L718Q Mutation as New Mechanism of Acquired Resistance to AZD9291 in EGFR-Mutated NSCLC

CASE REPORT

L718Q Mutation as New Mechanism of Acquired Resistance to AZD9291 in *EGFR*-Mutated NSCLC

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Introduction

AZD9291 (osimertinib) is an irreversible third-generation EGFR tyrosine kinase inhibitor (TKI) with impressive tumor responses in *EGFR*^{T790M}-mutated NSCLC.[1] The selectivity of AZD9291 for *EGFR* T790M depends on its covalent interaction with cysteine 797 in the adenosine triphosphate-binding cleft. Mutation at the *EGFR* C797 codon, such as the missense variant C797S, has been demonstrated as the principal mechanism of acquired resistance to third-generation EGFR TKIs.[2] Little is known about alternative mechanisms.[3] Herein, we reported a case with coexisting primitive L858R and acquired T790M mutations and development of resistance to AZD9291 through the occurrence of a new *EGFR* L718Q mutation that was previously reported in vitro but never confirmed in patients until now.[4]

Case Report

Advanced lung adenocarcinoma was diagnosed in a 71-year-old woman in February 2012. The TheraScreen test (Qiagen, Hilden, Germany) on DNA of a histological specimen of the tumor was positive only for L858R mutation on exon 21 of *EGFR* gene. First-line treatment with gefitinib was started in March 2012, with a partial response until January 2014, when progressive disease (PD) was demonstrated. Six cycles of chemotherapy with carboplatin-pemetrexed were then administered with PD. New pulmonary biopsies performed in July 2014 allowed identification of the T790M mutation. AZD9291 in a phase I clinical trial was started in August 2014 (Fig. 1*A* [left]), with the partial response maintained until September 2015 (Fig. 1*A* [middle]). A new nodal biopsy performed at the time of PD (Fig. 1*A* [right]) confirmed L858R and T790M mutations and excluded (by Sanger sequencing) the presence of the C797S *EGFR* resistance mutation. Potential *EGFR*-independent mechanisms of resistance such as the following were also excluded: anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) rearrangement; erb-b2 receptor tyrosine kinase 2 gene (*HER2*), *EGFR*, MNNG HOS Transforming gene (*MET*), and fibroblast growth factor receptor gene (*FGFR*) amplifications; and *KRAS* and *BRAF* mutations. Next-generation sequencing (NGS) was then performed on the same specimen, revealing a new *EGFR* mutation, c.2153T>A p.L718Q (Fig. 1*B*). Next-generation sequencing retrospectively performed on the AZD9291 pretreatment specimen confirmed the new occurrence of this mutation. With the limitation that negativity of the pre-AZD9291 sample could not be excluded for this mutation because of tumor heterogeneity, Sanger sequencing also confirmed these results (Fig. 1*C*).

Discussion

Recent studies revealed that acquired resistance to AZD9291 is mediated mainly by the *EGFR* C797S mutation or loss of the *EGFR* T790M mutation in patients with *EGFR*^{T790M}-mutant NSCLC.[2,4] Here, we present the first clinical report of resistance to AZD9291 mediated by the L718Q mutation in a patient with *EGFR*$^{L858R/T790M}$-mutant NSCLC.

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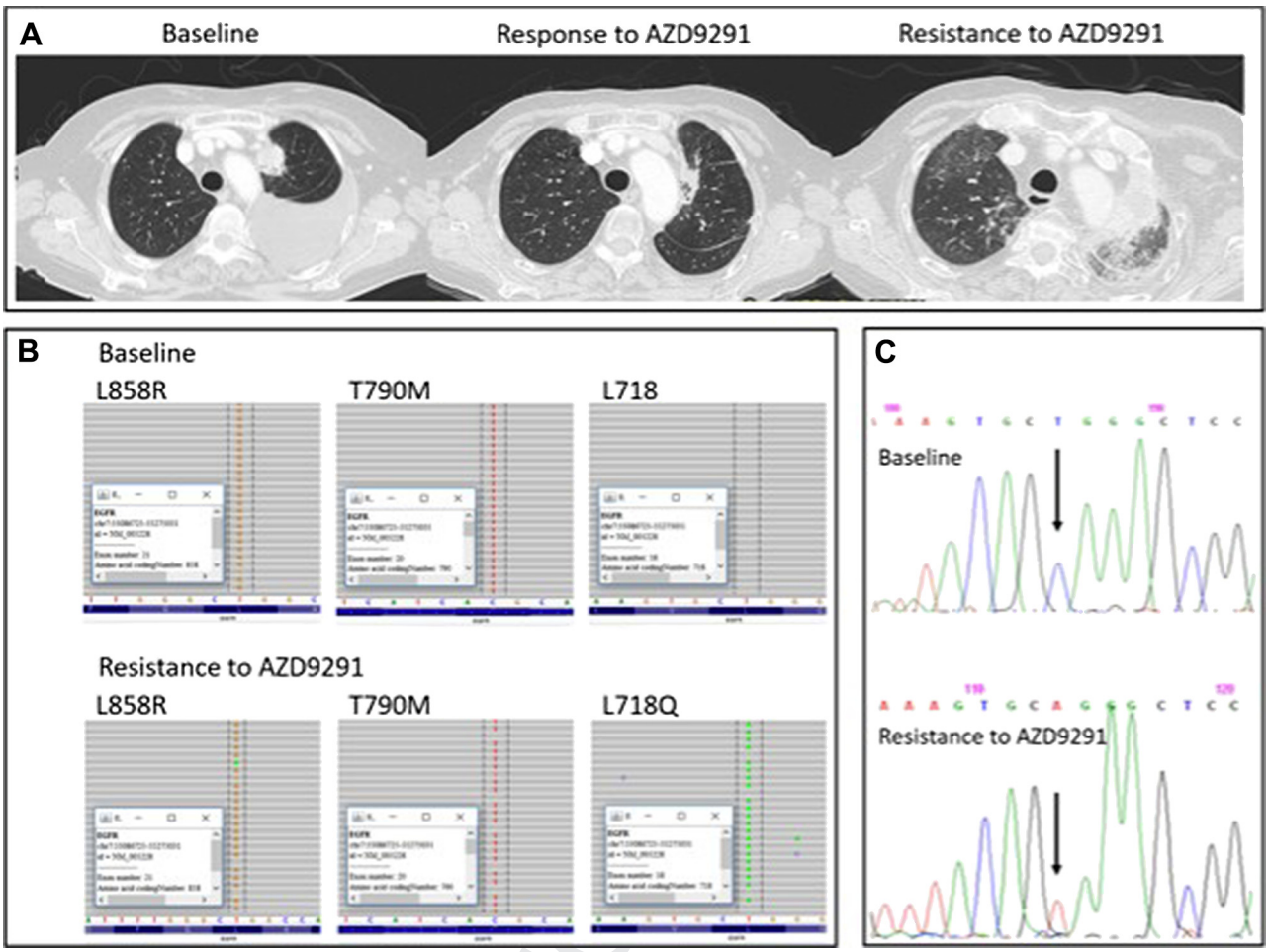

Figure 1. Acquired resistance to AZD9291. (*A*) Computed tomography of the chest showed the tumor at baseline before AZD9291 treatment (*left*), a partial response (*middle*), and progressive disease after treatment with AZD9291 (*right*). (*B*) The Integrative Genomics Viewer (Broad Institute, Cambridge, MA) revealed the mutational status of tumor at baseline (*top*) and after AZD9291 therapy (*bottom*). Next-generation sequencing was performed with TruSight Tumor 26 genes (Illumina, San Diego, CA) on the MiSeq platform (Illumina). (*C*) Sanger sequencing of EGFR exon 18 gene confirmed the occurrence of L718Q mutation in the tumor.

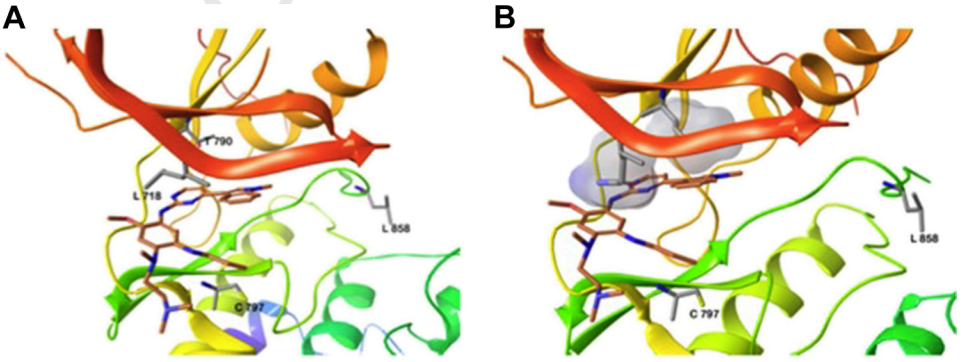

Figure 2. Interaction of AZD9291 with EGFR. (*A*) Crystal structure of wild-type EGFR in complex with AZD9291 (PDB code 4ZAU). L718 directly contacts the inhibitor in an arrangement that favors the formation of a covalent bond with C797. (*B*) Structural model illustrating the effects of the T790M and L718Q resistance mutations (*gray volumes*). Although the bulkier side chain of methionine at position 790 does not interfere with AZD9291 docking, the glutamine side chain at position 718 is expected to sterically interfere with the pose of AZD9291 that allows covalent bonding to C797.

Ercan et al. reported L718Q *EGFR* mutation as in vitro mechanism of resistance to AZD9291, although it occurred more frequently in WZ4002- and CO-1686–resistant models. With third-generation agents, triple-mutated L858R/T790M/L718Q Ba/F3 cells showed a high concentration that inhibits 50%, as in cells with L858R/L718Q.[4] Our clinical report confirms this preclinical evidence. Intriguingly, this preclinical study provided an in vitro demonstration that this triple mutation could potentially be sensitive to an irreversible quinazoline EGFR TKI such as afatinib.[4]

L718Q mutation could mediate drug resistance, likely through steric hindrance and affect drug binding. We built a crystallographic model demonstrating that the L718, which is located in the crystal structure of AZD9291 in complex with EGFR, is in direct contact with aniline ring and that the L718Q substitution likely interferes with irreversible binding of the compound, reducing the efficiency of covalent bond formation between the acrylamide warhead and C797 thiol group (Fig. 2).[5]

The identification of such new mechanisms of acquired resistance could allow better management of EGFR TKI therapy and provide alternative targets for development of new inhibitors.

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