

Response to crizotinib in a lung adenocarcinoma patient harboring a novel *SLC34A2-ROS1* fusion variant

Zheng Zhao,¹ Zhangjun Song,²
Xuwei Wang,³ Haifeng Sun,¹
Xiaomin Yang,² Yong Yuan,⁴
Pan Yu³

¹Third Department of Medical Oncology, Shanxi Provincial Cancer Hospital, ²Breast Surgery Center, Shanxi Provincial Cancer Hospital, Xi'an, ³Marketing Department, Novogene Bioinformatics Institute, Beijing, ⁴Pathology Department, Shanxi Provincial Cancer Hospital, Xi'an, People's Republic of China

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Correspondence: Zheng Zhao
Third Department of Medical Oncology,
Shanxi Provincial Cancer Hospital, 309 Yanta
West Road, Xi'an, Shanxi, 710061, People's
Republic of China
Email zhaozheng1513@163.com

Pan Yu
Novogene Bioinformatics Institute, 38 Xueqing
Road, Haidian District, Beijing, 100083, People's
Republic of China
Email yupan@novogene.com

Abstract: *ROS1* fusion is a common genetic alteration in non-small-cell lung cancer. Crizotinib, an anaplastic lymphoma kinase inhibitor, shows efficacy in the treatment of lung cancer cases with *ROS1* translocation. We report the response to crizotinib of a lung adenocarcinoma patient harboring a novel *SLC34A2-ROS1* fusion variant, which was different from the two common *SLC34A2-ROS1* fusion types reported in the literature. After crizotinib administration, overall recovery was good in this patient; the primary lesion was successfully treated, the lymph node metastases had disappeared, and the metabolism was normal.

Keywords: *SLC34A2-ROS1* fusion, crizotinib, lung adenocarcinoma, next-generation sequencing

Introduction

ROS1 fusion, a common genetic alteration in non-small-cell lung cancer (NSCLC),¹ is present in about 2.4% of lung adenocarcinoma cases. Several fusion types have been reported, including *CD74*, *SLC34A2*, *EZR*, *LRIG3*, *SDC4*, *TPM3*, *FIG GPOC*, *CCDC6*, *KDEL R2 LIMA1*, *MSN*, *CLTC*, *TMEM106B*, and *TPD52L1*.²⁻⁵ The application of reliable screening methods for gene rearrangement detection is key in identifying patients suitable for tumor-targeted therapy.^{5,6} To date, *ROS1* fusions in NSCLC are most commonly diagnosed by fluorescence in situ hybridization (FISH) or amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).⁶⁻¹⁰ However, ARMS-PCR and FISH are costly, less precise, and not suitable for detecting unknown significant mutations.¹¹⁻¹⁶ Next-generation sequencing (NGS) is an optional method for identifying unknown genes with potential benefit.^{10,17-19} Crizotinib, a tyrosine kinase inhibitor targeting ALK and MET, was approved by the US Food and Drug Administration (FDA) on March 11, 2016, for the treatment of advanced metastatic *ROS1*-rearranged NSCLC. This report describes a patient successfully treated with crizotinib, in whom a novel *SLC34A2-ROS1* fusion variant was identified. Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. Approval from an ethics committee was not required due to this being a retrospective study and the information regarding the case being anonymous.

Case report

A 48-year-old female patient with back pain was admitted to the Shanxi Provincial Cancer Hospital; she was suspected with left breast cancer. Subsequently, lymph node



metastasis of lung adenocarcinoma was considered in both sides of the neck and the left side of axillary lymph nodes after biopsy. She had 10 years of smoking history (1 pack/d) and no family history of genetic diseases. Biopsy samples were assessed for *EGFR*, *ALK*, and *ROS1* gene mutations by ARMS-PCR (Amoy Diagnostics Co., Ltd, Xiamen, People's Republic of China); however, all were negative. The patient received 2 cycles of chemotherapy (docetaxel 240 mg + cisplatin 240 mg). Because of progressive disease (PD), the treatment was discontinued on December 9, 2015. Positron emission tomography (PET)/computed tomographic (CT) imaging revealed that the tumor had developed multiple lymph node metastases. The chemotherapy regimen was replaced by 2 cycles of chemotherapy (pemetrexed 800 mg, intravenous [IV], d1; cisplatin 20 mg IV, d1–5, bevacizumab 0.5 IV d1) and a combined 1 cycle of local radiotherapy (4,400 cGy/22f/5w). CT assessment showed stable disease in the patient, and palliative treatment was performed by pemetrexed plus cisplatin chemotherapy. However, the chemotherapeutic treatment was discontinued because of severe bone marrow suppression.

With no effective treatment at hand, biopsy samples were assessed using the NGS-483 gene panel (Novogene Bioinformatics Technology Co., Ltd, Beijing, People's Republic of China). Biotinylated oligoprobes were designed along the no-repeat regions of 483 genes containing all the exons and some introns. The amplified exonic and intronic DNA was then sequenced on HiSeq X Ten sequencer (Illumina, San Diego, CA, USA) producing paired 150 bp reads. In the 483 genes, NGS revealed a new *ROS1* fusion (*SLC34A2-ROS1*) and the *TP53* p *E132Q* variation. Next, we assessed the causes of different detection results in the same samples. While analyzing *ROS1* fusion sequence data, a novel *SLC34A2-ROS1* fusion variant, different from the two common *SLC34A2-ROS1* fusion types, was discovered (Figure 1). According to the sequencing data, the novel breakpoint was chr6:117653720, chr4:25678781. To verify the breakpoint, we respectively designed a primer pair based on the *ROS1* gene's breakpoint downstream and left to the *SLC34A2* gene's upstream breakpoint. Direct Sanger sequencing demonstrated that the 3' untranslated region (3'UTR) of *SLC34A2* in exon 13 was disrupted and inverted to connect a position of intronic_e32_e31 of *ROS1* (Figure 2). The new breakpoint was not included in Catalogue Of Somatic Mutations In Cancer (COSMIC; <http://cancer.sanger.ac.uk/cosmic>) and was located in the *SLC34A2* gene of the last transcription region (extra exon 13, as the 3'UTR region, was not involved in translation). Therefore, conventional detection methods cannot be used.

On March 11, 2016, the FDA formally approved crizotinib (Pfizer) for the treatment of patients with advanced (metastatic) NSCLC harboring *ROS1* gene fusion. Would the patient with the novel *SLC34A2-ROS1* fusion variant showed no response to crizotinib? We predicted the protein domain of the new fusion gene, and the *ROS1* tyrosine kinase domain could not be destroyed.

Combined with the above information, the female patient was started on treatment with crizotinib (250 mg, 2/d, d1–30) from March 24, 2016. After 2 weeks, the breast mass was reduced, and the patient reported relieved pain and improved quality of life. Two months later, according to PET/CT, most metastatic lymph nodes had disappeared, with the metabolism returning to normal. Eleven months later, the original irregular nodules in the basal segment of the right lung's lower lobe were slightly narrowed, with reduced metabolism; the shape was uniform, with rough edges and no nodules or pleural adhesion. The lesion increase, which reflects the primary malignant lesion (pneumotype of the lower lobe of the lung), was slightly smaller and more active after treatment. The anterior mediastinum septum, the left lung, the upper and lower sides of the collarbone, and the left armpit had lymph node metastases. The lymph nodes of interstitial lesions violated the left vertebra of the neck; after treatment, the metastatic lymph nodes disappeared, with the metabolism returning to normal. In this case, the external pressure of lymph nodes, the left armpit, the cervical lymph and blood vessels, left upper extremities, left breast area edema, and swelling were relieved after treatment, and remained so. No new tumor metastases were found in other organs and bones in other parts of the body. The patient's overall recovery was good; the primary lesion was relieved, the lymph node metastasis had disappeared, and the metabolism was normal (Figure 3).

Discussion

Crizotinib has been approved by FDA for the treatment of advanced metastatic NSCLC with *ROS1* and *ALK* rearrangements.^{1,7,10,17} In the Phase I clinical trial of crizotinib, 50 NSCLC patients with *ROS1* fusion were enrolled, including 49 cases detected by FISH and 1 by reverse transcriptase-polymerase chain reaction (RT-PCR). The overall response rate (ORR) was 72%, with a median duration of response (DOR) of 17.6 months and a median progression-free survival (PFS) of 19.2 months.³

The *SLC34A2-ROS1* fusion variant is a common *ROS1* fusion in NSCLC accounting for the second highest percentage.^{4,5} It is likely generated from an intra-chromosomal

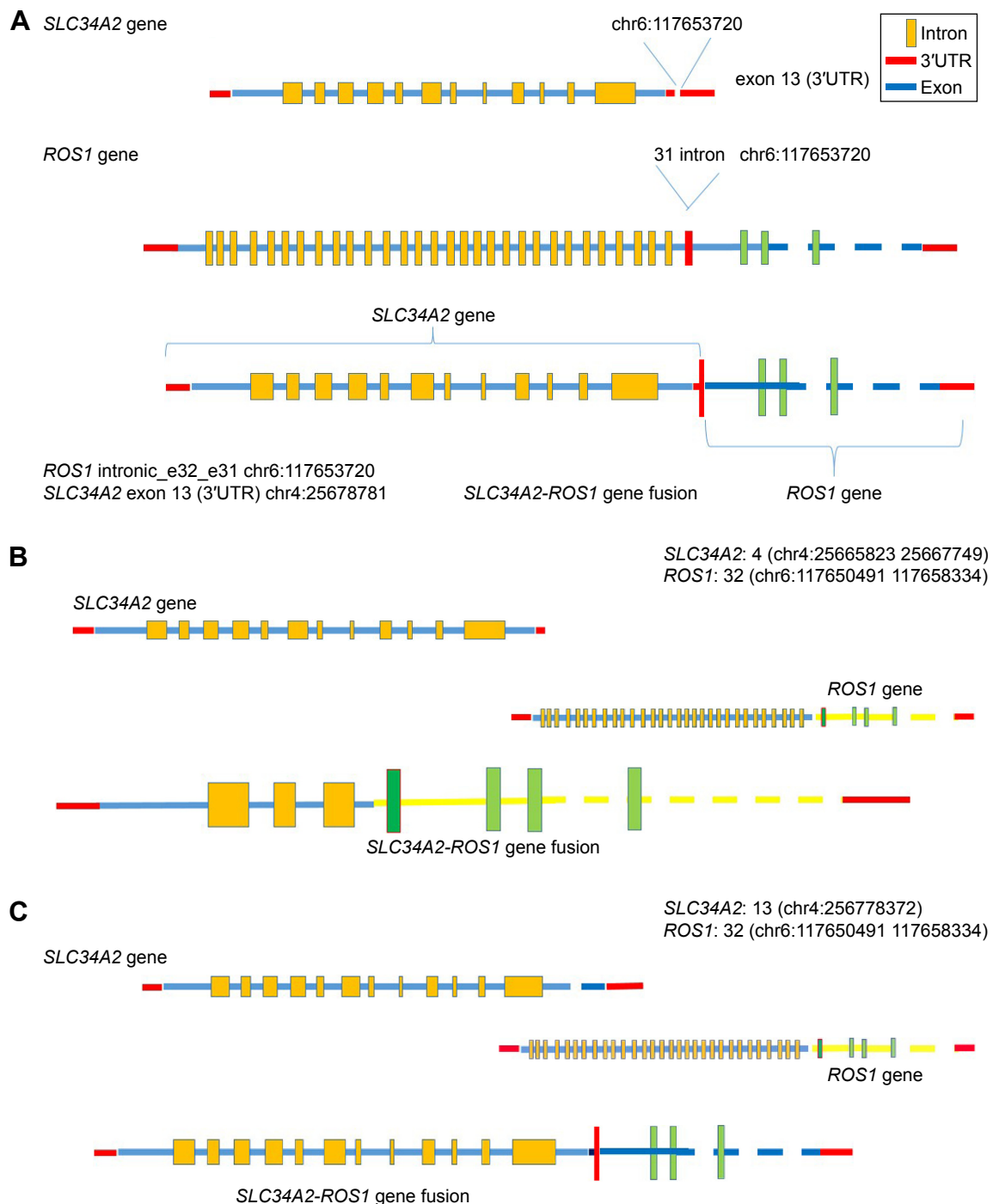


Figure 1 Structure of the novel *SLC34A2-ROS1* fusion gene.

Notes: **A** is the novel fusion gene and is different from the other two *SLC34A2-ROS1* fusion (**B** and **C**) variants. In the COSMIC database, two *SLC34A2-ROS1* fusion forms were found (**B** and **C**). In the novel *SLC34A2-ROS1* fusion, 3'UTR of *SLC34A2* in exon 13 was disrupted and inverted to connect a position of intronic_e32_e31 of *ROS1*.

Abbreviations: COSMIC, Catalogue Of Somatic Mutations In Cancer; 3' UTR, 3' untranslated region.

deletion and fusion, similar to *EML4-ALK* generation in NSCLC.^{1,8,13,15} NGS fortunately revealed a new *ROS1* fusion (*SLC34A2-ROS1*) and *TP53* p *E132Q* variation; *ALK* fusion mutation was not found in this sample. The novel *SLC34A2-ROS1* fusion variant (3'UTR of *SLC34A2* in exon 13 was disrupted and inverted to connect a position of intronic_e32_e31

of *ROS1*) differed from the two previously discovered *SLC34A2-ROS1* fusion types (Figure 1). Based on sequencing results, the novel breakpoint was chr6:117653720, chr4:25678781. After the administration of crizotinib for 2 weeks, the breast mass was reduced, pain was relieved, and the quality of life improved. After 2 months, PET/CT

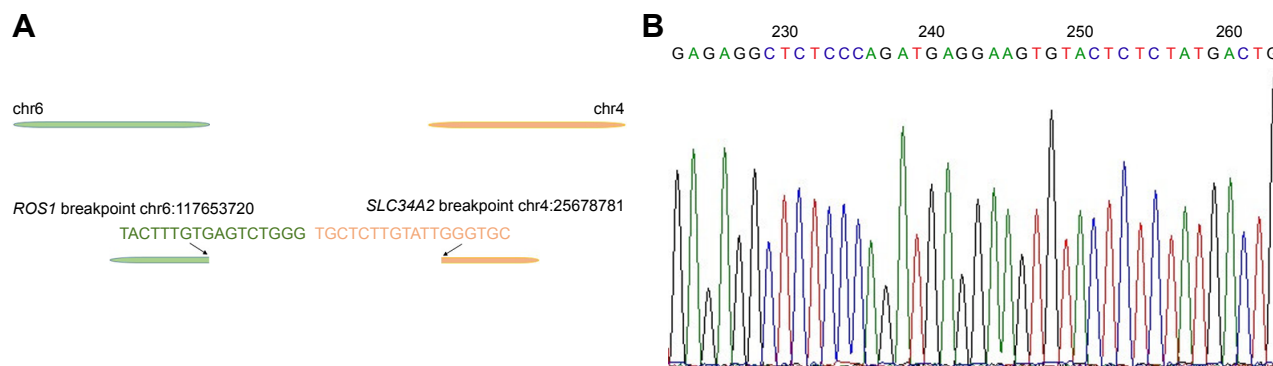


Figure 2 Accuracy verification of the novel *SLC34A2-ROS1* fusion by Sanger sequencing.

Notes: (A) Based on the results of the next-generation sequencing, we designed a primer pair based on the *ROS1* gene's breakpoint downstream and left to the *SLC34A2* gene's upstream breakpoint; (B) the mutations in the sample DNA were sequenced by Sanger sequencing and the breakpoints in the next-generation sequencing are identical.

indicated a progressive relief; 11 months later, overall recovery was good in this patient, with primary lesion successfully treated, no lymph node metastases, and normal metabolism. The patient also harbored a *TP53* pE132Q variation. Would this also explain why crizotinib was effective in this patient? The tumor suppressor gene *TP53* is the most frequently mutated in NSCLC. It encodes a 393-aa protein with three distinct domains. *TP53* pE132Q exists on the binding domain of TP53; however, it may not affect the function of proteins. In addition, this remains unclear as no relevant literature was found. These findings suggest that the novel *SLC34A2-ROS1* fusion shows good response to crizotinib. ARMS-PCR is a fast and sensitive method for evaluating the expression of known *ROS1* fusion variants,^{14,16} for which specific primers have been designed. In a previous study, the sensitivity and specificity of ARMS-PCR for detecting *ROS1* fusion were 100% and 85.1%, respectively.¹⁰ There are two possible reasons why we failed to detect the *ROS1* fusion in the patient's

sample: 1) only a small population of cancer cells might be carrying the *ROS1* fusion and 2) this fusion might occur at the transcriptional level, with no translation. In this report, the rare *ROS1* variants were not detected by ARMS-PCR, since ARMS-PCR is based on cDNA after mRNA transcription. The new breakpoint was not included in COSMIC and was located in the last transcription region of the *SLC34A2* gene; therefore, it could not be detected by ARMS-PCR. In the 483 genes, NGS revealed the new *ROS1* fusion (*SLC34A2-ROS1*); Direct Sanger sequencing demonstrated that the 3'UTR of *SLC34A2* in exon 13 was disrupted and inverted to connect a position of intronic_e32_e31 of *ROS1*. For further confirmation, a FISH assay (Guangzhou Anpingping [LBP] Pharmaceutical Technology Co., Ltd) was performed to check for this fusion variant; however, the results were negative. Also, we analyzed the reasons for the difference of detection result between the two methods. The gene fusion process is very complex; it is possible that in the upstream area *ROS1*

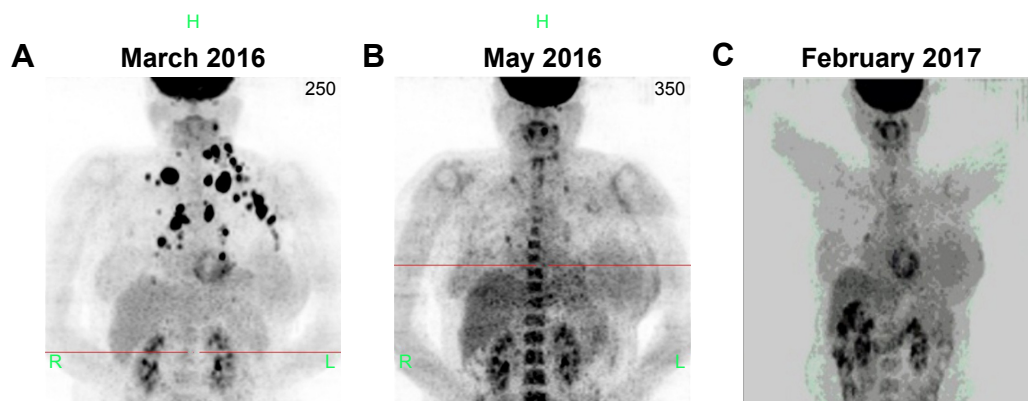


Figure 3 Computed tomography and positron emission tomography images of the right lower lobe.

Notes: Mediastinum, upper and lower bilateral clavicle area, left armpit, and lung lymph node metastases at treatment initiation (March 2016; A). After treatment with crizotinib for 2 months (May 2016; B), the metastatic lymph nodes had disappeared, with the metabolism returning to normal; 11 months later (February 2017; C). No new tumor metastases were found in other organs and bones in other parts of the body. The patient's overall recovery was good; the primary lesion was relieved, the lymph node metastasis had disappeared, and the metabolism was normal.

missing part of the area, by parts of *SLC34A2* gene insert this part again, without affecting the other areas in the FISH. The red probe can also be combined with a green probe, which is still negative when examined. A study shows that the same phenomenon exists in the *ALK* fusion.²⁰ There is a complete possibility of a rearrangement is no separation of the red and green probes. NGS is an optional method for the detection of all forms of gene variation. Thus, the multi-platform technology of detection can maximally prolong patient survival. To monitor the expression of this fusion from diagnosis throughout the treatment, liquid biopsy-circulating tumor DNA was used with the NGS 483 gene panel. Circulating tumor DNA samples of the patient, in July 2016 and January 2017, respectively, were assessed by the NGS 483 gene panel; interestingly, the *SLC34A2-ROS1* fusion variant had disappeared, in agreement with the PET/CT data.

Disclosure

Xuwei Wang and Pan Yu are employees of Novogene Bioinformatics Institute. The authors report no other conflicts of interest in this work.

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