CASE REPORT

Novel *PLEC-EML4-ALK* Double Fusion Underlying Crizotinib Resistance in a Metastatic Inflammatory Myofibroblastic Tumor: A Case Report

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ABSTRACT

ALK fusions are frequent oncogenic drivers in inflammatory myofibroblastic tumors. Treatment with crizotinib is effective in fusion-positive patients; however, acquired resistance remains a challenge. Here, we present a case of *EML4-ALK*-positive metastatic inflammatory myofibroblastic tumor that initially responded to crizotinib but developed resistance. The progressing lesion revealed the acquisition of a "double fusion" event in which *EML4-ALK* was additionally fused to *PLEC* to create a *PLEC-EML4-ALK* transcript. The double fusion was associated with an increase in *ALK* expression, mimicking the *ALK* fusion amplification that is a known mechanism of resistance to crizotinib in lung cancer. On transition to the more potent ALK inhibitor alectinib, the patient exhibited a dramatic response. Thus, the formation of a double fusion represents a novel and targetable mechanism of resistance to crizotinib.

Keywords: Case report; *ALK* fusion; Crizotinib; Inflammatory myofibroblastic tumor

Introduction

Inflammatory myofibroblastic tumor (IMT) is a genetically heterogeneous, ultrarare mesenchymal neoplasm. Approximately 85% of IMTs are characterized by genomic rearrangements resulting in gene fusions, the most common of which involve *ALK*.[1] The resulting fusion proteins function as oncogenic drivers and are successfully targeted by tyrosine kinase inhibitors.[2] However, resistance to tyrosine kinase inhibitors invariably develops. Here, we report a case of an adult patient with metastatic, *EML4-ALK*-positive IMT whose tumor developed resistance to the ALK inhibitor crizotinib through a novel double fusion involving *PLEC-EML4-ALK*. The double fusion was associated with higher expression of *ALK* mRNA, mimicking *ALK* fusion gene amplification, a known resistance mechanism to crizotinib in NSCLC.[3] To our knowledge, this is the first report of the formation of a double fusion as a mechanism of resistance to targeted therapy.

Case Presentation

A 60-year-old female patient presented to her local hospital in December 2021 with a several-month history of worsening cough and fever. Computed tomography (CT) of the chest revealed a large right pulmonary upper lobe mass, which was managed with surgical resection. The postoperative histologic examination was diagnostic

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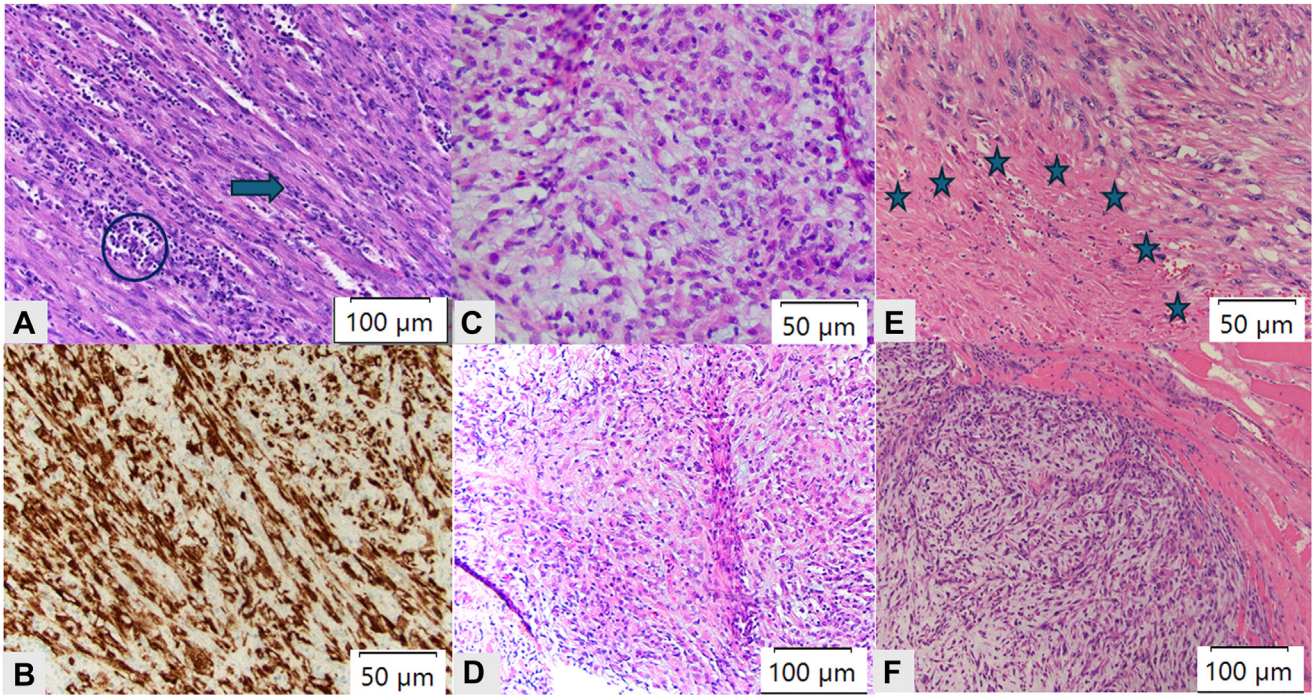

Figure 1. (*A*) Primary (treatment-naive) tumor from RUL lung (H&E 10×), March 2022. Tumor with enlarged nuclei (arrow) and infiltrating lymphocytes and plasma cells (circle) are visible. (*B*) ALK immunohistochemical staining in primary tumor from lung (20×). (*C*, *D*) Needle biopsy from right supraclavicular fossa (crizotinib-resistant) mass (H&E 20× and H&E 10×, respectively) February 2023. (*E*) Metastasectomy right apical lung, (H&E 20×), June 2023. Visible necrosis post-alectinib treatment (stars). (*F*) Metastasectomy right supraclavicular fossa mass (H&E 10×), June 2023. Tumor tissue post-alectinib treatment with visibly less treatment-induced necrosis compared with lung lesion (1*E*) and evidence of infiltration of the surrounding skeletal muscle. H&E, hematoxylin and eosin; RUL, right upper lung.

for IMT with positive surgical margins and evidence of pleural invasion (Fig. 1*A* and *B*). Three months after surgery, the patient presented with fever, cough, and weight loss. Imaging with CT and positron emission tomography confirmed the presence of locally recurrent disease and metastatic spread to the ileum, right supraclavicular fossa, and soft tissue of the right thigh. The ileal metastasis was removed for symptomatic relief and the patient was referred to our institution.

An RNA-based next-generation sequencing gene fusion assay (ArcherDx FusionPlex Solid Tumor, Integrated DNA Technologies, Inc., Coralville, IA) performed on the ileal metastasis identified an *EML4-ALK* fusion (*EML4* exon 2 to *ALK* exon 20). Reads supporting this fusion revealed fragments extending to *EML4* exon 1 (Fig. 2). The patient commenced treatment with the ALK inhibitor crizotinib (250 mg twice daily) with rapid clinical benefit and symptom resolution. The tumor remained stable by Response Evaluation Criteria in Solid Tumors 1.1 at all sites of disease until the completion of cycle 5 of treatment; at that time, the patient returned to the clinic with enlargement of the right supraclavicular fossa mass, causing difficulty swallowing and speaking. CT confirmed isolated disease progression of the supraclavicular mass whereas all other sites remained stable.

Next-generation sequencing-based fusion testing performed on a biopsy of the progressing supraclavicular lymph node lesion detected a *PLEC-EML4-ALK* "double fusion" (multiple splicing isoforms involving various regions of *PLEC* fused to exon 2 of *EML4*—all anticipated to be in-frame) (Figs. 1C and D, 2). Because reads supporting the *EML4-ALK* fusion in the pre-crizotinib sample revealed splicing of *EML4* exon 1 to exon 2, this additional fusion event was determined to be de novo and associated with crizotinib resistance. Quantitative reverse-transcriptase polymerase chain reaction revealed a roughly fourfold increase in *ALK* expression in the crizotinib-resistance sample (data not provided). No other potential mechanisms of resistance, including additional fusions, gene mutations, or copy number changes were observed. On progression, crizotinib was discontinued (after 24 wk of treatment), and the patient was started on the more potent ALK inhibitor alectinib (600 mg twice daily) with prompt and excellent clinical benefit. Imaging with CT and positron emission tomography after three cycles confirmed partial response per Response Evaluation Criteria in Solid Tumors 1.1. The patient underwent concurrent resections of the right supraclavicular fossa

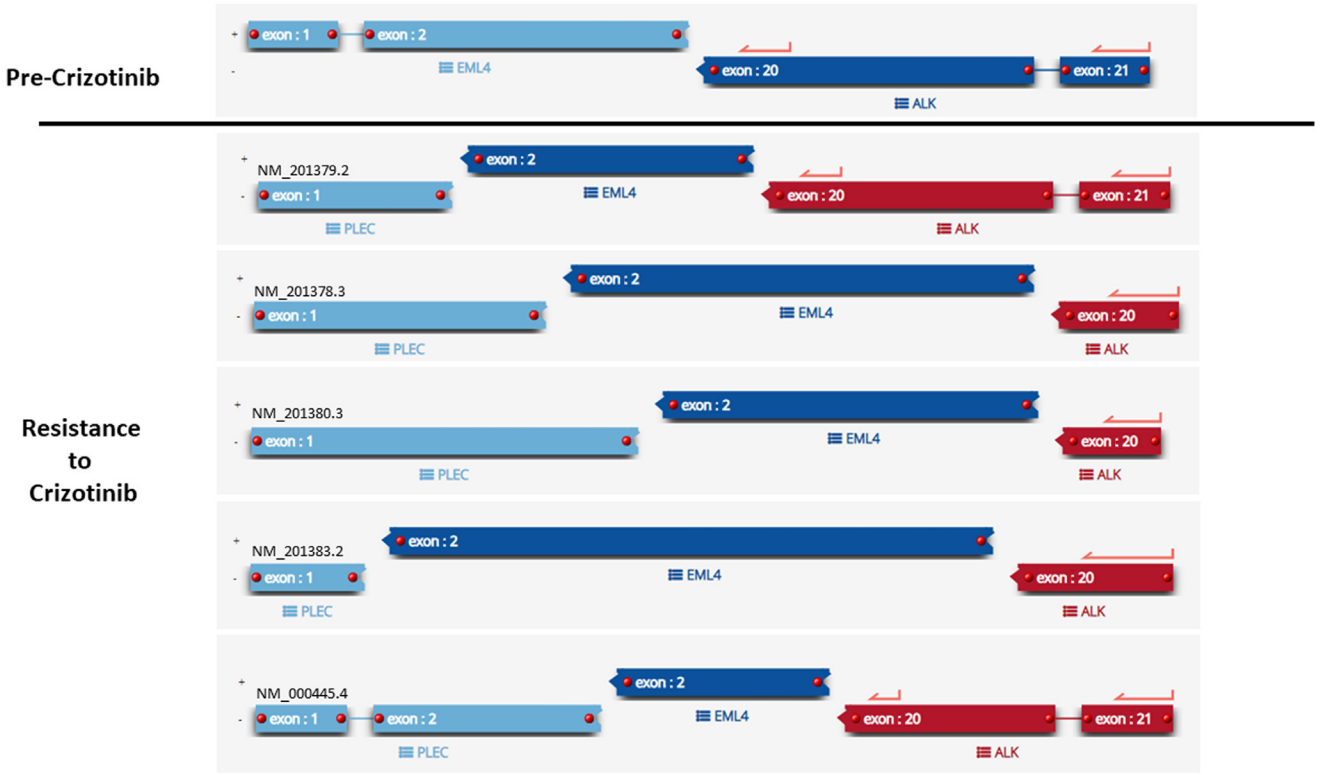

Figure 2. NGS-based gene fusion assessment. The pre-crizotinib sample (top) and post-crizotinib-resistance sample (bottom) were both assessed by means of the ArcherDx FusionPlex Solid Tumor NGS assay as part of clinical management. Screenshots from the ArcherDx Analysis user interface that schematically represent detected gene fusions are illustrated. In the pre-crizotinib sample, a standard *EML4*(exon 2)-*ALK*(exon 20) fusion was detected, with clear evidence of reads extending from *EML4* exon 2 into *EML4* exon 1. In the post-crizotinib-resistance sample, evidence of a secondary rearrangement fusing *EML4* exon 2 to *PLEC* was observed. Multiple unique isoforms of the *PLEC-EML4-ALK* double fusion were detected in which *EML4* exon 2 fused to a different exon of *PLEC* (specific reference sequence identifiers for *PLEC* are listed for each isoform above the *PLEC* component of fusion), likely indicative of alternative splicing. All isoforms were confirmed to be in-frame. NGS, next-generation sequencing.

mass and right apical lung mass. Pathologic assessment revealed differential responses to alectinib with enhanced necrosis in the lung mass (Fig. 1*E* and *F*). The *PLEC-EML4-ALK* fusion was detected in the lung mass. *EML4-ALK* was observed in the supraclavicular mass; however, the RNA was too fragmented to determine whether this was the typical *EML4-ALK* or *PLEC-EML4-ALK* double fusion. In addition, the supraclavicular mass revealed the well-described *ALK* resistance mutation p.G1202R. The disease rapidly progressed after surgery while alectinib treatment continued with local recurrence of the supraclavicular mass and evidence of new brain metastases. Alectinib was discontinued (after 26 wk of treatment), and the patient commenced radiation therapy to the brain lesions and lorlatinib (100 mg daily) treatment. The first scan after two cycles of lorlatinib revealed widespread disease progression. Because of worsening general conditions, the patient did not receive further treatment (15 total wk of lorlatinib treatment) and ultimately died 22 months after the initial diagnosis (Fig. 3).

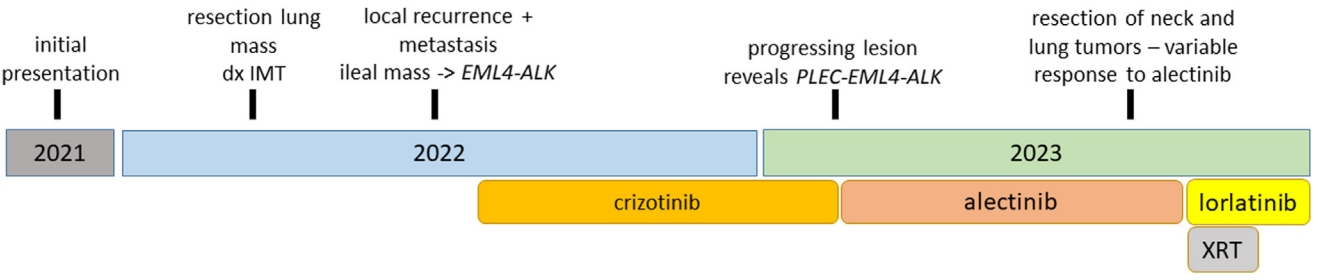

Figure 3. Timeline of clinical course. dx, diagnosis; IMT, inflammatory myofibroblastic tumor; XRT, radiation therapy.

Discussion

Fusion-targeted therapies have revolutionized care for patients with gene fusion-positive cancers, including IMTs. However, acquired resistance remains a therapeutic challenge. The patient presented here was affected by an aggressive *EML4-ALK*–positive IMT that initially responded to crizotinib. Resistance then occurred by means of an additional rearrangement event that created a "double fusion" that led to higher expression of the fusion product.

Genomic amplification of the *ALK* fusion gene is a known mechanism of resistance to crizotinib in NSCLC.[3] Presumably, the resulting overexpression of the ALK fusion protein reduces the ability of crizotinib to saturate its target, leaving sufficient fusion protein unbound by the drug to drive proliferative signaling. In NSCLC, more potent ALK inhibitors have been demonstrated to overcome the amplification-mediated resistance.[4] In this case, the double fusion resulted in a roughly fourfold increase in *ALK* fusion gene mRNA expression, potentially due to stronger activity of the *PLEC* promoter compared with the *EML4* promoter. We hypothesize that this increase in expression mimicked *ALK* fusion gene amplification. The rapid clinical benefit when the patient was switched to the more potent ALK inhibitor alectinib further supports this hypothesis. Limitations to this study include lack of molecular information on the primary tumor, lack of confirmation of an increase in ALK protein expression in the crizotinib-resistant tumor, and lack of transcriptome- and exome-wide assessment of the mechanism of resistance.

Conclusion

This case study represents the first report of an *ALK* "double-fusion" that resulted in overexpression as a mechanism of acquired resistance to crizotinib.

CRediT Authorship Contribution Statement

Alessandra Maleddu: Conceptualization, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing.

Trista K. Hinz: Formal analysis, Investigation.

Margaret A. Black: Investigation, Writing – original draft.

Dara L. Aisner: Supervision, Writing – original draft.

Carrie B. Marshall: Investigation, Writing – original draft.

Anthony D. Elias: Supervision, Writing – original draft.

Breelyn A. Wilky: Supervision, Writing – original draft.

Lynn E. Heasley: Supervision, Writing – original draft.

Kurtis D. Davies: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

Disclosure

Dr. Maleddu has served on advisory boards for Springworks and Deciphera. Dr. Aisner has received consulting fees from Jannssen, Nuvation Bio, and AbbVie, honoraria from Peerview Institute for Medical Education and Creative Educational Concepts, and travel support from Integrated DNA Technologies. Dr. Wilky has received research funding from Exelixis, consulting fees from Springworks, Deciphera, Epizyme, Adcendo, Polaris, Boehringer Ingelheim, AADi, and InhibRx, and travel support from Agenus. All other authors declare no conflict of interest.

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