

Analysis of Acquired Fusion Mutations in EGFR-TKIs-Resistant Patients with Advanced Lung Cancer

Yuan Yang¹, Nana Zhang², Liang Shi¹, Zhaoxin Chen¹, Baohua Lu¹ and Zhe Liu¹

¹Department of Oncology, Beijing Chest Hospital, Capital Medical University, and Beijing Tuberculosis and Thoracic Tumour Research Institute, Beijing, China

²Department of Pathology, Beijing Chest Hospital, Capital Medical University, and Beijing Tuberculosis and Thoracic Tumour Research Institute, Beijing, China

ABSTRACT

To determine the characteristics of non-small cell lung cancer (NSCLC) patients with fusion genes emerging after resistance to EGFR-TKIs. This study retrospectively collected 2800 cases of NSCLC with EGFR-sensitive mutations who underwent tissue-based next-generation sequencing (NGS) testing at least once. Patients with acquired fusion mutations, including *ALK*, *ROS1*, *RET*, etc., were included in the study, and clinical data, gene mutation status, treatment strategies, and follow-up were collected. Given the small sample size and exploratory design of this case series (n = 9), the findings were reported descriptively. There were six cases of *ALK* fusion, two cases of *RET* fusion, and one case of *ROS1* fusion. EGFR abundance decreased in five patients, while EGFR was detected as negative in the other four patients. When fusion mutations emerged, the use of *ALK/RET/ROS1*-TKIs or EGFR-TKIs combined with fusion-TKIs demonstrated efficacy. The progression-free survival ranged from 5 months to ≥ 39 months. One patient with acquired fusion gene mutations developed small-cell lung cancer transformation after *ALK*-TKIs resistance.

Key Words: Lung cancer, *ALK* fusion, *RET* fusion, *ROS1* fusion, EGFR-TKIs-resistance, Targeted therapy.

How to cite this article: Yang Y, Zhang N, Shi L, Chen Z, Lu B, Liu Z. Analysis of Acquired Fusion Mutations in EGFR-TKIs-Resistant Patients with Advanced Lung Cancer. *J Coll Physicians Surg Pak* 2026; **36(03)**:402-405.

INTRODUCTION

Due to the significant improvements in survival and quality of life brought by epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), they have been prioritised and widely used in EGFR-sensitive non-small cell lung cancer (NSCLC) patients. However, acquiring resistance after EGFR-TKIs is inevitable. The currently known resistance mechanisms include EGFR-dependent resistance, bypass or downstream activation, organisational or phenotypic transformation. The proportion of off-target resistance is higher than that of on-target resistance.¹

Acquired fusion gene mutations are a rare pathway activation mechanism that leads to resistance to EGFR-TKIs. Previous reports have shown that proto-oncogene tyrosine-protein kinase *ROS1* (*ROS1*), rearranged during transfection (*RET*), anaplastic lymphoma kinase (*ALK*), neurotrophic tyrosine receptor kinase 1 (*NTRK1*), fibroblast growth factor receptor 3 (*FGFR3*), and *BRAF* fusions mediate resistance to EGFR-TKIs.¹⁻³

This study retrospectively analysed cases in Beijing Chest Hospital to provide real-world data for such patients. This study aimed to determine the clinical-pathological features, drug selections, therapy responses, and prognoses of these unique patients.

METHODOLOGY

A retrospective study was conducted on 2,800 cases of NSCLC patients with EGFR-sensitive mutations who were admitted to the Department of Oncology, Beijing Chest Hospital, Capital Medical University, and Beijing Tuberculosis and Thoracic Tumour Research Institute, Beijing, China, from June 2019 to June 2024, with complete medical records. All patients received next-generation sequencing (NGS) testing either before EGFR-TKIs application or after resistance to EGFR-TKIs, with some undergoing both tests. This research specifically focused on patients with acquired fusion mutations, including *ALK*, *ROS1*, *RET*, *BRAF*, *FGFR3*, etc. The study period ranged from September 2024 to December 2024, during which data were gathered, and this investigation was conducted. The Ethical Committee of Beijing Chest Hospital approved this study (LW-2024-001).

According to fusion mutation cases selection criteria, the diagnosis of NSCLC was established through histopathological examination. At the time of first diagnosis, the presence of either exon 19 deletion (19del) or exon 21 mutation (21L858R) in the *EGFR* gene was confirmed using tissue-based NGS.

Correspondence to: Dr. Zhe Liu, Department of Oncology, Beijing Chest Hospital, Capital Medical University, and Beijing Tuberculosis and Thoracic Tumour Research Institute, Beijing, China

E-mail: doctorliuzhe@163.com

Received: March 02, 2025; Revised: September 16, 2025;

Accepted: January 09, 2026

DOI: <https://doi.org/10.29271/jcpsp.2026.03.402>

Before EGFR-TKIs treatment, *ALK/ROS1/RET/BRAF/FGFR3/NTRK1* fusion mutations were negative (NGS and immuno-histochemistry). Stage IV disease was defined based on the UICC lung cancer staging criteria (version 8). Treatment with EGFR-TKIs was followed by the development of acquired resistance as per David Jackman's criteria. NGS was repeated in the re-biopsy tumour lesions after resistance, and the fusion gene mutation was detected. Exclusion criteria included: patients with other active malignancies requiring concurrent treatment; patients with severe comorbidities such as cardiovascular diseases, autoimmune disorders, vasculitis, interstitial pneumonia, or HIV positivity; and patients who received chemotherapy before or concurrently with EGFR-TKIs therapy after the detection of EGFR-sensitive mutations.

The clinical and pathological characteristics of the selected patient cohort were comprehensively analysed. The assessment of curative effect was based on response evaluation criteria in solid tumours (RECIST1.1). The last follow-up period of this study was December 1, 2024. This study also incorporated analysis of mutation abundance, quantitatively defined as the proportion of mutated reads at a specific genomic location relative to the total number of reads at that same site.

Table I: Characteristics of nine patients.

Clinical features	Frequencies	Percentages	
Age	≤60 years	5	56%
	>60 years	4	44%
Gender	Male	3	33%
	Female	6	67%
Histological subtype	Adenocarcinoma	9	100%
Smoking history	Current	2	22%
	Never	7	78%
PDL-1	0%	2	22%
	1-49%	5	56%
EGFR type	>50%	2	22%
	19 DEL	5	56%
	L858R	4	44%

PDL-1: Programmed cell death ligand 1; EGFR: Epidermal growth factor receptor.

This small-scale case series (n = 9) was exploratory in nature; thus, all results are presented descriptively. Categorical variables are shown as absolute numbers and percentages. Due to the limited sample size, no hypothesis-testing statistical methods were employed. Statistical software SPSS version 26.0 (Armonk, NY: IBM Corp) was used for data analysis.

RESULTS

There were six cases of *ALK* fusion, two cases of *RET* fusion, and one case of *ROS1* fusion. There were no secondary mutations in the fusion genes of *NTRK1*, *BRAF*, and *FGFR3*.

The abundance of the *EGFR* gene decreased in five patients, and four patients tested negative for EGFR. All nine patients had lung adenocarcinoma. Five cases exhibited echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion, while one displayed striatin (*STRN*)-*ALK* fusion. Following the acquisition of *ALK* mutations, all cases achieved objective responses through treatment with *ALK*-TKIs or in combination with EGFR-TKIs. Patients with acquired *RET* and *ROS1* fusion mutations also achieved a partial response after undergoing fusion-TKIs. Patients' clinical features and pathological characteristics are shown in Table I. The patients' gene status, treatment plans, efficacy, and prognoses are shown in Table II. Regarding adverse events associated with second-line therapy, eight patients experienced Grade 1 events or higher. The most common adverse events included rash (5 cases, 55.6%), diarrhoea (4 cases, 44.4%), nausea (4 cases, 44.4%), and fatigue (2 cases, 22.2%), all of which were Grade 1 or 2 in severity. No Grade 3 or higher adverse events were reported.

Table II: Changes in NGS and treatment plans after resistance to EGFR-TKIs.

Case	New fusion genes (NGS abundance)	EGFR alterations after acquired fusion gene (NGS abundance)	Treatment after fusion detected (second-line therapy)	PFS of fusion-TKIs ±EGFR-TKIs	OS
Case1	<i>EML4-ALK V1</i> (2.98%)	EGFR 19 del (25.10%→18.28%)	Ensartinib+Osimertinib	>17 months	>39 months
Case2	<i>EML4-ALK V1</i> (2.30%)	EGFR exon 20 L858R (19.35%→0)	Alectinib followed by chemotherapy due to SCLC transformation	=21 months	=33 months
Case3	<i>EML4-ALK V2</i> (3.9%)	EGFR 19 del (22.4%→3.7%)	Ensartinib	>22 months	>42 months
Case4	<i>EML4-ALK V3</i> (7.94%)	EGFR L858R (3.4%→0) EGFR G719A (8.7%→0)	Alectinib	>32 months	>42 months
Case5	<i>EML4-ALK V3</i> (0.29%)	EGFR 19 del (12.4%→0)	Ensartinib	>18 months	>39 months
Case6	<i>STRN-ALK</i> (12.6%)	EGFR 19 del (56.98%→48.34%)	Alectinib+ Almonertinib	>20 months	>46 months
Case7	<i>CCDC6-RET</i> (1.86%)	EGFR 19 del (61.18%→57.03%)	Pralsetinib+ Almonertinib	=5 months	=43 months
Case8	<i>CCDC6-RET</i> (0.32%)	EGFR 19 del insP (41.25%→0)	Pralsetinib	=6 months	=51 months
Case9	<i>CD74-ROS1</i> (4.25%)	EGFR L858R (12.56%→1.94%)	Iruapinalib	>39 months	>41 months

EGFR: Epidermal growth factor receptor; NGS: Next generation sequencing; ALK: Anaplastic lymphoma kinase; TKIs: Tyrosine kinase inhibitors; PFS: Progression-free survival; OS: Overall survival; EML4: Echinoderm Microtubule-associated protein-Like 4 gene; STRN: Striatin; RET: Rearranged during transfection; ROS1: Proto-oncogene tyrosine-protein kinase ROS1; CCDC6: Coiled coil domain containing 6 gene; CD74: Cluster of differentiation 74.

DISCUSSION

From the basic clinical information of this research, it was found that female patients, non-smokers, and patients with high programmed cell death ligand 1 (PDL-1) expression had a higher proportion of secondary fusion gene mutations.

The incidence of primary fusion gene mutations is very low in NSCLC patients. In newly diagnosed advanced NSCLC, the proportion of *ALK* fusion is about 5%. *RET* fusions occur in 1-2% of NSCLC patients,⁴ and approximately 2.59% of patients carry the *ROS1* fusion gene in China.⁵

EGFR-TKIs treatment induces activation of bypass and/or downstream signalling pathways, promoting cell survival and proliferation, which is a form of EGFR-TKIs resistance.⁶ Previous studies have shown that *ALK/RET/ROS1* fusion mutations are acquired during EGFR-TKIs treatment.^{2,7-9} Secondary fusion mutations after EGFR-TKIs are even rarer. The overall changes in the status of secondary fusion gene mutations after resistance to EGFR-TKIs and the treatment plans remain unclear.

In this study, after acquired resistance to EGFR-TKIs, there were *ALK*, *RET*, *ROS1* mutations rather than *NTRK1*, *BRAF*, and *FGFR3*. There were six cases of *ALK* fusion mutations, including five *EML4-ALK* and one *STRN-ALK* fusion mutations. This research also screened two cases of *CCDC6-RET* fusion mutations and one case of cluster of differentiation 74 (CD74)-*ROS1* fusion gene mutation. Fusion gene partners of *ALK/RET/ROS1* were consistent with previous research, and no new rare fusion partners were found.

This research found that the abundance of the *EGFR* mutation was significantly reduced in five patients. The *EGFR* gene mutation in the other four patients were completely undetectable. This finding indicates that when acquired resistance occurs, the abundance of *EGFR* mutations is significantly reduced or even undetectable.

There are a few reports analysing the treatment approach and the effectiveness of therapy for acquired fusion mutations. The combination of *ALK*-TKIs and EGFR-TKIs has achieved therapeutic effects in previous case reports.¹⁰ In this study, the PFS of two patients treated with combined TKIs (case 1 and case 6) exceeded 17 and 20 months, respectively. At follow-up, they remained in a state of disease control. *In vitro* experiments have found that EGFR-TKIs activated *ALK* in solid tumours, which can induce EGFR-TKIs resistance. Gefitinib combined with *ALK*-TKIs had a more effective inhibitory effect on tumour cell activity.¹¹ In this study, four patients who received *ALK*-TKIs alone also achieved drug response.

In a multicentre retrospective real-world study, it was found that among acquired *RET* fusion patients who developed resistance after EGFR/*ALK*-TKIs therapy, immediate use of a pralsetinib-containing regimen after resistance was superior to delayed or non-immediate use of a pralsetinib-based regimen.¹²

In this study, *RET* patients were treated with pralsetinib, and partial response was achieved; however, the PFS was five and six months, respectively. This outcome may be related to the poor performance status of patients before subsequent treatment.

It is worth noting that one case of acquired *EML4-ALK* fusion occurred after treatment failure of alectinib. On re-biopsy, this patient presented with small-cell lung cancer transformation. Chemotherapy showed a degree of effectiveness. To the best of the authors' knowledge, this represents the first documented instance of *ALK* rearrangements followed by SCLC transformation as a source of resistance after EGFR-TKIs.

CONCLUSION

In the presence of fusion mutations, EGFR abundance may decrease or become undetectable. The use of fusion TKIs or EGFR-TKIs in combination with fusion TKIs in an optional treatment strategy. *ALK*-TKIs resistance can lead to small-cell lung cancer transformation in patients with secondary *ALK* fusion, indicating the importance of re-biopsy.

ETHICAL APPROVAL:

Ethical approval was obtained from the Ethical Committee of Beijing Chest Hospital, Capital Medical University, Beijing, China (Approval No. LW-2024-001).

PATIENTS' CONSENT:

Informed consent was obtained from all participants.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

YY, BL, ZL: Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, software, roles/writing of the original draft, and review.

NZ, LS, ZC: Data curation and resources.

All authors approved the final version of the manuscript to be published.

REFERENCES

1. Passaro A, Janne PA, Mok T, Peters S. Overcoming therapy resistance in EGFR-mutant lung cancer. *Nat Cancer* 2021; **2(4)**:377-91. doi: 10.1038/s43018-021-00195-8.
2. Piotrowska Z, Isozaki H, Lennerz JK, Gainor JF, Lennes IT, Zhu VW, *et al*. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with Osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov* 2018; **8(12)**:1529-39. doi: 10.1158/2159-8290.CD-18-1022.
3. Rosell R, Gonzalez-Cao M, Codony-Servat J, Molina-Vila MA, de Las Casas CM, Ito M. Acquired *BRAF* gene fusions in Osimertinib resistant EGFR-mutant non-small cell lung cancer. *Transl Cancer Res*. 2023; **12(3)**:456-60. doi: 10.21037/tcr-22-2888.

4. Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol* 2022; **40(6)**:611-25. doi: 10.1200/JCO.21.01626.
5. Yang X, Tang Z, Li J, Jiang J, Liu Y. Progress of non-small-cell lung cancer with ROS1 rearrangement. *Front Mol Biosci* 2023; **10**:1238093. doi: 10.3389/fmolb.2023.1238093.
6. Ferro A, Marinato GM, Mulargiu C, Marino M, Pasello G, Guarneri V, et al. The study of primary and acquired resistance to first-line osimertinib to improve the outcome of EGFR-mutated advanced Non-small cell lung cancer patients: the challenge is open for new therapeutic strategies. *Crit Rev Oncol Hematol* 2024; **196**:104295. doi: 10.1016/j.critrevonc.2024.104295.
7. Enrico D, Lacroix L, Chen J, Rouleau E, Scoazec JY, Loriot Y, et al. Oncogenic fusions may be frequently present at resistance of EGFR tyrosine kinase inhibitors in patients with NSCLC: A brief report. *JTO Clin Res Rep* 2020; **1(2)**:100023. doi: 10.1016/j.jtocrr.2020.100023.
8. Schrock AB, Zhu VW, Hsieh WS, Madison R, Creelan B, Silberberg J, et al. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. *J Thorac Oncol* 2018; **13(9)**:1312-23. doi: 10.1016/j.jtho.2018.05.027.
9. Zhao Z, Su C, Xiu W, Wang W, Zeng S, Huang M, et al. Response to pralsetinib observed in meningeal-metastatic EGFR-mutant NSCLC with acquired RET fusion: A brief report. *JTO Clin Res Rep* 2022; **3(6)**:100343. doi: 10.1016/j.jtocrr.2022.100343.
10. Wang LS, Chen SQ, Zhong X, Jiao XD, Liu K, Qin BD, et al. Acquired EML4-ALK fusion and EGFR C797S in cis mutation as resistance mechanisms to osimertinib in a non-small cell lung cancer patient with EGFR L858R/T790M. *Anticancer Drugs* 2023; **34(10)**:1146-50. doi: 10.1097/CAD.0000000000001489.
11. Ouyang X, Barling A, Lesch A, Tyner JW, Choonoo G, Zheng C, et al. Induction of anaplastic lymphoma kinase (ALK) as a novel mechanism of EGFR inhibitor resistance in head and neck squamous cell carcinoma patient-derived models. *Cancer Biol Ther* 2018; **19(10)**:921-33. doi: 10.1080/15384047.2018.1451285.
12. Hu J, Tang X, Guo R, Wang Y, Shen H, Wang H, et al. 37P pralsetinib in acquired RET fusion-positive advanced non-small cell lung cancer patients after resistance to EGFR/ALK-TKI: A China multi-center, real-world data (RWD) analysis. *J Thor Oncol* 2023; **18(4)**:S62.

