

Whacking a mole-cule: clinical activity and mechanisms of resistance to third generation EGFR inhibitors in *EGFR* mutated lung cancers with *EGFR*-T790M

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Abstract: Epidermal growth factor receptor (*EGFR*) mutations, especially *EGFR*-exon 19 deletions and *EGFR*-L858R, are the most frequent actionable genomic events in lung adenocarcinomas. Tumors arise due to constitutively activated *EGFR* signaling and are susceptible to *EGFR* tyrosine kinase inhibitors (TKIs). First generation *EGFR* TKIs (gefitinib and erlotinib) and the second generation *EGFR* TKI afatinib are approved worldwide. Although targeted therapies against *EGFR* mutants induce dramatic initial responses, acquired resistance (through multiple biological mechanisms) to erlotinib, gefitinib and afatinib emerges within the first 1-2 years of continued monotherapy. *EGFR*-T790M accounts for more than half of acquired resistance to first or second generation *EGFR* TKIs by modifying ATP affinity and drug binding kinetics. Two new studies have shown that two covalent pyrimidine inhibitors—AZD9291 and rociletinib of *EGFR*-T790M (i.e., third generation *EGFR* TKIs) shown remarkable clinical activity in patients with acquired resistance to erlotinib, gefitinib and afatinib when the tumor carries *EGFR*-T790M in conjunction with an activating mutation. However, and regrettably, acquired resistance to these third generation *EGFR* TKIs has already been reported in preclinical models and clinical specimens; such as a tertiary mutation at *EGFR*-C797S that prevents covalent binding of *EGFR* TKIs. The experience with sequential *EGFR* TKI monotherapy highlights tumor heterogeneity and adaptability (i.e., relentless game of whack-a-mole played between TKIs and cancer), and will help shape future clinical development of novel combinatory approaches to manage *EGFR* mutated lung adenocarcinomas.

Keywords: Epidermal growth factor receptor (*EGFR*); T790M; C797S; AZD9291; rociletinib; CO-1686; acquired resistance

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The mutational landscape of lung adenocarcinomas is complex and defined by heterogeneous subpopulations of tumors that can be addicted to oncogene-driven proliferative and anti-apoptotic signaling (1). Epidermal growth factor receptor (*EGFR*) mutations which were identified in 2004 (2-4)—are the poster children for the concept, as lung adenocarcinomas that harbor activating kinase domain *EGFR* mutations become addicted to deranged *EGFR* signaling and are susceptible to small-molecule compounds that disrupt *EGFR* activity (5). The

clinically-relevant and most frequent *EGFR* mutations are inframe deletions/insertions (around amino-acid residues 747 to 752) of exon 19 (these account for up to 40-50% of all *EGFR* mutations) and the L858R mutation (this accounts for up to 30-40% of all *EGFR* mutations) of exon 21 (5-7). The transcribed *EGFR* mutant proteins favor the active kinase state, induce sustained mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinases (PI3K) cascades, resulting in hyperproliferative and anti-apoptotic cell phenotypes (5). Acute inhibition

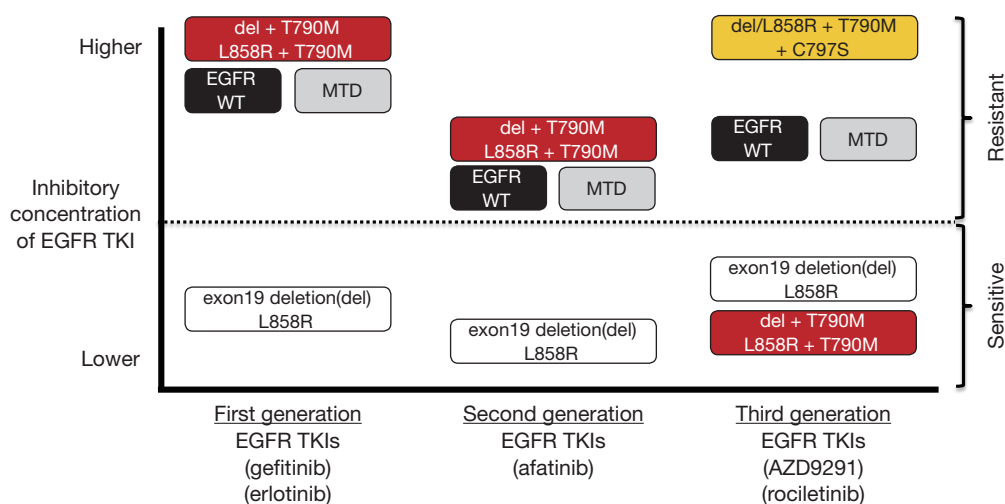


Figure 1 Pictorial graphical display of *in vitro* inhibitory concentrations of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) against various isolated EGFR proteins and the wild-type (WT) kinase. The y axis depicts lower (more sensitive to TKI) and higher (more resistant to TKI) inhibitory concentrations. First, second and third generation TKIs are shown, with maximum tolerated dose (MTD) matching the inhibitory doses for EGFR WT. The therapeutic window of each class of EGFR TKI represents *EGFR* mutated proteins that are inhibited below the MTD.

of EGFR through tyrosine kinase inhibitors (TKIs) in these oncogene addicted lung adenocarcinomas disrupts the intracellular signaling balance, leading to cell cycle arrest and apoptosis (8-10). The concept of “oncogene-addiction” (11) may be the shared basis of pathogenesis for all oncogenic kinase-driven tumors (12-14). These insights into the biology of *EGFR* mutations translated into the clinical real with the development of the first generation EGFR TKIs gefitinib and erlotinib, both of which are reversible ATP mimetic quinazoline derivatives (5,15,16); and also with the development of the second generation EGFR TKI afatinib, an irreversible inhibitor that binds to the C797 amino-acid residue of EGFR (17). First and second generation EGFR TKIs were originally developed to target the wild-type (WT) EGFR but are significantly more potent against common *EGFR* mutations and have a favorable therapeutic window (Figure 1) in tumors driven by EGFR-exon 19 deletions or EGFR-L858R (5). Over the last several years, a multitude of randomized clinical trials have compared an EGFR TKI (gefitinib, erlotinib or afatinib) against systemic platinum-based chemotherapies in advanced lung adenocarcinomas. In all of these trials, the response rates (RRs) with the EGFR TKIs exceeded 70% being >2 times higher than platinum-doublets, the median progression-free survival (PFS) times were significantly longer (with a median of approximately 10-12 months) than that with chemotherapy

and the median overall survival (OS) times augmented to over 24 months, especially in tumors with *EGFR*-exon 19 deletions (18)—despite a high rate of cross-over from chemotherapy to EGFR TKI (1,15-17). The combined data from these studies now define the clinical management of *EGFR* mutated lung cancers. Erlotinib, gefitinib and afatinib are approved worldwide for the first line treatment of lung adenocarcinomas with *EGFR*-exon 19 deletions or *EGFR*-L858R mutations (5,18).

The advances brought forth by first and second generation EGFR TKIs not only validated EGFR as an important target for lung cancer but also highlighted some of the limitations of these EGFR TKIs. Acquired resistance to erlotinib/gefitinib and afatinib therapy can come about through multiple biological mechanisms that highlight tumor heterogeneity and adaptability [i.e., a game of whack-a-mole analogy (Figure 2)]: (I) the gatekeeper kinase *EGFR*-T790M mutation that modifies ATP affinity, drug binding properties and shifts inhibitory curves (5); (II) activation of bypass signaling cascades that reactivate the MAPK and PI3K downstream pathways (5); and (III) phenotypic and genomic neuroendocrine transformation that silences the expression of or dependence on EGFR protein (19-21). By far, the selection of tumors harboring the original activating *EGFR* mutation with concurrent *EGFR*-T790M is the most common (>50-60%) mechanism of acquired resistance to

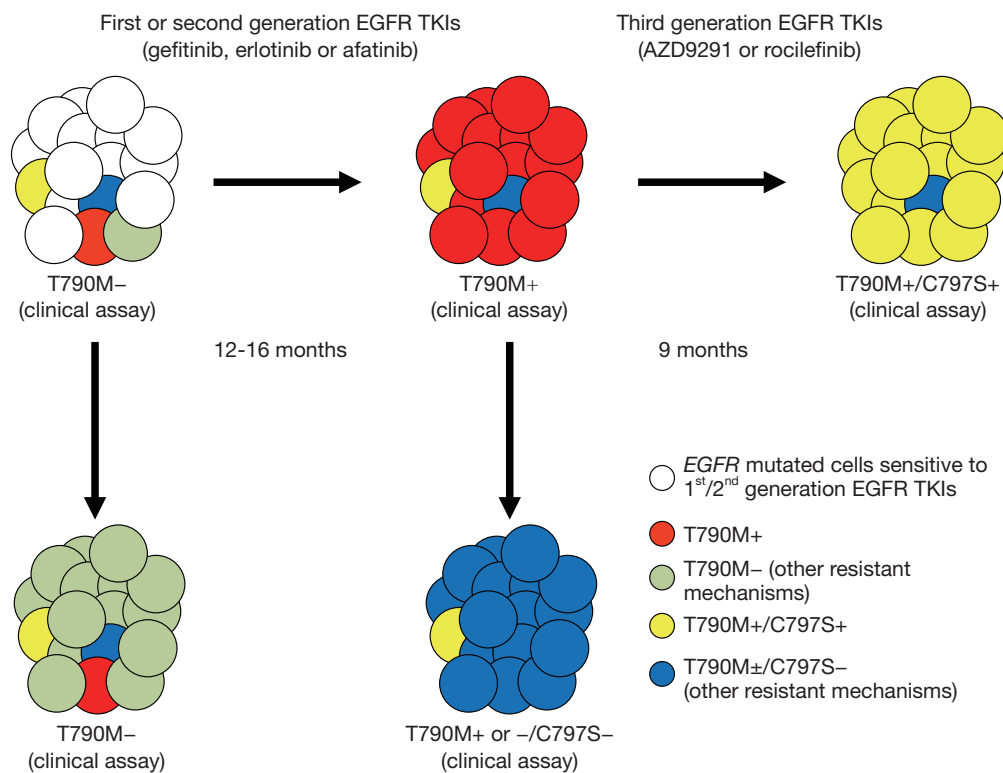


Figure 2 Clonal selection of heterogeneous mass of epidermal growth factor receptor (*EGFR*) mutated lung adenocarcinomas under pressure of EGFR tyrosine kinase inhibitors (TKIs). The graphical display portrays a hypothetical sequence of changes in clonal predominance upon long-term exposure/adaptation of the tumor mass to first/second and subsequently third generation EGFR TKIs. We highlight clones that have the secondary *EGFR*-T790M and the tertiary *EGFR*-C797S mutations. Note that pre-existing mutations may or may not be detected in the clinical setting. This figure attempts to make visualization of the relentless game of whack-a-mole that is constantly being “played” between TKIs and a highly heterogeneous/adaptable cancer.

first/second generation of EGFR TKIs (5,22-26). We and others first identified *EGFR*-T790M in 2005 (22,27), which leads to a threonine (T) to a methionine (M) amino-acid change at the 790 “gatekeeper” regulatory position of the EGFR kinase (22,27). In addition to its effect on ATP affinity and drug binding (5), *EGFR*-T790M can stimulate other oncogenic signals—such as the β -catenin pathway (23). Originally, *EGFR*-T790M was reported as an acquired mutation after exposure to first generation EGFR TKIs; however, recent progress with sensitive sequencing technologies has revealed that pre-existing *EGFR*-T790M clones can be detected in patients with TKI-naïve tumors (28); as indicated in *Figure 2*. In this context, emergence of *EGFR*-T790M may be due to selection of “*EGFR*-T790M-positive” clones under pressure from a first/second generation EGFR TKI (*Figure 2*).

Since we first reported that an irreversible EGFR TKI can inhibit *EGFR*-T790M *in vitro* (29), efforts has been made to identify potent irreversible (i.e., C797-binding) EGFR TKIs to overcome resistance caused by *EGFR*-T790M. However, the initial selected clinical compounds (i.e., second generation EGFR TKIs such as afatinib and dacomitinib) failed to induce responses in the clinical acquired resistance to gefitinib/erlotinib setting (5). These disappointing results can be explained by lack of EGFR mutant selectivity of second generation EGFR TKIs and their in-existent therapeutic window towards *EGFR*-T790M when compared to WT *EGFR* (*Figure 1*). Afatinib and others in the same class are extremely potent WT *EGFR* inhibitors and achievable serum/plasma levels (limiting toxicities include skin and gastrointestinal adverse events) in patients are unable to inhibit *EGFR*-T790M bearing lung

adenocarcinomas (5,6,30).

A major breakthrough in targeting EGFR-T790M occurred in 2009 with the identification of a novel class of covalent EGFR pyrimidine TKIs that are more selective for EGFR-T790M and EGFR TKI-sensitizing mutations than to WT EGFR (31). This class of TKIs against EGFR-T790M heralded the clinical development of third generation EGFR TKIs (*Figure 1*). The two compounds that have advanced the furthest are AZD9291 (AstraZeneca, with a proposed name of mereletinib) and rociletinib (Clovis Oncology, formerly named CO-1686). The impressive results from the expanded phase I first-in-human studies for both drugs were published in April 2015 (32,33). The phase I trial of AZD9291 (AURA) evaluated escalating doses of the drug in patients with advanced *EGFR* mutated lung cancer with resistance to treatment with the first generation EGFR TKIs (erlotinib/gefitinib) (32). A total of 253 patients were included in doses of AZD9291 of 20 mg up to 240 mg daily but a dose of 80 mg daily was considered as optimal to maximize efficacy and minimize skin/gastrointestinal adverse events observed at the higher doses (32). A total of 138 patients had tumors that were confirmed to harbor *EGFR*-T790M and 127 were evaluated for responses; with a RR of 61% (95% CI, 52-70%), disease control rate (DCR) of 95% (95% CI, 90-98%) and a median PFS of 9.6 months (95% CI, 8.3-not reached). As expected, tumors not harboring *EGFR*-T790M (61 evaluable patients) fared worse with a RR of 21% (95% CI, 12-34%), DCR of 61% (95% CI, 47-73%) and a dismal median PFS of 2.8 months (95% CI, 2.1-4.3 months). AZD9291 has been granted breakthrough therapy designation, orphan drug and fast track status by the United States Food and Drug Administration (FDA); and its approval with a companion diagnostic for *EGFR*-T790M is imminent based on results of the aforementioned AURA study and an ongoing phase II trial of AZD9291 80 mg daily for *EGFR*-T790M mutated lung adenocarcinomas (AURA-2 study). This third generation EGFR TKI is also being investigated in randomized trials after progression on gefitinib, erlotinib or afatinib against evidenced-based chemotherapies (AURA-3 study), as a first line therapy for *EGFR* mutated lung adenocarcinoma against gefitinib or erlotinib (FL-AURA study), and in combination with anti-PDL1 immunotherapies (MEDI4736), MEK inhibitors (selumetinib) or MET inhibitors (AZD6094) as part of the TATTON study. The phase I-II trial of rociletinib (TIGER-X) evaluated escalating doses of the drug in patients with advanced *EGFR* mutated lung cancer with

acquired resistance to first or second generation EGFR TKIs (33). A total of 130 patients were enrolled and received escalating doses of free-base and subsequently hydrogen bromide salt (HBr) drug formulations, with therapeutic doses considered to encompass 900 mg twice daily of free-base and 625,750 or 1,000 mg twice daily of HBr rociletinib (33). A total of 46 patients had tumors that were confirmed to harbor *EGFR*-T790M and were evaluated for responses; with a RR of 59% (95% CI, 45-73%), DCR of 93% and a median PFS of 13.1 months (95% CI, 5.4-13.1 months). Tumors not harboring *EGFR*-T790M (17 evaluable patients) fared worse with a RR of 29% (95% CI, 8-51%), DCR of 59% and a median PFS of 5.6 months (95% CI, 1.3-not reached). Interestingly, the predominant grade 3 adverse event was hyperglycemia thought to be secondary to a rociletinib metabolite that inhibits the type I insulin-like growth factor receptor (33); and the latter adverse event (often requiring anti-diabetic medications) in addition to concerns related to cardiac QT prolongation may hamper the rapid clinical development of this drug. Rociletinib has been granted breakthrough therapy designation by the FDA with data from the aforementioned TIGER-X and a global registration phase II trial in *EGFR*-T790M positive lung adenocarcinomas (TIGER-2 study) being evaluated for safety plus efficacy. This third generation EGFR TKI is also being investigated in randomized trials after progression on gefitinib, erlotinib or afatinib against evidenced-based chemotherapies (TIGER-3 study) and as a first line therapy for *EGFR* mutated lung adenocarcinoma against gefitinib or erlotinib (TIGER-1 study).

Despite the thrilling responses seen with AZD9291 and rociletinib in lung adenocarcinomas with acquired resistance to gefitinib, erlotinib or afatinib harboring the recalcitrant *EGFR*-T790M mutation (32,33), it is painfully evident that tumor plasticity and selection pressure continue to drive tumor adaptation and resistance to third generation EGFR TKIs (*Figure 2*). The clinical investigators of the AZD9291 clinical trials have convincingly shown that biological mechanisms of resistance to this drug can be readily identified in cell-free plasma DNA from patients (34). The most frequent (40% of 15 *EGFR*-T790M cases treated with AZD9291 in the AURA study) mechanism identified was the acquisition of the *EGFR*-C797S mutation in exon 20 of *EGFR*. These investigators and other show in preclinical models that *EGFR*-exon 19 deletion + T790M + C797S and *EGFR* - L858R + T790M + C797S generate proteins that are resistant to AZD9291, rociletinib and all irreversible EGFR TKIs (including quinazolone- and

pyrimidine-based compounds) by impairing covalent binding of these drugs to the C797 amino-acid residue of EGFR (34-36). Plasma samples also showed that another 33% of cases with AZD9291 progression only had *EGFR*-T790M and the original sensitizing mutation detected (Figure 2), and in another 27% of cases the *EGFR*-T790M was no longer detected (34). Although the plasma DNA was unable to evaluate for non-*EGFR* mutational mechanisms of acquired resistance, plentiful preclinical reports using third generation EGFR TKIs (including AZD9291) have consistently demonstrated bypass activation of the MAPK-ERK-RAS pathway (through *MAPK1* amplification, downregulation of negative regulators of ERK, *NRAS* mutation/amplification, *KRAS* amplification among others) as a major escape valve to EGFR inhibition (37,38). The clinical investigators of the rociletinib clinical trials have also demonstrated similarly that resistance to rociletinib in the TIGER-X study can be accompanied by putative bypass mechanisms in the presence or absence of *EGFR*-T790M or *EGFR*-T790M amplification (39). In addition, their group also reported neuroendocrine transformation of adenocarcinomas to small cell lung cancer with genotypic/phenotypic silencing of EGFR protein expression as a mechanism of resistance in 16% (2/12 cases) of rociletinib re-biopsies (39). Future reports of tumor and liquid biopsies of lung adenocarcinomas resistant to third generation EGFR TKIs will help define the true frequency of *EGFR*-C797S, MAPK pathway activation and small cell transformation as mechanisms of resistance to this new class of TKI (Figure 2).

EGFR mutated NSCLCs (those with exon 19 deletions or L858R) have come a long way in the last decade. Most patients with a new diagnosis of advanced *EGFR* mutated NSCLC in 2015 can expect to receive multiple lines of monotherapy with first, second and third generation EGFR TKIs and can anticipate a median OS that exceeds 2-3 years, which is undoubtedly a tremendous success given that a median OS in pre-EGFR TKI era was less than 1 year. However, the use of monotherapies with EGFR TKIs has also underscored the painful reality that a relentless game of whack-a-mole is constantly being played between TKIs and a highly heterogeneous/adaptable cancer; with the lung adenocarcinoma eventually winning out through mutations (*EGFR*-T790M and/or C797S), bypass mechanisms or histologic/genotypic transformation (Figure 2). The next decade of research milestones for *EGFR* mutated lung adenocarcinomas will need to address current unmet clinical needs; which include: the role of first, second and third

generation EGFR TKIs in the management of earlier stages (I-III) of NSCLC, the need for improved management of difficult-to-treat sanctuary sites such as the central nervous system (40), and the requisite for treatment strategies (most likely combination therapies with PI3K/MAPK inhibitors, immunotherapies or cytotoxic agents) that can delay or overcome acquired resistance to first, second and third generation EGFR inhibitors. We hope that we will eventually “catch all moles” (Figure 2) and “win” the game between TKIs and oncogenic kinase-driven tumors.

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Footnote

Provenance: This is a Guest Editorial commissioned by the Section Editor Hongbing Liu (Department of Respiratory Medicine, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China).

Conflicts of Interest: Daniel B. Costa has received consulting fees and honoraria from Pfizer Inc and Boehringer Ingelheim, respectively. Daniel B. Costa also conducts unremunerated clinical trials using AZD9291 (AstraZeneca) and rociletinib (Clovis Oncology). Susumu S. Kobayashi has received honoraria from Bristol-Myers Squibb. No other conflict of interest is stated.

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