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. 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. 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. 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. Almost all cases of cancer with associated oncogenic drivers will acquire resistance to TKIs after one to two years of initial treatment. 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 necrotic 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. 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, and is exclusive to other mutations (EGFR or KRAS). 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. 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.

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. E1990G and F1994L mutations could lead to crizotinib resistance as well, which has been confirmed in the same cells.

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.
kinase domains not only share phylogenetic 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 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       | UMA84 | 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.
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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Table 2 Liquid biopsy results identified by next-generation sequencing before crizotinib treatment

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

ND, no data.

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

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

ND, no data.
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