OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

CASE REPORT

A Novel ROS1-FBXL17 Fusion Co-Existing with CD74-ROS1 Fusion May Improve Sensitivity to Crizotinib and Prolong Progression-Free Survival of Patients with Lung Adenocarcinoma

OncoTargets and Therapy downloaded from https://www.dovepress.com/
For personal use only.

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Shaowei Lan[1,2,*]
Hui Li[1,2,*]
Ying Liu[3]
Jinhua Xu[3]
Zhicheng Huang[4]
Shi Yan[1,2]
Qiang Zhang[5]
Ying Cheng[1–3]

[1]Translational Oncology Research Lab, Jilin Provincial Cancer Hospital, Changchun 130012, People's Republic of China; [2]Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer, Jilin Provincial Cancer Hospital, Changchun 130012, People's Republic of China; [3]Department of Medical Thoracic Oncology, Jilin Provincial Cancer Hospital, Changchun 130012, People's Republic of China; [4]Department of Radiology, Jilin Provincial Cancer Hospital, Changchun 130012, People's Republic of China; [5]Burning Rock Biotech, Guangzhou 510000, People's Republic of China

*These authors contributed equally to this work

Purpose: The rearrangement of *ROS1* (C-ros oncogene 1) is an important driver of non-small cell lung cancer (NSCLC). Currently, only approximately 24 *ROS1* fusion partners have been shown to be sensitive to crizotinib. Although fusion partner determination is not required to treat patients with tyrosine kinase inhibitor, the correlation between *ROS1* phenotypes and efficacies still needs more researches. Furthermore, non-reciprocal/reciprocal *ROS1* translocations are rare and have not yet been reported. Thus, more novel *ROS1* fusion partners and non-reciprocal/reciprocal fusions need to be provided and supplemented to guide targeted therapy and prognosis for patients.

Case Presentation: Targeted next-generation sequencing panel was used to identify *ROS1* rearrangements in a Chinese patient with advanced lung adenocarcinoma. We identified a non-reciprocal/reciprocal *ROS1* translocation which contained a novel *ROS1-FBXL17* (F-box and leucine-rich repeat protein 17) fusion co-existing with the *CD74-ROS1* fusion and the patient was sensitive to crizotinib. The *ROS1* rearrangement was then validated using RT-qPCR. The progression-free survival (PFS) was 15.7 months which exceeded the highest PFS level (14.2 months) in the Chinese population reported recently. Thus, this non-reciprocal/reciprocal *ROS1* translocation patient had an excellent efficacy to crizotinib which was different from that in *ALK*. And it may be possible that the *ROS1-FBXL17* fusion in this patient synergistically promotes the sensitivity of the *CD74-RSO1* fusion to crizotinib.

Conclusion: The *ROS1-FBXL17* fusion may be a novel driver of NSCLC and we provide a non-reciprocal/reciprocal *ROS1* translocation mode very sensitive to crizotinib. Our study adds new data to the *ROS1* fusion database and provides a reference strategy for the clinical treatment of patients with double *ROS1* fusions or non-reciprocal/reciprocal *ROS1* translocation.

Keywords: *ROS1-FBXL17* fusion, *CD74-ROS1* fusion, non-reciprocal/reciprocal *ROS1* translocation, next-generation sequencing, intratumor heterogeneity, non-small cell lung cancer

Background

ROS1 (C-ros oncogene 1) rearrangement is a proven driver of non-small cell lung cancer (NSCLC) and occurs in approximately 1–3% of the patients with NSCLC worldwide.[1–4] Crizotinib, an oral tyrosine kinase inhibitor (TKI) that targets the *ROS1, ALK*, and *Met* receptor tyrosine kinases, is sensitive to the rearrangement of *ROS1*.[5–8] Many genes can fuse with *ROS1*, and currently, at least 24 partner genes

Correspondence: Ying Cheng
Jilin Provincial Cancer Hospital, 1066 Jinhu Road, Changchun, Jilin, People's Republic of China
Tel +86-431-80596051
Email chengying@csco.org.cn

© 2020 Lan et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

of *ROS1* have been identified in patients with NSCLC.[9–13] The application of next-generation sequencing (NGS) is reliable and helpful in the discovery of novel variants, including *ROS1* partners.[13–15] It is widely known that the *EML4-ALK* variants (V1-V7) or *EGFR* mutations (L858R/T790M/C797S/19 del.) have different TKIs efficacies between subtypes.[16–19] Furthermore, it had recently been confirmed that crizotinib-treated patients with non-reciprocal/reciprocal *ALK* translocation had a poor efficacy compared with patients carrying 3′-*ALK* fusion alone or with *EML4-ALK* fusion alone.[20] Therefore, it may also have different drug sensitivities and resistances between *ROS1* fusion subtypes. More important, the crizotinib efficacy in patients with non-reciprocal/reciprocal *ROS1* translocation has not been reported. Here, we identified a novel *ROS1-FBXL17* (F-box and leucine-rich repeat protein 17) fusion that co-existed with the *CD74-ROS1* fusion in one patient who was sensitive to crizotinib and was with a stable disease (SD) for more than 15 months, thus identifying a rare sensitive double fusion mode (a non-reciprocal/reciprocal translocation) for *ROS1*.

Case Report

In June 2018, a 51-year-old Chinese female showing symptoms such as productive cough (whitish sputum), chest pain, and dyspnea, was diagnosed with adenocarcinoma in the superior lobe of the right lung through systematic examination. The clinical stage of the cancer was determined as T3N2M0 (stage IIIb). Surgical resection of the superior lobe of the right lung was performed in Shanghai. However, a reexamination in our hospital for the patient in the 28th day after surgery found that the patient had pleural invasion, bilateral mediastinal and hilar lymph node metastasis, right supraclavicular lymph node metastasis, and right cervical lymph node metastasis (pT4N3M1c IVb). NGS was performed for an DNA sequence based 8 gene panel, including *EGFR, ALK, ROS1, MET, RET, BRAF, ERBB2*, and *KRAS*, using post-operative tissue samples, and no genetic alterations were found in any genes. The patient was administered one course of pemetrexed, combined with cisplatin chemotherapy, and achieved the SD state, according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1); however, the patient could not tolerate the side effects.

To determine whether the patient was suitable for treatment with immune checkpoint inhibitors, the immunotherapy biomarker programmed death ligand 1 (PD-L1, immunohistochemistry) and the tumor mutational burden (TMB, NGS) were detected using surgical specimens that were sliced consecutively. TMB was detected through a DNA sequence based 520 gene panel (520 genes closely associated to solid tumors) using the Illumina Novaseq 6000 Sequencing Platform and a Dx Oncoscreen plus TM kit (Burning Rock, China). This panel includes the driver genes involved in 8 gene panel. The results showed 5–10% PD-L1 positive cells and a TMB value of 5.6 mutations per megabase. Interestingly, NGS identified a *ROS1-FBXL17* fusion (R33: Fintergenic; mutation frequency: because *FBXL17* was not included in the panel, the accurate mutation frequency of the *ROS1-FBXL17* fusion could not be obtained.) coexisting with the *CD74-ROS1* fusion (C6: R33; mutation frequency: 27.70%), and *TP53, APC, MGA*, and *ZNF217* mutations. The novel *ROS1-FBXL17* rearrangement (2476 amino acids) was generated through the fusion of *ROS1* exons 1–32 on chr6: q22.1 (5′-3′, 1–1776 amino acids) to *FBXL17* exons 1–9 on chr5: q21.2 (5′-3′, 1–700 amino acid) (Figure 1A and C), while the *CD74-ROS1* rearrangement (778 amino acids) was generated through the fusion of *CD74* exons 1–6 on chr5: q33.1 (5′-3′, 1–207 amino acids) to *ROS1* exons 33–43 on chr6: q22.1 (5′-3′, 1786–2356 amino acids) (Figure 1B and C). In addition, RNA isolation and reverse transcription were performed in postoperative tissue samples, and real-time PCR (amplification refractory mutation system, ARMS) result confirmed the presence of the *CD74-ROS1* fusion for clinical requirement (Figure 1D). However, due to the shortage of tumor tissue, RNA sequencing could not be performed.

Before undergoing molecular targeting treatments, the patient underwent a computed tomography (CT) examination, which showed that the mediastinal lymph nodes were 1.35 cm (Figure 2A). The patient was then orally treated with crizotinib for more than 17 months (data cut-off: March 2, 2020). The mediastinal lymph nodes continued to shrink (from 0.65 cm to unmeasurable) without any other observable metastasis (Figure 2B–D). The efficacy evaluation in the lung indicated SD. However, magnetic resonance imaging (MRI) on January 10, 2020 showed a new lesion in the cerebellum (0.69 cm) compared with before (Figure 2F–H), and the patient received continued oral treatment with crizotinib. At the time of the assessment on March 3, 2020, the lesion was still growing in size (1.0 cm), confirming disease progression (Figure 2I).

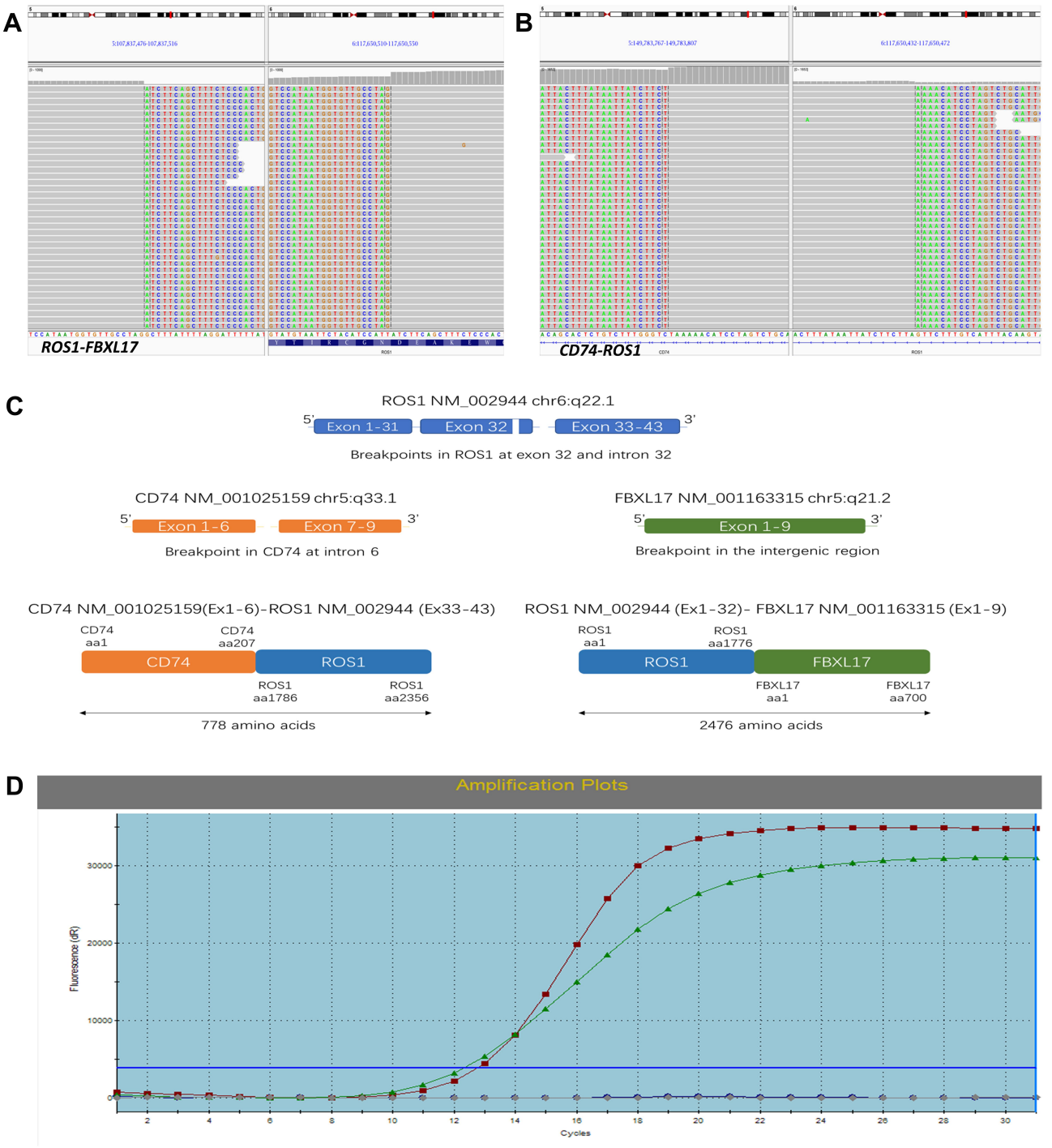

Figure 1 NGS and RT-qPCR were used to identify the *ROS1-FBXL17* and *CD74-ROS1* fusions in a patient with advanced lung adenocarcinoma. (**A**). The *ROS1-FBXL17* fusion. The junction point of *ROS1* is located on 6q22.1 and another junction point is in the intergenic region, close to *FBXL17*, on 5q21.3. (**B**). The *CD74-ROS1* fusion. The junction point of *ROS1* is located on 6q22.1 and that of *CD74* is on 5q33.1. (**C**). A schematic representation of the *ROS1-FBXL17* and *CD74-ROS1* fusion protein domain structures. Orange, *CD74*; green, *FBXL17*; blue, *ROS1*. The predicted fusion proteins of *ROS1-FBXL17* and *CD74-ROS1* are 2476 and 778 amino acids in length, respectively. (**D**). *ROS1* fusion was confirmed using RT-qPCR. Red line, samples from the patient; green line, positive control.

The patient presented with mild myelosuppression and there was no head discomfort or movement disorder. The progression-free survival (PFS) was 15.7 months. Then the patient received radiotherapy (30Gy/10 times/12 days) on head and the cerebellar lesion began to shrink. Thus far, the disease remains stable and the patient is still under crizotinib treatment. On October 13, 2020 the MRI showed a 0.64 cm lesion in the cerebellum (Figure 2J)

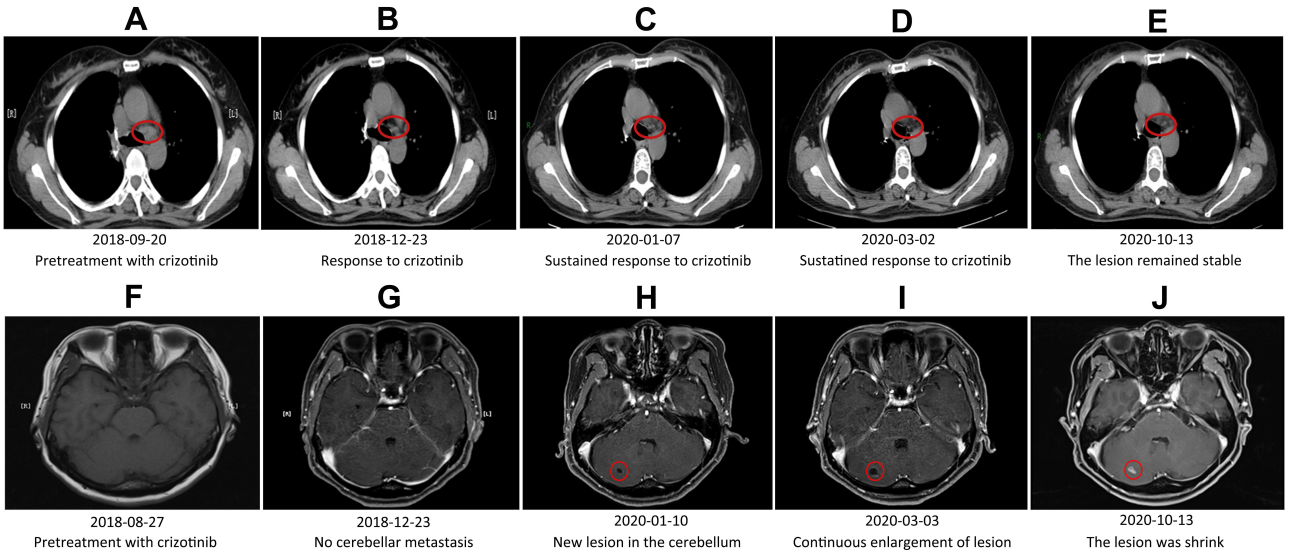

Figure 2 Computed tomography or magnetic resonance imaging showed a sustained response to crizotinib in the mediastinal lymph nodes and disease progression in the cerebellum. (**A**). After chemotherapy, the mediastinal lymph nodes showed swelling, for which crizotinib was administered as a treatment. (**B**). The mediastinal lymph nodes shrank significantly, without other measurable niduses after 3 courses of treatment with crizotinib. (**C**–**E**). A sustained response to crizotinib was observed, and the nidus was unmeasurable. (**F** and **G**). No metastatic lesions were observed in the cerebellum. (**H** and **I**). A new lesion was found in the cerebellum and disease progression was observed. (**J**). The cerebellar lesion was shrunk after received radiotherapy.

and CT showed the mediastinal lymph nodes remained stable (Figure 2E).

Discussion

To our knowledge, double genes fused with *ROS1* are rare and *FBXL17* is a novel partner for *ROS1*, making the right regimen for this mutation unclear. Although fusion variant determination was not required for patients treated with TKIs, the efficacies were different between fusion modes.[20] As compared with the *CD74-ROS1* fusion, the non-*CD74-ROS1* fusion might have a significantly longer PFS.[21] Therefore, identification of the mutant subtypes or non-reciprocal/reciprocal translocations are beneficial for both treatment and prognosis.

There is little information regarding the efficacy of crizotinib for patients harboring double genes fused with *ROS1*. A Chinese study in 2018 identified a patient who harbored a *CD74-ROS1* fusion that coexisted with the *SDC4-ROS1* fusion, and another patient who harbored a *SDC4-ROS1* fusion that coexisted with the *EZR-ROS1* fusion.[21] Crizotinib showed different efficacies in the two patients, which indicated that crizotinib may have different efficacies in double fusions of *ROS1*. The translocations in the two patients were both 3′-*ROS1*. But nothing was known about the efficacy of non-reciprocal/reciprocal *ROS1* translocation patients treated with crizotinib. Presence of non-reciprocal/reciprocal *ALK* translocation was predictive for worse survival and greater likelihood of baseline brain metastases in first-line crizotinib treated NSCLC patients.[20] In this case, we confirmed a patient with non-reciprocal/reciprocal *ROS1* translocation (*ROS1-FBXL17* fusion that coexisted with the *CD74-ROS1* fusion) had an excellent response to crizotinib. The PFS reached 15.7 months and exceeded the highest level (14.2 months) of the *ROS1* fusion reported in a recent study in Chinese population.[22] It is also higher than the median level (12.6 months) in Chinese patients with 3′-*ROS1* fusion or with *CD74-ROS1* fusion alone.[21] These suggested that the crizotinib efficacy in patients with non-reciprocal/reciprocal translocation in *ROS1* might be superior to that in *ALK*. The speculated reason may be that the biological effect of *ROS1* translocation is different from that of *ALK*, and the mechanism in *ROS1* may be more complex, or there may be some unknown mechanisms of concomitant mutation with *ROS1* that has not yet been discovered and revealed. However, further observations and more cases are needed to confirm these points. In addition, brain metastasis occurred after crizotinib treated over 15 months in this case. In the future, more studies are needed to explore whether patients with 5′-*ROS1* are also greater likelihood of baseline brain metastases, such as 5′-*ALK*.

It was not clear whether the *ROS1-FBXL17* fusion was functional and sensitive to crizotinib. Xu et al[12] have reported

a *ROS1-ADGRG6* rearrangement alone that is generated by the fusion of the exons 1–33 of *ROS1* on chr6: q22.1 to exons 2–26 of *ADGRG6* on chr6: q24.2 and is clearly sensitive to crizotinib. The main structural framework and the junction point on the *ROS1* of the *ROS1-FBXL17* fusion were similar to those of the *ROS1-ADGRG6* fusion; thus, we speculated that the *ROS1-FBXL17* fusion may have biological functions similar to those of *ROS1-ADGRG6*. Therefore, *ROS1-FBXL17* may be a novel driver mutation, or confer a positive and synergistic effect on the sensitivity of the *CD74-ROS1* fusion to crizotinib.

Furthermore, in this case, serial sectioning of a single postoperative tissue specimen was performed for targeted detection, using an 8 genes panel and a 520 genes panel successively. Interestingly, different results were obtained from the two experimental runs, each of which used different sections from the same paraffin block. The possible reasons for the difference included heterogeneity between slices,[23–25] formalin-fixed paraffin-embedded samples,[26] or differences in panels, or others. Thus, multi-point sampling and multiple detections may be important steps for determining the *ROS1* fusions, and for molecular subtyping, which can dictate the clinical treatment strategies for patients. Moreover, cytological samples or liquid biopsy is also a feasible strategy for patients with tissue sampling difficulties.

Conclusion

In summary, we identified a novel *ROS1-FBXL17* fusion that coexisted with the *CD74-ROS1* fusion in an advanced NSCLC patient sensitive to crizotinib. We provided a non-reciprocal/reciprocal *ROS1* translocation pattern very sensitive to crizotinib. The *ROS1-FBXL17* fusion may be functional and may promote the sensitivity of the *CD74* fusion to crizotinib but this needs further validation. Although fusion partners determination is not required for patients treated with crizotinib, due to the presence of intratumor heterogeneity and non-reciprocal/reciprocal translocations, molecular subtyping of *ROS1* fusions should be accurately and timely identified.

Ethics Statement

The study was approved by the Ethics Committee of Jilin Provincial Cancer Hospital. The patient provided written informed consent for the publication of any associated data.

Consent for Publication

All authors agreed to the publication of these data.

Acknowledgments

The authors would like to thank the patient for her positive attitude during clinical treatment. We also thank the doctors from the auxiliary departments who were involved in the accurate diagnosis of the patient's condition.

Funding

This research was supported in part by the Department of Science and Technology of Jilin Province (20190303157SF, 202002062JC and 202002063JC) and Scientific Research Funds of Jilin Province of Health and Family Planning Commission (2018J021 and 2018J023).

Disclosure

Qiang Zhang is an employee of Burning Rock Biotech. The authors report no other conflicts of interest in this work.

References

1. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol*. 2012;30(8):863–870. doi:10.1200/JCO.2011.35.6345
2. Cai W, Li X, Su C, et al. ROS1 fusions in Chinese patients with non-small-cell lung cancer. *Ann Oncol*. 2013;24(7):1822–1827. doi:10.1093/annonc/mdt071
3. Zhang Q, Wu C, Ding W, et al. Prevalence of ROS1 fusion in Chinese patients with non-small cell lung cancer. *Thorac Cancer*. 2019;10(1):47–53. doi:10.1111/1759-7714.12899
4. Shaw AT, Solomon BJ, Chiari R, et al. Lorlatinib in advanced ROS1-positive non-small-cell lung cancer: a multicentre, open-label, single-arm, Phase 1–2 trial. *Lancet Oncol*. 2019;20(12):1691–1701. doi:10.1016/S1470-2045(19)30655-2
5. Wiesweg M, Schuler M, Schildhaus HU. Crizotinib in ROS1 and MET deregulated NSCLC-letter. *Clin Cancer Res*. 2020;26(7):1774. doi:10.1158/1078-0432.CCR-19-3740
6. Lee A, Howell VM, Itchins M, Wheeler HR, Pavlakis N. ROS1-rearranged non-small-cell lung cancer, factor v leiden, and recurrent venous thromboses. *Clin Lung Cancer*. 2018;19(5):457–459. doi:10.1016/j.cllc.2018.05.015
7. Davies KD, Le AT, Theodoro MF, et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin Cancer Res*. 2012;18(17):4570–4579. doi:10.1158/1078-0432.CCR-12-0550
8. Ou SH. Crizotinib: a novel and first-in-class multitargeted tyrosine kinase inhibitor for the treatment of anaplastic lymphoma kinase rearranged non-small cell lung cancer and beyond. *Drug Des Devel Ther*. 2011;5:471–485. doi:10.2147/DDDT.S19045
9. Zhu YC, Zhou YF, Wang WX, et al. CEP72-ROS1: a novel ROS1 oncogenic fusion variant in lung adenocarcinoma identified by next-generation sequencing. *Thorac Cancer*. 2018;9(5):652–655. doi:10.1111/1759-7714.12617
10. Hicks JK, Boyle T, Albacker LA, Madison R, Frampton G, Creelan BC. Clinical activity of crizotinib in lung adenocarcinoma harboring a rare ZCCHC8-ROS1 fusion. *J Thorac Oncol*. 2018;13(8):e148–e150. doi:10.1016/j.jtho.2018.03.028
11. He Y, Sheng W, Hu W, et al. Different types of ROS1 fusion partners yield comparable efficacy to crizotinib. *Oncol Res*. 2019;27(8):901–910. doi:10.3727/096504019X15509372008132

12. Xu S, Wang W, Xu C, et al. ROS1-ADGRG6: a case report of a novel ROS1 oncogenic fusion variant in lung adenocarcinoma and the response to crizotinib. *BMC Cancer*. 2019;19(1):769. doi:10.1186/s12885-019-5948-y
13. Liu Y, Liu T, Li N, Wang T, Pu Y, Lin R. Identification of a novel WNK1-ROS1 fusion in a lung adenocarcinoma sensitive to crizotinib. *Lung Cancer*. 2019;129:92–94. doi:10.1016/j.lungcan.2018.12.011
14. Tsoulos N, Papadopoulou E, Metaxa-Mariatou V, et al. Tumor molecular profiling of NSCLC patients using next generation sequencing. *Oncol Rep*. 2017;38(6):3419–3429.
15. Shulskaya MV, Alieva AK, Vlasov IN, et al. Whole-exome sequencing in searching for new variants associated with the development of parkinson's disease. *Front Aging Neurosci*. 2018;10:136. doi:10.3389/fnagi.2018.00136
16. Lin JJ, Zhu VW, Yoda S, et al. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung cancer. *J Clin Oncol*. 2018;36(12):1199–1206. doi:10.1200/JCO.2017.76.2294
17. Woo CG, Seo S, Kim SW, et al. Differential protein stability and clinical responses of EML4-ALK fusion variants to various ALK inhibitors in advanced ALK-rearranged non-small cell lung cancer. *Ann Oncol*. 2017;28(4):791–797. doi:10.1093/annonc/mdw693
18. Li Y, Zhang T, Zhang J, et al. Response to crizotinib in advanced ALK-rearranged non-small cell lung cancers with different ALK-fusion variants. *Lung Cancer*. 2018;118:128–133. doi:10.1016/j.lungcan.2018.01.026
19. Castellanos E, Feld E, Horn L. Driven by mutations: the predictive value of mutation subtype in EGFR-mutated non-small cell lung cancer. *J Thorac Oncol*. 2017;12(4):612–623. doi:10.1016/j.jtho.2016.12.014
20. Zhang Y, Zeng L, Zhou C, et al. Detection of nonreciprocal/reciprocal ALK translocation as poor predictive marker in patients with first-line crizotinib-treated ALK-rearranged NSCLC. *J Thorac Oncol*. 2020;15(6):1027–1036. doi:10.1016/j.jtho.2020.02.007
21. Li Z, Shen L, Ding D, et al. Efficacy of crizotinib among different types of ROS1 fusion partners in patients with ros1-rearranged non-small cell lung cancer. *J Thorac Oncol*. 2018;13(7):987–995. doi:10.1016/j.jtho.2018.04.016
22. Liu C, Yu H, Chang J, et al. Crizotinib in Chinese patients with ROS1-rearranged advanced nonsmall-cell lung cancer in routine clinical practice. *Target Oncol*. 2019;14(3):315–323. doi:10.1007/s11523-019-00636-6
23. Lai GGY, Lim TH, Lim J, et al. Clonal MET amplification as a determinant of tyrosine kinase inhibitor resistance in epidermal growth factor receptor-mutant non-small-cell lung cancer. *J Clin Oncol*. 2019;37(11):876–884.
24. McLaughlin J, Han G, Schalper KA, et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol*. 2016;2(1):46–54. doi:10.1001/jamaoncol.2015.3638
25. Vukovic V, Nicklee T, Hedley DW. Microregional heterogeneity of non-protein thiols in cervical carcinomas assessed by combined use of HPLC and fluorescence image analysis. *Clin Cancer Res*. 2000;6(5):1826–1832.
26. Haile S, Corbett RD, Bilobram S, et al. Sources of erroneous sequences and artifact chimeric reads in next generation sequencing of genomic DNA from formalin-fixed paraffin-embedded samples. *Nucleic Acids Res*. 2019;47(2):e12. doi:10.1093/nar/gky1142

OncoTargets and Therapy Dovepress

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/oncotargets-and-therapy-journal