CASE REPORT

Repotrectinib Overcomes F2004V Resistance Mutation in *ROS1*-Rearranged NSCLC: A Case Report

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ABSTRACT

ROS1 tyrosine kinase inhibitors (TKIs) were found to provide a substantial clinical benefit for patients with advanced ROS1-positive (ROS1+) NSCLC. Nevertheless, TKI resistance inevitably develops with different mechanisms, preventing prolonged responses. For this reason, next-generation compounds are under clinical development. ROS1 F2004 substitutions have been previously detected on circulating tumor DNA of patients progressing to entrectinib. Hereby, we report the case of a patient with ROS1+ NSCLC in which F2004V-acquired mutation was detected on a site of disease progression, after entrectinib and crizotinib failure. A subsequent treatment with next-generation TKI repotrectinib led to disease response, providing the first clinical evidence of activity of repotrectinib against F2004V resistance mutation.

Keywords: Case report; Entrectinib; Repotrectinib; Resistance; ROS1 F2004V

Introduction

ROS1 gene fusions are targetable oncogenic alterations that occur in approximately 1% to 2% of NSCLC. The efficacy of *ROS1* tyrosine kinase inhibitors (TKIs) is prominent, with entrectinib and crizotinib being approved as standard treatments for patients with advanced NSCLC harboring *ROS1* fusions. Subsequent next-generation TKIs, such as lorlatinib and repotrectinib, were also found to have a strong therapeutic potential in both pretreated and untreated patients.

Anyway, TKIs are challenged by ensuing mechanisms of acquired resistance. The largest data come from post-crizotinib analyses where different *ROS1* point mutations can be found in 50% to 60% of resistant tumors. $ROS1^{G2032R}$ is the most frequent, followed by $ROS1^{D2033N}$, $ROS1^{S1986F}$, and $ROS1^{L2026M}$,[1] and a single case report

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described *ROS1*^{F2004C} acquisition, together with sarcomatoid transformation, after crizotinib failure.[2] Limited clinical data are available with regard to first-line entrectinib resistance mechanisms. Dimou et al.[3] described the case of a patient treated in the context of the phase 1 STARTRK-1 trial (NCT02097810) with evidence of *ROS1*^{F2004V} mutation in plasma circulating tumor DNA after entrectinib failure. In addition, dynamic monitoring by means of circulating tumor DNA sequencing of patients enrolled in STARTRK-2 trial (NCT02568267) revealed the presence of *ROS1*^{G2032R} and *ROS1*$^{F2004C/I}$ mutations in up to 28% of patients at progression.[4]

Hereby, we describe the identification of *ROS1*^{F2004V} that emerged on a site of disease progression after failure of sequential treatment with entrectinib and crizotinib and we provide the first clinical evidence of activity of repotrectinib against such resistance mutation.

Case Presentation

Briefly, a 49-year-old never-smoker woman was diagnosed with having stage IV lung adenocarcinoma harboring *CD74-ROS1* fusion, with lung, lymph node, bone, and multiple brain metastases. The timeline of clinical history with treatment sequence, local therapies, and molecular assessments is reported in Figure 1. Our patient was treated with entrectinib 600 mg daily for a total of 18 months, followed by crizotinib with a stable disease maintained for 7 months, and carboplatin with liver and brain progression as best response. At that point, a biopsy was performed on a liver metastasis of lung adenocarcinoma, allowing the identification by next-generation sequencing (NGS) analysis of *CD74-ROS1* fusion transcript together with an acquired *ROS1*^{F2004V} mutation. Subsequently, the patient began fourth-line repotrectinib therapy, 160 mg twice daily, within a compassionate use program. The treatment was well tolerated, and, after 2 months, brain and liver partial response with thoracic stability were documented. Nevertheless, after 5 months on repotrectinib, the patient experienced brain, subcutaneous, subcarinal, and bronchial disease progression determining partial occlusion of the left main bronchus. Notably, liver localizations contextually achieved a complete response. NGS analysis (Oncomine Focus Assay version 3.1 panel) on the adenocarcinoma from the bronchial sample confirmed the presence of *CD74-ROS1* fusion but absence of *ROS1*^{F2004V} mutation.

Discussion

Patterns of on-target resistance mutations differ between type I and type II ROS1 TKIs, which are characterized by binding the catalytically active or inactive conformation, respectively. Docking studies revealed

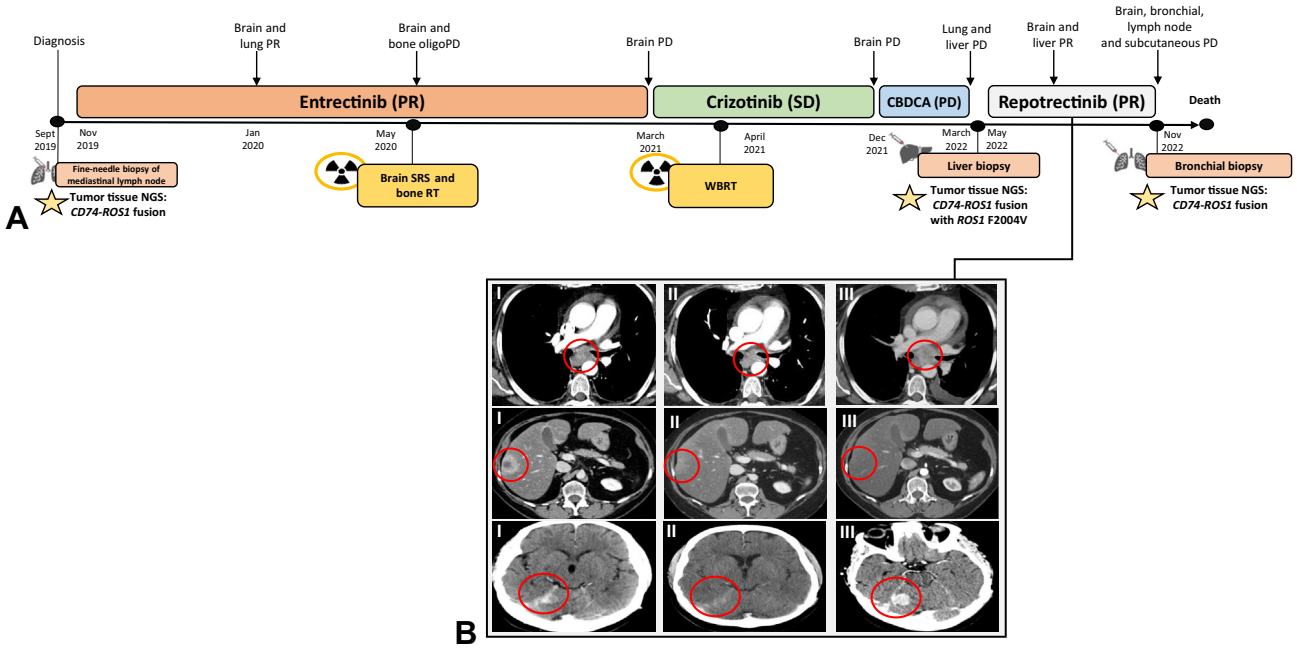

Figure 1. (*A*) Timeline of the clinical history, reporting treatment sequence, local therapies, and molecular assessments. (*B*) Computed tomography scans of the target lesions in the subcarina, liver, and brain (red circles) before (I), after 2 months (II), and after 5 months (III) of treatment with repotrectinib. Images illustrate complete response of liver metastasis and progression of subcarinal and brain metastases after the initial response. CBDCA, carboplatin; Dec, December; Jan, January; NGS, next-generation sequencing; Nov, November; PD, progressive disease; PR, partial response; RT, radiotherapy; SD, stable disease; SRS, stereotactic radiosurgery; WBRT, whole-brain radiotherapy.

that entrectinib binds with high affinity to adenosine triphosphate pocket of both active (type I) and inactive (type II) conformations. Thus, entrectinib may be susceptible to induce a subset of type I and type II binding mode resistance mutations. F2004 substitutions reside within the adenosine triphosphate pocket and emerged in vitro as liable for resistance against type II inhibitors cabozantinib and foretinib, but not against type I inhibitors (crizotinib, lorlatinib, repotrectinib, and brigatinib). In experiments of induced mutagenesis in BaF3 *CD74-ROS1* and *EZR-ROS1* cells by exposure to N-ethyl-N-nitrosourea, *ROS1*^{F2004C} developed as a recurrent entrectinib-resistant mutation, whereas next-generation macrocyclic inhibitors, lorlatinib and repotrectinib, retain potency against *ROS1*^{F2004C}.[5] These results are consistent with available clinical evidence, where F2004 substitutions appear mostly after progression to entrectinib[1,3,4] and are vulnerable to lorlatinib.[2,3]

In our clinical case, F2004V emerged after sequential treatment with entrectinib and crizotinib, and the next-generation inhibitor repotrectinib led to a complete response on the site of identification of such subpopulation of tumor cells. Anyway, a different cluster lacking *ROS1*^{F2004V} was responsible for disease progression after a few months.

Conclusions

Repotrectinib overcomes F2004V resistance mutation, which is more often inducible by entrectinib in ROS1-positive NSCLC. In this case, intratumor heterogeneity of resistance mechanisms prevented durable clinical benefit with ROS1 TKIs.

CRediT Authorship Contribution Statement

Elio Gregory Pizzutilo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing.

Alberto Giuseppe Agostara: Data curation, Formal analysis, Investigation, Resources, Supervision, Writing—original draft.

Laura Roazzi: Data curation, Roles/Writing—original draft.

Rebecca Romanò: Data curation, Roles/Writing—original draft.

Valentina Motta: Data curation, Formal analysis, Investigation, Visualization.

Calogero Lauricella: Data curation, Formal analysis, Investigation, Visualization.

Giovanna Marrapese: Data curation, Resources, Supervision.

Giulio Cerea: Supervision, Writing—review and editing.

Diego Signorelli: Supervision, Writing—review and editing.

Silvio Marco Veronese: Supervision, Writing—review and editing.

Laura Giuseppina Giannetta: Supervision, Writing—review and editing.

Andrea Sartore-Bianchi: Supervision, Writing—review and editing.

Salvatore Siena: Supervision, Writing—review and editing.

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