CASE REPORT

**Novel NLRC4-ALK and EML4-ALK double fusion mutations in a lung adenocarcinoma patient: A case report**

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**Keywords**
Crizotinib; double ALK fusions; EML4-ALK; lung adenocarcinoma; NLRC4-ALK.

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**Abstract**
Anaplastic lymphoma kinase (ALK) rearrangements have been reported in 5% to 6% of non-small cell lung cancer (NSCLC) patients. However, the concurrent existence of two ALK fusions within the same patient have rarely previously been reported. Moreover, considering the diversities of ALK mutations, it is necessary to evaluate the response of both double and new types of ALK fusions to ALK-tyrosine kinase inhibitors (ALK-TKIs). Here, we report a case of a 64-year-old Chinese woman who was diagnosed with lung adenocarcinoma (ADC) who concurrently harbored two types of ALK-rearrangements, including an unreported NLRC4-ALK fusion and EML4-ALK fusion. After surgery, the patient had a progression-free survival (PFS) of over 10 months with continuous crizotinib treatment after surgery. Our findings provide a better understanding of ALK-TKI in patients with two novel ALK concomitant fusions.

**Key points**
A lung adenocarcinoma patient harboring concurrent NLRC4-ALK and EML4-ALK fusion mutations benefited from crizotinib after surgery. Our findings provide important information for future treatment decision-making in patients with double ALK fusions.

**Introduction**
Non-small-cell lung cancer (NSCLC) has been estimated to account for 80% to 85% of the total number of lung cancers.¹ Anaplastic lymphoma kinase (ALK) gene rearrangements have been reported in 5% to 6% of NSCLC patients, especially in light or non-smokers.² So far, more than 30 types of ALK fusion partners (such as EML4, KIF5B and KLC1) have been identified in NSCLC.³ Crizotinib, a first-generation ALK-TKI, has been recommended as a first-line therapy for ALK-rearranged NSCLC, and has shown impressive single-agent activity in ALK-positive lung adenocarcinoma (ADC).⁴ Second-generation (alectinib, ceritinib, and brigatinib) and third-generation (lorlatinib) of ALK-TKIs have also been developed.⁵ In this report, we present for the first time an unreported NLRC4-ALK fusion mutation concurrently with EML4-ALK in an ADC patient.

**Case report**
In January 2019, a 64-year-old Chinese woman, who was a non-smoker, was referred to our hospital because of patchy shadows in the left upper lung on chest X-ray. Chest CT scan revealed a spiculated mass (2.8 cm × 2.1 cm) in the left upper lobe (Fig 1). She had no clinical symptoms of fever, cough, hemoptysis or dyspnea. Detection of serum tumor markers showed an increased level of cytokeratin 19 fragment (6.10 ng/mL; normal value, 0.00–3.00 ng/mL).
The patient was assessed as being acceptable for surgery after head CT and bone single-photon emission computed tomography (SPECT). On 21 February 2019, a pulmonary nodule (3.5 cm × 1.9 cm × 1.5 cm) and one of the pleural dissemination nodules (1 cm × 0.5 cm × 0.5 cm) were surgically removed. However, pleural effusion, pleural retraction and multiple implanted nodules were found during the operation. Postoperative pathology confirmed a stage IVa (pT2aN0M1a) ADC (Fig 2a,b).

To explore potential targeted therapies, next-generation sequencing (NGS) was performed on postoperative pulmonary nodule specimen using a 56 cancer-related gene panel. The coexistence of double ALK rearrangements were revealed, including an unreported NLRC4-ALK (N6:A20) fusion and a EML4-ALK (E20:A20, variant 2) fusion. In the novel NLRC4-ALK rearrangement, the exon 6 of NLRC4 fused to the exon 20 of ALK, with an abundance of 24.44% and the fusion points were at chr2 32 462 348 and chr2 29 447 458. EML4-ALK fusion was identified at an abundance of 15.33% (Fig 2c).

The patient received continuous oral crizotinib 250 mg twice daily as postoperative therapy from 10 March 2019,
and no obvious drug-related adverse effects were observed. Clinical and radiological follow-up showed no evidence of recurrent (Fig 3a–c). To date, over 10 months after surgery, the patient still showed stable disease.

**Discussion**

ALK gene arrangements are important driving oncogenes in NSCLC. Several different forms of ALK fusions have been reported, such as EML4-ALK, the most common ALK fusion in NSCLC, which harbors the 5’ end of EML4 fused to the entire ALK kinase domain and leads to constitutive ligand-independent kinase activation. However, ALK double fusions are rarely reported, and to our knowledge, only four cases have been previously reported, including EML6-ALK and FBXO11-ALK, DYSF-ALK and ITGAV-ALK, EML4-ALK and BCL11A-ALK, as well as PRKCB-ALK and EML4-ALK. In this report, we present the first case of novel NLRC4-ALK and EML4-ALK fusion mutations in ADC. When gene fusion happens, the expression of ALK kinase domain is regulated by the upstream regulatory element which derives from the fusion partner gene. Although there is no direct evidence to support NLRC4-ALK as a driver mutation, considering that NLRC4 has been reported to be highly expressed in lung tissues, there is a possibility that NLRC4-ALK rearrangement is a driver mutation.

ALK-TKIs have been widely used for ALK-positive patients, but the responses are heterogeneous for patient with different ALK fusions. Especially, when two kinds of ALK mutations exist simultaneously in one patient, the effectiveness of ALK-TKI treatment might be affected. In this case, the patient belonged to stage IV ADC accompanied with pleural metastasis; however, after 10 months of crizotinib treatment, no pleural dissemination was observed, which supported the effectiveness of crizotinib in patients with concomitant NLRC4-ALK and EML4-ALK mutations.

In conclusion, this report describes the first case of an ADC patient with an unreported NLRC4-ALK fusion and EML4-ALK fusion, with a PFS of over 10 months with continuous crizotinib treatment after surgery. Our report provides valuable information that patients with concurrent ALK double fusions could benefit from crizotinib, and provides a better understanding of ALK-TKIs in ADC with NLRC4-ALK rearrangement.

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**Disclosure**

No authors report any conflict of interest.
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