BRIEF REPORT

Response to Pralsetinib Observed in Meningeal-Metastatic *EGFR*-Mutant NSCLC With Acquired *RET* Fusion: A Brief Report

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Received 8 April 2022; revised 11 May 2022; accepted 13 May 2022
Available online - 19 May 2022

ABSTRACT

Introduction: *RET* is well known as an important driver gene in NSCLC. Moreover, *RET* is a rare acquired resistance mechanism to *EGFR*-mutant NSCLC. Only 36 NSCLC cases of coexistence of *EGFR* and *RET* were reported previously worldwide. So far, there have been no reports on the following: (1) whether combination of EGFR tyrosine kinase inhibitor (TKI) and RET TKI works for meningeal metastasis; (2) the concentrations of EGFR TKI and RET TKI in the cerebrospinal fluid (CSF) and plasma; and (3) whether *RET* fusions and *EGFR* mutation happened in the same clone or not.

Methods: We reported a patient with an *EGFR*-mutant NSCLC with acquired *RET* fusions and meningeal metastasis treated with pralsetinib and osimertinib; the specimen was analyzed by next-generation sequencing (Illumina NovaSeq 6000 platform). Symptom improvement and magnetic resonance imaging scan were used for effect evaluation. Furthermore, we determined the concentrations of pralsetinib and osimertinib in CSF and plasma by means of liquid chromatography tandem mass spectrometry. We also detected *RET* fusion and *EGFR L858R* mutation by methods of fluorescence in situ hybridization and immunohistochemistry with continuous sections to analyze whether *RET* fusions coexist with *EGFR* mutation in the same clone or not.

Results: The allele frequency of the *RET* fusion was detected to be 12.88%. This patient achieved a partial response, indicating pralsetinib combined with osimertinib may be clinically beneficial for meningeal metastasis in patients harboring acquired coexistent *RET* fusions. The concentrations of pralsetinib in the CSF and plasma were 704.76 nM and 91.31 μM, whereas those of osimertinib in the CSF and plasma were 23.70 nM and 2148.94 nM, respectively. *RET* fusion was found in the same clone of *EGFR L858R* mutation.

Conclusions: Our finding of this case indicated that *RET* fusion and *EGFR* mutation occur in the same population of cell clones, rather than in different cell clones. Combined pralsetinib may be an effective way to overcome the resistance, even for meningeal metastasis, owing to high CSF distribution of pralsetinib.

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Keywords: EGFR; RET fusion; Lung cancer; Meningeal metastasis; Pralsetinib; Cerebrospinal fluid

Introduction

EGFR and *RET* have already been widely acknowledged to be driver oncogenes in NSCLC, especially lung adenocarcinoma. Mostly, driver mutations are mutually exclusive; therefore, lung cancer cases with coexistent

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Disclosure: The authors declare no conflict of interest.

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Cite this article as: Zhao Z, Su C, Xiu W, et al. Response to pralsetinib observed in meningeal-metastatic *EGFR*-mutant NSCLC with acquired *RET* fusion: a brief report. *JTO Clin Res Rep*. 2022;3:100343.

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ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2022.100343

alterations of *EGFR* and *RET* rearrangements were rarely reported. Until now, only 36 cases of coexistence of *EGFR* and *RET* from 10 papers were reported previously,[1–11] and most cases had not received selective RET tyrosine kinase inhibitors (TKIs), such as pralsetinib and selpercatinib (Table 1). As far as we know, only two cases received combined treatments of pralsetinib with EGFR TKI and achieved partial response.[1] Nevertheless, it is still unclear whether this combined treatment is effective for meningeal metastasis. Here, we first report the clinical benefit of combined therapy with pralsetinib and osimertinib on a patient with *EGFR*-mutated meningeal-metastatic NSCLC harboring acquired coexisting *RET* fusion.

Case Report

A 43-year-old Chinese woman with no smoking history was diagnosed in August 2019 with stage IV lung cancer, and the pathologic examination result of the biopsy of the lymph node revealed metastatic adenocarcinoma with positive staining of pan-cytokeratin, TTF-1, Napsin A, Ki-67, and programmed death-ligand 1 and negative for CK5/6, P40, and LCA. The specimen was further analyzed by next-generation sequencing (Illumina NovaSeq 6000 platform) and revealed *EGFR L858R* mutation together with *EGFR V834L* mutation with which no other mutation coexisted at that time. After beginning with first-line treatment of gefitinib, the patient took progressive disease (PD) only after 3 months for the emergence of malignant pleural effusion. Liquid rebiopsy result of pleural effusion cells did not reveal acquired *T790M* mutation; thus, she took second-line treatment of osimertinib but progressed after 1 month. Then, the patient subsequently underwent combination chemotherapy of pemetrexed and carboplatin with bevacizumab, followed by maintenance therapy for 6 months. The patient developed PD at June 2020. Afterward, the fourth-line treatment of nab-paclitaxel and dacomitinib began in July 2020, but only dacomitinib was taken until May 2021 owing to the intolerance of the chemotherapy.

The patient then developed diplopia, headache, low back pain, and vomiting, with progressively enlarging bilateral neck mass. Subsequent enhanced computed tomography scan revealed multiple cysts in the liver, multiple bone metastases in the spine, and pelvis and sacral cysts. Extensively enhanced meninges and spinal meninges were revealed in the enhanced magnetic resonance scan, and physical examination revealed positive meningeal irritation sign, which together suggested meningeal metastases. The efficacy evaluation established PD. To identify the mechanism of underlying resistance, we performed a second computed tomography-guided needle biopsy of the lung and further analyzed by next-generation sequencing with a designed Genescope panel of 543 genes (Genecast, Beijing, People's Republic of China) and Illumina NovaSeq 6000 platform.[12] The sequencing results revealed an acquired *CCDC6-RET* fusion (C1;R12) with the allele frequency of 12.88% besides the *EGFR L858R* and *V834L* mutations (Fig. 1*A*). The patient then began to take the combination of osimertinib 80 mg daily and pralsetinib 400 mg daily since June 2021. Scans after 2 months revealed a response with the primary tumor shrinkage, and the follow-up efficacy evaluation established partial response (Fig. 1*B*). Symptoms such as headache and vomiting are remarkably relieved. Treatment is ongoing at the time of this writing.

Discussion

Although *RET* rearrangements and *EGFR* have been proved to activate driver alterations in NSCLC, the coexistence of both driver mutations is still very rare, even more rare for meningeal metastases. Here, we report a patient with meningeal-metastatic NSCLC with dual driver alterations. As far as we know, this is the first report of the efficacy of combined therapy with pralsetinib and osimertinib on meningeal metastases. Previous clinical trials reported the efficacy of pralsetinib and selpercatinib for patients with *RET*-rearranged NSCLC with central nervous system metastasis, but most were brain metastasis rather than meningeal metastasis. Only two case reports revealed efficacy of selective RET TKIs on meningeal metastasis for patients with *RET*-rearranged NSCLC up to now.[13,14] According to the two reported cases, selective *RET* inhibitors posed a positive effect to the patient's meningeal symptoms owing to the remission of the symptoms caused by intracranial hypertension, which suggests RET TKIs might have a potential effect on the meningeal metastasis, confirmed by the higher cerebrospinal fluid concentration to blood concentration ratio we measured for pralsetinib.

To clarify whether *RET* fusion coexisted with *EGFR* mutation in the same clone, we used fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) methods to detect the two mutations, respectively. The coexistence of *EGFR* and *RET-CCDC6* was proved by FISH and IHC methods as the same cluster of cells are singled out both in FISH and IHC (Fig. 2*A*–*F*). Furthermore, *KIF5B-RET* fusion is the most common *RET* arrangement in NSCLC, whereas most of the acquired *RET* fusions are *CCDC6-RET* as resistance to EGFR TKIs,[11] which indirectly proved that the *RET* fusion in this case was an acquired mutation.

The concentrations of osimertinib in the plasma and cerebrospinal fluid (CSF) have been reported previously, however, the concentrations of pralsetinib have never been reported before, neither in the plasma nor in the

Table 1. Reported Cases of the Coexistence of *EGFR* and *RET* Fusions[11]

Case	Age and Sex	Tissue or Plasma	*EGFR* Mutation	*RET* Fusion	T790M Status Before Fusion Detection	T790M Status After Fusion Detection	CNS Metastasis	*EGFR* TKI(s) Pre-Second CGP Biopsy	TKI(s) Treatment After Fusion Detection	Response (PFS)	Publications
1	43/F	T	L858R+V834L	CCDC6-RET	−	−	Meningeal metastasis	1. Gefitinib 2. Osimertinib 3. Dacomitinib	Osimertinib + pralsetinib	PR	This article
2	55/M	T	Del19	CCDC6-RET	NA	NA	−	Erlotinib	NA	NA	Klempner et al. 2015[10]
3	73/F	T	Del19	CCDC6-RET	NA	NA	−	Erlotinib	NA	NA	
4	44/M	T	Del19	CCDC6-RET	−	−	−	Afatinib	Erlotinib + cabozantinib	SD (2.5 mo)	Piotrowska Z et al. 2018[1]
5	60/F	T	Del19	CCDC6-RET	−	−	−	1. Afatinib 2. Osimertinib	Osimertinib + pralsetinib	PR (16 mo, ongoing)	
6	67/F	T	Del19	NCOA4-RET	−	−	−	Afatinib	Osimertinib + pralsetinib	PR (12 wk, ongoing)	
7	NA	P	Del19	CCDC6-RET	−	−	−	1. Erlotinib 2. Osimertinib	-	-	
8	69/M	T	Del19	CCDC6-RET	−	NA	−	Erlotinib	NA	NA	Schrock AB et al. 2018[3]
9	62/F	T	L858R	NCOA4-RET	−	NA	−	Afatinib	Afatinib + cabozantinib	SD (7 mo)	
10	70/F	T	L858R	TRIM24-RET	−	NA	−	NA	NA	NA	
11	46/M	P	Del19	TRIM24-RET	+	NA	−	1. Erlotinib 2. Osimertinib	NA	NA	
12	72/M	P	Del19	KIF5B-RET	−	NA	−	Icotinib	Cabozantinib + icotinib	PR (2 mo)	Zhu YC et al. 2018[4]
13	NA	T	Del19	CCDC6-RET	+	−	−	Osimertinib	NA	NA	Oxnard GR et al. 2018[6]
14	NA	P	Del19	NCOA4-RET	NA	NA	−	Osimertinib	NA	NA	Le X et al. 2018[7]
15	78/M	T	L858R+L747S	NCOA4-RET	+	NA	−	1. Erlotinib 2. Osimertinib	Osimertinib	PD	Offin M et al. 2018[8]
16	NA	P	Del19 or L858R	ERC1-RET	+	NA	−	Osimertinib	NA	NA	Zhou C et al. 2018[9]
17	52/F	NA	L858R	CCDC6-RET	−	−	−	Osimertinib	NA	NA	Xu H et al. 2019[2]
18	45/F	NA	Del19	CCDC6-RET	+	NA	−	Osimertinib	Osimertinib	NA	
19	51/M	NA	Del19	CCDC6-RET	+	−	−	Osimertinib	Osimertinib	NA	
20	46/F	NA	Del19	CDC123-RET	+	+	−	Osimertinib	1. Osimertinib 2. Capmatinib	NA	
21	80/F	NA	Del19	NCOA4-RET	+	−	−	Osimertinib	NA	NA	

(continued)

Table 1. Continued

Case	Age and Sex	Tissue or Plasma	*EGFR* Mutation	*RET* Fusion	T790M Status Before Fusion Detection	T790M Status After Fusion Detection	CNS Metastasis	*EGFR* TKI(s) Pre-Second CGP Biopsy	TKI(s) Treatment After Fusion Detection	Response (PFS)	Publications
22	54/M	NA	Del19	NCOA4-RET	+	+	−	Osimertinib	NA	NA	
23	NA	P	Del19	CCDC6-RET	+	NA	−	Erlotinib	NA	NA	Rich TA et al. 2019[5]
24	NA	P	L858R	NCOA4-RET	NA	NA	−	Erlotinib	NA	NA	
25	NA	P	Del19	CCDC6-RET	+	NA	−	Erlotinib	NA	NA	
26	NA	P	Del19	CCDC6-RET	−	NA	−	Erlotinib	NA	NA	
27	NA	P	L858R	CCDC6-RET	+	NA	−	Erlotinib	NA	NA	
28	NA	P	Del19	NCOA4-RET	−	NA	−	Erlotinib	NA	NA	
29	NA	P	Del19	CCDC6-RET	+	NA	−	1. Erlotinib 2. Afatinib 3. Osimertinib	NA	NA	
30	NA	P	Del19	CCDC6-RET	NA	NA	−	1. Afatinib 2. Osimertinib	NA	NA	
31	NA	P	Del19	NCOA4-RET	+	NA	−	1. Erlotinib 2. Osimertinib	NA	NA	
32	NA	P	Del19	CCDC6-RET	NA	NA	−	1. Erlotinib 2. Osimertinib	NA	NA	
33	NA	P	Del19	TRIM24-RET	−	NA	−	Osimertinib	NA	NA	
34	NA	P	Del19	CCDC6-RET	+	NA	−	Osimertinib	NA	NA	
35	NA	P	Del19	CCDC6-RET	+	NA	−	NA	NA	NA	
36	NA	P	Del19	NCOA4-RET	+	NA	−	NA	NA	NA	
37	NA	P	Del19	CCDC6-RET	+	NA	−	NA	NA	NA	

CNS, central nervous system; F, female; M, male; NA, not assessed; PFS, progression-free survival; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.

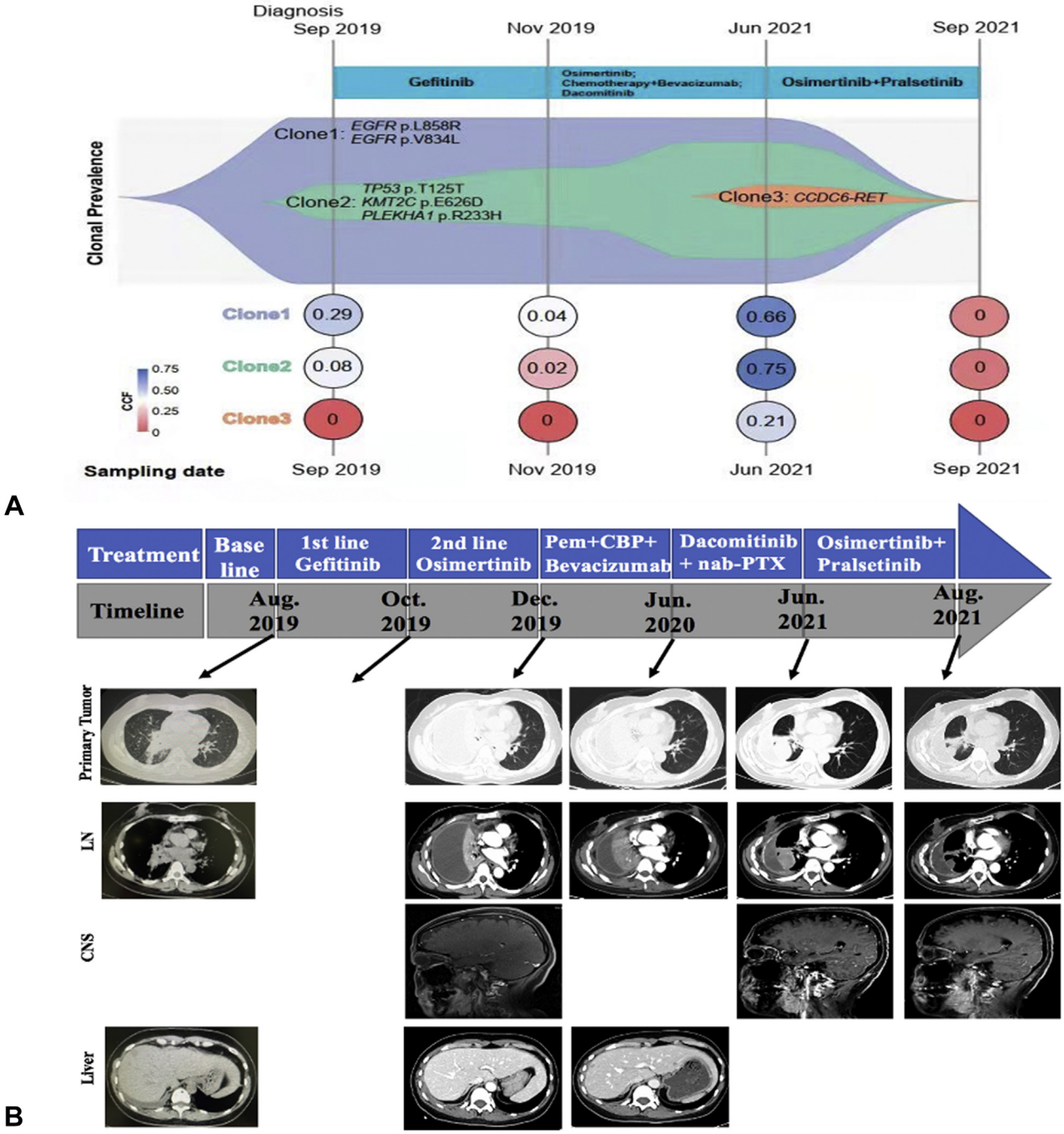

Figure 1. Clonal prevalence of three gene clones and imaging evaluation across the timeline. (*A*) Clonal prevalence of clone 1 (*EGFR*), clone 2 (TP53, KMT2C, PLEKHA1), and clone 3 (CCDC6-RET) is found in the sequence of sampling date and the anticancer treatments, vividly presenting the coexistence of CCDC6-RET and other gene mutations. (*B*) Sequence of anticancer treatments and their corresponding imaging evaluation across the timeline is found in sequence respectively. A remarkable shrinkage of the primary tumor could be noticed after the 2-month pralsetinib plus osimertinib treatment. Aug., August; CBP, carboplatin; CCF, cerebrospinal fluid; CNS, central nervous system; Dec., December; Jun., June; LN, lymph node; Nov., November; Oct., October; Pem, pemetrexed; PTX, paclitaxel; Sep., September.

CSF. We first measured the plasma and CSF concentrations of both pralsetinib and osimertinib in this patient. We collected plasma and CSF simultaneously on a voluntary basis from the patient after 4-month administration and then determined the concentrations by means of liquid chromatography tandem mass spectrometry. After setting the concentration gradients of the standard curve after pre-experiments, the liquid phase conditions were chosen to be column T3 with mobile phase of a: 0.1% formic acid ultrapure water and b: pure

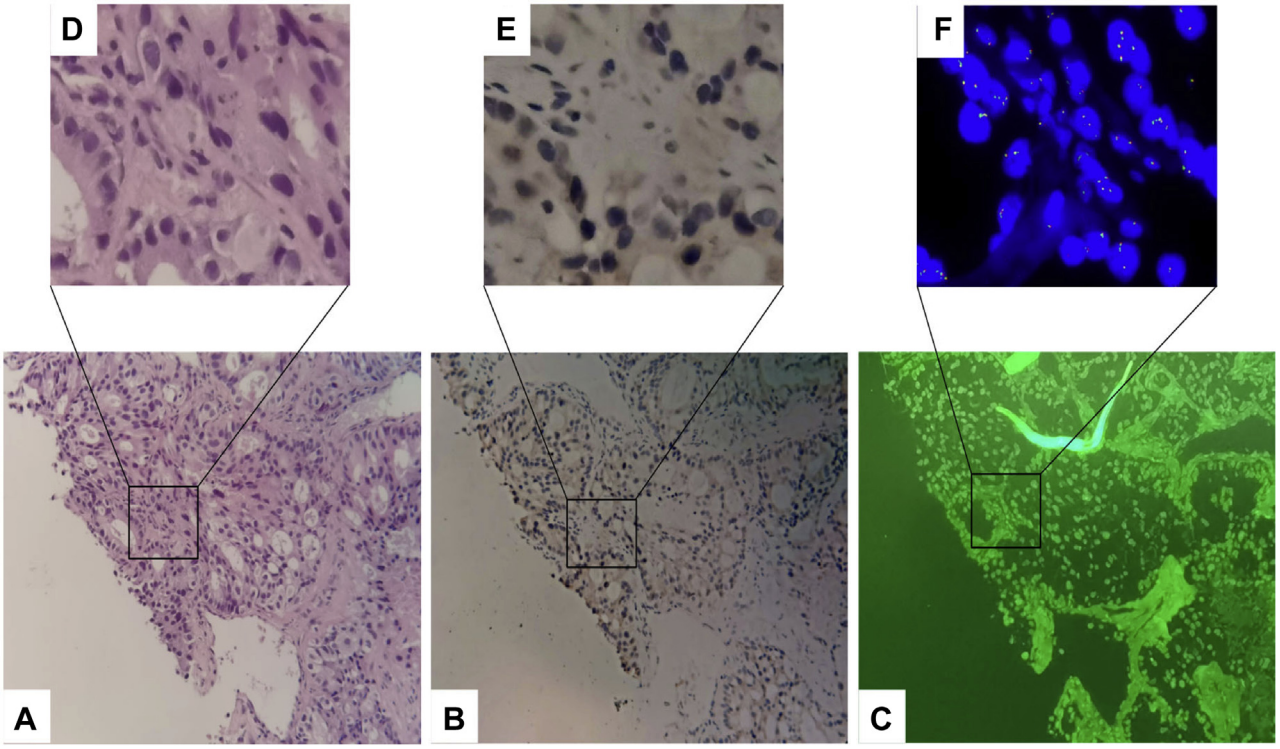

Figure 2. H&E staining, IHC analysis, and fluorescence in situ of the same clone. (*A*) H&E staining and immunohistochemical analysis (*B*) with PCK, TTF-1, Napsin A, Ki-67, PD-L1, CK5/6, P40, and LCA. The results revealed positive staining with PCK, TTF-1, Napsin A, Ki-67, and PD-L1 and negative for CK5/6, P40, and LCA, testing the *EGFR* L858R mutations. (*C*) RET fusions were tested by fluorescence in situ hybridization. (*D*, *E*, *F*) The same cluster of cells is singled out from three figures revealing *RET* fusion coexisted with *EGFR* mutation in the same clone. H&E, hematoxylin and eosin; PD-L1, programmed death-ligand 1.

acetonitrile. The plasma and CSF concentrations of osimertinib in this patient were 2148.94 nM and 23.70 nM, whereas the plasma and CSF concentrations of pralsetinib were 91.31 μM and 704.76 nM, respectively. After 4 months of continuous medications, we considered the detected concentrations to represent the steady-state concentrations.[15] Compared with the concentration that inhibits 50% of pralsetinib in *CCDC6-RET* (0.4 nM[15]), the concentrations of pralsetinib were much higher than concentration that inhibits 50% both in plasma and CSF in this case, suggesting pralsetinib does have better efficacy, even in the central nervous system. Blood and CSF drug concentrations have been reported to be possibly related to metabolic capability, drug interactions, individual differences, and brain radiotherapy.[16] Both pralsetinib and osimertinib are metabolized primarily by CYP3A4, so the concentration of osimertinib in combination may be different from single-agent setting. We first report the pralsetinib and osimertinib concentration in combination setting. Nevertheless, our findings facilitated a direct comparison of the differences in the blood and CSF distribution between pralsetinib and osimertinib, excluding the effects of metabolic factors and individual differences.

The EGFR mutation type in this case is *L858R* plus *V834L*, which is fairly rare, especially occurring with other activated driver alteration. As far as we know, it is the first report of a case that rare *EGFR L858R* plus *V834L* type seems with another driver gene. It was reported that *V834L* is predicted to result in steric hindrance of the drug near the anisole (methoxybenzene) group, and therefore develop the resistance to osimertinib.[7] Subsequently, the prediction had been proved in a case that primary resistance to osimertinib in a patient harboring both *L858R* and *V834L EGFR* mutations.[17] Together with this case, it might indicate that second-generation TKIs might perform a more lasting therapeutic effect.

Although the combining administration of osimertinib and pralsetinib seemed clinically beneficial to the patient in this case, two cases reported previously indicated that the full-dose combination of pralsetinib 400 mg with osimertinib 80 mg was intolerable as leukopenia and neutropenia could be observed.[1] Despite that, in this patient, full-dose combination of pralsetinib and osimertinib seemed acceptable because no obvious toxicities had been observed. Thus, dual drug combinations at adequate doses should be considered with caution.

In conclusion, this case together with the previous two papers[13,14] might serve as the basis for another possible choice for the meningeal metastasis of advanced *EGFR*-mutated NSCLCs with acquired RET mutations.

CRediT Authorship Contribution Statement

Zichen Zhao: Writing-original draft preparation.

Chao Su: Pharmacokinetic experiments.

Weigang Xiu: Figure visualization.

Weiya Wang: Fluorescence in situ hybridization and immunohistochemistry.

Shasha Zeng: Next-generation sequencing analysis.

Meijuan Huang: Formal analysis.

Youling Gong, **You Lu:** Reviewing and editing.

Yan Zhang: Supervision.

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