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Successful crizotinib monotherapy in *EGFR*-mutant lung adenocarcinoma with acquired *MET* amplification after erlotinib therapy

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ABSTRACT

MET is a driver oncogene in non-small-cell lung cancer (NSCLC), and its amplification is associated with acquired resistance to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors. A 56-year-old Japanese male with lung adenocarcinoma harboring an *EGFR* exon 21 L858R mutation received erlotinib to which he responded for 12 months. After disease progression, re-biopsy analyses revealed newly developed *MET* amplification. Neither *EGFR* exon 20 T790M mutation nor *MET* exon 14 mutations were detected. The MET inhibitor, crizotinib, showed a dramatic response. This is the first report of successful crizotinib single-agent therapy in *EGFR*-mutant NSCLC that acquired *MET* amplification during erlotinib therapy.

1. Introduction

MET is a driver oncogene in non-small-cell lung cancer (NSCLC) [1]. Crizotinib was initially invented as a MET inhibitor. Subsequently, its comparable inhibitory activity against anaplastic lymphoma kinase (ALK) and ROS1 was identified [2], and crizotinib is currently used as the first generation ALK inhibitor to treat patients with *ALK*-rearranged NSCLC. The efficacy and safety of crizotinib in NSCLC with aberrant MET signaling (including *MET* gene amplification and *MET* mutations) has yet to be fully elucidated, although some reports have suggested the treatment benefit of crizotinib [3–5]. *MET* gene amplification is a major cause of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI)-induced resistance in tumors with *EGFR* mutations [6–8]. When both the MET and EGFR signaling pathways were activated, two inhibitors were used to block each signaling [9,10]. In this report, we describe a dramatic response to crizotinib monotherapy in a lung adenocarcinoma patient who had EGFR-sensitive mutation and acquired *MET* amplification during erlotinib therapy.

2. Case report

A 56-year-old Japanese male former smoker was histologically diagnosed with stage IV lung adenocarcinoma based on bone metastasis biopsy specimen in March 2013. Mutational analysis with PCR-based assay (cobas® EGFR Mutation Test v2) revealed the *EGFR* exon 21 L858R mutation. He initially underwent four cycles of carboplatin/pemetrexed/bevacizumab, followed by 17 cycles of maintenance pemetrexed. However, his disease progressed by June 2014. An EGFR-TKI, erlotinib, was initiated and he continued to respond for 12 months. In November 2015, new lesions in the brain, parotid gland, skin, lung, abdominal lymph nodes, and bone were detected (clinical course is shown in Fig. 1A). A re-biopsy of parotid

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gland metastasis showed a persistent L858R mutation but not a T790M. Fluorescence *in situ* hybridization (FISH) analysis showed *MET* amplification that had not been observed in initial biopsy specimens (Fig. 2A). ALK and ROS1 were negative by immunohistochemical staining, and no mutations were detected in *MET* exon 14 by Sanger sequencing. He sequentially received two cycles of docetaxel and one course of nivolumab, but his disease progressed and he was hospitalized for his worsening general condition (Eastern Cooperative Oncology Group [ECOG] performance status of 4).

After he gave informed consent, crizotinib was initiated at 250 mg twice daily. Within a week, palpable lesions (skin and parotid gland metastases) rapidly shrank; computed tomography showed a dramatic response, with multiple lung metastases almost completely diminished (Fig. 1B). His performance status was improved to grade 1 and he was discharged. Crizotinib has been continued for more than 4 months.

3. Discussion

Although treatment with EGFR-TKIs is effective in patients with NSCLC with activating *EGFR* mutations, almost all patients acquire resistance to EGFR-TKIs. T790M, a secondary EGFR kinase domain mutation, is the most common mechanism of acquired resistance. *MET* amplification is another mechanism of acquired resistance to EGFR-TKIs, and is detected in 5–21% of cases [6–8,11]. We previously used FISH analysis to show *MET* gene amplification in 13.7% of resected NSCLC patients [11].

Although crizotinib is theoretically effective for patients with *MET* amplification [2], few reports demonstrate the treatment benefit in those who acquired *MET* amplification during EGFR-TKI therapy. We have summarized cases who had *EGFR*-mutant NSCLC with *MET* amplification and were treated with MET inhibitors in Table 1 [9,10,12]. Case 1 had double primary lesions [9]: one tumor in the left lower lobe harbored an *EGFR* exon19 deletion, and the other primary tumor in the right upper lobe harbored *MET* amplification. Combination therapy with crizotinib and erlotinib was started and controlled the disease well. Case 2 was diagnosed as having both *MET* amplification and an *EGFR* mutation in molecular analyses of a biopsy specimen taken at initial diagnosis [10]. Although erlotinib monotherapy failed to control the disease, addition of crizotinib to erlotinib yielded a good response. These two cases already had *MET* amplification before EGFR-TKI treatment. In contrast, our patient had an *EGFR* mutation and then newly developed *MET* amplification after erlotinib therapy, suggesting that *MET* amplification occurred as a mechanism of acquired resistance. Recently, Ou et al. also reported a patient who developed *MET* amplification after the third-generation EGFR-TKI, osimertinib therapy (Case 3) [12].

We consider our case is worth discussing in two points. First, our

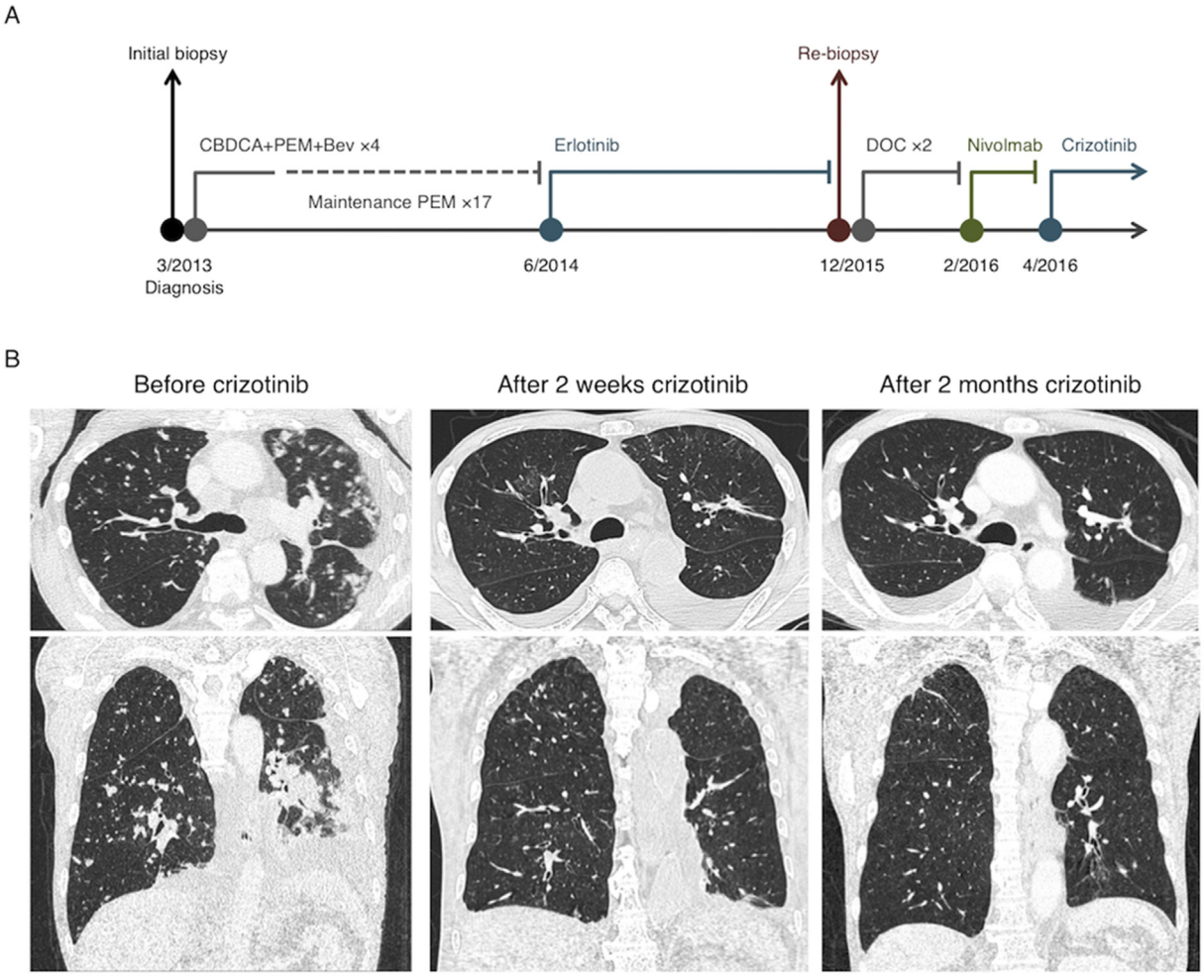

Fig. 1. (A) Clinical course. CBDCA: carboplatin, PEM: pemetrexed, Bev: bevacizumab, DOC: docetaxel. (B) Computed tomography images before and after treatment with crizotinib, respectively, showing dramatic response.

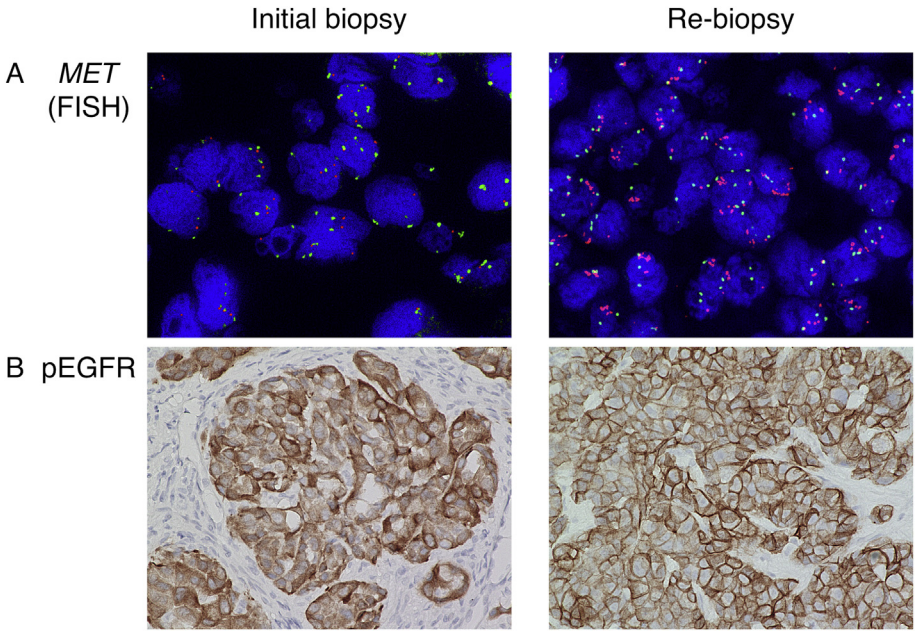

Fig. 2. Tumor histology at initial biopsy (left line) and re-biopsy (right line). Fluorescence *in situ* hybridization (FISH) with MET probe (red) and chromosome 7 centromere probe (green). Nuclei stained with 4′,6-diamidino-2-phenylinodole (blue) (× 100 magnification) (A). MET/centromere probe of chromosome 7 (CEP7) ratio increased from 0.4 at initial diagnosis to 2.1 at the time of progression; mean MET copy numbers similarly increased from 3.1 to 8.8 copies per cell. Immunohistochemical stains with phosphorylated EGFR (pEGFR; Tyr1068, dilution 1:200, clone D7A5; Cell Signal Technology) (B) (× 40 magnification). pEGFR were positive at initial diagnosis, which were still present at the time of progression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Previously reported cases of *EGFR*-mutant lung cancer with *MET* amplification that were treated crizotinib.

Case	Age	Sex	Histology	Smoking status	*EGFR* mutation	*MET* amplification	Technique	Interpretation for positive	Timing of detecting *MET* amplification	*MET* Exon 14 mutation	Therapy	Response
1 [9]	75	F	ADC	Former	Exon 19 deletion	Positive	FISH	*MET*/CEP7 ratio 6.5	initial diagnosis	WT	crizotinib + erlotinib	PR
2 [10]	73	F	ADC	Never	Exon 21 L858R	Positive	FISH, NGS	*MET*/CEP7 ratio > 15.0	initial diagnosis	WT	crizotinib + erlotinib	PR
3 [12]	73	F	ADC	Never	Exon 19 deletion	Positive	NGS	copy number 30	after osimertinib resistance	WT	crizotinib	SD
4 [present case]	56	M	ADC	Former	Exon 21 L858R	Positive	FISH	*MET*/CEP7 ratio 2.1	after erlotinib resistance	WT	crizotinib	PR

EGFR: epidermal growth factor receptor, ADC: adenocarcinoma, WT: wild type, NGS: next generation sequencing, FISH: fluorescence *in situ* hybridization, CEP7: centromere probe of chromosome 7, PR: partial response, SD: stable disease.

case harboring two aberrant oncogenes, EGFR-sensitive mutation and developed *MET* amplification, was treated with crizotinib monotherapy. Ideally, combination therapy with crizotinib and EGFR-TKI was initiated. Case 1 and case 2, who had *MET* amplification before EGFR-TKI treatment, were successfully treated with erlotinib and crizotinib. Case 3 was initially treated with crizotinib, and subsequently, osimertinib was added. In our case, the poor condition did not allow to initiate combination therapy. Involuntarily crizotinib monotherapy was administered, which induced dramatic tumor regression. We still detected an *EGFR* L858R mutation by the re-biopsy analysis. Furthermore, phosphorylated EGFR was still positive in the specimens at re-biopsy (Fig. 2B), implying that EGFR signaling remained to be activated. Because EGFR and MET have a common signaling pathway and compensate for each other, blockade of MET signaling might inhibit a key oncogenic pathway. Although accurate mechanism cannot be proposed, the *MET* amplification was considered as not a co-oncogenic driver with the EGFR mutation but a true oncogenic driver in the present case. The relationship between MET signaling and vascular endothelial growth factor-induced angiogenesis was reported [2,13]. And in a mouse model of melanoma, the blockade of MET signaling inhibited metastatic ability [14]. Second, the response to crizotinib monotherapy was rapid and obvious. His performance status was improved from grade 4 to 1 and he was discharged by walking. There were several differences between case 3 and ours; age, mutation type, and preceding therapy, which might lead to the different response. When selecting the appropriate chemotherapy regimen in patients with poor PS, not only efficacy but tolerability and safety are important factors. Our experience suggests that crizotinib monotherapy might be a feasible treatment option in those patients.

In conclusion, crizotinib monotherapy might be an effective treatment option to treat *EGFR*-mutant NSCLCs with acquired *MET* amplification. Further studies are warranted to clarify appropriate treatment for the EGFR-TKI-induced acquired resistance.

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Conflicts of interest

The authors declare no competing interests.

Author contributions

All authors contributed toward the conception and design, data analysis, drafting, and critically revising the paper, and agree to be accountable for all aspects of the work.

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