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BRAF V600E Mediates Crizotinib Resistance and Responds to Dabrafenib and Trametinib in a *ROS1*-Rearranged Non-Small Cell Lung Cancer: A Case Report

Juan Li,[a] Qifeng Wang,[b] Jun Ge,[a] Yuke Tian,[a] Wenxiu Yao[a]

Departments of [a]Medical Oncology and [b]Thoracic Radiotherapy, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu, People's Republic of China

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. *ROS1* fusion • *BRAF* V600E • Non-small cell lung cancer • Crizotinib resistance • Dabrafenib and trametinib

Abstract

Crizotinib, a multitargeted MET/ALK/ROS1 tyrosine kinase inhibitor, has been approved for the treatment of *ROS1* fusion–positive non-small cell lung cancers (NSCLCs). However, "on-target" or "off-target" resistance alterations often emerge that confer the drug resistance. Patients with *ROS1*-rearranged NSCLC who develop crizotinib resistance, especially those acquiring "off-target" resistance mutations, still lack effective therapeutic options for after crizotinib treatment. Herein, we reported a patient with stage IVb lung adenocarcinoma harboring *ROS1* fusion, who acquired a *BRAF* V600E and lost the *ROS1* fusion after progression on crizotinib. It was deduced that the V600E may originate from a subclone with an extremely low fraction that was independent of *ROS1* fusion–positive cells. The patient was subsequently treated with dabrafenib and trametinib combination and achieved a partial response lasting for more than 6 months. Our study revealed that *BRAF* V600E can confer the crizotinib resistance in *ROS1* fusion–positive NSCLC and presented the first case showing that the treatment with dabrafenib and trametinib can serve as an effective option for later-line treatment for this molecular-defined subgroup. ***The Oncologist*** 2021;26:e2115–e2119

Key Points

- Patients with *ROS1*-rearranged non-small cell lung cancer (NSCLC) who acquire "off-target" resistance mutations to crizotinib still lack effective therapeutic options for after crizotinib treatment.
- This report describes the case of a patient with *ROS1*-rearranged NSCLC who acquired a *BRAF* V600E and lost the *ROS1* fusion after crizotinib failure.
- The case was subsequently treated with dabrafenib and trametinib combination and achieved a partial response lasting for more than 6 months.
- This is the first article reporting that treatment with dabrafenib and trametinib may serve as an effective option for later-line treatment for patients harboring resistant *BRAF* V600E.

Introduction

ROS1 encodes a protooncogene receptor tyrosine kinase. Rearrangements of *ROS1* gene occur in 1%–2% of non-small cell lung cancers (NSCLCs) [1], characterizing a distinct molecular subgroup. The retained *ROS1* kinase domain fused with a partner gene often confers constitutive activation of the tyrosine kinase domain, and therefore drives oncogenesis [2]. Crizotinib, a multitargeted MET/ALK/ROS1 tyrosine kinase inhibitor (TKI), has demonstrated a promising objective response rate of 72%, median progression-free survival (PFS) of 19.3 months, and overall survival of 51.4 months in

Correspondence: Wenxiu Yao, M.D., Department of Medical Oncology, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, No. 55, Section 4, Renmin South Road, Chengdu, 610041, People's Republic of China. Telephone: 0086-028-85420847; e-mail: ywxhlx@sina.com Received April 29, 2021; accepted for publication August 18, 2021; published Online First on November 9, 2021. http://dx.doi.org/10.1002/onco.13979

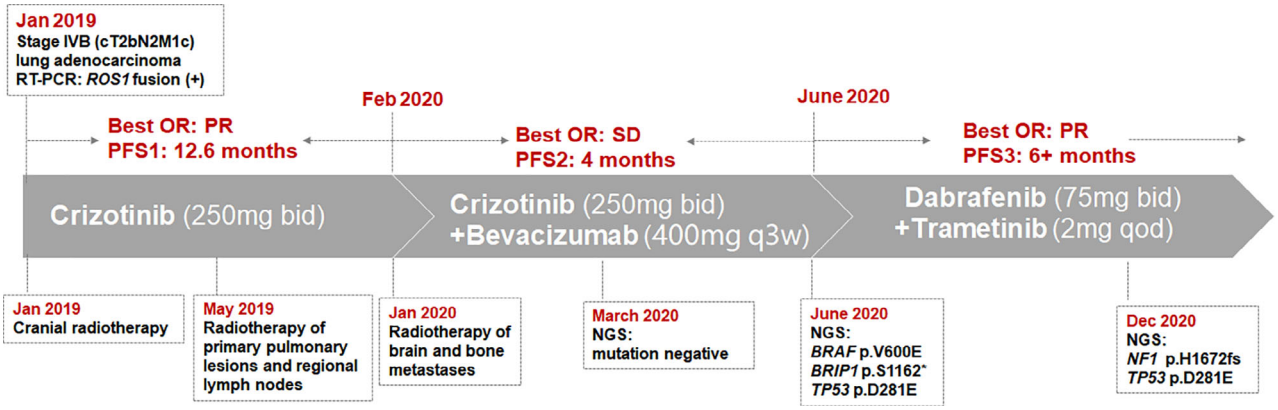

Figure 1. The timeline and treatment history of the patient.
Abbreviations: NGS, next-generation sequencing; OR, objective response; PFS, progressive-free survival; PR, partial response; RT-PCR, real-time polymerase chain reaction; SD, stable disease.

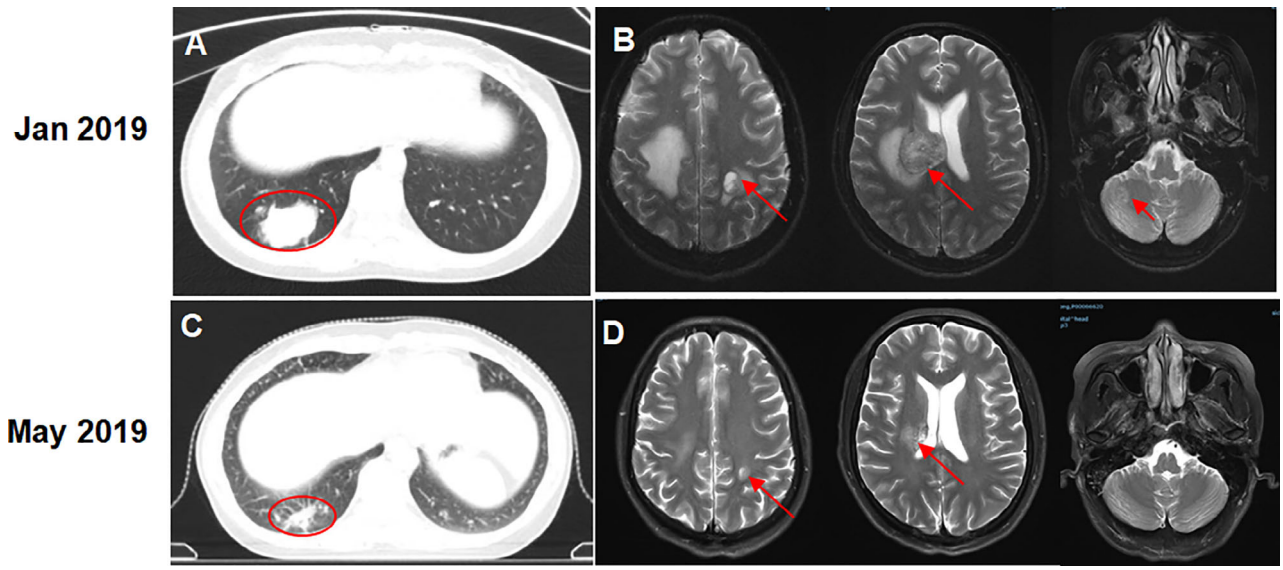

Figure 2. Responses to crizotinib treatment. **(A):** Lung lesion before treatment. **(B):** Brain metastatic lesions before treatment. **(C):** Lung lesion 5 months after treatment. **(D):** Brian lesions 5 months after treatment.

patients with advanced *ROS1* fusion–positive NSCLC [3] and now has been approved for the treatment of this molecularly-defined subgroup of NSCLCs.

Despite remarkable antitumor activity and survival advantage seen with crizotinib in patients with *ROS1* fusion–positive NSCLC, drug resistance often arises. The acquisition of ''on-target'' resistance mutations in *ROS1* has been found as the significant cause of resistance to crizotinib [4, 5]. A novel generation of TKIs against these *ROS1* mutations has been developed to overcome crizotinib resistance in *ROS1* fusion–positive NSCLCs and is currently under clinical investigation, including brigatinib, cabozantinib, ceritinib, etc. On the other hand, resistance mechanisms by activating bypass signaling pathways are less commonly reported [5, 6]. Patients who develop crizotinib resistance by acquiring these ''off-target'' resistance mutations still lack efficient therapeutic options for later-line treatment. Herein, we report a patient with stage IVb lung adenocarcinoma harboring *ROS1* fusion, who acquired *BRAF* p.V600E after progression on crizotinib. She was subsequently treated with dabrafenib and trametinib combination and achieved a durable partial response.

Case Presentation

The patient's treatment history and clinical course are summarized in Figure 1. In January 2019, a female patient, aged 44 years, was referred to our hospital and presented with coughing for 1 month as well as headache and dizziness for 2 weeks. She had no history of smoking or drinking. Her father had died from esophageal cancer. The patient had an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 2. An enhanced chest computed tomography (CT) scan revealed a soft tissue mass measuring 4.6 cm × 2.9 cm on the right lower lobe (Fig. 2A), accompanied by mediastinal and right lung hilum lymphadenopathy. Brain magnetic resonance imaging showed multiple enhancing nodules and masses in bilateral cerebral hemispheres and the right cerebellar hemisphere, with the largest lesion measuring 2.9×2.6 cm (Fig. 2B). The abdomen

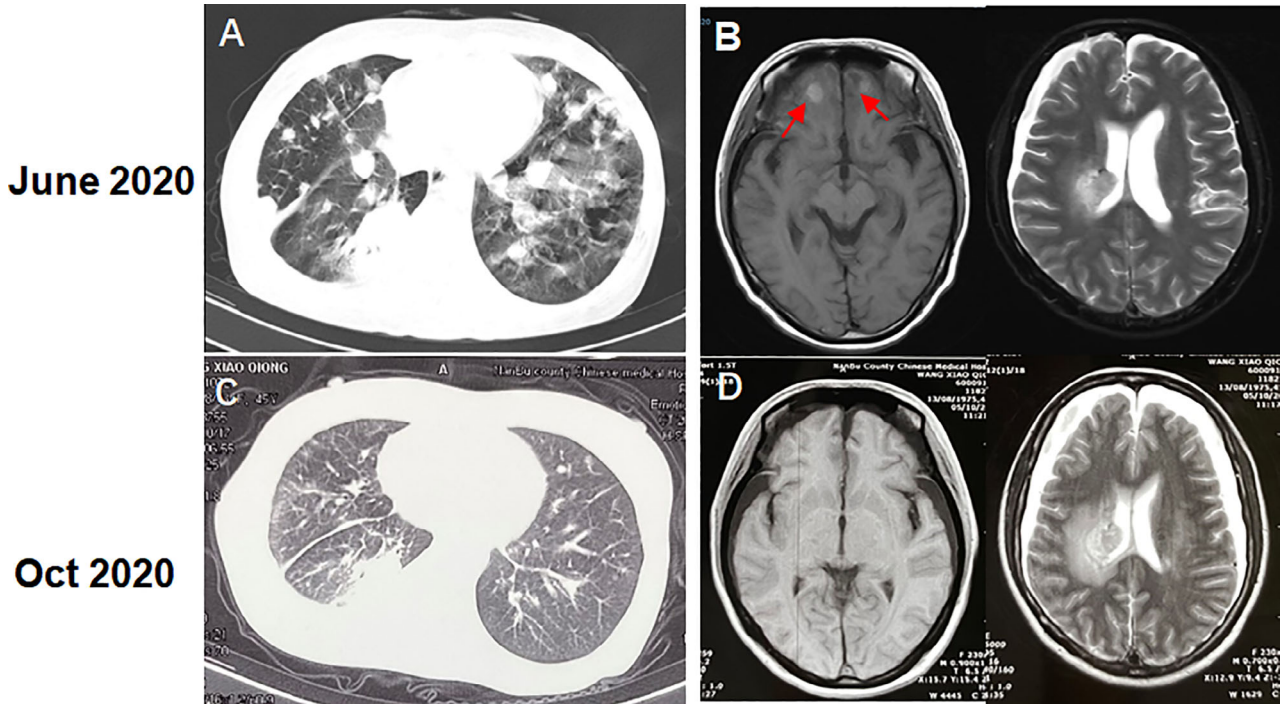

Figure 3. Responses to dabrafenib and trametinib combinatorial treatment. **(A):** Lung lesions before treatment. **(B):** Brain metastatic lesions before treatment. **(C):** Lung lesions 4 months after treatment. **(D):** Brian lesions 4 months after treatment.

enhanced CT and bone scanning detected no evidence of metastasis. The histopathological test of the biopsied pulmonary lesion indicated adenocarcinoma. The patient was diagnosed with a stage IVb (cT2bN2M1c) disease. Real-time polymerase chain reaction (RT-PCR) performed with the biopsy sample identified a *ROS1* fusion; no *EML4-ALK* fusion was detected. Amplification refractory mutation system polymerase chain reaction (ARMS PCR) was also performed but did not detect any mutations in *EGFR* exons 18–21, or *BRAF* V600.

Subsequently, the patient was treated with crizotinib (250 mg b.i.d.). Local radiotherapy was performed for brain metastatic lesions (GTV-T 50 Gy/10f), primary pulmonary lesions (GTV-T 60 Gy/20f), and regional lymph nodes (GTV-N 48 Gy/24f), respectively. The patient subsequently had symptoms alleviated. In May 2019, imaging tests revealed shrunken lung (Fig. 2C) and brain lesions (Fig. 2D) indicating a partial response (PR). The disease remained stable until January 2020, when the patient started to present with pain in her back. Imaging tests showed progression disease on brain lesions, stable disease (SD) on lung lesions, and the presence of a new metastatic lesion on the lumbar vertebra. Local radiotherapy was subsequently administrated for brain and lumbar vertebra lesions. The patient initiated a combinatorial regimen of crizotinib (250 mg b.i.d.) and bevacizumab (400 mg every 3 weeks) in February 2020 and achieved SD. Next-generation sequencing (NGS) was performed with the plasma sample collected in March 2020 using a 168-gene panel (Burning Rock Biotech, Guangzhou, China) but did not detect any mutation. After a PFS of 4 months, the patient's coughing and dyspnea symptoms were aggravated and the ECOG PS score increased to 3. Imaging tests revealed a mass (3.2 cm in diameter) on the right lower lobe and multiple nodules in both lungs (Fig. 3A) and bilateral pleural effusion and new lesions in bilateral frontal lobes (Fig.e 3B). The histopathological test of pleural effusion identified malignant cells. NGS with plasma and pleural effusion samples both identified *BRAF* p.V600E (allele frequency (AF), 0.06% in plasma; 2.85% in pleural effusion), *BRIP1* p.S1162* (AF, 1.53% in plasma; 5.68% in pleural effusion), and *TP53* p.D281E (AF, 1.17% in plasma; 6.93% in pleural effusion). *ROS1* fusion was not detected.

In June 2020, the patient started a combinatorial treatment of dabrafenib (75 mg b.i.d.) and trametinib (2 mg q.i.d.) and achieved PR 4 months later based on the shrinkage of lung nodules (Fig. 3C) and frontal lobe lesions (Fig. 3D). The coughing and dyspnea symptoms were largely relieved, and the ECOG PS improved to 1. NGS was performed with the patient's plasma sample collected in December 2020, when she remained as PR, and the results revealed the disappearance of *BRAF* p.V600E, retaining of *TP53* p.D281E (AF, 48.72%), and the emergence of *NF1* p.H1672fs (AF, 0.36%). The disease remained stable as of submission of the manuscript. The patient achieved a PFS of 6 months and counting.

Discussion

Several point mutations in the *ROS1* gene have been identified in crizotinib-resistant *ROS1*-rearranged NSCLCs [4], with *ROS1*-G2032R being the most common resistance mutation [7]. Besides, activation of bypass signaling pathways has also been linked to crizotinib resistance. It has been shown that epidermal growth factor receptor (EGFR) pathway activation can mediate crizotinib resistance by increasing the dependence on EGFR activity and reducing the dependence on ROS1 activity [8, 9]. Similarly, an activating mutation (p.D816G) in the KIT Proto-Oncogene, Receptor Tyrosine

Kinase (KIT) signaling pathway also serves as a ROS1-independent mechanism of resistance [10]. Cargnelutti et al. revealed that activation of the RAS pathway due to KRAS Proto-Oncogene *(KRAS)* amplification conferred the resistance to ROS1 inhibitors including crizotinib [11].

In the present study, we identified the acquisition of *BRAF* p.V600E after crizotinib progression in a *ROS1* fusion–positive NSCLC. *BRAF* encodes a key molecule in the MAP/ERK kinase signaling pathway, located downstream of ROS1. *BRAF* p.V600E has been known as an oncogenic driver observed in 1%–2% of NSCLCs [12]. Of note, in our case, *ROS1* fusion was neither detected in the plasma nor the pleural effusion after crizotinib resistance, whereas *BRAF* p.V600E was detected at a lower frequency of 0.06% in plasma and 2.85% in pleural effusion, compared with that of other mutations (>1% in plasma and >5% in pleural effusion). The results suggested that the *BRAF* p.V600E was more likely to originate from a subclone with an extremely low fraction that was independent of *ROS1* fusion–positive tumor cells. During the crizotinib treatment, the growth and proliferation of the cells with *ROS1*-fusion were inhibited, and cells with *BRAF* p.V600E were selected and evolved into the major resistance clone. Loss of *EML4-ALK* fusion has been reported in *ALK*-positive NSCLCs after crizotinib failure [13, 14]. Similarly, in vitro study and primary clinical evidence suggest that loss of activating *EGFR* mutation contributes to acquired resistance to EGFR TKI in lung cancer [15]. Therefore, the loss of activating alteration *ROS1*-fusion may also confer crizotinib resistance in our case. However, considering the lack of a rebiopsy after crizotinib resistance for confirmatory tissue testing and technical limitations of DNA sequencing on detecting complex genomic rearrangements, the possibility of false-negative results for *ROS1* fusion detection cannot be excluded.

The combined therapy with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib has demonstrated promising efficacy and manageable side effects in *BRAF* V600E–mutant NSCLC [12] and has recently been approved for clinical use. After the identification of *BRAF* p.V600E, our case was switched to the treatment with dabrafenib plus trametinib and showed a durable response along with the disappearance of *BRAF* V600E in plasma. To date, only two case reports have described the appearance of *BRAF* V600E as a mechanism mediating crizotinib resistance in *ROS1*-rearranged NSCLC [16, 17]. However, one patient switched to the dabrafenib single agent but passed away 15 days later, and the other died 11 days after the initiation of dabrafenib and trametinib therapy. Different from our case, these two patients retained detectable *ROS1* fusion after crizotinib resistance. Thus, it might be thought that combining a ROS inhibitor might be beneficial in such cases. Similarly, Meng et al. reported two *EGFR*-mutated cases acquiring *BRAF* V600E after osimertinib resistance, who received dabrafenib and trametinib combined with osimertinib. One of them achieved a durable response and the other progressed quickly [18]. Notably, our study reported the first case of crizotinib-induced *BRAF* V600E responding to the treatment with dabrafenib and trametinib in *ROS1*-rearranged NSCLC.

In conclusion, our study revealed that *BRAF* V600E can confer the crizotinib resistance in *ROS1* fusion–positive NSCLC and presented the first case suggesting that the treatment with dabrafenib and trametinib can serve as an effective option for later-line treatment for this molecular-defined subgroup.

Glossary of Genomic Terms and Nomenclature

NSCLC: non-small cell lung cancers
TKI: tyrosine kinase inhibitor
PFS: progression-free survival
NGS: next-generation sequencing
OR: objective response
PR: partial response
SD: stable disease
RT-PCR: real-time polymerase chain reaction
ECOG PS: Eastern Cooperative Oncology Group performance status
CT: computed tomography
ARMS PCR: amplification refractory mutation system polymerase chain reaction
ROS1: ROS proto-oncogene 1, receptor tyrosine kinase
KIT: KIT proto-oncogene, receptor tyrosine kinase
KRAS: KRAS proto-oncogene

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Author Contributions

Conception/design: Juan Li, Wenxiu Yao
Collection and/or assembly of data: Qifeng Wang, Jun Ge
Data analysis and interpretation: Yuke Tian
Manuscript writing: Juan Li
Final approval of manuscript: Juan Li, Qifeng Wang, Jun Ge, Yuke Tian, Wenxiu Yao

Disclosures

The authors indicated no financial relationships.

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