Response to Crizotinib Re-administration After Progression on Lorlatinib in a Patient With *ALK*-rearranged Non–small-cell Lung Cancer

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Clinical Practice Points

- Non–small-cell lung cancer (NSCLC) with anaplastic lymphoma kinase (ALK) gene rearrangement is sensitive to ALK-tyrosine kinase inhibitors (TKIs). First- and second-generation ALK-TKIs are effective for ALK-rearranged NSCLC; however, resistance to ALK-TKI treatment arises.
- Lorlatinib is a third-generation ALK-TKI and shows clinical activity for patients who have undergone previous ALK-TKI treatment. Although the response to lorlatinib was observed, eventually, acquired resistance to lorlatinib occurs, and post-lorlatinib treatment has not been determined.
- We present a case of ALK-rearranged NSCLC in a patient who responded to crizotinib re-administration after progression on lorlatinib. Although tumor heterogeneity, exposure of several therapies, and the limited small tissue samples can impact measurements of MET expression, phospho-MET was upregulated focally in post-lorlatinib tissue compared with pre-lorlatinib tissue, suggesting that resistance to lorlatinib and the subsequent response to re-administration of crizotinib after progression on lorlatinib might be partly related to MET pathway activation.
- Re-administration of crizotinib following lorlatinib might enhance the better prognosis of patients with ALK-rearranged NSCLC.

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Introduction

Chromosomal rearrangements of the anaplastic lymphoma kinase (*ALK*) gene are detected in approximately 5% of non–small-cell lung cancers (NSCLCs) and function as oncogenic driver genes.[1] First- and second-generation ALK-tyrosine kinase inhibitors (TKIs) were developed and showed clinical response for *ALK*-rearranged NSCLC.[2-4] However, resistance to those ALK-TKIs almost develops, resulting in clinical relapse.[5,6] Lorlatinib is a third-generation ALK-TKI and has demonstrated significant antitumor activity against ALK-rearranged NSCLC with previous ALK-TKI resistance.[7,8] Although lorlatinib response was observed, relapse on lorlatinib ultimately developed.[9-11] Mechanisms of lorlatinib resistance were reported,[9-11] but remained unknown. Moreover, post-lorlatinib treatment has not been determined.

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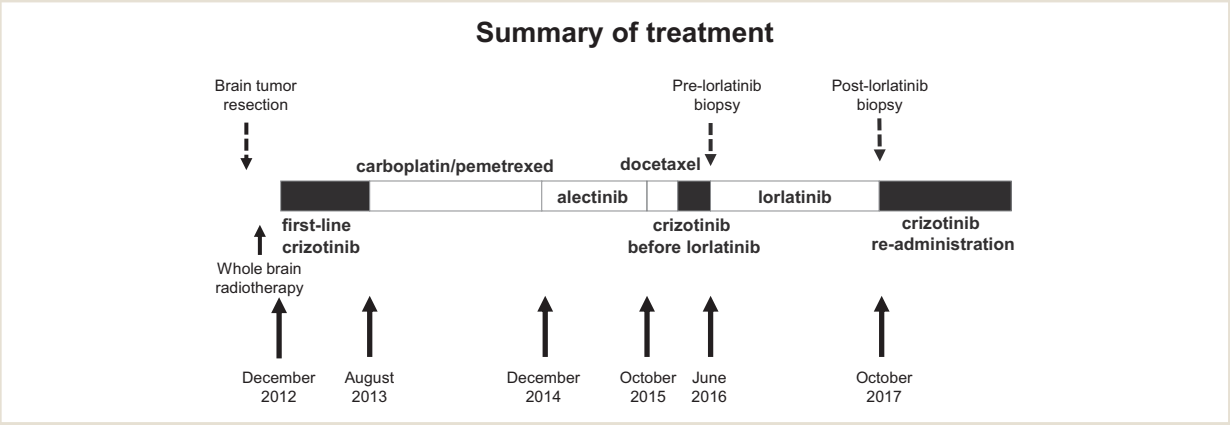
Figure 1 Summary of Patient Treatments



Here, we present a case of a patient who prolonged survival by sequential ALK-TKI treatment and responded to crizotinib re-administration after progression on lorlatinib.

Case Report

A 40-year-old woman was diagnosed with primary adenocarcinoma of the lung (cT1bN2M1c, cStage IVB, 8th edition of the International Union against Cancer/American Joint Committee on Cancer TNM staging system) in October 2012. Figure 1 shows a summary of the treatment. A magnetic resonance image of her brain showed multiple metastases, and subsequently, a large tumor in the right temporal lobe was resected. Adenocarcinoma with ALK rearrangement was detected in the resected brain tumor. After receiving whole brain radiotherapy, she underwent first-line therapy with crizotinib (250 mg twice daily) in December 2012 with a partial response (PR) for 8 months. After disease progression was observed in August 2013, she was treated with chemotherapy containing carboplatin (AUC = 6) and pemetrexed (500 mg/m^2) for 6 cycles,

Figure 2 A, Computed Tomography Images of Metastases of #2R and Right Adrenal Gland and Magnetic Resonance Imaging of Brain in June 2016. Response to Lorlatinib Was Observed on the Initial Imaging in July 2016. Isolated Progression in #2R Lymph Nodes Was Noted in March 2017. B, Computed Tomography Images of Metastases of #2R Lymph Nodes and Right Adrenal Gland and Magnetic Resonance Imaging of Brain. Response to Crizotinib Re-administration After Progression on Lorlatinib Was Observed, Lasting 14 Months

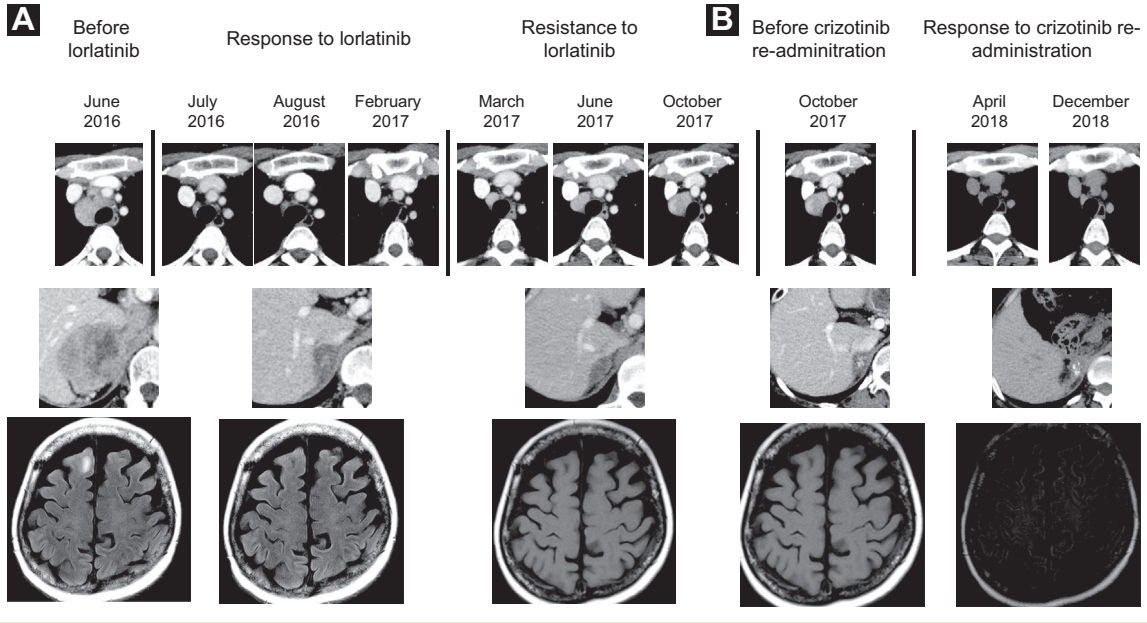
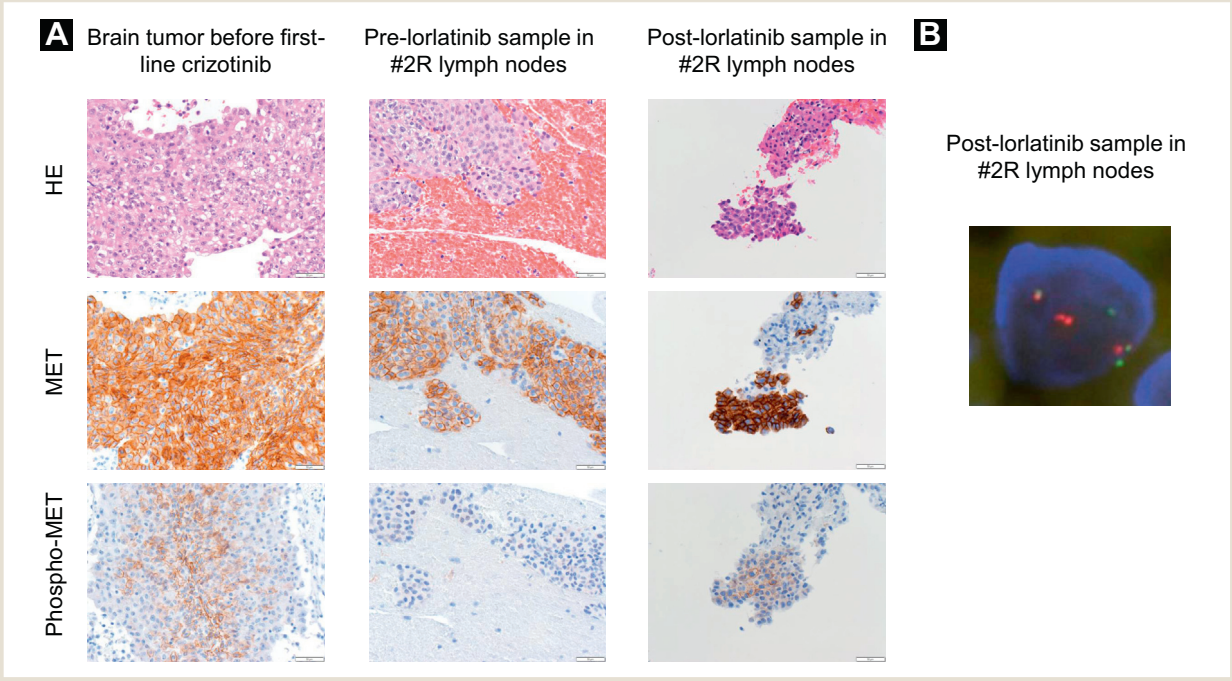


Figure 3 A, HE Stain, MET, and Phospho-MET in Representative Samples of Brain Tumor Before the First-Line Crizotinib, Pre-lorlatinib, and Post-lorlatinib Specimens. Pre-lorlatinib and Post-lorlatinib Samples Were Obtained From #2R Lymph Nodes. B, ALK Fluorescence in Situ Hybridization in Post-lorlatinib Samples. Gain of Split Green (5′) and Red (3′) ALK Signals per Each Tumor Cells was Observed



Abbreviation: HE = hematoxylin and eosin.

followed by maintenance pemetrexed for 11 cycles with PR. Sixteen months later, she experienced disease progression and was administered alectinib (600 mg once daily) in December 2014 with PR, which lasted 10 months. After the failure of alectinib and subsequent docetaxel treatment failed, crizotinib was administered. However, she experienced further disease progression with both sides of her supraclavicular lymph nodes, multiple mediastinal lymph nodes, and right adrenal gland and new brain metastasis in the right frontal lobe (Figure 2A). She enrolled in a phase II clinical trial of lorlatinib in June 2016,[7] and metastases in the #2R lymph node and right adrenal gland were evaluated as target lesions for Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. She experienced significant response in all the metastatic lesions that lasted 9 months. When isolated progression occurred in #2R lymph node in March 2017, the sum of the diameters had increased 25% from their nadir, and progressive disease was confirmed. Because treatment of beyond progressive disease was allowed in this clinical trial, but not local therapy, and the growth of #2R lymph node metastasis was slow without accompanying disease progression of the other metastases, lorlatinib treatment was continued for another 7 months (Figure 2A). Although local therapy might be proposed after discontinuation of lorlatinib treatment, the potential

Table 1 Results of Next Generation Sequencing

Gene	Alteration		Brain Tumor Resection	Pre-lorlatinib Biopsy	Post-lorlatinib Biopsy
ALK	Fusion	EML4-ALK, E20; A20	Positive	Positive	Positive
PIK3CA	SNV	p.Glu542Lys	7.3%	–	–
BRAF	SNV	p.Asp594Asn	3.7%	–	–
ERBB2	SNV	p.Ser310Phe	5.0%	–	–
ALK	SNV	p.Gly1269Ala	–	–	20%[a]
ALK	CNV		1.98	2.30[b]	4.65
MET	CNV		2.22	1.50[b]	2.85/2.61[a]

Abbreviations: ALK = anaplastic lymphoma kinase; CNV = copy number variation; SNV = single nucleotide variant.
[a]SCRUM-JAPAN (Cancer Genome Screening Project for Individualized Medicine in Japan) data.
[b]Data with low quality control.

regrowth of the multiple systemic metastases was a concern during the local therapy. Then we chose a systemic therapy. Cytotoxic chemotherapy was one of the important therapeutic options; however, the patient did not want to undergo chemotherapy because she experienced severe appetite loss and fatigue during the previous chemotherapy. Moreover, the previous chemotherapy was delayed owing to prolonged myelosuppression. Because only manageable mild diarrhea was observed during the previous crizotinib treatment and there was a report about crizotinib re-administration after lorlatinib resistance,[10] crizotinib administration was resumed in October 2017. Serial computed tomography showed significant radiologic response in #2R lymph node (Figure 2B). She is currently at 14 months of crizotinib administration with PR and without signs of disease progression.

To identify the mechanisms of lorlatinib resistance and response to crizotinib re-administration after progression on lorlatinib, we repeated the biopsy of #2R lymph nodes in pre-lorlatinib and post-lorlatinib treatment (Figure 1). Morphology was not changed (Figure 3A), and ALK rearrangement was detected in immunohistochemistry in brain tumor resection samples before first-line crizotinib treatment, pre-loratinib, and post-lorlatinib samples. MET was highly expressed in all samples (Figure 3A). Phospho-MET was observed in brain tumor resection before first-line crizotinib. In #2R lymph nodes, phospho-MET was not found in pre-loratinib samples and was up-regulated focally in post-loratinib samples (Figure 3A). We performed next generation sequencing (NGS) on formalin-fixed paraffin-embedded samples from the brain tumor resection, pre-lorlatinib samples, and post-lorlatinib samples using Oncomine Dx Target Test (Table 1), in which enchinoderm microtubule-associated protein-like 4 (EML4)-ALK variant 2 (E20;A20) was detected in all 3 samples. Furthermore, because low *ALK* copy number gain (CNG) (copy number variant = 4.65) was observed in the post-lorlatinib samples in NGS, ALK fluorescence in situ hybridization was examined, which confirmed ALK CNG (Figure 3B). There were no significant mutations in any of the samples. Moreover, we submitted fresh frozen specimen in the post-lorlatinib samples to the Cancer Genome Screening Project for Individualized Medicine in Japan (SCRUM-JAPAN), in which ALK mutation, low variant allele frequency (VAF) of G1269A (VAF = 20%) was observed in Oncomine comprehensive assay ver. 3. However, G1269A was not subject of data output in Oncomine Dx Target Test.

Discussion

We report the case of a patient with *ALK* rearrangement who exhibited a long survival by sequential treatment with several ALK-TKIs and had significant response to crizotinib re-administration after progression on lorlatinib.

First, crizotinib was reported to induce responses for untreated ALK-positive NSCLC.[2] More potent second-generation ALK inhibitors were developed for patients relapsing on crizotinib and recently showed better response compared with crizotinib in treatment-naive ALK-rearranged patients.[3,12] However, resistance to those TKIs always occurred.[5] Lorlatinib is a third-generation ALK-TKI and showed sensitivity for ALK resistance mutations that were acquired during the treatment of first- and second-generation ALK-TKIs.[7,8] Lorlatinib also demonstrated an antitumor effect on CNS metastases. In a phase II study, the response rate of lorlatinib and the intracranical response rate were 38.7% and 53.1%, respectively, in patients with 2 or more previous ALK-TKIs.[7] Our case, who received 3 previous ALK-TKIs, also showed significant response to lorlatinib in both extracranial and intracranial metastases, indicating that lorlatinib was a promising treatment in ALK-rearranged patients with CNS metastases even though several ALK-TKIs were previously administered.

Several mechansims of ALK-TKI resistance have been reported, including ALK-resistant mutations, activation of bypass signaling, and phenotypic changes such as small-cell lung cancer transformation and epithelial-to-mesenchymal transition.[6,13] Shaw et al reported a case who responded to crizotinib again after relapse on lorlatinib with ALK C1156Y-L1198F mutation.[10] In our post-lorlatinib sample, NGS in Oncomine comprehensive assay ver. 3 showed no sensitive mutations to crizotinib, but G1269A, which was predicted to be sensitive to lorlatinib and resistant to crizotinib based on IC_{50} values.[5] Yoda et al reported that one of the mechanisms of lorlatinib resistance was caused by multiple ALK compound mutations, but not single mutations, which were selected during the sequential ALK-TKI treatment.[9] They also reported that ALK G1269A was detected as single mutation in one case in a post-lorlatinib sample. However, ALK in cell lines established from this case was inhibited by lorlatinib, indicating that ALK-independent mechanisms were related to the lorlatinib resistance. Based on these reports, G1269A single mutation in our post-lorlatinib sample is not likely to induce lorlatinib resistance and crizotinib resensitization.

Low ALK CNG was observed in post-lorlatinib samples. Because high levels of *ALK* gene amplification or *ALK* CNG were reported as a resistance mechanism against crizotinib[14,15] and *ALK* amplification has not been reported in the resistance of lorlatinib, low ALK CNG in our case is not the main resistant mechanism against lorlatinib; however, it might contribute to induction of lorlatinib resistance in part.

MET amplification is demonstrated in *ALK*-rearranged patients with alectinib resistance or alectinib-resistant cell lines, which showed a response to crizotinib.[16,17] Owing to tumor heterogeneity, exposure to several therapies, and the limited small tissue samples, the changes observed in MET expression and the focally positive phopho-MET expression should be considered with caution.[18,19] However, MET activation might explain the lorlatinib resistance and response to re-administration of crizotinib after progression on lorlatinib. Crizotinib inhibits MET, ROS1, and RON kinase activity and has weak inhibitory activity against other receptor tyrosine kinases.[20-22] In our case, the mechanism of resistance to lorlatinib might be related to the induction of a bypass pathway, including MET, which can be inhibited by crizotinib.

Although indication of the resistance mechanisms of lorlatinib and resensitization of crizotinib after relapse on loratinib warrants further studies, our case suggests that re-administration of crizotinib following lorlatinib might enhance the better prognosis of patients with ALK-rearranged NSCLC.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; 448:561-6.
2. Solomon BJ, Mok T, Kim DW, et al, PROFILE 1014 Investigators. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; 371:2167-77.
3. Peters S, Camidge DR, Shaw AT, et al, ALEX Trial Investigators. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med* 2017; 377:829-38.
4. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK -rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet* 2017; 389: 917-29.
5. Gainor JF, Dardaei L, Yoda S, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer. *Cancer Discov* 2016; 6:1118-33.
6. Katayama R. Drug resistance in anaplastic lymphoma kinase-rearranged lung cancer. *Cancer Sci* 2018; 109:572-80.
7. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. *Lancet Oncol* 2018; 19:1654-67.
8. Zou HY, Friboulet L, Kodack DP, et al. PF-06463922, an ALK/ROS1 inhibitor, overcomes resistance to first and second generation ALK inhibitors in preclinical models. *Cancer Cell* 2015; 28:70-81.
9. Yoda S, Lin JJ, Lawrence MS, et al. Sequential ALK inhibitors can select for lorlatinib-resistant compound ALK mutations in ALK-positive lung cancer. *Cancer Discov* 2018; 8:714-29.
10. Shaw AT, Friboulet L, Leshchiner I, et al. Resensitization to crizotinib by the lorlatinib ALK resistance mutation L1198F. *N Engl J Med* 2016; 374:54-61.
11. Redaelli S, Ceccon M, Zappa M, et al. Lorlatinib treatment elicits multiple on- and off-target mechanisms of resistance in ALK-driven cancer. *Cancer Res* 2018; 78: 6866-80.
12. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus crizotinib in ALK-positive non-small-cell lung cancer. *N Engl J Med* 2018; 379:2027-39.
13. Lin JJ, Riely GJ, Shaw AT. Targeting ALK: precision medicine takes on drug resistance. *Cancer Discov* 2017; 7:137-55.
14. Doebele RC, Pilling AB, Aisner DL, et al. Mechansims of resistance to crizotinib in patients with *ALK* gene rearranged non-small cell lung cancer. *Clin Cancer Res* 2012; 18:1472-83.
15. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc Natl Acad Sci U S A* 2011; 108:7535-40.
16. Gouji T, Takashi S, Mitsuhiro T, Yukito I. Crizotinib can overcome acquired resistance to CH5424802: is amplification of the MET gene a key factor? *J Thorac Oncol* 2014; 9:e27-8.
17. Isozaki H, Ichihara E, Takigawa N, et al. Non-small cell lung cancer cells acquire resistance to the ALK inhibitor alectinib by activating alternative receptor tyrosine kinases. *Cancer Res* 2016; 76:1506-16.
18. Cai W, Lin D, Wu C, et al. Intratumoral heterogeneity of *ALK*-rearranged and *ALK/EGFR* coaltered lung adenocarcinoma. *J Clin Oncol* 2015; 33:3701-9.
19. Kwon D, Koh J, Kim S, et al. MET exon 14 skipping mutation in triple-negative pulmonary adenocarcinomas and pleomorphic carcinomas: an analysis of intratumoral MET status heterogeneity and clinicopathological characteristics. *Lung Cancer* 2017; 106:131-7.
20. Kigota A, Togahi Y, Hayashi H, et al. Activated MET acts as a salvage signal after treatment with alectinib, a selective ALK inhibitor, in ALK-positive non-small cell lung cancer. *Int J Oncol* 2015; 46:1025-30.
21. Tanimoto A, Yamada T, Nanjo S, et al. Receptor ligand-triggered resistance to alectinib and its circumvention by Hsp90 inhibition in EML4-ALK lung cancer cells. *Oncotarget* 2014; 5:4920-8.
22. Cui JJ, Tran-Dubé M, Shen H, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). *J Med Chem* 2011; 54:6342-63.