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REVIEW

Mechanisms of resistance to third-generation EGFR tyrosine kinase inhibitors

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Abstract **The tyrosine kinase inhibitors (TKI) of the epidermal growth factor receptor (EGFR) are becoming the first line of therapy for advanced non-small cell lung cancer (NSCLC). Acquired mutations in *EGFR* account for one of the major mechanisms of resistance to the TKIs. Three generations of EGFR TKIs have been used in clinical applications. AZD9291 (osimertinib; Tagrisso) is the first and only FDA approved third-generation EGFR TKI for T790M-positive advanced NSCLC patients. However, resistance to AZD9291 arises after 9–13 months of therapy. The mechanisms of resistance to third-generation inhibitors reported to date include the *EGFR* C797S mutation, *EGFR* L718Q mutation, and amplifications of *HER-2, MET,* or *ERBB2*. To overcome the acquired resistance to AZD9291, EAI045 was discovered and recently reported to be an allosteric EGFR inhibitor that overcomes T790M- and C797S-mediated resistance. This review summarizes recent investigations on the mechanisms of resistance to the EGFR TKIs, as well as the latest development of EAI045 as a fourth-generation EGFR inhibitor.**

Keywords EGFR; tyrosine kinase inhibitor; AZD9291; EAI045

Introduction

An increasing number of effective targeted agents with novel mechanisms of action are being used in clinical applications for the treatment of advanced non-small cell lung cancer (NSCLC) [1–5]. The small molecule tyrosine kinase inhibitors (TKIs) are small molecules that bind to the epidermal growth factor receptor (EGFR); these inhibitors are becoming the first line of therapy for advanced NSCLC [6–8]. Deletion of exon 19 (del.19) and the L858R mutation in exon 21 are common EGFR-activating mutations that confer sensitivity to the first- and second-generation EGFR TKIs, such as gefitinib, erlotinib, and afatinib [9–13]. The acquired T790M mutation in exon 20 of *EGFR* is the most common mechanism of resistance to second-generation EFGR TKIs [14–16]. The mutant-selective irreversible inhibitors, AZD9291 (osimertinib or mereletinib; Tagrisso), rociletinib (CO-1686), HM61713 (BI1482694), ASP8273, EGF816, and PF-06747775 are highly active against T790M-positive NSCLC [17–20]. However, resistance is inevitable. This review summarizes the possible mechanisms of resistance and the relatively new discovery of a fourth-generation EGFR TKI named EAI045.

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Mechanisms of resistance to the first- and second-generation EGFR TKIs

Activating mutations in the kinase domain of *EGFR*, which leads to increased kinase activity and ligand independency, serve as oncogenic drivers in NSCLC. L858R, which is a single point substitution in exon 21 (45% mutations), and the exon 19 deletion (del.19; 45% mutations) are the two most-representative activating mutations [14–16]. Patients with NSCLC harboring these mutations demonstrate superior response to first-generation EGFR TKIs, gefitinib and erlotinib (response rate 50%–80%). The responses typically last for 6–12 months before resistance develops [14]. Various resistance mechanisms were elaborated, including EFGR amplification, additional genetic lesions other than EGFR, activation of parallel pathways (e.g., MET amplification), and /or downstream signaling pathways (e.g., PI3K/AKT/mTOR) [7,15]. Afatinib (BIBW2992) was shown to be active against EGFR mutants [21]. Afatinib is a second-

generation EGFR TKI which has been approved for the treatment of advanced NSCLC [10,13]. The T790M mutation in exon 20 of *EGFR* is the most common mechanism of resistance to the second-generation EGFR TKIs [2]. To conquer the T790M mutation, several third-generation EGFR TKIs were developed [2]. AZD9291 (osimertinib; Tagrisso) has been approved for T790M-positive NSCLC.

Mechanisms of resistance to third-generation EGFR TKIs

The C797S mutation in the tyrosine kinase domain of *EGFR* is a leading mechanism of resistance to the third generation of irreversible EGFR inhibitors targeting the T790M mutation [22–26]. Thress *et al.* [23] sequenced cell-free DNA (cfDNA) extracted from paired pre- and post-treatment plasma of patients who progressed after therapy on AZD9291 in the AURA study; results showed that 1 out of 7 patients acquired the *EGFR* C797S mutation. The resistance to AZD9291 was further validated in a constructed cell line harboring the C797S mutation. Subsequent research with ddPCR, which is a more sensitive method, revealed the C797S mutation in 6 (40%) out of 15 patients who developed acquired resistance to AZD9291. Two separate case reports also confirmed that the C797S mutation was responsible for resistance to AZD9291 [25] and HM61713 [27].

The mechanism of resistance to third-generation EGFR-TKIs was characterized in cell lines resistant to WZ4002, CO-1686, and AZD9291 [28]. Three major drug-resistance-related *EGFR* mutations were identified: *L718Q, L844V,* and *C797S.* All three mutations could cause resistance to WZ4002 and CO-1686, but only the C797S mutation leads to AZD9291 resistance.

MGH121 Res#1 is a novel cell line reported to be resistant to third-generation TKIs [24]. C797S was one of the acquired mutations. The investigators introduced a L858R/T790M/C797S mutant construct to the cells; these cells were shown to be resistant to all EGFR TKIs. The investigators performed detailed analyses on the effect of the presence of T790M and C797S in the same allele (i.e., *cis*) or in different alleles in the same cell (i.e., *trans*) on TKI sensitivity. This study elegantly showed that del.19/T790M was resistant to second-generation TKIs, whereas del.19/C797S was resistant to third-generation inhibitors. When T790M and C797S were *cis* in the same gene, the cells were resistant to all EGFR TKIs. The precise determination of allelic mutations should be considered in future studies to guide clinical decisions [26].

HER2 amplification was reported as another resistance mechanism [22], which was discovered in a NSCLC patient with disease progression after 12 months of AZD9291 treatment in the AURA trial. *HER2* amplification was identified without the C797S mutation in the re-biopsy tumor tissue. *CMET* amplification without the T790M or C797S mutation was reported in a separate case of NSCLC after 10 months of AZD9291 treatment [22].

The *EGFR* L718Q mutation was reported most recently to be responsible for resistance to AZD9291 in a 71-year-old woman with advanced lung adenocarcinoma harboring the L858R mutation in exon 21 of *EGFR* [29]. The patient progressed through first-line gefitinib and second-line carboplatin–pemetrexate. New tumor biopsies were found to have the T790M mutation. AZD9291 was given as the third-line therapy. After 13 months of AZD9291 treatment, the patient progressed from the partial response. A new nodal biopsy was performed; Sanger sequencing confirmed the L858R and T790M mutations but the results were negative for *C797S, HER-2, MET, ALK, KRAS,* and *BRAF* alterations. Next-generation sequencing (NGS) of the same specimen revealed EGFR L718Q mutation, which was further proven to be an acquired mutation after AZD9291 therapy [29].

Given the appearance of more cases of AZD9291 resistance, the mechanisms of resistance to third-generation EGFR TKIs has become increasingly heterogeneous (Table 1). In a recent study, specimens from seven patients in two clinical trials treated with third-generation EGFR TKIs AZD9291 (n = 5, NCT01802632) or rociletinib (n = 2, NCT02147990) were analyzed for new mutations [30]. The amplification of the *MET, ERBB2*, or the *KRAS* G12S mutations was identified after progression with AZD9291 or CO-1686 treatment [30]. The *MET* and *ERBB2* mutations are suggested to be EGFR-independent bypass-track mechanisms of resistance to third-generation EGFR inhibitors. Among these EGFR-independent resistance mechanisms, the gene amplification and protein overexpression of MET are common mechanisms of resistance to first- and third-generation EGFR TKIs [15,22,30].

EAI045 is a fourth-generation EGFR inhibitor for overcoming the C797S mutation

To overcome the commonly-acquired C797S mutation, a library of approximately 2.5 million compounds was screened in a recent study against the L858R/T790M *EGFR* mutant kinase peptide [31]. Thus, the EGFR allosteric inhibitor-001 (EAI001) was discovered. With further optimization, EAI045 was found to be the most selective inhibitor, with the highest selective inhibition of the *EFGR* mutant over the wild-type *EGFR*. The combination of EAI045 and cetuximab caused a marked tumor shrinkage in a mouse model carrying the *EGFR* mutant with L858R/T790M/C797S. Thus, EAI045

Table 1 Patients with NSCLC who developed resistance to third-generation EGFR TKIs

Case report	Pretreatment EGFR mutations	Prior treatments	Third generation EGFR TKI	Gene status at resistance	Reference
60 YF	del.19	Chemo, erlotinib Radiotherapy, afatinib/cetuximab Chemo/erlotinib	AZD9291 PFS = 9 months	del.19 and T790M and C797S and TSC2N486I	[25]
57 YF	del.19	Gefitinib, chemo Afatinib/nimotuzumab	HM61713 PFS = 17 months	del.19 and T790M and C797S	[27]
71 YF	L858R	Gefitinib	AZD9291 PFS = 13 months	L858R and T790M and L718Q	[29]
71 YF	del.19	Chemo, erlotinib	CO-1686	del.19 and T790M and HER2 A	[30]
64 YF	L861Q and T790M and HER2 A	NA	AZD 9291 PFS = 7 months	EGFR L861Q and T790M	[30]
54 YF	del.19 and T790M and MET amp	Chemo, erlotinib	CO-1686 PFS = 3 months	NA	[30]
57 YM	L858R and T790M	Gefitinib, chemo	AZD9291 PFS = 4 months	EGFR L858R and T790M and MET amp	[30]
60 YM	del.19 and T790M	Erlotinib, chemo Radiotherapy	AZD9291 PFS = 18 months	del.19 and T790M and MET amp and HER2 A	[30]
56 YF	del.19 and T790M	Gefitinib, radiotherapy Erlotinib, afatinib, afatinib/cetuximab Pemetrexate/erlotinib	AZD9291 PFS = 22 months	del.19 and T790M and C797S and MET amp	[30]
51 YF	del.19 and T790M	Gefitinib, chemo Afatinib, afatinib/cetuximab	AZD9291 PFS = 9 months	del.19 and KRAS G12S	[30]

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; del. 19, EGFR exon 19 deletion; amp, amplification; chemo, chemotherapy; NA, not available; PFS, progression free survival.

represents the first fourth-generation EGFR TKI that can overcome the T790M and C797S mutations.

Discussion and future perspectives

Given the availability of AZD9291 (osimertinib; Tagrisso) for clinical use, more and more resistance cases are expected. The wider use of cfDNA for mutation analysis will enhance the detection of acquired *EGFR* mutations [32–37].

The mechanisms of resistance to third-generation EGFR-TKIs are heterogeneous [26]. Thus far, the C797S mutation is the most commonly-acquired mutation that confers resistance to third-generation EGFR TKIs. EAI045 represents the first purposefully engineered molecule to overcome the T790M and C797S mutations. Several new classes of anticancer agents are under active development, such as the BCL-2 inhibitors [38–41], SINE inhibitors [42–45], and non-coding RNAs [46,47]. Given the heterogeneity of the resistance mechanisms [48], a combination of EGFR TKIs with other agents is required to overcome the diverse resistant clones. Among these agents, cetuximab [31,49], the immune checkpoint inhibitors [3,50–52], the MET and MEK inhibitors [53,54], and other chemotherapeutic agents are candidates for combination with EGFR TKIs. A biomarker for the combination treatment is the key element for formulating therapy regimens [55–58].

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Compliance with ethics guideline

Shuhang Wang, Yongping Song, Feifei Yan, and Delong Liu declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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