A Case of Lung Adenocarcinoma Response to Alectinib Harboring a Rare *EML4*-*ALK* Variant, Exon 6 of *EML4* Fused to Exon 18 of *ALK*

Lirong Liu, MM^{1,2,*}; Fangfang Hou, MM^{1,2,3,*}; Yufeng Liu, MM³; Wenzhu Li, MM^{1,2}; and Haibo Zhang, MD^{1,2,4,5}

ABSTRACT

More than 20 types of *ALK* fusion variant subtypes have been identified, including different fusion partner genes or *EML4*-*ALK* fusions with different breakpoints. However, different *ALK* fusions show different sensitivities to *ALK*-tyrosine kinase inhibitors (*ALK*-TKIs) and the emergence of rare fusions brings great challenges to the target therapy in clinic. We report a rare *EML4*-*ALK* (E6;A18) fusion in a patient with lung adenocarcinoma that responded well to alectinib. This is the second case of this rare variant reported but the first report of response to an *ALK*-TKI. This evidence is the first to show that alectinib may be effective for this rare fusion type of non-small cell lung cancer, and these findings have important implications for drug selection in patients with this subtype. Further studies are needed to understand the function of this variant.

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***ALK* rearrangement** is found in approximately 5% of patients with non-small cell lung cancer (NSCLC).¹ The overall response rate and progression-free survival of patients with *ALK*-positive NSCLC are significantly improved in the development and clinical application of *ALK* inhibitors, including the first-generation (crizotinib), second-generation (eg, alectinib, ceritinib, brigatinib), and even third-generation *ALK* inhibitors (lorlatinib).^{2–6} More than 20 types of *ALK* fusion variant subtypes have been identified, including different fusion partner genes or *EML4*-*ALK* fusion with different breakpoints. However, different *ALK* fusions show different sensitivities to *ALK*-tyrosine kinase inhibitors (*ALK*-TKIs).^{7,8} There is only one publication of *EML4*-*ALK* (E6;A18) fusion, a rare fusion type, that showed resistance to crizotinib in a 48-year-old male patient with lung adenocarcinoma.⁹ Here, we report a rare *EML4*-*ALK* (E6;A18) fusion with stable protein expression in a patient with lung adenocarcinoma who responded well to alectinib.

This study was approved by the ethics committee of The Second Affiliated Hospital of Guangzhou University of Chinese Medicine. Written informed consent was obtained from the patient for the publication of case details and images.

Case Presentation

In March 2020, a 64-year-old woman who had never smoked and had no significant medical history was initially admitted to our hospital because she had been experiencing shoulder and neck pain for 2 months. Cervical MRI indicated bone destruction of the C5 vertebral body, which was considered to be a metastatic tumor. ¹⁸F-FDG PET/CT revealed left lower lobe atelectasis. There were hypermetabolic nodules in the left hilar suggestive of lung cancer with obstructive pneumonia (Figure 1A). The left hilar nodules were diagnosed as adenocarcinoma by endobronchial ultrasound transbronchial needle aspiration under bronchofiberscopy. The final diagnosis was clinical stage IV (T2bN2bM1c) lung

¹Department of Oncology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine; ²Department of Oncology, Guangdong Provincial Hospital of Traditional Chinese Medicine; ³The Second Clinical Medical College of Guangzhou University of Chinese Medicine; ⁴Guangdong-Hong Kong-Macau Joint Laboratory on Chinese Medicine and Immune Disease Research; and ⁵Guangdong Provincial Key Laboratory of Clinical Research on Traditional Chinese Medicine Syndrome, Guangzhou, Guangdong, China.

*These authors contributed equally and are co-first authors.

Figure 1. Dynamic monitoring of the patient's response to alectinib. (A1–A3) PET/CT shows hypermetabolic nodules in left hilar suggestive of lung cancer with obstructive pneumonia and bone and right adrenal metastases. (B1–B3) CT after 2 cycles of carboplatin/pemetrexed with bevacizumab shows left lower lobe atelectasis aggravated with a large amount of pleural effusion. (C1–C3) CT shows partial response after 8 weeks of alectinib. (D1–D3) CT shows continuous response when taking alectinib for 6 months.

adenocarcinoma according to the lung cancer TNM classification.¹⁰ We identified a rare fusion of *EML4* with *ALK* through next-generation sequencing (NGS; BGISEQ-500 platform [MGI Tech Co. Ltd], commercial panel, only DNA-based) technology in which exon 6 of *EML4* was fused to exon 18 of *ALK* (E6;A18) (Figure 2). Immunohistochemistry (Ventana ALK [D5F3] Assay, Roche Diagnostics) showed that ALK was positive (Figure 3B). To our knowledge, only one case of this rare variant has been reported,⁹ and that patient died 2 weeks after starting treatment with crizotinib, suggesting that patient was resistant to crizotinib and had a poor prognosis.

We first gave our patient 2 cycles of pemetrexed (500 mg/m² intravenously on day 1 every 3 weeks) and carboplatin (area under the plasma concentration–

time curve, 5 mg/mL per min intravenously on day 1 every 3 weeks) in combination with bevacizumab (7.5 mg/kg intravenously on day 1 every 3 weeks). However, chest CT showed left lower lobe atelectasis aggravated with a large amount of pleural effusion (Figure 1B). The clinical condition of the patient worsened. Her ostalgia worsened and she experienced an obvious cough and shortness of breath. Alectinib (600 mg orally twice daily) was started in May 2020 with a rapid clinical response. One week after taking alectinib, her cough and shortness of breath were significantly relieved. The ostalgia was gradually alleviated and she was able to stop the analgesics after 3 weeks. As of this writing, the patient has taken alectinib for more than 9 months, with a continuous partial response noted in September 2020 by CT (Figure 1C, D).

Figure 2. A fusion variant of *EML4* intron 6 with *ALK* intron 17 was identified by NGS analysis of genomic DNA and supposed to cause a rare *EML4*-*ALK* fusion transcript in which exon 6 of *EML4* was fused to exon 18 of *ALK* (E6:A18). **(A)** Paired-end sequencing data from tumor tissue samples indicated somatic intrachromosomal *EML4*-*ALK* fusion as demonstrated by Integrative Genomics Viewer program (Broad Institute; <https://igv.org/app>).¹⁶ **(B)** Alignment of NGS reads to supposed-reference sequence. **(C)** Schematic diagram of the predicted domains of the *EML4*-*ALK* fusion protein compared with the 2 most common *EML4*-*ALK* variants, V1 and V3. **(D)** Secondary structure prediction for the acquired region in Phyre2 software.

Abbreviations: ALK, anaplastic lymphoma kinase; CC, coiled coil domain; Fxa inhibition, coagulation Factor Xa inhibitory site; NGS, next-generation sequencing; SS, secondary structure; TMD, transmembrane domain; WD, tryptophan and aspartic acid.

Figure 3. (A) Ultrasound-guided fine-needle aspiration cytology of the left hilar nodules showed adenocarcinoma cells (hematoxylin-eosin, original magnification $\times 100$). (B) Immunohistochemistry showed that ALK(D5F3) was positive.

Discussion

This is the first report that describes the rare variant *EML4-ALK* (E6;A18) response to alectinib. The unique previous publication of a case involving an *EML4-ALK* (E6;A18) fusion revealed that a 48-year-old male patient with lung adenocarcinoma with *EML4-ALK* (E6;A18) was resistant to crizotinib. His disease progressed rapidly and he died 2 weeks after taking crizotinib.⁹ Based on this case report, we recommended chemotherapy as the first-line treatment for our patient; unfortunately, chemotherapy was ineffective.

Without more options, we focused on the rare *ALK* fusion again. In the previous *EML4-ALK* (E6;A18) case report, the investigators proposed that the transmembrane domain in exon 18—containing variants may be related to the differences in *EML4-ALK* protein stability and resistance to crizotinib.⁹ However, there was no fluorescence in situ hybridization or immunohistochemical analysis to verify the fusion gene or protein expression after the investigators revealed a novel *EML4-ALK* fusion (E6;A18) by Sanger sequencing of reverse transcriptase-PCR product. With the same breakpoint of *ALK* exon 18, different fusion partner, Chen et al¹¹ reported a noncoding partner (Chr2 30297612) with exon 18 of *ALK* in a patient with lung adenocarcinoma who had stable *ALK* fusion protein expression and experienced a response to crizotinib. Based on the stable overexpression of the *ALK* protein in the tumor cells of our patient (Figure 3), and the better efficacy of alectinib reported in the ALEX, J-ALEX, and ALESIA studies,^{12–14} we administered alectinib to our patient. The symptoms of cough, shortness of breath, and pain were relieved within 1 week, and the imaging evaluation at the eighth week showed ongoing partial response and revealed good therapeutic effect over 9 months to the cutoff date.

This is the second reported case of a patient carrying a rare variant *EML4-ALK* (E6;A18) fusion. Unlike the first patient with *EML4-ALK* (E6;A18) resistant to crizotinib, our case showed for the first time that alectinib is effective for this rare variant *EML4-ALK* (E6;A18) fusion. Compared with the canonical *EML4-ALK* fusion, this new fusion (E6;A18) acquires 85 amino acids, from positions 229 to 314. The added region contains 12 amino acids derived from introns and 73 amino acids derived from exons. Similar to the Chr2 30297612-*ALK* (Chr2:30297612; A18) fusion case, there was stable *ALK* fusion protein expression in our *EML4-ALK* (E6;A18) fusion case, which indicates that the transmembrane domain in exon 18 may not destabilize *EML4-ALK* (E6;A18) protein structure. Indeed, *EML4-ALK* (E6;A18) had complete *EML4* trimerization domain. The trimerization domain of *EML4* is essential for *ALK* autophosphorylation and activation.⁷ Therefore, we speculate that *EML4-ALK* (E6;A18) fusion with immunohistochemistry-verified protein overexpression could benefit from *ALK*-TKI therapy. A secondary structure prediction in Phyre2 software (Structural Bioinformatics Group) for the new sequence found that the acquired region 229–314 was displayed as several beta sheets followed by an alpha helix (Figure 2D).¹⁵ In addition, ligand binding site prediction for the acquired peptide region 229–314 showed no binding potential. Therefore, the newly added 85 amino acids may not have a particularly significant impact on the function of fusion protein. However, further studies are needed to understand the function of this variant. In addition to this method, an NGS platform that incorporates RNA sequencing could also be useful in querying expression of rare fusion proteins. Combination detection is strongly recommended for this rare fusion to optimize the treatment strategy.

Conclusions

This is the second case of a patient with lung adenocarcinoma harboring a rare *EML4-ALK* (E6:A18) fusion reported, but the first report of response to an ALK-TKI. Our data suggested that alectinib may be effective for this rare fusion type of NSCLC and provided evidence for drug selection in patients with this subtype for the first time.

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Correspondence: Haibo Zhang, MD, Department of Oncology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, No. 111 Dade Road, Yuexiu District, Guangzhou 510120, China.
Email: haibozh@gzucm.edu.cn

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