Heterogeneity-based, multiple mechanisms in the resistance to osimertinib (AZD9291): A case report

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Keywords
Epidermal growth factor receptor T790 mutation; heterogeneity; MET amplification; osimertinib; resistance.

Abstract
Osimertinib is a novel, irreversible, mutant-selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor targeting EGFR mutations and the EGFR T790M mutation. Here, we report a woman with EGFR-mutated lung adenocarcinoma who, after 23-month treatment with gefitinib, developed the EGFR T790M mutation, which converted the T790M status from positive to negative before osimertinib treatment and developed MET amplification, leading to rapid progression on osimertinib in two months. Subsequent treatment with crizotinib and c-Met inhibitor plus gefitinib also failed to improve the clinical outcome, suggesting the potential existence of another resistance mechanism. Our findings revealed the underlying multiple and heterogeneous mechanisms in resistance to osimertinib, suggesting combination strategies should be considered post-osimertinib progression.

Introduction
Osimertinib (AZD9291) is a third-generation and mutation-selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) used in patients with EGFR T790M mutant non-small cell lung cancer (NSCLC) who failed prior treatment with EGFR TKIs. However, acquired resistance to osimertinib inevitably occurs. Here, we report multiple and heterogeneous mechanisms involved in the resistance to osimertinib, which induce rapid disease progression.

Case Report
The patient was a 63-year-old, never-smoker, Asian woman with stage IV lung adenocarcinoma harboring EGFR Del19 mutation (by ARMS). She was treated with gefitinib for 23 months with partial response as the best response and progressed in October 2015. Re-biopsy of the lung found an EGFR Del19 mutation and an exon 20 T790M mutation by the PCR method using cobas® EGFR Mutation Test v2 assay (Roche Molecular Diagnostics, Pleasanton, CA, USA). The patient was then enrolled in the phase 3 study of AZD9291 (AURA 3) and assigned to the chemotherapy group (pemetrexed/cisplatin), achieving partial response after two-cycle treatment, and progressed after four-cycle chemotherapy and two-cycle pemetrexed maintenance. The EGFR Del19 mutation (ARMS) and c-Met amplification (FISH+ and IHC 2+) was detected, but not the T790M mutation. This patient was
then crossed over to receive osimertinib 80 mg once daily according to the AURA 3 protocol, and rapidly progressed in two months with progressed disease as the best tumor response, while shrinkage of the intracranial tumor was seen. Liquid molecular analysis by PCR showed an activating EGFR Del19 mutation, but no EGFR T790M or C797S mutation, while c-Met amplification was found in a tumor biopsy of the supraclavicular lymph nodes. The patient was then enrolled in a clinical trial evaluating a new c-Met inhibitor, BPI-9016, in June 2016, and achieved stable disease; however, the duration of the clinical benefit only lasted for three months. Post-progression next-generation sequencing results revealed the EGFR Del19 mutation without any signal of other biomarkers (including KRAS, NRAS, BRAF, PIK3CA, HER-2, RET, PDGFRA, and C-KIT). Crizotinib plus gefitinib was suggested; however, concerned about the potential toxicity, she started single-agent crizotinib, and continued to receive a c-Met inhibitor plus gefitinib after progression on crizotinib. During these treatments, the tumor in the lung was well controlled, while significant progress was seen in the liver. The patient died in December 2016 (Fig 1).

Discussion

Osimertinib (AZD9291) is a mutant-selective, third-generation EGFR TKI that is designed to overcome the acquired T790M-mediated TKI resistance. In the phase 1 component of AURA, osimertinib demonstrated an objective response rate of 61% and a progression-free survival was 9.6 months in patients with T790M-positive NSCLC.1 A pooled analysis of two subsequent phase 2 studies of osimertinib further confirmed these findings, the overall response rate was 66% and the median progression-free survival was 11.0 months in 411 T790M-positive NSCLC patients.2 More recently, the phase 3 AURA 3 study found that patients with T790M-positive NSCLC who were treated with osimertinib had a better overall response rate and longer progression-free survival than did those receiving chemotherapy after first-line therapy with EGFR TKI.3 Furthermore, the benefit of osimertinib was also shown in patients with central nervous system metastases by a longer duration of progression-free survival.1 Based on these studies, osimertinib is recommended as standard therapy for patients with metastatic EGFR T790M mutation-positive NSCLC, who have progressed on or after EGFR-TKI
therapy. However, like early-generation EGFR TKIs, development of clinical resistance to osimertinib has also been reported. So far, several mechanisms of resistance have been identified in osimertinib and other third-generation EGFR TKIs, including acquired EGFR C797S mutation, loss of EGFR T790M mutant clones, activation of bypass pathway, and histological transformation.

In this case, we identified an activating T790M mutation at the time of acquired resistance to gefitinib; however, after chemotherapy, the wild-type T790 became the dominant driver of tumor growth. The main explanation is the coexistence of positive T790M and wild-type T790 clones before osimertinib treatment. The true biopsy of a T790M “positive” tumor is more than a binary positive/negative variable. The coexistence of positive and wild-type T790 clones within a tumor makes osimeritinib suppress the growth of T790-positive cells, while the T790 wild-type clones escape and mediate the development of resistance.

Furthermore, the degree of this heterogeneity is predictive of response: a tumor with a higher fraction of T790M-positive cells at baseline is associated with a better response to third-generation EGFR TKIs. In addition, chemotherapy may affect the EGFR mutation status in tumor cells due to the different sensitivities of EGFR-mutated and wild-type tumor cells to chemotherapy; it is possible that similar a mechanism exists in EGFR T790-positive tumor cells. Alternative pathway activation, MET amplification, was also identified in our case. However, heterogeneous responses were noted after single c-Met inhibitor or combination of c-Met inhibitor and EGFR TKI, while no other biomarkers were found. These findings indicated the potential existence of multiple mechanisms of resistance within a tumor, such as histological transformation to small cell carcinoma. Last but not least, the heterogeneity of biopsy status between primary and metastatic tumor lesions should also be noted. In this case, although the biopsy was conducted in the lung and in the lymph nodes pre- and post-chemotherapy, respectively, the rapid overall treatment failure to osimertinib suggested that the T790M mutation status in the lymph nodes was consistent with that in the lung.

Increasing attention has been paid to the application of plasma genotyping. A lack of available tissue for molecular assessment is commonly seen in real-word practice; single-site biopsies might not be representative of the overall predominant resistance mechanisms due to the heterogeneity of metastases. Furthermore, liquid biopsy has potential applications for dynamic monitoring of the efficacy of targeted therapies and early detection of resistance mutations. Oxnard et al. reported that an equivalent outcome was seen in patients with positive T790M in plasma to those with positive T790M in tissue, supporting the use of plasma genotyping. In the current study, liquid biopsy was used once as surrogate samples after progression on multiple-line treatments, the lack of temporal monitoring of the EGFR T790M by plasmatic test is a major limitation of this report.

Our findings provide evidence for the coexistence of multiple mechanisms in the resistance to osimertinib within a tumor; furthermore, the resistance mechanism may vary between tumor lesions in one patient. Currently, combination trials of osimertinib and other pathway inhibitors are being investigated (TATTON, NCT02143466). This report further supports the combined regimens of T790-targeted agents and other drugs to target T790-wild-type cells or other bypass pathway when making clinical decision.

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Disclosure

No authors report any conflict of interest.

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