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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 there are three targeted therapies (erlotinib, afatinib, and crizotinib) approved by the Food and Drug Administration (FDA) to treat patients with specific genetic abnormalities in NSCLC. As mutational screening expands to include a greater number of genes, it will become more difficult to interpret the results, particularly if mutations are found in multiple genes or in 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. We also discuss the current NCCN guidelines for mutational testing in NSCLC and the inherent difficulties with the interpretation of mutational results when multiple mutations are found in a single gene or across multiple genes.

Keywords

EGFR; *PIK3CA*; non-small cell lung cancer; germline; targeted therapeutics; tyrosine kinase inhibitors; acquired resistance; personalized medicine

Case Report

A 67 year-old Caucasian male 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 x-ray 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 three left sided lung nodules between 1.0 cm and 1.5 cm in size. A brain MRI showed no evidence of disease. A CT-guided biopsy of both the right lung mass and a left lung nodule was performed, and pathology revealed a moderately-differentiated adenocarcinoma with an

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acinar and lepidic growth pattern. The malignant cells stained positively with CK7 and TTF-1 suggesting pulmonary origin. A diagnosis of stage IV (T2bNxM1a) adenocarcinoma of the lung was made.

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 as the T790M mutation has been shown to be one mechanism by 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 patient might yet derive a benefit from erlotinib therapy. Therefore, 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 one month showed stable disease, however a repeat CT scan after 4 months of erlotinib therapy showed progression of disease with the right-sided lung mass now measuring 7.0 cm in size along with enlarging mediastinal and hilar lymphadenopathy.

The patient continued to take erlotinib 150 mg daily and was referred to our institution for discussions on additional therapeutic options. Concomitant with his initial consultation, he underwent repeat biopsy and additional tumor molecular profiling. At our institution, standard clinical genotyping is performed using the SNaPshot 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 was designed to test for 38 somatic mutations in 8 genes (*AKT1*, *BRAF*, *EGFR*, *KRAS*, *MEK1*, *NRAS*, *PIK3CA*, and *PTEN*). In parallel, there is a PCR-based sizing assay which 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 prior to *EGFR* TKI therapy together with the allele frequency of the T790M mutation from the SNaPshot results (Figure 1) made us consider the possibility of a germline *EGFR* mutation.

Interestingly, on further questioning, the patient's mother died at age 50 of lung cancer. She had smoked 1 pack of cigarettes per day for 15 years, but quit smoking 10 years before her diagnosis. The patient's maternal grandfather died of multiple myeloma that was diagnosed at age 67, but there was no other family history of cancer. The patient is married and has two adult children and three 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/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 had 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 had progression of disease after 3 months of therapy.

He was then treated with carboplatin (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 had 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 one month showed a dramatic decrease in FDG avidity of most of his lesions and he had stable disease by CT scan at 2 months, 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 Guidelines^{®5} and the NCCN Biomarker Compendium^{®6} currently recommend testing for *EGFR* mutations and *ALK* gene rearrangements at diagnosis in non-squamous NSCLC to guide therapy in the metastatic setting. In addition, testing for *ROS1* gene rearrangements can also be considered given reports of crizotinib efficacy in this cohort of lung cancer patients.^{7,8} 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 mutations⁹ 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 cancer¹¹ and there have been responses to the multitargeted TKI cabozantinib in patients with *RET* mutant lung cancer.¹² Consequently, by expanding

mutational testing beyond *EGFR* and *ALK*, we may be able to identify patients for mutation-specific early stage clinical trials that are available at many NCCN 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 to conventional single gene mutation testing. Our institution was one of the first cancer centers in the United States to institute standard clinical genotyping of all lung cancer. As discussed above, our current platform for molecular testing involves the SNaPshot assay, a multiplexed PCR based assay which simultaneously detects mutations in 9 genes that are relevant to existing or emerging targeted therapeutics. Newer next generation sequencing based clinical diagnostic platforms, such as the FoundationOne™ test from Foundation Medicine® offers 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 in order 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 our knowledge of *EGFR* gene mutations. For some time, *EGFR* T790M mutations were thought to be present only at 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 35–40%.^{14,15} The more widespread use of highly sensitive mutation testing platforms are thus 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 if the concurrent mutations are found in the same cells or in separate clones of tumor cells. In addition, there is no method to definitively distinguish between 'driver' mutations and 'passenger' mutations'.

One resource to provide clinicians and patients with up to date information on distinct mutations in a number of cancer subtypes is Mycancergenome.org.¹⁶ This website 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, there is a comprehensive compiled list of targeted therapies in various stages of clinical development.

In the case described above, our testing showed mutations in both the *EGFR* gene and the *PIK3CA* gene, 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 if this mutation was a possible mechanism of resistance to erlotinib, as has been reported⁴, or if it was present at the time of diagnosis. While *PIK3CA* mutations have been shown to confer resistance to *EGFR* TKI in a small series of patients without *EGFR*

mutations¹⁸, the response to erlotinib was varied in 3 patients with both *PIK3CA* mutations and *EGFR* sensitizing mutations.¹⁹ In our 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.

We had hoped that the combination of afatinib + 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 highlight 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 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 the development of 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 our 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 two 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 that we develop the knowledge to decipher the results of these tests to deliver effective care to our patients.

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Figure 1.
SNaPshot testing of biopsy of right upper lobe nodule 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.