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

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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 an effective way to overcome the resistance, even for meningeal metastasis, owing to high CSF distribution of pralsetinib.

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.

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...
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, 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. 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 3. The patient subsequently underwent combined 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 Genescast panel of 543 genes (Genecast, Beijing, People’s Republic of China) and Illumina NovaSeq 6000 platform. 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. 1A). 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. 1B). 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. 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. 2A–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, 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                                 | NA                                 | Erlotinib                                      | NA           | NA             | Klempner et al. 2015 \(^{10}\) |
| 3    | 73/F        | T               | Del19       | CCDC6-RET  | NA                                 | NA                                 | NA                                 | Erlotinib                                      | NA           | NA             | Piotrowska Z et al. 2018 \(^{3}\) |
| 4    | 44/M        | T               | Del19       | CCDC6-RET  | --                                 | --                                 | --                                 | Afatinib                                      | Erlotinib + cabozantinib Osimertinib + pralsetinib | SD (2.5 mo) |               |
| 5    | 60/F        | T               | Del19       | CCDC6-RET  | --                                 | --                                 | --                                 | 1. Afatinib 2. Osimertinib                 | 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               | -            | -              | Schrock AB et al. 2018 \(^{7}\) |
| 8    | 69/M        | T               | Del19       | CCDC6-RET  | --                                 | NA                                 | --                                 | Erlotinib Erlotinib                        | NA           | NA             | Zhu YC et al. 2018 \(^{4}\) |
| 9    | 62/F        | T               | L858R       | NCOA4-RET  | --                                 | NA                                 | --                                 | Afatinib Afatinib + cabozantinib           | SD (7 mo)    |               | Oxnard GR et al. 2018 \(^{6}\) |
| 10   | 70/F        | T               | L858R       | TRIM24-RET | --                                 | NA                                 | --                                 | 1. Erlotinib 2. Osimertinib               | NA           | NA             |               |
| 11   | 46/M        | P               | Del19       | TRIM24-RET | +                                  | NA                                 | NA                                 | Erlotinib Osimertinib                     | NA           | NA             | Offin M et al. 2018 \(^{8}\) |
| 12   | 72/M        | P               | Del19       | KIF5B-RET  | --                                 | NA                                 | --                                 | Icotinib Cabozantinib + icotinib          | PR (2 mo)    |               | Zhou C et al. 2018 \(^{7}\) |
| 13   | NA          | T               | Del19       | CCDC6-RET  | +                                  | --                                 | --                                 | Osimertinib                                 | NA           | NA             | Xu H et al. 2019 \(^{2}\) |
| 14   | NA          | P               | Del19       | NCOA4-RET  | NA                                 | NA                                 | --                                 | Osimertinib                                 | NA           | NA             |                   |
| 15   | 78/M        | T               | L858R+L747S | NCOA4-RET  | +                                  | NA                                 | --                                 | 1. Erlotinib 2. Osimertinib               | Osimertinib PD |               | Le X et al. 2018 \(^{7}\) |
| 16   | NA          | P               | Del19 or L858R | ERC1-RET | +                                  | NA                                 | --                                 | Osimertinib                                 | NA           | NA             |                   |
| 17   | 52/F        | NA              | L858R       | CCDC6-RET  | --                                 | --                                 | --                                 | Osimertinib                                 | NA           | NA             |                   |
| 18   | 45/F        | NA              | Del19       | CCDC6-RET  | +                                  | NA                                 | --                                 | Osimertinib Osimertinib                   | NA           | NA             |                   |
| 19   | 51/M        | NA              | Del19       | CCDC6-RET  | +                                  | --                                 | --                                 | Osimertinib Osimertinib                   | NA           | NA             |                   |
| 20   | 46/F        | NA              | Del19       | CDC123-RET | +                                  | +                                  | --                                 | Osimertinib 1. Osimertinib 2. Capmatinib | NA           | NA             |                   |
| 21   | 80/F        | NA              | Del19       | NCOA4-RET  | +                                  | --                                 | --                                 | Osimertinib                                 | NA           | NA             |                   |

(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          | Rich TA et al. 2019^5 |
| 23   | NA          | P                | Del19         | CCDC6-RET  | +                                   | NA                                 | --            | Erlotinib                         | NA                                       | NA          |             |
| 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                      | NA                                       | NA          |             |
|      |             |                  |               |            |                                     |                                     |               | 2. Afatinib                       | NA                                       | NA          |             |
|      |             |                  |               |            |                                     |                                     |               | 3. Osimertinib                    | NA                                       | NA          |             |
| 30   | NA          | P                | Del19         | CCDC6-RET  | NA                                 | NA                                 | --            | 1. Afatinib                       | NA                                       | NA          |             |
|      |             |                  |               |            |                                     |                                     |               | 2. Osimertinib                    | NA                                       | NA          |             |
| 31   | NA          | P                | Del19         | NCOA4-RET  | +                                   | NA                                 | --            | 1. Erlotinib                      | NA                                       | NA          |             |
|      |             |                  |               |            |                                     |                                     |               | 2. Osimertinib                    | NA                                       | NA          |             |
| 32   | NA          | P                | Del19         | CCDC6-RET  | NA                                 | NA                                 | --            | 1. Erlotinib                      | NA                                       | NA          |             |
|      |             |                  |               |            |                                     |                                     |               | 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.
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
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. Compared with the concentration that inhibits 50% of pralsetinib in CCDC6-RET (0.4 nM), 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. 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. Subsequently, the prediction had been proved in a case that primary resistance to osimertinib in a patient harboring both L858R and V834L EGFR mutations. 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. 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\textsuperscript{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**

**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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