Recent updates on third generation EGFR inhibitors and emergence of fourth generation EGFR inhibitors to combat C797S resistance

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Abstract

EGFR T790M mutation leads to resistance to most of clinically available EGFR TKIs. Third-generation EGFR TKIs against the T790M mutation have been in active clinical development, which includes osimertinib, rociletinib, HM61713, ASP8273, EGF816, and PF-06747775. On the other hand recently EGFR C797S mutation was reported to be a leading mechanism of resistance to the third-generation inhibitors. The C797S mutation appears to be an ideal target for overcoming the acquired resistance to the third generation inhibitors. This review summarizes the third generation inhibitors, synthesis, their mechanism of resistance and latest development on the discovery of a fourth-generation EGFR TKIs and U to Y allosteric strategies to combat the C797S EGFR resistance problem.

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T790M EGFR
C797S EGFR
Fourth generation EGFR inhibitors

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1. Introduction

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) gefitinib, erlotinib, and afatinib are effective for the treatment of EGFR mutant Non-Small Cell Lung Cancers (NSCLCs) [1–5]. Although most patients with EGFR mutant NSCLC respond to these therapies, the responses are not permanent, and patients typically develop resistance after an average of one year on treatment [6]. There are several mechanisms of acquired resistance to erlotinib, including the development of a “gatekeeper” point mutation, T790M, which prevents the TKI from effectively inhibiting EGFR [7–11] and is observed in over 50% of resistant biopsies [12–14].

Second generation EGFR inhibitors, including afatinib (BIBW2992) and dacomitinib (PF00298804) are irreversible EGFR inhibitors that bind to Cys797 and have been shown in preclinical experiments to effectively inhibit EGFR with activating mutations (Exon 19 deletion or L858R) as well as those with the T790M resistance mutation [15–17]. However, their activity in patients with erlotinib-resistant cancers harboring T790M has been minimal [18,19]. The discordance between laboratory and clinical results is likely due to a poor therapeutic window. These drugs are equally potent against wild type EGFR and EGFR T790M, and thus the toxicity resulting from inhibiting wild type EGFR (rash and diarrhea) precludes the use of doses that would be needed to effectively suppress T790M [20].

More recently, third generation EGFR TKIs including WZ4002, CO1686, AZD9291 and EGFR816 have been developed to target mutant EGFR harboring T790M [21–23]. This class of inhibitor also binds covalently to Cys797, and largely spares WT EGFR, thereby decreasing toxicity and permitting the use of doses that fully suppress T790M. This large therapeutic window likely accommodate the greater than 50% response rates observed in clinical trials with CO1686 and AZD9291 in erlotinib resistant, T790M-positive NSCLCs (Fig. 1) [24].

Based on these promising results, both drugs have received FDA, “breakthrough therapy designation” and this class of inhibitors is on the verge of becoming widely implemented for treatment of this patient population [25]. However, as with first and second generation compounds, resistance has emerged: there have been recent reports of C797S mutation or loss of T790M mutation in cell free plasma DNA samples from patients who have developed resistance and a second separate report of C797S mutation in biopsy samples from single patient Fig. 2 [26,27]. Additionally, studies with third generation cell lines have shown that the allelic context of the activating gatekeeper and C797S mutations affects the sensitivity to three generation of inhibitors with no EGFR TKIS alone or in combination able to suppress activity when mutation are in cis as shown in Fig. 2 [28]. These data suggest that there is a need for drugs which can overcome EGFR (C797S) resistance obstacle in Non Small Cell Lung Cancer (NSCLC). This review summarizes the third generation inhibitors, their mechanism of resistance and latest development on the discovery of a fourth-generation EGFR TKIs and U to Y allostERIC strategies to combat the C797S EGFR resistance problem.

2. Third generation

The third generation TKIs such as osimertinib (AZD9291), rociletinib (CO1686), HM61713, EGFR816 and ASP8273 are mutant selective and EGFR wild type (WT) sparing, targeting sensitising EGFR mutations as well as T790M EGFR (Table 1) [29]. Furthermore they have very low inhibitory effect on WT EGFR thus overcoming the toxicity limitation seen with the first and second generation EGFR TKIs [29]. WZ4002 was one of the earliest compounds investigated. In vitro, WZ4002 was 30–100 times more potent against EGFR T790M and 100 times less potent against WT EGFR and similar potency was seen in vivo using T790M driven murine lung models. WZ4002 is currently not in clinical development [30].

2.1. Rociletinib (CO1686)

Rociletinib, also known as AVL301 and CO1686, is an orally available small molecule, irreversible inhibitor of epidermal growth factor receptor (EGFR) with potential antineoplastic activity. EGFR inhibitor CO1686 binds to and inhibits mutant forms of EGFR, including T790M, thereby leading to cell death of resistant tumor cells. Compared to other EGFR inhibitors, CO1686 inhibits T790M, a secondary acquired resistance mutation, as well as other mutant EGFRs and may have therapeutic benefits in tumors with T790M-mediated resistance to other EGFR tyrosine kinase inhibitors [31].

It has a 22-fold selectivity over WT EGFR. In NSCLC cell lines containing EGFR mutations, rociletinib demonstrates the following cellular pEGFR IC50: 62 nM in NCI-1975 (L858R/T790M), 187 nM in HCC827 (exon 19 deletion), 211 nM in PC9 (exon 19 deletion). In cell lines expressing WT EGFR, cellular pEGFR IC50 are: >4331 nM in A431, >2000 nM in NCI-H1299, and >2000 nM in NCI-H1358 [29,30].

In a Phase I/II study (TIGER-X), rociletinib was administered to patients with EGFR mutated NSCLC who had disease progression during treatment with a previous line of EGFR TKI therapy. The Phase I trial was a dose escalation study to assess safety, side-effect profile and pharmacokinetic properties of rociletinib, and the Phase II trial was an expansion arm to evaluate efficacy [32]. T790M positivity was confirmed before enrolment in the Phase II portion. At the dose of 500 mg BID, the objective response rate in 243 centrally confirmed tissues from T790M positive patients was 60% and the disease control rate was 90%. Rociletinib also showed activity in centrally confirmed T790M negative patients with the overall response rate being 37%. The common dose-limiting adverse event was grade 3 hyperglycemia occurring in 17% of patients at a dose of 500 mg BID. Grade 3 QTc prolongation was observed in 2.5% of the patients at the same dose. Treatment-related adverse events leading to drug discontinuation was seen in only 2.5% of patients at 500 mg BID [31,32].

Rociletinib hydrobromide had been filed in the U.S. for the treatment of EGFR mutant non-small cell lung cancer (NSCLC), but rejected by FDA in April 2016. Breakthrough therapy designation was assigned by FDA for this indication in 2014 [29–32].

Baevsky et al., reported the synthesis of rociletinib (Scheme 1), where they reacted 2,4-dichloro-5-trifuoromethyl-pyrimidine (1)
with N-Boc protected 1,3-diaminobenzene (2) under the basic condition (n-ButOH, DIPEA). Intermediate (3) deprotected to form intermediate (4) using acid in presence of TFA. Compound (4) acylated with acryloyl chloride (5) to form intermediate (6), which was reacted with 1-(4-(4-amino-3-methoxyphenyl)-piperazin-1-yl)ethan-1-one (7) to form rociletinib (8) [33].
2.2. Osimertinib (AZD9291)

Osimertinib (previously known as mereletinib or AZD9291, trade name Tagrisso) is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) drug developed by AstraZeneca Pharmaceuticals for mutated EGFR cancers [34–36]. It is a mono-anilino-pyrimidine compound that is structurally different from other third generation EGFR TKIs (Table 1). Osimertinib targets the cysteine-797 residue in the ATP binding site of the EGFR kinase irreversibly through covalent bond formation [29,30]. It is structurally different from the first and second-generation EGFR TKIs. This compound is an irreversible mutant-selective EGFR TKI (exon 19 deletion EGFR IC$_{50}$ = 12.92 nM, L858R/T790MEGFR IC$_{50}$ = 11.44 nM, wild-type EGFR IC$_{50}$ = 493.8 nM) [29,30]. It is the only approved EGFR TKI currently indicated for patients with metastatic EGFR T790M mutation-positive NSCLC [29,30]. Two circulating active metabolites, a desmethyl indole analog AZ5104 and an N-demethylated analog AZ7550 were observed in both pre-clinical as well as clinical settings [29,30]. Compared with osimertinib, AZ5104 showed better potency against the activating mutant (IC$_{50}$ of 2 nM in PC9 cell line) and the double mutant (IC$_{50}$ of 2 nM in H1975 cell line), and IC$_{50}$ of 33 nM in LoVo cell line with WT EGFR. AZ7550 showed a very similar potency (IC$_{50}$ of 28 nM in PC9 cell line, IC$_{50}$ of 45 nM in H1975 cell line) and selectivity profile (WT EGFR; IC$_{50}$ of 786 nM in LoVo cell line) to osimertinib [37–40]. In a pharmacokinetic study in mice, osimertinib demonstrated good bioavailability, moderate clearance and wide tissue distribution with a half-life of 3 h following a single oral dose of 25 mg/kg. The half-lives of the circulating metabolites in plasma were similar to osimertinib and the total exposure levels (AUC) were approximately 68% and 33% compared to parent for AZ7550 and AZ5104, respectively. It is interesting to note that the level of metabolites (AZ7550 and AZ5104) relative to osimertinib is significantly lower in human than that in mouse [29,30].

In November 2015, after a Priority Review, the US FDA granted accelerated approval to osimertinib for the treatment of metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, which has progressed on or after EGFR tyrosine

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Table 1

Third generation EGFR tyrosine kinase inhibitors.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Research Code</th>
<th>Status</th>
<th>Company</th>
<th>CAS No.</th>
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<td>Approved</td>
<td>AstraZeneca</td>
<td>1421373-65-0</td>
<td>NCT02151981</td>
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<tr>
<td>Olmutinib</td>
<td>[Image]</td>
<td>HM61713</td>
<td>Approved</td>
<td>Hannmi, Boehringer Ingelheim, Zai Lab</td>
<td>1353550-13-6</td>
<td>NCT01588145</td>
</tr>
<tr>
<td>Rocletinib</td>
<td>[Image]</td>
<td>AVL301, CO1686</td>
<td>Phase II/III Rejected by FDA April 2016</td>
<td>Avila Therapeutics and Co-developed by the Originator and Clovis Oncology</td>
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<td>[Image]</td>
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<td>Phase II (Active)</td>
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<td>[Image]</td>
<td>ASP8273</td>
<td>Phase II (Active)</td>
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<tr>
<td>Brigatinib</td>
<td>[Image]</td>
<td>AP26113</td>
<td>Phase I/II</td>
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</tr>
<tr>
<td>Avitinib</td>
<td>[Image]</td>
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<td>Phase II (Active)</td>
<td>ACEA Bioscience</td>
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<tr>
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The FDA approval made reference to two clinical trials, in which an EGFR T790M mutation was confirmed by a Cobas EGFR mutation test; osimertinib was given as 80 mg once daily [29,30]. The European Commission (EC) gave a similar approval in Feb 2016 after two Phase II studies (AURA extension and AURA2). Osimertinib is being compared to chemotherapy in the AURA3 phase III trial [29,30,41-43].

Butterworth et al., reported the synthesis of osimertinib as depicted in Scheme 2, where 1H-indole (9) is reacted with 1,2-dichlorehane (10) in presence of methyl magnesium bromide to afford 3-(2-chloropyrimidin-4-yl)-1H-indole (11). Methylation of 11 using iodomethane gave 3-(2-chloropyrimidin-4-yl)-1-methylindolide (12). Further reaction of 3-(2-chloropyrimidin-4-yl)-1-methylindolide (12) and 4-fluoro-2-methoxy-5-nitroaniline (13) using 4-methylbenzenesulfonic acid hydrate afford N-(4-fluoro-2-methoxy-5-nitrophenyl)-4-(1-methylindol-3-yl) pyrimidin-2-amine (14) as a yellow solid. N1,N1,N2-trimethylethane-1,2-diamine (15) was added to a suspension of N-(4-fluoro-2-methoxy-5-nitrophenyl)-4-(1-methylindol-3-yl) pyrimidin-2-amine (14) and DIPEA, this mixture was heated in a microwave and purified by flash silica chromatography to afford compound (26).

2.3. Olmutinib (HM61713)

HM61713 is an irreversible kinase inhibitor and covalently binds to a cysteine residue near the kinase domain of mutant EGFR. HM61713 has a half-life of over 24 h for EGFR inhibition [29,30,44-48]. HM61713 caused potent inhibition in cell lines H1975 (L858R and T790M) and HCC827 (exon 19 deletion) [49-52]. HM61713 has a low potency for NSCLC cell line H358 harboring wild-type EGFR (GI50 of 2225 nM) [29,30]. In the in vivo studies of xenograft models with grafts of H1975 and HCC827, HM61713 was active against the tumors without showing any side effects [29,30].

Olmotinib was approved by the Korean Ministry of Food and Drug Safety (MFDS) on May, 2016 [53,54]. It was originally discovered by Hanmi, then licensed to Boehringer Ingelheim the development and global commercialisation rights, except South Korea, China and Hong Kong in July 2015, and licensed to ZAI Lab the exclusive rights in China (including Hong Kong and Macau) in November 2015 [55]. Olmutinib is an EGFR tyrosine kinase inhibitor (TKI), for the treatment of patients with locally advanced or metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer [56].

Cha et al., reported the synthesis of olmutinib (Scheme 3) [57]. Compound (19) is subjected to condensation reaction with urea (20) in an organic solvent (e.g. N,N-dimethylformamide) to obtain condensed compound (21). Compound (21) thus obtained is reflux with stirring in the presence of chlorinating agent (phosphorous oxychloride or thionyl chloride) to obtain a chlorinated compound (22). Compound (22) followed by a reaction in an organic solvent in the presence of an inorganic base with 3-nitrophenol (23) at a temperature ranging from room temperature to 100 °C, inducing a substitution at the C-4 position of the compound (24). Compound (24) reacted with 4-(4-methylpyperazin-1-yl)iline (25) in an alcohol solution or organic acid in the presence of palladium catalyst gave a compound (26). Compound (26) is subjected to reduction mediated with iron, to obtain compound (27). Aniline compound (27) is subjected to a reaction with an acryloyl chloride (5) in an organic solvent, at a low temperature gave olmutinib (28) [57].

2.4. Nazartinib (EGF816)

Nazartinib, also known as EGF816, is an orally available, irreversible, third-generation, mutant-selective epidermal growth inhibitor (TKI) therapy [29,30].
factor receptor (EGFR) inhibitor, with potential antineoplastic activity [58]. EGF816 covalently binds to and inhibits the activity of mutant forms of EGFR, including the T790M EGFR mutant, thereby preventing EGFR-mediated signaling [59]. This may both induce cell death and inhibit tumor growth in EGFR-overexpressing tumor cells. EGF816 preferentially inhibits mutated forms of EGFR including T790M, a secondarily acquired resistance mutation, and may have therapeutic benefits in tumors with T790M-mediated resistance when compared to other EGFR tyrosine kinase inhibitors [60]. EGF816 target profiles suggest that it represents an alternative and better therapy option against T790M mutations [29,30].

EGF816 (Novartis Pharmaceuticals) has preclinical activity targeting sensitizing EGFR mutants as well as T790M mutants with 60-fold selectivity over WT EGFR [29,30]. EGF816 is in phase II clinical trials by Novartis for treatment of non-small cell lung cancer (NSCLC) in combination with nivolumab and it is also in phase I/II clinical trials for the treatment of solid tumors [61–64].

Lelais et al., reported the synthesis of nazartinib as given in Scheme 4 [65]. 1-Chloro-2-fluoro-3-nitrobenzene (29) reacted with Boc protected azocan-3-amine (30) in DMF to give Boc protected (R)-N-(2-chloro-6-nitrophenyl)azepan-3-amine (31). A mixture of Boc protected (R)-N-(2-chloro-6-nitrophenyl)azepan-3-amine (31) was reduced using Zn in AcOH to afford (R)-tert-butyl 3-((2-amino-6-chlorophenyl)amino)azepane-1-carboxylate (32), which was further reacted to give tert-butyl (R)-3-(2-amino-7-chloro-1H-benzo[d]imidazol-1-yl)azepane-1-carboxylate (33). 2-methylisonicotinic acid (34) is added to the 33 using HATU as catalyst to afford-((R)-tert-butyl)-3-(7-chloro-2-(2-methyl

![Scheme 2. Synthesis of osimertinib.](image)
sonicotinamido)-H-benzo[d]imidazol-1-yl)azepane-1-carboxylate (35) as a light yellow foam. A solution of 35 in MeOH was treated with HCl in dioxane for Boc deprotection to afford the title compound (36). Compound 4-(dimethylamino)-but-2-enoic acid hydrochloride (38) was added to compound 37 in DMF to afford the title compound nazartinib (39).

2.5. Naquotinib (ASP8273)

ASP8273 is another small molecule, irreversible TKI inhibitor that inhibits the kinase activity of EGFR mutations including T790M, with limited activity against EGFR wild-type (WT) NSCLC [29]. In the in vitro enzymatic and cell-based assays, ASP8273 were evaluated against EGFR mutants (L858R, exon 19 deletion, L858R/T790M, and del19/T790M) and WT EGFR [30]. ASP8273 was found by mass spectrometry to covalently bind to a mutant EGFR (L858R/T790M) via cysteine residue 797 in the kinase domain of EGFR with long-lasting inhibition of EGFR phosphorylation for 24 h. In the NSCLC cell lines harboring the above EGFR mutations, ASP8273 had IC₅₀ values of 8–33 nM toward EGFR mutants, more potently than that of WT EGFR (IC₅₀ value of 230 nM). In mouse xenograft models, ASP8273 induced complete regression of the tumors after 14 days of treatment [66]. ASP8273 was found to suppress the signaling pathway through ERK and Akt. ASP8273 even showed activity in mutant EGFR cell line which is resistant to other EGFR TKIs including AZD9291 and CO1686 [67]. Therefore, ASP8273 represents a unique agent active in NSCLC with EGFR T790M mutation.

ASP-8273 is in phase III clinical trials for the oral treatment of epidermal growth factor receptor (EGFR) mutated non-small cell lung cancer (NSCLC) [68]. Synthesis of naquotinib is not disclosed yet.

2.6. PF06747775

PF06747775 is another small molecule inhibitor of EGFR T790M. This molecule is being studied in phase I/II clinical trial (NCT02349633) in advanced NSCLC patients with EGFR mutations (del 19 or L858R ± T790M) [29]. The agent will be administered as continuous daily dosing in 21-day cycles. The starting dose of PF06747775 will be 25 mg PO daily [30]. Patient enrolled need to have confirmed sensitizing EGFR mutations and had progressed on prior EGFR TKI. In phase II component, all patients need to have confirmed T790M mutation [69]. Behenna et al., reported the synthesis of PF06747775 (Scheme 5) [70,71].
treated with potassium tert-butoxide to give the title compound (PF-06747775) \[46\] \[70,71\].

2.7. Avitinib (AC0010)

Avtinib is in phase I/II clinical trials by ACEA Biosciences (Hangzhou) for the treatment of non small cell lung cancer (NSCLC) \[72\]. Avtinib, also known as AC0010 or AC0010MA, is an orally available, irreversible, epidermal growth factor receptor (EGFR) mutant-selective inhibitor, with potential antineoplastic activity \[72\]. Upon oral administration, avtinib covalently binds to and inhibits the activity of mutant forms of EGFR, including the drug-resistant T790M EGFR mutant, which prevents signaling mediated by mutant forms of EGFR. This may both induce cell death and inhibit tumor growth in EGFR-mutated tumor cells. EGFR, a receptor tyrosine kinase that is mutated in a variety of cancers, plays a key role in tumor cell proliferation and tumor vascularisation \[73\]. As this agent is selective towards mutant forms of EGFR, its toxicity profile may be reduced when compared to non-selective EGFR inhibitors, which also inhibit wild-type EGFR \[73\]. Synthesis of Avitinib is not disclosed yet.

2.8. Brigatinib (AP26113)

Brigatinib, also known as AP-26113, is an orally active, potent and selective Dual ALK/EGFR inhibitor. AP26113 binds to and inhibits ALK kinase and ALK fusion proteins as well as EGFR and mutant forms \[74\]. This leads to the inhibition of ALK kinase and EGFR kinase, disrupts their signaling pathways and eventually inhibits tumor cell growth in susceptible tumor cells \[75,76\]. In addition, AP26113 appears to overcome mutation-based resistance. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development; ALK dysregulation and gene rearrangements are associated with a series of tumors \[77\]. Brigatinib has been filed by Ariad in US for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive locally advanced or metastatic non-small cell lung cancer (NSCLC) who has been resistant to crizotinib \[78\]. Brigatinib received Breakthrough Therapy designation from the FDA for the treatment of patients with ALK-NSCLC whose tumors are resistant to crizotinib, and was granted orphan drug designation by the FDA for the treatment of ALK-NSCLC \[79\]. The Company is seeking accelerated approval for brigatinib from the FDA and plans to request a priority review of the application \[80,81\].

Rozamus et al., reported the synthesis of brigatinib as given in Scheme 6 \[82\]. 2,4,5-trichloropyrimidin-4(3H)-one (48) in a DMF was reacted with 4-(dimethylphosphoryl)aniline (47) to give the final product 49. To a 4-fluoro-2-methoxy-1-nitrobenzene (51) in DMF was added 1-methyl-4-(piperidin) piperazine (50) and potassium carbonate to afford the 52. A reduction of (52) with palladium gives the compound 53. Addition of 2,5-dichloro-N-[4-(dimethylphosphoryl)phenyl]pyrimidin-4-amine (49) in 2-methoxyethanol to 2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]aniline (53) gave brigatinib (54).

3. Mechanism of resistance to third generation inhibitors

Whilst the third generation EGFR TKIs are highly active, progression still occurs. Several mechanisms of acquired resistance to osimertinib and rociletinib have been described in in vitro and in
the clinical setting. C797S mutation located within the tyrosine kinase domain of EGFR was reported to be a leading mechanism of resistance to the third generation irreversible EGFR inhibitors targeting T790M mutation [83–86]. HER2 amplification [83], CMET amplification [83] and EGFR L718Q mutation [87] were also responsible for acquired resistance to third-generation EGFR inhibitors without T790M or C797S mutation. Amplifications of MET, HER2, or KRAS G12S mutation was recently identified in NSCLC patients who progressed with treatment of AZD9291 or CO1686 [88] as shown in Fig. 3.

3.2. L718Q, L844V and C797S mutation

Ercan et al., identified 3 major drug resistance mutations. EGFR L718Q, L844V and C797S cause resistance to both WZ4002 and rociletinib (CO1686) while, in contrast, only EGFR C797S leads to osimertinib (AZD9291) resistance. Cells containing an EGFR sensitizing mutation, Del 19 or L858R, in conjunction with L718Q, L844V or C797S retain sensitivity to quinazoline based EGFR inhibitors gefitinib and afatinib. The C797S mutation, in the presence of Del 19 or L858R and T790M, causes resistance to all current EGFR inhibitors, but L858R/T790M/C797S remains partially sensitive to cetuximab which leads to disruption of EGFR dimerization (Fig. 3) [90].

3.3. C797S mutation

3.3.1. C797S mutation mediates resistance to osimertinib (AZD9291)

In the first-in-human phase I/II AURA trial of AZD9291, systemic progression in NSCLC patients was seen after treatment for a median of 9.6 months [91]. Characterization of the mechanisms of resistance in 22 patients who became resistant to AZD9291 was reported [92]. These patients with progression on AZD9291 in the AURA trial had paired pre-treatment and post treatment plasma samples.

Through the study of T790M-positive patients with acquired resistance to AZD9291, three molecular subtypes of AZD9291 resistance were revealed: (1) acquired C797S together with a T790M mutation [93]. This novel mutation is a ‘tertiary’ substitution mutation at the AZD9291 binding site, changing cysteine 797...
to serine (EGFR C797S), which results in the blocking of drug binding. (2) T790M mutation without a C797S mutation and (3) loss of the T790M mutation without a C797S mutation. The report also discovered that in some cases, two different nucleotide mutations (T to A and G to C) leading to C797S amino acid mutation occurred in the same patients. Since only 6 out of 15 cases acquired C797S mutation, additional mechanisms of resistance to AZD9291 must be present (Fig. 3) [94].

3.3.2. C797S mutation mediates resistance to olmutinib (HM61713)

Song et al., reported the first case of C797S mutation as resistance mechanism of olmutinib (HM61713) that blocks EGFR inhibition and promotes resistance to third-generation EGFR TKIs. This case also highlights the importance of repeat biopsy to evaluate the underlying resistance mechanism in patients in whom resistance to third-generation EGFR TKIs develops and to make treatment decisions. Acquired resistant C797S mutation was known to be one of the resistance mechanisms of osimertinib (AZD9291), which has not been reported for HM61713 yet (Fig. 3) [95].

3.3.3. Cis and trans C797S

Niederst et al., demonstrate that the allelic context in which C797S was acquired may predict responsiveness to alternative treatments. If the C797S and T790M mutations are in trans, cells will be resistant to third generation EGFR TKIs, but will be sensitive to a combination of first and third generation TKIs. If the mutations are in cis, no EGFR TKIs alone or in combination can suppress activity. If C797S develops in cells wild type for T790 (when third generation TKIs are administered in the first line setting), the cells are resistant to third generation TKIs, but retain sensitivity to first generation TKIs. The context in which the C797S develops with respect to the other EGFR alleles impacts the efficacy of subsequent treatments (Fig. 3) [96].

3.4. HER2 and MET amplification mediates resistance to osimertinib (AZD9291)

Since some patients who progressed on AZD9291 were negative for the C797S mutation, additional resistance mechanisms must be
present. In a case report, a 54-year-old male with stage IV adenocarcinoma was found to have acquired T790M mutation after progression from second-line treatment with gefitinib [97]. The disease progressed after 12 months of AZD9291 treatment on the AURA trial. HER2 amplification was identified without C797S mutation from the tumor biopsy.

The second patient from the same report was a 60-year-old female, a never-smoker, who was diagnosed with stage IV adenocarcinoma with pleural metastasis [98]. Mutation analysis revealed the known EGFR activating mutation in exon 21, L858R. She received erlotinib and gefitinib sequentially for 12 months. After disease progression, T790M was identified by NGS and she was treated with AZD9291 on the AURA trial [99]. She had partial tumor regression and remained progression free for 10 months. Re-biopsy of the AZD9291 resistant tumor identified an EGFR activating mutation and CMET amplification without T790M or C797S mutation.

These two cases indicated that in refractory NSCLC without T790M or C797S mutations, additional gene mutations or amplifications of tyrosine kinases other than EGFR can be the mechanisms of resistance. Additional treatment targeting the mutations will be needed (Fig. 3) [99].

3.5. RAS signalling dependence of osimertinib (AZD9291)

Eberlein et al., an increased dependence on RAS signalling was reported including novel NRAS mutation (E63K), gain of copy number of WT NRAS and KRAS in pre-clinical studies of acquired resistance to osimertinib. MEK inhibition (with selumetinib) in addition to osimertinib resulted in regression of tumor in transgenic model (Fig. 3) [100].

4. Discovery of fourth generation allosteric C797S inhibitors

To search for an allosteric inhibitor that binds to EGFR away
from the ATP-binding site, jia et al., obtained human EGFR mutant peptides spanning residues 696–1022 (including wild type, L858R, L858R/T790M, T790M, and T790M/V948R mutant) [101]. The purified EGFR L858R/T790M mutant enzyme was used to screen a library of 2.5 million compounds at 1 μM ATP concentration. 1322 top hits were found from this round of screening. The top hits were then assayed for their IC50 values at both 1 μM and 1 mM ATP to separate the ATP competitive from non-competitive compounds. The top hits were also screened against the wild-type EGFR to select out those with more specificity for the mutant EGFR.

The first compound, EAI001 (EGFR allosteric inhibitor-1) (Fig. 4), was discovered with such potency and selectivity for mutant EGFR (half maximal inhibitory concentration (at 1 mM ATP, IC50 0.024 μM for L858R/t790M, IC50 > 50 μM for wild-type EGFR)). However, it only had modest potency against individual L858R and T790M mutants. After medicinal-chemistry-based optimization of EAI001, EAI045 inhibitor was found to have high potency and selectivity for L858R/T790M mutation (Fig. 4). The profound selectivity for the EGFR mutant was confirmed through profiling a panel of 250 protein kinases. EAI045 was therefore confirmed to be an allosteric, non-ATP competitive inhibitor of mutant EGFR [101].

In L858R/T790M-mutant NSCLC cell line H1975 cells, EAI045 decreased but did not completely abolish the EGFR autophosphorylation. In stably transfected NIH-3T3 cells harboring the L858R/T790M EGFR mutant, EAI045 showed the same activity. In L858R-mutant H3255 cells, EAI045 exhibited moderate activity (Fig. 4) [101].

In the HaCaT cells, a keratinocyte cell line with wild-type EGFR, EAI045 did not show any activity of inhibiting EGFR phosphorylation. In stably transfected NIH-3T3 cells harboring the L858R/T790M EGFR mutant, EAI045 showed the same activity. In L858R-mutant H3255 cells, EAI045 exhibited moderate activity (Fig. 4) [101]. In the HaCaT cells, a keratinocyte cell line with wild-type EGFR, EAI045 did not show any activity of inhibiting EGFR phosphorylation. These again confirmed the selectivity of EAI045 for mutant EGFR. Since dimerization of EGFR is required for its activation, these investigators hypothesized that the allosteric inhibitor was inactive for those asymmetric dimers/dimers between wild type and mutant EGFR peptides. The investigators confirmed that EAI045 was markedly more active in dimerization-defective EGFR mutants. When combined with cetuximab, a monoclonal antibody that can block EGFR dimerization by preventing EGF ligand binding, EAI045 markedly inhibited the proliferation of Ba/F3 cells bearing L858R/T790M mutation. These in vitro studies proved that EAI045 is active and selective for T790M-harboring EGFR mutants that are in a monomer state [101].

In a genetically engineered mouse model of L858R/T790M-mutant-driven lung cancer, the efficacy of EAI045 was tested alone and in combination with cetuximab. Remarkable tumor regression was observed in L858R/T790M-mutant mice treated with the combination of EAI045 and cetuximab. No response was seen in those mice treated with EAI045 alone. The same effect was seen in both L858R/T790M/C797S engineered Ba/F3 cells and in mice carrying the L858R/T790M/C797S tumor xenografts. These assays clearly showed that EAI045 can overcome resistance from acquired T790M and C797S mutations [101].

5. U to Y allosteric strategy to combat C797S EGFR resistance

The cocrystal structure (PDB code 3IKA and 4ZAU) of the EGFR T790M kinase domain in complex with WZ4002 and AZD9291 indicated that a “U-shaped” configuration of a pyrimidine core, together with an aniline ring bearing a hydrophilic group and an acrylamide moiety, is propitious to bind with EGFR target (Figs. 4 and 5). A great numbers of small molecules were designed and synthesized as selective EGFR inhibitors against the EGFR T790M mutation based on this binding mechanism.

Similarly we studied the binding interaction of allosteric inhibitors EAI045 using the PDB ID SD41 [105]. EAI045 was an allosteric, non-ATP competitive inhibitor of mutant T790M EGFR, having Y shaped configuration as shown in (Figs. 4 and 5). Focusing on these molecules and reviewing their design strategies (U to Y shaped configuration) along with their typical anticaner activity will provide valuable clues for the further development of more active EGFR T790M inhibitors.

6. Conclusion and future perspectives

The tyrosine kinase inhibitors are the first treatment of choice for most advanced NSCLC. T790M mutation of exon 20 of the EGFR is the most common mechanism of acquired resistance to the first and second generation EGFR TKIs. AZD9291 (osimertinib), rociletinib (CO686) and HM61713 (B11482694) are third-generation EGFR inhibitors targeting the T790M mutation. AZD9291 has been approved for T790M positive advanced NSCLC. However, resistance arises rapidly, generally over a period of 9–13 months. Biomarker studies and mutation analysis are playing more and more important roles in guiding cancer diagnosis and clinical development of novel therapeutic agents. Through these approaches, EGFR C797S mutation was quickly identified to be a leading mechanism of resistance to second-generation inhibitors. Additional mechanisms of resistance have been reported. The C797S mutation appears to be an ideal target for overcoming the acquired resistance to the third-generation inhibitors. Additional mechanisms of resistance have been reported. The C797S mutation appears to be an ideal target for overcoming the acquired resistance to the third-generation inhibitors. EAI045 is so far the first allosteric TKI purposefully engineered to overcome T790M and C797S mutations. Mutations in C797S are not supposed to affect the efficacy of EAI045, because the residue is away from the allosteric binding pocket. The allosteric inhibitor however is ineffective alone due to...
receptor dimerization. However, combination with cetuximab renders EA045 fully active against T790M and C797S. The clinical efficacy of this compound remains unknown at the moment. C797S mutation is not the only mechanism for resistance to third-generation EGFR TKIs. Albeit less common, EGFR L718Q mutation represents another mechanism of resistance to AZD9291. Hence it is possible to develop TKIs targeting this particular mutant. Combination of EGFR TKI with other agents should be one of the future directions to overcome the diverse resistant tumor clones. Besides cetuximab, MET and MEK inhibitors may be of value in combination therapy. Immune checkpoint blockers have been approved for therapy of a variety of advanced cancers. These may be considered for combination therapy to overcome the resistance to TKIs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.05.027.

Abbreviations

EGFR  epidermal growth factor receptor
TKIs  tyrosine kinase inhibitors
WT  wild type
NSCLC  Non small cell lung cancer
THF  tetrahydrofuran
H  hours

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