EGFR-Mutant Non–Small Cell Lung Cancer in the Era of Precision Medicine: Importance of Germline EGFR T790M Testing

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Abstract

With the rapid development of precision medicine, next-generation sequencing (NGS) has provided the ability to uncode tumors at the DNA level. Identifying *EGFR* mutations and other molecular changes has become more crucial in the management of non–small cell lung cancer (NSCLC) than ever before. Although the histologic subtypes in patients with advanced NSCLC remain valid in determining treatment options, the detection of specific molecular signatures such as de novo T790M with sensitizing *EGFR* mutations could be more useful than the histologic subtype itself. Germline T790M mutation should be suspected and tested for when multiple biopsies show de novo T790M mutations or when de novo T790M is found in patients with a family history of lung cancer. This case report presents a 60-year-old woman with bilateral NSCLC with 3 different distinct histologic diagnoses. Evaluating the molecular profile using NGS completely changed the diagnosis, prognosis, and management of this rare presentation of NSCLC.

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Precision medicine has rapidly been applied by oncologists who treat non–small cell lung cancer (NSCLC). In patients with newly diagnosed advanced-stage NSCLC, it is now considered standard of care to offer treatment regimens based on molecular status, but the implications of de novo T790M are not yet widely known. When multiple biopsies show T790M mutations, suspicion of and testing for germline T790M mutations are crucial in providing precise and tailored management for patients with NSCLC and their potentially affected family members.

Methods

Next-generation sequencing (NGS) using the FoundationOne test (Foundation Medicine, Inc., Cambridge, MA) was performed 3 times in the case presented. In this method, hybrid-capture–selected libraries are se-

quenced to high uniform depth (targeting >500x coverage by non-PCR duplicate read pairs, with >99% exons at coverage >100x) on the Illumina HiSeq2000 sequencing platform.¹

Genomic DNA isolation and *EGFR* T790M real-time PCR was performed at the Detroit Medical Center University Laboratories, Molecular Genetics Diagnostic Laboratory, a CLIA-licensed and College of American Pathologist–accredited laboratory. Genomic DNA was extracted from peripheral whole blood with the Quick-Gene DNA Whole Blood Kit S (Fujifilm, Tokyo, Japan) according to the manufacturer's recommendations. Ten nanograms of genomic DNA was amplified with the *EGFR* mutation analysis kit (EntroGen, Inc, Woodland Hills, CA) on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher Scien-

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tific, Waltham, MA) according to manufacturer's recommendations.

Case Presentation

A 60-year-old African American woman presented with abdominal pain. She underwent a CT scan, which revealed a right upper lobe mass and a left upper lobe lung mass (Figure 1). She had no history of smoking. Lung biopsy from the right upper lobe revealed adenocarcinoma, and the left lung biopsy revealed adenosquamous carcinoma. Tumor molecular profile was tested with NGS. The right lung sample was positive for *EGFR* exon 18 mutation (G719R) and T790M. The left lung sample showed *EGFR* exon 19 deletion (E746 del) and T790M. At diagnosis, she did not have any metastases outside the thoracic area and her brain MRI at baseline was also negative. She was considered to have stage IV disease based on bilateral lung masses and was initiated on afatinib at an outside institution. Her best response was stable disease, and she continued afatinib for 12 months. She then experienced disease progression with a new left adrenal metastasis (Figure 2). Adrenal gland biopsy confirmed the diagnosis of metastatic NSCLC with squamous histology. Brain MRI was again negative for metastatic disease. She was offered palliative chemotherapy with carboplatin and paclitaxel. She then presented to our institution.

Given the deferring histology, we obtained NGS on the adrenal gland biopsy, which reported similar molecular signature with *EGFR* exon 19 deletion (E746 del) and T790M. She was started on osimertinib and achieved a partial response within 2 months

of starting this new third-generation *EGFR* tyrosine kinase inhibitor (TKI). Because NGS showed multiple lesions with T790M, the possibility of a germline mutation was suspected. Further questioning revealed that her sister had a history of NSCLC in her 50s and had died of the disease several years prior. A peripheral whole blood sample from our patient confirmed the presence of a germline mutation T790M. She was referred to the genetics clinic for further counseling and testing for hereditary lung cancer syndrome. Her 3 children were also referred for genetic counseling.

Discussion

Historically, Histology Mattered

Lung cancer is the most common cancer worldwide. In 2012, there were 1.8 million new cases, with 1.6 million deaths worldwide.² In the United States, adenocarcinoma is the most common type of NSCLC, and squamous cell carcinoma (SCC) is the second most frequent histologic subtype. Histologic diagnosis of adenocarcinoma requires evidence of either neoplastic gland formation, pneumocyte marker expression (TTF-1, napsin), or intracytoplasmic mucin, whereas the diagnosis of SCC is based on the presence of keratin production by tumor cells and/or intercellular desmosomes or immunohistochemistry consistent with SCC (eg, expression of p40, p63, CK5, or CK5/6, desmoglein).³

Distinguishing SCC from other NSCLCs, particularly adenocarcinoma, has become increasingly important for patients with advanced-stage disease,⁴

Figure 1. Left upper lung mass (A) before osimertinib (3.5 x 3.0 cm) and (B) 17 weeks posttreatment (1.3 x 1.4 cm).

Figure 2. Left adrenal mass (A) before osimertinib (4.7 x 3.2 cm) and (B) 17 weeks posttreatment (3.1 x 2.1 cm).

because certain agents are contraindicated for patients with squamous histology due to decreased efficacy (ie, pemetrexed)⁵ or the potential for increased toxicity (ie, bevacizumab).⁶

EGFR and the Era of Molecular Medicine

Over the past decade, there have been tremendous advancements in the management of advanced NSCLC, particularly in molecularly targeted therapy.

In the IPASS trial, which randomized 1,217 patients to either gefitinib or carboplatin plus paclitaxel, progression-free survival was significantly prolonged with gefitinib (median, 9.5 vs 6.3 months; hazard ratio [HR] for progression, 0.48) in patients whose tumors contained an *EGFR* mutation, but was significantly shorter (median, 1.5 vs 6.5 months; HR for progression, 2.85; 95% CI, 2.05–3.95) in patients without this mutation.⁷ Similar results were also demonstrated in a meta-analysis that included data from 13 phase III trials with 2,620 patients (1,475 *EGFR* mutation–positive and 1,145 mutation–negative),⁸ and carrying the *EGFR* mutation became known to be the most critical prognostic marker and indication for the use of *EGFR* inhibitors.

These studies led to the approval of erlotinib in advanced NSCLC with activating *EGFR* mutations. For patients with advanced NSCLC with tumors harboring the activating *EGFR* mutations (exon 19 deletions, L858R point mutation in exon 21), treatment with *EGFR* TKIs such as erlotinib, gefitinib, or afatinib is indicated for initial management.

A secondary mutation in *EGFR*, most commonly the T790M (mutation involves the substitution of methionine for threonine at position 790) has been associated with acquired resistance to *EGFR* TKIs, and is known to occur in approximately 50% of patients who develop resistance to *EGFR* TKIs. Osimertinib was developed to specifically target T790M.^{9–11}

What About De Novo T790M?

De novo *EGFR* T790M mutation, or T790M mutation detected before TKI treatment, occurs in <1% of all lung cancers and approximately 2% of all *EGFR*-mutant lung cancers.¹² Germline *EGFR* T790M mutations are found in approximately 50% of all patients with de novo *EGFR* T790M.¹³ This carries a significant implication, especially with regard to screening family members. Oxnard et al¹⁴ recommend that all patients with baseline *EGFR* T790M identified in their pretreatment tumor tissue be referred to clinical genetics to discuss *EGFR* T790M germline testing.

Gazdar et al¹⁵ studied a family with germline T790M mutations over 5 generations and combined their observations with data obtained from a literature search. Based on their analysis, T790M germline mutations occurred in approximately 1% of NSCLC cases. Both sporadic and germline T790M mutations were predominantly adenocarcinomas. Inheritance was dominant, and mutations favored female sex and were occasionally multifocal. Of lung cancer tumors arising in T790M germline mutation carriers, 73%

had a concomitant activating *EGFR* gene mutation. These are all findings consistent with those from our case presentation. Of importance, Gazdar et al¹⁵ estimated a 31% risk for lung cancer in T790M germline mutation carriers who were never-smokers.

Although we have started to identify the association between germline *EGFR* T790M with the development of lung adenocarcinoma, management, such as how to screen for the development of lung cancer, how tumors respond to TKI therapy, and whether to even consider chemoprevention with TKIs, remains an area of further research.⁸ Based on a retrospective study by Yu et al,¹⁶ patients with tumors harboring de novo T790M mutation seem to have a poor response to erlotinib monotherapy, with a response rate of only 8%. Response to osimertinib in patients with de novo T790M is an area of further investigation. The primary objective of the ongoing INHERIT trial is to determine the prevalence of germline *EGFR* mutations in patients with lung cancer with *EGFR* T790M mutations and in relatives of germline *EGFR* mutation carriers, and to better characterize this rare familial lung cancer syndrome (ClinicalTrials.gov identifier: NCT01754025).¹⁷

The Role of NGS in Precision Medicine

NGS has given us the ability to obtain a more comprehensive and precise picture of the tumor at the genetic level and is often both time-saving and cost-effective. Current NGS technology was developed using traditional Sanger sequencing and its variants. Typically, DNA or RNA is fragmented into smaller pieces. From the fragments (or microRNAs), libraries are constructed and sequenced at a high coverage. The sequenced reads are aligned to a reference genome and the results are analyzed statistically and interpreted.^{1,18}

Regardless of the technique used, determination of tumor molecular status has now become the standard of care in NSCLC. It is recommended that all advanced metastatic NSCLC be evaluated for *EGFR*-activating mutations, *ALK* translocation, and *ROS1* translocations, because targeted therapies are available for these molecular changes.^{19,20} As of October 2016, the FDA approved use of first-line pembrolizumab in patients with high PD-L1 expression of tumor proportion score $\geq 50\%$. Currently, checking for tumor PD-L1 is also considered the standard of care in advanced metastatic NSCLC.¹⁵

Treating the Patient-Molecular Status Versus Histology

Although some mutations, such as *EGFR* and *KRAS*, are more common in adenocarcinoma, alterations in the *FGFR* kinase family are more common in squamous cell histology.²¹ These molecular changes are known to occur regardless of histology and are often associated with therapeutic predictive values.^{22,23} For example, the Lung Cancer Mutation Consortium reported that a driver alteration was detected in 63% of fully genotyped cases of lung adenocarcinoma. The drivers found in this study were as follows: *KRAS*, 182 (25%); sensitizing *EGFR*, 107 (15%); *ALK* rearrangements, 56 (8%); other *EGFR*, 43 (6%); 2 genes, 29 (4%); *BRAF*, 16 (2%); *HER2*, 15 (2%); *PIK3CA*, 6 (1%); *MET* amplification, 5 (1%); *NRAS*, 5 (1%); *MEK1*, 1 ($<1\%$); and *AKT1*, 0 (0%). Among 938 patients with treatment information, 264 with a driver alteration treated with a targeted agent had a median survival of 3.5 years, 313 with a driver who did not receive targeted therapy had a median survival of 2.4 years, whereas 361 without an identified driver had a median survival of 2.1 years ($P<.0001$).²⁴

Molecular changes may have more than treatment implications. NGS can simultaneously detect multiple gene variants, which may at times deliver surprising results that could potentially alter patient management. For the present patient, all 3 biopsies had concomitant T790M mutations, leading to the suspicion of germline T790M. Indeed, the T790M mutation was detected in the patient's whole blood, consistent with hereditary lung cancer syndrome.¹⁰ She and her family members were referred to the genetics clinic for further counseling and testing.

Conclusions

Our case describes a never-smoker female patient who had sensitizing *EGFR* mutations and de novo T790M mutations detected on biopsy specimens. She had 3 different histologic subtypes. This led to the identification of a rare germline T790M mutation known to be associated with familial lung cancer syndrome, highlighting the importance of testing for germline *EGFR* T790M testing. The detection of specific molecular signatures such as these could be more useful than the histologic subtype itself.

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