Case Report: Detection of c797s as a Mechanism of Resistance in a Patient With Lung Cancer With *EGFR* Mutations

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Abstract

We present here the case of a patient with exon 19 *EGFR*-sensitive mutation who, after months of experiencing benefit with tyrosine kinase inhibitors (TKIs), developed resistance to TKIs because of the development of *EGFR* T790M mutation that was initially missed. The patient was subsequently treated with palliative chemotherapy until he had disease progression (PD). However, after chemotherapy was initiated, the T790M mutation was identified by liquid biopsy and confirmed with a new tissue biopsy. Later, after starting osimertinib, the patient achieved disease stabilization until he developed a c797s mutation with PD. He did not respond to immunotherapy and subsequently died. Pertinent issues regarding diagnosis and therapy are discussed here.

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Introduction

Non–small cell lung cancer (NSCLC) was the first epithelial neoplasm treated with targeted therapy, based on the discovery of *EGFR* mutations and their predictive value and response to tyrosine kinase inhibitors (TKIs). The prevalence of *EGFR* mutations is strongly correlated with ethnicity: 10% to 20% in Caucasians, 30% to 40% in Asians, and, as described in a recent study, 26% in Latin American patients.[1,2]

Despite the initial success of targeted therapy (overall response rate [ORR] 60% to 83% for erlotinib and 71% for gefitinib), patients will develop resistance generally after 1 year of treatment. The mechanism of resistance includes the acquisition of additional mutations in the EGFR receptor, such as T790M (60% of cases), the activation of additional driver genes, and histological transformation to small cell lung cancer.[1,3]

As an example of daily oncology practice, we describe here the case of a patient who had an *EGFR* mutation that made him sensitive to TKI therapy (deletion in exon 19), and the difficulty of documenting a resistant (T790M) mutation. The natural disease history of such patients keeps evolving with further progression of disease and the onset of new mutations such as c797s. The case demonstrates the underlying need for more molecular monitoring with tissue or liquid biopsies. Also, we give an example of the use of liquid biopsies in assisting in the identification of resistance mutations.

Case Report

The patient was a 54-year-old male nonsmoker with a past medical history significant for hypertension, hyperlipidemia, anxiety, and stage IV lung cancer (adenocarcinoma, with an *EGFR* mutation [exon 19 deletion]) diagnosed with tissue biopsy. He originally presented with multiple pulmonary nodules, multiple subcentimeter lesions on brain MRI, and metastatic lesions to the thoracic and lumbar spine. He was started on erlotinib 150 mg daily and received stereotactic brain radiation. Subsequent imaging revealed that the lesions in his chest and brain were stabilized or reduced in size for the next 10 months. The patient subsequently reported experiencing pain. MRI scan showed worsening on spine lesions (T11-L1), for which he underwent a short course of intensity-modulated radiation therapy; therapy with erlotinib continued. However, 3 months later an MRI of the brain revealed new brain metastases and systemic disease progression (PD) on PET scan.

The patient expressed interest in enrolling in a clinical trial: AURA 3 (NCT02151981) for osimertinib (Tagrisso) versus chemotherapy. His tumor was biopsied again and sent for central testing. It was reported that the tumor did not have the T790M mutation in tissue and he was not accepted in the clinical trial. We started him on palliative chemotherapy with carboplatin and pemetrexed for 5 cycles until he again had PD.

Next-generation sequencing (NGS) through a liquid biopsy revealed positive results for T790M. We conducted a second test of his previously negative tissue biopsy that also revealed positive results for T790M mutation. There were no other actionable mutations such as *ALK* or *MET*.

The patient was started on osimertinib and he was stable for 14 months until systemic and brain PD was exhibited in the PET scan and brain MRI. We found an EGFR c797s–resistance mutation in his blood. The patient was started on palliative nivolumab and ipilimumab; however, therapy eventually failed and he died from complications of pneumonia.

Discussion

Tyrosine kinases are part of a large multigene family that is

crucial for signal transmission cascades.[4] Many extracellular receptors of growth factors have an intrinsic TKI activity that is triggered by the process of binding their ligands. Phosphorylation of downstream effectors usually produces conformational changes in the EGFR receptor and exposes catalytic sites with the effect of signal amplification.[5] Docking of TKIs in the catalytic sites is either favored by some mutations (sensitizing mutations) or conversely disfavored (resistance mutations such as T790M and c797s). The study of resistance mutations led to the design of third-generation TKIs like osimertinib.

We classify resistance as primary or acquired. Primary resistance is present when the tumor cells bear an intrinsic mechanism of resistance, and therefore do not respond to the original treatment. In acquired resistance, the tumor cells develop mechanisms of resistance under forces of natural selection or selective pressure.[6] Primary resistance is developed during the process of clonal evolution of cancer cells; it exists in the absence of or the modification of drug target or the expression of mechanisms to escape that create drug resistance. Although the process is not fully understood, several *EGFR* mutations exhibit ex novo resistance to EGFR TKI, including L7475 and D761V in exon 19; T790M, V769M, and insertions in exon 20; and T854A and A871E in exon 21.[7] T790M ex novo (before exposure to TKI) are variable in frequency, according to various studies, but the presence of this mutation before the treatment does not preclude the use of first-generation TKIs. The role that third-generation TKIs will play in this scenario is still not clear, beyond their potent activity in T790M-acquired mutation after TKI resistance. A prognostic role for T790M ex novo mutation was described together with a predictive value for a therapeutic benefit with pemetrexed.[8] Jackman et al defined the criteria of acquired resistance for EGFR TKIs; they include prior monotherapy with EGFR TKIs in the presence of typical sensitizing mutations, or tumor progression within 30 days after achieving complete response, partial response (PR), or stable disease (≥6 months) to TKIs despite the uninterrupted treatment.[9] In the clinical setting, treatment with TKIs could be continued after evidence of resistance, thus obtaining a prolonged period of disease control in some cases.[10] Acquisition of T790M mutation is the most frequent mechanism of resistance; it occurs in about 50% to 60% of cases.[11] Among other mechanisms that lead to resistance to EGFR TKI, it is important to consider c-MET amplification and the less-frequent presence of *ALK* translocations.[7]

Finding the resistance mutation can be a challenge. First, we do not frequently perform biopsies in patients with lung cancer, and second, after several years of conducting small biopsies with endobrachial ultrasound, we are experiencing an increase in CT-guided core biopsies. We also have to consider costs and morbidity for the patients when we order tissue biopsies.

As an alternative, liquid biopsies are easier and cheaper, and results are reported more quickly. As such, there is a great interest among oncologists to further develop this field. Liquid biopsies represent a new technology, and we all need to feel comfortable learning its role in our practice, either by complementing or replacing tissue biopsies with liquid biopsies. Data from Lanman et al[12] using NGS in plasma versus tissue sequenced at 5 institutions in stage III-IV solid-tumor cancers showed that cell-free plasma DNA NGS sensitivity is 85% of that found in tissue NGS, likely because not all tumor DNA may be shed into circulation. However, the tissue DNA NGS sensitivity is 80.7% versus that of cell-free DNA (cfDNA) in plasma NGS, likely because cfDNA picks up intra- and inter-tumor heterogeneity that is missed by needle or forceps biopsies of tissue. Specificity was 99.6% versus 99.7%, and accuracy was 99.3% for both.

During the American Society of Clinical Oncology 2016 Annual Meeting, Wakelee et al[13] presented data comparing liquid biopsy results from blood and urine with tissue biopsy results; the patients had NSCLC with T790M treated with rociletinib. They reported that *EGFR* T790M sensitivity is 80.9% for blood and 81.1% for urine, with tissue biopsy as the reference. Notably, only 57% of the patients were positive by all 3 types of sampling: Some patients were detected only by urine, blood, or tissue. Another important finding was that the objective response rate (ORR) to rociletinib was similar in all 3 types of patients (32% to 36%).

Regarding the use of agents, among third-generation TKIs that are specific to T790M mutations, only osimertinib has received the approval by the US and European regulatory agencies.[14] Despite osimertinib's availability we still start therapy with the other TKIs, such as first-generation gefitinib or erlotinib, or second-generation afatinib, which has a dual EGFR/HER2 inhibition activity and it is approved for the treatment of lung tumors bearing the *EGFR* L858R mutation or exon 19 deletions.[15] Osimertinib is still not approved for frontline therapy.

The LUX-Lung 5 trial showed that afatinib plus paclitaxel improved progression-free survival (PFS) (HR, 0.60; $P = .003$) and ORR (32.1% vs 13.2%; $P = .005$) compared with chemotherapy in patients with acquired resistance to TKIs and who progressed on afatinib after initial benefit.[16] The specific activity against T790M mutation, however, is very limited. The LUX Lung-4 trial, which investigated patients who progressed while receiving the TKI afatinib, showed a confirmed ORR of 8.2%.[17]

Osimertinib is a third-generation irreversible TKI with selective activity against T790M mutation.[14] It was approved based on the results of 2 phase II trials (AURAex and AURA 2), achieving ORR of 66% among 411 patients with *EGFR* T790M; the median PFS was 11 months and the disease control rate was 91%.[18] A phase III trial (FLAURA) comparing osimertinib versus gefitinib or erlotinib in patients with advanced NSCLC showed promising activity in treatment-naïve patients with common *EGFR* mutations.[19] Rociletinib (CO-1686) irreversibly binds mutant *EGFR*. In a phase I trial,[20] rociletinib showed promising activity, with an ORR of 60% in T790M-positive patients versus 37% in the

T790M-negative group. However, the real-world ORR was less than described by authors (34% for the 625-mg arm and 28% for the 500-mg arm)[21] and the pharmaceutical company stopped its development.[22]

New drugs such as olmutinib (BI 1482694), AP26113, ASP8273, EGF816, and PF-06747775 are currently under development. Unfortunately, new mechanisms of resistance, such as the c797s mutation as seen in our patient, are also appearing. In a study by Oxnard et al, 15 out of 67 (22%) patients had detectable c797s mutations. These 15 patients had detectable T790M (T790-positive/c797s-positive). Additionally, this mutation was more common with *EGFR* exon 19 del (13/43; 30%) than those with L858R (2/24; 8%; $P = .06$).[23] Among the patients, 32 out of 67 (48%) had no detectable T790M in plasma despite presence of the original *EGFR* mutation, suggesting overgrowth of an alternate resistance mechanism, such as *MET* or HER2 amplification or *BRAF* V600E mutation. MET amplification is found in 4% of new lung cancer tumors and is present in 20% of patients with acquired *EGFR* mutations.[24]

The case report presented here illustrates a typical case of a patient with exon 19 *EGFR*-sensitive mutation, who, after months of experiencing benefit with TKIs, developed resistance to TKIs because of the development of an *EGFR* T790M mutation. It was a challenge to confirm the diagnosis because the tissue biopsy was negative, and the patient had to endure chemotherapy until a liquid biopsy found the *EGFR* T790M mutation; a second opinion from a tissue biopsy confirmed the mutation. Usually the patient would undergo another line of chemotherapy, without taking the extra step to get a second opinion or to order a liquid biopsy after negative tissue for T790M was reported. Repeat biopsy can reveal crucial information relevant to treatment decisions, but only, obviously, if it is performed. Fortunately, the use of liquid biopsy became an important tool in this case.[25]

It is worrisome that despite the fact that close to 60% of patients who fail TKI therapy have T790M, not all of these patients are currently being treated with osimertinib. This shortcoming might be due to not enough repeat biopsies being ordered, or due to the unavailability of liquid biopsy testing. Whatever the cause, there is much progress to be made in this regard for the benefit of our patients.

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