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CASE REPORT

Identification of a Rare *BAIAP2-ROS1* Fusion and Clinical Benefit of Crizotinib in the Treatment of Advanced Lung Adenocarcinoma: A Case Report

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Objective: Previous studies have shown that fusion partners have a potential role in influencing different tumorigenic abilities of ROS1 fusion variants, as well as potential differential responses to crizotinib. Therefore, it is important to accurately identify the type of ROS1 rearrangement in NSCLC for clinical treatment selection.

Materials and Methods: Deep-coverage targeting solid tumor 31 cancer-related genes panel was used to capture DNA-based NGS information to detect gene fusion. RNA fusion panel based on hybrid capture sequencing was performed to verify gene fusions from total RNA which isolated from formalin fixed paraffin-embedded (FFPE) tissue blocks.

Results: Using DNA-targeted NGS method, we identified a novel *BAIAP2-ROS1* fusion in a 71-year-old non-smoking female patient with stage IVB lung adenocarcinoma. Rearrangement consisted of *BAIAP2* in exon1-exon13 of chr17: q23 and *ROS1* in exon35-exon43 of chr6: q22, which were further confirmed by RNA-based NGS methodology. A complete kinase domain in *ROS1* fusion was preserved. The patient subsequently received crizotinib and showed significant tumor reduction until 17 months, who got benefit from targeted therapy.

Conclusion: This study discovered a novel *BAIAP2-ROS1* rearrangement; it provides more knowledge of *ROS1* fusion in clinical personalized treatment. The good response to crizotinib therapy emphasizes the importance of DNA-based and RNA-based NGS in rare fusion identification in clinical practice.

Keywords: *BAIAP2-ROS1* fusion, advanced lung adenocarcinoma, sensitive to crizotinib, next-generation sequencing

Introduction

With the wide application of next-generation sequencing (NGS) technology in clinical practice, more and more rare fusion variant type has been found in non-small cell lung cancer (NSCLC). *ROS1* fusion gene is detected in cancer patient with a certain mutation rate and incidence (1–2%).[1] The incidence of *ROS1* fusion is mainly concentrated in younger Asian patients with no smoking history and histological type of adenocarcinoma, and it rarely coexists with other driver gene mutations. *ROS1* gene can rearrange with multiple genes, and the most frequent fusion partner is *CD74*.[2] When *ROS1* fuses with other genes, the kinase domain was usually retained and would activate signaling pathways that are critical to the occurrence and progression of cancer, such as PI3K/AKT/mTOR and JAK/STAT pathway.[3,4]

Currently, two targeted drugs, crizotinib and entrectinib, have been approved by FDA for the treatment of patients with *ROS1* fusion, while the current development of *ROS1* inhibitors is still relatively limited. The objective response rate (ORR) of this treatment can reach more than 70%, which brings clinical benefits to *ROS1*-

831

positive advanced NSCLC.[5] Although some new small molecule tyrosine kinase inhibitors (TKI) have been continuously developed after crizotinib, the median progression-free survival (PFS) of these TKIs was 9–12 months.[6] Drug resistance has become the most common bottleneck to the clinical application of *ROS1* inhibitors. At present, there is an urgent need for stronger and more selective *ROS1* inhibitors to overcome these problems.[7] Therefore, accurate identification of *ROS1* rearrangement is the very first key step for clinical treatment selection.

Case Presentation

A 71-year-old female was admitted to the hospital on December 5, 2019 due to cough and chest tightness for 3 days. Chest CT (computed tomography) showed lung cancer was considered. Multiple nodules were found in both lungs, accompanied by enlarged right hilar and mediastinal lymph nodes, and right pleural effusion with adjacent lung parenchyma insufficiency (Figure 1A). The size of the target lesion in the lower right lobe is about 7.0×6.0cm (Figure 1B). The CEA value was 3.1ng/mL. After 18 days of the treatment with crizotinib, mediastinal lymph nodes are reduced (Figure 1C). Lung lesions are also reduced (Figure 1D). At the time of 12.5 months of crizotinib treatment, hilar lymph nodes decreased (Figure 1E), and the pleural effusion decreased (Figure 1F). After 14 months of crizotinib treatment, mediastinal lymph nodes changed (Figure 1G) and the right pleural effusion increased (Figure 1H). After 17 months of oral crizotinib, the mediastinal lymph nodes were enlarged (Figure 1I). The left pleural effusion was significantly increased (Figure 1J). The pathological results changed by the timeline of medication (Figure 1K). Subsequently, a lung biopsy was performed under the guidance of CT. Metastatic carcinoma of the right parietal pleura combined with the source of immunohistochemical lung adenocarcinoma was diagnosed (Figure 1L). Finally, through genetic testing, the diagnosis was revised: stage IV right lung adenocarcinoma, *ROS1* positive.

With written informed consent, the formalin fixed paraffin embedded (FFPE) tissues in primary tumor tissues were detected by deep coverage NGS test targeting 31 tumor associated gene. Deep-coverage targeting solid tumor 31 cancer-related genes panel was used to capture DNA-based NGS information to detect gene fusion. RNA fusion panel based on hybrid capture sequencing was performed to verify gene fusions from total RNA which isolated from formalin fixed paraffin-embedded (FFPE) tissue blocks. The mutation profile showed a rearrangement between chromosome 17 and chromosome 6 with a mutant allele frequency of 69%. Sequences support of *BAIAP2-ROS1* fusion was detected by DNA-Seq (Figure 2A) and RNA-Seq (Figure 2B) methods. Schematic of genome rearrangement of *BAIAP2-ROS1* involving fusion breakpoints at mRNA and protein levels (Figure 2C). The fusion of *BAIAP2-ROS1* in tissue was determined which was formed by exon1-exon13 fragment of *BAIAP2* and exon35-exon43 fragment of *ROS1* (Figure 2A and C). The rearrangement was further verified by RNA fusion plate hybrid capture sequencing (Berry Oncology Corporation) technology[8] to confirm the existence of *BAIAP2-ROS1* (B13: R36) fusion mutation (Figure 2B and C). As the complete *ROS1* kinase domain was retained in this novel fusion, constitutive kinase activity and oncogenic transformation were achieved.

Based on the identification of this new *ROS1* fusion, from December 30, 2019, the patient took crizotinib twice a day at a dose of 250 mg (Figure 1K). After 18 days of the treatment with crizotinib, the lesions in both lungs and mediastinum were reduced (Figure 1C and D). Partial response (PR) was achieved according to the RECIST guideline. The lesion size in the lower lobe of the right lung was about 3.9×2.7cm and the lymph nodes were reduced by 2.0cm. CEA value was 3.30 ng/mL. The right pleural effusion accompanied by insufficiency of the right lung was relived. The patient's cough and chest tightness improved. PR was achieved after 12.5 months crizotinib treatment, the imaging map showed that the target lesion was a cord-like high-density shadow, the amount of pleural effusion was reduced, and the hilar lymph nodes were reduced (Figure 1E and F). CEA value was 4.2ng/mL. After 14 months of oral administration of crizotinib, the right pleural effusion increased and the lesion was evaluated as stable disease (SD) (Figure 1G and H). CEA value was 5.88 ng/mL. Finally, after 17 months of oral crizotinib, the target lesion was about 2.6 cm x 2.0 cm in the right lower hilum, and the left pleural effusion was significantly increased, and the lesion was PD (Figure 1I and J). CEA value was 5.54 ng/mL. The
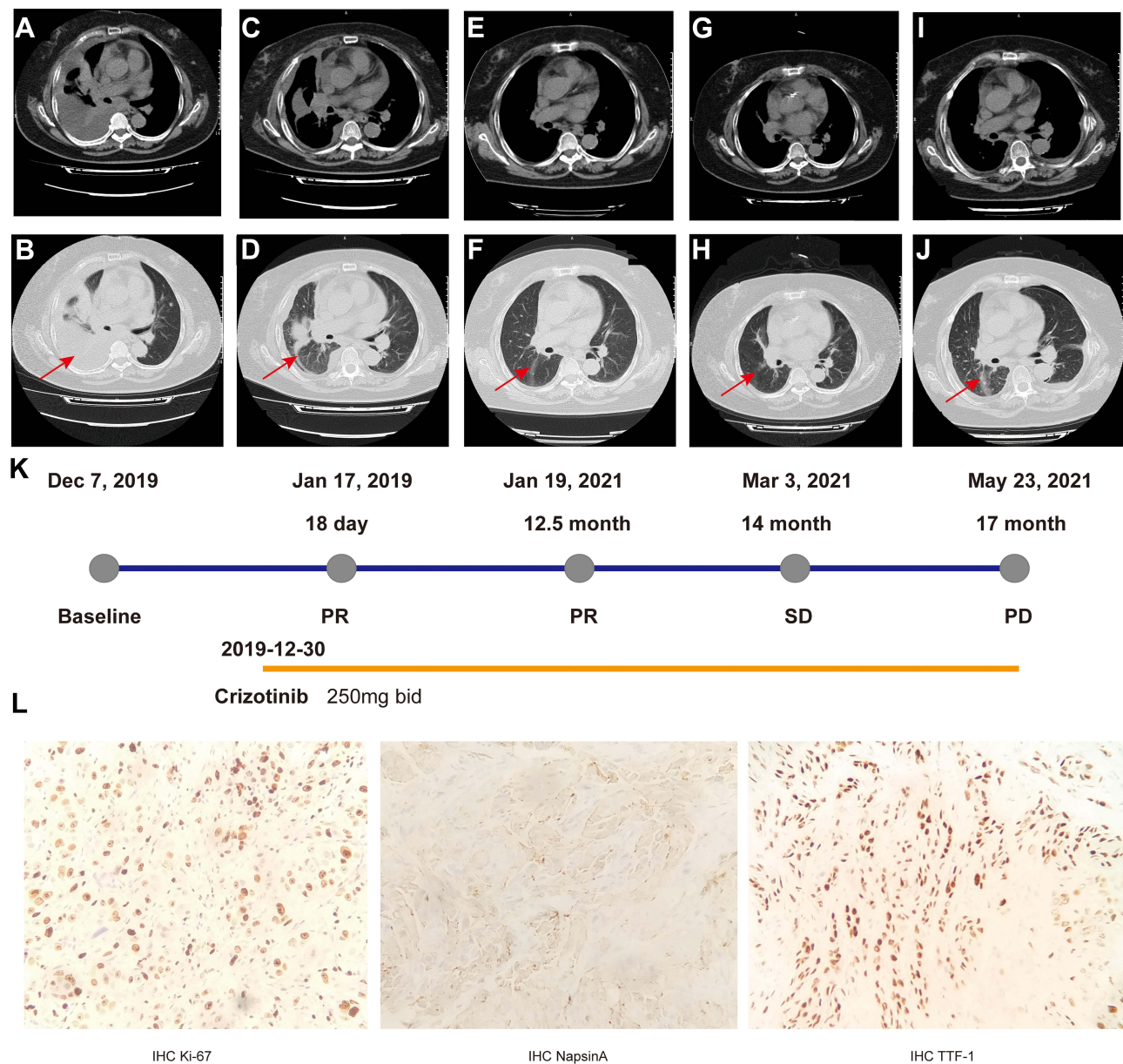
Figure 1 Timeline of clinical treatment course and radiologic features. (**A**) Computed tomography (CT) scan of mediastinal lymph nodes at baseline. (**B**) CT scan of lung masses at baseline. (**C**) CT scan of mediastinal lymph nodes after 18 days of crizotinib treatment. (**D**) CT scan of lung masses after 18 days of crizotinib treatment. (**E**) CT scan of mediastinal lymph nodes after 12.5 months of crizotinib treatment. (**F**) CT scan of lung masses after 12.5 months of crizotinib treatment. (**G**) CT scan of mediastinal lymph nodes after 14 months of crizotinib treatment. (**H**) CT scan of lung masses after 14 months of crizotinib treatment. (**I**) CT scan of mediastinal lymph nodes after 17 months of crizotinib treatment. (**J**) CT scan of lung masses after 17 months of crizotinib treatment. (**K**) Timeline of the patient's clinical treatment course and response to cancer drugs during the patient's clinical course. Red arrows indicate the tumor and lymph node sites. (**L**) Pathological examination of the patient tumor tissue. Hematoxylin and eosin (HE) staining of the biopsy specimen showed a poorly differentiated tumor tissue. IHC experiments showed positive expressions of Ki-67, Napsin A, TTF-1, and negative expression of P40 (×200), revealing an adenocarcinoma origin of the lung cancer.
Abbreviations: NGS, next-generation sequencing; PR, partial response; PD, progressive disease; SD, stable disease.

patient accidentally fell and injured the right femur and ribs at 15th month of medication because of severe osteoporosis. The patient had been taking crizotinib during her convalescence, the lesion was controlled again and assessed as PD at 17 months. But the patient's physical condition was not so well and passed away one month later (July 2021). The results of clinical data showed that the novel *BAIAP2-ROS1* fusion was sensitive to the first-generation tyrosine kinase inhibitor, crizotinib, and the patient had no obvious drug-related adverse reactions during the follow-up period of medication.
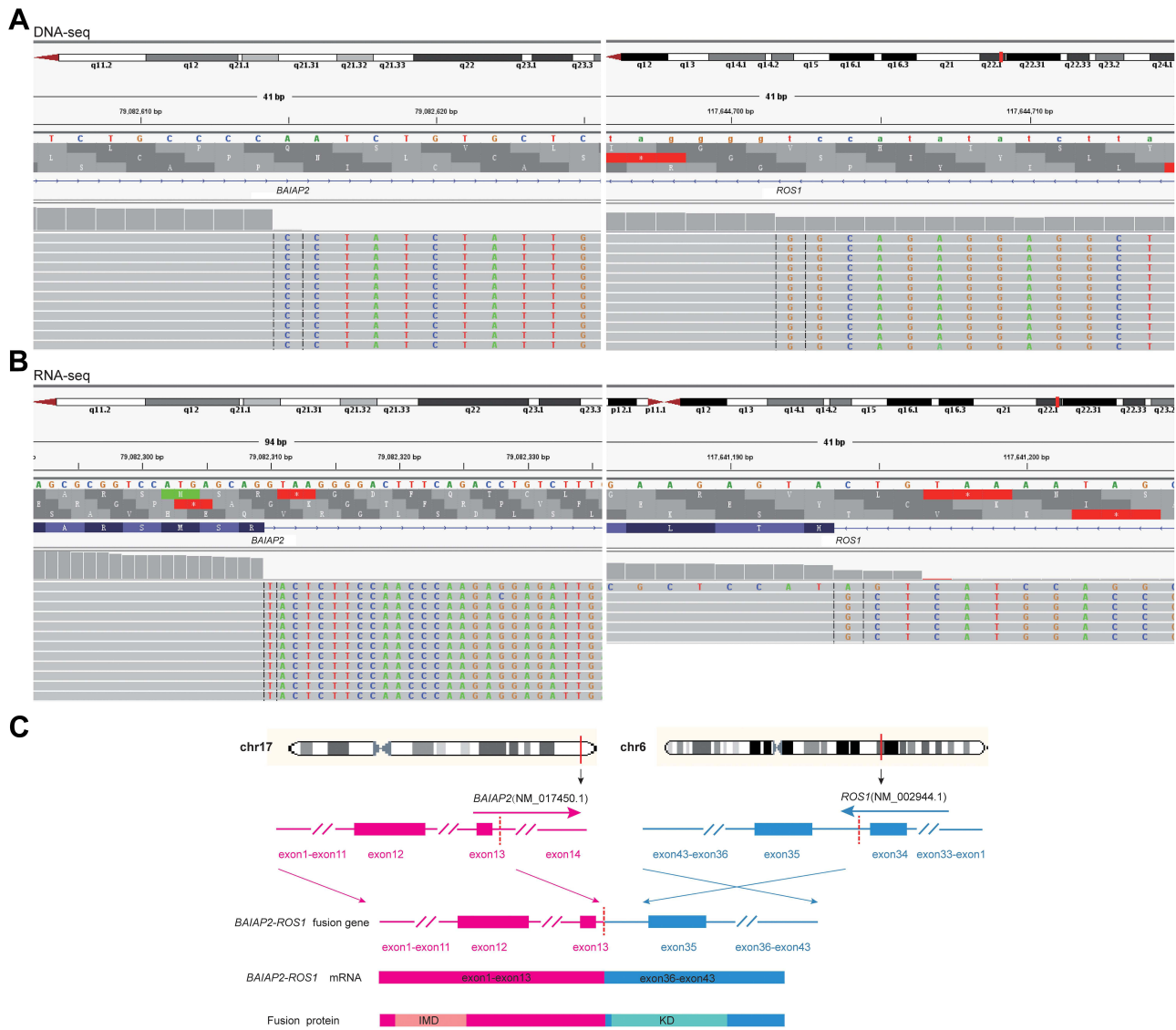
Figure 2 Identification and verification of a novel *BAIAP2-ROS1* fusion. (**A**) DNA sequencing reads indicating *BAIAP2* and *ROS1* fusion regions were visualized by the Integrative Genomic Viewer (IGV) software. The fusion breakpoints are localized at chr17: q23: 79,082,614 and chr6: q22.1: 117,644,699, respectively. (**B**) RNA sequencing reads indicating *BAIAP2* and *ROS1* fusion regions were visualized by the Integrative Genomic Viewer (IGV) software. The fusion breakpoints are localized at chr17: q23: 79,082,304 and chr6: q22.1: 117,641,192, respectively. (**C**) Schematic of genomic rearrangement involving the fusion breakpoints in mRNA and protein level. The transcript resulted in exons 1–13 of *BAIAP2* containing coiled-coil domains fused to exons 35–43 of *ROS1* including kinase domain. Pink, *BAIAP2*; Blue, *ROS1*; IMD, IRSp53 and MIM (missing in metastases) homology Domain.
Abbreviation: KD, kinase domain.

Discussion

In recent years, DNA-based next-generation sequencing (NGS) has been widely used as an important diagnostic method for detecting *ROS1* rearrangements, besides fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). DNA-based NGS fusion detection method has advantage on detecting the exons and introns of genes, detecting multiple gene fusions in one test, and determining the fusion partners and breakpoints,[9] which could eliminate false positives for *ROS1* fusion detected by IHC. RNA-based NGS technology could exactly detect gene rearrangement performed in the transcription process, and many studies have proved that the combination of DNA and RNA-based NGS technology can comprehensively inspect gene fusion/rearrangement and significantly improve the detection rate of rare fusion.[1,10] Simultaneous determination of targets and rare and effective fusion mutations through NGS methodology can improve

possible missed detections and unclear fusion genes in traditional detection methods, effectively improve the detection rate of fusion genes, and offer help to doctors in clinical diagnosis and treatment.[11]

This is the first reported case of *BAIAP2-ROS1* (B13:R35) fusion in advanced NSCLC patient. This study found a novel *ROS1* rearrangement fused with a new gene partner, *BAIAP2*, which was sensitive to crizotinib in a female advanced lung adenocarcinoma patient. The BAIAP2-ROS1 fusion was determined by targeted DNA 31 panel next-generation sequencing methodology and verified by RNA fusion panel based on hybrid capture sequencing (Berry Oncology Corporation). Subsequent crizotinib treatment yielded a favorable 17-month response, superior to many ROS1-fusion crizotinib treatments, with the majority of cases developing resistance within 9–12 months.[12,13] This report provided the clinical evidence on this novel *ROS1* rearrangement and offered drug-sensitive support for targeted therapy in lung adenocarcinoma with this new-found gene fusion. It also expands NSCLC's treatment of *ROS1* rearrangement and emphasizes the importance of genetic testing for precise treatment decision.

Ethics Statement
The study was approved by the Ethical Committee of Medical Research, NingHai First Hospital of NingBo City.

Consent for Publication
Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure
Wendy Wu, Yan Lei, Linwei Li and Xiaoxing Su are the employees of Berry Oncology Corporation. The other authors declare no conflict of interest.

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