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Development of epidermal growth factor receptor tyrosine kinase inhibitors against EGFR T790M. Mutation in non small-cell lung carcinoma

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Abstract: Individualized therapies targeting epidermal growth factor receptor (EGFR) mutations show promises for the treatment of non small-cell lung carcinoma (NSCLC). However, disease progression almost invariably occurs 1 year after tyrosine kinase inhibitor (TKI) treatment. The most prominent mechanism of acquired resistance involves the secondary EGFR mutation, namely EGFR T790M, which accounts for 50%–60% of resistant tumors. A large amount of studies have focused on the development of effective strategies to treat TKI-resistant EGFR T790M mutation in lung tumors. Novel generations of EGFR inhibitors are producing encouraging results in patients with acquired resistance against EGFR T790M mutation. This review will summarize the novel inhibitors, which might overcome resistance against EGFR T790M mutation.

Keywords: EGFR-TKIs; EGFR mutations; T790M; AZD9291; CO-1686

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1 Introduction

Global Cancer Statistics [1] show lung cancer accounts for about 13% of total cancer diagnoses; an estimated 1.8 million new lung cancer cases occurred in 2012. Lung cancer was the leading cause of cancer-related mortality among males in 2012. Among females, lung cancer was the leading cause of cancer death in more developed countries, and the second leading cause of cancer death in less developed countries. In China [2], Lung cancer remained the most common cancer and the leading cause of cancer death in 2011. The crude lung cancer incidence rate for lung cancer was 48.32/100 000, accounting for 19.31% of all new cancer cases. The crude lung cancer mortality rate in 2011 was 39.27/100 000, accounting for 25.04% of cancer deaths. NSCLC accounts for approximately 80% of all lung cancers [3]. Undoubtedly, with distinct mechanisms of action and toxicity, molecular targeted therapy is very effective in patients with advanced NSCLC who have specific genetic alterations. The development and clinical application of inhibitors that target the EGFR provide important insights for new NSCLC therapies [4].

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are effective clinical therapies for NSCLC patients with EGFR mutation [5-11]. Over 75% of patients harboring these mutations have dramatic or significant clinical and radiographic responses within days of treatment with EGFR-TKIs and show improved progression-free survival (PFS) [5, 9, 10, 12-15]. However, the vast majority of patients ultimately develop disease progression following successful treatment with an EGFR-TKI. The most common mechanism of acquired resistance, detected in 50% of patients, is a secondary mutation in EGFR at position T790 (T790M) after an initial response to Gefitinib or Erlotinib. This mutation leads to an increase in adenosine-5′-triphosphate (ATP) affinity, thus making it more difficult for reversible EGFR-TKIs gefitinib and erlotinib to bind the EGFR-TKI domain [16].

Currently, the development of effective strategies to treat TKI-resistant lung tumors is a major clinical need,

and efforts have focused on targeting EGFR T790M. Although the second-generation EGFR inhibitor afatinib can inhibit EGFR T790M in vitro, its use in patients is limited by skin and gastrointestinal toxicities at the doses required to achieve inhibition of EGFR T790M [17]. More recently, the third-generation mutant-selective EGFR inhibitors like AZD9291 and rociletinib (CO-1686) have emerged as potential therapeutics to block the growth of EGFR T790M-positive in NSCLC [18, 19]. These drugs have potent activity against both the common EGFR mutations (exon 19 deletions and the L858R mutation) that confer sensitivity to EGFR- TKIs and the T790M mutation that confers resistance. Most importantly, unlike the first-(gefitinib and erlotinib) and second-generation (afatinib) EGFR-TKIs, both AZD9291 and rociletinib have a significantly increased potency for EGFR T790M mutants than for wildtype (WT) EGFR.

2 T790M mutation in NSCLC

The most common mechanism of resistance to first-generation EGFR tyrosine kinase inhibitors (gefitinib and erlotinib) is a mutation in the "gatekeeper" residue (the ATP binding site on the kinase), where methionine replaces threonine (T790M). T790M mutation in EGFR accounts for approximately 50%–60% of all lung cancer cases with acquired resistance to the current clinical EGFR tyrosine kinase inhibitors [20, 21]. The development of a T790M restores the EGFR tyrosine kinase domain affinity to ATP, and therefore gefiinib is displaced from the binding pocket, and the 'driving' signal for proliferation is switched on again [22-25].

Yun et al. [16] reported that T790M mutants bind gefitinib with low nanomolar affinity. They first measured binding of gefitinib to the WT and T790M mutants by using a direct binding assay in which intrinsic fluorescence of EGFR is quenched by titration with the inhibitor. The T790M mutant binds gefitinib with Kd=4.6nM, considerably weaker than the WT kinase (Kd=35.3nM). Strikingly, the T790M mutation restores the ATP affinity to near WT levels in the L858R/T790M double mutant (Km[ATP]=8.4 M, as compared with Km[ATP]=148 M for the L858R mutant). They also find that the T790M mutation activates the kinase 5-fold as compared with the WT enzyme. In effect, the increased ATP affinity is the primary mechanism by which the T790M mutation confers drug resistance. The diminished ATP affinity of the oncogenic mutants open a ''therapeutic window,'' which renders them more easily inhibited relative to the WT EGFR and other kinases on which the inhibitors might have activity. The T790M secondary mutation effectively closes this window by restoring ATP affinity to WT levels.

3 Treatment of T790M mutation

The first-generation EGFR-TKIs, gefitinib and erlotinib, are effective as first-line treatment of advanced NSCLC harboring activating EGFR mutations (deletions in exon 19 and exon 21 L858R mutation). But the efficacy of these agents is often limited because of the emergence of drug resistance conferred by a second mutation, T790M. The second and third-generation EGFR- TKIs were designed to have more potent inhibition of EGFR and to overcome EGFR T790M [19]. EGFR-TKIs are summarized in Table 1.

Table 1: Representative EGFR-TKIs currently in use or development. edited from [26].

First genertion (target WT EGFR)	Second generation (irreversible inhibitors of EGFR and HER 2)	Third generation (EGFR mutant-specific, irreversible inhibitors)
Erlotiniba	**Neratinib**	**Rociletinib (Clovis)**
Gefitinib	Afatiniba	AZD9291 (AstraZeneca)
Icotinib	Dacomitinib	HM61713 (Hanmi)
		EGF816 (Novartis)
		ASP8273 (Astellas)
		WZ4002(selleck)

[a]FDA approved for treatment of lung cancer.

4 First-generation TKIs

The first-generation EGFR-TKIs provide significant clinical benefit in women, in patients who had never smoked, had pulmonary adenocarcinomas or who were of Asian origin. FDA initially approved gefitinib in May 2003 for the treatment of NSCLC with EGFR mutations, and in June 2005 the FDA withdrew approval for use in new patients due to lack of evidence that it extended life [27]. On July 13, 2015, FDA approved gefitinib as a first-line treatment for NSCLC. Moreover, gefitinib is officially approved for this treatment in dozens of countries worldwide. Erlotinib is another EGFR tyrosine kinase inhibitor that has a similar mechanism of action to gefitinib [28]. The gefitinib or erlotinib as first- or second-line therapy or maintenance therapy improved objective response rate (ORR) and PFS

for patients with advanced NSCLC, but unable to prolong overall survival (OS) [29, 30]. Furthermore, these first-generation TKIs have shown high incidence of adverse events: mild to moderate skin toxicity (rash, itching, and dry skin), gastrointestinal reactions (diarrhea and nausea), and fatigue [31, 32]. Nevertheless, people initially responding to gefitinib or erlotinib therapy invariably develop resistance, thereby limiting median PFS to 14 months and a median OS of 27 months [33]. In short, all patients inevitably develop acquired resistance to these agents, and secondary EGFR mutations are the major contributors.

5 Second-generation TKIs

The second-generation EGFR-TKIs include afatinib and dacomitinib. Unlike gefitinib and erlotinib, these agents covalently bind EGFR at cysteine 797, leading to more efficient EGFR inhibition in preclinical models. Afatinib has been approved as first-line treatment of advanced NSCLC harboring activating EGFR mutations [34]. Although the irreversible EGFR inhibitor afatinib can inhibit EGFR T790M in vitro, its use in patients is limited by skin and gastrointestinal toxicities at the doses required to achieve inhibition of EGFR T790M. Dacomitinib treatment is associated with promising PFS as first-line therapy in patients with EGFR-mutant non-small-cell lung cancer [35]. The second-generation EGFR-TKIs increase the efficacy over first-generation EGFR-TKIs and overcome the adverse events.

5.1 Afatinib

Afatinib (BIBW 2992) is an orally, irreversible EGFR-TKI. It is an ATP-competitive aniline-quinazoline derivative harboring a reactive acrylamide group. Afatinib is able to block EGFR, HER2 and HER4 kinases, forming covalent and irreversible bonds, and acting also on cancer cell harboring T790M mutations [36]. The irreversible, covalent binding of afatinib leads to a longer suppression of the receptor kinase activity than the reversible first-generation EGFR-TKIs, because the kinase activity is suppressed until the synthesis of new receptors [37].

Afatinib is not only active against wide-type EGFR, but also against EGFR mutant models with EGFR-activating mutations, including T790M. During phase 1 studies [38], six out of 12 patients had tumour size reductions; three achieving prolonged stable disease (SD). One of three patients, harboring T790 M resistance mutations with an adenocarcinoma of the lung diagnosed 2.9 years ago and resistant to gefitinib and erlotinib, was progression-free for 310 days and had a maximum tumour size reduction of –7.7%. In a phase II study [39], patients with stage IIIB to IV pulmonary adenocarcinoma progressed after 12 weeks of prior erlotinib and/or gefitinib. Of 61 evaluable patients, five had ORR. Median PFS was 4.4 months, and median OS was 19.0 months. Two patients had acquired T790M mutations: L858R T790M, and deletion in exon19 T790M; they had stable disease for 9 months and 1 month, respectively. In EGFR mutation–negative patients, the ORR was 27% (3 of 11), which was higher than in EGFR mutation–positive (4.5%; 2 of 44) patients. In a phase III study [40, 41], median OS of LUX-Lung 3 was 28.2 months in the afatinib group and 28.2 months in the pemetrexed-cisplatin group; median OS of LUX-Lung 6 was 23.1 months in the afatinib group and 23.5 months in the gemcitabine-cisplatin group. Although afatinib did not improve OS in the whole population, OS was improved with the drug for patients with del19 EGFR mutations. However, the low response rates in these trials made investigators doubt its ability to overcome acquired EGFR T790M. Possible explanation included the presence of multiple resistance mechanisms [34, 42].

5.2 Dacomitinib

Dacomitinib (PF-00299804) is an irreversible inhibitor of EGFR, HER2, and HER4. In preclinical cell lines and xenograft studies, dacomitinib demonstrated activities against both activating EGFR mutations and EGFR T790M [43, 44]. In a phase I trial [45, 46], fifty-seven patients with NSCLC were treated with Dacomitinib. Four patients, all previously treated with gefitinib or erlotinib (2 with exon 19 deletions, 1 with exon 20 insertion, 1 mutational status unknown), had a partial response to dacomitinib. In a phase II study [47], of six patients with EGFR T790M, 3 had SD≥6 weeks (9, 12, and 12 weeks, respectively), 3 had progressive disease (PD), 1 had some degree of tumor shrinkage and the other increased in size. Of the 36 patients with SD as best overall response (BOR) (median duration, 15 weeks), 10 patients (28%) had prolonged clinical benefit (SD, 6 months). The 6 patients with documented T790M had a median PFS of 7 weeks, which was similar to that of patients with EGFR wild-type tumors (8 weeks). In the above study, the overall response rate for patients was 5% but cannot reach BOR according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0. The patients with known EGFR T790M did not respond to dacomitinib therapy despite efficacy in preclinical models. These

could be due to the presence of concurrent drug resistance mechanisms (such as MET amplification) [48], or to the inability of dacomitinib to fully inhibit EGFR in tumors harboring EGFR T790M at the doses currently under clinical investigation [45].

Dacomitinib did not increase OS and therefore cannot be recommended for treatment of patients with advanced non-small-cell lung cancer previously treated with chemotherapy and an EGFR tyrosine-kinase inhibitor. In a phase 3 trial [49], Dacomitinib did not improve OS compared with placebo (median 6.83 months for dacomitinib vs 6.31 months for placebo; however, patients in the dacomitinib group had longer PFS than those in the placebo group (median 2.66 months vs 1.38 months, respectively). In the other phase 3 trial [50], dacomitinib was not superior to erlotinib in an unselected patient population with advanced non-small-cell lung cancer or in patients with KRAS wild-type tumours. Median PFS was 2.6 months (95% CI 1.9–3.0 for erlotinib and 1.9–2.9 for dacomitinib) in both the dacomitinib group and the erlotinib group. Further study of irreversible EGFR inhibitors should be restricted to patients with activating EGFR mutations.

6 Third-generation TKIs

More recently, third-generation EGFR inhibitors, such as AZD9291, WZ4002 and CO-1686, have been developed. In preclinical studies, these compounds are active against cell lines and murine models harboring T790M mutations and spare wild-type EGFR in vitro and in vivo [51].These drugs have potent activity against both the common EGFR mutations (exon 19 deletions and the L858R mutation) that confer sensitivity to EGFR-TKIs and the T790M mutation that confers resistance. Most importantly, unlike the first-(erlotinib and gefitinib) and second-generation (afatinib and dacomitinib) EGFR-TKIs, the third-generation EGFR-TKIs were highly active in patients with lung cancer with the EGFR T790M mutation who had had disease progression during prior therapy with EGFR-TKIS [52-54]. For patients, this has the potential to translate into reduced skin and gastrointestinal toxicities related to inhibition of wild-type EGFR.

6.1 AZD9291

AZD9291, as a monoanilino-pyrimidine compound, is a novel, irreversible EGFR-TKI, has proved to be more effective against both EGFR-TKI sensitizing and resistance T790M mutations in preclinical models. This phase I clinical study showed that AZD9291 has robust effiacy and is well tolerated in EGFR mutant NSCLC patients with acquired resistance to EGFR-TKIs.

Jänne PA and coworkers [19, 55-57] conducted a phase 1 study to determine the safety and efficacy of AZD9291 in patients with advanced EGFR-mutated NSCLC in whom resistance to treatment with EGFR tyrosine kinase inhibitors had developed. AZD9291 was proved more effective against both EGFR-TKI sensitizing and resistance T790M mutations in preclinical models. AZD9291 was administered orally, at doses of 20-240 mg once daily in patients with advanced lung cancer who had radiologically documented disease progression after previous treatment with EGFR tyrosine kinase inhibitors. The results showed that overall response rate was 51% (123/239). The response rate with AZD9291 was higher among those with the EGFR T790M mutation (61%; 95% CI, 52 to 70) than among those without this mutation (21%; 95% CI, 12 to 34). The median PFS was 9.6 months (95% CI, 8.3 to not reached) in EGFR T790M–positive patients and 2.8 months (95% CI, 2.1 to 4.3) in EGFR T790M–negative patients. AZD9291 proved to be clinically effective by the higher overall response rate (61%) in NSCLC patients with positive EGFR T790M and higher overall disease control rate (96%) in T790M positive patients. No dose limiting toxic effects were observed during the 28-day evaluation period at any dose level. Adverse events were ≥10% of patients overall of any grade and of grade 3 or higher. The most common adverse events were diarrhea (47% of patients), rash (grouped term; 40%), nausea (22%), and decreased appetite (21%). Adverse events of diarrhea and rash increased in frequency in a dose-dependent manner. It should be noted that duration of responses to AZD9291 were much shorter in T790M-negative patients, and responses in T790M-negative were more likely to be seen in patients who had not been on another EGFR-TKI immediately prior to AZD9291, suggesting that these responses may be a nonspecific 'EGFR-TKI retreatment effect'[58]. AZD9291 is in further phases of studies, details of which are summarised in Table 2.

6.2 Rociletinib (CO-1686)

The rociletinib (CO-1686) is a novel, irreversible and orally delivered kinase inhibitor that specifically targets the mutant forms of EGFR including T790M while exhibiting minimal activity towards the wild-type receptor. Oral administration of CO-1686 as single agent induces tumor regression in EGFR mutated NSCLC tumor xenograft and transgenic models [59]. CO-1686 takes advantage

Table 2: Ongoing clinical studies for third-generation tyrosine kinase inhibitors. edited from [68]

	Phase	Primary endpoint	Status	Thr790Met status	Key features
AZD9291					
AURA-2 (ClinicalTrials.gov, number NCT02094261)	2	Objective response rate	Ongoing but not recruiting	Positive	Failed EGFR-TKI; EGFR mutant
AURA-3 (ClinicalTrials.gov, number NCT02151981)	3	PFS	Recruiting	Positive	Failed first-line EGFR -TKI; EGFR mutant; standard group: platinum-based doublet chemotherapy
FLAURA (ClinicalTrials.gov, number NCT02296125)	3	PFS	Recruiting	Positive/negative	First-line; EGFR mutant; standard group: gefitinib/erlotinib
ClinicalTrials.gov, number NCT02143466	1	Safety and tolerability	Recruiting	Positive/negative	Failed EGFR-TKI; EGFR mutant; AZD9291 in combination with either MEDI4736 or AZD6094 or selumetinib
Rociletinib					
TIGER-1 (ClinicalTrials.gov, number NCT02186301)	2	PFS	Recruiting	Positive/negative	First-line, randomised; EGFR mutant; standard group: erlotinib
TIGER-2 (ClinicalTrials.gov, number NCT02147990)	2	ORR	Recruiting	Positive	Single group; EGFR mutant; failed first-line EGFR-TKI
TIGER-3 (ClinicalTrials.gov, number NCT02322281)	3	PFS	Not yet recruiting	Positive/negative	Failed EGFR-TKI and platinum doublet chemotherapy; EGFR mutant; standard group: single-agent chemotherapy
HM61713					
ClinicalTrials.gov Identifier: NCT02444819	2	overall response rate	Recruiting	Positive	First-line; EGFR Mutation
ClinicalTrials.gov Identifier: NCT02485652	2	ORR	Recruiting	Positive	Failed EGFR-TKI; EGFR mutant
ClinicalTrials.gov Identifier: NCT01894399	1	Plasma/Urine PK parameters	Not yet recruiting	No	Healthy Korean, Japanese and Caucasian Randomized
ClinicalTrials.gov Identifier: NCT01588145	1	Safety and tolerability	Recruiting	Positive	advanced NSCLC; EGFR mutant
EGF816					
ClinicalTrials.gov Identifier: NCT02323126	2	PFS	Recruiting	Positive	Adult Patients; EGFR Mutated Combination With Nivolumab
ClinicalTrials.gov Identifier: NCT02335944	1/2	DLT; ORR	Recruiting	Positive	NSCLC; EGFR mutant in Combination With INC280
ClinicalTrials.gov Identifier: NCT02108964	1/2	DLT; ORR	Recruiting	Positive	Adult Patients With EGFR mut Solid Malignancies

ASP8273					
ClinicalTrials.gov Identifier: NCT02500927	2	adverse events	Recruiting	Positive	EGFR-TKI naïve Patients; EGFR mutant
ClinicalTrials.gov Identifier: NCT02192697	1/2	Safety and tolerability	Active, not recruiting	Positive	NSCLC; EGFR mutant
ClinicalTrials.gov Identifier: NCT02113813	1	Safety and tolerability	Recruiting	Positive	NSCLC; EGFR mutant

PFS=progression free survival; TKI=tyrosine kinase inhibitor; ORR=objective response rate; DLT=dose limiting toxicity

of increased residence time at EGFR by alkylating Cys797 and thereby preventing toxic effects. Utilizing proteolytic digestion and nano-LC-MS/MS analysis, Engel J and coworkers [60]confirmed the alkylation of Cys797. Sequist LV et al. [61, 62] reported that rociletinib produced similar results, with a response rate of 59% (95% CI, 45 to 73) among patients with the EGFR T790M mutation and 29% (95% CI, 8 to 51) among those without this mutation. The estimated median PFS was significantly longer in those with the EGFR T790M mutation (13.1 months; 95% CI, 5.4 to 13.1) than in those without it (5.6 months; 95% CI, 1.3 to not reached). Hyperglycaemia was the most frequent grade 3 adverse event, occurring in 20 (22%) of 92 patients. Hyperglycemia observed with rociletinib was easily managed with oral hypoglycemic therapy. Currently, phase 2 and 3 with rociletinib is ongoing (Table 2)

6.3 HM61713

HM61713 is a novel, oral, selective inhibitor for EGFR mutations including both activating mutations and T790M, but not EGFR wild-type. HM61713 was studied in a phase I Korean study enrolling patients with EGFR mutated NSCLC that had progressed on prior TKI therapy (NCT01588145) [63]. A total of 93 patients have been enrolled in both dose escalation (up to 500 mg/day) and expansion cohorts (35:58 respectively). Drug-related adverse events reported in ≥10% of patients were skin exfoliation, nausea, diarrhea, rash, decreased appetite and pruritus. Disease control rate was 76.5% and 73.1%. Among 27 patients who had T790M mutation at baseline biopsy, 18 patients showed decreased size in the target lesions and all the unconfirmed partial responses observed were T790M mutation positive cases. In conclusion, HM61713 showed good safety profile and promising anti-tumor activity in patients with EGFR mutated NSCLC who failed to EGFR-TKIs, especially in patients with T790M mutation. Phase I /II Trial with HM61713 is in progress (Table 2).

6.4 EGF816

Shailaja Kasibhatla et al. [64] developed a covalent mutant-selective EGFR inhibitor, EGF816 that potently inhibits both activating EGFR mutations as well as the T790M resistance mutation while sparing wild-type. EGF816 exhibits excellent anti-tumor activity in the relevant patient derived tumor cell lines at well-tolerated doses and is expected to provide long term duration of responses compared to current EGFR-TKI therapy in the clinic. Other study [65] has shown EGF816 potently inhibits both activating (L858R and Ex19Del) and T790M resistant mutations in various cellular assays; it is selective against a large panel of kinases in both Ambit and BaF3 profiling, and more importantly is selective against WT EGFR. EGF816 is efficacious in mutant EGFR-driven xenograft models. Phase 1 and 2 with EGF816 is ongoing (Table 2).

6.5 ASP8273

ASP8273 is a small molecule, irreversible TKI that inhibits the kinase activity of EGFR activating mutations and T790M resistance mutation, with higher potency than wild type EGFR in vitro. Preclinical data [66] suggest that ASP8273 achieved complete tumor regressions in mouse NSCLC xenograft models with EGFR activating mutations or T790M resistance mutation. Dose administration ranges from 10.3 to 18.4 hours across all dose levels. Preliminary results for antitumor activity show that 4/7 patients (57%) with T790M resistance mutation at 200 mg showed partial responses based on RECIST 1.1. All patients harboring T790M mutation are still on study without PD. In the presentation, further update will be published. Phase 1 and 2 with ASP8273 is ongoing (Table 2).

6.6 WZ4002

Zhou W et al. [51] identify that WZ4002 is a novel structural class of EGFR kinase inhibitors that are effective in vitro and in vivo models harboring the EGFR T790M mutation. WZ4002 suppress the growth of PC9GR4 cell lines (contain EGFR delE746_A750/T790M) and inhibit EGFR phosphorylation. They further determined WZ4002 is effective in vivo by using mouse lung cancer models harboring either EGFR L858R/T790M or Del E746_A750/T790M. Sakuma Y et al. [67] found that WZ4002 similarly down regulated the phosphorylation levels of EGFR and its main downstream molecules, Akt and ERK 1/2, in H1975 SR cells, as well as HCC827 cells in suspension. WZ4002 is 30- to 100-fold more potent against the T790M EGFR mutation, and up to 100-fold less potent against wild-type EGFR, compared with the other currently available EGFR- TKIs. WZ4002 is a pyrimidine-based EGFR-TKI. Consequently, WZ4002 is a promising drug in the treatment of EGFR-mutant lung adenocarcinomas. To date, there are still no clinical studies with WZ4002.

7 Acquired resistance to Third-generation TKIs

Multiple mutations in the EGFR gene are a major cause for the failure of the third-generation TKIS in the treatment of patients harboring T790M NSCLC who initially responded to this therapy. Ercan D et al. [69] performed an N-ethyl-N-nitrosourea (ENU) mutagenesis screen in EGFR-mutant (sensitizing alone or with concurrent EGFR T790M) Ba/F3 cells and selected drug-resistant clones. They identified 3 major drug resistance mutations. EGFR L718Q, L844V, and C797S cause resistance to both WZ4002 and CO-1686 while, in contrast, only EGFR C797S leads to AZD9291 resistance. Cysteine 797 is the site of covalent binding for all three of these agents. Covalent binding is required to overcome the increased ATP affinity mediated by T790M. 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.

Mutations at the EGFR C797 codon, located within the kinase-binding site, were very recently reported to be a potential mechanism of resistance to AZD9291 in T790M-positive patients. Niederst MJ et al. [70] identify the C797S EGFR mutation in cells made resistant to a third-generation inhibitor and demonstrate that it is sufficient to promote resistance to third-generation TKIs. Yu HA et al. [71] reported a tertiary acquired mutation identified in a clinical lung cancer sample. They describe herein a patient whose tumor acquired an EGFR C797S mutation after treatment with a third-generation EGFR-TKI. The acquired EGFR C797S should confer resistance to all third-generation EGFR-TKIs, similar to the emergence of EGFR T790M and its cross-resistance to all first-generation EGFR-TKIs. Thress KS et al.[23]studied cell-free plasma DNA (cfDNA) collected from subjects with advanced lung cancer whose tumors had developed resistance to the AZD9291. They found that acquired EGFR C797S mutation mediates resistance to AZD9291 in non–small cell lung cancer harboring EGFR T790M

It is well established that resistance to EGFR-TKIs can occur via activation of pathways, like MET, that bypass EGFR to signal downstream. Planchard D et al. [72] identified HER2 and MET amplification as a potential mechanism of acquired resistance to third-generation EGFR-TKIs such as AZD9291 in two patients with EGFR T790M-positive NSCLC by fluorescence in situ hybridization and comparative genomic in situ. Yosuke Togashi et al. [73] reported that β-Catenin might be a novel therapeutic target in EGFR T790M mutation NSCLC. They found that the inhibition of the β-Catenin signaling enhanced the sensitivity to EGFR-TKIs in EGFR-mutated NSCLC carrying the T790M mutation. Walter AO and colleagues [59] found that NSCLC cells with acquired resistance to CO-1686 exhibited signs of epithelial-mesenchymal transition (EMT) and increased sensitivity to AKT inhibitors. Chan S et al. [74] reported that IGF1R amplification was related to the secondary acquired resistance against the 2nd or 3rd generation EGFR inhibitor therapies. They have successfully identified a 2, 4-diarylamino-pyrimidines compound 8g, which strongly suppresses the proliferation of CO-1686-resistant H1975-IGF1R cancer cells, suggesting it is promising for future anticancer drug discovery.

8 Conclusion and perspective

Lung cancer remains among the most commonly occurring cancers in the world with the majority of the cases being of NSCLC. As in other malignancies, molecularly targeted therapies are being increasingly studied and are being more and more approved for clinical use in case of

NSCLC. Among the molecularly targeted therapies available for advanced NSCLC, EGFR-TKIs occupy a central place and form part of the standard treatment algorithms. These agents are especially useful in the treatment of selected subgroups of patients harboring mutations in the EGFR gene apart from the common use of chemotherapy and radiation.

The first-generation EGFR-TKIs such as gefitinib and erlotinib provide significant clinical benefit in certain subgroups namely female gender, non-smoker status, Asian ethnicity and adenocarcinoma. However, clinical outcomes of the first-generation EGFR inhibitors erlotinib and gefitinib were rather disappointing, especially in terms of OS. Despite the initial response, emergence of acquired resistance to these drugs is almost inevitable. The development of drug resistance, primarily due to the T790M point mutation, leads to the total loss of potency in almost all NSCLC patients. Second-generation TKIs, such as the recently approved afatinib and dacomitinib would probably be able to clinically block the growth of NSCLC tumors harboring the T790M mutation but clinical application was limited because of poor selectivity and low reaction rate. Third-generation TKIs offer a new hope in terms of selectivity against acquired resistance. Initial clinical results revealed promising response rates with only mild side effects. However, these clinical studies are still in an early stage of evaluation and hence no reliable prognosis can be made. Unfortunately, evidences for the existence of EGFR mutants that are resistant to third-generation EGFR inhibitors are already present, although the mechanism is not yet clear.

In summary, acquired drug resistance limits the long-term clinical success of targeted therapies for patients with EGFR-mutant NSCLC. Although third-generation inhibitors against EGFR T790M mutation show promises in overcoming acquired resistance to EGFR-TKI, fourth-generation inhibitors targeting against acquired resistance to third-generation inhibitors need to be developed in the future.

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