Case Report

Detection of an EML4-ALK fusion mutation secondary to epidermal growth factor receptor-tyrosine kinase inhibitor (*EGFR*-TKI) therapy for lung cancer: a case report

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Background: For epidermal growth factor receptor-mutant (*EGFR*-mutant) advanced non-small cell lung cancer (NSCLC) patients, *EGFR*-tyrosine inhibitors such as gefitinib, erlotinib, and osimertinib, are recommended as the preferred first-line treatment. Unfortunately, relevant drug resistance is often inevitable and for first and second generation *EGFR*-tyrosine kinase inhibitors (TKIs), drug resistance most commonly (50–60% of cases) occurs at the secondary point mutation T790M. Second-line treatments may include administering the third generation of *EGFR*-TKIs, such as osimertinib and almonertinib. In a few relevant studies, rearrangement of the anaplastic lymphoma kinase (*ALK*) gene was detected in patients with T790M mutation after drug resistance to osimertinib re-occurred following administration as a second-line treatment. The studies concluded that *ALK* rearrangement is a rare but critical drug resistance mechanism for osimertinib. However, to date, it remains unclear whether almonertinib also triggers the same *ALK* rearrangement. The current case study is the first one detailing the detection of an *ALK* rearrangement after almonertinib resistance in advanced *EGFR*-mutant NSCLC, which contributes to the limited body of literature examining *ALK* rearrangement as a mechanism of resistance to *EGFR*-TKIs in advanced *EGFR*-mutant NSCLC.

Case Description: Herein, we present a 35-year-old female patient with *EGFR*-mutant advanced NSCLC in the last trimester of pregnancy. The patient was administered multiple treatments, including first-line icotinib and second-line almonertinib. According to the next-generation sequencing (NGS) assay after almonertinib resistance, the development of an *EML4-ALK* fusion mutation was considered to be a potential mechanism of almonertinib resistance. Subsequently, the patient received a combination of almonertinib and crizotinib, and at the last follow-up, the treatment showed a curative effect and then maintained a one-month stable disease.

Conclusions: This case report suggests that *ALK* rearrangement may be a potential mechanism of almonertinib resistance. The combination of *ALK* TKI therapy and *EGFR* TKI may be a viable strategy for almonertinib-resistant NSCLC patients induced by *ALK* rearrangement.

Keywords: Epidermal growth factor receptor (*EGFR*); anaplastic lymphoma kinase (*ALK*); almonertinib; acquired drug resistance; case report

Submitted Jun 01, 2022. Accepted for publication Jul 07, 2022.
doi: 10.21037/apm-22-744
View this article at: https://dx.doi.org/10.21037/apm-22-744

Introduction

Lung cancer has the highest morbidity and mortality of all malignancies worldwide, and approximately 85% of lung cancer patients have non-small cell lung cancer (NSCLC). Furthermore, more than half of the patients are in an advanced stage at diagnosis (1). Identifying the relevant driver genes and selecting the appropriate targeted drugs is vital to improving the prognosis of patients with

advanced NSCLC. Epidermal growth factor receptor (*EGFR*) mutations occur in around 50% of relevant cases and is one of the most common driver genes in lung cancer. The most common gene mutations include *EGFR* 19del and *EGFR* 21L858R. In patients with advanced NSCLC and sensitive mutations, administration of *EGFR*-tyrosine kinase inhibitors (TKIs) has shown significantly better therapeutic effect compared to conventional chemotherapy. Globally, treatment guidelines recommend *EGFR*-TKIs as the best option for first-line treatment. However, most patients develop drug resistance at around 9–14 months after administration of first-line treatment with first- or second-generation *EGFR*-TKIs. The most common mechanisms for acquired drug resistance include secondary point mutations (e.g., T790M mutation), bypass activation (e.g., *c-MET* amplification), human epidermal growth factor receptor 2 (*HER2*) mutation, and histological type transformation (2,3). The T790M mutation is the most common mechanism for acquired drug resistance (4), occurring in around 60% of cases. The third generation of *EGFR*-TKIs, such as osimertinib, can overcome the acquired drug resistance caused by the T790M mutation and can achieve a better curative effect on the disease. However, inevitably, drug resistance will re-occur (5). For the third generation of TKIs, drug resistance mechanisms include secondary *EGFR* point mutation, T790M loss, *EGFR* amplification, abnormal bypass activation, and histological phenotype transformation. Additionally, in some cases, oncogene fusion may also be a potential mechanism for drug resistance. In the AURA3 study (6), amongst the osimertinib resistant cases, one patient presented with a *RET-ERC1* gene fusion and another had a *NTRK1-TPM3* gene fusion. The FLAURA study (7) identified one case of *ALK* gene fusion. Almonertinib, a third generation *EGFR*-TKI that was independently developed in China, has been shown in the APOLLO study (8) to be safe and effective for use in T790M positive patients with locally advanced or metastatic NSCLC. However, there are few studies examining the mechanisms of resistance developed after almonertinib administration. This current report details a young woman diagnosed with advanced lung adenocarcinoma during pregnancy who had undergone biopsies following treatment with icotinib and almonertinib. An *ALK* gene rearrangement was identified using dynamic detection, and this is the first case report detailing the detection of an *ALK* rearrangement serves as a rare molecular mechanism to almonertinib resistance, which contributes to the limited body of literature examining *ALK* rearrangement as a mechanism of resistance to *EGFR*-TKIs in advanced *EGFR*-mutant NSCLC. We present the following article in accordance with the CARE reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-744/rc).

Case presentation

The patient is a 35-year-old Han Chinese female, who was 8 months pregnant. She had no history of smoking and had previously been healthy. She underwent a cesarean section on May 13, 2020 and was admitted to our hospital 2 days after the procedure due to an intermittent cough she had experienced for more than half a year. Physical examination showed multiple enlarged lymph nodes palpable on both sides of her neck, which were tough and mildly tender. The patient presented with a relatively large node on the right side of her neck, approximately 2 cm in diameter. She had low breathing sounds in her lower lungs but no wet or dry rale, and no pleural friction sounds were heard. On auxiliary examination there was a tumor marker carcinoembryonic antigen 81.95 ng/mL, soluble cytokeratin 298.60 ng/mL, and neuron-specific enolase 42.43 ng/mL. Others were within the corresponding normal ranges. A contrast-enhanced computed tomography (CT) scan of the chest and abdomen showed changes after the cesarean section. A mass was observed in the inferior lobe of the left lung, with a lobulation approximately 6.5 cm long present at the margin. There were multiple enlarged lymph nodes throughout the body, multiple low-density shadows about 3 cm long in the liver, multiple areas with uneven bone density, and bilateral pleural effusion accompanied with atelectasis of the adjacent lung tissue. There was a small amount of pericardial effusion, and also abdominal effusion. A magnetic resonance imaging (MRI) scan of the head showed no obvious abnormalities. Bone imaging scans found multiple abnormal punctate groupings all over the body, which were determined as multiple bone metastases. Color-guided ultrasound was used to perform a right supraclavicular lymph biopsy and pathological findings revealed adenocarcinoma, exfoliative cell examination from the bilateral pleural fluid identified cancer cells, suggestive of metastatic lung adenocarcinoma. A peritoneal wash was sent for testing during the caesarean delivery, and the results suggested that malignancy could not be excluded. In addition, heterogeneous cells were observed. Genetic testing of the lymph node tissue (by next-generation sequencing) showed an in-frame deletion mutation in *EGFR*

exon 19, with an abundance of 79.00%. The *EGFR* copy number was amplified (copy number =8.76), while the other gene mutations such as *ALK*, *ERBB2*, *BRAF*, *MET*, *RET*, *ROS1*, *KRAS*, and *PIK3CA* were not detected. The primary diagnosis was determined to be left lung adenocarcinoma of State IVB (T3N3M1c), with liver, bilateral pleural, and bone metastasis, in addition to a *EGFR* exon 19 deletion.

From May 20, 2020, the patient was treated with 0.125 g icotinib hydrochloride, 3 times daily. After 1 month of treatment, partial response (PR) was detected. The chest CT revealed that the mass in the lower lobe of the left lung was reduced by 43%, from 6.5 to 3.7 cm in long diameter. All target lesions shrunk by about 50%. There were no obvious adverse side effects. Subsequently, the patient was regularly re-examined and continuously evaluated as a PR case. In November 2020, the patient presented with enlarged cervical lymph nodes on both sides of the cervical lymph node. A color Doppler ultrasonography showed that the size of the lumps was approximately 2.0 cm × 0.9 cm. Following a re-examination by chest and abdomen enhanced CT, newly enlarged lymph nodes under the axilla were discovered. She was considered to have progressive disease (PD), the progression-free survival (PFS) of using icotinib as first-line treatment was 5 months. An *EGFR* T790M genetic test was performed on the patient's blood and the results indicated positive T790M mutation (abundance: 3.36%). As second-line treatment, 110 mg almonertinib was administered once daily. Initial outcome evaluation suggested PR. In April 2021, enlarged cervical lymph nodes were detected. Following a chest and abdomen CT scan, a low-density shadow was observed in the spleen with a high possibility of metastasis. In addition, there were increased and enlarged lymph nodes throughout the body, as well as increased and enlarged liver metastases. A head MRI scan found no signs of brain metastasis. The outcome was evaluated as PD [lung lesion: stable disease (SD); metastasis: PD]. As second-line treatment, almonertinib PFS was 5 months. Genetic testing in another biopsy of the left supraclavicular lymph nodes showed p.E746-A750del in-frame deletion mutation in the *EGFR* exon 19, with an abundance of 43.20%. There was amplification of the *EGFR* copy number (copy number =2.4). A gene rearrangement involving *ALK*, namely echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* (E2:A20), with an abundance of 7.36%, was detected. Almonertinib combined with crizotinib was administered as the third-line treatment. At the last follow-up on May 26, 2021, the patient was considered to be in partial remission (PR). The detailed treatment regimen and adverse events of this patient are described in *Figure 1*. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

The development of targeted therapy has created a breakthrough in the treatment of NSCLC, which has not only significantly prolonged patient survival, but also improved their quality of life. Currently, for mutation positive patients with NSCLC, targeted therapy is the regimen for first-line treatment.

EGFR-TKIs are the standard regimen for first-line treatment in patients with NSCLC of *EGFR* sensitive mutations. Osimertinib has been approved for second-line treatment in patients with advanced NSCLC who show positive T790M after *EGFR*-TKI treatment, and for first-line treatment for *EGFR*-sensitive mutation. Some studies have shown that osimertinib-resistant patients who show positive T790M after undergoing posterior line treatment, the initial L858R mutation is more prone to C797S mutation. Meanwhile, for the initial 19del, T790M loss and activation of the *EGFR*-independent resistance pathway are more likely to occur, e.g., the most common *MET* amplification (9). At present, there are few reports on *ALK* gene rearrangement as the mechanism of osimertinib resistance, which accounts for only 0.13% of the total cases which has been reported (10). Hou *et al.* performed a retrospective analysis on 7 cases of secondary *ALK* rearrangement after developing osimertinib resistance (11). The results showed that dual-targeted therapy was beneficial after osimertinib resistance mediated by *ALK* gene rearrangement had occurred, and suggested that *ALK* gene rearrangement might be the underlying cause of osimertinib resistance. Many cases of *ALK* gene rearrangement occurring after osimertinib resistance have been reported (12), and for these cases, positive outcomes were achieved after patients were subsequently treated with *ALK*-TKIs.

Almonertinib (13,14) is a third generation *EGFR*-TKI approved for global use. Its design is based on a modification of the structure of osimertinib, whereby the

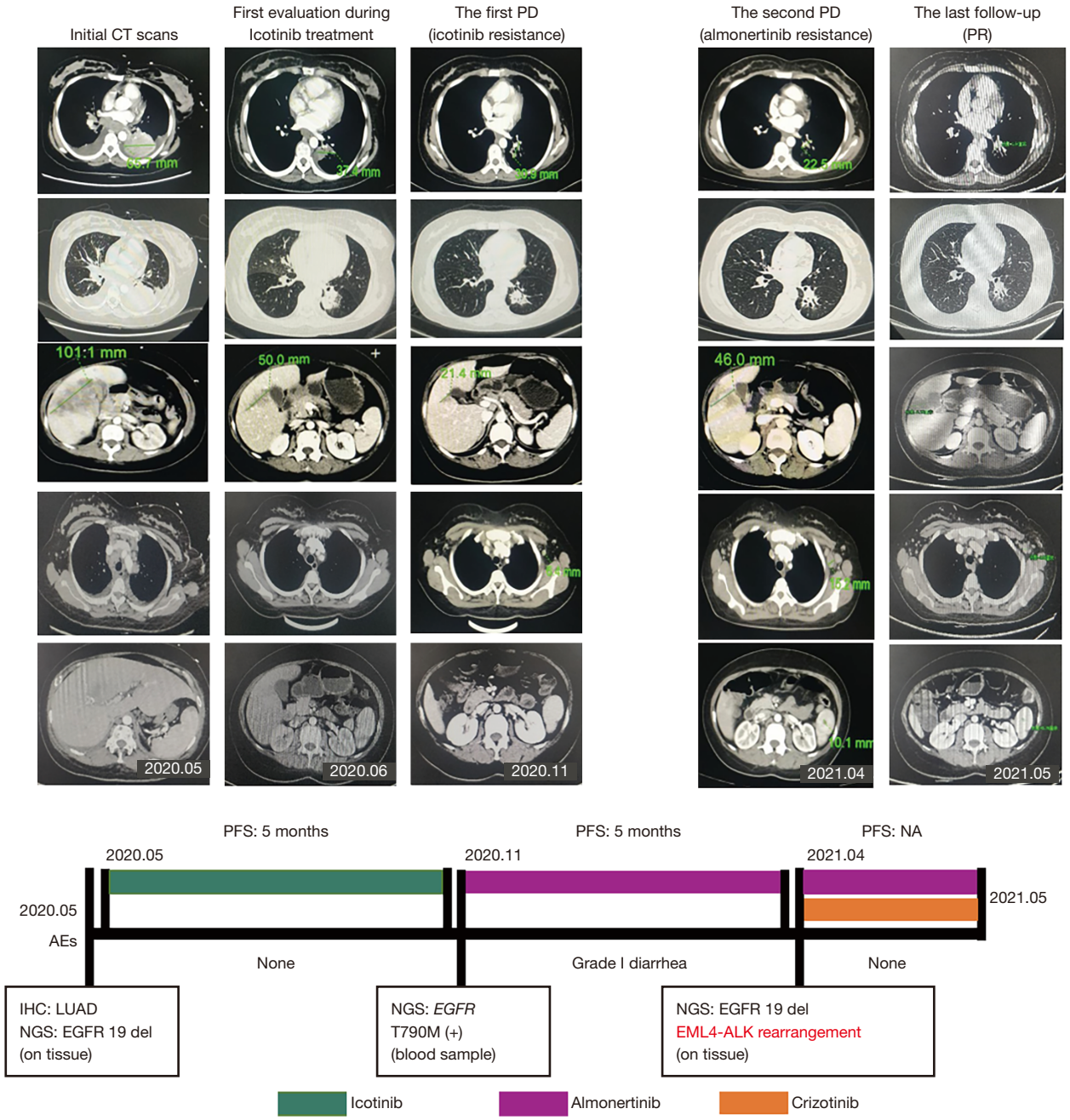

Figure 1 Treatment history of the patient in this case study. PD, progressive disease; PR, partial remission; CT, computed tomography; PFS, progression-free survival; NA, not applicable; AE, adverse event; IHC, immunohistochemistry; LUAD, lung adenocarcinoma; NGS, next generation sequencing; *EGFR*, epidermal growth factor receptor.

methyl group at the indole ring is replaced by a cyclopropyl group, thus enhancing the selectivity and inhibitory effects on the T790M mutation, and increasing the permeability of the blood-brain barrier. The APOLLO phase II clinical study (8) included a total of 244 patients with advanced NSCLC from China, who were *EGFR* T790M positive after undergoing first-line treatment with *EGFR*-TKIs. The results showed positive outcomes and demonstrated

the safety of second-line treatment with almonertinib for *EGFR* T790M positive patients. Second-line treatment with almonertinib resulted in significantly reduced lesions and the progression-free survival was 5 months. Further genetic testing found loss of T790M mutation and EML4-*ALK* fusion mutation. At present, there are no reports investigation the mechanisms of almonertinib-related drug resistance. This current case report suggests that *ALK* rearrangement may be a potential cause of almonertinib-related drug resistance.

It should be noted that this patient was pregnant when diagnosed with advanced lung cancer. Yang *et al.* (15) examined 64 cases of pregnancy complicated with lung cancer and found that 54 patients (84.38%) were confirmed in the middle or late trimester, suggesting that most patients are often misdiagnosed in early pregnancy, and both the gestational age and tumor stage are often advanced at diagnosis, resulting in poor prognosis. A study conducted by Niikawa *et al.* (16) involving 59 cases with NSCLC, demonstrated that 43 patients had significantly higher estradiol levels in lung cancer tissues compared to normal lung tissues. In addition, for estrogen receptor positive cases, the estradiol level was positively correlated with tumor size and Ki-67 expression level in the tumor tissue.

During pregnancy, the maternal hormone levels may be elevated and immune function compromised, thus accelerating tumor growth. Estrogen receptors can be divided into two subtypes: estrogen receptor α (ERα) and estrogen receptor β (ERβ). ERβ is highly expressed in lung cells and bronchial epithelial cells. Nose *et al.* (17) evaluated 447 specimens after lung adenocarcinoma surgery and demonstrated that *EGFR* mutations were associated with a high expression of ERβ in the nucleus. Among the patients studied, 67% were positive for *EGFR* gene mutation. However, the relationship between estrogen and lung cancer must be explored further in future studies.

The patient in this current case report was diagnosed with lung cancer in her last trimester of pregnancy. Genetic testing indicated an *EGFR* 19del, and the association of estrogen levels in the body with the lung cancer gene mutation could not be excluded. After multi-line targeted therapies, the patient's overall survival reached up to 13 months. Therefore, for pregnant patients with lung cancer, early detection and timely treatment may prolong their survival. In addition, during diagnosis and treatment, appropriate treatment regimens should be selected according to the patient's individual conditions and gestational age.

There were some limitations to this case report. Firstly, the estrogen and progesterone levels were not monitored during pregnancy, so the relationship between estrogen levels and gene mutation cannot be concluded, more research on the association are needed. Secondly, at the time of the patient's first progression, we only sent the peripheral blood for *EGFR* T790M gene testing instead of comprehensive genetic testing, the plasma assay of *EGFR* T790M single-point may insufficient to identify the resistance of EGFR-TKIs, since concurrent driver gene resistance impairs icotinib's efficacy. And also whether there was an ALK fusion gene rearrangement or not at the first progression remains uncertain. Thirdly, the re-biopsy site only represents a partial pathology of the resistance, and the mechanism of resistance may differ from one site to another.re-biopsy of only one site might not always be appropriate. In this case report, we only sent cervical lymph node tissue samples during the initial diagnosis and subsequent progress, but did not submit lung primary lesion tissue samples. It is not possible to determine whether there is heterogeneity between the lung primary lesion and lymph node samples. The use of NGS to detect ctDNA in blood samples for supplementary biopsy may enhance the credibility and better guide treatment. This highlights the need for comprehensive next generation sequencing (NGS) assays.

Conclusions

This is the first case report detailing the detection of an *ALK* rearrangement after almonertinib resistance in advanced *EGFR*-mutant NSCLC, and the combination of *ALK* TKI therapy and almonertinib may be a viable strategy in this setting. This case suggested that *ALK* rearrangement may be an underlying mechanism of resistance to almonertinib.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-744/rc

Conflicts of Interest: All authors have completed the

ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-744/coif). The 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 this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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(English Language Editor: J. Teoh)

Cite this article as: Ren KH, Qin WW, Wang Y, Peng JC, Hu WX. Detection of an EML4-ALK fusion mutation secondary to epidermal growth factor receptor-tyrosine kinase inhibitor (*EGFR*-TKI) therapy for lung cancer: a case report. Ann Palliat Med 2022;11(7):2503-2509. doi: 10.21037/apm-22-744

