

## CASE REPORT

**CEP72-ROS1: A novel ROS1 oncogenic fusion variant in lung adenocarcinoma identified by next-generation sequencing**You-cai Zhu<sup>1\*</sup>, Yue-fen Zhou<sup>2\*</sup>, Wen-xian Wang<sup>3</sup> , Chun-wei Xu<sup>4</sup> , Wu Zhuang<sup>5</sup>, Kai-qi Du<sup>1</sup> & Gang Chen<sup>3</sup>

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**Introduction**

Lung cancer morbidity and mortality are increasing in both developed and developing countries worldwide.<sup>1,2</sup> Oncogenic *ROS1* rearrangements have become an established molecular target in lung cancer. *ROS1* rearrangements are identified in 1–2% of non-small cell lung cancer (NSCLC) patients.<sup>3</sup> NSCLC was the second solid tumor found to harbor *ROS1* rearrangement. Nowadays, a total of

**Abstract**

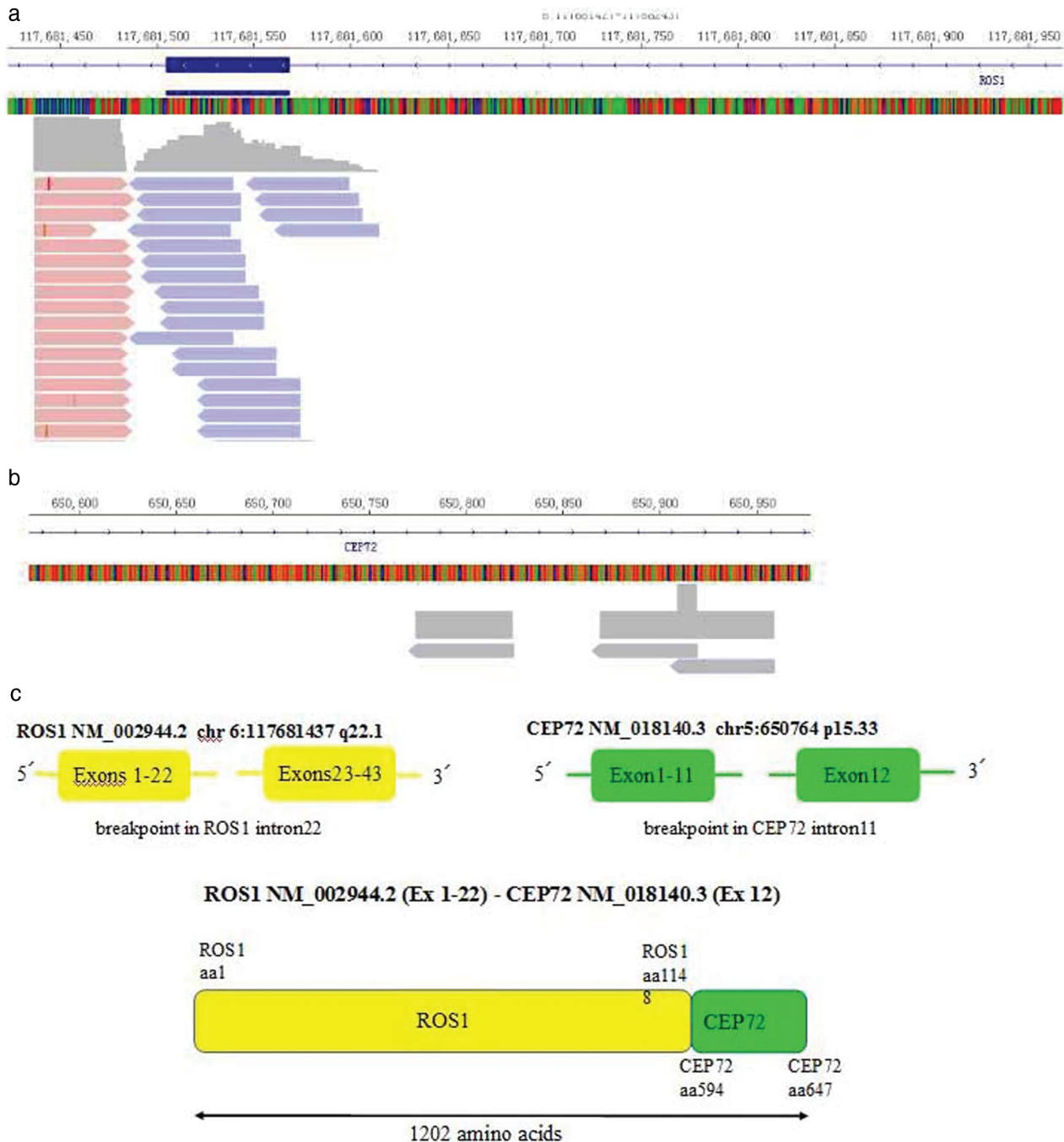
*ROS1* rearrangement is a validated therapeutic driver gene in non-small cell lung cancer (NSCLC) and represents a small subset (1–2%) of NSCLC. A total of 17 different fusion partner genes of *ROS1* in NSCLC have been reported. The multi-targeted MET/ALK/*ROS1* tyrosine kinase inhibitor (TKI) crizotinib has demonstrated remarkable efficacy in *ROS1*-rearranged NSCLC. Consequently, *ROS1* detection assays include fluorescence in situ hybridization, immunohistochemistry, and real-time PCR. Next-generation sequencing (NGS) assay covers a range of fusion genes and approaches to discover novel receptor-kinase rearrangements in lung cancer. A 63-year-old male smoker with stage IV NSCLC (TxNxM1) was detected with a novel *ROS1* fusion. Histological examination of the tumor showed lung adenocarcinoma. NGS analysis of the hydrothorax cell-blocks revealed a novel *CEP72-ROS1* rearrangement. This novel *CEP72-ROS1* fusion variant is generated by the fusion of exons 1–11 of *CEP72* on chromosome 5p15 to exons 23–43 of *ROS1* on chromosome 6q22. The predicted *CEP72-ROS1* protein product contains 1202 amino acids comprising the N-terminal amino acids 594–647 of *CEP72* and C-terminal amino acid 1–1148 of *ROS1*. *CEP72-ROS1* is a novel *ROS1* fusion variant in NSCLC discovered by NGS and could be included in *ROS1* detection assay, such as reverse transcription PCR. Pleural effusion samples show good diagnostic performance in clinical practice.

17 *ROS1* fusion partner genes have been reported, including *CD74*, *SLC34A2*, *GOPC*, *CCDC6*, *SDCA*, *TPM3*, *EZR*, *LRIG3*, *KDEL2*, *LIMA1*, *MSN*, *CLTC*, *TPD52L1*, *TMEM106B*, *FAM135B*, and *SLC6A17*.<sup>4–7</sup> In general, we use surgery or percutaneous lung biopsies to detect gene status. However, in some cases, we are unable to obtain tumor tissue. Approaches for examining molecular biomarkers in body fluids, such as effusion or serum, may be

clinically helpful for predicting the response to TKI treatment.

Crizotinib, an ALK/ROS1/MET inhibitor, was the first targeted agent approved by the United States Food and Drug Administration (US FDA) for the treatment of advanced ROS1-rearranged NSCLC based on a phase I crizotinib trial. It demonstrated an objective response rate

(ORR) of 72% and median progression-free survival (PFS) of 19.2 months in advanced ROS1-rearranged NSCLC.<sup>8</sup> Because of precise detection assays, such as next-generation sequencing (NGS), more and more novel ROS1 fusion partner genes are being identified in lung cancer. However, the clinical significance of these variants requires further investigation. The efficacy of crizotinib treatment in some



**Figure 1** Paired-end sequencing data indicated somatic intrachromosomal (a) CEP72 and (b) ROS1, demonstrated by Integrative Genomics Viewer program. (c) Schematic representation of the translocation involving CEP72 and ROS1 and the predicted domains of the CEP72-ROS1 fusion protein.

of the abovementioned *ROS1* rearrangements has not yet been reported.

Herein, we report a novel *ROS1*-rearranged (*CEP72-ROS1*) NSCLC fusion variant in a smoking patient who presented with malignant pleural effusion of lung adenocarcinoma (stage IV).

## Case report

Our case involves a 63-year old male smoker (> 100 pack-year) with a one-week history of chest distress and dyspnea. The patient was diagnosed with stage IV NSCLC (malignant pleural effusion) in December 2014. We found adenocarcinoma cells from pleural effusion. Immunohistochemistry (IHC) of pleural effusion analysis demonstrated positivity in TTF-1 and negativity in CK 5/6 and P63. We detected the gene mutation status of the pleural effusion, which showed wild-type epidermal growth factor receptor variants using an amplification refractory mutation system and *ALK* gene negative using reverse transcription PCR assay (AmoyDx, Xiamen, China). NGS analysis of the hydrothorax cellblocks revealed a novel *CEP72-ROS1* rearrangement (Geneplus, Beijing, China). This novel *CEP72-ROS1* fusion variant is generated by the fusion of exons 1–11 of *CEP72* on chromosome 5p15 to exons 23–43 of *ROS1* on chromosome 6q22. The predicted *CEP72-ROS1* protein product contains 1202 amino acids comprising N-terminal amino acids 594–647 of *CEP72* and C-terminal amino acid 1–1148 of *ROS1* (Fig 1).

Unfortunately, the patient experienced disease progression but could not be treated with chemotherapy because of decreased performance status or with *ROS1* inhibitors because of the cost, and thus died shortly after.

## Discussion

To our knowledge, *CEP72-ROS1* fusion in lung cancer has not previously been reported, thus this is the first report of novel *ROS1* fusion. *CEP72* is located on chromosome 5p15 and contains 12 exons. The product of this gene is a member of the LRR superfamily of proteins, including 647-amino acid. The protein is localized to the centrosome, a non-membranous organelle that plays critical roles in microtubule organization, cell division, and cilium formation.<sup>9</sup> The *CEP72* protein contains a “coiled-coil” domain (476–620 aa) that is common among the fusion protein partners of receptor tyrosine kinase (RTK) rearranged NSCLC. Functionally, *CEP72* has been linked to genetic modifiers of ubiquitin-positive frontotemporal dementia (UP-FTD) and frontotemporal dementia and/or amyotrophic lateral sclerosis 1 (FTDALS1). Interestingly, the *TMEM106B* gene, which has been linked as a genetic modifier of frontotemporal lobar degeneration (FTLD), is also

reported to be translocated to proto-oncogene tyrosine-protein kinase (*ROS1*) in *ROS1*-rearranged NSCLC,<sup>5</sup> and the *HIP1* gene is reported to be translocated to *ALK* in *ALK*-rearranged NSCLC at three different breakpoints.<sup>10</sup> Three genes, *CEP72*, *TMEM106B*, and *HIP1*, were all related to various neurological diseases; whether *ALK* and *ROS1* rearranged NSCLC patients with these fusion protein partners present with certain unique clinicopathological characteristics remains to be determined.

*ROS1* testing in NSCLC samples is often only considered after the patient is determined as negative for *EGFR* mutation and *ALK* rearrangement.<sup>11</sup> Nowadays, *ROS1* is a validated therapeutic target for NSCLC. In a phase I study, the multi-targeted MET/*ALK*/*ROS1* inhibitor crizotinib demonstrated remarkable efficacy in *ROS1*-rearranged NSCLCs, and consequently gained approval by the US FDA. Testing for the *ROS1* gene in advanced NSCLC should be routine. There are currently no approved companion assays for *ROS1* fusion NSCLC. Based on experience with *ALK*, commonly used methods for *ROS1* fusion detection include fluorescence in situ hybridization, immunohistochemistry, reverse transcription PCR, and NGS.<sup>12</sup> Developments in NGS have created a new method for the simultaneous detection of a large number of gene fusions with known and unknown genes and gene mutations.<sup>13,14</sup> We thought the types of *ROS1* fusion were various and IHC assay was a prescreening test.<sup>15</sup> In addition, NGS could identify novel fusions and increase the list of actionable variants for NSCLC patients.

Malignant pleural effusion (MPE) presents in approximately 50% of patients with advanced NSCLC. MPEs often contain tumor cells and biomarkers and can represent an alternative to tumor tissues in clinical practice. PCR and fluorescence in situ hybridization have successfully detected *EGFR* mutations and *ALK* rearrangements in MPE samples, respectively.<sup>15–17</sup> In our case, *ROS1* fusion was detected in MPE using NGS, demonstrating that MPE detection is a feasible method to detect *ROS1* gene status. In the future, it is worth exploring the use of MPE for *ROS1* or other gene detection in NSCLC patients and the predictive value of MPE-based detection for clinical use. The results of this case report create awareness of a novel type of *ROS1* translocation in NSCLC patients. NGS assay could provide a novel diagnostic approach. Research into the biology of different *ROS1*-driven lung cancers for the development of new therapeutic strategies is required.

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## Disclosure

No authors report any conflict of interest.

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