BRIEF REPORT

Combination Osimertinib and Gefitinib in C797S and T790M *EGFR*-Mutated Non–Small Cell Lung Cancer

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ABSTRACT

Introduction: Osimertinib, a third-generation EGFR tyrosine kinase inhibitor has demonstrated efficacy in tumors harboring the *EGFR* T790M resistance mutation. Inevitably, resistance to third-generation inhibitors results in disease progression, with the *EGFR* C797S mutation being one of several resistance pathways identified to date. On the basis of preclinical data, we report what is the first known case of a patient harboring the T790M and C797S mutations in *trans* treated with combination gefitinib and osimertinib.

Methods: On development of progressive disease after multiple therapies, the patient's plasma was sequenced using the Oncomine Lung cfDNA Assay (Thermo Fisher Scientific, Waltham, MA). Subsequent monitoring of circulating tumor DNA in plasma was performed by droplet digital polymerase chain reaction.

Results: Sequencing showed that the T790M and C797S mutations were in *trans*. Within 2 weeks of commencement of combination therapy, rapid clinical improvement occurred. Accompanying this, a rapid decline in the C797S mutation subclone in plasma was detected. However, the levels of the *EGFR* exon 19 deletion driver mutation and the T790M resistance mutation in the circulating tumor DNA continued to rise and the patient died from progressive disease 6 weeks after commencement of combination therapy. There were no adverse events seen with the combination therapy.

Conclusion: This is, to the best of our knowledge, the first reported case of combination EGFR tyrosine kinase inhibitor therapy tailored to the allelic conformation of T790M and C797S mutation that resulted in brief clinical improvement without toxicity.

Keywords: Osimertinib; Gefitinib; C797S mutation; *EGFR* mutation; Non–small cell lung cancer

Introduction

EGFR-mutated NSCLC is exquisitely responsive to targeted tyrosine kinase inhibitor (TKI) therapy with erlotinib, gefinitib, or afatinib. However, resistance to the EGFR TKIs invariably occurs, most frequently because of the T790M mutation, which increases the receptor affinity for adenosine triphosphate (ATP) binding and hence reduction in primary activity of ATP-competitive TKIs.[1]

Osimertinib, a third-generation TKI, is a potent inhibitor of activating *EGFR* mutations and the T790M resistance mutation. The phase III AURA 3 study confirmed a significant progression-free survival (PFS) benefit (hazard ratio = 0.32, 95% confidence interval:

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0.21–0.39) of osimertinib compared with platinum-pemetrexed chemotherapy in T790M-positive patients after progression while taking a first-generation TKI.[2] In the original AURA dose expansion study, the median PFS noted with osimertinib was 9.6 months.[3] On the AURA 3 study, the median PFS was 10.1 months.[2]

The C797S mutation occurs in exon 20. The cysteine at position 797 is used by third-generation TKIs such as osimertinib to covalently bind with the receptor. Hence the *EGFR* C797S mutation leads to ineffective binding and subsequent resistance to osimertinib.[4]

Unlike T790M, which is the most common mechanism of resistance to first-generation TKIs, the C797S mutation was initially reported in only six of 19 patients progressing during osimertinib therapy (31%).[5] In our own series of patients, the C797S mutation was detected in four of 10 patients with the T790M mutation, but it occurred in *trans* in only one.

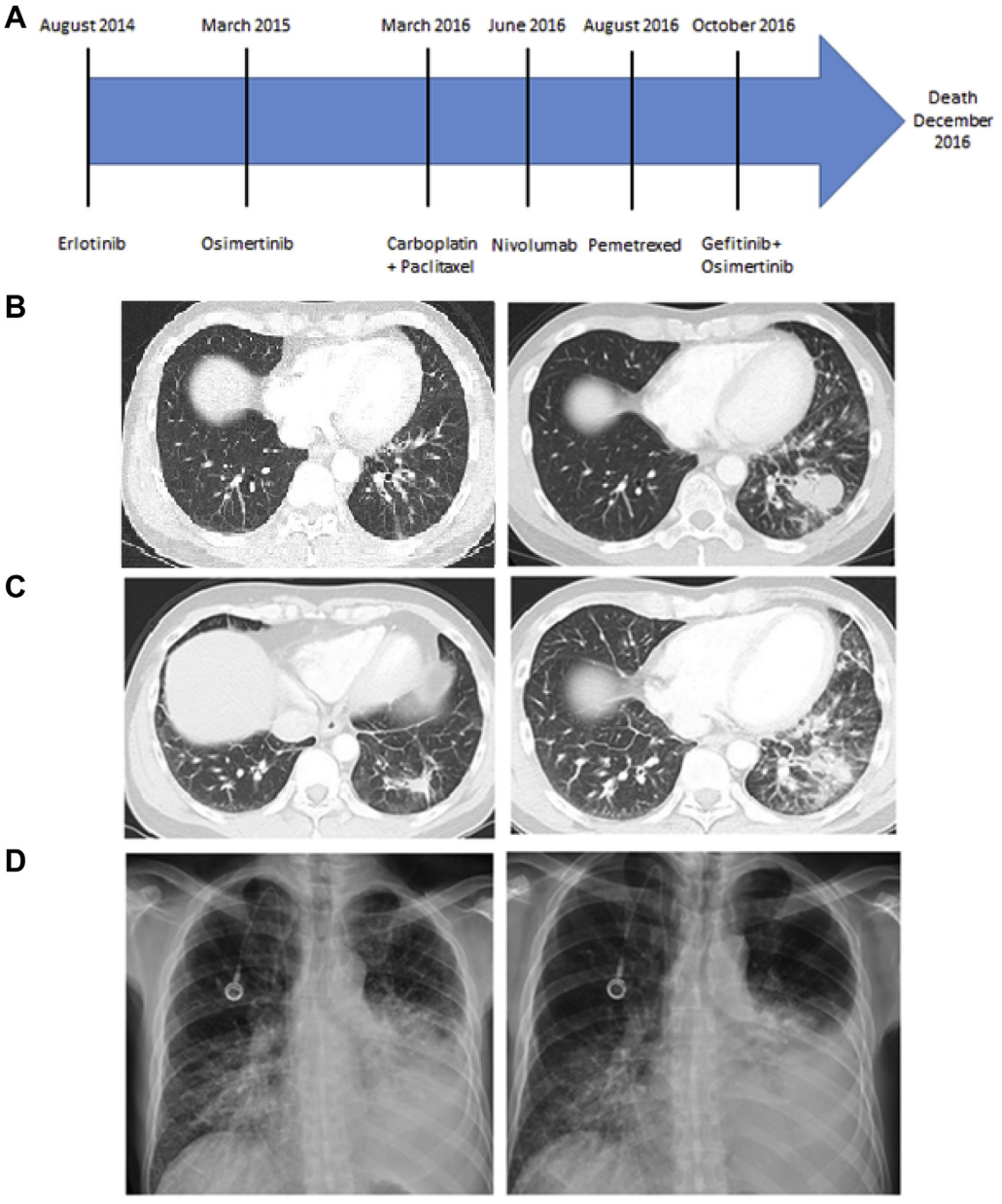

Figure 1. Sequence of the patient's anticancer treatment and radiological imaging before and after therapies. (*A*) Time line of anticancer therapies received by the patient. (*B*) Computed tomography (CT) scan (*left*) shows a left lower lobe lung primary 3 months into erlotinib therapy in November 2014. CT scan (*right*) shows progressive disease during erlotinib therapy in March 2015. (*C*) CT scan (*left*) shows response in the lung 3 months into osimertinib therapy in June 2015. CT scan (*right*) shows progressive disease with lymphangitis carcinomatosis in March 2016. (*D*). Chest radiograph (*left*) obtained before commencement of combination gefitinib and osimertinib therapy. Chest radiograph (*right*) obtained 2 weeks after combination gefitinib and osimertinib therapy.

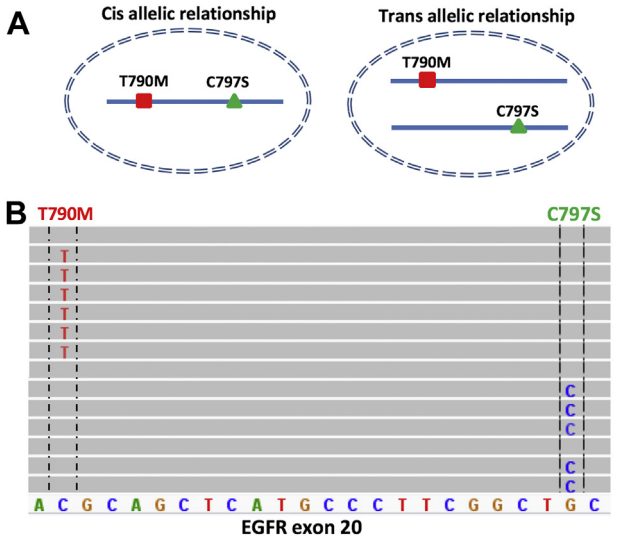

Figure 2. Allelic relationship of *EGFR* T790M and C797S mutations. (*A*) Diagram showing the *cis* (on the same DNA strand) and *trans* (on different DNA strands) allelic relationships of the *EGFR* T790M and C797S mutations. (*B*) Deep sequencing data on the patient's plasma DNA showing the *EGFR* T790M and C797S mutations detected in a *trans* allelic relationship.

Intriguing preclinical data in a T790M-positive cultivated first-generation– and third-generation–resistant cell line demonstrated that the configuration of the T790M and C797S mutations affected the cellular response to therapy.[6] If the two *EGFR* mutations were in *cis* (on the same DNA strand), the cells were refractory to combination first- and third-generation TKIs. However, when the two mutations were in *trans* (on different DNA strands), the combination of first- and third-generation EGFR inhibitors led to inhibition in EGFR signalling and cell death.

With this background, we report a patient with metastatic *EGFR*-mutant NSCLC treated with the combination of gefitinib and osimertinib after development of the T790M and C797S mutations in the *trans* allelic context.

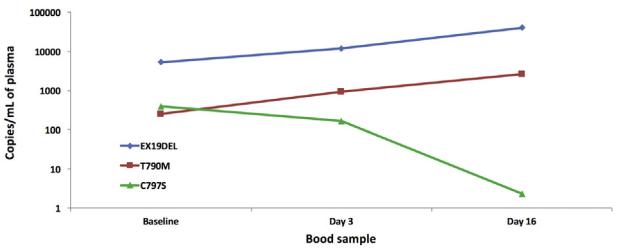

Figure 3. Monitoring of the *EGFR* mutations in blood. Three blood samples were collected before and during the combination therapy consisting of gefitinib and osimertinib (days 3 and 16). The levels of *EGFR* exon 19 deletion (ex19del), T790M, and C797S mutations were measured by using droplet digital polymerase chain reactions assays. Levels of the *EGFR* ex19del and T790M steadily increased, whereas the level of the C797S mutation rapidly decreased.

Case Report

A 41-year-old man of Asian ethnicity with lymphangitic pulmonary disease and metastases to the mediastinum and bone presented in August 2014 with symptomatic cough and dyspnoea on exertion. He began receiving erlotinib, 150 mg, (Fig. 1*A*) after the detection of an exon 19 deletion within the *EGFR* gene, which resulted in a radiological response and rapid clinical benefit lasting 6 months (Fig. 1*B*). Subsequent tissue biopsy at the time of relapse confirmed the original exon 19 deletion in addition to an acquired T790M in exon 20.

The patient then received osimertinib, 80 mg, as part of the AURA3 trial; he achieved a rapid clinical and radiological response lasting 8 months (Fig. 1*C*). Because of ongoing clinical benefit, the patient continued receiving osimertinib for a further 4 months but was switched to carboplatin and paclitaxel after worsening lymphangitis. Again, chemotherapy resulted in a partial response after two cycles but disease progression after the fourth cycle.

The patient went on to receive nivolumab, 3 mg/kg; however, after four cycles, his condition worsened and he required hospitalization for dyspnea on minimal exertion and a debilitating cough. High-resolution chest computed tomography (CT) demonstrated worsening of the lymphangitis carcinomatosis. Restaging brain, abdominal, and pelvic CT revealed new metastases in the brain, liver, and pleura. The patient received one cycle of pemetrexed chemotherapy, but his condition continued to deteriorate.

To study the cause of disease progression after osimertinib, a blood sample was collected and double-spun to separate plasma. Plasma DNA samples isolated using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) were tested for the *EGFR* exon 19 deletion, T790M, and C797S mutations using droplet digital polymerase chain reaction assays. An *EGFR* C797S (c.2390G>C) mutation was detected. To determine the *cis* or *trans* allelic relationship of the C797S with T790M (Fig. 2*A*), the patient's plasma DNA was deep sequenced by using the Oncomine Lung cfDNA Assay (Thermo Fisher Scientific, Waltham, MA). A C797S (c.2390G>C) mutation in addition to the original exon 19 deletion and T790M mutations was detected in the *EGFR* gene. However, the C797S and T790M mutations were detected on different DNA strands, indicating the *trans* allelic relationship (Fig. 2*B*).

As the C797S mutation was detected in *trans* with the T790M mutation, the patient was treated with a combination of gefitinib and osimertinib. Within 3 days, his clinical situation improved and he was discharged. His

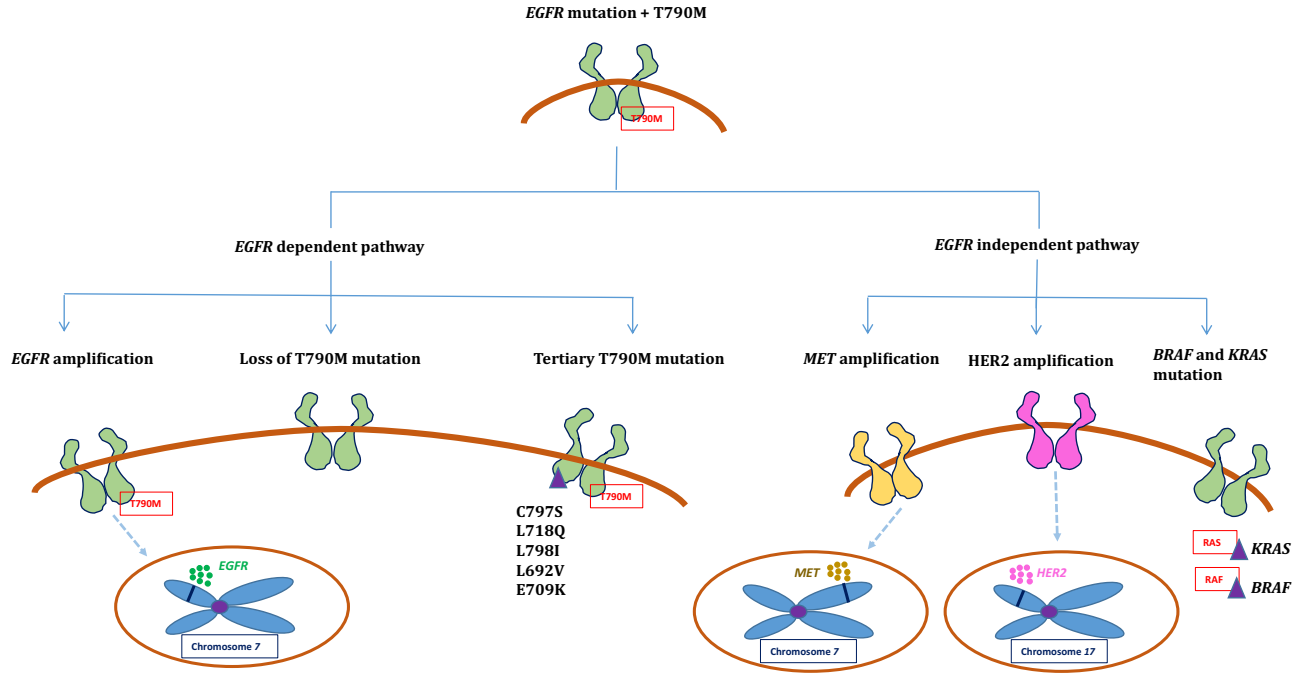

Figure 4. Mechanisms of resistance to osimertinib, a third-generation EGFR tyrosine kinase inhibitor, including *EGFR*-dependent and *EGFR*-independent pathways. *MET*, Mesenchymal to Epithelial Transition gene; *HER2*, erb-b2 receptor tyrosine kinase 2 gene.

condition was reviewed 2 weeks later, and his dyspnea and cough were well controlled. A chest radiograph showed some improvement of the lymphangitis carcinomatosis (Fig. 1*D*). However, a month after commencement of combination TKI therapy, the patient re-presented to the hospital with relapse of his cough and dyspnea. A chest CT scan showed significant progression of the lymphangitis carcinomatosis; the patient's condition continued to deteriorate, and he died 2 weeks later (6 weeks after commencement of the combination TKI and 2 years and 2 months after the diagnosis of metastatic lung cancer). Two blood samples were collected during the combination therapy to monitor the levels of the *EGFR* exon 19 deletion, T790M, and C797S mutations. The level of the C797S mutation was substantially reduced during the combination therapy, whereas the *EGFR* exon 19 deletion and T790M mutations steadily increased (Fig. 3).

Discussion

Lung tumors responding to osimertinib inevitably develop resistance through *EGFR*-dependent and *EGFR*-independent mechanisms (Fig. 4). The *EGFR*-dependent resistance mechanisms include acquired tertiary *EGFR* mutations such as *EGFR* C797S and loss of the T790M mutation. The acquired *EGFR* C797S mutation that affects the binding site of osimertinib was predicted in preclinical models[6,7] and is the most common resistance mechanism found.[5,8] Thress et al. identified *EGFR* C797S mutations in six of 19 patients with lung cancer who had progressed while taking osimertinib (31%).[5]

Although *EGFR* C797S mutations are the most commonly detected, other *EGFR* mutations that interfere with the covalent binding of osimertinib can also cause resistance to osimertinib. Bersanelli et al. identified an *EGFR* L718Q mutation in an osimertinib-resistant lung tumor.[9]

Loss of the *EGFR* T790M mutation with retention of the initial activating *EGFR* mutation was reported in four of 15 patients with osimertinib-resistant T790M-mutant lung cancer.[5] Our group previously reported such a case, in which the plasma level of *EGFR* exon 19 deletion mutation rapidly increased without detectable *EGFR* T790M mutation in the blood when the patient progressed during osimertinib treatment.[10]

Several *EGFR*-independent mechanisms of resistance to osimertinib that activate bypass pathways have also been reported. Mesenchymal to Epithelial Transition gene (*MET*) amplification is as an important mechanism of resistance to first-generation EGFR TKIs through phosphorylation of erb-b2 receptor tyrosine kinase 3 and downstream activation of the phosphoinositide 3-kinase/AKT pathway. Recent preclinical and clinical studies have also demonstrated that *MET* amplification causes resistance to osimertinib.[11,12] In addition, *BRAF* and *KRAS* mutations, as well as transformation to small cell carcinoma, have also been identified as mechanisms of acquired resistance to osimertinib.[13–15]

Although resistance to third-generation EGFR TKIs inevitably occurs, strategies for managing this scenario have largely been limited to chemotherapy. On the basis of preclinical data, we report for (to the best of our

knowledge) the first time, the clinical efficacy of combination first- and third-generation EGFR TKIs in a patient with the C797S mutation occurring in *trans* with the T790M mutation. Although initial clinical benefit occurred, our patient progressed rapidly and died within a short time. The combination TKIs were well tolerated, with no rash, diarrhea, transaminitis, or pneumonitis (which are adverse events known to occur with each TKI individually). Our results of monitoring circulating tumor DNA suggest multiple *EGFR*-mutant clones detectable within the plasma. The initial decline in C797S paralleled the clinical improvement; however, the steady rise in the plasma T790M and activating exon 19 deletion levels indicate rapid progression of another resistant clone.

Recently, a novel TKI that inhibited the triple mutation consisting of the activating *EGFR,* T790M, and C797S mutations was reported.[16] In this preclinical study, a genetically engineered L858R/T790M/C797S mouse was treated with the combination of cetuximab, an antibody that blocks EGFR dimerization, and EAI045, an allosteric inhibitor that synergizes with cetuximab and overcomes the enhanced ATP affinity conferred by the T790M mutation and is not affected by the C797S mutation, as the residue is remote from the allosteric binding pocket. After two doses of treatment, significant tumor shrinkage was noted.

Conclusion

The fluidity of the cancer genome enables rapid evolution of resistance mechanisms on exposure to targeted therapies. Development of the C797S mutation to osimertinib is a relatively new finding, with newer therapeutic strategies looking to target this mutation. Tailoring a therapeutic approach according to the allelic conformation of the T790M and C797S mutations demonstrated some efficacy in our patient, although the improvement was brief. As tumors evolve in the host and become exposed to subsequent drug therapies, resistant subclones emerge. This temporal heterogeneity, which differs between patients undergoing the same therapies, can result in single or multiple resistance mechanisms. The durability of the response to therapy likely depends on the relative quantities of each. Although our patient did not obtain substantial benefit, more data are needed to determine whether the efficacy of combination EGFR TKIs can be taken beyond a novel in vitro observation.

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