BRIEF REPORT

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EGFR Thr790Leu as a Potential Resistance Mechanism to First-Generation EGFR Tyrosine Kinase Inhibitor May Respond to Osimertinib in Patients With Lung Adenocarcinoma

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ABSTRACT

Introduction: It has been well established that *EGFR* Thr790Met is one of the major resistance mechanisms to first- and second-generation EGFR tyrosine kinase inhibitors (TKIs). Nevertheless, whether *EGFR* Thr790Leu (T790L), which shares the mutation site of Thr790 with *EGFR* Thr790Met, mediates resistance to EGFR TKIs remains elusive. The treatment options for patients harboring this rare mutation have not been reported.

Methods: Capture-based targeted ultradeep sequencing was performed on tumor and plasma samples collected at various treatment milestones from three patients with advanced lung adenocarcinoma undergoing targeted therapy.

Results: Needle biopsy of lymph node metastasis from patient 1 revealed *EGFR* T790L at disease progression on first-line treatment of gefitinib. Patient 2 had *EGFR* T790L identified from needle biopsy of lung tissue at disease progression on icotinib treatment. This patient was subsequently treated with osimertinib and achieved stable disease with a progression-free survival of 9 months. For patient 3, at disease recurrence after surgery, resected lung tumor tissue was retrieved for molecular profiling and revealed *EGFR* exon 19 deletion and *EGFR* T790L. The patient subsequently received osimertinib treatment and continued to benefit for 16 months and counting. She has maintained stable disease at the time of submission of this manuscript.

Conclusions: We revealed for the first time that *EGFR* T790L may serve as a potential resistance mechanism to first-generation EGFR TKIs. We also report the first clinical evidence of efficacy generated by osimertinib in patients with lung adenocarcinoma harboring primary or acquired *EGFR* T790L, shedding light on treatment options for this subset of patients.

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Introduction

Therapies targeting *EGFR*, such as EGFR tyrosine kinase inhibitors (TKIs), are the first-line treatments for patients with advanced or metastatic NSCLC carrying activating *EGFR* mutations. Despite the initial response with an average progression-free survival (PFS) of less than a year, drug resistance inevitably develops in almost all patients.[1] The most common resistance mechanism is *EGFR* Thr790Met (T790M), accounting for 50% of resistance cases.[2,3] As a "gatekeeper," the residue T790 of EGFR locates at the entrance of the adenosine triphosphate (ATP)-binding pocket. Because of the bulky methionine sidechain, T790M leads to a change of conformation and development of steric hindrance, thereby affecting EGFR TKI binding to the ATP-kinase

pocket.[4] The acquired resistance of *EGFR* T790M has been extensively studied; however, whether *EGFR* Thr790Leu (T790L), located at the same amino acid as T790M, also plays a similar role in mediating resistance to EGFR TKI remains elusive. The treatment options for patients harboring this rare mutation have not been reported. In this study, we report three patients with NSCLC carrying acquired or primary *EGFR* T790L, of whom two were treated and benefited from osimertinib treatment.
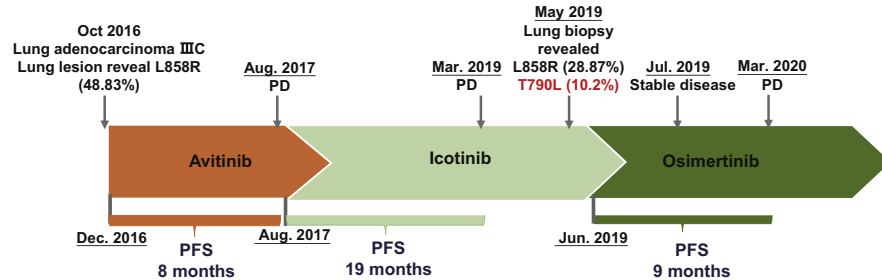
Materials and Methods

Sample Preparation

For patient 1, samples of metastasis in the right supraclavicular lymph node were obtained by means of needle biopsy during initial diagnostic procedure and at progressive disease on gefitinib treatment. Lung tumor tissues of patient 2 and patient 3 were obtained by
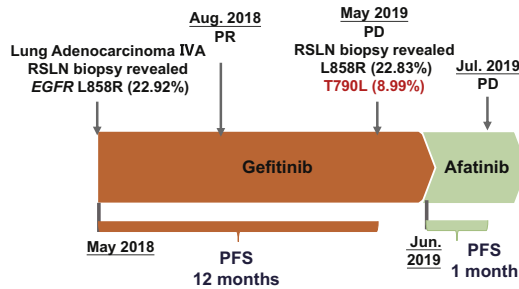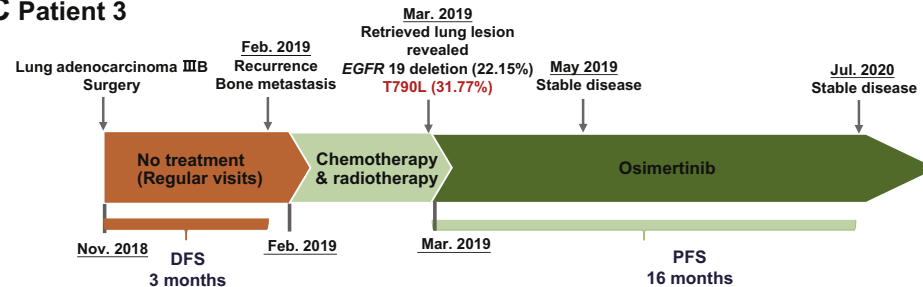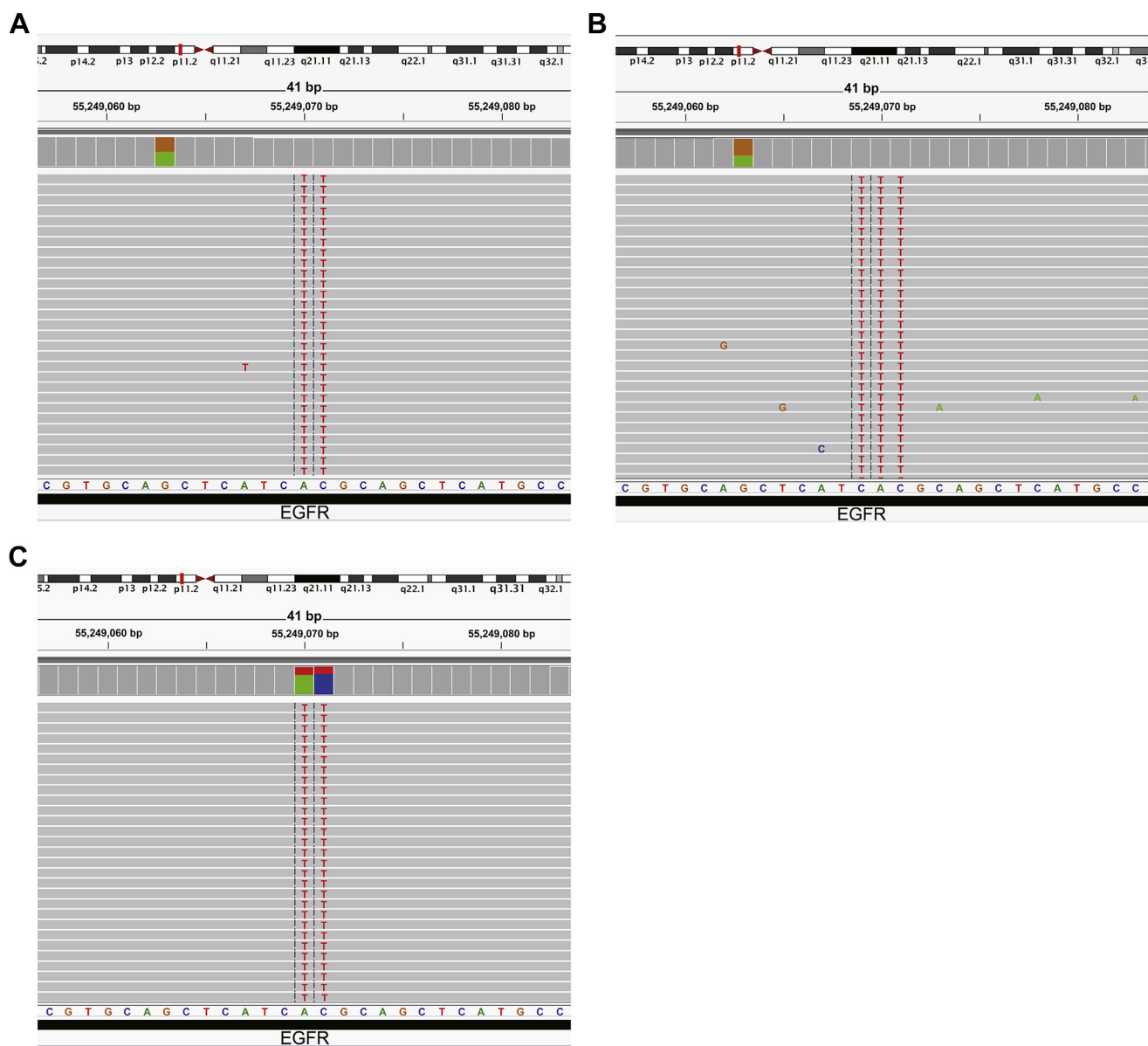

Figure 1. Treatment history of (*A*) patient 1, (*B*) patient 2, and (*C*) patient 3. Aug., August; Dec., December; SD, stable disease; DFS, disease-free survival; Feb., February; Jul., July; Jun., June; Mar., March; Nov., November; Oct, October; PD, progressive disease; PFS, progression-free survival; PR, partial response; RSLN, right supraclavicular lymph node; T790L, Thr790Leu.

Figure 2. Allelic context of *EGFR* T790L of three patients. (*A*) At disease progression on gefitinib, biopsy of metastasis in the right supraclavicular lymph node from patient 1 revealed *EGFR* T790L (c.2368_2369delinsTT, AF = 8.99%). (*B*) At disease progression on icotinib, lung biopsy from patient 2 revealed *EGFR* T790L (c.2367_2369delinsTTT, AF = 10.2%). (*C*) The lung tumor lesion from patient 3 revealed *EGFR* T790L (c.2368_2369delinsTT, AF = 31.77%). AF, allelic frequency; bp, base pair; T790L, Thr790Leu.
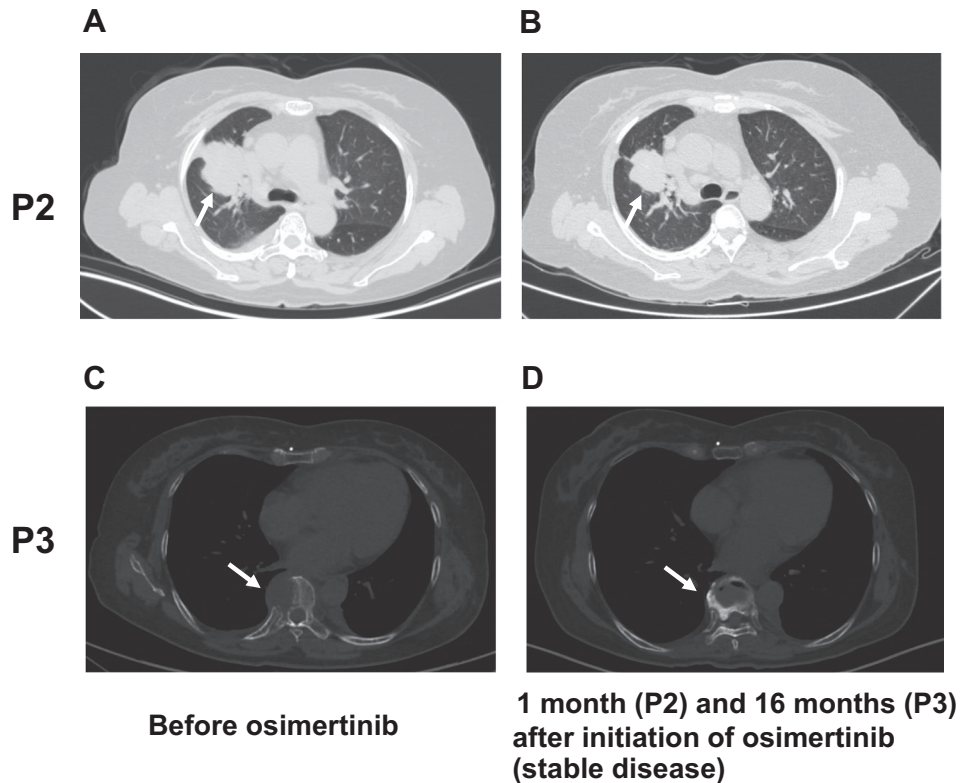
means of needle biopsy and pulmonary lobectomy, respectively. Informed consent was obtained from each patient, and the present study was approved by the Ethics Committee of Shanghai Chest Hospital (IS2138). Tumor tissue DNA and cell-free DNA from plasma were extracted as previously described.[5]

Targeted Somatic Sequencing

Capture-based ultradeep targeted sequencing was performed for somatic mutation profiling using a panel consisting of 68 cancer-related genes as previously described.[5]

Case Presentation

Patient 1. A 71-year-old woman was diagnosed with stage IV lung adenocarcinoma in May 2018. Molecular profiling performed on needle biopsy of the right supraclavicular lymph node metastasis identified *EGFR* mutation L858R with an allelic frequency (AF) of 22.92%. The patient was treated with gefitinib, a first-generation EGFR TKI. Computed tomography (CT) scan revealed partial response accompanied with reduced pleural effusion. In May 2019, her disease progressed and the rebiopsy of metastasis in the right supra-clavicular lymph node identified an acquired *EGFR*

A

B

P2

C

D

P3

Before osimertinib

1 month (P2) and 16 months (P3) after initiation of osimertinib (stable disease)

Figure 3. CT scan results of the lung (P2) (*A*) before and (*B*) after 1 month of osimertinib treatment. CT scan results of the thoracic vertebra (P3) (*C*) before and (*D*) after 16 months of osimertinib treatment. CT, computed tomography; P2, patient 2; P3, patient 3.

T790L (c.2368_2369delinsTT, AF = 8.99%) and a pre-existing *EGFR* L858R (AF = 22.83%) (Figs. 1*A* and 2*A*). Because of economic reasons, she could not afford off-label use of osimertinib. She was subsequently treated with afatinib 30 mg once daily. Nevertheless, no clinical benefit was observed with disease progression recorded in July 2019.

Patient 2. A 55-year-old woman, nonsmoker, was diagnosed with stage IIIC lung adenocarcinoma. *EGFR* L858R (AF = 48.83%) was detected from a biopsy of the pulmonary right upper lobe tumor in October 2016. She was enrolled in a phase 1 avitinib, a pyrrolopyrimidine-based irreversible EGFR TKI clinical trial (AC201401AVTN01) in December 2016. The patient achieved stable disease in March 2017, and her disease progressed in August 2017 with a PFS of 8 months. The patient was switched to icotinib 125 mg three times daily, achieving a PFS of 19 months. At disease progression, capture-based targeted sequencing was performed on both plasma and repeat biopsy of the pulmonary right upper lobe tumor. Biopsy sample revealed an acquired *EGFR* T790L (c.2367_2369delinsTTT, AF = 10.2%) along with a pre-existing L858R (AF = 28.87%) (Fig. 2*B*), but plasma sample only revealed *EGFR* L858R. Subsequently, she was treated with osimertinib 80 mg once daily and achieved stable disease after 1 month of treatment (primary lesion shrank from 5.1 × 4.2 cm to 3.9 × 3.1 cm in diameter by CT scan) with a PFS of 9 months. The treatment history of this patient is summarized in Figure 1*B*. CT scan results before osimertinib treatment and 1 month after the treatment are found in Figure 3*A* and *B*.

Patient 3. A 58-year-old woman diagnosed with stage IIIB (T3N2M0) lung adenocarcinoma underwent left lung inferior lobectomy and systemic lymph node dissection in November 2018. In February 2019, the patient experienced recurrence accompanied with pain on the right side of chest and back, bone metastasis in thoracic vertebra, and multiple nodules in the right lung. She received cisplatin and pemetrexed chemotherapy and local thoracic vertebra radiotherapy (30 Gy/10 fractions) in February 2019 and March 2019, respectively. In addition, targeted sequencing was performed on tumor tissue collected during the lung lobectomy in November 2018 and plasma samples obtained in March
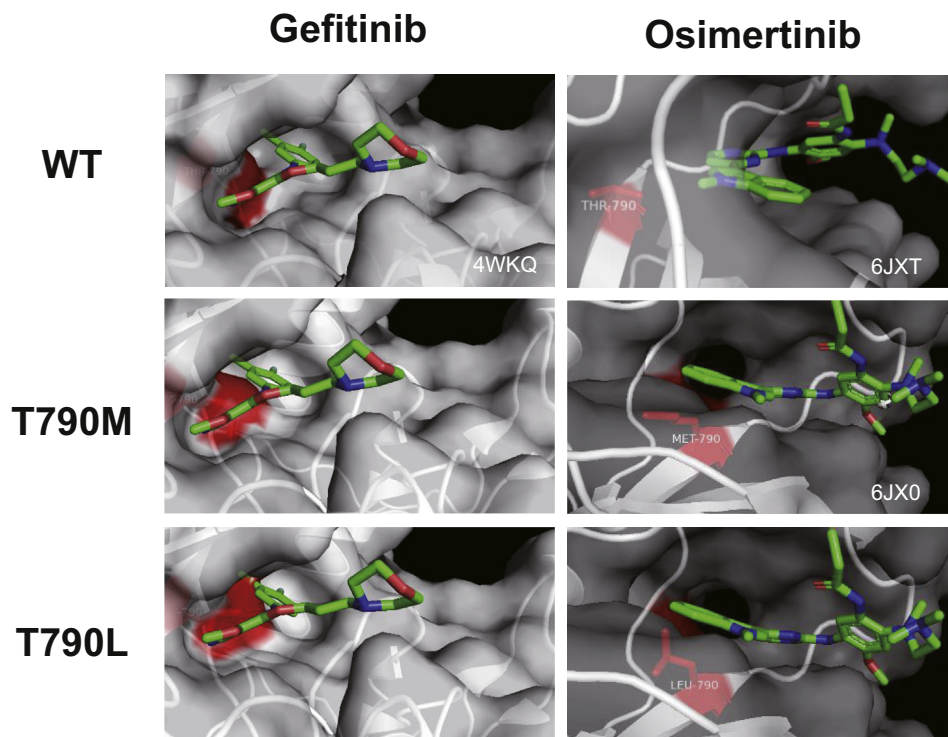
Figure 4. Protein structure prediction of *EGFR* T790M or T790L in complex with EGFR TKIs. In silico structure modeling predicts that the *EGFR* mutation T790L and T790M may lead to spatial conflictcion (hot red) at a similar level for TKI bindings. Coordinates of WT *EGFR* and gefitinib complex (PDB id: 4WKQ) (top left), WT *EGFR* and osimertinib complex (PDB id: 6JXT) (top right), and *EGFR* T790M and osimertinib complex (PDB id: 6JX0) (middle right) were obtained from the PDB and modeled by PyMOL software. Structures of *EGFR* T790M and gefitinib complex (middle left) and *EGFR* T790L and gefitinib complex (bottom left) were predicted by the PyMOL software on the basis of coordinates of wild-type *EGFR* and gefitinib complex (PDB id: 4WKQ). Structures of *EGFR* T790L and osimertinib complex (bottom right) were predicted by the PyMOL software on the basis of coordinates of *EGFR* T790M and osimertinib complex (PDB id: 6JX0). PDB, Protein Data Bank; T790L, Thr790Leu; T790M, Thr790Met; TKI, tyrosine kinase inhibitor; WT, wild type.

2019. Lung tumor tissue revealed *EGFR* exon 19 deletion (AF = 22.15%) and *EGFR* T790L (c.2368_2369delinsTT, AF = 31.77%) (Fig. 2*C*), both of which were not detected from the plasma sample. Accordingly, the patient was administered with osimertinib 80 mg once daily. She achieved stable disease and has remained on the treatment for 16 months and counting. The treatment history of the patient is summarized in Figure 1*C*. CT scan results before osimertinib treatment and 16 months after the treatment are found in Figure 3*C* and *D*.

Structural Prediction of EGFR T790M or T790L in Complex With EGFR TKI

To confirm that *EGFR* T790L mediates resistance to first-generation EGFR TKI and T790L interacts with osimertinib, we performed in silico structural modeling to investigate the conformational changes induced by *EGFR* T790M and T790L. Gefitinib binds to wild-type EGFR properly. Both T790L and T790M induce distortion with increased spatial conflictcion (hot red), thus interrupting the binding of gefitinib and subsequently

leading to drug resistance. The binding affinity between *EGFR* T790L and osimertinib is similar to that of T790M, suggesting comparable efficacy of osimertinib to T790L (Fig. 4).

Discussion

This study revealed that *EGFR* T790L is a potential mechanism of resistance to first-generation EGFR TKI. We also proposed a potential treatment option—osimertinib—for patients with advanced NSCLC harboring *EGFR* T790L. In case 1, the patient acquired *EGFR* T790L with pre-existing L858R at disease progression on first-line treatment of gefitinib. In case 2, the patient acquired *EGFR* T790L along with pre-existing *EGFR* L858R after a durable response (stable disease) of 19 months PFS achieved by second-line treatment of icotinib, further implying *EGFR* T790L may be the underlying resistance mechanism to first-generation EGFR TKI. Nevertheless, we cannot conclude whether avitinib or icotinib induced *EGFR* T790L in this case. In cases 2 and 3, capture-based targeted sequencing was performed on both tumor tissue sample and plasma sample at disease progression or

recurrence. Only tissue sample revealed *EGFR* T790L in both cases. The discordance observed between paired tissue and plasma sample can be partly attributed to the limited amount of circulating-tumor DNA present in patients, resulting in a considerable percentage of patients with no mutation detected from their blood samples.

EGFR T790L locates at the same amino acid position with T790M, which induces steric hindrance, thus affecting EGFR TKI binding to ATP-kinase pocket.[4] It is the most common acquired resistance to first- or second-generation EGFR TKIs. *In silico* analysis revealed that both *EGFR* T790L and T790M induce distortion with increased spatial conflliction (hot red in Fig. 4), thus interrupting the binding of gefitinib and subsequently leading to drug resistance. Furthermore, the binding affinity between *EGFR* T790L and osimertinib is similar to that of T790M, suggesting comparable efficacy of osimertinib to T790L. Of note, in this study, two patients carrying primary or acquired *EGFR* T790L with *EGFR* L858R or exon 19 deletion received osimertinib treatment and achieved stable disease with a PFS of 9 months and 16 months and counting, respectively. This study provides first clinical evidence that osimertinib exerts encouraging durable efficacy in a patient with advanced NSCLC harboring *EGFR* T790L.

The T790 gatekeeper mutations have recently been explored through computer simulation.[6] This study found five possible drug-resistant mutations at residue T790 in *EGFR* that are well tolerated structurally and energetically, but T790L was not included. The reason is that this study assumed that only single-nucleotide changes are allowed. Nevertheless, in the three cases of the current report T790L are two or three nucleotide changes, including c.2368_2369delinsTT and c.2367_2369delinsTTT (Fig. 2), which are beyond the scope of the hypothesis of this study. To date, we have not observed any resistance causing mutations at residue T790 other than T790M and T790L in the clinic. On the basis of our database, we have more than six hundred patients with first-generation EGFR TKI-relapsed NSCLC whose tumor samples were sequenced by NGS so far. In addition, T790L mutation has never been reported in previous literatures. Therefore, the frequency of acquired *EGFR* T790L in patients with EGFR TKI

relapse may be lower than 0.3% by our estimate. Nevertheless, much larger scale assessment for frequency of this mutation will need to be conducted and externally validated and allied *in vitro* to draw significant conclusions in the future.

To the best of our knowledge, this is the first report that *EGFR* T790L was identified in patients with NSCLC and that it can serve as an acquired resistance mechanism after the exposure to first-generation EGFR TKIs. We also reported the first clinical evidence that lung adenocarcinoma harboring primary or acquired *EGFR* T790L responded to osimertinib, shedding light on the treatment options for patients with advanced NSCLC harboring this rare mutation.

Acknowledgments

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