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CASE REPORT

Patient harboring a novel *PIK3CA* point mutation after acquired resistance to crizotinib in an adenocarcinoma with *ROS1* rearrangement: A case report and literature review

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Abstract

ROS1 rearrangement occurs in 1–2% of non-small cell lung cancer (NSCLC) cases. These patients would benefit from treatment with the anaplastic lymphoma kinase inhibitor, crizotinib; however, resistance to crizotinib inevitably develops in such patients despite an initial response. The mechanism of acquired resistance to crizotinib in patients with NSCLC with *ROS1* rearrangement has not yet been identified. Herein, we report a case of a 66-year-old woman diagnosed with adenocarcinoma. PCR revealed no *EGFR* or *ALK* mutations. After the patient underwent several rounds of chemotherapy, crizotinib was administered. The disease explosively progressed six months later. A novel *PIK3CA* gene point mutation (p.L531P) was detected by next generation sequencing. This case is the second report of bypass activation conferred crizotinib resistance in a patient with NSCLC with *ROS1*-rearrangement, but is the first to confirm that activation of the *mTOR* signaling pathway leads to acquired crizotinib resistance.

Introduction

Lung cancer has the highest morbidity and mortality worldwide. Non-small cell lung cancer (NSCLC) is the most common histologic type of lung cancer, with about 80–85% of lung cancer patients diagnosed with NSCLC.[1–3] With the rapid development of molecular diagnostic technology, our understanding of NSCLC is no longer only concerned with histological aspects, but also the molecular profile of NSCLC, which has been gradually revealed in recent years. More and more oncogenic drivers have been discovered, leading to effective targeted therapy for advanced NSCLC patients with associated oncogenic drivers. The identification of activating mutations within the kinase domain of *EGFR* has led to the widespread use of kinase inhibitors in this genetically defined subset of lung cancers.[4–6] Rearrangement occurs in another targeted gene, *ROS1*, in approximately 1–2% of NSCLC cases. The multiple targeted tyrosine kinase inhibitor (TKI), crizotinib, is highly active in patients with lung cancer who harbor this oncogenic driver.[7–9] However, as acquired resistance to EGFR-TKIs is inevitable, patients with *ROS1* rearrangement will develop acquired resistance to crizotinib after a median progression-free survival period of 19.2 months.[10] Almost all cases of cancer with associated oncogenic drivers will acquire resistance to TKIs after one to two of years initial treatment.[11,12] Drug-resistance has not only become a major limitation in the clinical application of TKIs, but is also an urgent problem affecting the survival

of advanced NSCLC patients. It is encouraging that the molecular mechanisms of acquired resistance to TKIs are gradually being identified. With developing research, new therapeutic strategies to overcome resistance are being used to prolong life in advanced NSCLC patients. The patient reported herein was diagnosed with lung adenocarcinoma and harbored a *ROS1* rearrangement. She initially showed a response to crizotinib treatment, but the disease ultimately progressed. We identified an acquired mutation in her blood that led to *mTOR* pathway activation, which conferred resistance to crizotinib.

Case report

A 66-year-old woman experiencing a cough, but no hemoptysis or fever, was evaluated. A chest computed tomography (CT) scan taken in March 2015 revealed a 24 mm tumor in the right lower lung. Space occupying lesions were found on physical examination two years previously, but no significant medical history was reported. Imaging examinations, including brain magnetic resonance imaging and positron emission tomography-CT, were normal. Blood laboratory testing showed a carcinoembryonic antigen level beyond the normal limits (Fig 1).

Pathology of a needle biopsy of the tumor on the left clavicle revealed an adenocarcinoma (April 13, 2015) (Fig 2, Table 1), and the tumor was stage IIIB. The neoplastic cells stained positive for thyroid transcription factor -1 (clone, SPT24), NapsinA (clone, OTI3E5), cytokeratin 7 (CK7; clone, EP16), and negative for P63 (clone, UMAB4), and CK5/6 (clone, D5/16B4) (all dilutions, 1:100) (Table 1). No *EGFR* or *ALK* mutations were detected by PCR in a formalin-fixed, paraffin-embedded tumor specimen. Chemotherapy was administered with pemetrexed (0.75 g) and carboplatin (550 mg) on April 15, 2015 and May 7, 2015. A CT scan revealed that the pulmonary lesions had stabilized (Fig 1). Second-line chemotherapy with pemetrexed (0.75 g), carboplatin (550 mg), and bevacizumab (375 mg) was administered from May 28, 2015 (Fig 1). Two cycles of chemotherapy later, a partial response of the pulmonary lesions was observed on a CT scan. After another two cycles of second-line chemotherapy, the patient received pemetrexed (0.75 g) and bevacizumab (375 mg) as maintenance therapy from August 24, 2015 (Fig 1). After four cycles of maintenance therapy, progression of the pulmonary lesions was observed on a CT scan on November 20, 2015.

In order to consider new therapeutic strategies, next-generation sequencing was performed on the blood sample. A variant of the *ROS1* translocation, *CD74-ROS1* fusion, was detected (Fig 3, Table 2). The patient was administered oral crizotinib from January 20, 2016, and a partial response of the pulmonary lesions was subsequently observed on a CT scan on March 3, 2016. Unfortunately, chest tightness and shortness of breath occurred in June 2016. A CT scan on July 8, 2016 showed that the pulmonary lesions had rapidly progressed (Fig 2), indicating acquired resistance to crizotinib. Nedaplatin and apatinib were respectively administered, but the patient's condition continued to deteriorate. Next-generation sequencing was carried out on blood samples once more, and a novel point mutation (p.L531P) of the *PIK3CA* gene was detected (Fig 3, Table 3).

This mutation activated the *mTOR* signaling pathway, therefore everolimus, an *mTOR* signaling pathway inhibitor, was administered, however the disease had substantially progressed and the patient died on August 16.

Discussion

Crizotinib is a kind of small molecular ATP-competitive ALK inhibitor. It blocks the c-Met signal transduction pathway by inhibiting the c-Met kinase, thereby inhibiting expression of the *ALK* fusion gene, and refraining the growth of tumor cells.[13,14] Patients with NSCLC with *ROS1* rearrangement also benefit from treatment with ALK inhibitors. *ROS1* rearrangement is commonly detected in young non-smoking patients with adenocarcinoma,[7] and is exclusive to other mutations (*EGFR* or *KRAS*).[15] Crizotinib improves prognosis in patients with NSCLC with *ROS1* rearrangement, with an objective response rate of 72% and median progression-free survival of 19.2 months. However, acquired resistance to this targeted therapy is inevitable. The mechanism of acquired resistance to crizotinib in patients with NSCLC with *ROS1* rearrangement has not yet been completely identified. It is thought to be a result of the activation of other signaling pathways or ROS1 tyrosine kinase mutations, generally.

Two types of ROS1 tyrosine kinase mutations that lead to acquired crizotinib resistance in NSCLC patients with *ROS1* rearrangement have been discovered clinically. One type lies at the gatekeeper residue of the ROS1 tyrosine kinase, such as an L2026M mutation, which interferes with the combination of ROS1 tyrosine kinase and crizotinib directly and confers crizotinib resistance.[16,17] The other is located in the solvent-front region of the kinase domain adjacent to the crizotinib-binding site, including L1951R, G2032R, D2033N, S1986Y, and S1986F, which confer resistance to crizotinib through steric interference.[16,18–22]

Researchers have also discovered other ROS1 tyrosine kinase mutations that confer crizotinib resistance in vitro. L2155S and G2101A mutations have been identified to confer crizotinib resistance in Ba/F3 cells that express cDNA that encodes to *CD74-ROS1*.[19] E1990G and F1994L mutations could lead to crizotinib resistance as well, which has been confirmed in the same cells.[17] ROS1 and ALK

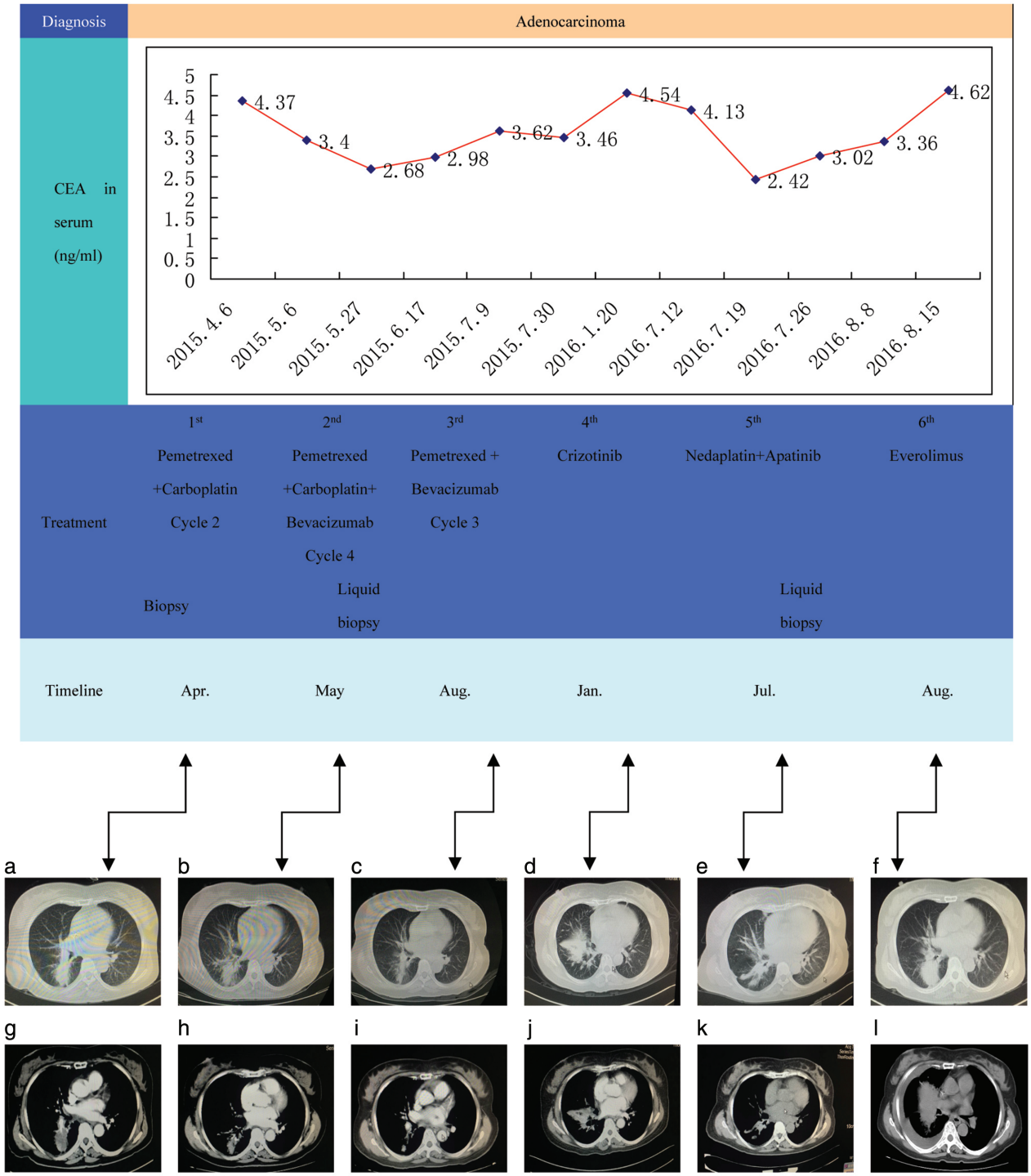

Figure 1 Lung adenocarcinoma treatment with different chemotherapy or target therapy regimens and results of monitoring the carcinoembryonic antigen (CEA) levels. The color-coded boxes to the left of each panel explain the relevant data. Lung computed tomography (CT) scans from (**a**) April 2015, (**b**) May 2015, (**c**) August 2015, (**d**) January 2016, (**e**) July 2016, and (**f**) August 2016. CT scans of the mediastinum from (**g**) April 2015, (**h**) May 2015, (**i**) August 2015, (**j**) January 2016, (**k**) July 2016, and (**l**) August 2016.

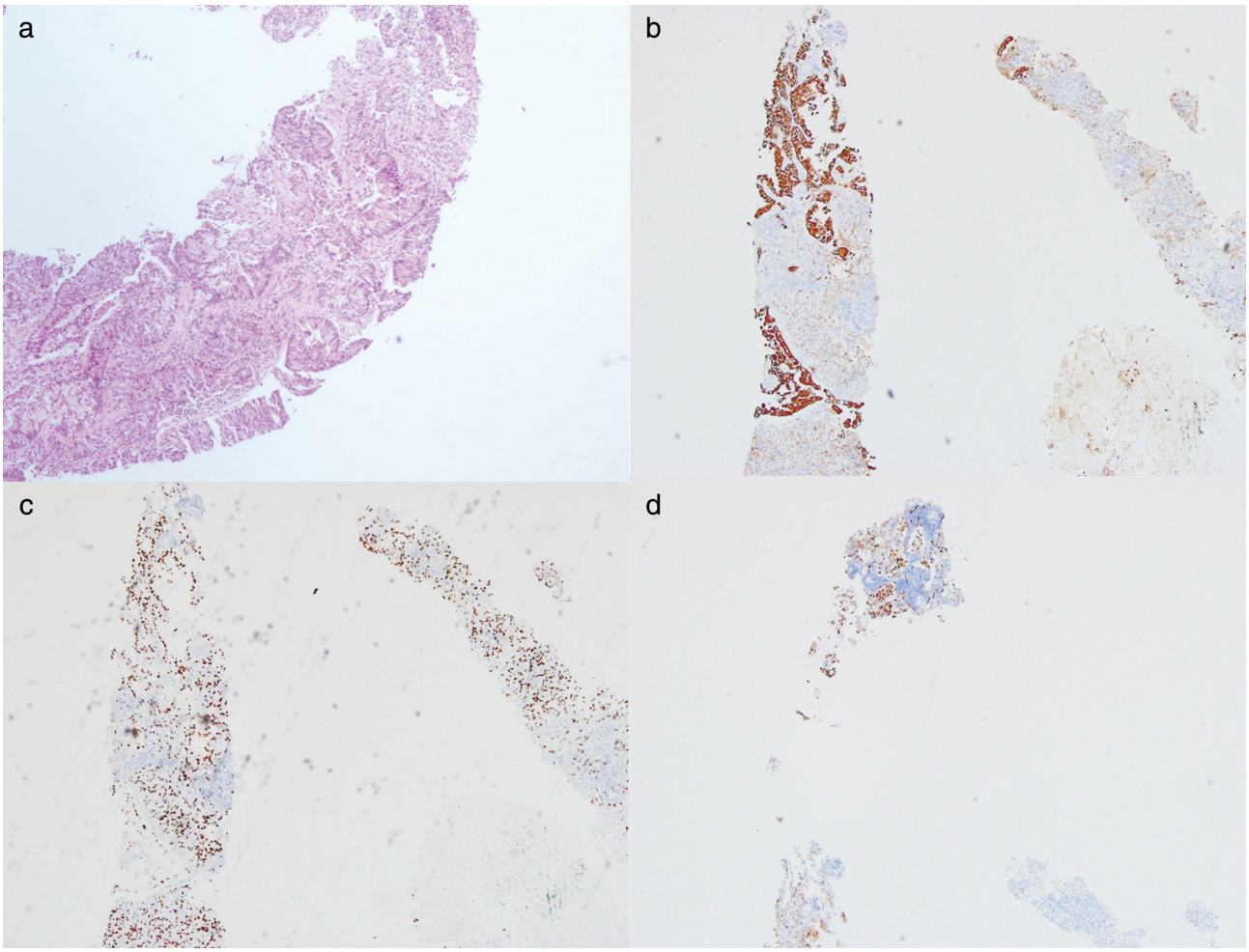

Figure 2 (**a**) Hematoxylin and eosin staining revealed lung adenocarcinoma (×100). Immunohistochemical examination revealed that the tumor cells were positive for monoclonal (**b**) anti-thyroid transcription factor-1, (**c**) anti-cytokeratin 7, and (**d**) anti-NapsinA antibodies of adenocarcinoma (×100).

Table 1 Primary antibodies used for immunohistochemical staining

Antibody	Clone	Dilution	Purchased from
TTF-1	SPT24	1:100	Zymed Laboratories, Inc.
NapsinA	OTI3E5	1:100	Zymed Laboratories, Inc.
P63	UMAB4	1:100	Zymed Laboratories, Inc.
CK7	EP16	1:100	Zymed Laboratories, Inc.
CK5/6	D5/16B4	1:100	Zymed Laboratories, Inc.

CK, cytokeratin; TTF, thyroid transcription factor.

kinase domains not only share phylogenic origin and structure homology, but also may have similar mutational hotspots and TKI sensitivity, therefore 1981Tins, L1982F, and F2004C/V mutations are predicted to cause crizotinib resistance, which are homologous to the 1151Tins, L1152R, F1174C/V mutations in the ALK gene, respectively.[22]

Multiple signaling pathways control cell growth and proliferation. Dziadziuszko *et al.* first reported that the activation of bypass signaling pathways causes an abnormal proliferation signal that leads to the malignant proliferation of cells that subsequently avoid the ROS1 pathway and confer crizotinib resistance.[23] A D816G mutation in the *KIT* gene was detected in an NSCLC patient with *ROS1* rearrangement and the disease progressed after an initial response to crizotinib. The mutation led to autophosphorylation and activation of the downstream signaling pathway. Dziadziuszko *et al.* also confirmed that the D816G mutation in the *KIT* gene conferred crizotinib resistance to the cell line, which expresses the *ROS1* fusion gene.[23] EGFR signaling pathway activation has also been reported to cause acquired resistance to crizotinib, but has only been confirmed in cell lines.[20,24] Our case is the second to demonstrate bypass activation conferred crizotinib resistance in an NSCLC patient with *ROS1* rearrangement who had initially benefited from crizotinib treatment. It is the first

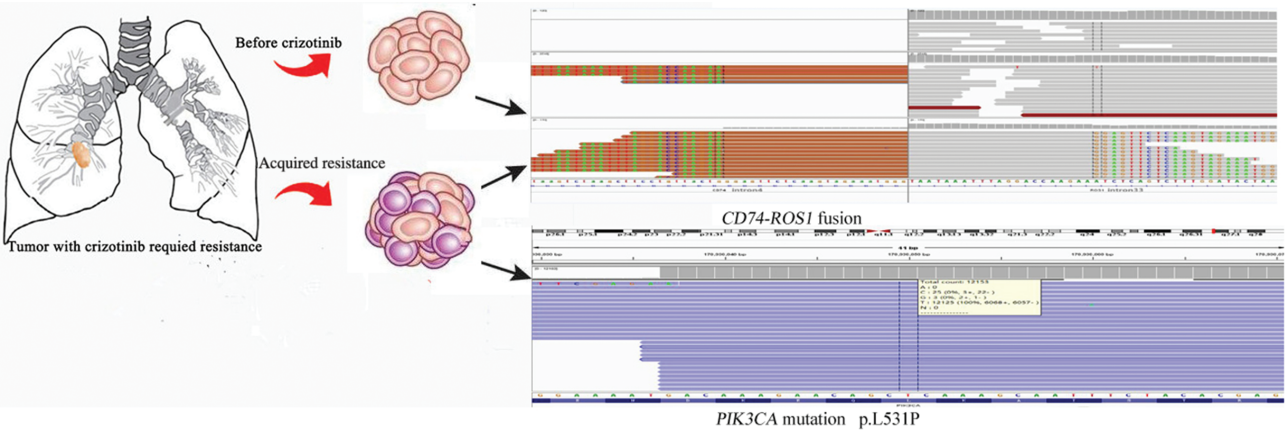

Figure 3 Schema shows the tumor with dual drivers of the *CD74-ROS1* fusion gene by next generation sequencing before crizotinib treatment, but with another driver gene-*PIK3CA* mutation (p.L531P) after acquired resistance of crizotinib.

Table 2 Liquid biopsy results identified by next-generation sequencing before crizotinib treatment

Gene	Type	Gene	Type
ROS1 fusion	*CD74-ROS1*	*TP53*	C176F
TP53	G245S	ND	ND

ND, no data.

Table 3 Gene mutation identified by next-generation sequencing after acquired resistance to crizotinib

Gene	Type	Gene	Type
ROS1 fusion	*CD74-ROS1*	*PIK3CA*	L531P
TP53	C176F	*TP53*	G245S

report to confirm that *mTOR* signaling pathway activation can lead to acquired resistance to crizotinib in a clinical setting.

There is no standardized therapeutic strategy for NSCLC patients with *ROS1* rearrangement after acquired resistance to crizotinib. Chemotherapy was used in this case once rapid disease progression occurred after targeted therapy acquired resistance, according to therapies used in *ALK* positive NSCLC patients in a similar condition. However, chemotherapy was not successful and the disease directly progressed. The administration of bevacizumab resulted in a partial response and slowed disease progression, after which apatinib, another vascular endothelial growth factor receptor-resistant medicine was used and next-generation sequencing was carried out. A novel point mutation in the *PIK3CA* gene was detected, which activated the *mTOR* signaling pathway. Consequently, everolimus, an *mTOR* signaling pathway inhibitor, was administered. However the disease had progressed too far and the patient died from circulatory failure. In conclusion, progression-free survival was five months and overall survival was 16 months.

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Disclosure

No authors report any conflict of interest.

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