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Novel SLC12A2-ROS1 Fusion in Non-Small Cell Lung Cancer with a Significant Response to Crizotinib: The Importance of Choosing the Appropriate Next-Generation Sequencing Assay

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

Key Words. ROS1 • Lung cancer • Fusion gene • Next-generation sequencing • Crizotinib • Molecular diagnosis

Abstract

Identifying the druggable target is crucial for patients with nonsquamous advanced non-small cell lung cancer (NSCLC). This article adds to the spectrum of *ROS1* fusion cases described in NSCLC. We describe a novel SLC12A2-ROS1 rearrangement that has not been previously reported in other cancers: a fusion that has clinical and radiological sensitivity to crizotinib. Fluorescence in situ hybridization detected the SLC12A2-ROS1 fusion and it was confirmed through hybrid capture-based next-generation sequencing (NGS); however, the fusion could not be detected by amplicon-based assay. The success of implementing NGS into routine clinical practice depends on the accuracy of testing. The test's methodological features should then be considered because they significantly affect the results. Given this patient's response to crizotinib, identifying patients with undescribed *ROS1* fusions has important therapeutic implications. ***The Oncologist*** 2021;26:e908–e912

Key Points

- This is the first known description of an SLC12A2-ROS1 fusion. Considering the patient's clinical features and tumor response observed after crizotinib therapy, the authors confirm that this new rearrangement has relevant clinical impact for patients with non-small cell lung cancer.
- The success of implementing next-generation sequencing (NGS) into routine clinical practice depends on the accuracy of the testing. Different assays and NGS platforms can achieve differing results. Each assay's limitations need to be considered to ensure the quality of precision medicine in clinical practice.

Patient Story

A 21-year-old nonsmoking woman with no relevant medical history started with progressive dyspnea, constitutional syndrome, and hemoptysis in July 2018. Active bacterial infection and tuberculosis were ruled out. The patient then underwent a thoracic computed tomography (CT) scan, which revealed the presence of multiple lung nodules and signs of lymphangitic carcinomatosis, suggesting a primary lung tumor. To obtain a pathology sample to confirm the suspected diagnosis, the patient underwent fiberoptic bronchoscopy with bronchoalveolar lavage. The immunohistochemical (IHC) analysis showed positivity for thyroid transcription factor-1 (TTF-1), with the pathology diagnosis

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compatible with a stage IV lung adenocarcinoma. The results of the molecular testing for *EGFR* and *ALK* were negative, with a programmed death-ligand 1 (PD-L1) value >1%. Considering the patient's age and tumor sample paucity, the patient underwent an amplicon-based next-generation sequencing (NGS) study through an Oncomine Focus Assay w2.2 (OFA, Thermo Fisher Scientific, Waltham, MA). However, no druggable mutations were found. Given the patient's clinical severity and significant deterioration in her general and respiratory condition, the patient started treatment with a platinum doublet plus pembrolizumab. Nevertheless, after three cycles of therapy, the patient's clinical condition worsened (performance status of 2); she required oxygen therapy, and the follow-up CT scan showed disease progression.

The patient was then referred to our hospital for a second opinion (Fig. 1A). Considering her clinical profile, we extended the molecular study with additional assays. No RET and ALK fusions were detected by fluorescence in situ hybridization (FISH) analysis (Agilent Technologies, Santa Clara, CA), IHC study for BRAF V600E were negative (Ventana, F. Hoffmann-La Roche, Basel, Switzerland), and no EGFR variants were reported (TheraScreen RGQ PCR, Agilent Technologies, Santa Clara, CA). However, a FISH analysis revealed a *ROS1* rearrangement (Agilent Technologies, Santa Clara, CA) and we therefore decided to start the patient on crizotinib therapy [1].

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Genotyping Results and Interpretation of the Molecular Results

The identification of druggable targets in advanced non-small cell lung cancer (NSCLC) is critical because it offers the best therapeutic option in terms of antitumor response and survival. Currently, performing molecular testing of the *EGFR*, *ALK*, *ROS1*, and *BRAF* genes is critical for patients with nonsquamous advanced NSCLC, for patients with no previous history of smoking, and for those under the age of 50 years, regardless of the histological type [2]. In this case report, there was a critical need for combining the clinical profile and performing the appropriate molecular diagnostic procedure to reach a decision regarding the most appropriate therapy. The molecular tumor board can assist physicians with the NGS testing results, making it possible to explain the disagreements in the different molecular testing assays [3]. This approach can also result in the identification of new molecular biomarkers.

Despite the negative *ROS1* result in the amplicon-based NGS assay, FISH showed an unbalanced translocation with the presence of signals corresponding to the extreme 3' of the gene (Agilent Technologies, Santa Clara, CA) in 90% of the tumor cells. Based on the manufacturer recommendations, we considered a positive *ROS1* translocation (Fig. 2A). Because of the discrepancy between the OFA and FISH assays, we performed a hybrid capture-based NGS technique with the TruSight Oncology 500 panel (TSO500, Illumina, San Diego, CA), identifying an undescribed SLC12A2-ROS1 fusion (Fig. 2B). We also performed a germline genetic study but found no alterations.

Functional and Clinical Significance of the SLC12A2-ROS1 Translocation

ROS1 rearrangements include various fusion partners in the 5′ position with different breakpoints described in the exons 32, 34, 35, and 36 [4–8]. In all reported cases, the *ROS1* kinase domain was conserved, resulting in a fusion protein with aberrant ligand-independent activation [9]. As a result of the diversity of the *ROS1* rearrangement, identifying novel fusions is challenging [10]. RNA sequencing hybrid capture NGS panels and precise bioinformatics pipelines enable clinicians to focus on target genes while preserving the ability to identify novel fusion partners. The detailed breakpoint can also be identified, which enables clinicians to check for the presence of the functional domains (tyrosine kinase for *ROS1*) in the final configurations. In our patient, the fusion breakpoints occurred at the end of exon 15 for *SLC12A2* and the start of exon 36 for *ROS1* (Fig. 2C). Using this approach, the list of fusion partners is expected to grow as more samples are examined [11].

Potential Strategies to Target the Pathway and Implications for Clinical Practice

Each assay's limitations need to be kept in mind to ensure the quality of precision medicine in clinical practice, both for the classic molecular techniques and for newer ones based on the genomic sequencing of the tumor. Insufficiently experienced and qualified professionals can lead to incorrect choices and improper use of NGS techniques [12]. For correct clinical reports, it is essential that we know the sample-dependent (tumor cellularity and the genomic material's quality, quantity, and integrity) and technique-dependent (DNA- or RNA-based panel, amplicons or hybrid capture, limit of detection) issues and their respective biases. Similarly, a final check by a specialist bioinformatician can ensure compliance with all quality requirements for producing clinical reports.

To ensure the absence of fusion genes, an RNA-based panel with hybrid capture-based NGS needs to be performed. If a DNA-based panel is selected, there is the risk of an undetected rearrangement being present if the breakpoint is located in an uncovered intronic region. Because of these limitations in fusion detection approach with DNA-based panels, targeted RNAseq assays should be used even if there are negative results by DNAseq assays to guarantee accurate detection of actionable gene rearrangements [13]. If amplicon-based assays are selected, novel fusion partners will not be detected and the rearrangement can only be approximated through an imbalance of probes when the design allows for it; the breaking point will remain unclear and therefore whether the complete tyrosine kinase domain is included in the final configuration will be unclear as well. Although the OFA and the TSO500 are assays with DNA- and RNA-based panels for low input and formalin-fixed paraffin-embedded samples, the main difference lies in the technology used: amplicon-based in OFA and hybrid capture-based in TSO500. Previously, studies showed that the amplicon-based sequencing assays missed unreported fusions [11, 14–16]. Therefore, partners

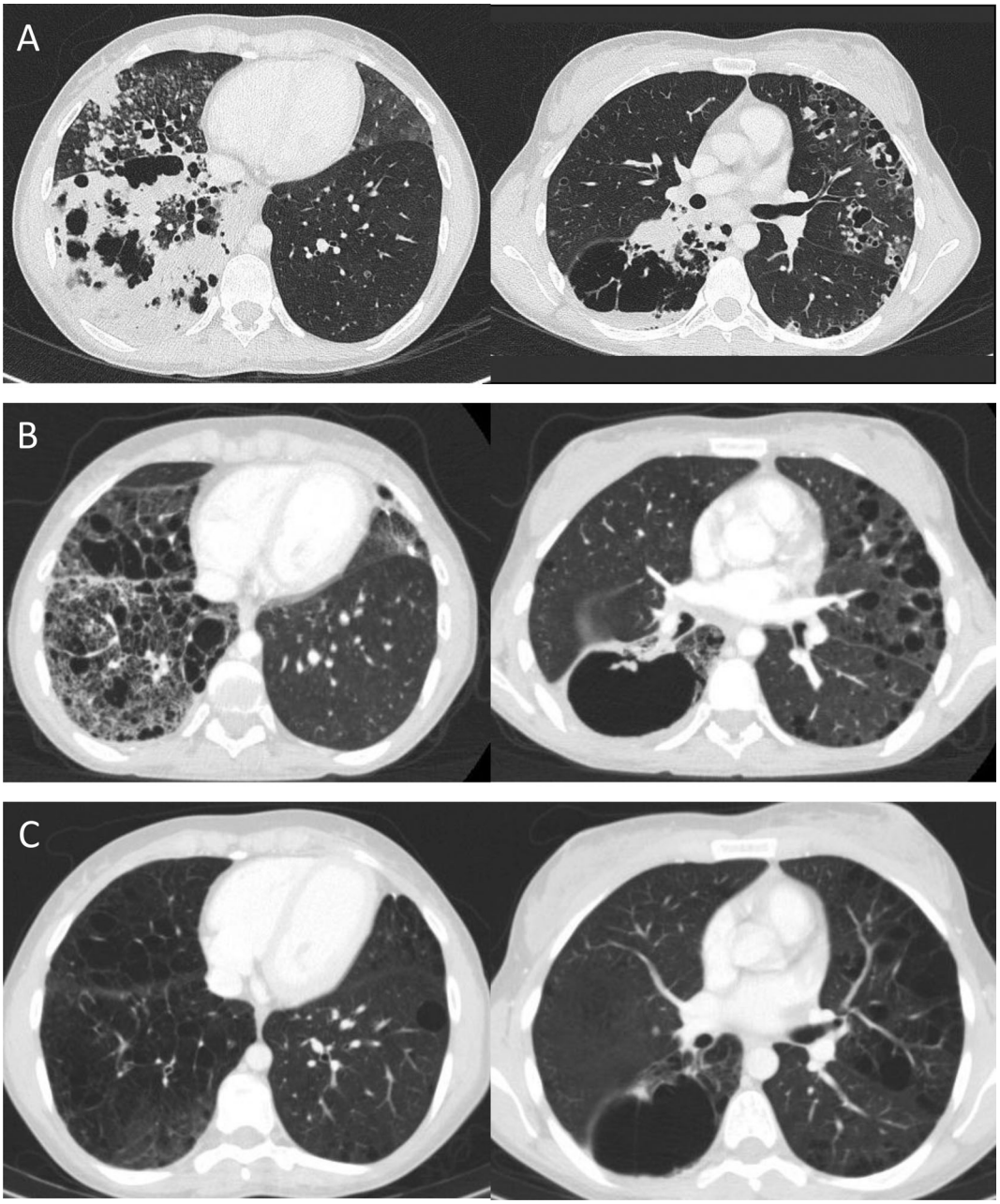

Figure 1. Thoracic computed tomography (CT) scans in the evolution of lung disease with crizotinib therapy. **(A)**: Baseline CT scan at the start of therapy. Bilateral pulmonary nodules with multiple cystic images are present in all lung lobes. In the left lower lobe, the presence of a large cystic lesion stands out. **(B)**: Re-evaluation CT scan after 6 weeks of crizotinib therapy. Disappearance of pulmonary nodules and resolution of cystic lesions can be seen, persisting in the left lower lobe. **(C)**: CT scan at 2 years shows resolution of practically all cystic lesions, with only a residual left lower lobe lesion.

must be previously known in order to be directly captured with amplicon technology. An assay that captures both known and new fusion genes will avoid false negatives.

Knowing these issues is therefore essential because the correct choice and implementation of assays ensure complete molecular characterization of the tumor [11, 17]. Hence, we considered that the TSO500 assay is suitable for clinical practice because it allows detection of new fusion partners when at least one of the fusion genes is included in the capture of the RNA-based panel. Although this approach is insufficient to detect fusions in novel genes, it exceeds the requirements in clinical practice because the gene rearrangements of interest are only those with targeted therapy.

We presented this case report in our Molecular Tumor Board, where the need to combine the clinical profile and perform the appropriate molecular diagnostic procedure has been critical for the treatment decision-making process. This approach has resulted in the identification of a new molecular *ROS1* rearrangement and has been a key factor in establishing the appropriate treatment and obtaining a clinical benefit for the patient. Without this multidisciplinary

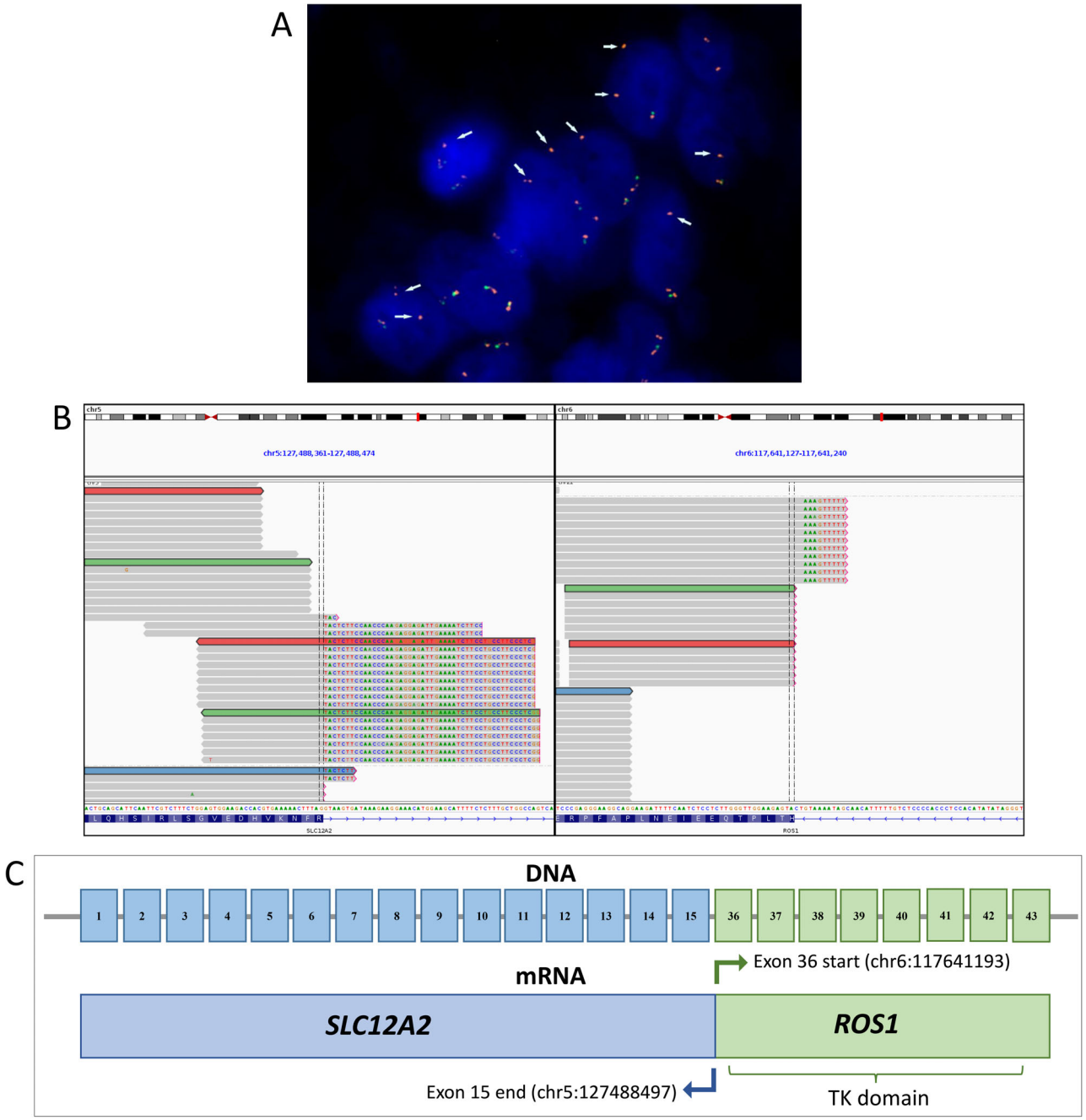

Figure 2. Molecular and next-generation sequencing (NGS) analysis of the tumor. **(A)**: A break-apart fluorescent in situ hybridization probe reveals an isolated 3′ROS1 probe (orange indicated by arrows) in a non-small cell lung cancer formalin-fixed paraffin-embedded specimen. Normal cells show fused orange and green signals. Nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI); ROS1 3′ is labeled with orange fluorochrome and ROS1 5′ is labeled with green fluorochrome. **(B)**: NGS reads for the two breakpoints in genomic context (left *SLC12A2*, right *ROS1*). The colored reads (red, green, and blue) are part of the same pair: one read of the pair aligns in *SLC12A2* and the other in *ROS1*. **(C)**: Schematic representation of the novel SLC12A2-ROS1 rearrangement. Fusion breakpoints occurred in 15 (*SLC12A2*) and 36 (*ROS1*) exons. The tyrosine kinase (TK) domain was preserved in the final configuration.

approach, the patient's progression was likely to have been unfavorable.

Patient Updated

The patient's condition improved immediately after starting the crizotinib therapy, with no further need for oxygen therapy after a few days and a disappearance of the dyspnea. Treatment tolerance was good, and the only adverse event was grade 1 nausea, which was controlled with metoclopramide. The radiological studies showed a partial response at week 6, with a reduced number of pulmonary nodules and an improvement in the honeycomb pattern (Fig. 1B). Two years later, the patient is leading a normal life, continues to take crizotinib, and maintains clinical and radiological control of the disease (Fig. 1C).

Considering the patient's clinical features and the tumor response observed after crizotinib therapy, which is comparable to the response reported for other *ROS1* fusion partners [18], we can confirm that this new rearrangement has relevant clinical impact in patients with NSCLC.

This case provides several important considerations. First, the exceptional presentation of NSCLC at an early age might cause a delayed diagnosis because of this possibility not being considered and with the previous ruling out of other nonmalignant diseases. Second, although the clinical profile should not be the only consideration for selecting patients to identify druggable targets, the search for biomarkers should be a priority for patients who present with this disease at an early age, given the high probability of druggable molecular alterations in the tumor. The lack of an identified therapeutic target when the probability of its presence is high forces clinicians to consider alternative detection techniques. Finally, although routine use of NGS is recommended for advanced NSCLC [19], the methodological features should be considered because they significantly affect the results. The use of the hybrid-capture approach in conjunction with a comprehension individualized bioinformatics study could detect rearrangements missed by amplicon-based strategies, making it possible to characterize novel fusion partners [11].

Glossary of Genomic Terms and Nomenclature

ROS1: ROS proto-oncogene 1, receptor tyrosine kinase
SLC12A2: solute carrier family 12 member 2
NGS: next-generation sequencing
FISH: fluorescence in situ hybridization
CT: computed tomography
OFA: Oncomine Focus Assay
TSO500: TruSight Oncology 500

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Disclosures

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