CE Case Report

A Patient With Metastatic Lung Adenocarcinoma Harboring Concurrent *EGFR* L858R, *EGFR* Germline T790M, and *PIK3CA* Mutations: The Challenge of Interpreting Results of Comprehensive Mutational Testing in Lung Cancer

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Abstract

Mutational testing has moved to the forefront as an integral component in the management of patients with non–small cell lung cancer (NSCLC). Currently 3 targeted therapies (erlotinib, afatinib, and crizotinib) are approved by the FDA to treat patients with specific genetic abnormalities in NSCLC. As mutational screening expands to include a greater number of genes, the results will become more difficult to interpret, particularly if mutations are found in multiple genes or genes that are not actionable at the time of testing. This case report summarizes the diagnosis and treatment of a patient with NSCLC that harbored multiple potentially targetable driver mutations. It also discusses the current NCCN Clinical Practice Guidelines in Oncology for mutational testing in NSCLC and the inherent difficulties with interpreting mutational results when multiple mutations are found in a single gene or across multiple genes. (*J Natl Compr Canc Netw* 2014;12:6–11)

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Summarize the diagnosis and treatment of a patient with NSCLC that harbored multiple potentially targetable driver mutations
- Explain the role of multiplexed gene sequencing platforms and next-generation sequencing testing in tailoring effective treatments to individual patients
- Discuss examples showing that it is difficult to plan effective treatment for a patient with multiple potentially targetable driver mutations

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Case Report

A 67-year-old Caucasian man with no smoking history experienced the onset of a nonproductive cough in early 2011. After an episode of small-volume hemoptysis, he presented to his primary care provider for assessment. A chest radiograph showed a large right lung mass with several smaller left-sided lung nodules. A follow-up CT scan showed a 5.6-cm mass in the right lower lobe, enlarged right hilar and mediastinal lymphadenopathy, and 3 left-sided lung nodules between 1.0 and 1.5 cm. A brain MRI showed no evidence of disease. A CT-guided biopsy was performed on the right lung mass and a left lung nodule, and pathology revealed a moderately differentiated adenocarcinoma with an acinar and lepidic growth pattern. The malignant cells stained positively with CK7 and TTF-1, suggesting a pulmonary origin. Stage IV (T2b-NxM1a) adenocarcinoma of the lung was diagnosed.

Standard molecular testing of exons 18–21 of the epidermal growth factor receptor (*EGFR)* gene performed at an outside institution revealed the presence of both an *EGFR* L858R missense mutation in exon 21 and a T790M point mutation in exon 20. His local oncologist was surprised to find the T790M mutation in this treatment-naïve patient, because the T790M mutation has been shown to be one mechanism through which lung cancers develop resistance to *EGFR* tyrosine kinase inhibitor (TKI) therapy.[1,2] The oncologist hypothesized that the clone expressing the T790M mutation might be low prevalence given the patient's lack of previous therapy, and therefore decided that the patient might yet derive benefit from erlotinib therapy.

The patient was started on erlotinib, 150 mg, with a plan to perform early imaging to gauge response. Shortly after the initiation of therapy, the patient noted improvement in his presenting symptoms. The first interval CT scan after 1 month showed stable disease; however, a repeat CT scan after 4 months of erlotinib therapy showed disease progression, with the right-sided lung mass now measuring 7.0 cm, along with enlarging mediastinal and hilar lymphadenopathy.

The patient continued to take erlotinib, 150 mg daily, and was referred to the authors' institution to discuss additional therapeutic options. Concomitant with the initial consultation, he underwent repeat biopsy and additional tumor molecular profiling. At this institution, standard clinical genotyping is performed using the SNaPshot Multiplex System assay. This assay is a PCR-based platform that can be performed using material from formalin-fixed, paraffin-embedded tumor samples. The current lung SNaPshot panel at this institution was designed to test for 38 somatic mutations in 8 genes (*AKT1*, *BRAF*, *EGFR*, *KRAS*, *MEK1*, *NRAS*, *PIK3CA*, and *PTEN*). In parallel, a PCR-based sizing assay assesses for *EGFR* exon 19 deletions, *EGFR* exon 20 insertions, and *HER2* exon 20 insertions.[3] *ALK* testing is performed separately with fluorescence in situ hybridization, the FDA-approved companion diagnostic for crizotinib therapy.

Results from the SNaPshot assay revealed not only the previously known *EGFR* L858R and T790M mutations, but also a *PIK3CA* H1047L mutation. *PIK3CA* mutations have been described as mechanisms of acquired resistance to first-generation *EGFR* TKI therapy.[4] The presence of the T790M mutation before *EGFR* TKI therapy, together with the allele frequency of the T790M mutation from the SNaPshot results (Figure 1), caused the authors to consider the possibility of a germline *EGFR* mutation.

Interestingly, further questioning revealed that the patient's mother died of lung cancer at 50 years of age. She had smoked 1 pack of cigarettes per day for 15 years, but quit smoking 10 years before her diagnosis. Furthermore, the patient's maternal grandfather died of multiple myeloma that was diagnosed at 67 years of age. No other family history of cancer was revealed. The patient is married and has 2 adult children and 3 young grandchildren. After consultation with genetic counselors, the patient decided not to undergo additional testing from peripheral blood to confirm the presence of the possible germline *EGFR* T790M mutation, nor did his children request further genetic testing.

The patient subsequently enrolled in a phase I/II clinical trial evaluating the combination of afatinib, 40 mg daily, and cetuximab, 500 mg/m^2, every 2 weeks in patients with *EGFR*-mutant lung cancer with acquired resistance to *EGFR* TKI therapy. The patient tolerated therapy reasonably well; he developed a rash requiring doxycycline therapy, and required a dose reduction of cetuximab and afatinib 3 weeks after starting therapy. CT scans showed stable disease after the first and second month, but unfortunately he experienced disease progression after 3 months of therapy.

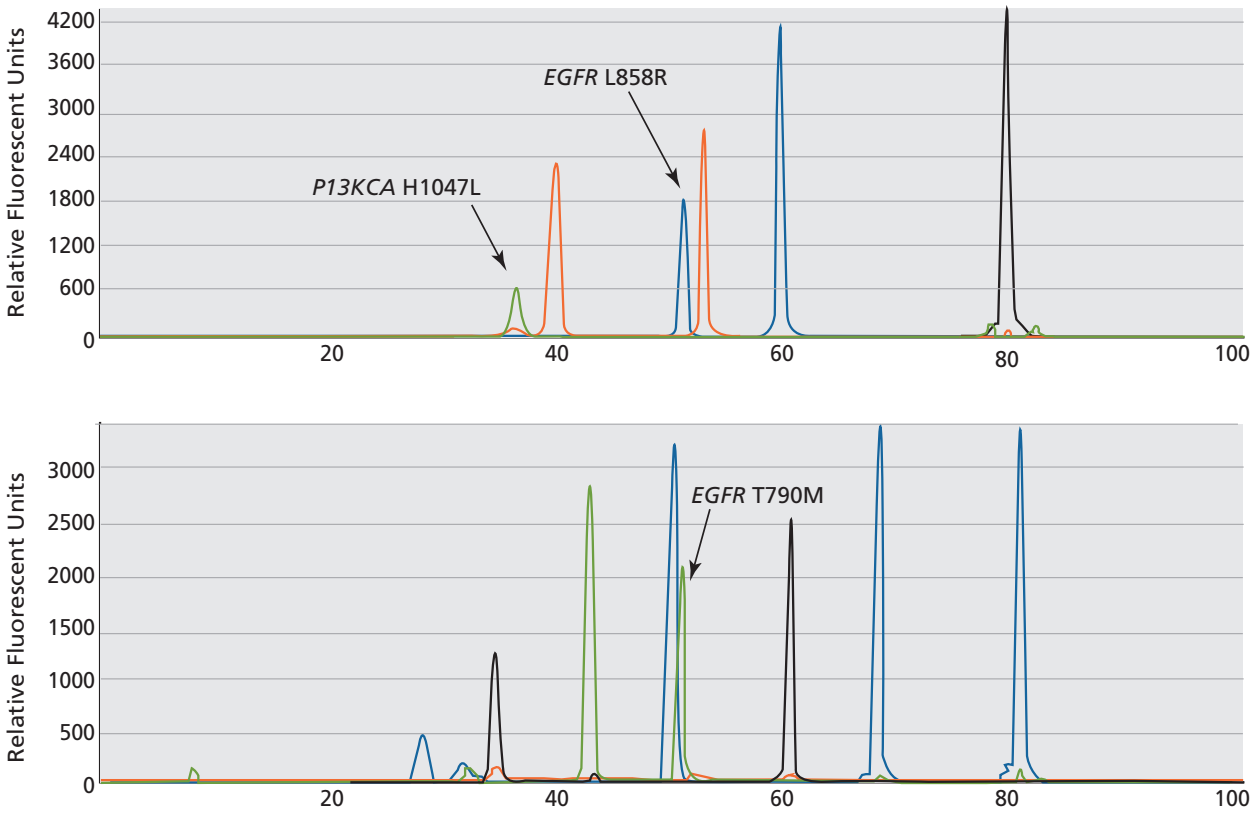

Figure 1 SNaPshot testing of right upper lobe nodule biopsy after 3 months of erlotinib therapy. Multiplexed panel detecting mutational status of representative gene loci. Solid arrows indicate mutant peaks at *EGFR* and *PI3KCA* gene loci.

He was then treated with carboplatin (area under the curve [AUC] 6), pemetrexed (500 mg/m^2), and bevacizumab (15 mg/kg) every 3 weeks for 4 cycles, with stable disease as best radiographic response. He tolerated chemotherapy well and went on to receive maintenance therapy with pemetrexed (500 mg/m^2) and bevacizumab (15 mg/kg) every 3 weeks. He completed 6 cycles of this maintenance regimen but then experienced disease progression, with the development of several new right-sided lung nodules.

Soon thereafter he began therapy on a phase I clinical trial of an oral PI3K inhibitor. His clinical course was unremarkable except for the development of hyperglycemia (an on-target side effect) that was well controlled with oral hypoglycemic agents. A repeat PET scan after 1 month showed a dramatic decrease in FDG avidity of most of his lesions. At 2 months he had stable disease by CT scan; but after 3 months of therapy, he became increasingly dyspneic, and workup showed a new, large, malignant right pleural effusion.

The patient had an indwelling tunneled pleural catheter placed to manage the effusion and was started on docetaxel (75 mg/m^2). He has had stable disease after 3 cycles of therapy and continues to be active with some mild dyspnea on exertion and cough. His current ECOG performance status is 1.

Discussion

This case illustrates the potential complexity involved in the interpretation of mutational testing results in patients with lung cancer. The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines)[5] for Non–Small Cell Lung Cancer (NSCLC) and the NCCN Biomarkers Compendium[6] currently recommend testing for *EGFR* mutations and *ALK* gene rearrangements at diagnosis in nonsquamous NSCLC to guide therapy in the metastatic setting (to view the most recent versions of these guidelines and the compendium, visit NCCN.org). In addition, testing for *ROS1* gene rearrangements can also be considered given reports of crizotinib ef-

ficacy in this cohort of patients with lung cancer.[7,8] The NCCN Guidelines do not specifically recommend other routine mutational testing.[5] However, larger mutational testing platforms can provide information on additional potentially targetable driver mutations, such as *BRAF*, *RET*, and *HER2*, each of which occurs at low frequency in NSCLC and for which there are ongoing clinical trials. Early reports suggest that targeting these mutations may prove fruitful in NSCLC. Dabrafenib, a *BRAF* inhibitor, showed activity in 17 patients with *BRAF* V600E mutations,[9] and case reports have documented responses of *BRAF*-mutant lung cancer to the *BRAF* inhibitor vemurafenib.[10] In addition, afatinib, which inhibits both *HER2* and *EGFR*, has shown efficacy in patients with *HER2*-mutant lung cancer,[11] and responses have been documented to the multitargeted TKI cabozantinib in patients with *RET*-mutant lung cancer.[12] Consequently, expanding mutational testing beyond *EGFR* and *ALK* may enable identification of patients eligible for mutation-specific early-stage clinical trials that are available at many NCCN Member Institutions.

Multiplexed gene sequencing platforms and next-generation sequencing testing expand on the number of genetic mutations screened and, as a result, increase the amount of subsequent data available to direct therapy compared with conventional single gene mutation testing. The authors' institution was one of the first cancer centers in the United States to institute standard clinical genotyping of all lung cancer. The current platform for molecular testing involves the SNaPshot assay, a multiplexed PCR-based assay that simultaneously detects mutations in 9 genes that are relevant to existing or emerging targeted therapeutics. Newer next-generation sequencing–based clinical diagnostic platforms offer mutational analysis of hundreds of cancer-related genes,[12,13] and other private companies are expected to bring similar testing platforms to the market in the coming years. Deciphering the data from these panels will be paramount to tailor effective treatments to individual patients. With the increased use of these tests, a greater number of patients will be found to have at least one mutation, a single patient will be more likely to have multiple mutations in the same specimen, and the identified mutations may be present within the same gene. One example of the effect of next-generation sequencing is demonstrated from the current knowledge of *EGFR* gene mutations. For some time, *EGFR* T790M mutations were thought to be present only at the time of acquired resistance to *EGFR* TKI therapy; however, using deep sequencing techniques, several series report the rate of *EGFR* T790M mutations in pretreatment specimens concurrent with *EGFR* TKI sensitizing mutations to be as high as 35% to 40%.[14,15] The more widespread use of highly sensitive mutation testing platforms are therefore likely to identify a greater number of patients that will not benefit from *EGFR* TKI therapy.

When a patient's tumor is found to have more than one mutation in one gene or across several genes, effective treatment planning can be difficult. In most cases, it is also not clear whether the concurrent mutations are found in the same cells or in separate clones of tumor cells. In addition, no method exists to definitively distinguish between driver and passenger mutations.

One resource that provides clinicians and patients with up-to-date information on distinct mutations in several cancer subtypes is Mycancergenome.org.[16] This Web site is freely available to practitioners and patients across the globe and is continuously updated by experts in the field. Users can learn which therapies may be effective against specific mutations and can find clinical trials that are available at institutions worldwide. In addition, a comprehensive compiled list of targeted therapies in various stages of clinical development is available.

In the present case described, testing showed mutations in both the *EGFR* and *PIK3CA* genes, which has been previously reported.[17,18] The *PIK3CA* mutation was not tested at diagnosis, and was found only after initial erlotinib therapy. Therefore, it is not clear whether this mutation was a possible mechanism of resistance to erlotinib, as has been reported,[4] or if it was present at the time of diagnosis. Although *PIK3CA* mutations have been shown to confer resistance to *EGFR* TKI therapy in a small series of patients without *EGFR* mutations,[18] the response to erlotinib was varied in 3 patients with both *PIK3CA* mutations and *EGFR*-sensitizing mutations.[19] In the present case, the presence of the *PIK3CA* mutation may not have been the cause for the lack response to erlotinib given the initial presence of the *EGFR* T790M mutation.

The authors had hoped that the combination of afatinib and cetuximab would overcome the lack of

response to the initial *EGFR* TKI, as seen in preliminary results of a clinical trial.[20] Unfortunately, the patient did not derive benefit from this treatment regimen. Likewise, his best response with a *PI3K* inhibitor in a phase I clinical trial was stable disease. The lack of response to targeted therapies despite the presence of activating somatic mutations highlights the difficulties in using results of mutational testing to choose patients appropriately for mutation-specific therapies.

Interestingly, the patient's testing showed the likely presence of a germline *EGFR* T790M mutation, which has been previously reported.[21–23] With the expansion of mutational screening to a wider audience and the high sensitivity of the testing, a larger number of patients will be found to have possible germline or inherited mutations. Importantly, these results will have the potential to influence the care of not only the patient but also the patient's family members. Until sufficient knowledge is gained regarding the prognostic and predictive values of germline mutations in lung cancer, physicians will struggle to counsel patients and their families regarding the risk for developing cancer and/or the efficacy of screening or prevention programs to mitigate their particular risk.

Conclusions

Although *EGFR* mutations have been reported to be present with concomitant *PIK3CA* mutations, this is the first reported case to the authors' knowledge of a patient with both a possible germline *EGFR* T790M mutation and a somatic *PIK3CA* mutation. As a consequence of mutational screening with a multiplexed platform, the patient has participated in 2 separate clinical trials using targeted therapies, and is alive with a good quality of life 23 months after the diagnosis of stage IV NSCLC.

The treatment of NSCLC in 2013 requires knowledge of somatic mutations in the individual patient. Multiplexed next-generation sequencing assays are increasingly being used to evaluate for therapeutic options and to screen patients for mutation-directed clinical trials. Over the coming years, it will be critical to develop the knowledge to decipher the results of these tests to deliver effective care to patients.

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Posttest Questions

1. Known mechanisms of acquired resistance of *EGFR*-mutated lung cancer to *EGFR* TKI therapy include all of the following except:
   a. Small cell histologic transformation
   b. PIK3CA mutation
   c. EGFR T790M mutation
   d. KRAS mutation
   e. MET amplification
2. *EGFR* mutations can be inherited germline mutations.
   a. True
   b. False
3. Larger mutational testing platforms can provide information on additional potentially targetable driver mutations including:
   a. HER2
   b. BRAF
   c. RET
   d. All of the above