Primary Resistance to ALK Inhibitors in a Patient with Nonsmall Cell Lung Cancer with ALK Rearrangement: A Case Report with Review of Literature

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Abstract
Anaplastic lymphoma kinase inhibitors (ALKi) are the standard of care for metastatic ALK-rearranged nonsmall cell lung cancer (NSCLC). Though most patients respond well to ALK, seldom there are instances where the disease progresses rapidly. Here, we present a case of a 41-years-old male diagnosed as NSCLC with ALK rearrangement. Despite being started on first- and second-generation ALK-targeted therapy, he had rapid disease progression ultimately succumbing to the disease within 3 months of diagnosis. We suspect that our patient has a variant of ALK, making him resistant to both first- and second-line targeted therapy. Subjecting such nonresponders to next-generation sequencing and identifying the variants might help to recognize a subset of patients among ALK+ NSCLC who will need intense monitoring and early institution of other therapies for a better outcome.

Keywords
► EML4-ALK fusion protein
► nonsmall cell lung cancer
► resistance

Introduction
Lung cancer is the number one reason for cancer-related death globally,1 with nonsmall cell lung cancer (NSCLC) accounting for approximately 80 to 85% of cases. The rearrangement of the echinoderm microtubule-associated protein-like 4 (EML4)–anaplastic lymphoma kinase (ALK) gene was initially detected as an oncogenic incentive of NSCLC in 2007.2 ALK- EML4 fusion has been established as a therapeutic target and several ALK inhibitors (ALKi) have emerged as front-line therapy in advanced ALK-positive (+) lung cancer patients which can effectively suppress the oncogenic activity of ALK rearrangement. These patients usually show dramatic responses to ALK-directed targeted therapies allied with tyrosine kinase inhibitors (TKIs). Crizotinib was the first ALK-directed TKI approved for the same.3 Other than crizotinib, numerous second-generation (e.g., ceritinib, alectinib, brigatinib)4 and third-generation (e.g., lorlatinib)5 ALK-directed TKIs are also available.

The ALK gene belongs to the insulin receptor superfamily and encodes for the ALK protein. It is positioned on the short arm of chromosome 2 (2p23). ALK is a transmembrane
tyrosine kinase receptor with an extracellular domain, a transmembrane segment, and a cytoplasmic receptor kinase segment. ALK expression is said to occur in the nervous system throughout embryogenesis and declines after birth. In adulthood, a lesser percentage of ALK proteins are created only in uncommon, dispersed neural and endothelial cells and in pericytes in the brain.

ALK rearrangement is identified in approximately 5% of NSCLC (ALK+ NSCLC). The fusion of the EML4 gene and the ALK gene occurs by a small inversion within chromosome 2p. This translocation fuses the N-terminus of EML-4 with the kinase domain of ALK to create the EML4-ALK fusion gene which leads to catalytically active kinase function and carcinogenicity. The breakpoint of the ALK gene is always on exon 20, while the EML4 breakpoint location may differ, generating fusion protein variants.6–Table 1 describes the different ALK variants based on the fusion partners, including EML and other rare fusion partners.2,7–16

The whole fusion protein stability, inhibitor-induced degradation, and drug sensitivity are influenced by the stability of the EML4-ALK gene. At present, more than 15 EML4-ALK fusion variants have been identified with the most common being variant 1 (EML4 breakpoint exon 13), variant 2 (EML4 breakpoint exon 20), and variant 3a/b (EML4 breakpoint exon 6a/b) seen in 43, 6, and 40% of cases, respectively.6–16

Emerging data indicates that there may be biological and clinical implications of ALK fusion variants in ALK-positive lung cancer. The V3 variant of ALK is found to present a more aggressive disease and inferior response to therapy. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are routinely done to detect ALK rearrangements fail to identify these variants.17 We hereby report a case of ALK+ NSCLC started on ALK-directed targeted therapy but showed primary resistance to first- and second-line TKIs. This study was approved by the institutional review board and the patient’s consent was obtained.

Case Presentation

A 41-year-old gentleman, nonsmoker, presented to the institute with cough and breathlessness for 1 month and low backache with difficulty in walking for 15 days. His history was unremarkable. Clinical examination revealed reduced air entry on the right side with paraplegia. Chest X-ray revealed opacity in the right hilar region. Contrast-enhanced computed tomography (CECT) scan of the thorax detected a heterogeneous enhancing mass in the right lung lower lobe of 6.8 × 6.4 cm with underlying collapse and metastatic nodules in the left lung, moderate right pleural effusion, and mediastinal lymphadenopathy (►Fig. 1A–C). Magnetic resolution imaging of the spine revealed metastasis involving multiple vertebral bodies. The patient underwent a positron emission tomography scan which revealed a pleural-based soft tissue mass of 7.9 × 5.3 cm involving the right hiliar region obliterating the right middle and lower lobe bronchus (►Fig. 2A). There were multiple parenchymal and subpleural nodules in both lungs along with mediastinal, right supraclavicular lymphadenopathy, and right pleural effusion. There were lytic lesions in the left scapula, sternum, bilateral iliac bones, bilateral acetabulum, multiple vertebrae, and partial collapse of the D7 vertebra with intraspinal extension (►Fig. 2B, C). CT-guided core needle biopsy from the right hiliar mass revealed adenocarcinoma. D5F3 IHC identified the existence of ALK rearrangement. Epidermal growth factor receptor (EGFR) was wild-type and there was no ROS-1 rearrangement. A chest drainage tube was inserted to drain the pleural effusion. The patient received palliative radiotherapy of 30 Gy in 10 fractions over 2 weeks to the D7 vertebra and then was planned for ALK-directed targeted therapy with tab crizotinib 250 mg twice daily. However, even after 20 days of starting crizotinib, there was no evident clinical improvement. Therefore, CECT thorax was repeated, showing progressive disease owing to growth in the dimension of the lung lesions and multiple brand-new skeletal metastases. In view of progression, ALK rearrangement was reconfirmed by FISH as the patient was unwilling to go for next-generation sequencing (NGS). Treatment was changed to second-line ALK-directed therapy with the tablet ceritinib 450 mg once daily. After 1 month dyspnea worsened, mandating intensive care support. On reassessment, CECT thorax showed progressive disease in the form of an increase in the size of the lung lesions and recurrent pleural effusion (►Fig. 3). The patient was started on chemotherapy with pemetrexed and carboplatin, but he eventually succumbed.

Table 1 ALK fusion partners in NSCLC

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<th>Variant</th>
<th>Symbol</th>
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Abbreviations: ALK, anaplastic lymphoma kinase; NSCLC, nonsmall cell lung cancer.
**Fig. 1** Computed tomography (CT) scan of thorax showing (A, B) right lower lobe lung mass with underlying collapse, (C) moderate right pleural effusion.

**Fig. 2** Positron emission tomography-computed tomography (PET-CT) showing (A) pleural-based soft tissue mass of 7.9 × 5.3 cm involving the right hilar region obliterating the right middle and lower lobe bronchus with right pleural effusion. (B, C) Lytic lesions involving the left scapula, sternum, multiple vertebrae, bilateral iliac bones, and acetabulum, partial collapse of D7 vertebra with intraspinal extension.
2 weeks after the first cycle of chemotherapy, that is within 2 months of initiation of ALK-targeted therapy (Fig. 4).

Discussion

TKI sequencing therapy combined with local treatments has improved survival among metastatic ALK+ NSCLC patients. Currently, median survival exceeds 5 years after two ALKi. While mechanisms of acquired resistance to ALKi in ALK+ NSCLC are well described, including both ALK-dependent mechanisms, such as TK domain mutations or amplification of ALK gene,^{18} and ALK-independent ones, such as EGFR, MET, and PI3K mutations or amplifications, few patients, however, present with failure to respond to upfront TKI treatment. This suggests presence of certain primary resistance mechanisms which are not well understood.^{19} These patients could potentially benefit from a thorough histological reevaluation to exclude the presence of any other histological components mixed with adenocarcinoma. Some case reports have described instances of primary resistance to ALKi in ALK+ NSCLC, where patients had coexisting MYC amplification^{20} or KRAS mutation.^{21}

The expression of specific EML4-ALK variants may dictate the degree of the benefit obtained from ALKi. The patients with EML4-ALK variant 3 rearrangements were found to manifest poorer results contrasted to their counterparts with variant 1 and variant 2. In addition, tumors with variant 3 rearrangements displayed increased belligerent demeanor, advanced stage at early presentation, increased propensity for metastasis, higher frequency of atypical metastatic locations, and premature failure after treatment with TKI or platinum-based chemotherapy, thus showing a shorter overall survival.^{22} Noh et al also observed an elevated incidence of metastatic illness within freshly identified ALK+ NSCLC cases possessing V3 compared with other variants.^{23}

It is reported that the stability of the EML4-ALK protein is influenced by the existence of a tandem atypical β-propeller in the EML protein (TAPE) domain. V3a/b or V5a/b are short variants that deficit a TAPE domain and may be least TKI reactive than the lengthier TAPE containing variants such as V1 or V2.^{24} V3-positive patients are said to progress faster through multiple TKI treatment lines primarily through the development of distinct secondary ALK resistance mutations, particularly ALK G1202R. This mutation impedes appropriate drug binding thus generating resistance to first- as well as second-generation ALKi.^{6}

However, no substantial variation in the efficacy of crizotinib amidst patients with the EML4-ALK fusion variants was evident in a few studies.^{25} The ALEX study also reported a similar level of benefit with both alectinib and crizotinib in the first-line setting in patients with variants 1 and 3a/b.^{4} Our patient had lung and spinal metastasis at presentation and had accelerated progression during the course of treatment with resistance to both first- and second-line ALK-directed therapies, which could be due to the presence of any of the variants of ALK rearrangement, associated with poor prognosis and aggressive disease course. Routine ALK testing by FISH and IHC might miss such cases upfront. The development of NGS techniques will probably enable the detection of ALK rearrangement variants and emergent mutation screening in one test. It can help in the identification of higher-risk cases that should be kept under more aggressive surveillance and may preferentially be offered access to other experimental treatments.^{22} Our attempt to perform NGS on the paraffin block (posthumously, after taking relatives’ consent) was futile due to insufficient tissue, reemphasizing the importance of upfront NGS testing rather than sequential testing.

Conclusion

The presence of ALK rearrangement in general portends a favorable response to ALK-directed targeted therapies in

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**Fig. 3** Computed tomography (CT) thorax showing a right hilar soft tissue mass, satellite nodule in the posterior segment of the right upper lobe (arrow), skeletal metastatic lesions.

**Fig. 4** Rapid progression despite anaplastic lymphoma kinase (ALK)-targeted therapy in an ALK-rearranged advanced nonsmall cell lung cancer (NSCLC).
most cases. But there might be a subset of patients who present with aggressive disease associated with failure of upfront ALK-directed targeted therapies where the cause needs further evaluation. Such cases warrant a rebiopsy and a comprehensive histological evaluation to rule out a remote possibility of small cell transformation. This case report underscores the significance of conducting baseline molecular profiling in advanced lung cancer. However, given our constraints, particularly financial limitations, decision making between sequential testing and upfront NGS becomes a daunting task. Further studies are warranted to assess the possibility of small cell transformation. This case report a comprehensive histological evaluation to rule out a remote needs further evaluation. Such cases warrant a rebiopsy and upfront ALK-directed targeted therapies where the cause 

Declaration of Patient Consent Form
Consent was obtained from the patient.

Ethics Approval
This study was approved by the institutional Ethics Committee, with Ref.no/DRI/IMS.SH/SoA/2021/097 dated 07.07.2021 (first approval) and Ref.no/IEC/IMS.SH/SoA/438 dated 13.10.2022 (approval renewal); and was conducted in accordance with the Declaration of Helsinki.

Declaration of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors’ Contributions
All authors contributed equally to the writing, data, and figures of the final manuscript. The authors confirm that all authors have read and approved the manuscript.

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Conflict of Interest
None declared.

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