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

Philip E. Lammers, MD; Christine M. Lovly, MD; and Leora Horn, MD, MSc

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)

NCCN: Continuing Education

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

Communication of Financial Relationships

Kerrin M. Green, MA, Assistant Managing Editor, JNCCN—Journal of the National Comprehensive Cancer Network

Ms. Green has disclosed that she has no relevant financial relationships.

Deborah J. Moonan, RN, BSN, Manager, CE Supporter Outreach

Ms. Moonan has disclosed the following relationship with commercial interests: AstraZeneca: Stockholder/Former Employee.

Kristina M. Gregory, RN, MSN, OCN, Vice President, Clinical Information Operations

Ms. Gregory has disclosed that she has no relevant financial relationships.
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 (T2bN2M1a) 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. 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. 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. 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 germ-line 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/m², 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.
He was then treated with carboplatin (area under the curve [AUC] 6), pemetrexed (500 mg/m²), 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/m²) 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/m²). 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) for Non–Small Cell Lung Cancer (NSCLC) and the NCCN Biomarkers Compendium currently recommend testing for EGFR mutations and ALK gene rearrangements at diagnosis in non–squamous 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-
Comprehensive Mutational Testing in Lung Cancer

The NCCN Guidelines do not specifically recommend other routine mutational testing. 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, and case reports have documented responses of BRAF-mutant lung cancer to the BRAF inhibitor vemurafenib. In addition, afatinib, which inhibits both HER2 and EGFR, has shown efficacy in patients with HER2-mutant lung cancer, and responses have been documented to the multitargeted TKI cabozantinib in patients with RET-mutant lung cancer. 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, 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%. 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. 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. 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, 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, the response to erlotinib was varied in 3 patients with both PIK3CA mutations and EGFR-sensitizing mutations.

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.\textsuperscript{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.\textsuperscript{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 diagnostic results of these tests to deliver effective care to patients.

References

Comprehensive Mutational Testing in Lung Cancer


Instructions for Completion

To participate in this journal CE activity: 1) review the learning objectives and author disclosures; 2) study the education content; 3) take the posttest with a 66% minimum passing score and complete the evaluation at http://education.nccn.org/node/38071; and 4) view/print certificate. After reading the article, you should be able to answer the following multiple-choice questions. Credit cannot be obtained for tests completed on paper. You must be a registered user on NCCN.org. If you are not registered on NCCN.org, click on “New Member? Sign up here” link on the left hand side of the Web site to register. Only one answer is correct for each question. Once you successfully answer all posttest questions you will be able to view and/or print your certificate. Software requirements: Internet.

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