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Review Article

T790M Mutation Related Resistance of First Generation EGFR Inhibitors, their Treatment with Novel EGFR inhibitors in NSCLC and Their Side Effects: A Review

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ABSTRACT

The epidermal growth factor receptor (EGFR) and members of its family play a considerable role in carcinogenesis through their involvement in proliferation, apoptosis, enhanced cell motility, and neoangiogenesis. Improper activation of EGFR TK results in increased malignant cell survival, proliferation, invasion and metastasis. EGFR overexpressions observed in tumors from more than 60% of patients with metastatic non-small-cell lungcancer (NSCLC) and are correlated with poor prognosis. The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs), Gefitinib and Erlotinib, are reversible competitive inhibitors of the tyrosine kinase domain of EGFR that bind to its adenosine-5 triphosphate-binding site. Most NSCLC patients who initially respond to gefitinib and erlotinib eventually become resistant and experience progressive disease. The point mutation T790M accounts for about one half of these cases of acquired resistance. In this review, we discuss recent advances in the understanding of acquired TKI resistance in *EGFR*-mutant lung cancer and review therapeutic progress with second generation TKIs and combinations of targeted therapies. Various second-generation EGFR TKIs like Neratinib, Afatinib, Tesevatinib, Dacomotinib, TAE226, AZD9291, HM61713are currently being evaluated and may have the potential to overcome T790M-mediated resistance, but these are also associated with various side effects. Overcoming of this resistance as well as discovery of new potential markers and inhibitors is the main goal of ongoing research in NSCLC.

Keywords: *EGFR, Gefitinib, Erlotinib, T790M mutation, EGFR TKIs, NSCLC*

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INTRODUCTION

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (TKs), referred to as the HER or ErbB family, consists of four members—EGFR (HER1/ErbB1), HER2(ErbB2), HER(ErbB3) and HER4(ErbB4)—that regulate many developmental, metabolic and physiological processes. The epidermal growth factor receptor and members of its family play a considerable role in carcinogenesis through their involvement in proliferation, apoptosis, enhanced cell motility, and neoangiogenesis. In tumor cells, the TK activity of EGFR may be dysregulated by various oncogenic mechanisms, including *EGFR* gene mutation, increased gene copy number and EGFR protein overexpression [1]. Improper activation of EGFR TK results in increased malignant cell survival, proliferation, invasion

and metastasis. EGFR overexpressions observed in tumors from more than 60% of patients with metastatic non-small-cell lungcancer (NSCLC) and is correlated with poor prognosis [2]. These findings have provided a rationale for the development of novel anticancer agents that target EGFR. Therefore, the predictive and prognostic significance of EGFR overexpression in non–small cell lung cancer (NSCLC) has over the years become important, resulting in the development of numerous targeted therapies [3, 4, 5, 6, 7]. The discovery of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has led to a new paradigm of lung cancer treatment. The use of EGFR TKIs for NSCLC began in 2003. EGFR TKIs were first administered to non-selected patients with advanced NSCLC who had failed all approved chemotherapies [8]. Two anilinoquinazoline epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors: Gefitinib and Erlotinib have gained approval for use in unselected patients with NSCLC [9, 10].

Treatment with the reversible EGFR TK inhibitors (TKIs), gefitinib and erlotinib, results in dramatic antitumor activity in a subset of patients with NSCLC. Sequencing of the *EGFR* gene revealed that a majority of tumors responding to EGFR TKIs harbored mutations in the TK domain of *EGFR* [11, 12]. This article reviews the types of activating and resistance *EGFR* mutations and the pivotal role they have in the sensitivity and resistance of NSCLC tumors to gefitinib and erlotinib. In this review, we discuss recent advances in the understanding of acquired TKI resistance in *EGFR*-mutant lung cancer and review therapeutic progress with second generation TKIs and combinations of targeted therapies.

EGFR signaling

EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα). ErbB2 has no known direct activating ligand, and may be in an activated state constitutively or become active upon heterodimerization with other family members such as EGFR. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer [13]. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the erbB receptor family, such as ErbB2, to create an activated heterodimer. EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine residues in the C-terminal domain of EGFR occurs [14]. This autophosphorylation by ATP elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiates several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation [15]. Such proteins modulate phenotypes such as cell migration, adhesion, and proliferation. Activation of the receptor is important for the innate immune response in human skin [16]. The kinase domain of EGFR can also cross-phosphorylate tyrosine residues of other receptors it is aggregated with, and can itself be activated in that manner.

Mechanism of action of EGFR inhibitors

In recent years, quinazolines have emerged as a versatile template for inhibition of a diverse range of receptor tyrosine kinases. The most widely studied of these is the EGFR, with the small-molecule inhibitor Gefitinib (Iressa) being the first agent from this class to be approved for the treatment of Non-Small Cell Lung Cancer [17, 18]. These compounds act via competing with ATP for binding at the catalytic domain of tyrosine kinase [19].

Problem associated with marketed EGFR TKIs

As Gefitinib is a selective chemotherapeutic agent, its tolerability profile is far superior to previous cytotoxic agents. Adverse drug reactions do still occur however, but may be preferable to the fatal consequences of not taking the therapy.

Other common adverse, effects (≥1% of patients) include: diarrhoea,

nausea, vomiting, anorexia, stomatitis, dehydrati on, skin reactions, paronychia (skin infection that occurs around the nails), asymptomatic elevations of liver enzymes, asthenia, conjunctivitis, blepharitis (inflammation of eyelash follicles).

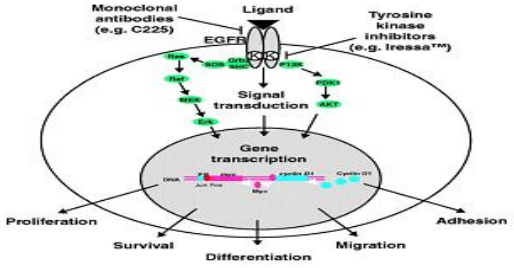

Figure 1: EGFR signalling pathway and site of action of EGFR TK inhibitors

Acne is reported very commonly. Infrequent adverse effects (0.1–1% of patients) include: interstitial lung disease, corneal erosion, aberrant eyelash and hair growth [20]. Gefitinib and Erlotinib treatment cause mutation which is responsible for resistance to these drugs.

Gefitinib and Erlotinib treated patients with NSCLC were identified to have somatic mutations in TK domain of ErbB1 mostly in exons 19 and 21 , these comprise small in-frame deletions around the ATP binding site of TK domain. However, the cells containing an activating mutation are interestingly more sensitive to ErbB1 inhibition. In contrast, a secondary mutation has been observed in Gefitinib and Erlotinib-responsive advanced NSCLC patients. This mutation resulted threonine to methionine change at position 790 in the kinase domain of ErbB1 (T790M) and this unlikely mutation is reported to have resistance to gefitinib and erlotinib treatment [21].

Mechanisms of Acquired Resistance to Gefitinib and Erlotinib

Several mechanisms of resistance to Erlotinib and Gefitinib have been described in laboratory-based models:-

a. A mutant form of EGFR termed EGFRvIII has an in-frame deletion mutation that produces a truncated 150 kDa protein, which is constitutively phosphorylated in a ligand-independent manner [22].

b. EGFR-dependent tumors that are initially sensitive to EGFR TKIs acquire a mutation at threonine 790. Substitution of this residue in EGFR with a bulky methionine may cause resistance by steric interference with binding of TKIs, including gefitinib and erlotinib [21].

c. Tumors can become resistant when individual tumor cells undergo an oncogenic shift, which has been noted with several other RTKs, including HGF receptor, AXL and IGF1R [23].

d. In addition to IGF1R as a mechanism of escape, downregulation of the IGF- binding proteins IGFBP3 and IGFBP4, have been implicated in resistance to TKIs. These proteins are crucial for regulating the levels of IGF1R ligands, and loss leads to overactivation of the receptor [24].

e. Mutations in both PTEN have been implicated in impaired response to TKI therapy

f. Mutations in both Ras have been implicated in impaired response to TKI therapy [25].

g. Cells that developed acquired resistance to gefitinib *in vivo* were shown to have increased VEGF production leading to altered angiogenesis and enhanced escape from cetuximab therapy [26].

h. VEGFR1 has also been implicated in the contribution to resistance to EGFR TKIs [27].

Among the resistance causing factors, the secondary point mutation T790M, which substitutes methionine for threonine at amino acid position 790 of EGFR gene domain, might play the most important role. Furthermore, most clinical reports indicated that T790M accounted for half of the acquired resistant TKI cases [28].

Secondary mutation: egfr t790m

The first identified mechanism of acquired resistance to EGFR TKIs was the *EGFR* T790M mutation in 2005. Patients with NSCLC harboring either exon 19 deletions or the L858R mutation that progressed after a period of response to Gefitinib or Erlotinib. In postprogression biopsies, the original *EGFR* mutation and the novel T790M in exon 20 were identified. When T790M was introduced in vitro to sequences containing wild-type *EGFR*, exon 19 deletion–*EGFR*, or L858R-*EGFR*, the resulting proteins were significantly more resistant to Gefitinib in the constructs containing T790M [29, 31]. The inhibitory concentrations to Erlotinib and Gefitinib in T790M-containing constructs exceeded 5 μM, which is a concentration more than 100-fold higher than that required to inhibit exon 19 deletions or L858R-EGFR [32]. A NSCLC cell line with the L858R-T790M mutation was significantly more resistant to Gefitinib or Erlotinib than lines with L858R and an exon 19 deletion [29, 31, 33]. The T790M mutation is most often seen in cis; however, it can occur in trans, to L858R or exon 19 deletions [29, 33]. *EGFR*-mutated cell lines that have L858R-T790M or exon 19 deletions-T790M continue to be dependent on EGFR, because alternative EGFR inhibitors halt cell proliferation and lead to apoptosis [31, 33, 34]. Some *EGFR*-mutated NSCLC cell lines (H3255 and PC-9) that are exposed to incremental concentrations of Gefitinib in culture end up acquiring T790M [33, 35] and mouse lung cancer models of L858R-T790M confirmed that these tumors are resistant to Gefitinib and Erlotinib [36]. These preclinical and clinical data support T790M as a main mechanism of resistance to EGFR TKIs. How T790M affects the hypersensitivity of activating *EGFR* mutations is still not completely clear. Initially, it was speculated, based on the crystallographic structure of the kinase domain of EGFR, that the bulkier methionine residue of the "gatekeeper" T790M changed the ATP binding pocket of the

kinase, therefore blocking the engagement of Erlotinib or Gefitinib [29]. However, more recently, it was demonstrated that T790M affected minimally the binding of Gefitinib to L858R-EGFR. Instead, L858R-T790M-EGFR had increased affinity to ATP when compared with L858R alone, which is predicted to decrease binding of Gefitinib and Erlotinib because these drugs are ATP-competitive kinase inhibitors [37]. These findings will certainly affect the development of the next generation of EGFR inhibitors with the ability to overcome T790M. In the original reports, preprogression samples lacked T790M, and it was thought that this abnormality was acquired only after exposure to Gefitinib or Erlotinib [29, 30]. A further EGFR mutation called T790M accounts for 60% of this acquired resistance. No currently approved therapies target T790M [38].

Novel epidermal growth factor receptor tyrosine kinase inhibitors

Initial steps have begun to use preclinical data for rationale design of clinical trials of patients with acquired resistance to Gefitinib or Erlotinib. In the original publication of EGFR-T790M, the authors had demonstrated that an irreversible EGFR inhibitor (CL-387,785, an ATP mimetic that covalently bind to Cys-797 of EGFR) was able to in vitro inhibit T790M in cis to activating exon 19 deletion mutation at lower concentrations than Gefitinib or Erlotinib [29, 31]. Following that original observation, many groups have shown that other irreversible and second-generation EGFR inhibitors in vitro can partially overcome L858R-T790M and exon 19 deletions-T790M EGFR mutations [39, 34]. In some cases, the drugs tested in vitro have already entered the clinical arena.{ Riely GJ.} The above-mentioned findings have spawned phase II trials to address if these novel EGFR inhibitors will be useful in patients with *EGFR* T790M and/or acquired resistance to gefitinib or erlotinib. Insights gained from the treatment of patients with metastatic NSCLC with gefitinib and erlotinib are dramatically changing drug development and treatment strategies, as well as clinical outcomes. Because acquisition of the secondary resistance point mutation T790M reduces the efficacy of ATP-competitive inhibitors, one strategy for preventing or overcoming EGFR TKI resistance would be to identify novel agents that bind and inhibit EGFR by a distinct, non-ATP competitive mechanism. A second strategy may be to irreversibly inhibit the binding of ATP to the TK domain with an irreversible rather than a reversible inhibitor [34, 41, 42].

Neratinib [HKI-272]

Neratinib [HKI-272] was one of first compounds to be tested in this specific population. This is an irreversible pan-ErbB TKI (EGFR, ErbB2, ErbB3) that in vitro and in vivo has activity against classic EGFR mutations (L858R, exon 19 deletions), exon 20 EGFR insertions (which are more resistant to gefitinib/erlotinib), amplified or mutated ErbB2, and compound EGFR mutations with T790M [34, 40, 41]. Neratinib (HKI-272), an irreversible HER family inhibitor targeting EGFR/HER-1, HER-2 and HER-4, was initially tested in a phase I trial of 72 patients with advanced ErbB2 or ErbB1/EGFR IHC positive tumors [43]. Maximum tolerated dose (MTD) was determined to be 320 mg and the most common related adverse event at this dose was diarrhea. Strikingly, a long-lasting disease control (defined as stable disease for >24 weeks) was observed in 43% of refractory NSCLC patients.

Afatinib (BIBW2992)

Afatinib is an irreversible TKI that might potentially overcome the resistance of first-generation EGFR TKIs. Afatinib is a second generation of EGFR TKI that showed great efficacy against tumors bearing the EGFR T790M mutation, but it failed to show the improvement on overall survival of lung cancer patients with EGFR mutations possibly because of novel acquired resistance mechanisms. Currently, there are no therapeutic options available for lung cancer patients who develop acquired resistance to afatinib. To identify novel resistance mechanism(s) to afatinib, we developed afatinib resistant cell lines from a parental human-derived NSCLC cell line, H1975, harboring both EGFR L858R and T790M mutations. We found that activation of the insulin-like growth factor 1 receptor (IGF1R) signaling pathway contributes to afatinib resistance in NSCLC cells harboring the T790M mutation. IGF1R knockdown not only significantly sensitizes resistant cells to afatinib, but also induces apoptosis in afatinib resistance cells. In addition, combination treatment with afatinib and linsitinib shows more than additive effects on tumor growth in in vivo H1975 xenograft. Therefore, these finding suggest that IGF1R inhibition or combination of EGFR-IGF1R inhibition strategies would be potential ways to prevent or potentiate the effects of current therapeutic options to lung cancer

patients demonstrating resistance to either first or second generation EGFR TKIs [44].

Multiple phase I studies identified, afatinib in 50 mg once daily the maximum tolerated dose (MTD) with main toxicities represented by diarrhea and skin rash [45].

The use of afatinib associated with cetuximab represents a new possibility of therapy following progression on gefitinib or erlotinib. We present two patients who acquired resistance to first-generation TKI and who underwent combination treatment with afatinib plus cetuximab as third-line therapy. Both patients presented partial response, and the time duration of disease control was 8 months and 10 months. The combined use of afatinib plus cetuximab emerges as a new possibility for the treatment of patients with advanced NSCLC harboring mutated EGFR after progression on first-generation EGFR TKIs with consequently acquired resistance to TKIs.

Toxicity is also a significant concern with the dual EGFR inhibition, because of the potential for overlapping side effects. In the study, the most common adverse events included rash (90%), diarrhea (71%), nail effects (57%), and stomatitis (56%). The grade 3 events were of 20%, 6%, 0%, and 1%, respectively. Less common side effects were fatigue, nausea, xerosis, pruritus, and headache [46]. Thus, close evaluation of these patients is required with early intervention for toxicities, which may include dose delays or reductions.

XL647(Tesevatinib)

The secondary EGFR T790M mutation is found in 50% of patients with acquired resistance. XL647, an oral small molecule inhibitor of multiple receptor tyrosine kinases, including EGFR, VEGFR2, HER2, and EphB4, in NSCLC patients known or suspected of having tumors harboring T790M [47]. The most common adverse effects are diarrhea, nausea, and fatigue [48].

Dacomitinib (PF-00299804)

Dacomitinib is an orally administered, highly selective irreversible small molecule inhibitor of the HER family of tyrosine kinases (HER1, HER2, and HER4) [42,49]. In preclinical human tumor xenograft models that express and/or overexpress HER family members, dacomitinib showed antitumor effects. Antitumor effects were shown against tumors with *EGFR*-activating mutations, found in 30–50% of Asian patients with lung cancer [50], and also against tumors with *T790M*, a mutation detected in the tumors of approximately 50% of patients with lung adenocarcinoma who develop acquired resistance to gefitinib or erlotinib [21,30,51]. Dacomitinib appeared to be generally well tolerated in Japanese patients; the safety profile was consistent with that observed in Western and Dose-limiting toxicities (DLTs) observed at any dose, and adverse effects(AEs) were generally of grade 1/2 severity and manageable. No grades 4 AEs were reported. Most common AEs (primarily skin and gastrointestinal toxicities of rash, diarrhea, paronychia, stomatitis, and dry skin) were consistent with the toxicities of other EGFR tyrosine kinase inhibitors [56, 57, 58]. For comparison, the most commonly reported AEs in the phase II dacomitinib versus erlotinib study of advanced NSCLC [54] were diarrhea, dermatitis acneiform, stomatitis, mucosal inflammation, and paronychia; similarly, the Korean phase I/II study in refractory NSCLC [55] reported diarrhea, dermatitis acneiform, paronychia, and stomatitis as the most common AEs. In each of these studies, AEs were primarily of grade 1 or 2 severity and manageable with standard supportive care.

Rociletinib (CO-1686)

Rociletinib (CO-1686) is a new and potent oral EGFR inhibitor designed to selectively target both the initial activating EGFR mutations as well as the T790M resistance mutation. This compound spares wild-type EGFR and this means that it causes far fewer toxic side effects than other EGFR inhibitors," Adverse side effects of rociletinib were manageable and included asymptomatic hyperglycemia, nausea, and diarrhea, and these were mostly grade 1 or 2. Only two patients had any form of rash, which was grade 1 and transient. The most common, grade 3 adverse event was hyperglycemia, which was observed in 14% of patients [38].

AZD9291

AZD9291 is a novel oral, potent, and selective third-generation irreversible inhibitor of both $EGFRm^{+}$ sensitizing and T790M resistance mutants that spares wild-type EGFR. This mono-anilino–pyrimidine compound is structurally distinct from other third-generation EGFR TKIs and offers a pharmacologically differentiated profile from earlier generation EGFR TKIs [59].

AZD9291 is an irreversible inhibitor of EGFR T790M mutations. In about 60% of patients with NSCLC, resistance to first-generation EGFR inhibition has been associated with an acquired mutation in EGFR T790M. In all patients treated with AZD9291 at all doses, diarrhea was reported by 50% and rash by 46%. The most common adverse events were primarily low-

grade. Grade 3/4 treatment-related adverse events occurred in 17% of patients [60].

HM61713

HM61713, an orally active novel epidermal growth factor receptor (EGFR) mutant selective inhibitor, is considered a 3rd-generation EGFR mutation tyrosine kinase inhibitor which excelled in reducing resistance and side-effects after administration with previous EGFR TKIs Iressa® and Tarceva® HM61713 is a novel, oral, mutant-selective inhibitor of EGFR and T790M, but not for EGFR wild-type. This open label phase I trial was showed the patients with T790M mutation-positive the regression rate was 29.2%, with a disease control rate of 75%. The primary side effects were nausea, headache, and rash . HM61713 caused mild side effects and can be controlled easily. The efficacy of HM61713 is not better than the other two—AZD9291 and CO-1686 [61].

TAE226

TAE226, a bis-anilino pyrimidine compound, has been developed as an inhibitor of focal adhesion kinase (FAK) and insulin-like growth factor-I receptor (IGF-IR). In this study, we investigated the effect of TAE226 on non-small-cell lung cancer (NSCLC), especially focusing on the EGFR mutational status. TAE226 was more effective against cells with mutant EGFR, including the T790M mutant, than against cells with wild-type one. TAE226 also showed higher affinity of about 15-fold for the L858R/T790M mutant than for the wild-type one by kinetic interaction analysis. The antitumor effect against EGFR-mutant tumors including T790M mutation was confirmed in mouse models without any significant toxicity. In summary, we showed that TAE226 inhibited the activation of mutant EGFR and exhibited anti-proliferative activity against NSCLCs carrying EGFR mutations, including T790M mutation. Obvious side effects, including the loss of body weight, did not occur in the vehicle-treated group or in the groups treated with 30mg/kg or 60mg/kg of TAE226, while a loss of body weight was observed in mice treated with 90 mg/kg of TAE226. The tumor volume in both xenografts significantly decreased over time and in a dose-dependent manner, compared with the vehicle-treated mice. Furthermore, both xenografts of EGFR-TKI-sensitive (PC-9) and EGFR-TKI-resistant (RPC-9) cell lines showed similar anti-tumor effects in response to TAE226 in vivo [62].

CONCLUSION

The rapid pace of preclinical work in the field of *EGFR* mutations and mechanisms of resistance to gefitinib and erlotinib continues to shed light on potential clinical approaches for this NSCLC subtype. The *EGFR* T790M mutation accounts for 50% of cases, other secondary resistance mutations are rare. Several novel targeted therapies are currently in clinical development for patients with advanced NSCLC. The irreversible EGFR TKIs are one class of agents that may have the potential to prevent and overcome resistance that emerges during treatment with gefitinib and erlotinib. Results of ongoing phase III studies on this class of compounds in erlotinib-resistant NSCLC populations are eagerly awaited.

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