/72734520#section=2D-Structure; Nazartinib: CID 72703790; https://pubchem.ncbi.nlm.nih.gov/compound/72703790#section=2D-Structure; Olmutinib: CID 54758501; https://pubchem.ncbi.nlm.nih.gov/compound/54758501#section=2D-Structure; Alflutinib: CID 118861389; https://pubchem.ncbi.nlm.nih.gov/compound/118861389#section=2D-Structure; Almonertinib: CID 121280087; https://pubchem.ncbi.nlm.nih.gov/compound/121280087#section=2D-Structure; Lazertinib: CID 121269225; https://pubchem.ncbi.nlm.nih.gov/compound/121269225#section=2D-Structure; Lazertinib: CID 121269225; https://pubchem.ncbi.nlm.nih.gov/compound/121269225#section=2D-Structure; Naquotinib: CID 71667668; https://pubchem.ncbi.nlm.nih.gov/compound/71667668#section=2D-Structure; EAI045: CID 121231412; https://pubchem.ncbi.nlm.nih.gov/compound/121231412#section=2D-Structure; TQB3804: CID 138911391; https://pubchem.ncbi.nlm.nih.gov/compound/138911391#section=2D-Structure; Brigatinib: CID 68165256; https://pubchem.ncbi.nlm.nih.gov/compound/68165256#section=2D-Structure).

Availability of data and materials

Not applicable.

Authors' contributions

JH, YG and CX were responsible for the study conception and design. Administrative support was provided by YG and CX. JH and ZH contributed to the provision of study materials or patients. JH, ZH and LH collected and assembled the data, and JH, ZH and LH were responsible for data analysis and interpretation. All authors helped to write the manuscript and have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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