OncoTargets and Therapy

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CASE REPORT

Effective treatment of pulmonary adenocarcinoma harboring triple EGFR mutations of L858R, T790M, and *cis*-C797S by osimertinib, bevacizumab, and brigatinib combination therapy: a case report

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Osimertinib is commonly used in pulmonary adenocarcinoma patients who are resistant to first-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and carry the T790M mutation. However, the use of osimertinib may result in the development of further resistance, most commonly via the *cis*-C797S mutation. Herein, we report a case of a lung cancer patient harboring triple EGFR mutations of L858R, T790M, and *cis*-C797S who was treated with a combination of osimertinib, bevacizumab, and brigatinib. The above 3 mutations were detected by circulating tumor DNA analysis after osimertinib treatment. Subsequently, the patient received combination therapy of osimertinib and bevacizumab; the partial relief obtained was negated by later disease progression. The regimen was then changed to osimertinib, bevacizumab, and brigatinib combination therapy. Partial remission was observed, and a significant reduction in EGFR mutations was detected. This case represents the first evidence that 1) bevacizumab combined with osimertinib can significantly relieve tumor growth and respiratory symptoms in non-small-cell lung cancer patients with osimertinib resistance and 2) the clinical use of osimertinib, bevacizumab, and brigatinib is effective as combination therapy for pulmonary adenocarcinoma in the presence of triple EGFR mutations of L858R, T790M, and *cis*-C797S. These combination therapies may provide potential novel treatment options for pulmonary adenocarcinoma patients.

Keywords: pulmonary adenocarcinoma, EGFR-T790M, EGFR *cis*-C797S, drug resistance mechanisms

Introduction

Most pulmonary adenocarcinoma patients acquire drug resistance after receiving first-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs).[1] The EGFR T790M mutation is the primary cause of this drug resistance,[2] and although the third-generation TKI osimertinib can overcome this mutation,[3] an additional EGFR mutation (C797S) is often generated.[4] A previous study showed that for patients resistant to first-generation EGFR-TKIs, combined application of bevacizumab (a monoclonal antibody against vascular endothelial growth factor [VEGF]) can significantly prolong progression-free survival (PFS).[5] However, there is no clinical evidence to date regarding whether bevacizumab combination is effective in patients resistant to osimertinib.

Recent in vitro and in vivo studies have shown that when T790M/C797S mutations are in the *cis* configuration, ie, located on the same DNA strand, brigatinib

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http://dx.doi.org/10.2147/OTT.S170358

(a dual-target inhibitor of anaplastic lymphoma kinase [ALK]/EGFR) can inhibit the growth of cancer cells harboring triple EGFR mutations of L858R/T790M/*cis*-C797S and delay acquired resistance to osimertinib.[6] However, this effect has not been reported in clinical practice. Herein, we for the first time report effective treatment of a case of advanced pulmonary adenocarcinoma with triple EGFR mutations of L858R/T790M/*cis*-C797S by the combination therapy of osimertinib, bevacizumab, and brigatinib.

Case report

In February 2011, a 66-year-old woman with no history of smoking presented with a central poorly differentiated adenocarcinoma (nonmucinous bronchioloalveolar type, $cT_4N_2M_{1a}$, stage IV, Figures 1A and 2) in the right lung accompanied by mediastinal lymph node and pleural metastases in the right lung. In April 2011, the patient received targeted gefitinib treatment (250 mg daily [qd] by mouth [po]), and her condition was partially improved after 1 month (May 2011, Figure 1B). However, the pulmonary lesion increased after 20 months of continuous treatment (January 2013, Figure 1C). The patient then received discontinued chemotherapy with pemetrexed and carboplatin, but the pulmonary lesion continually increased, with the tumor eventually occupying almost the entire right lung (January 2016, Figure 1D). Considering that the patient may have acquired drug resistance to gefitinib, we altered the therapy to osimertinib (80 mg qd po). After 1 month of treatment, the size of the pulmonary lesion was dramatically reduced (February 2016, Figure 1E). However, 1 year later, a CT scan showed that the pulmonary lesion had again increased and that the disease had progressed (February 2017, Figure 1F). We thus detected circulating

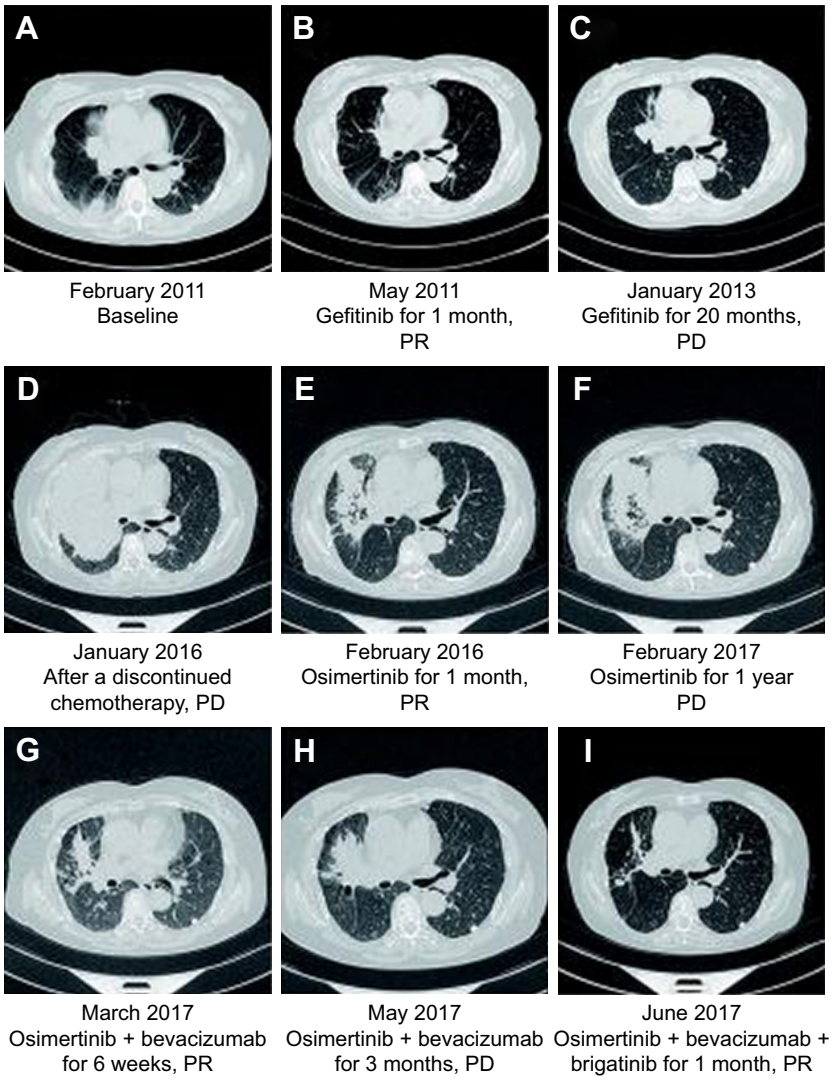

Figure 1 (**A–I**) Chest CT scans at various time points.
Abbreviations: PR, partial response; PD, progressive disease.

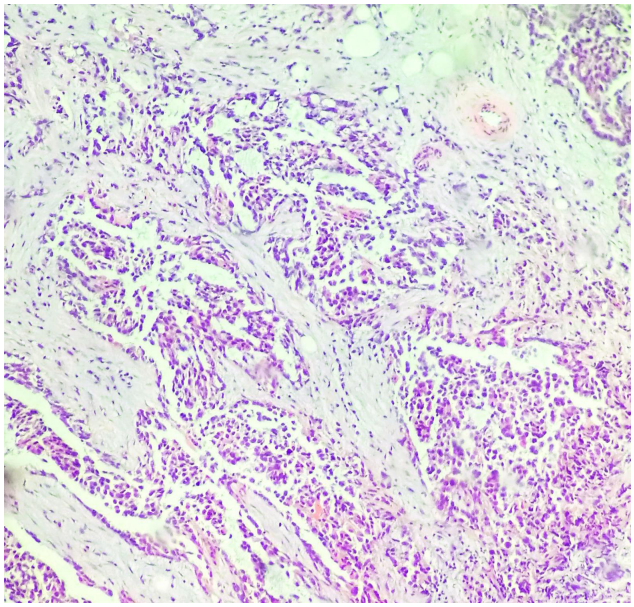

Figure 2 Representative histopathological image of the tumor (H&E staining, 10×).

tumor DNA (ctDNA) in plasma, and the results showed an L858R mutation in EGFR exon 21 (abundance 6.0%), a T790M mutation in exon 20 (abundance 1.7%), and a *cis*-C797S mutation in exon 20 (abundance 0.6%) (Figure 3A). A previous Phase II clinical study indicated that bevacizumab combined with the first-generation TKI erlotinib significantly prolonged the PFS of non-small-cell lung cancer (NSCLC) patients carrying EGFR mutations.[7] Therefore, the patient was subsequently administered combination therapy of osimertinib (80 mg qd po) and bevacizumab (15 mg/kg every 3 weeks [q3w]). After 6 weeks (2 cycles), the pulmonary lesion was smaller, and the symptoms of chest tightness and shortness of breath were significantly relieved (March 2017, Figure 1G). However, after 3 months of treatment (4 cycles), the pulmonary tumor had enlarged again, with disease progression (May 2017, Figure 1H). Based on a recent study showing that combination therapy of brigatinib with an EGFR-TKI could overcome osimertinib resistance in the

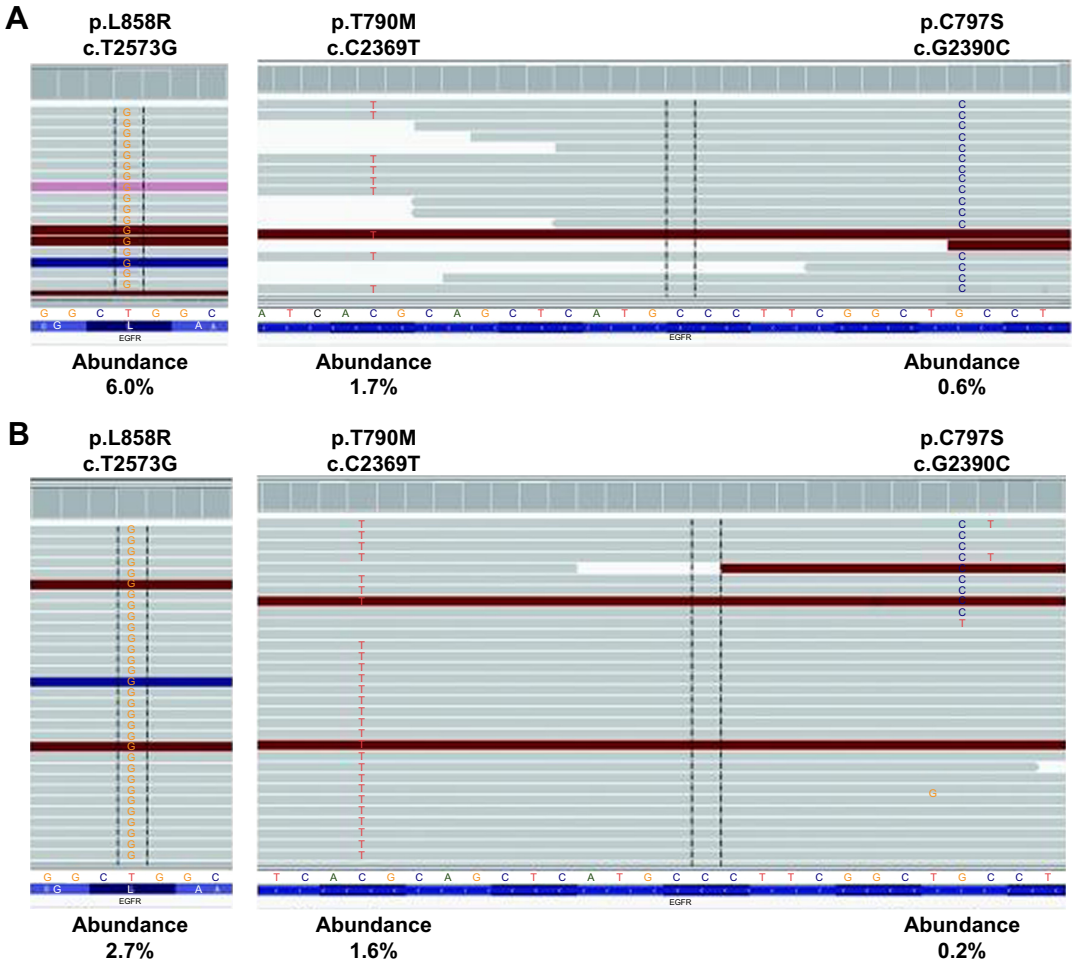

Figure 3 EGFR mutation analysis of the patient's plasma before (**A**) and after (**B**) brigatinib treatment.
Note: The mutation abundance was calculated by a commercial service Pulmocan® provided by Geneseeq (Nanjing, People's Republic of China).

presence of T790M and *cis*-C797S mutations, the patient was administered combined targeted treatment of osimertinib (80 mg qd po), bevacizumab (15 mg/kg q3w) and brigatinib (100 mg qd po). Partial remission was observed after 1 month of treatment (June 2017, Figure 1I). Two months later, ctDNA detection was carried out again, and the results showed reduced abundance of the L858R mutation in EGFR exon 21 (abundance 2.7%), T790M mutation in exon 20 (abundance 1.6%), and C797S mutation in exon 20 (abundance 0.2%) (Figure 3B). These results indicate that the addition of brigatinib enhanced the effectiveness of the treatment. The patient has currently not exhibited significant adverse reactions and is continuing to receive the combined targeted treatment of osimertinib, bevacizumab, and brigatinib.

Discussion

Approximately 30%–50% of NSCLC patients in the Asian population carry EGFR gene mutations.[8] For NSCLC patients with EGFR-sensitive mutations, EGFR-TKI-targeted therapy has better therapeutic efficacy than combined platinum drug-based traditional chemotherapy and can significantly prolong PFS.[9] However, acquired drug resistance is prone to occur after the use of EGFR-TKIs, and the T790M mutation in exon 20 of the EGFR gene is the main factor leading to acquired resistance to first-generation EGFR-TKIs.[10] The third-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 EGFR. However, the C797S mutation blocks this binding, resulting in further acquired drug resistance.[4] Much recent research effort has focused on counteracting the C797S mutation-based acquired drug resistance of osimertinib.

Studies have suggested that tumor VEGF levels increase after acquired drug resistance to EGFR-TKIs.[11,12] As a result, the dependence of tumor cells on EGFR signaling will decrease, whereas their dependence on the VEGF pathway will increase.[11] As an anti-VEGF antibody, bevacizumab in combination with EGFR-TKIs might improve the antitumor effect because they target different pathogenesis pathways, such as angiogenesis and EGFR activation. Osimertinib plus bevacizumab showed better effects in mouse xenograft tumors of RPC-9 cells.[12] Moreover, a Phase II clinical trial (JO25567 trial) showed that erlotinib (a first-generation TKI) combined with bevacizumab significantly prolonged the PFS of patients with NSCLC harboring EGFR-sensitive mutations compared with erlotinib alone.[7] However, as a third-generation TKI, the efficacy of the combination of osimertinib and bevacizumab has not been confirmed in relevant clinical practice. In the current case, when the patient acquired resistance to osimertinib, a combination of osimertinib and bevacizumab was administered after a careful consideration of the patient based on JO25567.[7] As we expected, the symptoms were relieved after 2 cycles of treatment, and a CT scan showed that the pulmonary lesion was significantly reduced. These results suggest that the antitumor effect may increase with a combination of a third-generation TKI and an anti-VEGF antibody.

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 more sensitive to the combination therapy of first- and third-generation EGFR-TKIs; however, such a mutation structure is rare.[13] Indeed, data thus far suggest that EGFR T790M and C797S mutations are often present in *cis*, and there is currently no effective treatment option for such cases. Recent in vivo and in vitro experiments have shown that as a dual-target inhibitor of ALK/EGFR, brigatinib can suppress the growth of tumor cells carrying triple EGFR mutations of L858R/T790M/*cis*-C797S and delay osimertinib-induced acquired resistance.[6] Surprisingly, such a rationale was clinically confirmed in the current case, whereby the addition of brigatinib significantly improved the patient's condition after the disease had progressed during osimertinib and bevacizumab combination treatment. In a previous study, Uchibori et al[6] carried out a computational simulation and structure–activity relationship analysis to explore the affinity and binding mode of brigatinib. The results showed that brigatinib can competitively affect the ATP-binding site of the EGFR kinase domain. The chloro, phosphine oxide group, and the methoxy group of brigatinib worked as key elements that contributed to its superior efficacy for triple-mutant EGFR.[6] In addition, brigatinib can effectively suppress the phosphorylation of EGFR and its downstream signaling pathways, such as AKT and ERK1/2, in EGFR mutated-cells.[6] However, whether bevacizumab plays an important role in the therapeutic effects remains elusive, and additional in-depth research is required to address this question.

In the present study, we collected plasma samples from a patient with advanced pulmonary adenocarcinoma during the targeted treatment process, and ctDNA was examined to guide the EGFR-TKI-targeted therapy. The ctDNA results revealed the L858R mutation in exon 21 (abundance 6%), T790M mutation in exon 20 (abundance 1.7%), and C797S mutation in exon 20 (abundance 0.6%) when the disease

had progressed during osimertinib treatment (Figure 3A). This result confirmed that this patient developed *cis*-C797S mutation-based acquired drug resistance ~1 year after using osimertinib, which was consistent with a previous study.[4] Subsequently, combination therapy with osimertinib and bevacizumab was administered. However, after a temporary stabilization, the patient's disease began to progress again. According to a recent study, brigatinib can ameliorate the osimertinib-induced drug resistance caused by the *cis*-C797S mutation and increase the anticancer effect of osimertinib, and therefore, the drug regimen was changed to the combination of osimertinib, bevacizumab, and brigatinib. After 6 weeks of treatment, a CT scan showed that the pulmonary lesion had significantly decreased. ctDNA was subsequently detected, and the results showed a decreased abundance of EGFR mutations: L858R (abundance 2.7%), T790M (abundance 1.6%), and *cis*-C797S (abundance 0.2%) (Figure 2B). It is noteworthy that the abundance of the *cis*-C797S mutation was significantly reduced (~0.33-fold, Figure 4). A previous study demonstrated that dynamic changes in mutation abundance can reflect the efficacy of EGFR-TKIs and that a rapid decrease in mutation abundance predicts a better EGFR-TKI response.[14] Thus, we speculate that brigatinib increased the therapeutic efficacy by targeting the EGFR *cis*-C797S mutation; the results of this current case are consistent with previous experimental studies.[6]

In summary, we report the first clinical evidence that bevacizumab combined with osimertinib significantly relieved tumor growth and respiratory symptoms in NSCLC patients with osimertinib resistance. In addition, we demonstrate for the first time that clinical application of osimertinib, bevacizumab, and brigatinib combination therapy for the treatment of pulmonary adenocarcinomas carrying triple EGFR mutations of L858R, T790M, and *cis*-C797S is effective. Currently, there is no effective therapy for EGFR-TKI drug resistance caused by the C797S *cis*-mutation. Therefore, these combination therapies may provide potential novel treatment options for patients.

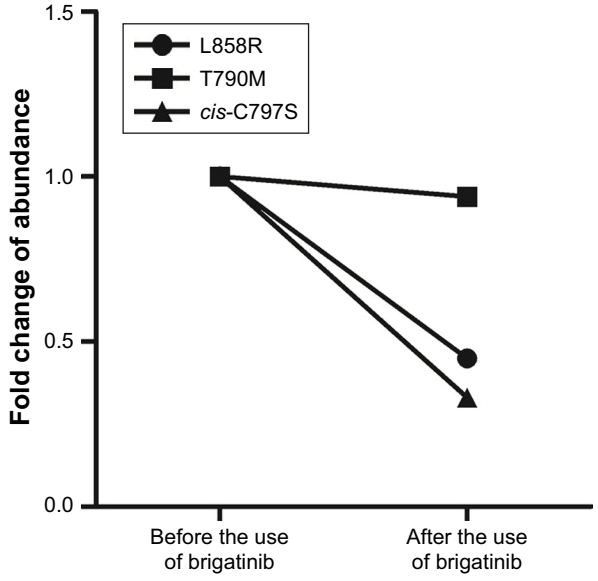

Figure 4 Fold change in the abundance of each EGFR mutation.

Acknowledgments

We thank the patient in this report and her family. A written informed consent has been provided by the patient to have the case details and any accompanying images published. This work was supported by grants from the Science Research Project of Department of Education of Liaoning Province (No L2016011), the Doctoral Scientific Research Fund of the Second Hospital of Dalian Medical University (DY2YBSQD201501), and the Dalian Medical Science Research Project (1711099).

Disclosure

The authors report no conflicts of interest in this work.

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