OPEN

A review of research progress on mechanisms and overcoming strategies of acquired osimertinib resistance

Fanjie Qu, Yi Zhou and Weiwei Yu

Targeted therapy with epidermal growth factor receptor tyrosine kinase inhibitors(EGFR-TKIs) is the standard first-line treatment for advanced EGFR-mutated non-small cell lung cancer (NSCLC). Third-generation EGFR-TKIs, represented by osimertinib, have been approved to overcome the EGFR T790M mutation in patients who are resistant to first- or second-generation TKIs, which brings more survival benefits for patients with advanced NSCLC. However, resistance to the third generation of EGFR-TKIs is still inevitable. Acquired drug resistance is the main reason for limiting the long-term effectiveness of targeted therapy in EGFR-mutated NSCLC patients. The mechanism of EGFR-TKI resistance of the third generation has become a focus of research in the field of targeted therapy. In this review, we summarize the research progress in resistance mechanisms of advanced NSCLC to osimertinib and the potential overcoming strategies and hope to provide a clinical basis and ideas for precision treatment of NSCLC. *Anti-Cancer Drugs* 33: e76–e83

Anti-Cancer Drugs 2022, **33**:e76–e83

Keywords: acquired drug resistance, epidermal growth factor receptor tyrosine kinase inhibitor, non-small cell lung cancer, osimertinib, resistance mechanisms

Department of Oncology, Dalian Third People's Hospital, Dalian, China

Correspondence Yi Zhou, MD, Department of Oncology, Dalian Third People's Hospital, No. 40, QianShan Road, Dalian, Liaoning Province 116033, China
Tel: +86 411 86526893; e-mail: zhou9yii@163.com

Received 31 July 2021 **Revised form accepted** 4 August 2021

Introduction

Molecular targeted therapy has successfully ushered in a new era of precision therapy for advanced nonsmall cell lung cancer (NSCLC). The discovery of epidermal growth factor receptor (EGFR) mutations significantly changed the treatment paradigm of patients with EGFR-mutant NSCLC. In these patients, the treatment of choice as first-line therapy is first- or second-generation EGFR-tyrosine kinase inhibitors (EGFR-TKIs), because patients with EGFR mutation-positive EGFR-TKIs have significantly better efficacy than conventional chemotherapy; however, most patients develop resistance after 9–14 months of first-or second-generation EGFR-TKIs. The most common mechanism of acquired resistance to EGFR-TKIs is the development of a second mutation in exon 20 of EGFR (T790M) [1]. Osimertinib is a third-generation EGFR-TKI designed to overcome T790M-mediated resistance. AURA3 studies have shown that osimertinib had significantly greater efficacy than platinum therapy plus pemetrexed in patients with T790M-positive advanced NSCLC (including investigator-assessed progression-free survival) in whom the disease had progressed during first-line EGFR-TKI therapy [1].

Osimertinib is an oral, third-generation, irreversible EGFR-TKI that selectively inhibits both EGFR-TKI-sensitizing and EGFR T790M resistance mutations [2]. A phase 3 trial [3,4] compared the first-line osimertinib with other EGFR-TKIs in patients with EGFR mutation-positive advanced NSCLC in the FLAURA trial, which showed longer progression-free survival (PFS) and longer overall survival (OS) with osimertinib than with the comparator EGFR-TKIs.

Third-generation TKI drugs show excellent clinical efficacy in patients with EGFR positive and EGFR T790M mutations, but the development of drug resistance is inevitable and heterogeneity of the tumor determines the diversity of resistance. Mechanisms of resistance to third-generation TKIs include an EGFR-dependent pathway (such as new EGFR mutations, T790M reduction/disappearance and EGFR amplification, etc.) and EGFR-independent pathways (such as bypass pathway activation and histological transformation). At present, the third-generation targeted drug resistance mechanism represented by osimertinib has become a hot topic in the research field and understanding the resistance mechanism of the most common third-generation EGFR-TKI and new drugs or schemes to overcome resistance is helpful to improve the therapeutic effect on NSCLC and improve the quality of life of the patients. In this article, we review the principle mechanisms of acquired resistance and the potential strategies to overcome osimertinib.

Resistance mechanism dependent on the epidermal growth factor receptor pathway

New epidermal growth factor receptor-C797S mutation

Mutation of C797S in EGFR is a novel mechanism of acquired resistance to third-generation TKIs. The development of acquired resistance to third-generation

0959-4973 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/CAD.0000000000001242

EGFR-TKIs, involving the cysteine residue at codon 797 mutation, has been observed. Tumors in these patients developed resistance to the EGFR-TKI AZD9291, and the study of the cell-free plasma DNA (cfDNA) of the patients found that 6 of the 15 patients acquired the C797S mutation [5]. Cells resistant to a third-generation TKI acquired an additional EGFR mutation, C797S, which prevented the suppression of EGFR [5,6]. It has been previously reported that 22% of NSCLC patients have T790M-Positive lung cancer and acquired resistance to osimertinib due to the C797S mutation [7]. Subsequent studies found that the allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors affects sensitivity to subsequent treatment strategies [5]. EGFR C797S and T790M mutations are mainly cis isomers (located on the same chromosome), accounting for 85% of the mutations. Approximately10% of patients have the C797S/T790M trans configuration (located on different chromosomes), and a small number of patients have only the C797S mutation without the combined T790M mutation; that is, C797S occurs in T790M wild-type cells [5]. Next-generation sequencing of tumor biopsies after osimertinib resistance was performed. Genotyping of plasma cell-free DNA revealed 82% of T790M cis C797S, 10% trans, 6% isolated mutations and 2% unknown mechanisms [7]. Preliminary analysis of plasma samples from 73 patients who progressed after second-line treatment with osimertinib from AURA3, 10 (14%) acquired EGFR secondary mutations in C797S [8]. However, in the plasma sample analysis of 91 patients who progressed after first-line treatment with osimertinib from Flaura, 6 (7%) acquired EGFR secondary mutation in C797S [9]. It can be seen that there are differences in the mechanism of drug resistance of osimertinib as first-line and second-line treatment for NSCLC.

Other new epidermal growth factor receptor mutations

In addition to C797S, some new mutations as EGFR-dependent mechanisms of resistance to osimertinib have been confirmed successively, such as L792X, G796S, L718Q, S768I, G796R, G796D, G724S, etc [8–16]. Other rare EGFR point mutations associated with drug resistance observed in the AURA3 and FLAURA studies were L792X (3%), G796S (1%), L718Q (1%) mutations, and S768I (1%) [8–9]. Another study found that EGFR G796/C797, L792 and L718/G719 mutations were found in osimertinib-resistant patients with 24.7, 10.8 and 9.7%, respectively. L792 and L718 mutants markedly increased the half-inhibitory concentration (IC50) of osimertinib *in vitro*, among which the L718Q mutation conferred the greatest resistance to osimertinib [10].

The G796S/R mutation can abolish the covalent binding of osimertinib to the EGFR [10,11]. Secondary mutations in L792, L718 and G719 residues lead to resistance by changing the spatial structure of the connection between osimertinib and EGFR [10]. Zheng *et al*. [12] presented a case of a Chinese NSCLC patient who developed resistance to osimertinib and discovered de novo EGFR G796D mutation as a potential mechanism.

In-vitro studies showed that most of the G796D positive cells appeared after the T790M positive clones were weakened by osimertinib [11–13]. These findings highlight tumor heterogeneity and clonal evolution during the development of drug resistance.

The EGFR G724S mutation is a very rare driver mutation. Oztan *et al*., [14] detected the EGFR G724S mutation in two patients with EGFR-driven lung adenocarcinoma who had progressed after osimertinib treatment. Fassunke *et al*.,[15] identified an association between the selection of EGFR T790M-negative but EGFRG724S-positive subclones and osimertinib resistance. They demonstrated that EGFRG724S limits the activity of third-generation EGFR inhibitors both *in vitro* and *in vivo*. Structural analyses and computational modeling indicate that EGFRG724S mutations may induce a conformation of the glycine-rich loop, which is incompatible with the binding of third-generation TKIs.

In addition, multiple secondary EGFR mutations were found in 30% of osimertinib-resistant patients, which presents a challenge for subsequent treatment [10].

Decrease or disappearance of T790M and epidermal growth factor receptor gene amplification

The decrease or disappearance of the T790M mutation is also one of the reasons for the secondary drug resistance of the third-generation TKI drugs; Thress *et al*., [5] reported that four cases lost the T790M mutation in 15 patients with resistance to osimertinib treatment. Preliminary analysis of plasma samples from 73 patients who progressed after second-line treatment with osimertinib from AURA3, 36 (49%) patients had no detectable T790M [8]. In the study by Oxnard *et al*., [7] the probability of T790M deletion was 68% (28/41) in resistant patients. They found time to treatment discontinuation was shorter in patients with T790M loss (6.1 vs. 15.2 months), suggesting the emergence of preexisting resistant clones; and in studies of serial plasma levels of mutant EGFR, loss of T790M at resistance was associated with a smaller decrease in levels of the EGFR driver mutation after 1–3 weeks of therapy (100 vs. 83% decrease; $P = 0.01$) [7]. Lin *et al*., [17] found that loss of the Thr790Met mutation but the presence of EGFR-activating mutations in plasma was associated with the shortest PFS (median 26 months). Oh *et al*., [18] found that PFS was shorter in the T790M-loss group than in the T790M-persistent group (4.4 vs. 7.7 months) through molecular pathologic data of biopsy samples obtained after resistance to osimertinib.

The loss of T790M may be a result of third-generation EGFR-TKI treatment, or it may be one of the causes of drug resistance. In T790M-preserved cases, the most

common resistance mechanism is mostly related to the C797S mutation or mesenchymal-epithelial transition factor (MET) amplification; patients with T790M deletion are often mostly independent of the EGFR signaling pathway and exhibit different resistance mechanisms [19]. Repeat biopsy after acquiring resistance to osimertinib is helpful to direct further treatment strategies.

EGFR amplification and copy number alterations are also important resistance mechanisms. Selective amplification of the EGFR-exon19del allele may represent a novel resistance mechanism to osimertinib [20]. Le *et al*., [19] found that EGFR amplification occurred in 19% (8/42) of patients with osimertinib resistance. The reason for resistance caused by EGFR amplification may be related to the relatively insufficient concentration of TKI drugs.

Resistance mechanism independent of epidermal growth factor receptor pathway

Mesenchymal-epithelial transition factor amplification

The most common alternative pathway in bypass activation is MET amplification, which accounts for 5–10% of acquired EGFR-TKI resistance cases, and plays an important role in the resistance mechanism of both the first and third generations of EGFR-TKIs.Planchard *et al*., [21] were the first to find that MET amplification was present in oxitinib-resistant patients, accompanied by the disappearance of the T790M mutation.The AURA3 study showed that Met amplification was detected in 19% (14/73) of second-line osimertinib resistance, which is the second most common resistance mechanism besides the C797S mutation [8]. Le *et al*., [19] showed that MET amplification was the second most common alteration(14%). Lin *et al*., [17] found that there were no statistically significant differences in median overall survival (MOS), PFS and survival after disease progression among patients with and without MET amplification [14].

MET amplification promotes gefitinib resistance by driving the ERBB3-dependent activation of PI3K, a pathway thought to be specific to EGFR/ERBB family receptors [22]. In the Flaura study, MET amplification occurred in 15% (14/91) of first-line osimertinib resistance, which surpassed the proportion of C797S mutations in 7% (6/91) [9]. Thus, MET amplification is the most common mechanism of resistance when osimertinib is used as first-line therapy.

Human epidermal growth factor receptor 2 amplification

Human epidermal growth factor receptor 2 (Her2) amplification plays an important role in the resistance mechanisms of both the first and third generations of EGFR-TKIs. HER2 amplification was detected by genetic analysis of osimertinib-resistant patients [21]. The AURA3 study showed that Her2 amplification was detected in 5% (4/73) of second-line osimertinib resistance, and one patient acquired MET and HER2 amplification simultaneously [8]. In the Flaura study, HER2 amplification occurred in 2% (2/91) of first-line osimertinib resistance [9].

HER2 is a member of the ErbB family, HER2 and EGFR indirectly activate PI3K, and HER2 amplification is associated with acquired EGFR-TKI resistance, occurring in 12% of NSCLC patients who develop resistant EGFR mutations. Preclinical studies have shown that HER2 overexpression reduces the sensitivity of PC9GR cell lines (T790M positive) to osimertinib [23]. HER2 amplification led to first-generation and second-generation EGFR-TKI resistance, and it was nearly 10% in patients with T790M mutations that progressed after osimertinib treatment [24].

PIK3CA mutations

PIK3CA mutations occur in lung adenocarcinomas, usually concurrent with EGFR, KRAS and ALK[25]. Some data suggest that sensitivity to EGFR-TKI is decreased in EGFR-mutant lung cancers harboring a PIK3CA mutation, and in EGFR-mutant and KRAS-mutant lung cancers, a concurrent PIK3CA mutation was associated with a decrease in MOS [26]. PIK3CA mutations have also been reported in patients treated with osimertinib, and patients with osimertinib resistance may develop PIK3CA mutations while preserving or losing the T790M mutation [7]. The incidence of PIK3CA amplification in drug-resistant patients was 10% (4/41) reported by Oxnard *et al*., [7]. In the AURA3 study, the incidence of PIK3CA amplification in osimertinib-resistant patients was 4% (3/73) [8].

Other bypasses

Cell line studies have identified the RAS pathway as important in emerging osimertinib resistance, including mutations in NRAS and copy number gains. The BRAF V600E mutation is also a known acquired resistance mutation [27]. Significant KRAS amplification was observed in osimertinib-resistant cell lines by Nakatani *et al*., [28]. Mutations in KRAS, such as G12S and Q61K, have also been associated with osimertinib resistance [7,23]. Oxnard *et al*., [7] reported two cases of osimertinib resistance with BRAF co-mutation with EGFRT790M. In the AURA3 study, one patient (1%) developed KRAS (G12D) gene mutations and two patients (3%) developed BRAFV600E mutation after osimertinib resistance [8]. In the Flaura study, KRAS mutations in patients who were treated with first-line osimertinib resistance are included: A146T (1%), G12C (1%), G12D (1%), A146T (1%), G12C (1%), G12D (1%) and BRAF V600E mutation occurred in 3% of patients (3/91)[9].

Insulin-like growth factor-1 receptor pathway, phosphatase and tension homology deleted on chromosome

ten gene deletion and amplification of fibroblast growth factor 2-fibroblast growth factor receptor 1 may lead to secondary resistance to osimertinib. Kim et al., [29] found localized amplification of fibroblast growth factor receptor (FGFR1) and increased expression of FGF2 in the tumor tissue of a patient who was resistant to osimertinib. Park *et al*., [30] found abnormal activation of insulin-like growth factor-1 receptor and deletion of insulin-like growth factor binding protein-3 in drug-resistant WZ4002 cell lines, and downregulation of International Genetics Federation (IGF)1R by shRNA, as well as inhibition of IGF1R activity, could restore the sensitivity to WZ4002. This suggests that the abnormal activation of IGF1R might be one of the mechanisms of drug resistance in third-generation EGFR-TKIs.The above data highlight that the genotype mutations in lung cancer patients will change with the change in drug therapy, so it is necessary to dynamically monitor the gene mutation spectrum of lung cancer patients in clinical practice, which may help to determine the appropriate follow-up therapy.

Histologic transformation and others

Histologic transformation from NSCLC to small-cell lung cancer (SCLC) has been described as one of the major resistance mechanisms for EGFR-TKIs and can seriously affect patient outcomes[31]. SCLC transformation of NSCLC may occur after treatment with EGFR-TKIs, accounting for 3–10% of the resistance mechanism of first-generation EGFR-TKIs, and 1% of the resistance mechanism of third-generation EGFR-TKIs [32]. Lee *et al*., [33] applied whole-genome sequencing for nine tumors acquired at various time points from four patients and detected genetic predictors for small-cell transformation and found that EGFR-TKI-resistant lung adenocarcinomas (LADCs) and SCLCs share a common clonal origin and undergo branched evolutionary trajectories, and inactivation of both Rb and p53 was strikingly more frequent in the small-cell-transformed group than in the non-transformed group [33]. Thus, the evaluation of RB1 and TP53 status in EGFR-TKI-treated LADCs is informative in predicting small-cell transformation, Lin *et al*., [17] performed ctDNA detection on 40 osimertinib-resistant patients, among which two patients underwent SCLC transformation, Marcoux *et al*., [34] found that EGFR-mutant NSCLCs can undergo SCLC transformation, and they demonstrated that this occurs at an average of 17.8 months, where the median survival since the time of SCLC transformation was 10.9 months after diagnosis and cases are often characterized by Rb1, TP53 and PIK3CA mutations.

Transformation of squamous cell carcinoma (SCC) has also been reported in osimertinib-resistant patients. Lin *et al*., [17] performed ctDNA detection in 40 oxitinib-resistant patients, among which one patient had SCC transformation. In addition to two cases of SCLC transformation, one case of SCC transformation was detected in 22 patients with osimertinib resistance in the AURA study [17]. Roca *et al*., [35] presented a pooled analysis of the outcomes of EGFR-mutated ADK patients with a changed phenotype to squamous cell carcinoma (SqCC) following TKI. The re-biopsy after third-generation TKI revealed SqCC histology along with the basal EGFR mutation, while T790 M disappeared; they found that most patients were female (82%), 41% were former smokers; median time to SqCC onset was 11.5 months, and the median survival after SqCC diagnosis was 3.5 months regardless of the treatment received [35].

In conclusion, tissue biopsy is also very important to determine whether there is a change in histological type for patients with EGFR-TKIs resistance, in addition to liquid biopsy to identify relevant mutated genes [31].

Epithelial-mesenchymal transition (EMT) beyond EGFR mutations per se is a common mechanism for acquired resistance to EGFR TKIs [7,36]. Weng *et al*., [36] reported that NSCLC cells with acquired resistance to gefitinib or osimertinib (AZD9291) exhibit EMT features, with a decrease in E-cadherin and increase in vimentin and stemness, without possessing any EGFR secondary mutations.

AURA3 and Flaura studies showed that the frequency of cell cycle gene alterations was 11 and 12%, respectively, in patients treated with osimertinib as first-line or second-line treatment [8,9]. Both cyclin E1 (CCNE1) amplification and cyclin-dependent kinases (CDK6) amplification occurred in 7% (5/73) of the AURA3 studies, which are the most common cell cycle gene alterations [10]. The most common cell cycle gene alteration in the Flaura study was CDK6 amplification (3%) [9]. Le *et al*., [19] also showed the deletion of CCND1, CCNE1, and CDKN2A [19]. Changes in cell cycle genes may be involved in the development of oxitinib resistance.

These data provide clinical evidence of the heterogeneity of acquired resistance to oxitinib in advanced NSCLC and the need for clinical trial strategies to overcome or prevent multiple resistance mechanisms

Treatment strategy after oxitinib resistance

Treatment strategies targeting resistance mechanisms dependent on the epidermal growth factor receptor pathway

First- and third-generation TKIs may also be combined effectively, but this is only likely if the C797S and T790M mutations occur, and mutation of C797S in EGFR is a common mechanism of acquired resistance to third-generation TKIs. The C797S mutation, which impairs the covalent binding between the cysteine residue at position 797 of EGFR and osimertinib, induces resistance to osimertinib. Niederst *et al*., [6] demonstrated that the allelic context in which C797S was acquired may predict responsiveness to alternative treatments. First- and third-generation TKIs may be combined effectively, but this is only likely if the C797S and T790M mutations occur in trans. If the mutations are cis, no EGFR-TKIs alone or in combination can

suppress the activity. If C797S develops in wild-type T790 cells, the cells are resistant to third-generation TKIs but retain sensitivity to first-generation TKIs [6].

Jia *et al*., [37] describe the rational discovery of EAI045, an allosteric inhibitor that targets selected drug-resistant EGFR mutants. The compound inhibits L858R/T790M-mutant EGFR with low-nanomolar potency in biochemical assays. However, as a single agent, it is not effective in blocking EGFR-driven proliferation in cells which interact in an asymmetric manner in the active state. They observed cetuximab with EAI045, a novel EGFR resistance mutation selective allosteric inhibitor was also effective in a mouse model of the triple-mutant EGFR L858R/T790M/C797S [37]. Ercan *et al*., [13] also reported that the C797S mutation, in the presence of Del19 or L858R and T790M, causes resistance to all current EGFR inhibitors, but L858R/T790M/C797S remains partially sensitive to cetuximab, which leads to the disruption of EGFR dimerization.

Brigatinib is an ALK and EGFR inhibitor that can inhibit del19/C797S/T790M mutation *in vitro* and *in vivo*, and Uchibori *et al*., [38] revealed the key component in brigatinib to inhibit the triple-mutant EGFR and demonstrated that combination therapy with brigatinib and anti-EGFR antibody is a powerful candidate to overcome triple-mutant EGFR.

Wang *et al*., [39] reported the first clinical evidence of the efficacy of combination therapy consisting of first- and third-generation EGFR-TKIs targeting concomitant EGFR T790M and C797S in trans. They also revealed that the clonal progression of C797S from trans to cis during disease progression may serve as a potential resistance mechanism.

In addition to C797S, new drug-resistant mutations have been confirmed successfully, and secondary L792 mutations may be sensitive to gefitinib [10], while patients with L718Q resistant mutations can choose the first or second generation of EGFR-TKIs [13]. Yang *et al*., [10] reported that 30% of osimertinib-resistant patients had multiple secondary mutations in EGFR, which also posed a challenge for subsequent treatment. Patients who retain the initial EGFR mutation and lose T790M may be re-selected for treatment with first-generation EGFR-TKIs [40]. Other studies have suggested that the loss of T790M does not mean the re-sensitization of first-generation TKIs, but often means the overexpression of competitive mutation sites (such as KRAS mutation, RET fusion and FGFR fusion) [7].

Strategies for targeting resistance mechanisms independent of the epidermal growth factor receptor pathway

Mesenchymal-epithelial transition factor inhibitors

The current study has demonstrated that MET amplification and hyperactivation is a resistance mechanism to both first and third-generation EGFR-TKIs, because both erlotinib and osimertinib-resistant HCC827 cell lines possessed an amplified MET gene and were cross-resistant to osimertinib or erlotinib. MET inhibition overcame the resistance of these cell lines to osimertinib both *in vitro* and *in vivo*, and the current study has demonstrated that MET amplification and hyperactivation is a resistance mechanism to both first and third generation EGFR-TKIs, because both erlotinib- and osimertinib-resistant HCC827 cell lines possessed an amplified MET gene and were cross-resistant to osimertinib or erlotinib. MET inhibition overcame the resistance of these cell lines to osimertinib both *in vitro* and *in vivo*. Hence, we suggest that MET inhibition is an effective strategy to overcome the resistance of certain EGFR-mutated NSCLCs with MET amplification to osimertinib [24]. Hence, we suggest that MET inhibition is also an effective strategy to overcome the resistance of certain EGFR-mutated NSCLCs with MET amplification to osimertinib, and Ou *et al*., [41] demonstrated that MET amplification is also a potential resistance mechanism to osimertinib. Tumor biopsy revealed the emergence of a high level of MET amplification (30 copies) after osimertinib treatment [41]. Patients treated with the single-agent MET inhibitor crizotinib had a transient symptomatic benefit [41]. Deng *et al*., [42] also believed that the combination of c-Met inhibitors crizotinib and osimertinib was an effective treatment option [42]. Martine *et al*., [43] detected MET gene amplification after prolonged treatment with osimertinib in a brain metastatic lesion from an NSCLC patient presenting with an EGFRT790M mutation. Importantly, the combination of capmatinib (c-MET inhibitor) and afatinib (ErbB-1/2/4 inhibitor) completely suppressed tumor growth in mice orthotopically injected with cells derived from this brain metastasis.

HER2 inhibitors

Her2 inhibitors alone or in combination with third-generation EGFR-TKIs are potential therapeutic strategies for drug resistance caused by HER2 amplification. La Monica *et al*., [44] found that T-DM1 exerted an additive effect when combined with osimertinib in terms of inhibition of cell proliferation, cell death and antibody-dependent cell-mediated cytotoxicity induction in PC9 and H1975 cell lines. They demonstrated that HER-2 amplification was associated with osimertinib-resistance and that T-DM1 co-administration is a potential strategy to overcome this resistance [44].

Other pathway inhibitors

Although PIK3CA mutations generally suggest a poor prognosis in patients with NSCLC, the concurrent PIK3CA mutation did not affect the benefit of EGFR-TKI monotherapy in EGFR-mutant lung cancers [26].

Third-generation EGFR-TKIs combined with mitogen-activated extracellular signal-regulated kinase inhibitors are a potential therapeutic strategy for drug

resistance caused by activation of the RAS-mitogen-activated protein kinase (MAPK) signaling pathway, and preclinical studies have found that significant KRAS amplification was observed in osimertinib-resistant cell lines, and concomitant inhibition of MAPK kinase and EGFR could overcome osimertinib resistance [28]. Eberlein *et al*., [27] reported that acquired resistance to AZD9291 is associated with an increased dependence on RAS signaling. *In vitro*, a combination of AZD9291 and selumetinib prevented the emergence of resistance in cells, and *in vivo*, concomitant dosing of AZD9291 with selumetinib caused regression of AZD9291-resistant tumors in an EGFRm/T790M transgenic model [27]. A study that reported an acquired mutation, BRAF V600E, was found in the patient at the time of progression while being treated with osimertinib. Cells grown from malignant pleural effusion were sensitive to the BRAF V600E inhibitor and were more vulnerable to combination treatment with osimertinib [45].

Other strategies

Chemotherapy

Chemotherapy after drug resistance is also an option if the patient's physical condition permits, especially for patients with SCLC transformation. However, there is currently a lack of adequate research, and there is evidence that platinum combined with etoposide chemotherapy responds well to SCLC transformation-mediated drug resistance [31]. Oh *et al*., [18] reported that two patients with SCLC transformation responded well to subsequent chemotherapy. Marcoux *et al*., [34] also found that the responses of patients with SCLC transformation to platinum-etoposide and taxanes are frequent, but checkpoint inhibitors yielded no responses [34]. Therefore, for patients who have no targetable mutations after drug resistance or whose resistance mechanism is unknown, cisplatin/carboplatin plus paclitaxel/albumin paclitaxel/pemetrexed, with or without bevacizumab, can be used for 4–6 cycles, followed by maintenance therapy.

Immune checkpoint inhibitors

Although immune checkpoint inhibitors (ICIs) have made huge advances in shifting the treatment paradigm in NSCLC, their role in EGFR-mutant disease is unclear, and despite the significant antitumor activity of pembrolizumab in NSCLC, clinical benefits have been less frequently observed in patients whose tumors harbor EGFR mutations compared to EGFR wild-type patients, and a meta-analysis revealed that in EGFR-mutant advanced NSCLC, ICIs do not improve OS over that with docetaxel [46]. A randomized controlled trial assessed the efficacy of pembrolizumab in patients with previously treated, programmed cell death ligand 1 (PD-L1)-positive, advanced NSCLC, and found that pembrolizumab prolongs OS and has a favorable benefit-to-risk profile in patients with previously treated, PD-L1-positive, advanced NSCLC [47]. Whether immunotherapy or immunization combined with third-generation TKIs can be the treatment strategy for patients resistant to third-generation TKIs still needs further study; pembrolizumab lacks efficacy in TKI-naïve, PD-L1+, EGFR-mutant patients with advanced NSCLC, including those with PD-L1 expression ≥50%, and it is not an appropriate therapeutic choice in this setting [48].

The third arm of TATTON investigated the combination of osimertinib with durvalumab and reported early data. In pretreated T790M-positive and T790M-negative patients, the response rate was 67 and 21%, respectively. The combined rate of interstitial lung disease was 38%, and as such, this arm is currently on hold [49]. Immunotherapy combined with chemotherapy may offer new hope for third-generation TKIS-resistant patients.

Takehiro reported a case of pembrolizumab and salvage chemotherapy in EGFRT790M-positive NSCLC with high PD-L1 expression, which showed a PD-L1 expression level that increased from <25 to >90% after eighth-line osimertinib therapy. He was treated with pembrolizumab as a ninth-line treatment and gemcitabine as a 10th-line treatment, which produced a good response [50]. Therefore, ICI and chemotherapy should be considered even in EGFR-mutated patients after failure of EGFR-TKIs, especially in patients with high PD-Li expression [50].

Conclusion

Clinical trials have confirmed the efficacy and safety of third-generation EGFR-TKI in patients with T790M mutation-positive lung cancer. Not only do these agents have activity in T790M mutant lung cancer but many have the advantage of wild-type inhibition. However, resistance to the third generation of EGFR-TKIs is still inevitable. Acquired drug resistance is the main reason for limiting the long-term effectiveness of targeted therapy in EGFR-mutated NSCLC patients. The mechanism of resistance of third-generation EGFR-TKIs in the treatment of advanced NSCLC is very complex. Understanding the mechanism of resistance to osimertinib is of great significance for the selection of subsequent treatments. Only by starting from the mechanism and studying new targeted drugs or new combination therapies for drug resistance targets can a more reasonable treatment strategy be developed for drug-resistant patients.

Acknowledgements

Conception and design: Qu and Zhou. All authors have read and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest.

References

1 Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, *et al*.; AURA3 Investigators. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med* 2017; **376**:629–640.

2 Mazza V, Cappuzzo F. Treating EGFR mutation resistance in non-small cell lung cancer - role of osimertinib. *Appl Clin Genet* 2017; **10**:49–56.
3 Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, *et al.*; FLAURA Investigators. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med* 2018; **378**:113–125.
4 Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, *et al.*; FLAURA Investigators. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med* 2020; **382**:41–50.
5 Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, *et al.* Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *NatMed* 2015; **21**:560–562.
6 Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, *et al.* The allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. *Clin Cancer Res* 2015; **21**:3924–3933.
7 Oxnard GR, Hu Y, Mileham KF, Husain H, Costa DB, Tracy P, *et al.* Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive Lung cancer and acquired resistance to osimertinib. *JAMA Oncol* 2018; **4**:1527–1534.
8 Papadimitrakopoulou VA, Wu YL, Han JY, *et al.* Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. *Ann Oncol* 2018; **29**(suppl 8):LBA51.
9 Ramalingam SS, Cheng Y, Zhou C, Ohe Y, Imamura F, Cho BC, Lin MC, *et al.* Mechanisms of acquired resistance to first-line osimertinib: preliminary data from the phase III FLAURA study. *Ann Oncol* 2018; **29**(suppl 8):LBA50.
10 Yang Z, Yang N, Ou Q, Xiang Y, Jiang T, Wu X, *et al.* Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non-small cell lung cancer patients. *Clin Cancer Res* 2018; **24**:3097–3107.
11 Ou SI, Cui J, Schrock AB, Goldberg ME, Zhu VW, Albacker L, *et al.* Emergence of novel and dominant acquired EGFR solvent-front mutations at Gly796 (G796S/R) together with C797S/R and L792F/H mutations in one EGFR (L858R/T790M) NSCLC patient who progressed on osimertinib. *Lung Cancer* 2017; **108**:228–231.
12 Zheng D, Hu M, Bai Y, Zhu X, Lu X, Wu C, *et al.* EGFR G796D mutation mediates resistance to osimertinib. *Oncotarget* 2017; **8**:49671–49679.
13 Ercan D, Choi HG, Yun CH, Capelletti M, Xie T, Eck MJ, *et al.* EGFR mutations and resistanceto irreversible pyrimidine-based EGFR inhibitors. *Clin CancerRes* 2015; **21**:3913–3923.
14 Oztan A, Fischer S, Schrock AB, Erlich RL, Lovly CM, Stephens PJ, *et al.* Emergence of EGFR G724S mutation in EGFR-mutant lung adenocarcinoma post progression on osimertinib. *Lung Cancer* 2017; **111**:84–87.
15 Fassunke J, Müller F, Keul M, Michels S, Dammert MA, Schmitt A, *et al.* Overcoming EGFR (G724S)-mediated osimertinib resistance through unique binding characteristics of second-generation EGFR inhibitors. *PLoS One* 2018; **9**:4655.
16 Bersanelli M, Minari R, Bordi P, Gnetti L, Bozzetti C, Squadrilli A, *et al.* L718Q mutation as new mechanism of acquired resistance to AZD9291 in EGFR-mutated NSCLC. *J Thorac Oncol* 2016; **11**:e121–e123.
17 Lin CC, Shih JY, Yu CJ, Ho CC, Liao WY, Lee JH, *et al.* Outcomes in patients with non-small-cell lung cancer and acquired Thr790Met mutation treated with osimertinib: a genomic study. *Lancet Respir Med* 2018; **6**:107–116.
18 Oh DK, Ji WJ, Kim WS, Choi CM, Yoon SK, Rho JK, Lee JC. Efficacy, safety, and resistance profile of osimertinib in T790M mutation-positive non-small cell lung cancer in real-world practice. *PLoS One* 2019; **14**:e0210225.
19 Le X, Puri S, Negrao MV, Nilsson MB, Robichaux J, Boyle T, *et al.* Landscape of EGFR-dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-mutant NSCLC. *Clin Cancer Res* 2018; **24**:6195–6203.
20 Knebel FH, Bettoni F, Shimada AK, Cruz M, Alessi JV, Negrão MV, *et al.* Sequential liquid biopsies reveal dynamic alterations of EGFR driver mutations and indicate EGFR amplification as a new mechanism of resistance to osimertinib in NSCLC. *Lung Cancer* 2017; **108**:238–241.
21 Planchard D, Loriot Y, André F, Gobert A, Auger N, Lacroix L, Soria JC. EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients. *Ann Oncol* 2015; **26**:2073–2078.
22 Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, *et al.* MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; **316**:1039–1043.
23 Ortiz-Cuaran S, Scheffler M, Plenker D, Dahmen L, Scheel AH, Fernandez-Cuesta L, *et al.* Heterogeneous mechanisms of primary and acquired resistance to third-generation EGFR inhibitors. *Clin Cancer Res* 2016; **22**:4837–4847.
24 Shi P, Oh YT, Zhang G, Yao W, Yue P, Li Y, *et al.* Met gene amplification and protein hyperactivation is a mechanism of resistance to both first and third generation EGFR inhibitors in lung cancer treatment. *Cancer Lett* 2016; **380**:494–504.
25 Chaft JE, Arcila ME, Paik PK, Lau C, Riely GJ, Pietanza MC, *et al.* Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol Cancer Ther* 2012; **11**:485–491.
26 Eng J, Woo KM, Sima CS, Plodkowski A, Hellmann MD, Chaft JE, *et al.* Impact of concurrent PIK3CA mutations on response to EGFR tyrosine kinase inhibition in EGFR-mutant Lung cancers and on prognosis in oncogene-driven lung adenocarcinomas. *J Thorac Oncol* 2015; **10**:1713–1719.
27 Eberlein CA, Stetson D, Markovets AA, Al-Kadhimi KJ, Lai Z, Fisher PR, *et al.* Acquired resistance to the mutant-selective EGFR inhibitor AZD9291 is associated with increased dependence on RAS signaling in preclinical models. *Cancer Res* 2015; **75**:2489–2500.
28 Nakatani K, Yamaoka T, Ohba M, Fujita KI, Arata S, Kusumoto S, *et al.* KRAS and EGFR amplifications mediate resistance to rociletinib and osimertinib in acquired afatinib-resistant NSCLC harboring Exon 19 Deletion/T790M in EGFR. *Mol Cancer Ther* 2019; **18**:112–126.
29 Kim TM, Song A, Kim DW, Kim S, Ahn YO, Keam B, *et al.* Mechanisms of acquired resistance to AZD9291: a mutation-selective, irreversible EGFR inhibitor. *J Thorac Oncol* 2015; **10**:1736–1744.
30 Park JH, Choi YJ, Kim SY, Lee JE, Sung KJ, Park S, *et al.* Activation of the IGF1R pathway potentially mediates acquired resistance to mutant-selective 3rd-generation EGF receptor tyrosine kinase inhibitors in advanced non-small cell lung cancer. *Oncotarget* 2016; **7**:22005–22015.
31 Minari R, Bordi P, Del Re M, Facchinetti F, Mazzoni F, Barbieri F, *et al.* Primary resistance to osimertinib due to SCLC transformation: issue of T790M determination on liquid re-biopsy. *Lung Cancer* 2018; **115**:21–27.
32 Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, *et al.* Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; **3**:75ra26.
33 Lee JK,Lee J, Kim S, Kim S, Youk J, Park S, *et al.* Clonal history and genetic predic- tors of transformation into small-cell carcinomas from lung adenocarcinomas. *JClinOncol* 2017; **35**:3065–3074.
34 Marcoux N, Gettinger SN, O'Kane G, Arbour KC, Neal JW, Husain H, *et al.* EGFR-Mutant adenocarcinomas that transform to small-cell Lung cancer and other neuroendocrine carcinomas: clinical outcomes. *J Clin Oncol* 2019; **37**:278–285.
35 Roca E, Pozzari M, Vermi W, Tovazzi V, Baggi A, Amoroso V, *et al.* Outcome of EGFR-mutated adenocarcinoma NSCLC patients with changed phenotype to squamous cell carcinoma after tyrosine kinase inhibitors: a pooled analysis with an additional case. *Lung Cancer* 2019; **127**:12–18.
36 Weng CH, Chen LY, Lin YC, Shih JY, Lin YC, Tseng RY, *et al.* Epithelial-mesenchymal transition (EMT) beyond EGFR mutations per se is a common mechanism for acquired resistance to EGFR TKI. *Oncogene* 2019; **38**:455–468.
37 Jia Y, Yun CH, Park E, Ercan D, Manuia M, Juarez J, *et al.* Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. *Nature* 2016; **534**:129–132.
38 Uchibori K, Inase N, Araki M, Kamada M, Sato S, Okuno Y, *et al.* Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. *Nat Commun* 2017; **8**:14768.
39 Wang Z, Yang JJ, Huang J, Ye JY, Zhang XC, Tu HY, *et al.* Lung adenocarcinoma harboring EGFR T790M and In trans C797S responds to combination therapy of first- and third-generation EGFR TKIs and shifts allelic configuration at resistance. *J Thorac Oncol* 2017; **12**:1723–1727.
40 Engel J, Richters A, Getlik M, Tomassi S, Keul M, Termathe M, *et al.* Targeting drug resistance in EGFR with covalent inhibitors: a structure-based design approach. *J Med Chem* 2015; **58**:6844–6863.
41 Ou SI, Agarwal N, Ali SM. High MET amplification level as a resistance mechanism to osimertinib (AZD9291) in a patient that symptomatically responded to crizotinib treatment post-osimertinib progression. *Lung Cancer* 2016; **98**:59–61.
42 Deng L, Kiedrowski LA, Ravera E, Cheng H, Halmos B. Response to dual crizotinib and osimertinib treatment in a Lung cancer patient with MET amplification detected by liquid biopsy who acquired secondary resistance to EGFR tyrosine kinase inhibition. *J Thorac Oncol* 2018; **13**:e169–e172.

43 Martinez-Marti A, Felip E, Matito J, Mereu E, Navarro A, Cedrés S, *et al*. Dual MET and ERBB inhibition overcomes intratumor plasticity in osimertinib-resistant-advanced non-small-cell lung cancer (NSCLC). *Ann Oncol* 2017; **28**:2451–2457.
44 La Monica S, Cretella D, Bonelli M, Fumarola C, Cavazzoni A, Digiacomo G, *et al*. Trastuzumab emtansine delays and overcomes resistance to the third-generation EGFR-TKI osimertinib in NSCLC EGFR mutated cell lines. *J Exp Clin Cancer Res* 2017; **36**:174.
45 Ho CC, Liao WY, Lin CA, Shih JY, Yu CJ, Yang JC. Acquired BRAF V600E mutation as resistant mechanism after treatment with osimertinib. *J Thorac Oncol* 2017; **12**:567–572.
46 Lee CK, Man J, Lord S, Links M, Gebski V, Mok T, Yang JC. Checkpoint inhibitors in metastatic EGFR-mutated non-small cell Lung cancer-A meta-analysis. *J Thorac Oncol* 2017; **12**:403–407.
47 Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, *et al*. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**:1540–1550.
48 Lisberg A, Cummings A, Goldman JW, Bornazyan K, Reese N, Wang T, *et al*. A phase II study of pembrolizumab in EGFR-mutant, PD-L1+, tyrosine kinase inhibitor naïve patients with advanced NSCLC. *J Thorac Oncol* 2018; **13**:1138–1145.
49 Ahn MJ, Yang J, Yu H, *et al*. Osimertinib combined with durvalumab in EGFR-mutant non-small cell lung cancer: results from the TATTON phase Ib trial. *J Thorac Oncol* 2016; **11**(suppl 4):S115.
50 Tozuka T, Seike M, Minegishi Y, Kitagawa S, Kato T, Takano N, *et al*. Pembrolizumab and salvage chemotherapy in EGFR T790M-positive non-small-cell lung cancer with high PD-L1 expression. *Onco Targets Ther* 2018; **11**:5601–5605.