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Case Report

A novel double fusion of *EML4-ALK* and *PLEKHA7-ALK* contribute to rapid progression of lung adenocarcinoma: a case report and literature review

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Abstract
A 40-year-old male with *EML4-ALK* (E6:A20) fusion variant 3 and previously unreported *PLEKHA7-ALK* (P3:A20) fusion in lung adenocarcinoma exhibited resistance to alectinib and chemotherapy. Subsequent next-generation sequencing (NGS) from the plasma specimen revealed the co-existing mutation in the *KEAP1* gene, which may represent an intrinsic resistance to ALK-TKI. Furthermore, the presence of double fusion *PLEKHA7-ALK* (P3:A20) may also have played a critical role in the resistance to alectinib. *KEAP1* mutation (p.E244K) was also founded in this patient which may lead to resistance to standard chemotherapy. The patient was then treated with brigatinib, which effectively halted the rapid progression. Unfortunately, the patient deceased to uncontrollable, rapidly progressing pleural effusion and pulmonary embolism, resulting in an overall survival of 9 months. This represents the rare case of NSCLC with a double fusion of *EML4-ALK* and *PLEKHA7-ALK*, exhibiting resistance to alectinib and chemotherapy. Our case suggests that the double fusion of *EML4-ALK* and *PLEKHA7-ALK* and co-existing *KEAP1* mutation may serve as an adverse prognostic factor. Additionally, brigatinib may offer a potential treatment option for lung adenocarcinoma patients with *PLEKHA7-ALK* (P3:A20) fusion.

Keywords *ALK* fusion · Brigatinib · *PLEKHA7-ALK* · *EML4-ALK* · NGS

1 Introduction

The presence of Anaplastic Lymphoma Kinase (*ALK*) rearrangement is estimated to occur in approximately 5–7% of non-small cell lung cancer (NSCLC) cases [1]. The introduction of ALK tyrosine kinase inhibitors (ALK-TKI) has led to a marked improvement in the overall survival (OS) of patients with advanced NSCLC with *ALK* rearrangement. Alectinib, brigatinib, lorlatinib, and ceritinib are currently recommended as first-line treatments for NSCLC patients with *ALK* rearrangement [2]. However, the efficacy of ALK-TKI treatment may be heavily influenced by the specific partners involved in the *ALK* fusion. While the majority of patients benefit from ALK-TKI therapy [3], a small subset experience primary resistance, posing a challenge for clinicians [4]. This paper presents a case of a NSCLC patient with a rare *EML4-ALK* (E6:A20) fusion in conjunction with a *PLEKHA7-ALK* (P3:A20) fusion and *KEAP1* mutation, a rare co-occurrence in NSCLC patients.

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1.1 Case introduction

On May 21, 2021, a 40-year-old Chinese male patient with no history of smoking or familial cancer was referred to a local hospital due to a persistent cough. A chest CT scan revealed a 0.8 × 0.6 cm nodule with increased density in the lower lobe of the right lung. Following a negative finding from fiberoptic bronchoscopy, a CT-guided percutaneous biopsy of the nodule in the right lung was performed, and pathology confirmed the presence of lung adenocarcinoma. FDG-PET/CT imaging indicated multiple metastases in the lymph nodes, right hilum, sacrum, left ilium, and right hip (Fig. 1). Consequently, the patient was diagnosed with stage IV lung adenocarcinoma (cT1aN2M1c) according to the 8th edition of the TNM of lung cancer staging by the Union for International Cancer Control (UICC) and International Association for the Study of Lung Cancer (IASLC). Subsequent analysis using the amplification refractory mutation system (ARMS) based on fluorescent quantitative PCR revealed *ALK* fusions.

The patient commenced oral alectinib at a dosage of 600 mg twice daily from June 13, 2021, and continued this treatment for a month. However, on July 19, 2021, a chest CT scan revealed progressive disease based on the RECIST 1.1 criteria, as indicated by an increase in the longest diameters of the lower right lung lesion. Subsequently, the patient sought further treatment at our hospital. Immediate analysis of 520 related genes in specimen of primary tumor using next-generation sequencing (NGS) revealed the presence of the *EML4-ALK* (E6:A20) fusion, along with an unreported *PLEKHA7-ALK* (P3:A20) fusion (Fig. 2), and a *KEAP1* mutation whose mutation sequence is p.E244K. Furthermore, immunohistochemistry analysis showed a tumor proportion score (TPS) of 0% for PD-L1 expression. Consequently, ALK-TKI has been discontinued and the treatment plan was revised to include standard chemotherapy consisting of pemetrexed (900 mg), carboplatin (700 mg), and bevacizumab (500 mg) administered via intravenously every three weeks. On September 12, 2021, a chest ultrasound revealed a pleural effusion of 53 mm on the right side, and the patient had dyspnea, which necessitated thoracocentesis. Subsequently, cis-platinum (20 mg) and bevacizumab (200 mg) were injected into the right thoracic cavity. Despite two months of intracavitary chemotherapy, a chest CT on September 15, 2021, indicated progressive disease. Consequently, the patient commenced oral brigatinib at a dosage of 180 mg per day, starting from September 15, 2021, for a duration of two months. Imaging examinations revealed no significant enlargement of the longest diameter of the mass in the right lung or the bone metastasis; however, the pleural effusion showed no signs of improvement. As Anlotinib, a novel multi-targeting tyrosine kinase inhibitor, has shown activity in NSCLC with ALK fusion. Since patients have progressed or relapsed after receiving at least two previous chemotherapy regimens, we decided to offer this patient anlotinib as a fourth line treatment [5]. Since December 4, 2021, in an effort to alleviate the pleural effusion, the patient received oral anlotinib at a dosage of 12 mg once daily, administered from day 1 to day 14, every

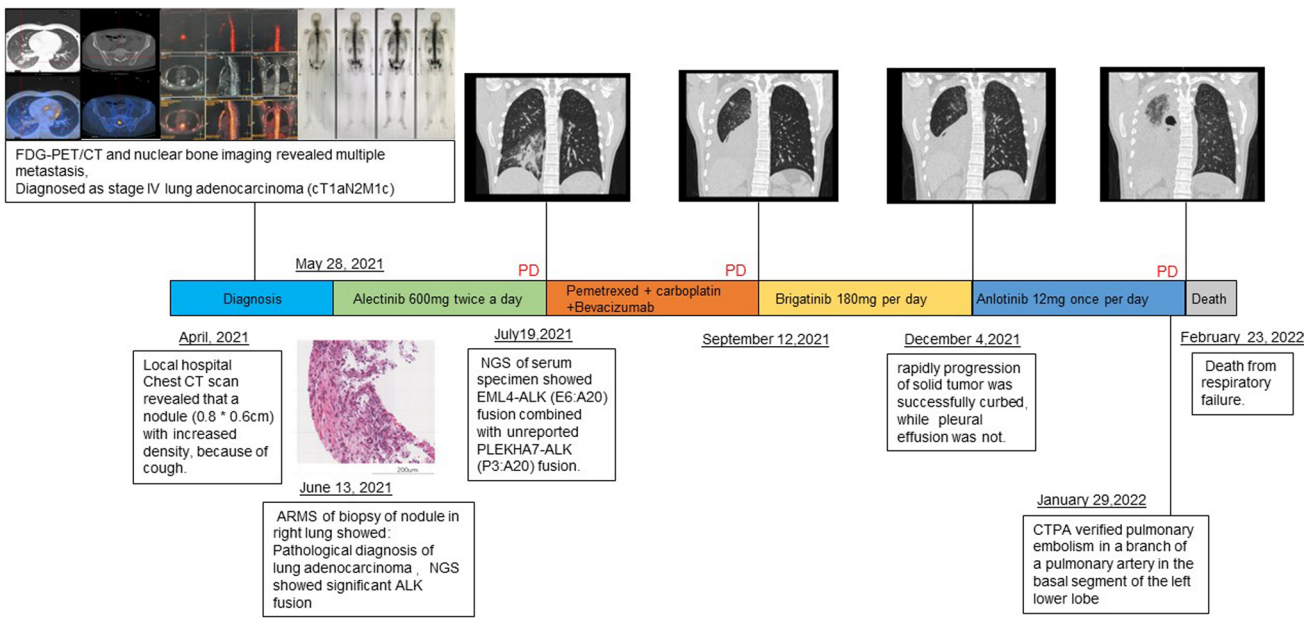

Fig. 1 Patient's CT images and treatment history. CTPA: computer tomography pulmonary angiography, ARMS: amplification refractory mutation system, NGS: next generation sequencing

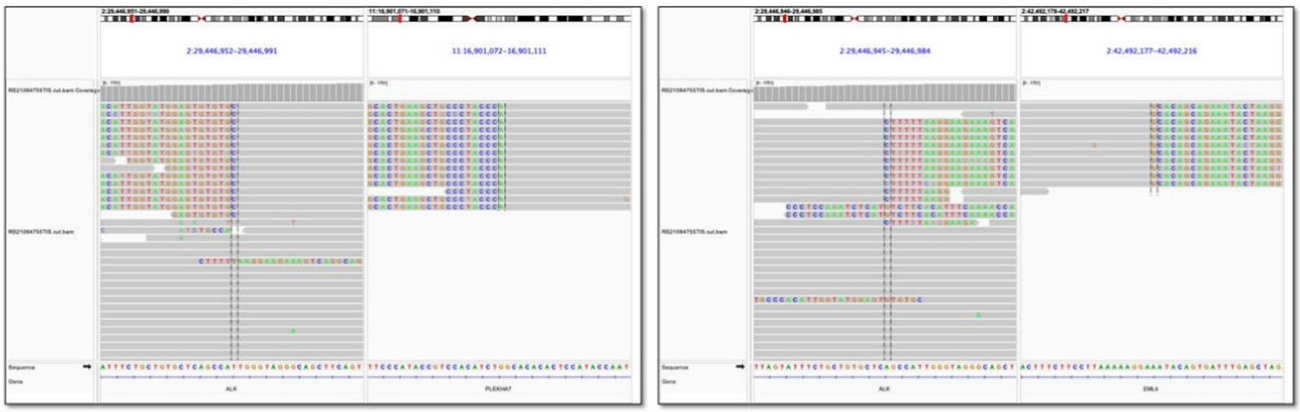

Fig. 2 DNA sequencing showed *EML4-ALK* (E6:A20) fusion and *PLEKHA7-ALK* (P3:A20) fusion

3 weeks. Unfortunately, a subsequent chest ultrasound on January 11, 2022, revealed the recurrence of pleural effusion. The patient reported progressively worsening dyspnea, and a computer tomography pulmonary angiography (CTPA) confirmed the presence of pulmonary embolism (PE). Despite receiving palliative treatment including hydrocortisone, oxygen therapy, nebulization and heparin anticoagulation, the dyspnea continued to worsen. Regrettably, the patient passed away on February 23, 2022 (Fig. 1).

2 Discussion

ALK fusion accounts for approximately 5–7% in NSCLC [1]. In 2013, the efficacy of the first targeted ALK-TKI, crizotinib, was found to be superior than chemotherapy in the first line in NSCLC with *ALK* fusion, resulting in a significant improvement in overall survival (OS) [6]. In recent years, NSCLC patients with *ALK* rearrangement have been reported to have a median survival of more than 7 years [7]. However, the OS of the patient in this case was only 9 months, potentially resulting from the unprecedented double fusions of *EML4-ALK* and *PLEKHA7-ALK* and co-existing *KEAP1* mutation.

The primary activation mechanism of *ALK* in lung cancer is rearrangement, involving more than 90 identified fusion partners [8]. The most frequent fusion partner of *ALK* is the echinoderm microtubule-associated protein-like 4 (*EML4*), followed by kinesin family member 5B (*KIF5B*), TRK-fused gene (*TFG*), kinesin light chain 1 (*KLC1*), protein tyrosine phosphatase non-receptor type 3 (*PTPN3*), and striatin (*STRN*) [6]. However, the fusion partners beyond these are rare. Similarly, the occurrence of double fusions of *ALK* is extremely uncommon, with only a few case reports published, such as *NLRC4-ALK* and *EML4-ALK* [9], *EML4-ALK* and *BCL11A-ALK* [10]. Nevertheless, the double fusions of *EML4-ALK* and *PLEKHA7-ALK* are never reported before.

We identified and reported for the first time a case of double fusions of *EML4-ALK* and *PLEKHA7-ALK*. The *PLEKHA7-ALK* fusion has only been previously reported as concomitant double-fusion of *PLEKHA7-ALK* and *INPP5D-ALK* and as an acquired gene fusion in a single case report (Fig. 3) [3]. *PLEKHA7* is coding adherens junction protein with diverse functions in mammalian cells [11]. The specific physiological function of *PLEKHA7* is not yet fully understood. Notably, Schrock et al. found that *PLEKHA7-ALK* fusion represented a novel acquired resistance mechanism to Osimertinib, although after a 4-month combination treatment of alectinib and osimertinib, *PLEKHA7-ALK* fusion was not identified in repeat blood-based ctDNA testing [12]. Furthermore, Pei Li et al. found that patients with *PLEKHA7-ALK* and *INPP5D-ALK* showed good sensitivity to alectinib [3]. *ALK*-mutations, L1196M and G1269A, are the most common ALK-TKI-resistance on crizotinib, while the G1202R mutation may more frequently occur as an acquired resistance mechanism for brigatinib, ceritinib, and alectinib [13]. In addition, the activation of certain signaling pathways represents another important resistance mechanism, including amplification of *KIT*, *MAPK*, *IGF-1R-IRS-1* pathway, *EGFR* signaling, *MET* amplification, and *BRAF* V600E mutation 3. Despite five rounds of NGS tests, the first one was obtained from primary tumor, and the following four were obtained from blood, no relevant ALK-TKI resistance alterations were identified beyond *KEAP1* mutation. However, the patient exhibited resistance to alectinib at the commencement of targeted therapy. We speculate that the *PLEKHA7-ALK* fusion might represent a new intrinsic resistance mechanism to alectinib.

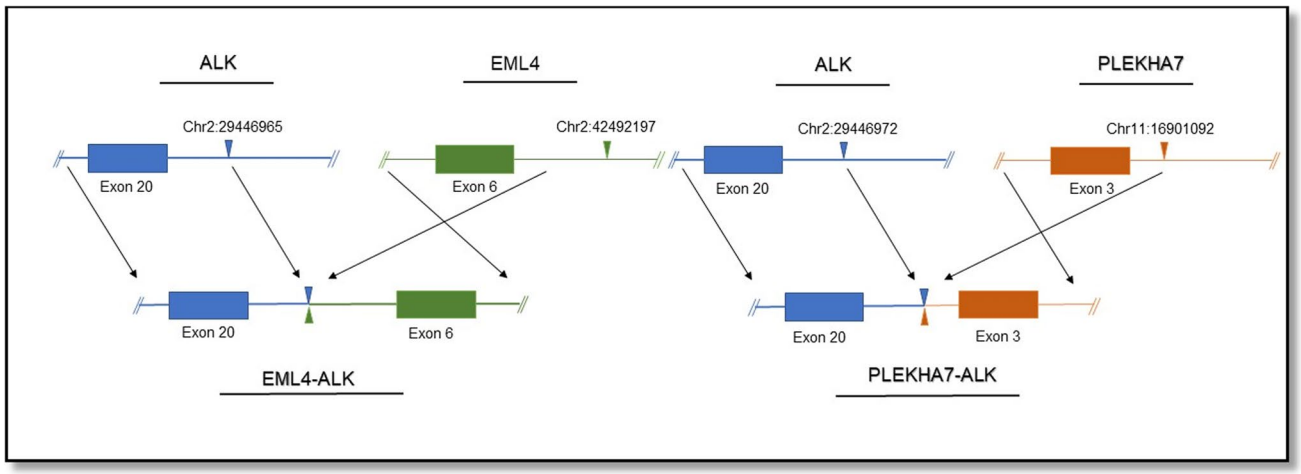

Fig. 3 ALK gene and the EML4 gene map on chromosome 2p. EML4 is disrupted at chr2 42,492,197 and is ligated to chr2 29,446,965 of ALK. ALK gene and the PLEKHA7 gene map on chromosome 2p, PLEKHA7 is disrupted at chr11 16,901,092 and is ligated to chr2 29,446,972 of ALK

KEAP1 plays a crucial role in the oxidative stress response. Recent studies have associated *KEAP1* mutation with chemotherapy resistance and worse OS compared to wild-type NSCLC patients [14]. However, the impact of *KEAP1* mutation in patients undergoing immunotherapy remains controversial [15]. In this case, the patient also exhibited resistance to standard chemotherapy, potentially due to the presence of a *KEAP1* mutation. Brigatinib, the second generation of ALK-TKI, has been used as a first-line treatment in advanced NSCLC with *ALK* fusion and has shown potent efficacy against many *ALK* resistance mutations [16]. Following the initiation of brigatinib, the rapid progression of the solid tumor was miraculously brought under control. Therefore, we believe that brigatinib may offer a potential treatment for NSCLC patients with primary *PLEKHA7-ALK* fusion. Unfortunately, malignant pleural effusion remained uncontrolled throughout the entire treatment. Malignant pleural effusion has always posed a significant challenge in treatment and is often indicative of a worse OS [17]. The therapy for malignant pleural effusion mainly focuses on alleviating symptoms, including thoracentesis, thoracic drainage, pleurodesis, and indwelling pleural catheter [18]. However, the combination of bevacizumab with cis-platinum thoracic perfusion has emerged as a novel approach for control, which may offer a better prognosis in treating malignant pleural effusion [19]. Rintaro et al. found that bevacizumab plus chemotherapy and thoracic perfusion is highly effective in NSCLC patients with malignant pleural effusion [20]. Despite this, the injection of cis-platinum and bevacizumab into the patient's right cavity failed to control the malignant pleural effusion.

In conclusion, we have reported a case of a NSCLC patient with unreported double *ALK* fusions of *PLEKHA7-ALK* and *EML4-ALK* co-existing with *KEAP1* mutation. Several clinical studies have shown that the second generation of ALK-TKI is beneficial for most NSCLC patients with *EML4-ALK* fusion [21]. In this case, we suspect that the double fusion of *EML4-ALK* and *PLEKHA7-ALK* reduces the tumor's responsiveness and sensitivity to ALK-TKI. The coexisting mutation site of *KEAP1* (p.E244K) may also have an impact on prognosis [22].We hypothesize that primary *PLEKHA7-ALK* fusion may represent a new primary resistance mechanism to alectinib. The use of brigatinib effectively slowed down disease progression [23], offering a potential effective treatment for NSCLC patients with primary *PLEKHA7-ALK* fusion. However, brigatinib failed to control the malignant pleural effusion, which recurred after thoracentesis. Therefore, the treatment of such patients requires further research and development.

2.1 Statement

The clinical data of Shanghai Changzheng Hospital (the Second Affiliated Hospital of Naval Medical University) were retrospectively included in this study. This study was approved by the Ethics Committee of the Second Hospital Affiliated to Naval Medical University and involved human subjects. Each subject received informed consent and the study complied with the ethical guidelines of the latest edition of the Helsinki Declaration.

Author contributions Zhongzhao Wang wrote the main text, Yang Luo conduct data sorting and analysis, Heng Gong visualized the drawing and analysis, Yang Chen and Hao Tang provided supervision and guidance as well as a critical review of the manuscript.

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Data availability Data supporting the findings of this study may be obtained from the corresponding author upon reasonable request.

Declarations

Consent for publication The consent for publication was obtained from the next of kin [as the patient is deceased].

Competing interests The authors declare no competing interests.

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