Combined Use of Crizotinib and Gefitinib in Advanced Lung Adenocarcinoma With Leptomeningeal Metastases Harboring *MET* Amplification After the Development of Gefitinib Resistance: A Case Report and Literature Review

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Clinical Practice Points

- MET amplification could be detected in the cerebrospinal fluid of patients who experienced progression with epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) treatment.
- MET amplification was an important cause of acquired resistance to EGFR-TKIs in patients with leptomeningeal metastases.
- Combination therapy with MET inhibitors might be promising for controlling leptomeningeal metastases with acquired resistance to EGFR-TKIs.

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Introduction

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have become the first-line choice for the treatment of patients with advanced non-small-cell lung cancer (NSCLC) harboring *EGFR* activating mutations.[1] However, the majority of these patients inevitably experienced acquired resistance in < 1 year,[2] and the progression of central nervous system metastasis, including brain metastases (BMs) and leptomeningeal metastases (LMs) is frequently observed during EGFR-TKIs treatment. Among LMs, *MET* amplification is an important cause of acquired resistance to EGFR-TKIs.[3] However, the clinical efficacy of combined use of EGFR-TKIs and MET inhibitors for controlling leptomeningeal carcinomatosis with acquired resistance to gefitinib is unknown.

Here, we describe the application of combination therapy with MET inhibitors to treat a patient who experienced progressive disease (PD) with LMs after gefitinib treatment. To the best of our knowledge, this is the first case of such a combination therapy in a patient with NSCLC with LMs harboring *MET* amplification after the development of EGFR-TKIs resistance.

Case Report

A 45-year-old female with no history of smoking presented with dry cough and chest pain in November 2016. A chest computed tomography (CT) revealed a 37 × 42 mm nodule in the upper lobe of the right lung with multiple small nodules, enlarged mediastinal lymph nodes, and ipsilateral pleural effusion. Magnetic resonance imaging (MRI) of the vertebrae showed multiple bone metastases. The contrasted brain MRI examination revealed no abnormalities in the leptomeninges or brain. A CT-guided percutaneous needle biopsy of the lung lesion in the right upper lobe revealed adenocarcinoma (Figure 1A_1-A_2). *EGFR* L858R mutation was detected in the biopsy specimen by amplification refractory mutation system analysis. ALK and ROS1 were negative by immunohistochemistry. The patient was diagnosed with advanced *EGFR* L858R mutant

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lung adenocarcinoma (cT4N1M1c, stage IVB). She was treated with gefitinib, 250 mg daily, from November 2016. After 2 months of treatment, chest CT showed a dramatic reduction in the tumor size, indicating the achievement of a partial response (PR) according to the Response Evaluation Criteria in Solid Tumors, version 1.1. The patient continued receiving gefitinib with good tolerance. Repeated chest CT every 2 months continued to show a PR (Figure $1B_1$-B_2).

The patient experienced PD after 8 months (Figure $1D_1$-D_2), with symptoms of memory loss, headache, and nausea. The contrasted brain MRI examination in September 2017 revealed multiple small nodules in bilateral hemispheres and leptomeninges, suggesting LMs and BMs (Figure $1C_1$). We performed next generation sequencing (NGS) for both plasma circulating tumor DNA (ctDNA) and cerebrospinal fluid (CSF). Genomic DNA was profiled using a capture-based targeted sequencing panel, commercially available from Burning Rock Biotech (Guangzhou, China). This panel covers the selected exons and introns of 168 cancer-related genes, spanning 170 kb of the human genome.

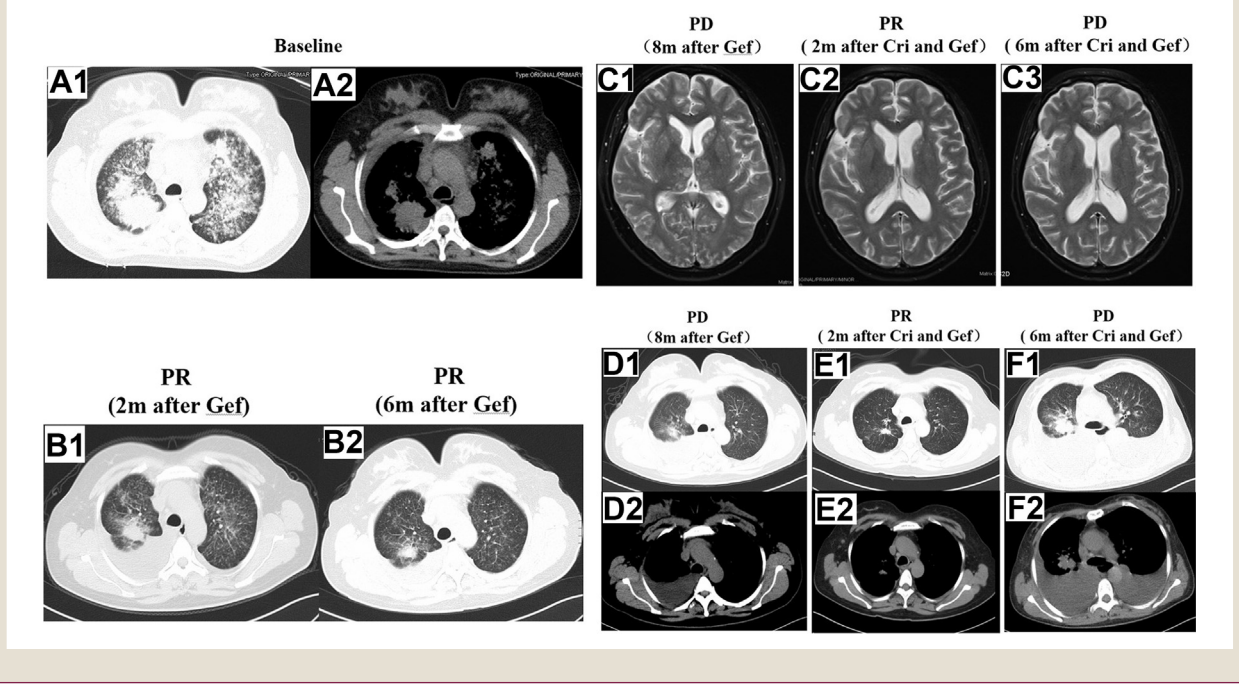
The plasma ctDNA was positive for the *EGFR* L858R mutation (allele frequency, [AF], 0.25%), and there was no distinct acquired resistance mutation in the plasma ctDNA (Figure 2A). However, in the CSF ctDNA, both *MET* amplification (copy number [CN], 3.31) and the *EGFR* L858R mutation (AF, 58.11%) were detected (Figure 2B). The *MET* exon 14 skipping mutation was not detected. The patient was treated with gefitinib combined with crizotinib, a MET inhibitor. Subsequently, her headache diminished over 2 weeks. Two months later, repeated brain MRI showed the disappearance of multiple nodules in both the brain and leptomeninges (Figure $1C_2$), and chest CT showed a dramatic reduction in multiple small nodules and the pleural effusion (Figure $1E_1$-E_2), indicating the achievement of a PR. The patient was continued on the combination therapy.

However, chest CT in April 2018 showed an increase in the size of the primary right upper lobe lesion with increased pleural effusion (Figure $1F_1$-F_2), indicating PD. However, the nodules in the brain remained stable, as determined by brain MRI (Figure $1C_3$). The progression-free survival (PFS) duration of the combination therapy was 6 months. The second NGS analysis showed that *EGFR* L858R mutation was positive in the CSF ctDNA (AF, 30.27%), but *MET* amplification was not detected (Figure 2C).

Discussion

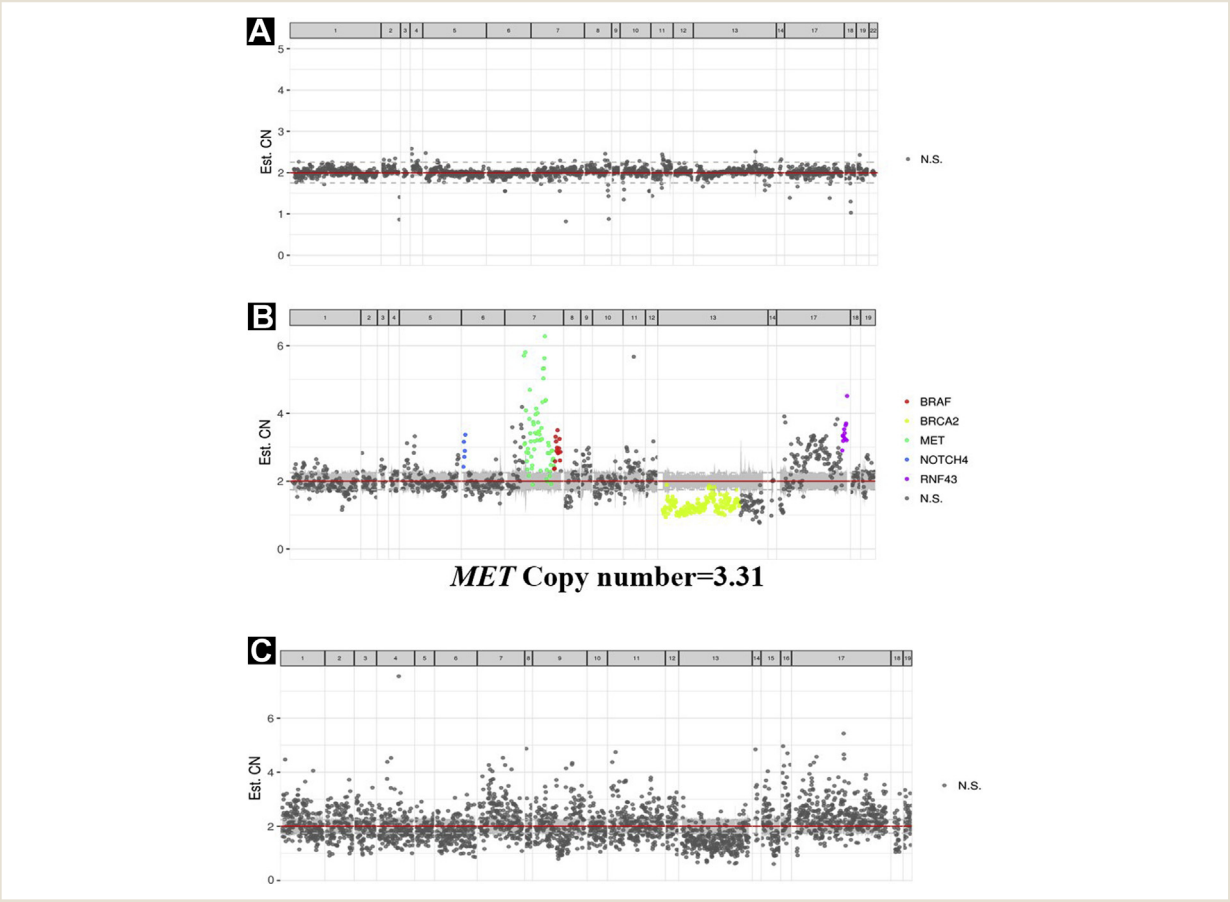
MET amplification is a major cause of acquired resistance to the first-generation EGFR-TKIs, such as erlotinib and gefitinib.[4,5] In recent decades, *MET* amplification induced after osimertinib treatment has been reported in a small number of cases, suggesting that *MET* amplification can also be responsible for osimertinib resistance.[6] Although crizotinib is effective for patients with *MET* amplification,[7] few reports have demonstrated the treatment benefit in those who acquired *MET* amplification during treatment with EGFR-TKIs. We have summarized the clinical outcomes of treatment with MET inhibitors in patients with *MET* amplification after the development of EGFR-TKIs resistance in previous studies.[8-13]

Figure 1 **Chest Computed Tomography Scan Images and Brain magnetic Resonance Scan Images before and after for the Response to Epidermal Growth Factor Receptor-tyrosine Kinase Inhibitor Treatment. A, Computed Tomography Images of the Patient before Gefitinib. B, Clinical Response to the Gefitinib. C, Clinical Response to the Gefitinib and Crizotinib in Leptomeninges/brain Metastases. D-F, Clinical Response to the Gefitinib and Crizotinib Combination in Primary Tumor and Pleural Effusion**



Abbreviations: Cri = crizotinib; Gef = gefitinib; PD, progressive disease; PR, partial response.

Figure 2 ***MET*** **Copy Number Amplification Detected by Next Generation Sequencing in Plasma and Cerebrospinal Fluid (CSF). A, No Copy Number Gain in Plasma Circulating Tumor DNA (ctDNA) after gefitinib Resistance. B,** ***MET*** **Copy Number is 3.31 in CSF ctDNA after gefitinib Resistance. C, No Copy Number Gain in CSF ctDNA after 6 Months of Crizotinib and Gefitinib Combination Therapy**



The patients' characteristics and clinical information are listed in Table 1. The rebiopsy specimens included parotid gland, lung, plasma, pleural effusion, and exclusive CSF samples in our case. All patients had received crizotinib after developing EGFR-TKIs resistance; 7 achieved a PR, and the others achieved stable disease (SD). Here, we report the clinical efficacy of combination therapy in patients with LMs harboring *MET* amplification after developing gefitinib resistance.

Approximately one-third of patients develop central nervous system (CNS) metastases, including BMs and LMs after the initial response to EGFR-TKIs.[14] Because leptomeningeal lesions are difficult to access, the mechanisms of acquired EGFR-TKIs resistance in the CNS are largely unknown. A liquid biopsy of the CSF is a reliable method for determining the genetic characteristics of LMs, because ctDNA from the CSF represents the genomic alterations of the CNS lesions more accurately than ctDNA from plasma.[15] Li et al[16] found unique copy number variations in patients with LMs, as determined by CSF ctDNA. The *MET* copy number gain was the most frequent copy number variation and was identified in 11 of 23 patients after EGFR-TKIs failure.[16]

The presence of *MET* amplification after progression in our case was different between the CSF and plasma NGS analyses. However, a good response was observed in both the LMs and primary tumors, possibly owing to intertumoral heterogeneity.[17] One retrospective pooled analysis of the PROFILE 1005 (The Phase II trial of *ALK*-rearranged NSCLC; NCT00932451) and 1007 (The Phase III trial of *ALK*-rearranged NSCLC; NCT0093283) trial data assessed the benefit of crizotinib in BMs.[18] Wang et al[19] also indirectly confirmed the clinical efficacy of crizotinib in a rare case of NSCLC with BMs. Giulio et al[20] suggested that the CNS may benefit from crizotinib even with very low concentrations of the drug in the CSF. Other determinants might play a role regarding the activity of crizotinib against CNS metastasis. A previous study suggested that the *MET* copy number gain was associated with gefitinib resistance in *EGFR*-mutant LMs, as determined by in vitro and in vivo experiments,[3] suggesting that might be an important cause of acquired resistance to EGFR-TKIs in patients with LMs/BMs. Nanjo et al[3] established tumor cell lines from gefitinib-resistant LM tumors, referred to as PC-9/LMC-GR cells. PC-9/LMC-GR cells acquired gefitinib resistance via MET activation associated with an increase in the *MET* copy number. In PC-9/

Table 1 Clinical Characteristics and Treatment Outcomes of Patients With NSCLC With Acquired *MET* Amplification After EGFR-TKIs Resistance

Article	Age/Gender	Smoking Status	Histologic Type	*EGFR* Mutation	EGFR-TKIs	Response	Rebiopsy Specimen	Technique	MET Inhibitors	Response/PFS
Yoshimura et al, 2017[8]	56/M	Former	AC	L858R	Erlotinib	PR/12m	Parotid land	FISH	Crizotinib/monotherapy	PR/4m
Ou et al, 2016[9]	73/F	Never	AC	19Del	Osimertinib	PR/9m	Lung	NGS	Crizotinib/combination	SD/2m
Li et al, 2017[10]	36/F	Never	AC	L858R	Erlotinib	SD/6m	Plasma	NGS	Crizotinib/combination	PR/2m
Wang et al, 2018[11]	73/M	Never	AC	19Del	Icotinib	PR/11m	Pleural effusion	NGS	Crizotinib/monotherapy	PR/4m
	50/F	Never	AC	19Del	Icotinib	PR/23m	Pleural effusion	NGS	Crizotinib/monotherapy	PR/6m
Wang et al, 2018[12]	51/M	Unknown	AC	19Del	Osimertinib	Unknown	Plasma	NGS	Crizotinib/combination	PR/1m
	44/F	Unknown	AC	19Del	Osimertinib	SD/5m	Plasma	NGS	Crizotinib/combination	PR/3m
Suryavanshi et al, 2017[13]	54/M	Unknown	AC	L858R	Gefitinib	PR/9m	Lung	FISH	Crizotinib/monotherapy	SD/2m
	59/M	Unknown	AC	L858R	Gefitinib	PR/4m	Lung	FISH	Crizotinib/monotherapy	PR/3m
Present patient	45/F	Never	AC	L858R	Gefitinib	PR/8m	CSF	NGS	Crizotinib/combination	PR/6m

Abbreviations: AC = adenocarcinoma; CSF = cerebrospinal fluid; EGFR-TKIs = epidermal growth factor receptor-tyrosine kinase inhibitors; F = female; FISH = fluorescence in situ hybridization; M = male; NGS = next generation sequencing; PFS = progression-free survival; PR = partial response; SD = stable disease.

LMC-GR cells, whereas EGFR phosphorylation was inhibited, increased MET or AKT phosphorylation was not inhibited by gefitinib. These results suggested that MET activation might be involved in gefitinib resistance in PC-9/LMC-GR cells. However, the mechanism described above was demonstrated only in cell lines.

Crizotinib alone remarkably inhibited MET and AKT phosphorylation. Gefitinib plus crizotinib inhibited the phosphorylation of EGFR and MET almost completely. A previous study[3] also demonstrated that the combined use of EGFR-TKIs and crizotinib dramatically regressed LMs with acquired resistance to gefitinib in both cell lines and mouse models. These findings suggest that combination therapy with MET inhibitors might be promising for controlling LMs with acquired resistance to EGFR-TKIs. However, such combination therapy has not been confirmed in previous clinical experiences.

Conclusion

This case highlights *MET* amplification as an important cause of acquired resistance to EGFR-TKIs in patients with LMs. Our case also suggests that combined therapy with MET inhibitors may be a promising strategy for overcoming such resistance. However, the clinical efficacy and safety of such combination therapy must be validated in large-scale clinical trials.

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Disclosure

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

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