CASE BASED DISCUSSIONS

A patient with complex multiple genomic *ALK* alterations

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Received 16 October 2015
Revised 11 December 2015
Accepted 22 December 2015
Published Online First 2 February 2016

To cite: Liu X, Rice SJ, Jamis-Dow CA, *et al*. *Thorax* 2016;**71**:383–385.

INTRODUCTION

Lung cancer is a disease with a heterogeneous complement of mutations.[1] Although point mutations and deletions are among the most common types of mutations in lung cancer, translocations in the *ALK* gene, which occur in approximately 5% of lung adenocarcinomas, exist predominantly in non-smokers. *ALK* translocations gained notoriety recently because they are targets for the kinase inhibitor crizotinib (Xalkori).[1] Crizotinib has exhibited profound efficacy and has obtained FDA (United States Food and Drug Administration) approval for use in patients with non-small cell lung cancer (NSCLC) with *ALK* translocation, as determined by a break-apart fluorescent in situ hybridisation (FISH) assay.[2] Here we report a case where a patient with a complicated *ALK* genotype, including an *EML4-ALK* variant 5a/b translocation and *ALK* tandem duplication with response to crizotinib treatment.

CASE PRESENTATION

A 70-year-old female patient with complaints of progressive dyspnoea underwent a chest CT scan, which revealed a 6 cm spiculated mass with extrinsic compression of the trachea and the right main stem bronchus. PET-CT (positron emission tomography-CT) scan confirmed the findings of the CT scan and the mass was metabolically active, and there was presence of metastases in the lymph nodes. Histological evaluation along with immunostaining revealed primary lung adenocarcinoma. An MRI of the brain revealed nodular intraparenchymal metastatic deposits in the left cerebellar hemisphere, left inferior cerebellar vermus and the left superior parietal cortex. The patient received palliative radiation to the lung mass and gamma knife treatment for the brain metastasis. Genetic profiling performed on the biopsied tissue using Foundation Medicines' comprehensive genomic profiling, reported alterations in eight genes including premature stop codons in *TP53*, *ARID1A*, *BRD4* and *SETD2*, single nucleotide polymorphisms in the *TERT* promoter, *MAP2K4*, *U2AF1* and an *EML4-ALK* translocation. Closer evaluation of the sequencing data showed a complex genomic environment around the *ALK* gene, which included a rare *EML4-ALK* variant 5a/b translocation (<2% of *EML4-ALK* translocations in lung cancer[2]) and a tandem duplication of the *ALK* gene with breakpoints in *ALK* exon 19 and *LOC728730* intron 3 (figure 1A teal and green bar, respectively). The tissue was subsequently submitted for FISH analysis to confirm an *EML4-ALK* translocation. Approximately 65% of the cells were positive for an *EML4-ALK* translocation, which correlated with the sequencing results (figure 1B, C).

Based on the presence of the *EML4-ALK* translocation, the patient was treated with crizotinib (Xalkori) 250 mg daily. The patient tolerated the treatment very well; no adverse events were reported. A follow-up CT after 5 months of therapy showed a partial response to the crizotinib treatment indicated by a decrease in tumour size from 5.9×3.2 cm, following radiation therapy, to 2×1.4 cm post-treatment (figure 1D). The patient continues to respond to the crizotinib treatment, but brain metastases have recurred, which is known to occur despite crizotinib treatment.[3] The patient will undergo a second round of gamma knife therapy for the brain metastases.

DISCUSSION

Crizotinib has achieved remarkable clinical success in patients with lung cancer with *ALK* translocations. *ALK* translocations can occur with various partners, but the most common translocation in lung cancer occurs with *echinoderm microtubule-associated protein like 4* (*EML4*) protein. Many *EML4-ALK* translocations variants have been reported, each involve the positioning of the *ALK* kinase domain (exons 20–29) downstream of *EML4* gene at different breakpoints. Although the kinase domain of ALK drives signalling, the EML4 coiled–coiled domain is thought to be important for homodimerisation and stability of the fusion protein. Preclinical models evaluating the most common *EML4-ALK* variants 1, 2 and 3a/b have indicated that these variants can show differential sensitivities to crizotinib.[4] A recent small (n=61) retrospective study reported no difference in objective response rate or progression-free survival with crizotinib treatment between the different *EML4-ALK* variants.[5] It is clear that further clinical investigations are warranted to provide a more clear view of how *EML4-ALK* variant responds to ALK-inhibitors.

Here we report the case of a patient with lung adenocarcinoma who had a tumour displaying a complex genomic organisation around the *ALK* gene as determined by comprehensive genomic profiling. FISH analysis correlated with the genomic profiling by detecting an *ALK* translocation. Based on the sequencing and FISH data, crizotinib seemed to be an ideal treatment for this patient. However, little was known regarding how the genetic complexity of the tumour would affect its response to

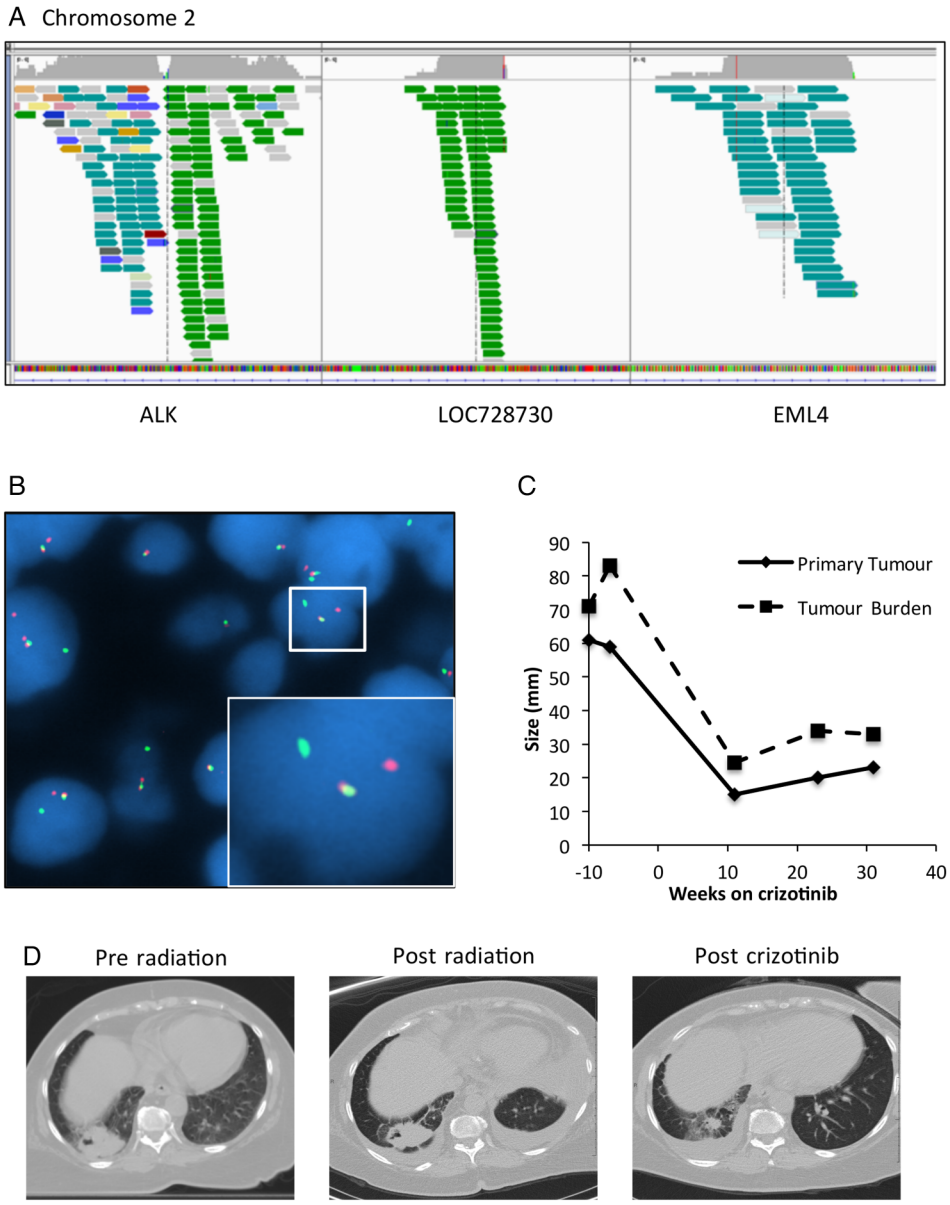

Figure 1 Molecular characterisation of the lung adenocarcinoma case presented. (A) Sequencing data showing the EML4-ALK translocation (teal reads in left and right panels) and tandem duplication (green reads in left and centre panels). (B) Fluorescent in situ hybridisation analysis performed at Mayo Cytogenetics Core Facility. The white box indicates the area of the inlay, which shows a cell with a normal ALK allele (yellow, combined green and red probes) and a translocated ALK allele (separate green and red probes). (C) A graph showing the patients response to crizotinib. Zero indicates the day the patient started crizotinib treatment. (D) The patient's response to crizotinib therapy. A representative CT scan of the patient's EML4-ALK-positive tumour prior to radiation treatment (preradiation), following radiation treatment (postradiation) and 5 months after crizotinib treatment (post-crizotinib).

crizotinib. It has been noted that increased *ALK* copy number is associated with crizotinib resistance, therefore the magnitude and duration of the patient's response to the treatment was unclear in spite of being positive for an *ALK* translocation by FISH. Prior to this report, little was known about how a tumour with the *EML4-ALK* variant 5a/b translocation, which occurs in <2% of lung cancers,[2] would respond to crizotinib in vivo. This variant is among the shortest, fusing *EML4* exon 2 to exon 20 of the *ALK* gene. Animal data suggests that this variant has the ability to transform cells and should respond to the treatment[2]; however, this case was complicated by the presence of tandem duplications within the *ALK* gene.

This case highlights the applicability of next-generation sequencing (NGS) for identifying patients carrying tumours with *EML4-ALK* translocations and other genetic variations in the *ALK* genomic region. Although FISH is an approved standard method to identify *ALK* alterations, NGS offers expanded functionality by detecting translocations in addition to sequence information from multiple other genomic regions, which could be applicable to the treatment of patients. In the present case, eight genetic alterations were identified in the patient by NGS compared with only *ALK* rearrangement by FISH. The additional mutation information could help to direct treatment if alterations in genes known to provide resistance to crizotinib are detected. Additionally, tumours that are negative for *ALK* translocations by FISH can actually possess crizotinib sensitive *ALK* translocations. Here, we report that a tumour with a complex *ALK* genotype, identified by NGS, was sensitive to crizotinib.

It should be noted that although the primary tumour responded to the crizotinib, new brain metastases developed during the course of treatment. Development of brain metastases is a common occurrence despite crizotinib treatment, which may be a result of the drugs poor blood–brain barrier permeability.[3] While on crizotinib, patients can develop new brain metastases as a result of acquired drug resistance.[3]

Acknowledgements We would like to thank Darlene Knutson, Sara Kloft-Nelson and Dr Patricia T. Greipp, DO, from the Mayo Cytogenetics Core, who performed the ALK FISH analysis, and Eric Sanford of Foundation Medicine.

Competing interests SA is an employee and has equity interest in Foundation Medicine.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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