The emergence of various genetic alterations mediated the Osimertinib resistance of a patient harboring heterozygous germline EGFR T790M: a case report

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Abstract: Epidermal growth factor receptor (*EGFR*) T790M is the major mechanism mediating resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Despite the high frequency of *EGFR* activating mutations among East Asian lung cancer patients, germline T790M has been the subject of very little research. Questions remain as to whether germline T790M develops resistance to Osimertinib and if so, through which mechanisms. This study examined a patient harboring germline *EGFR* T790M who acquired resistance to Osimertinib therapy. After the failure of first-line icotinib therapy, which was administered for only 3 months, targeted next-generation sequencing of plasma samples collected at icotinib progression and the re-analysis of the baseline tissue biopsy sample revealed *EGFR* T790M with allelic frequencies approximating 50%. Lymphocyte genomic deoxyribonucleic acid (DNA) sequencing confirmed the germline heterozygous status of the T790M mutation. In addition to the *EGFR* T790M, a concurrent *EGFR* L858R was detected from the baseline tissue sample. Osimertinib therapy was initiated resulting in a partial response within 1 month of the commencement of the therapy. After 15.2 months of Osimertinib therapy, disease progression was evaluated due to the presence of pleural effusion. The targeted sequencing of plasma and pleural effusion samples revealed the emergence of *EGFR* G719A, tumor protein p53 (*TP53*) Q136X, and the co-amplification of Cyclin D1, fibroblast growth factor (*FGF*) 19, *FGF3*, and *FGF4*. This case highlights the importance of conducting next-generation sequencing–based molecular testing during both diagnostic and disease progression assessments to reveal sensitizing mutations and mutations that could mediate primary and acquired resistance to targeted therapeutics.

Keywords: EGFR T790M; familial lung cancer; germline T790M; Osimertinib resistance

Submitted Nov 05, 2020. Accepted for publication Dec 23, 2020.
doi: 10.21037/atm-20-7626
View this article at: http://dx.doi.org/10.21037/atm-20-7626

Introduction

Despite harboring activating mutations in epidermal growth factor receptor (*EGFR*), 20–30% of patients with *EGFR*-mutant non-small cell lung cancer (NSCLC) lack an objective response or only respond to EGFR tyrosine kinase inhibitor (TKI) therapy for less than 3 months due to the existence of genetic alterations that mediate primary resistance to EGFR-TKIs (1,2). *EGFR* T790M is commonly acquired during EGFR-TKI therapy and accounts for the majority of secondary resistance to first- and second-generation EGFR-TKI; however, 2% of patients harbor either somatic or germline T790M before any exposure to EGFR-TKIs, resulting in primary resistance (1,3,4). Germline *EGFR* T790M has been reported in non-smokers and is associated with inherited lung cancer susceptibility (3-6). The *EGFR* activating mutation rate is between 40–50% among Chinese NSCLC patients (7); however, germline *EGFR* T790M is rare (6,8). *EGFR* T790M-mediated resistance can effectively be reversed by the third-generation EGFR-TKI Osimertinib (9,10). Similar

to clinical findings about the benefits of Osimertinib therapy for patients with acquired T790M from first- or second-generation EGFR-TKI therapy (9,10), first-line Osimertinib therapy has been shown to benefit NSCLC patients who harbor either somatic T790M at baseline or germline T790M with concomitant *EGFR* sensitizing mutation (8,11). In this paper, we discuss a Chinese patient with advanced lung adenocarcinoma harboring germline *EGFR* T790M who developed Osimertinib resistance despite a durable partial response. We present the following article in accordance with the CARE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-7626).

Case presentation

In October 2018, a 57-year-old non-smoking female patient presented with an intermittent dry cough and chest tightness at our institution. Enhanced computed tomography (CT) scans of the patient's chest revealed soft-tissue nodules in the basal segment of the right lobe of the lung, the presence of bilateral ground-glass nodules, and the slight enlargement of the right hilar and mediastinal lymph nodes (*Figure 1A*). The patient was diagnosed with Stage IV (T4N2M1a) lung adenocarcinoma. Following the guidelines for molecular testing for all lung cancer patients, tissue biopsy samples were sent for molecular testing. An allele-specific polymerase chain reaction assay was used to confirm the *EGFR* L858R mutation, after which the patient was administered with icotinib at the standard daily dose of 125 mg achieving stable disease (*Figure 1B*).

A review of the patient's CT scans at 3 months revealed the enlargement of primary lung lesions, evaluated as progressive disease by the investigators (*Figure 1C*). Plasma samples collected at icotinib progression were sent for targeted sequencing with a panel consisting of 168 genes (Burning Rock Biotech, Guangzhou, China). The analysis of the plasma sample only detected *EGFR* T790M. The targeted sequencing of the archived tissue biopsy sample from baseline detected *EGFR* L858R, *EGFR* T790M, and catenin beta 1 (*CTNNB1*) S37F. Interestingly, the allelic fraction (AF) of *EGFR* T790M detected from both the tissue and plasma samples consistently approximated 50% (*Figure 1*), which raised the suspicion that the T790M was a germline mutation. *EGFR* testing of the lymphocyte genomic DNA confirmed the germline heterozygous status of the T790M mutation. Further investigations of the patient's family history revealed that the patient's father and paternal aunt had died of lung cancer in 2004 and 2006, respectively (*Figure 2*). Her living family members were currently all cancer-free. Osimertinib was then administered at 80 mg once daily starting from April 1, 2019. Within two months of Osimertinib therapy, a 33.6% shrinkage of the primary lesions was observed, and was deemed by the investigators to be a partial response based on the Response Evaluation Criteria in Solid Tumors Version 1.1 (*Figure 1D,E*). No adverse events were observed. On June 23, 2020, after approximately 15.2 months of Osimertinib therapy, the primary lesions remained stable; however, the accumulation of pleural fluid was observed on the left lung lobe, indicating disease progression (*Figure 1F*). The targeted sequencing of the pleural effusion and plasma samples collected at Osimertinib progression identified the *EGFR* T790M and the emergence of various mutations, including *EGFR* G719A, tumor protein p53 (*TP53*) Q136X, and the co-amplification of Cyclin D1 (*CCND1*), fibroblast growth factor (*FGF*) *19*, *FGF3*, and *FGF4* (*Figure 1*). Pleural effusion cytology showed adenocarcinoma features.

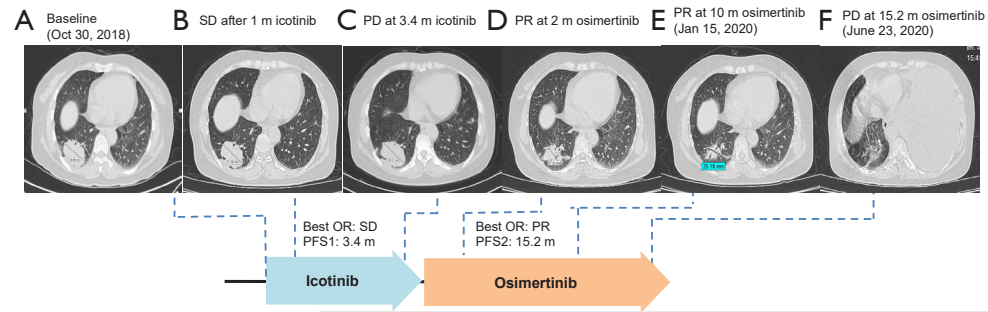
Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was provided by the patient. The patient has given her consent for the publication of her case.

Discussion

Primary resistance is defined as a lack of tumor regression after 3 months of uninterrupted EGFR-TKI therapy (1). Numerous studies have associated various genetic alterations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (12), mesenchymal-to-epithelial transition (*MET*) (1), anaplastic lymphoma kinase (*ALK*) (1), human epidermal growth factor receptor 2 (*HER2*) (6) and in the kinase domain of *EGFR*, including T790M (1,6), as playing an important role in mediating primary resistance to EGFR-TKI. These genetic alterations impair the response of and confer resistance to inhibition via EGFR-dependent and EGFR-independent signaling pathways similar to acquired mechanisms of EGFR-TKI resistance (13). Compared to acquired EGFR-TKI resistance, the molecular mechanisms mediating primary resistance are less well understood.

In our patient, the disease control for 3 months of icotinib therapy was mediated by the subpopulation



Gene mutation	ARMS-PCR of tissue biopsy at baseline	Targeted sequencing				
		Before osimertinib therapy			At osimertinib PD	
		Plasma at icotinib PD	Baseline tissue biopsy right lobe (reanalysis)	White blood cell	Plasma	Pleural effusion on left lobe
EGFR L858R	Positive	ND	32.31%	ND	ND	ND
EGFR T790M	Not tested	50.00%	50.51%	Detected	Heterozygous germline	41.66%
CTNNB1 S37F	Not tested	ND	14.24%	ND	ND	ND
EGFR G719A	Not tested	ND	ND	ND	20.36%	36.64%
TP53 Q136*	Not tested	ND	ND	ND	26.51%	75.28%
CCND1 copy number amplification	Not tested	ND[G]	ND	ND	CN: 2.8[H]	CN: 3.3
FGF19 copy number amplification	Not tested	ND[G]	ND	ND	CN: 2.8[H]	CN: 3.6
FGF3 copy number amplification	Not tested	ND[G]	ND	ND	CN: 3.1[H]	CN: 3.9
FGF4 copy number amplification	Not tested	ND[G]	ND	ND	CN: 3.0[H]	CN: 4.1

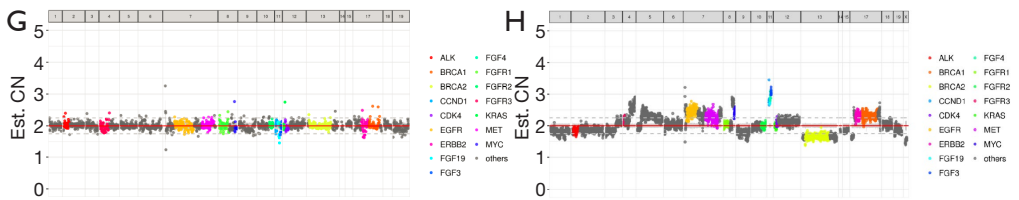


Figure 1 Clinical summary of the patient. Thoracic computed tomography (CT) scans of primary lung lesions at (A) baseline measured at a diameter of 56.96 mm; (B) size reduction to 51.09 mm after 1 month of icotinib treatment; (C) enlargement of the lesion to 56.01 mm at disease progression (PD) from icotinib therapy; reduction to (D) 37.20 mm within 2 months, and (E) 25.18 mm at 10 months of Osimertinib therapy; and (F) the stable primary lesions with the left lobe filled with pleural effusion at progression after 15.2 months of Osimertinib therapy. An illustrated summary of the treatment received by the patient, including the best objective response (OR) and progression-free survival (PFS), in each line of treatment. Table summarizing the following: *EGFR* mutation detected using allele-specific polymerase chain reaction from baseline tissue biopsy sample; mutations and their corresponding allelic fractions detected using targeted sequencing with a 168-gene panel (OncoScreen Target, Burning Rock Biotech) from the plasma sample at PD from icotinib (March 27, 2019) (second column), archived tissue biopsy (third column), and plasma sample (fifth column), and pleural effusion (sixth column) obtained at PD from Osimertinib (June 29, 2020); and EGFR genotyping of white blood cell samples (fourth column). ND, not detected; EGFR, Epidermal growth factor receptor. Illustrations of the copy number variations in specified genes depicts normal gene copy numbers (CN) at the start of osimertinib therapy (G) and the acquisition of coamplification of *CCND1*, *FGF19*, *FGF3*, and *FGF4* at osimertinib progression (H).

of *EGFR* L858R-mutant clones. However, due to the existence of germline *EGFR* T790M, icotinib had limited activity. However, subsequent Osimertinib therapy was able to overcome the primary resistance to icotinib by targeting the tumor cells harboring the T790M mutation, resulting in remarkable tumor regression. Interestingly, both the plasma and tissue biopsy samples from our patient consistently harbored the T790M mutation at an AF of approximately 50%, indicating a heterozygous germline mutation. In support of these findings, another study also demonstrated the accuracy of plasma genotyping in identifying the germline status of T790M as a heterozygous

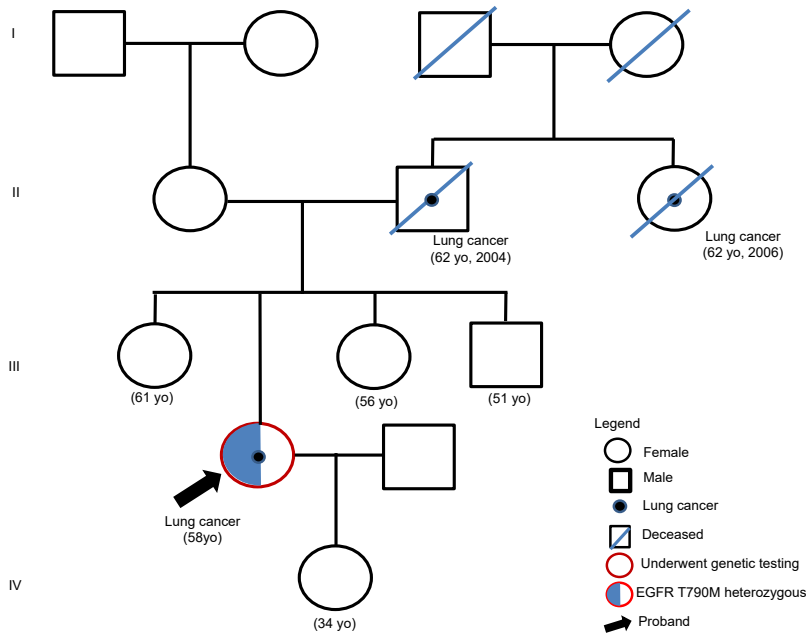


Figure 2 Pedigree analysis illustrating the patient's family history of lung cancer, and the detection of heterozygous EGFR T790M mutation in the patient. EGFR, Epidermal growth factor receptor.

or homozygous mutation if the AF approximates 50% or 100%, respectively, which is far greater than the AF of common *EGFR* driver mutations (14). The heterozygosity of the T790M mutation and the patient's family history of lung cancer strongly suggested inherited lung cancer in an autosomal dominant inheritance pattern. However, the mutation status of the deceased father and paternal aunt were unavailable.

Lung cancer typically develops sporadically through the acquisition of somatic mutations; conversely, inherited lung cancer is rare (6). Due to the possibility of familial lung cancer predisposition associated with germline T790M, and as half of the patients who harbor T790M at baseline are carriers of the germline T790M mutation, the National Comprehensive Cancer Network guidelines recommend additional testing and genetic counseling for those in whom T790M has been detected before exposure to EGFR-TKI (15). The low incidence of germline T790M in East Asians may be due to the widespread use of traditional methods of EGFR testing. As targeted sequencing has become integrated as a routine diagnostic procedure in clinical oncology in recent years, it is also likely that baseline detection of T790M will increase. Targeted sequencing facilitates individualized therapy by revealing not only the mutations that impart sensitivity to currently available targeted therapeutics but also other concurrent mutations that could mediate resistance.

EGFR-dependent and -independent mechanisms of Osimertinib resistance have been well elucidated (16); however, the mechanisms of Osimertinib resistance in certain subsets of patients remain unknown. In our patient, three main genetic alterations were acquired during Osimertinib therapy, *EGFR* G719A, *TP53* Q136X, and the amplification of chromosomal region 11q13 that contains *CCND1*, *FGF19*, *FGF3*, and *FGF4*. At least one of these alterations was the oncogenic driver of disease progression as observed by the development of pleural effusion in the other lobe. In a previous study, durable partial response with front-line Osimertinib therapy was reported in a patient with advanced lung adenocarcinoma who harbored *EGFR* G719A and germline *EGFR* T790M (11); thus, *EGFR* G719A was ruled out as the driver of Osimertinib resistance in our patient. Despite the lack of evidence implicating *TP53* Q136X as an EGFR-independent resistance mechanism to Osimertinib or any other EGFR-TKI, this nonsense mutation in the exon 4 of *TP53* has been reported as one of the *TP53* mutations that confer poor prognoses for patients with *EGFR*-mutant advanced NSCLC (17). It has been suggested that the detection of concomitant *TP53* mutations before the initiation of EGFR-TKI therapy can

reduce the efficacy of EGFR-TKIs, leading to a significantly shorter progression-free survival (18). This suggests that *TP53* Q136X is one of the contributors in driving disease progression, but not the major driver.

The amplification of chromosomal region 11q13, which contains numerous genes, including *CCND1*, *FGF19*, *FGF3*, and *FGF4*, has been reported in 5.6% of cancer patients (22/391), a majority of whom had breast cancer (68%, 15/22) (19,20). *CCND1* amplification in breast tumors has been associated with increased tumor aggressiveness and implicated in poor prognosis and treatment failure (21). The co-amplification of *FGF3*, *FGF4*, *FGF19*, and *CCND1* has been associated with a significantly higher median number of alterations (19), has been found in cancer patients who experienced hyperprogression with immunotherapy, and may accelerate tumor growth (22). FGF and FGF receptor (FGFR) signaling regulates essential biological processes, including wound healing and angiogenesis (19,20). Aberrant FGFR signaling brought about by genetic alterations in FGF/FGFR has been implicated in the development and/or progression of cancer, and has thus been considered as therapeutic targets in various solid tumors (19,20). The co-amplification of *FGF3/FGF19/EMSY* at chromosome 11q13.3–13.5 locus was detected in a patient with *EGFR*-mutant lung adenocarcinoma at Osimertinib progression (16). Thus, the evidence suggests that the amplification of chromosome 11q13 plays a role in cancer progression. The exact Osimertinib resistance mechanism could not be pinpointed in our patient; however, the increased genetic heterogeneity could synergistically upregulate EGFR-independent pathways that drive Osimertinib resistance and promote disease progression. We speculate that the selective pressure brought upon by Osimertinib therapy promoted the growth of other clones harboring the various genetic alterations that we identified from the samples obtained at Osimertinib progression.

To the best of our knowledge, this is the first report of the emergence of Osimertinib resistance in a patient harboring germline heterozygous *EGFR* T790M. This study also highlighted the importance of conducting targeted next-generation sequencing-based molecular testing during both diagnostic and disease progression assessments to gain an understanding of the genetic landscape for therapeutic decisions and treatment monitoring.

Acknowledgments

The authors would like to thank the patient and her family for their participation in this study. We would also like to thank the investigators, study coordinators, operation staff, and the whole project team who worked on this case. We are also grateful to Qiaolin Kang and Yan Zheng of Burning Rock Biotech for their assistance in data collection.
Funding: None.

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-7626

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-7626). AL is an employee of Burning Rock Biotech. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was provided by the patient. The patient has given her consent for the publication of her case.

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(English Language Editor: L. Huleatt)

Cite this article as: Liu B, Qin J, Yin Y, Zhai L, Liu G, Lizaso A, Shi D. The emergence of various genetic alterations mediated the Osimertinib resistance of a patient harboring heterozygous germline EGFR T790M: a case report. Ann Transl Med 2021;9(1):80. doi: 10.21037/atm-20-7626