Case Report

Complex genetic alterations contribute to rapid disease progression in an *ALK* rearrangement lung adenocarcinoma patient: a case report

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Abstract: Anaplastic lymphoma kinase tyrosine kinase inhibitors (ALK-TKIs) have been found to significantly improve the quality of life and survival in *ALK*-positive non-small cell lung cancer (NSCLC) patients. However, the duration of responses is limited by drug resistance. Genetic heterogeneity of *ALK*-positive tumors could potentially explain the differences in individual patient outcomes. We performed next-generation sequencing (NGS) on plasma samples, pleural effusion samples, and tissue re-biopsy obtained at various treatment milestones from an *ALK* rearrangement lung adenocarcinoma patient undergoing targeted therapy. The liver metastases of the *EML4-ALK* NSCLC patient presented rapid progression after 3.5 months of alectinib, while the other lesions showed good partial response. Targeted NGS identified the newly emerged *MET* amplification except for *EML4-ALK* in plasma ctDNA and liver lesions. Subsequently, a clinical benefit was achieved one month after the commencement of crizotinib, a dual ALK and MET inhibitor; however, the patient experienced disease progression another month later. Several rounds of ALK-TKI combination therapy were tried but failed. Concurrent genetic alterations, including loss-of-function mutations in *FBXW7* and *MLL3*, may mainly contribute to poor prognosis in the patient. It highlighted the molecular profiling by using NGS can be useful in identifying the heterogeneity across lesions and the resistance mechanism of targeted treatments.

Keywords: Anaplastic lymphoma kinase (ALK); *MET* amplification; resistance mechanism; next-generation sequencing (NGS); case report

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Introduction

Anaplastic lymphoma kinase (*ALK*) gene rearrangements have been reported in approximately 5% of non-small cell lung cancers (NSCLCs) and function as oncogenic driver event (1,2). The second-generation ALK inhibitor alectinib demonstrated superior efficacy and lower toxicity compared to the first-generation ALK inhibitor crizotinib in advanced *ALK*-rearranged NSCLCs (3), establishing alectinib as the new standard first-line therapy. The sequential therapy of ALK-tyrosine kinase inhibitors (TKIs) allows long survivals up to more than 7 years (4,5). Despite responding to ALK-TKIs initially, rapid progression may have occurred, thereby limiting the prolonged effectiveness of ALK-TKIs. Genetic heterogeneity of *ALK*-positive tumors could potentially explain the differences in individual patient outcomes (6). Here, we present a case of an *ALK*-rearranged

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NSCLC patient who had initially benefited from alectinib and crizotinib before the disease rapidly progressed. We identified some concurrent mutations by panel sequencing which possibly conferred resistance to ALK-TKIs. We present the following case in accordance with the CARE reporting checklist (available at https://dx.doi.org/10.21037/tcr-20-3473).

Case presentation

A 39-year-old man, a former smoker (10 cigarettes/day), with no significant past medical and family history, presented to our hospital for chest and back pain that had persisted for 2 weeks. A chest computed tomography (CT) scan revealed a lesion in the upper lobe of his right lung. Pathologic analysis of bronchoscopy biopsy confirmed lung adenocarcinoma in April 2019. The clinical stage was IV due to extensive metastases (bilateral lung, liver, retroperitoneal lymph nodes, etc.). The tumor tissue was sent for genomic testing using targeted DNA sequencing (1,021 cancer-related genes) as well as pleural effusion circulating tumor DNA (ctDNA) (Supplementary Method), as described previously (7,8). Echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* (E13:A20) fusion was identified with a mutant allele frequency (AF) of 15.5% in tissue and 31.2% in ctDNA (*Table 1*). The patient received alectinib (600 mg twice a day) since May 2019 and achieved partial response to liver metastasis [Response Evaluation Criteria in Solid Tumors (RECIST) v1.1] after 1 month (*Figure 1*). However, the CT scan showed a dramatic progression of liver metastasis after 3.5 months of treatment, while the other lesions showed good partial response. Then the patient was treated with radiofrequency ablation of liver tumors, but the liver lesions continued to grow rapidly. Meanwhile, the next-generation sequencing detected both *EML4-ALK* rearrangement and *MET* amplification in plasma ctDNA and liver lesions (*Table 1*). Crizotinib (250 mg twice a day), a dual inhibitor of ALK and MET, was then administered in September 2019. Symptoms such as chest and back pain significantly improved within the first month, and the diameter of the liver metastases decreased from 66 to 50 mm. But unfortunately, the patient developed multiple low-density nodules in the liver in November 2019. To explore new therapeutic strategies, we used ctDNA analysis to track the evolution of resistance during treatment. The result revealed retained *EML4-ALK* fusion (AF =14.5%) without the amplification of *MET* (*Table 1*). Since the patient experienced further disease progression with new bone lesions, pemetrexed, cisplatin, and bevacizumab were given as third-line treatment. After two cycles of chemotherapy, liver metastases, especially those in the left lobe, progressed again, so the patient switched to a combination treatment of alectinib and cabozantinib (60 mg twice a day), a multikinase inhibitor with activity against MET, in March 2020. He achieved stable disease (SD) after two months, and cabozantinib discontinued due to grade 3 hand-foot syndrome (HFS). At that time, a second biopsy specimen showed low PD-L1 expression with a tumor proportion score (TPS) of 1–2% by immunohistochemistry (Dako 22C3). The patient had shown increased ctDNA gene mutation frequencies while the amplification of *MET* was still not detected (*Table 1*). Based on the results from the phase III ALTER-0303 trial (Clinical Trial Registry ID: NCT 02388919) of anlotinib in China, anlotinib (12 mg) and alectinib were administered, but the patient's condition continued to deteriorate. Repeated analyses indicated the presence of an inactivating mutation in *FBXW7* (p.M268Dfs*18) during the disease (*Table 1*) which may be sensitive to mTOR inhibitor (9). After a discussion with the patient and approval from his insurer, he was treated with lorlatinib (100 mg) and everolimus (10 mg) in May 2020. After 20 days of treatment, the diameter of the liver metastases decreased from 225 to 167 mm. However, the disease had substantially progressed and the patient died on June 15, 2020. *Figure 1* illustrated the flow of treatments and image evaluation.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

Discussion

Though the number of *ALK*-rearranged lung cancer patients is relatively small, multiple ALK-TKIs have a relatively longer progression-free survival (PFS) period and have been approved for clinical use. Alectinib, a second-generation ALK-TKI, improves prognosis of treatment-*naive ALK*-positive NSCLCs, with an objective response rate of 82.9% and median PFS of 34.8 months (3,10). However, resistance to TKIs is inevitable and the mechanism of acquired resistance to alectinib in patients with *ALK* rearrangement has not yet been completely identified. In our case, the patient showed a mixed response to liver metastasis and primary lung tumors after initial alectinib treatment

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Table 1 Results of next-generation sequencing during treatment.

Gene	Alteration	Amino acid changes/ functional area	Variant allele frequency/copy number							Significant
			Baseline		Resistance to Alectinib		Resistance to Crizotinib	Alectinib plus Cabozantinib Progression		
			Lung tumor	Pleural effusion supernatant ctDNA	Liver tumor	Plasma ctDNA	Plasma ctDNA	Liver tumor	Plasma ctDNA	
TP53	SNV	p.N131del	13.00%	31.80%	45.40%	25.10%	15.40%	42.80%	59.20%	VUS
SETD2	SNV	p.E1115*	7.40%	20.00%	38.90%	17.50%	10.30%	32.40%	46.60%	Likely pathogenic
MLH1	SNV	p.I216V	7.90%	18.00%	25.00%	14.20%	11.20%	39.90%	50.00%	VUS
FBXW7	SNV	p.M268Dfs*18	13.00%	16.90%	40.40%	20.30%	7.80%	23.40%	29.60%	Likely pathogenic
POLD1	SNV	p.R910W	5.20%	12.50%	15.80%	10.20%	5.80%	17.40%	17.60%	VUS
MLL3	SNV	p.W430*	ND	12.40%	ND	ND	5.10%	21.70%	33.60%	Likely pathogenic
EML4-ALK	Fusion	E13; A20	15.50%	31.20%	44.70%	19.40%	14.50%	35.50%	53.60%	Pathogenic
ALK-ZFP36L2 (intergenic)	Fusion	A19; intergenic	8.50%	20.10%	26.00%	13.30%	10.50%	21.30%	28.60%	VUS
MET	CNV		ND	ND	14	7.2	ND	ND	ND	Pathogenic

ND, not detected.

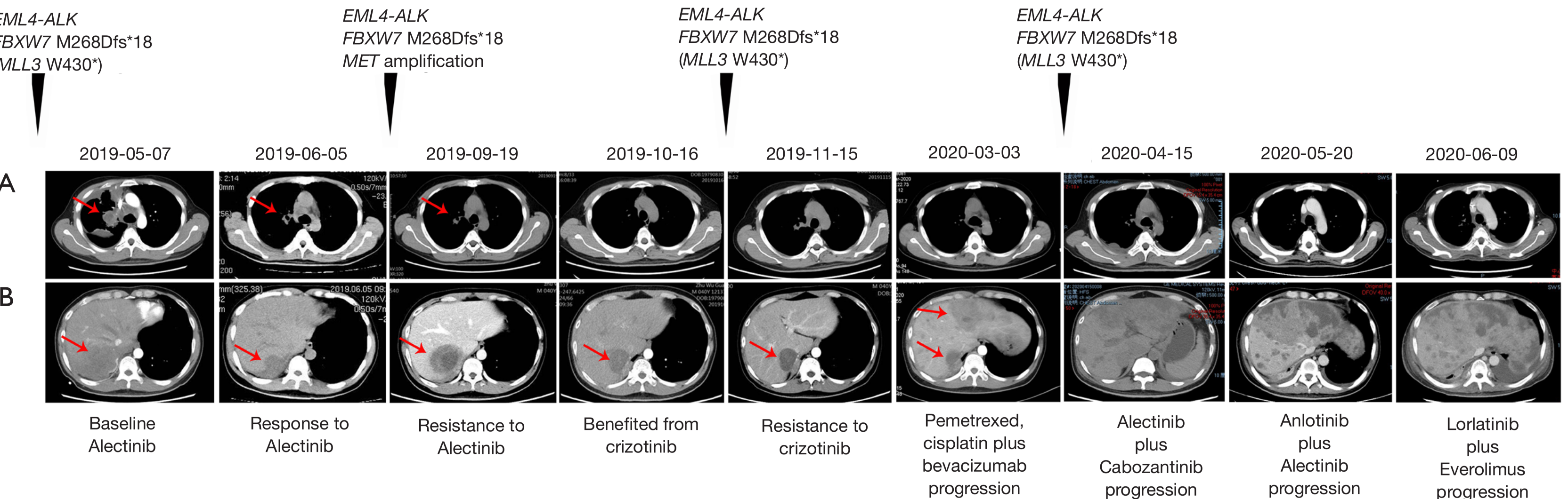

Figure 1 Clinical response to ALK-TKIs therapy of primary and liver metastasis lesions (relevant changes indicated by arrows). (A) Computed tomography (CT) scans of the chest from May 2019 to June 2020; (B) Liver CT scans from May 2019 to June 2020.

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(*Figure 1*). This phenomenon may attribute to intertumoral genetic heterogeneity. To clarify the resistance mechanism, a panel sequencing was performed in the liver metastasis. In addition to the *EML4-ALK* fusion previously discovered in the primary lesion, *MET* amplification was detected. Previous studies have found evidence that cMET activation through *MET* gene amplification can potentially confer resistance to alectinib but not to crizotinib (11,12). Our case showed a clinical benefit of crizotinib despite of drug resistance that occurred rapidly after. A negative result for the amplification of *MET* was found in the cfDNA at that time, which may be due to non-shedding of the amplified *MET*, real elimination of the amplified clone, or too little ctDNA in the plasma sample. Considering the absence of the amplification of *MET* was confirmed in rebiopsies from hepatic biopsies after the progression of alectinib and cabozantinib, we cannot exclude that crizotinib caused or contributed to the disappearance of the *MET* amplification tumor clone, as we did observe a response of some hepatic lesions to this drug.

Except for *MET* amplication, some key variants were list in table1, especially those present from baseline throughout the time course. Their pathogenicity and association with ALK-TKIs resistance were assessed using the public databases and published literature, such as ClinVar, Catalogue of Somatic Mutations in Cancer (COSMIC), and PubMed. Previous studies suggested a potential role of *TP53* mutations in poor therapeutic response and outcome in *ALK/TP53* co-mutated patients (6,13,14). The deletion of *FBXW7* in NSCLC was also found associated with poor overall survival (15,16). FBXW7 is a member of the F-box protein family, which controls proteasome-mediated degradation of oncoproteins such as rapamycin (mTOR), c-Myc, cyclin E, Mcl-1, Jun, and Notch 1 (17). *In vitro* studies showed that the loss of FBXW7 leads to resistance to gefitinib and crizotinib (16,18). Villaruz *et al.* reported a case that harbored an *FBXW7* mutation without EGFR-mutant or ALK rearrangement responded to the mTOR inhibitor temsirolimus (9). In our case, a combination of ALK-TKI and mTOR inhibitor did not seem to overcome ALK-TKI resistance, potentially due to posterior line of therapy or different choice of mTOR inhibitor. Moreover, Ye *et al.* reported a PI3K/Akt- and MEK/Erk-independent resistance mechanism by which loss of FBXW7 leads to targeted therapy resistance via stabilization of anti-apoptotic protein Mcl-1 (18), suggesting that FBXW7-mediated activation of multiple signaling pathways might contribute to ALK-TKIs resistance. Notably, FBXW7 inactivation is known to partly induce TKI-resistance by promoting epithelial-mesenchymal transition (EMT) (16). EMT has recently been implicated in resistance to lorlatinib in patient-derived cell lines (19) and to alectinib and lorlatinib in a patient (20). Interestingly, we identified a concomitant nonsense mutation in *MLL3* at relapse on Crizotinib. Some studies demonstrated the function of mutant *MLL3* in facilitating tumor EMT (21) and involvement in lung cancer development and survival (22). Together, these observations indicate that EMT induced by the deletion of FBXW7 and MLL3 may represent the main mechanism of resistance to ALK-TKIs. There are not enough samples to confirm the presence of EMT by using markers such a positive immunostaining for vimentin and loss of E-cadherin expression. But we found that the third tumor rebiopsy taken from hepatic metastases had almost completely lost the expression of the adenocarcinoma-marker CK7 and TTF1. It is worth-noting that mutations crossing take place between *MET* and *MLL3*, revealing the resistance heterogeneity and selection of tumor evolution.

In addition to the above speculation, non-reciprocal *ALK* translocation with the retaining of the 5' region of the ALK gene was also observed in the patient samples (*Table 1*), which was reported as a poor predictive marker in first-line crizotinib-treated *ALK*-rearranged NSCLCs (23). In that study, three patients who harbored non-reciprocal/reciprocal *ALK* translocation also did not benefit from alectinib therapy after the failure of crizotinib. However, it is still poorly understood how 5'-*ALK* DNA could contribute to poor prognosis of patients.

In conclusion, we report the case of an *EML4-ALK* fusion-positive NSCLC patient, who progressed rapidly during the different lines of ALK-TKIs therapy in a year. Genetics variations concurrent with *EML4-ALK* from tissues and ctDNA during disease may be accounted for the treatment response and prognosis of the patient. Molecular profiling by using NGS can be useful for monitoring tumor heterogeneity and clonal evolution during ALK-TKIs treatment in NSCLCs. Treatment strategises for these patients need further research.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

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Supplementary Method

DNA extraction, targeted capture, and NGS

Genomic DNA of tissue samples was extracted by using the QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. 4–5 mL of plasma was used to isolate cell-free DNA (cfDNA) by using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, CA). Peripheral blood lymphocytes (PBLs) were used to extract germline genomic DNA with the DNeasy Blood Kit (Qiagen, Valencia, CA). Indexed NGS libraries were constructed from sheared DNA using the DNA Library Preparation Kit for MGISeq-2000 (BGI, Shenzhen, China). All libraries were hybridized to custom-designed biotinylated oligonucleotide probes (Roche NimbleGen, Madison, WI, USA) covering 1021 genes. DNA sequencing was performed using the MGISeq-2000 Sequencing System (BGI, Shenzhen, China) per the manufacturer's guideline.

Sequencing data analysis and variant interpretation

The clean reads were mapped to the reference human genome (GRCh37) using Burrows–Wheeler Aligner (BWA), after removal of terminal adaptor sequences and low quality data. Somatic small insertions and deletions (InDels) and single nucleotide variants (SNVs) were called with MuTect and GATK. Copy-number variants (CNVs) were identified with CONTRA 2.0.8 software, and structural variants (SV) were identified using BreakDancer. All final candidate variants were manually verified in the Integrative Genomics Viewer (IGV).

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