

IN THE SPOTLIGHT

Shades of T790M: Intratumor Heterogeneity in *EGFR*-Mutant Lung CancerEiki Ichihara^{1,2} and Christine M. Lovly^{1,2,3}

Summary: In the setting of recent exciting clinical results and numerous ongoing trials, Piotrowska and colleagues explore mechanisms of acquired resistance to the mutant-specific *EGFR* inhibitor rociletinib, and demonstrate that loss of T790M, *EGFR* amplification, and small-cell transformation are all clinically relevant mechanisms of drug resistance. The authors provide a new paradigm for using quantitative assessment of the *EGFR* T790M:activation mutation allele frequency ratio to prognosticate responses to rociletinib and also demonstrate that plasma-based assessments of circulating tumor DNA can be used to monitor drug response and the emergence of drug resistance. *Cancer Discov*; 5(7); 694-6. ©2015 AACR.

See related article by Piotrowska et al., p. 713 (6).

The discovery of *EGFR* mutations in non-small cell lung cancer heralded the dawn of molecularly targeted therapy in this disease. Indeed, numerous phase III studies have now documented that patients with *EGFR*-mutant tumors derive significant benefit from treatment with first- or second-generation *EGFR* tyrosine kinase inhibitors (TKI), such as erlotinib, gefitinib, and afatinib (1). However, acquired resistance develops on average 1 year after the initiation of TKI therapy. The most common mechanism of acquired resistance is a secondary *EGFR* T790M mutation, which is found in about 50% to 60% of tumors at the time of resistance (1).

In order to overcome T790M-mediated resistance, third-generation *EGFR* TKIs, including rociletinib (2) and AZD9291 (3), have been developed. These agents can inhibit both activating *EGFR* mutations and the secondary T790M mutation, and recent exciting clinical trial data have demonstrated a response rate of approximately 60% in patients with T790M-positive tumors (4, 5). Unfortunately, the depth and duration of response varies, suggesting that, even among T790M-positive tumors that respond to treatment with third-generation *EGFR* inhibitors, not all the tumor cells are homogeneously sensitive to these inhibitors. In addition, “secondary acquired resistance” to these agents has already emerged (Fig. 1), and there still exists a significant percentage of T790M-positive tumors that do not respond to third-generation *EGFR* inhibitors at all.

In this issue of *Cancer Discovery*, Piotrowska and colleagues (6) identify heterogeneous mechanisms through which tumors

can evade the effects of the third-generation *EGFR* TKI rociletinib. They successfully captured paired pretreatment and rociletinib-resistant biopsy samples from 12 patients, including 1 patient who had two distinct biopsies at the time of resistance. All 12 patients had previously been treated with erlotinib or afatinib and had documentation of a T790M-positive biopsy before initiation of rociletinib. The authors found that half of the T790M-positive *EGFR*-mutant lung cancers treated with rociletinib lost the T790M mutation upon progression (6/13 biopsies) but still retained the original activating *EGFR* mutation. Loss of T790M mutation upon progression has also been reported with another third-generation *EGFR* TKI, AZD9291 (7).

Among the 6 patients whose tumors lost T790M upon progression (so called T790-wild-type progression), the authors identified transformation to small cell lung cancer histology in 2 patients and *EGFR* amplification in 3 patients as resistance mechanisms. Notably, they did not identify any additional *EGFR* mutations, including the previously described *EGFR* C797S mutation (7), in their cohort.

To further study T790M heterogeneity, the authors established a cell line from a T790M-positive malignant pleural effusion of a patient with acquired resistance to the second-generation *EGFR* TKI afatinib. They isolated eight single-cell clones from this novel cell line and, interestingly, detected T790M in only five of the eight clones, suggesting that both T790M-positive and T790M-negative cells coexist simultaneously after acquiring resistance to afatinib. In fact, heterogeneity of T790M-positive clones is not limited to tumors with acquired resistance to first- or second-generation *EGFR* TKIs, but also observed in TKI-naïve *EGFR*-mutant lung cancer. Though its frequency is somewhat controversial, previous studies have shown that T790M-mutated cells may preexist as a minor population in *EGFR* TKI-naïve lung cancers (8). Therefore, it is possible that intratumor heterogeneity of the T790M mutation preexists in treatment-naïve *EGFR*-mutant lung cancer and becomes apparent under selection by first- or second-generation *EGFR* TKIs.

Importantly, the authors demonstrate that the pretreatment fraction of T790M-positive clones affected the response

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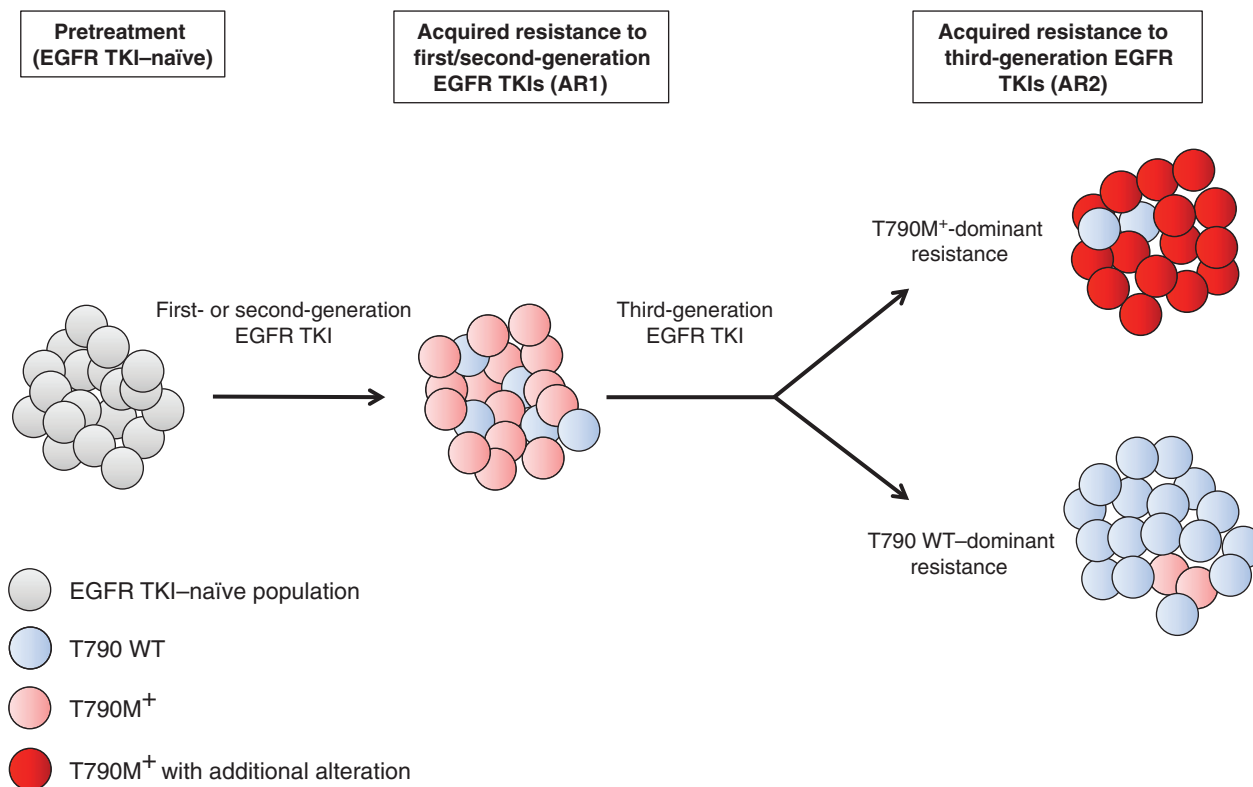


Figure 1. Schematic representation of acquired resistance to the third-generation EGFR inhibitor rociletinib. A first- or second-generation EGFR TKI eliminates the majority of EGFR TKI-naïve clones with *EGFR*-activating mutations (e.g., *EGFR* exon 19 deletion, *EGFR* L858R). After acquiring resistance to first- or second-generation EGFR TKIs [acquired resistance 1 (AR1)], both T790M-positive and T790-wild-type clones may coexist within the “T790M-positive” tumor, but at present, only a binary assessment for reporting T790M mutational status (T790M present/T790M absent) is used in a clinical setting. The original activating *EGFR* mutation is thought to be present in all the cells. Within the AR1 tumor, the T790-wild-type clones presumably harbor other (non-T790M) resistance mechanisms, such as *PIK3CA* mutation, *HER2* amplification, *IGF1R* activation, small cell transformation, etc. The heterogeneous “T790M-positive” tumor can be effectively treated with a third-generation EGFR TKI, such as rociletinib, which has documented efficacy against *EGFR*-activating and T790M mutations. After acquiring resistance to the third-generation EGFR TKI [acquired resistance 2 (AR2)], some tumors will have T790M-dominant resistance, with the original T790M clones now harboring additional alterations that mediate resistance to the third-generation EGFR TKI. In contrast, some tumors will have T790-wild-type (WT) resistance, with outgrowth of clones that contain the original *EGFR* mutation but lack T790M.

to rociletinib in their study cohort. Excluding patients with *EGFR* amplification and with an *EGFR*-activating mutation fraction of >60% in the pre-rociletinib biopsy, the authors calculated the ratio of pretreatment T790M:activation mutation allele frequency and correlated this ratio with maximal tumor shrinkage. Twenty-five patients were included in this analysis, which demonstrated that higher baseline fraction of T790M was significantly associated with greater response to rociletinib. These results imply that replacing the current binary assessment of T790M status (T790M present vs. T790M absent) in tumor samples with a quantitative T790M: activation mutation allele frequency may represent a prognostic stratification for patients treated with rociletinib.

These new findings raise several interesting questions. First, what is mediating rociletinib resistance in those patients whose tumors retained the T790M mutation? Neither “tertiary” *EGFR* mutations, such as C797S, which are known to confer resistance to third-generation EGFR TKIs, nor *MET* amplification were detected in any of the tumors examined. Furthermore, the authors did not report on the development of MAPK pathway reactivation, including *NRAS* mutations

and amplification of *KRAS*, *NRAS*, or *MAPK1*, which were found to mediate resistance to AZD9291 in preclinical models (9). As additional rociletinib-resistant tumors are systematically evaluated, these resistance mechanisms may emerge.

Second, why is the T790M mutation lost in half of the resistant tumors? This is presumably due to selection of preexisting T790-wild-type clones during rociletinib treatment. However, why were only T790-wild-type clones selected despite the fact that rociletinib inhibits not only T790M-positive EGFR but also T790-wild-type EGFR with activating mutations? One possible explanation is that rociletinib is more potent against activating *EGFR* mutations with T790M compared with those without T790M. Previous studies have shown that rociletinib inhibits the kinase activity of EGFR exon 19 deletion with T790M better than that of EGFR exon 19 deletion without T790M (94% vs. 65%), whereas there was no difference in L858R EGFR with or without T790M (89% vs. 87%; ref. 2). Given that all the cases except one in the current study harbored exon 19 deletion with T790M, this may be a possible mechanism. Indeed, the authors demonstrated that a higher pretreatment proportion of T790M-positive cells was associated with better

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response to rociletinib. However, differential activity of rociletinib against exon 19 deletion with or without T790M does not entirely explain why T790M is lost during rociletinib treatment, because one patient whose initial tumor harbored L858R/T790M mutations developed resistance to rociletinib and lost T790M while retaining L858R. Thus, another possible explanation is outgrowth of T790-wild-type clones that harbor additional, EGFR-independent resistance mechanisms (Fig. 1). Considering that T790-wild-type clones in a “T790M-positive” tumor should also be resistant to first- or second-generation EGFR TKIs, these surviving T790-wild-type clones likely harbored additional resistance mechanisms (i.e., “bypass” activation of alternative signaling pathways or histologic transformation) before rociletinib treatment. These EGFR-independent mechanisms of resistance would not be sensitive to rociletinib. In fact, two of the six T790-wild-type tumors that became resistant to rociletinib acquired *RBI* alterations leading to small-cell transformation, suggesting that *RBI* alterations occurred in T790-wild-type clones (6). This notion is also supported by the authors’ plasma circulating tumor DNA data. Rociletinib initially decreased both *EGFR* deletion 19 and T790M in all the three cases examined, presumably reflecting the decreased number of T790M-positive clones, but in two cases, only the *EGFR* exon 19 deletion increased at radiographic progression while T790M remained low, indicating that T790-wild-type clones were outgrowing the T790M clones. In the remaining patient, both exon 19 deletion and T790M began to re-expand at progression, likely due to a secondary alteration that occurred within T790M-positive cells.

Third, and finally, the authors convincingly show that a higher pretreatment proportion of T790M-positive cells, as assessed by the *EGFR* T790M:activation mutation allele frequency ratio, was associated with better response to rociletinib. Therefore, how can such quantitative assessment be employed in the clinic to replace the current binary assessment of T790M status?

Overall, in the authors’ present (6) and simultaneously reported studies (10), they provide timely data that advance our knowledge of resistance to third-generation EGFR TKIs and uncover a role for tumor heterogeneity in predicting response to this exciting class of agents. These studies also offer a rationale for subsequent therapeutic strategies to overcome rociletinib resistance, including the intriguing possibility of using first- or second-generation EGFR TKIs to overcome resistance to third-generation inhibitors. To evaluate these hypotheses, further studies are warranted.

Disclosure of Potential Conflicts of Interest

C.M. Lovly reports receiving commercial research grants from Astra-Zeneca and Novartis, and is a consultant/advisory board member

for Novartis and Sequenom. No potential conflicts of interest were disclosed by the other author.

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