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How and when to use genetic markers for non-small cell lung cancer

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Abstract

Purpose of review—Many driver mutations that determine the malignant behavior of lung cancer have been identified in recent years. The promise of therapies targeted to the specific molecular pathways altered by such mutations has made genetic testing in non-small cell lung cancer (NSCLC) attractive to clinicians. We review recent research on clinically relevant genetic and molecular tests for patients with NSCLC, with an emphasis on the tests linked to actionable mutations that influence therapy and improve outcomes.

Recent findings—Mutations in the epidermal growth factor receptor (EGFR) and translocations involving the anaplastic lymphoma kinase (ALK) gene have been shown to be common driver mutations in lung adenocarcinoma. The presence or absence of these mutations has been demonstrated to predict response to targeted therapy in many recent studies. Targeted therapies for patients with mutations in the EGFR domain or the EML4-ALK translocation have been shown to be effective and are approved for use. Ongoing studies continue to define the extent of their utility and may continue to expand their indications. Sufficient tissue for genetic analysis can be obtained from cytologic samples, including those obtained from endobronchial ultrasound-guided transbronchial needle aspiration.

Summary—Genetic testing for driver mutations is useful in identifying patients with NSCLC who are likely to respond to targeted therapy. These tests are best used in patients with adenocarcinoma who have advanced-stage cancer.

Keywords

Lung cancer; genetic test; targeted therapy; epidermal growth factor receptor; anaplastic lymphoma kinase

Introduction

Lung cancer is responsible for more deaths than any other form of cancer in the United States; more than 150,000 people died from lung cancer in 2008 alone. Non-small cell lung cancer (NSCLC) is much more common than small cell lung cancer (1). Historically, all types of NSCLC were treated in a similar manner, determined primarily by clinicopathologic stage, and patients with advanced NSCLC received cytotoxic chemotherapy as the mainstay of treatment (2). As recent research has improved knowledge of the molecular pathways that determine the behavior of cancer cells, it has become clear that NSCLC is a biologically heterogeneous group of cancers driven by differing molecular

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pathways. As the mutations causing the oncogenic alterations to these pathways have been discovered and genetic tests for them have become available, researchers have been developing treatments that target these aberrant pathways in the hope that such targeted treatments will provide better outcomes with fewer adverse effects than traditional cytotoxic chemotherapy (3)*.

In this review, we will first discuss the characteristics of a useful genetic marker in patients with NSCLC. We will then review the genetic tests that have been linked to driver mutations in NSCLC for which targeted treatment is available. Finally, we will briefly discuss the approved targeted treatments for NSCLC that harbors these mutations. A full discussion of the many other mutations that have been found to play a role in malignant behavior in NSCLC for which effective targeted treatments have not yet been discovered or approved will not be provided in this review.

Characteristics of a Useful Genetic Marker

Not all mutations harbored in cancer cells make useful genetic markers. Useful genetic markers are those that indicate driver mutations, can be identified accurately using genetic testing, are common in the population of interest, and point to an oncogenic pathway for which effective targeted therapy exists

A genetic marker is useful only if it is associated with an oncogenic pathway whose disruption would result in inhibited cellular growth and survival. Most of the genetic mutations found in cancer cells do not contribute to the development of cancer. These “passenger mutations” occur incidentally, are not of themselves responsible for malignant transformation of the cell, and are not important for maintaining the malignant phenotype (4). Although passenger mutations may often be related to pathways important to the growth and survival of the cell, they are typically found downstream from other genes that stimulate multiple pathways promoting growth and survival. Mutations in these genes, or “driver mutations,” initiate the transformation of a benign cell to a malignant cell. Driver mutations are not found in the germline genome, and the transformed cell comes to rely on the driver mutation's signaling for survival, a process known as oncogene addiction (4-6). For this reason, driver mutations are often good candidates for targeted therapy. Disrupting the signaling of driver mutations can have far-reaching effects on multiple metabolic pathways within the cancer cell. Disrupting the pathway regulated by a single passenger mutation is generally less useful because driver mutations continue to stimulate multiple additional pathways promoting growth and survival (4, 6).

In addition to identifying a driver mutation, a useful genetic marker should have an accurate test available that identifies the presence of the mutation of interest. Various methods are used to test samples of cancers for mutations, including polymerase chain reaction, immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH). However, not every type of genetic test predicts tumor response to targeted therapy for each specific mutation, and the correct test must be chosen to properly identify the sought-after mutation. For example, the 2 most commonly tested markers in NSCLC are those for mutations in the epidermal growth factor receptor (EGFR) and for the echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK) translocation. Mutational analysis is currently the preferred test for EGFR mutations, whereas the EML4-ALK translocation is best identified via FISH (7, 8)*.

Many types of histologic and cytologic samples have been used to test for EGFR mutations and the EML4-ALK translocation. Histologic specimens obtained via surgery or core needle biopsy have been historically preferred because of the large amount of tissue provided, but biopsies of this size are difficult to obtain in some cases (9)*. Nakajima et al first reported

the use of cytologic specimens obtained during endobronchial ultrasound (EBUS) for EGFR mutation testing, and several other groups have confirmed the utility of cytologic specimens for this purpose (10-12). Billah et al reported only a 4% rate of insufficiency for EGFR mutation testing of tissue obtained via EBUS (9)*. A more recent large multicenter study of 774 cytologic specimens obtained via EBUS reported that EGFR mutation analysis was possible in 90% of specimens for which it was requested (13)*. ALK fusion genes can also be identified on cytologic specimens obtained via EBUS (14). Even multiple gene mutation analyses are feasible using cytologic specimens (15, 16). The literature supports the use of cytologic specimens of good quality, including those taken during bronchoscopy, for molecular testing in NSCLC.

A useful genetic marker must also be found commonly enough for its use to be practical. Genetic testing is costly, and testing large numbers of patients when the prevalence of the mutation of interest is vanishingly low would produce relatively little benefit for the cost involved. One way to enrich the population chosen to undergo genetic testing in lung cancer is to narrow the group of patients to be tested to a more manageable size with a higher prevalence of the mutation of interest. For example, the genetic alteration in EGFR that is susceptible to targeted therapy is found almost exclusively in non-squamous NSCLC. The prevalence of the EGFR mutation is less than 5% in patients with squamous cell cancer, and is estimated to be about 15-20% for patients with adenocarcinoma (17-19)*. The group of patients referred for EGFR mutation analysis should also include patients with histologically adenosquamous tumors, because small studies have demonstrated a significant response of adenosquamous tumors with the EGFR mutation to agents targeting EGFR (20)*. Using tumor histologic findings to determine when genetic testing should be performed can allow the clinician to use genetic testing in a population in which the prevalence of a genetic mutation justifies the expense of the test.

Finally, a useful genetic marker must indicate an oncogenic pathway for which an effective targeted therapy exists. It is reasonable to test for a broad array of mutations that may not be associated with a targeted therapy as part of epidemiologic studies of lung cancer or to assess patients for entry into a clinical trial. However, it is much less useful to do so in general clinical practice because most mutations have no effective targeted therapy, making the information that would be obtained not actionable. It is more appropriate and less costly to perform genetic testing only for mutations that mark oncogenic pathways that are susceptible to available and effective targeted treatments. The genetic markers in NSCLC that predict susceptibility to currently available targeted therapy are few and are found almost exclusively in adenocarcinoma of the lung.

The following sections review the genetic markers that are considered useful markers in NSCLC, with a brief discussion of the currently available targeted therapies for patients with these mutations.

EGFR Mutations

Mutations in the EGFR family of receptor tyrosine kinases are found in 10-23% of lung adenocarcinomas and rarely if ever in squamous cancers (18, 19, 21, 22)*. EGFR tyrosine kinases are involved in regulating multiple cellular activities, including growth, migration, and survival (21). The 2 most common EGFR mutations are small deletions in exon 19 and the L858R missense mutation in exon 21. Together these mutations account for more than 90% of EGFR mutations found in lung adenocarcinoma, and both mutations activate cellular survival pathways that inhibit apoptosis and have been shown to be transforming mutations in cultured cells (19, 23-26)*. Because cells with transforming EGFR mutations have also

been shown to be sensitive to the effects of targeted EGFR tyrosine kinase inhibitors (TKIs), tumors with EGFR mutations are good subjects for targeted therapy (26-30).

Although EGFR mutations are reported to be more common in never-smokers, women, and Asians, EGFR mutations are not found exclusively in these groups (18, 31, 32). Two large studies recently assessed the genetic and clinical characteristics of patients with lung adenocarcinoma. D'Angelo et al examined 2142 lung adenocarcinoma specimens for EGFR mutations and found that although EGFR mutations were more common in specimens from never-smokers and women, significant numbers of specimens from smokers and men also harbored the mutations (22)*. Dogan et al examined 3026 lung adenocarcinoma specimens and found that it was impossible to clinically identify a subgroup of patients with adenocarcinoma of the lung that had a low enough (less than 1%) prevalence of EGFR mutations to obviate the need for genetic testing (19)*.

No strong consensus has been reached on the best way to test for EGFR mutations. FISH and IHC can be used to measure the EGFR gene copy number, but neither method has yet been consistently shown to produce results that correlate with sensitivity to therapy targeted to EGFR (7)*. One recent study did compare FISH, IHC, and mutation analysis by direct sequencing in specimens from 40 patients with NSCLC treated with EGFR TKI. Only results from EGFR mutation analysis by direct sequencing were found to correlate with response to therapy in this study, so the mutation analysis method is currently recommended for testing for EGFR mutations (31).

Mutations in EGFR also predict responsiveness to treatment with EGFR TKIs (30, 33-38)*, and 2 medications in this class, gefitinib and erlotinib, are currently available. Both are orally administered EGFR TKIs that are approved for the treatment of advanced NSCLC for the indications shown in Table 1 (39-41). Several recent studies have continued to refine criteria used to select patients for testing for EGFR mutations as well as potentially expand the indications for using EGFR TKIs to treat NSCLC. The most important of these for gefitinib was the Iressa Pan-Asia Study, which compared gefitinib with carboplatin plus paclitaxel in 1217 previously untreated patients with advanced NSCLC in Asia. Patients were eligible to participate in the study if they had clinical features associated with response to EGFR TKIs in prior studies: histologically indicated adenocarcinoma and nonsmoker or former light smoker. Although no difference in mean progression-free survival duration was noted between the study groups, differences in outcome were observed when the patients were stratified by EGFR mutation status. Among patients whose NSCLC harbored the EGFR mutation, the mean progression-free survival duration was longer among those treated with gefitinib than among those treated with carboplatin-paclitaxel. Conversely, among patients whose NSCLC did not harbor the EGFR mutation, the mean progression-free survival duration was shorter among those treated with gefitinib than among those treated with carboplatin-paclitaxel (see Figure 1). These findings demonstrate the primacy of the EGFR mutation status in determining which patients responded to treatment with gefitinib. All of the patients in this study had clinical characteristics that were thought to predict a good response to treatment with gefitinib, but only EGFR mutation status accurately predicted response to targeted therapy (42). A subsequent, similar study in Korea confirmed these findings in a smaller group of patients (43)*.

Other recent studies assessing gefitinib in patients selected by mutation status rather than by clinical characteristics have shown promising results. The Western Japan Oncology Group compared gefitinib with cisplatin plus docetaxel in 172 patients with advanced NSCLC with the EGFR mutation and found that patients in the gefitinib group had longer progression-free survival durations than those in the cisplatin-docetaxel group (44). The North-East Japan Study Group also compared gefitinib with carboplatin-paclitaxel in patients with

advanced NSCLC with the EGFR mutation and found that those in the gefitinib group had longer progression-free survival durations and better quality of life than those in the carboplatin-paclitaxel group (45). Although neither study was able to demonstrate a significant improvement in overall survival durations in those treated with gefitinib, this may have been due to crossover of patients in the control group to the gefitinib group after they experienced disease progression (44, 45).

Other recent trials have assessed extended indications for gefitinib. One retrospective case-control study suggested a survival benefit for patients with lung adenocarcinoma who have experienced recurrence after complete resection (46). Another recent trial demonstrated improved progression-free survival durations in patients with stage III or IV adenocarcinoma who received gefitinib as maintenance therapy after an initial response to standard chemotherapy and who had clinical characteristics predicting the presence of an EGFR mutation (47)*. Additional trials will be needed to confirm the utility of both of these potential indications.

Erlotinib was initially shown to improve overall survival rates in unselected patients with advanced NSCLC whose disease progressed despite prior first- or second-line chemotherapy. These patients all had stage III or IV NSCLC with residual disease after having received 1 or 2 standard chemotherapeutic regimens and were not eligible for further chemotherapy. Overall survival durations, progression-free survival durations, and response rates were all significantly greater in the group receiving erlotinib compared with the group receiving a placebo. Subgroup analysis suggested similar clinical predictors of response to erlotinib to the clinical predictors of response to gefitinib (48).

Although trials have not shown erlotinib to be as effective of a first-line treatment as standard chemotherapy for unselected patients with NSCLC (49)*, other recent trials in China and Europe have shown that first-line treatment with erlotinib resulted in better progression-free survival than first-line treatment with standard chemotherapy in patients with advanced NSCLC that harbored the EGFR mutation (50, 51)*. In the OPTIMAL study, Zhou et al randomized 165 patients in China with stage IIIB or IV NSCLC who were known to have EGFR mutations to receive initial treatment with either erlotinib or gemcitabine plus carboplatin. Patients in the erlotinib group had longer progression-free survival durations and fewer adverse effects than patients in the chemotherapy group (50)*. Rosell et al assessed erlotinib as a first-line treatment for advanced NSCLC in European patients in the EORTC trial: 173 patients with advanced NSCLC with an EGFR mutation were randomized to receive initial treatment with either erlotinib or cisplatin plus docetaxel. The median progression-free survival duration was significantly longer in the erlotinib group, and the erlotinib group also had a lower rate of severe treatment-related adverse events (51)*. These trials suggest a role for erlotinib as a first-line treatment for selected patients with NSCLC, although formal approval for this indication in the United States is still pending.

Other recent studies have explored additional indications for erlotinib. One group retrospectively analyzed a cohort of patients with stage I or II lung adenocarcinoma with an EGFR mutation who underwent complete resection as primary therapy. Patients who received erlotinib as adjuvant therapy had slightly improved disease-free survival durations, but randomized trials will be needed to confirm this finding (52)*. Erlotinib has also been reported to be effective in patients with adenosquamous carcinoma with an EGFR mutation (20)*, and a phase II trial has suggested that erlotinib may also sensitize tumors to radiotherapy (53). Erlotinib has not been shown to be effective in patients with an EGFR mutation whose disease has already demonstrated resistance to gefitinib (54), and a recent phase II trial did not demonstrate additional benefit from administering chemotherapy to

selected patients who had a high probability of harboring an EGFR mutation and were already receiving erlotinib as a first-line treatment for lung adenocarcinoma (55)*. The common thread in all of these studies is the importance of EGFR mutation analysis in predicting the response to targeted therapy. More research in this field should continue to clarify and possibly expand the indications for targeted EGFR TKIs.

EML4-ALK Translocation

A more recently characterized mutation is an inversion of the short arm of chromosome 2 that can lead to the EML4-ALK translocation (56). This translocation causes constitutive activation of the ALK receptor tyrosine kinase and has been shown to be transforming in cells and in vivo (56, 57)*. The EML4-ALK translocation is found in 2-7% of lung adenocarcinomas (57-60). It is more common in patients younger than 55 years and nonsmokers, and it is nearly always found in non-squamous NSCLC (56, 61, 62). The current standard for genetic testing for the EML4-ALK translocation is the break-apart FISH assay. This test is favored currently because it was the exclusive test used in the initial trials of crizotinib and its results correlate to response to targeted therapy. The FISH assay can identify any rearrangement involving ALK, allowing it to identify rare and previously unseen ALK rearrangements. This means that it has a lower false negative rate than the currently available reverse transcriptase polymerase chain reaction test for ALK, which cannot identify previously unknown ALK rearrangements (8).

The presence of the EML4-ALK translocation predicts sensitivity of NSCLC to crizotinib, an orally administered inhibitor of the ALK kinase as well as several other kinases within the cell (3, 62). Crizotinib received accelerated US Food and Drug Administration approval in 2011 for use in patients with advanced NSCLC who were confirmed to have the EML4-ALK translocation according to FISH assay results (8). Kwak et al evaluated 82 patients with ALK translocations in the first published trial of crizotinib for the treatment of NSCLC. An overall response rate of 57% was demonstrated in this single-arm, open-label study (62). Another phase I trial reported in abstract form in 2011 reported a 61% overall response rate (63). A retrospective analysis demonstrated improved overall survival durations after treatment with crizotinib among patients with advanced NSCLC with the ALK translocation compared with ALK-positive controls, but prospective studies are lacking (64)*. Other ongoing prospective trials are assessing survival benefits of treatment with crizotinib. Although less information is available regarding crizotinib than for EGFR TKIs, the activity of crizotinib against ALK and several other kinases that may act as driver mutations holds great promise.

Practical Clinical Application of Genetic Testing in NSCLC

As outlined above, the ideal useful genetic marker in NSCLC indicates a driver mutation that responds to targeted therapy and is present in a significant proportion of the population to be tested, and for which an accurate test is available. The practicing clinician has little influence on which genetic tests or targeted therapies are available; however, the clinician can decide which patients with NSCLC to test for genetic markers. A simple, clinically based algorithm as outlined in Figure 2 can help physicians to identify patients for whom the above detailed genetic tests should be ordered.

First, the clinical stage of the tumor should be considered. None of the available targeted therapies are currently indicated for limited-stage lung cancer; surgery remains the mainstay of treatment for limited-stage NSCLC (65). When surgery or surgery followed by adjuvant chemotherapy is the initial plan for definitive treatment, testing for genetic markers predicting response to targeted therapy is not clearly indicated. However, between 9% and 40% of patients who appear to have limited-stage disease at the time of diagnosis experience

cancer recurrence after complete resection (66-68). Targeted therapy may be useful for certain patients who experience such recurrences, but clinical factors alone cannot predict which patients are likely to respond to targeted treatment (8, 19). For this reason, the practice of tissue banking, if available, is a good way to allow for genetic testing at a later date without the need for a repeat biopsy for patients who experience recurrence after resection.

For patients who have advanced or metastatic NSCLC at the time of diagnosis, the histologic type of the tumor should also be used as an early screening step to determine which patients should receive genetic testing. Because the currently available genetic tests that predict response to available targeted therapies are few and largely restricted to adenocarcinoma of the lung, patients with adenocarcinoma (or non-squamous NSCLC) should be considered for additional genetic testing. Patients with squamous cell lung cancer have not yet been conclusively shown to derive benefit from targeted therapy, so genetic testing should be deferred for these patients. However, patients with squamous cell lung cancer may also benefit from tissue banking; if ongoing research leads to new targeted therapies for squamous cell lung cancer, then genetic testing could be performed at a later date if needed without a repeat biopsy.

Patients who have locally advanced or metastatic adenocarcinoma of the lung should undergo genetic testing. EGFR mutations are much more common than the EML4-ALK translocation in lung adenocarcinoma, and the 2 mutations are very unlikely to be found in the same tumor (18, 19, 21, 22, 60). Therefore, a good argument can be made for performing EGFR testing first because it is more likely to be positive, which would avoid the cost of performing the EML4-ALK test. If EGFR mutation analysis is positive for a mutation predicting susceptibility to targeted therapy, then no additional testing is needed and the patient can receive treatment with erlotinib or gefitinib. If EGFR mutation testing is negative, then testing the tumor for the EML4-ALK translocation using the break-apart FISH assay is the next appropriate step. If FISH assay results for the EML4-ALK translocation are positive, then the patient can then receive treatment with crizotinib. If the test results are negative, then standard cytotoxic chemotherapy is appropriate.

It should also be noted that genetic testing in patients with NSCLC that does not fit the parameters of this recommendation (e.g., limited stage or squamous tumors) may be appropriate in the context of clinical trials.

Conclusion

NSCLC is a heterogeneous disease with varying biological behavior driven by differing genetic alterations that determine malignant behavior. Although many of the mutations involved in oncogenic pathways have been found, few of these mutations are truly useful genetic markers for clinical practice, and currently the useful mutations are found primarily in lung adenocarcinoma. Outside the setting of clinical trials, clinicians should order only genetic tests that yield information that can lead to a change in treatment and thereby potentially improve outcomes. We recommend genetic testing for locally advanced and metastatic adenocarcinomas of the lung as outlined above in Figure 2. In appropriately selected patients, we recommend testing for EGFR mutations and then for the EML4-ALK translocation if EGFR testing is negative to determine whether targeted therapy with EGFR TKIs or crizotinib is indicated. We also recommend tissue banking, if it is available, for patients with early-stage lung cancer or squamous cell lung cancer so that tissue is available for future testing in case of recurrence or new advances in targeted therapy. As clinical trials of more therapies targeted toward driver mutations in NSCLC are conducted, more such genetic tests will likely be incorporated into routine clinical practice.

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Key points

- Useful genetic tests identify driver mutations which are common in the population of interest and indicate a pathway for which effective targeted therapy exists
- The identified useful genetic tests in non-small cell lung cancer are found predominantly in adenocarcinoma of the lung
- Clinical characteristics in the absence of mutation testing are not sufficient to predict response to targeted therapy, but clinical characteristics can be used to select patients who are appropriate for genetic testing
- Erlotinib and gefitinib target cancers harboring the EGFR mutation and crizotinib targets those harboring the EML4-ALK translocation

Figure 1.

Results from the Iressa Pan-Asia Study [42] showing that in patients with non-small cell lung cancer harboring in the epidermal growth factor receptor (EGFR) mutation, the mean progression-free survival duration was longer among those treated with gefitinib than among those treated with carboplatin plus paclitaxel (B). However, the opposite was true among patients whose cancer did not harbor the EGFR mutation (C). (Figure reprinted with permission.)

Figure 2.
Suggested algorithm for genetic testing in patients with non-small cell lung cancer.

Table 1
Indications for approved targeted therapies in patients with non-small cell lung cancer (NSCLC) [33-35]

Targeted therapy	Indications
Gefitinib	Formerly for advanced NSCLC that has not responded to either platinum-based or docetaxel chemotherapy; in the United States now restricted to clinical trials or for patients who have benefitted from previous treatment with gefitinib
Erlotinib	For advanced or metastatic NSCLC that has not responded to at least 1 other chemotherapy regimen or maintenance therapy for stage IIIB/IV NSCLC that has not progressed after 4 cycles of standard chemotherapy
Crizotinib	For advanced NSCLC harboring an anaplastic lymphoma kinase translocation