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Case Report

Crizotinib rechallenge reverses *MET* amplification-mediated resistance to ALK inhibitors in a patient with advanced *ALK*-rearranged non-small cell lung cancer: a case report

Rixiang Wang[1], Weizhuang Chen[2], Wenrui Jiao[2], Feng Ren[3], Hongcheng Wu[2], Shibo Wu[2]

Departments of [1]Pharmacy, [2]Respiratory Medicine, [3]Medical Imaging, Lihuili Hospital, Ningbo Medical Center, Ningbo 315041, China

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Abstract: Anaplastic lymphoma kinase (ALK) rearrangements are effectively targeted by ALK tyrosine kinase inhibitors. However, the clinical benefits of targeted inhibitors are limited by the emergence of drug resistance. Moreover, treatment strategies after developing resistance to third-generation ALK inhibitors are limited. Herein, we report a male patient with *EML4-ALK*-rearranged lung adenocarcinoma who received sequential targeted therapies of first-, second- and third-generation ALK inhibitors including crizotinib, ensartinib, brigatinib and lorlatinib for nearly 4.5 years. Analysis of rebiopsy of the lung lesion using targeted next-generation sequencing (NGS) revealed the detection of *ALK* G1269A and *MET* amplification at progression on fifth-line chemotherapy. Crizotinib was re-administered as sixth-line therapy achieving short but profound treatment response for 2 months. Our case provides clinical evidence that rechallenge of crizotinib is effective in reversing *MET* amplification-mediated resistance from sequential ALK inhibitor therapy. Moreover, it also highlights the necessity of performing targeted NGS in understanding the underlying mechanism of resistance to targeted therapies and guiding the optimal therapeutic strategies that could improve the treatment outcomes of patients with advanced *ALK*-positive NSCLC.

Keywords: Acquired resistance, ALK inhibitors, case report, MET, rebiopsy

Introduction

Anaplastic lymphoma kinase (ALK) rearrangements have been reported in 3-7% of non-small cell lung cancer (NSCLC) patients [1-3]. The clinical benefits afforded by different generation of ALK tyrosine kinase inhibitor (TKI) are severely limited by the development of resistance within the first year or two after treatment is initiated [4, 5]. The development of resistance is mediated by a variety of mechanisms, including secondary mutations within the ALK tyrosine kinase domain and activation of alternative signaling pathways [5-7].

To identify the resistance mechanism, repeat biopsies are always needed. A growing number of studies have emphasized the importance of targeted next-generation sequencing (NGS) in elucidating the mechanism of acquired resistance to targeted therapies and guiding subsequent treatment strategies to improve treatment outcomes of patients with actionable mutations [8].

In this study, we report a patient with *EML4-ALK*-rearranged lung adenocarcinoma who received multiple lines of ALK TKI therapies, including sequential administration of first-, second- and third-generation ALK TKIs. After disease progression on fifth-line chemotherapy, NGS analysis of lung tissue biopsy specimen showed co-existence of *ALK* rearrangement, *ALK* secondary mutation and *MET* amplification. *MET* amplification has been reported as one of the bypass mechanisms in acquiring resistance to ALK TKI therapy, which could be effectively reversed by crizotinib therapy [5, 9]. This case highlights the importance of rebiopsy after disease progression and the utility of

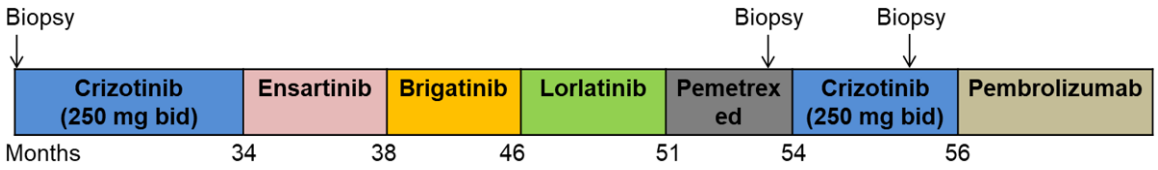

Figure 1. Illustration of the various treatments the patient received for anaplastic lymphoma kinase (ALK)-rearranged non-small-cell lung cancer and the duration of each treatment in months. Arrows indicate the time points wherein biopsy was performed.

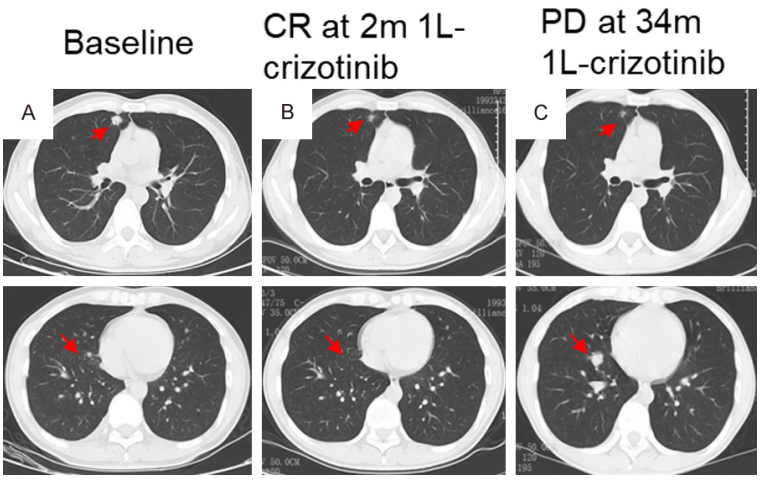

Figure 2. Computed tomographic (CT) images of the patient at baseline (A), at evaluation of complete response (CR) after two months of first-line (1 L) crizotinib therapy (B), and at evaluation of progressive disease (PD) at 34 months of 1 L crizotinib therapy due to enlargement of one of the lung lesions (bottom panel, C). Red arrows indicate the lesions.

targeted NGS in exploring the underlying mechanism of resistance to targeted therapies and guiding treatment decisions in a patient with advanced *ALK*-positive NSCLC. We present the following case in accordance with the CARE guideline.

Case report

In October 2014, a 33-year-old man with no history of smoking was diagnosed with stage IV lung adenocarcinoma, clinical stage T4N0M1a (with pleural involvement). **Figure 1** summarizes his treatment timeline. At initial diagnosis, no *EGFR* and *ROS1* mutations were detected. Meanwhile, fluorescence in situ hybridization (FISH) analysis was positive for *EML4-ALK* rearrangement. He received first-line crizotinib at 250 mg twice daily and achieved complete response of primary lung lesions evaluated by the investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) (**Figure 2A**, **2B**). After 34 months of response to crizotinib therapy, review of CT scan revealed stable disease in one of the lesions (upper panel, **Figure 2C**), but significant enlargement in one of the lung lesions (bottom panel, **Figure 2C**). He was then enrolled in a clinical trial of ensartinib, a new-generation ALK TKI for crizotinib-resistant *ALK*-positive NSCLC. He received ensartinib at 225 mg once daily and achieved partial response with manageable toxicity lasting for 4 months. After ensartinib failure, he received brigatinib 180 mg orally once daily for 8 months as third-line therapy followed by lorlatinib at a daily dose of 75 mg divided into morning intake of 50 mg and evening intake of 25 mg for 5 months as fourth-line therapy. After failure of sequential treatment of ALK TKIs, pemetrexed (500 mg/m^2) monotherapy was administered for 4 cycles from April to June 2019 without benefit due to rapidly worsening of the disease (**Figure 3A**). To explore other treatment strategies, capture-based targeted NGS was performed on the lung biopsy specimen using a commercial gene panel targeting 168 cancer-related genes (Burning Rock Biotech Ltd, Guangzhou China). NGS results revealed the detection of *EML4-ALK* fusion, *MET* amplification (copy number [CN] = 3.8), *ALK* G1269A, and *TP53* R273C. Based on the molecular profile, we decided to re-administer crizotinib at standard dose of 250 mg twice daily as sixth-line therapy. A review of CT after 4 weeks of crizotinib re-administration showed a significant reduction in tumor burden (**Figure 3B**). His disease was controlled until 2 months later when CT revealed the worsening of lung lesions and enlarging mass beside the sternum

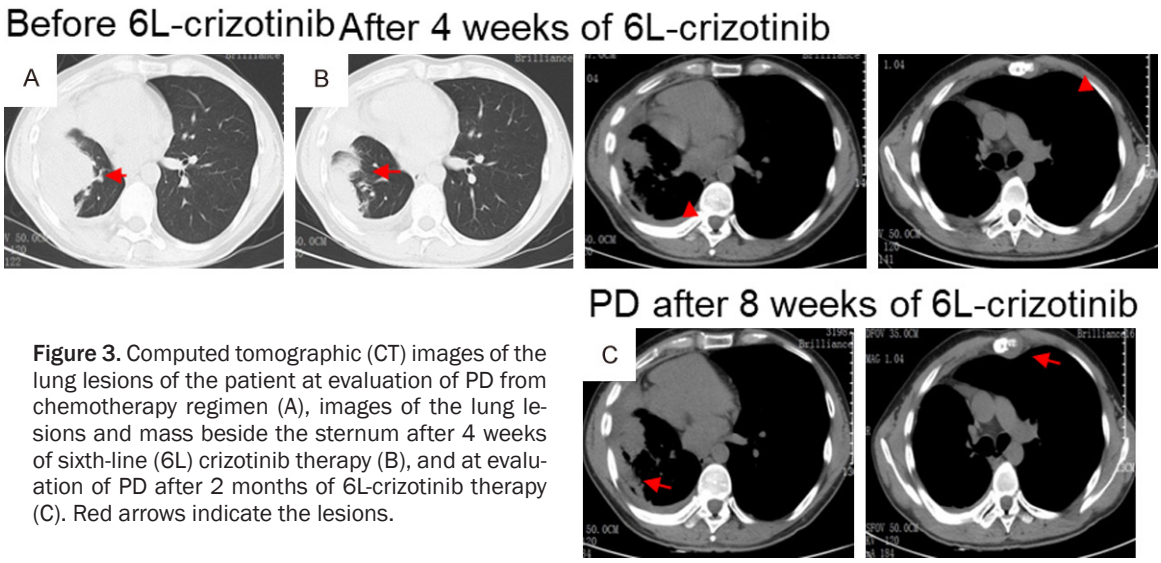

Figure 3. Computed tomographic (CT) images of the lung lesions of the patient at evaluation of PD from chemotherapy regimen (A), images of the lung lesions and mass beside the sternum after 4 weeks of sixth-line (6L) crizotinib therapy (B), and at evaluation of PD after 2 months of 6L-crizotinib therapy (C). Red arrows indicate the lesions.

(**Figure 3C**). A biopsy was also performed on the mass beside the sternum. NGS revealed the detection of a new mutation in *SETD2* (c.2601_2604del p.Phe867fs), in addition to the other genetic alterations detected from the lung lesion including *EML4-ALK*, *ALK* G1269A, *TP53* R273C and *MET* amplification (CN = 6.3). After progression, he is currently treated with PD-1 inhibitor pembrolizumab at a dose of 200 mg on a 21-day cycle.

Discussion

The patient received multiple lines of ALK TKI to target his *EML4-ALK*-positive lung cancer. Commonly, resistance to crizotinib develops within the first year or two after treatment is initiated [6]. Our patient had clinical benefit from crizotinib for approximately 3 years, which was significantly longer than the median progression-free survival of patients receiving first-line crizotinib therapy [4]. The development of resistance to ALK TKIs is a dynamic and clonal process and is mediated by a variety of different mechanisms, including secondary mutations within the ALK tyrosine kinase domain and activation of alternative signaling pathways [5-7]. Despite the diversity of resistance mechanisms, most crizotinib-resistant tumors remain *ALK*-dependent and sensitive to more potent second- or third-generation ALK TKIs such as ceritinib, brigatinib and lorlatinib [5, 10-12]. After failure of crizotinib, our patient received ensartinib for 4 months, brigatinib for 8 months and lorlatinib for 5 months sequentially. Unfortunately, he did not consent to perform rebiopsy after resistance to crizotinib, ensartinib, brigatinib and lorlatinib due to economic reasons; hence, the underlying mechanisms of resistance during those treatment time points were unknown. Nevertheless, he benefitted from the sequential administration of ALK TKIs. However, due to the lack of next-generation ALK TKI that could reverse the resistance of third-generation inhibitor lorlatinib, he was administered with chemotherapy with no benefit.

NGS analysis of repeat biopsy specimen is becoming more essential in exploring the underlying mechanisms of acquired resistance to targeted therapies and guiding the precise therapeutic decisions in patients with advanced *ALK*-positive NSCLC. In addition to the *EML4-ALK* mutation that was previously discovered in the primary lesion, *MET* amplification was detected in the same lesion. *MET* amplification is one of the bypass mechanisms mediating the resistance to ALK targeted therapy [5, 9]. Since we did not perform rebiopsy in any other time point, we cannot identify the specific time of emergence of *MET* amplification. In our case, re-administration of crizotinib as sixth-line therapy after identification of *MET* amplification achieved short but profound objective response, suggesting that administration of chemotherapy resensitized the tumor to crizotinib. However, response to crizotinib was limited due to the co-occurrence of *ALK* G1269A. *ALK* G1269A is one of the commonly reported sec-

ondary mutations that mediate crizotinib resistance in NSCLC [7, 13].

Currently, no best practice guidelines recommend rebiopsy and biomarker testing after development of resistance to targeted inhibitors to guide therapeutic strategies for subsequent-line treatment. After resistance develops to one targeted inhibitor, targeted NGS analysis could provide clinically relevant insights into the underlying mechanisms of resistance to targeted therapies and guide optimal therapeutic strategies that could improve the treatment outcome of the patient.

In conclusion, our case provides clinical evidence that rechallenge of crizotinib is effective in reversing *MET* amplification-mediated resistance from sequential ALK inhibitor therapy. It also highlights the importance of performing targeted NGS analysis in guiding the disease management of patients with advanced *ALK*-positive NSCLC.

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Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Disclosure of conflict of interest

None.

Address correspondence to: Shibo Wu, Department of Respiratory Medicine, Lihuili Hospital, Ningbo Medical Center, No. 57, Xingning Road, Ningbo 315041, China. Tel: +86-0003-2449-2903; E-mail: woshibo2015@yeah.net

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