Case report

Effective treatment of pulmonary adenocarcinoma harboring triple EGFR mutations of L858R, T790M, cis-G796s/cis-C797s by osimertinib, brigatinib, and bevacizumab combination therapy: A case report

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ABSTRACT

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) were widely used in advanced non-small cell lung cancers (NSCLCs) with EGFR sensitive mutation and greatly improved the patient survival. With the widespread use of EGFR TKI, TKI resistance is increasingly emerging in the clinic. Osimertinib, a 3rd EGFR-TKI, commonly was used in patients who were resistant to early-generation EGFR-TKIs carrying T790M mutation. After using of osimertinib, it might result in the development of further resistance, for example, the cis-C797S mutation. Herein, we report an effective treatment for a case of advanced pulmonary adenocarcinoma patient with triple EGFR mutations of L858R/T790M/cis-C797S and L858R/T790M/cis-G796S by the combination therapy of osimertinib, brigatinib, and bevacizumab after the combination of brigatinib and cetuximab failed. The plasma circulating tumor DNA (ctDNA) monitoring provided information of EGFR mutation evolution and guided appropriate therapy regimen during the progression. After the combination therapy worked, a significant reduction of the 3 EGFR mutations was detected. The side effect was acceptable during the whole period of therapies.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide in human, 5-year Overall Survival (OS) for newly diagnosed patients less than 20% [1]. Over the last decades, targeted drug therapy for lung cancer has made significant strides, especially with the development of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). However, most advanced non-small cell lung cancer (NSCLCs) patients acquired drug resistance after receiving 1st or 2nd generation EGFR TKIs. The EGFR T790M mutation was the primary cause of drug resistance [2], and although osimertinib which is the third-generation TKI could overcome T790M mutation [3], additional resistance to osimertinib were often generated consequently. EGFR Cis-C797S/G mutation was the most common resistance mutation to osimertinib [4] and was resistant to multiple EGFR-TKIs [5–7]. EGFR cis-G796S/R mutation was a rare osimertinib-induced resistance mutation and could coexist with cis-C797S/G [4,8]. Existence of cis-G796S/R mutation significantly increased the complexity of acquired resistance mutations to osimertinib [8].

Osimertinib resistance was a major challenge in EGFR mutations targeted therapy. Much researches focused on counteracting EGFR

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C797S mutation. In recent years, some case reports found that the combination of brigatinib and anti-EGFR antibody [9] or osimertinib, bevacizumab, and brigatinib [10] may be effective against the EGFR 19del/T790M/cis-C797S and EGFR L858R/T790M/cis-C797S mutations. At present, there were few treatment options for advanced NSCLCs carrying triple EGFR mutations of L858R/T790M/cis-C797S. For more complex mutations, L858R/T790M/cis-C797S and L858R/T790M/cis-G796S, no effective targeted therapy has been reported. In this paper, we report an effective treatment of a case of advanced pulmonary adenocarcinoma with triple EGFR mutations of L858R/T790M/cis-C797S and L858R/T790M/cis-G796S by the combination therapy of osimertinib, brigatinib, and bevacizumab.

2. Case report

A 74-year-old Chinese woman with no history of smoking was diagnosed with lung adenocarcinoma accompanied by adrenal
metastasis, pleural metastasis and lymph node metastasis (poorly differentiated nonmucinous bronchioloalveolar type, cT4N3M1, stage IV) in February 2017. Mutation of EGFR was detected from tumor tissue by polymerase chain reaction (PCR) and the EGFR exon 21 L858R was positive. Then the patient received gefitinib treatment (250 mg daily [qd] by mouth [po]), which resulted in radiological remission and rapidly clinical benefit.

However, the pulmonary lesion increased after 9 months of continuous treatment (November 2017). Considering that the patient might have acquired drug resistance to gefitinib, a biopsy by tracheoscopy was performed. The biopsy pathology still suggested adenocarcinoma and EGFR PCR test suggested EGFR L858R was positive. Whereafter, we detected circulating tumor DNA (ctDNA) in plasma, and the results showed only L858R mutation (abundance 0.14%, Fig. 1A). Due to the poor condition, the patient did not receive chemotherapy. Then she received traditional Chinese medicine treatment for 12 months until the symptoms of dyspnea and hemoptysis increased significantly. Pulmonary lesion was larger than before in Chest CT. In November 2018, the results of ctDNA in plasma showed an L858R mutation in EGFR exon 21 (abundance 36.31%) and a T790M mutation in exon 20 (abundance 15.68%, Fig. 1B). Consequently, we altered the therapy to osimertinib (80 mg qd po). At the beginning of treatment, as the rapid production of pleural effusion, we perfused endostar (anti-angiogenic drug, 45 mg 3 times per week for 1 week) into the pleural cavity of the patient. After 2 months of treatment, the patient’s symptoms were relieved and the lung lesions were reduced again. Unfortunately, in July 2019, a CT scan showed that the pulmonary lesion had increased again and that the disease had progressed. Histopathological biopsy suggested adenocarcinoma for the third time, and the immunohistochemistry of programmed cell death ligand 1 (PDL-1) indicated tumor proportion score (TPS) was <1%. In the meantime, ctDNA analysis found an L858R mutation in EGFR exon 21 (abundance 0.51%), a T790M mutation in exon 20 (abundance 0.37%), a cis-C797s mutation in exon 20 (abundance 5.08%) and a cis-C796s mutation in exon 20 (abundance 0.71%, Fig. 1C). At first, we tried the treatment a combination of brigatinib (90 mg qd po) and cetuximab (600 mg monthly, by intravenous injection) for 2 months, disappointingly, the treatment failed to work.

In October 2019, ctDNA detection was carried out again, and the results showed an L858R mutation in EGFR exon 21 (abundance 4.06%), a T790M mutation in exon 20 (abundance 4.31%), a cis-C797s mutation in exon 20 (abundance 3.19%) and a cis-C796s mutation in exon 20 (abundance 0.68%). Based on the change of EGFR mutation abundances (Fig. 2), we adjusted the treatment regimen to osimertinib (80 mg qd po), brigatinib (90 mg qd po) and bevacizumab (15 mg/kg every 3 weeks, by intravenous injection). Partial remission was observed after 6 weeks (2 cycles) of treatment. The patient experienced mild diarrhea at the first month and the diarrhea disappeared after using montmorillonite powder. Over 3 months later the patient developed a hypertension, after irbesartan was added, the blood pressure returned to normal soon. 4 months later, ctDNA detection showed reduced abundance of the L858R mutation (abundance 0.97%), T790M mutation (abundance 0.57%), cis-C797S mutation (abundance 0.18%) and cis-C796s mutation (abundance 0.14%, Fig. 2) and partial response was achieved 4 months after initial treatment (Fig. 3). The patient had stable disease until May 2020 (progression free survival [PFS] lasting 8 months).

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

3. Discussion

EGFR-TKIs have become one of the most important therapeutics for the treatment of NSCLC. However, most patients treated with 1st or 2nd generation EGFR-TKIs developed acquired resistance after about 8–14 months, and approximately 60% of patients had a T790M acquired resistance mutation [11]. The second mutation (T790M) increased the ATP-binding affinity of the mutated protein and hence reduced the activity of ATP-competitive TKIs [12]. The 3rd generation EGFR-TKI, osimertinib, has an extremely strong affinity for EGFR containing the T790M mutation, with a strong antitumor effect via covalent binding to the cysteine at position 797 of

![Fig. 2. Dynamic changes of EGFR mutations in plasma during treatment.](image-url)
EGFR [13]. Nevertheless, resistance to second-line osimertinib has also been described in patients and in more than 30% of cases is due to the emergence of additional mutations in EGFR such as C797S/G, G796S/R, L792F/H, L718Q/V, and G724S [14, 15]. The C797S mutation was the most common resistance mutation to osimertinib, which was detected in approximately 20–30% of osimertinib acquired resistance cases [4]. The C797S mutation removes the cysteine side-chain with which osimertinib reacts covalently, thus preventing drug binding to EGFR. There were only a few studies have been conducted on EGFR G796S mutation. It was reported as a rare osimertinib-induced resistance mutation and often coexists with other drug-resistant mutations (such as C797S) [4, 8]. The G796S is located under the phenyl aromatic ring of osimertinib and occupies the solvent front position homologous with anaplastic lymphoma kinase (ALK) G1202R and ROS1 G2032R. Computer models predicted that EGFR G796S mutation might sterically interfere with the osimertinib aromatic ring and disable the binding of osimertinib to the kinase domain [8].

For the C797S mutation, it has been reported that the treatment strategy is determined by whether the C797S and T790M mutations are on the same DNA strand. When the EGFR T790M and C797S mutations are present in trans, they are sensitive to the combination therapy of 1st and 3rd generation EGFR-TKIs; however, such mutation structure was rare [5]. Indeed, studies suggest that the vast majority of T790M and C797S mutations were in cis [4], and these cancer cells were refractory to both 1st and 3rd generation TKIs, and combination 1st and 3rd generation TKIs [16]. It remained unclear which therapeutic strategies might be effective at overcoming resistance to osimertinib caused by cis-C797S. Strategies for managing this scenario was largely limited to chemotherapy. Recently, Wang et al. reported that the combination of brigatinib and cetuximab could conquer EGFR T790M and cis-C797S with a remarkable PFS and minor toxicity [9]. Another case reported by Zhao et al., which showed that L858R/T790M/cis-C797S responds to the brigatinib, osimertinib and bevacizumab combination therapy [10]. With this background, our patient, with a multi-resistant mutation, might have alternative treatment options other than chemotherapy.

Brigatinib, a 2nd generation ALK inhibitor, was used to treat crizotinib-resistant ALK-positive metastatic NSCLC [17]. Brigatinib had activity against a number of kinases. In in vitro kinase assays, brigatinib had potent activity against ALK, including the mutant variants G1202R, C1156Y et al. The drug also demonstrated activity against ROS1 and EGFR L858R and to a lesser extent EGFR T790M resistance mutation (L858R/T790M) [18]. In 2017, Uchibori et al. [9] performed a drug screening in order to investigate therapeutic strategies for treating patients with triple EGFR mutants (L858R/T790M/cis-C797S): In vivo and in vitro experiments shown that brigatinib could suppress the growth of tumor cells carrying the triple EGFR mutations and delay-induced acquiring resistance by osimertinib. They carried out a computational simulation and structure-activity relations hip analysis to explore the affinity and binding mode of brigatinib. The results shown that brigatinib can competitively affect the ATP-binding site of the EGFR kinase domain. In addition, the combination of brigatinib and anti-EGFR antibody showed more preferable activity than brigatinib monotherapy without toxicity. Cetuximab was a chimeric monoclonal immunoglobulin G1 antibody, blocked EGFR signaling and might mediate antitumor immune mechanisms [19, 20]. Cetuximab was mainly used against colorectal cancer [21] and head and neck cancer [22]. In the treatment of NSCLC, the benefit of Cetuximab in combination with chemotherapy was limited which was independent of the presence or absence of EGFR mutation [23]. Nevertheless, as mentioned above, the combination of cetuximab and brigatinib shown an activity to inhibit multiple EGFR mutations. It was supposed that cetuximab likely induces synergistic growth inhibition of cancer cells through the degradation of total and cell surface EGFR [9].

In this case, the patient was treated with gefinitib and osimertinib, and then developed osimertinib resistance by EGFR L858R/
T790M/cis-C797S and L858R/T790M/G796S mutations. As the immunohistochemistry of lung adenocarcinoma tissue shown PD-L1 TPS<1% and the patient did not choose chemotherapy, we firstly tried the treatment of combination of brigatinib and cetuximab which has indicated to be effective in both preclinical trials and clinical case report. The recommended dosing regimen of brigatinib for ALK positive NSCLC was 90 mg daily for 1 week, which was then increased to 180 mg daily if tolerated. In our case, the dose escalation of brigatinib was not chosen owing to safety concerns when using combination therapy. Unfortunately, the combination therapy did not work and the disease had progressed. A ctDNA examination after the combination therapy failed revealed that the abundance of cis-C797S (5.1%–3.19%) and cis-G796S (0.71%–0.68%) got decreased and the abundance of L858R (0.51%–4.06%) and T790M (0.37%–4.31%) got increased. This result might indicate that there were several mutation types in the tumor cells, for instance L858R, L858R/T790M, L858R/T790M/cis-C797S, L858R/T790M/G796S and the like. Meanwhile, the presence of G796S mutation made our treatment more difficult. The decrease of cis-C797S abundance might suggest a response to brigatinib and the increase of L858R and T790M might requires previous osimertinib. Coupled with the case report mentioned above showed that L858R/T790M/cis-C797S responds to the brigatinib, osimertinib and bevacizumab combination therapy. Combining brigatinib, osimertinib and bevacizumab was administered after the failure of combination of brigatinib and cetuximab.

Bevacizumab, a humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), regresses preexisting tumor blood vessels and blocks the formation of new ones [24]. Combining bevacizumab and platinum-based chemotherapy for lung cancer patients improves PFS and OS [25]. Similarly, erlotinib plus bevacizumab improved PFS compared with erlotinib alone for patients with EGFR mutant NSCLC [26]. However, recently in one randomized clinical trial comparing osimertinib plus bevaczumab vs osimertinib alone, the combination arm failed to show prolongation of PFS in patients with advanced lung adenocarcinoma with EGFR T790M mutation [27]. Although the bevacizumab could enhance the effect of EGFR-TKIs in treating NSCLCs with EGFR mutations remains elusive, it seems that when EGFR-TKIs resistance happened, combining bevacizumab and EGFR-TKIs appeared to prolong antitumor activity. Some studies found that VEGF level of tumor patient increased after acquiring drug resistance to EGFR-TKIs [28, 29]. Therefore, when the dependence of tumor cells on EGFR signaling decreased, their dependence on the VEGF pathway would increase [28]. As a result, bevacizumab, an anti-VEGF antibody, combining with EGFR-TKIs might improve the antitumor effect because they antagonised both angiogenesis and EGFR activation. In vitro studies confirmed that it became refractory to erlotinib after long-term administration of erlotinib, erlotinib plus bevacizumab prolonged antitumor activity [29].

As we expected, the symptoms were relieved after 2 cycles of combination of brigatinib, osimertinib and bevacizumab was administered, and a CT scan showed that the pulmonary lesion was significantly reduced. After the combination therapy getting a response, we collected plasma samples from this patient and ctDNA was examined. The ctDNA results revealed a decreased abundance of EGFR mutations: L858R (4.06% → 0.51%), T790M (4.31% → 0.57%), cis-C797S (3.19% → 0.18%) and cis-G796S (0.68% → 0.14%). Previous study has demonstrated that dynamic changes in mutation abundance can reflect the efficacy of EGFR-TKIs and that a decrease in mutation abundance predicts a better EGFR-TKI response [30]. Therefore, we speculated that combining bevacizumab and brigatinib increased the therapeutic efficacy of EGFR cis-C797S and G796S mutations.

In summary, the case report demonstrate that clinical application of brigatinib, osimertinib and bevacizumab combination therapy for the treatment of pulmonary adenocarcinomas carrying triple EGFR mutations of L858R, T790M, cis-C797S/cis-G796S is effective. Currently, treatment regimens for osimertinib-induced resistance cis-C797S and cis-G796S mutation are limited. Hence, this combination therapy may provide a potential novel treatment option for them.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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