

Ineffectiveness of Crizotinib in a Non-Small-Cell Lung Cancer with Novel ALK- LIMS1 Fusion: A Case Report

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Abstract: Anaplastic lymphoma kinase (ALK) rearrangements have been reported in 3–7% of non-small-cell lung cancers (NSCLC). ALK has been reported to be fused with a variety of genes in NSCLC. Significant clinical activity was achieved by ALK inhibitors in patients with NSCLC harbouring ALK translocations. We reported on a 48-year-old male Chinese patient with advanced lung adenocarcinoma harboring a novel ALK-LIMS1 who showed no response to crizotinib. The tissue was assayed by immunohistochemistry (IHC) for ALK and showed diffuse expression of ALK. Next-generation sequencing (NGS) was performed on the peripheral blood and tissue. The previous tumor tissue showed diffuse expression of ALK. Tissue and the later peripheral blood revealed a ALK-LIMS1 fusion. The patient failed to benefit from crizotinib (250 mg, twice a day), with a progression-free survival of two months. We identified a new ALK-LIMS1 fusion from an advanced lung adenocarcinoma which was primary resistant to crizotinib. Our case suggested that the coexistence of mutations and the non-dominant clone, as well as the rearrangement of ALK fusion, did not result in expressed ALK kinase domain that might lead to no response to ALK-TKIs.

Keywords: non-small-cell lung cancer, next-generation sequencing, ALK- LIMS1, crizotinib

Introduction

Targeted therapy has become the preferred first-line treatment option for NSCLC with driver alterations. ALK rearrangements are shown in approximately 3–7% of all metastatic NSCLC.¹ Patients with ALK fusion could receive significant clinical benefit from treatment with ALK inhibitors. The first-generation ALK tyrosine-kinase inhibitor (TKI) crizotinib and the second-generation TKI alectinib, brigatinib, ceritinib are established as standard first-line therapies for patients with advanced NSCLC confirmed as ALK-positive. The first-generation crizotinib has a reported objective response rate (ORR) of 82.7% and a disease control rate of over 90%, with median progression-free survival of 13.0 months and overall survival of 36.0 months.² The latest data showed significantly prolonged PFS with alectinib (median PFS 34.8 months) and median treatment duration was longer, at 28.1 months.³ Here, we report a case of advanced lung adenocarcinoma harboring a novel LIM zinc finger domain containing 1 (LIMS1)-ALK by NGS. Crizotinib was tested for this patient but he showed no response to it. The current detection methods of ALK include ICH, FISH, NGS and RNA. Importantly, results of large cohorts in clinical investigations indicate that some non-small-cell lung cancer cases (0.2–21%) show discordant results between IHC and FISH, implying that a single assay strategy can lead to inadequate selection of patients. Particular attention should be paid to cases with borderline results in FISH analysis, which have been found to be related to discrepant findings, thus adding IHC analysis might be recommended. In turn, IHC findings show similar pitfalls in regard to discrepancies, though few instances have been reported.⁴

Case Report

A 48-year-old male Chinese patient presented in our hospital who had a former cigarette smoking history of more than twenty years. The patient was diagnosed with a well-differentiated adenocarcinoma in the middle lobe of the right lung in 2014. The patient was successfully treated with complete surgical resection and received treatment of nedaplatin plus paclitaxel for 4 cycles. Routine follow-up after the treatment was performed on this patient. As a patient with cough, chest tightness, and shortness of breath, he received a computed tomography (CT) scan, which showed metastases in the lung, bone, liver and right pleural effusion in December 2017. The pleural effusion was characterized by immunohistochemical test and showed TTF-1 (+), CK (+), NapsinA (+), MOC31 (+), P40 (+), P63 (-), WT-1 (-), Calmodulin (-), CD56 (+). Cytological examination of the pleural effusion showed tumor cells, which were considered as adenocarcinoma with neuroendocrine differentiation of the lung. EGFR mutations were not detected in the pleural effusion. The patient rejected any treatment after this recurrence. Three months later, the patient got a pain in abdomen and CT revealed multiple metastases as before, but the tumor had grown. Subsequently, the patient had to undergo chemotherapy and Avastin. However, the tumor rapidly progressed after the treatment.

IHC was performed with the Ventana D5F3 (Oro Valley, Arizona) revealing the diffuse expression of ALK in the tumor tissue from the previous surgery (Figure 1). At the same time, the previous surgical tumor tissue was detected by NGS based on a pan-cancer 8-gene panel. ALK-LIMS1 was detected. Due to the progress of the patient's condition, peripheral blood obtained from the patient was tested by next-generation sequencing based on a pan-cancer 1021-gene panel. The gene alterations were detected in the liquid biopsy, and a total of five-point mutations and a fusion were detected for this patient: KIT p.E561K with a mutant allele frequency (MAF) of 29.5%, TP53 p.N310Tfs*35 with a MAF of 17.7%, AXL p.E66K (0.7%), NTRK3 p.L835M (0.7%), IL6ST p.D171N (0.6%) and ALK-LIMS1 with a MAF of 12.1%. As a result, there were no actionable driver mutations detected which could be treated with targeted therapy. Interestingly, a novel fusion gene fused by ALK-LIMS1 was detected. The ALK-LIMS1 was fused by intron 20 of ALK to intron 1 of LIMS1 and retained the promotor region and the entire intracellular kinase domain, respectively (Figure 2A and B). This was the first discovery of this novel partner gene for ALK fusion in NSCLC worldwide. The patient, considering his chemotherapy insensitive status, received crizotinib (250 mg, twice a day) from December 2018. Two months later, the right upper lung lesion and liver (Figure 3) progressed and the left pleural and pelvic effusions appeared. Disease progression was thus considered and crizotinib showed no response in the patient. The patient died in March 2019. The treatment timeline and tumor evaluation are presented in Figure 4.

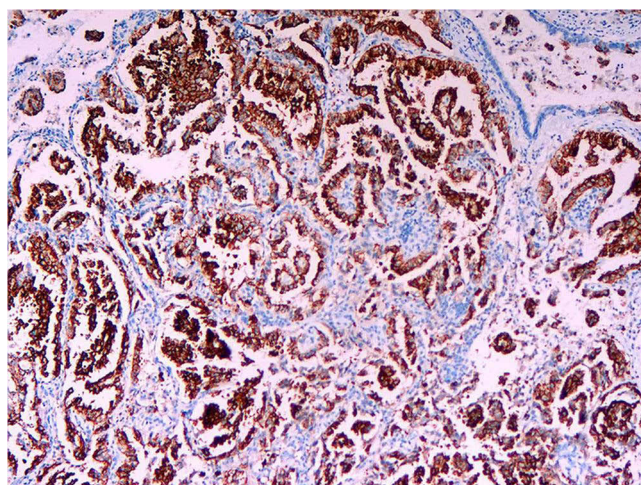


Figure 1 Immunohistochemical staining by D5F3 (Ventana, Oro Valley, Arizona) shows diffuse expression of ALK in the cytoplasm of tumor cells (original magnification $\times 100$).

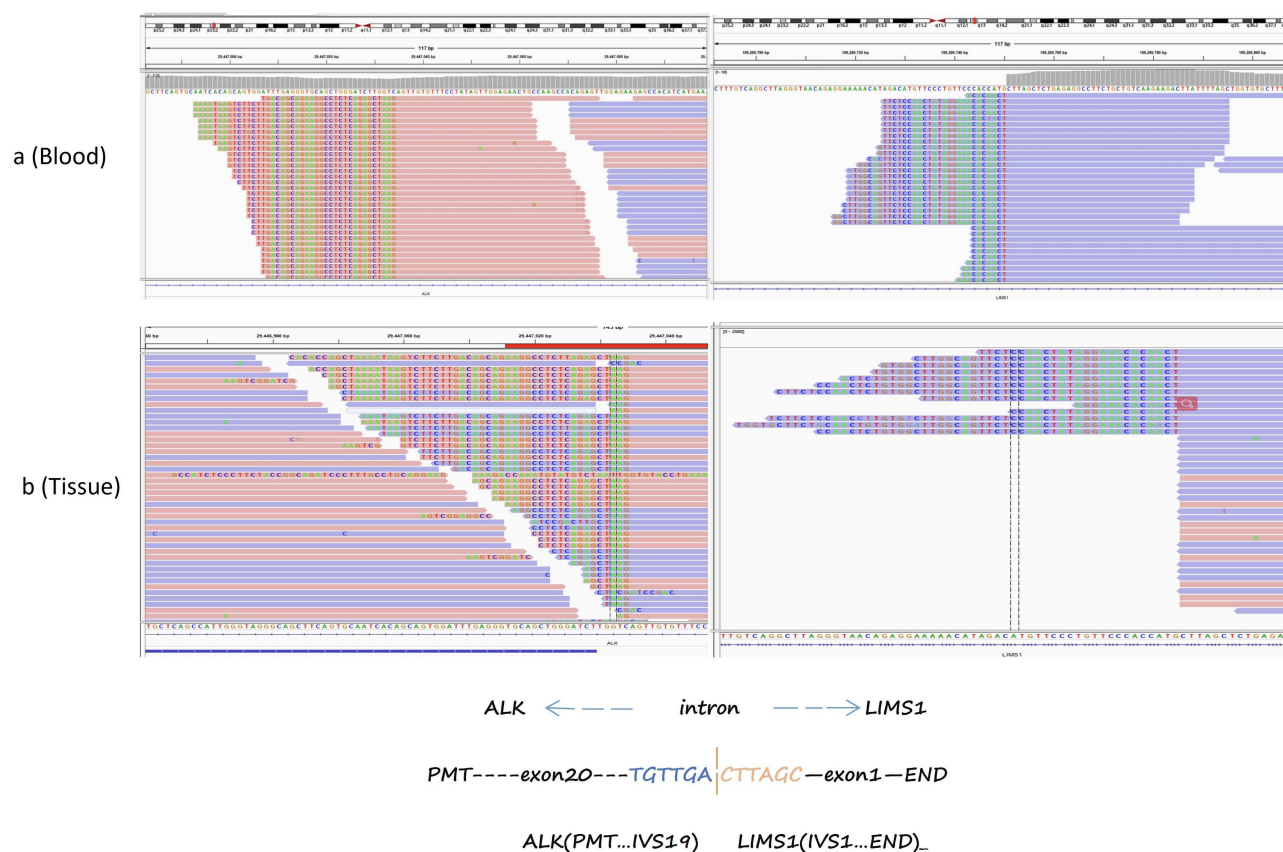


Figure 2 The novel LIMS1-ALK fusion. Sequencing reads of LIMS1 and ALK are shown by the Integrative Genomics Viewer.

Abbreviations: PMT, promoter; IVS, intervening sequence.

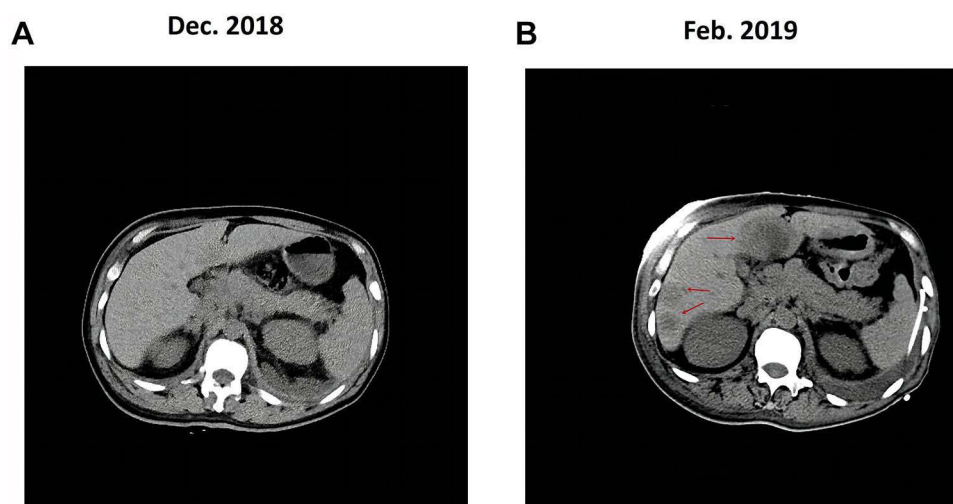


Figure 3 Images of liver computed tomography (CT) scans of the patient before (A) and after (B) crizotinib.

Discussion

ALK has been reported to be fused with a variety of genes in NSCLC. We discovered a novel ALK-LIMS1 fusion from a young male with advanced lung adenocarcinoma with no other actionable mutation. The ALK fusion was initially detected by IHC in the patient's surgical tissue, and was validated by NGS DNA sequencing, which identified the

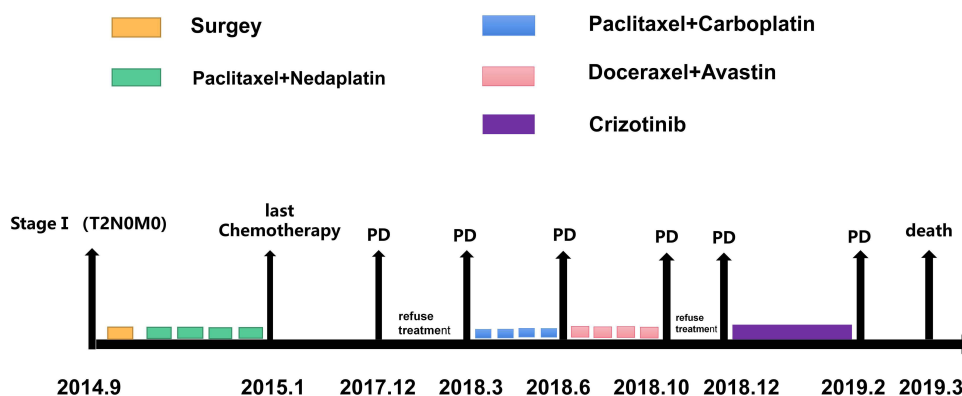


Figure 4 The timeline from diagnosis of NSCLC to different stages of therapeutic regime and tumor evaluation and death.

chaperone gene LIMS1 and the breakpoint. The ALK-LIMS1 fusion was also validated in the liquid biopsy sample at the time of recurrence. LIMS1, also named PINCH-1 (a particularly interesting new cysteine-histidine rich protein), was reportedly expressed in several cancers, such as lung cancer, colon cancer and breast cancer.^{5–7} A recent research reported that LIMS1 is highly expressed in lung adenocarcinoma and promotes proline synthesis through regulation of mitochondrial dynamics.⁵ In addition to being verified at the level of DNA and protein expression, RNA sequencing is also helpful for a better understanding of ALK-LIMS1. And some reports show that some uncommon (non-EML4 partner) ALK fusions are detected at the DNA level of expressed EML4-ALK transcripts. RNA sequencing can further determine whether this fusion can produce functional fusion proteins.⁸ Unfortunately, the NGS RNA sequencing failed because of the surgical tissue quality problem.

Patients diagnosed with ALK-activating NSCLC will achieve a clinical efficacy base on treatment with ALK inhibitors. Crizotinib, a small-molecule inhibitor of ALK, demonstrated significant clinical activity in patients with NSCLC harbouring ALK translocations. A PROFILE 1014 trial has a reported objective response rate (ORR) of 74.0% and median progression-free survival (mPFS) of 10.9 months and the probability of 1-year survival was 84% with crizotinib as first-line treatment for advanced ALK-positive NSCLC.⁹ A PROFILE 1015 trial has a reported ORR of 59.8% and mPFS of 8.1 months with crizotinib for a patient with locally advanced or metastatic ALK-positive lung cancer who had received one or more prior platinum-based regimen.¹⁰ The patient carrying novel CUX1-ALK, detected by NGS and the oncogenic ability of the fusion gene was further validated in cells, showed a superior response to crizotinib, with a PFS of 20 months.^{5–11} A case of TNIP2-ALK fusion (analyzed by both IHC and NGS from blood and aspiration biopsy) in advanced lung adenocarcinoma responded to crizotinib, with an OS at least of 12 months.⁵ As reported, resistance to crizotinib usually occurred after approximately 10 months of treatment.¹¹ Actually, the patient failed to have sustained benefit from crizotinib. One possible reason was that the ALK-LIMS1 fusion occurred at the genomic level and did not result in ALK expression, leading to the ineffectiveness of crizotinib. Another possible reason was clonal architecture in this patient. As mentioned above, the ALK-LIMS1 was more likely because of the subclonal variation with a MAF of 12.1%. However, the KIT p.E561K was 29.5%, which might play a dominant clone in the tumor. An advanced NSCLC patient with STRN-ALK fusion reported limited clinical activity to crizotinib.¹² In the case, biopsy specimen identified STRN-ALK with a MAF of 0.04%, PIK3CA p.G106V with a MAF of 53.63%, and KRAS p.G12C with a MAF of 76.83%. Thus, the patients with a lower abundance of actionable mutation may benefit less from targeted agents. Besides, a study mentioned that patients with TP53 mutations were associated with reduced PFS (3.7 versus 10.8 months) treated with ALK inhibitors.¹³ These suggested that the coexistence of mutations and the non-dominant clone might result in resistance to ALK-TKIs. What's more, these suggest that patients with ALK fusion cannot be simply treated with ALK inhibitors based on gene detection or immunohistochemistry. On the one hand, it is necessary to evaluate the rearrangement position of ALK fusion. On the other hand, we need to use a variety of technologies to comprehensively analyze the molecular map of patients to provide more comprehensive medical evidence for finding the most appropriate treatment strategy.

Five ALK-TKIs are approved for advanced NSCLC with ALK rearrangement: crizotinib, alectinib, ceritinib, lorlatinib, brigatinib. Different ALK-TKI have different mechanisms, efficacy and safety. As reported, some ALK-TKIs are effective for tumors that are resistant to other ALK-TKIs. In the present case, the first-generation crizotinib failed to bring clinical efficacy. The second- and third- generation ALK-TKI may be effective. But the present case survival time was only one month after the crizotinib failed. In another case, the patient harboring STRN-ALK fusion was resistant to first-line therapy alectinib, while crizotinib exhibited clinical activity, though limited.⁸ More ALK inhibitors should be tried if possible.

Conclusion

We identified a new ALK-LIMS1 fusion which was likely to demonstrate primary resistance to crizotinib. The ALK-LIMS1 may provide a reference for crizotinib resistance. Our study suggested that coexistence of mutations and non-dominant clones, as well as the rearrangement of ALK fusion might be associated with primary resistance. Additional studies are required to verify this suggestion.

Statement of Ethics

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images.

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Disclosure

The authors report no conflicts of interest in this work.

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