Biomarkers in Non-Small Cell Lung Cancer: Opportunity and Challenge

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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Abstract

Lung cancer, a major global public health issue, is the leading cause of cancer death in the United States. In spite of the importance of this disease, there has been only a 3% improvement in 5-year survival over the past 30 years. While there have been some recent promising developments in screening and diagnosis, there is an urgent need to improve on therapy so that more people can be cured or have a longer life. In the field of biomarkers for non–small cell lung cancer we are beginning to characterize lung tumors by their molecular signature and design therapy accordingly. This article will address biomarkers for non–small cell lung cancer, with an emphasis on those that are either already used in clinical practice or being studied in current clinical trials. Epidermal growth factor receptor (EGFR), Kirsten rat sarcoma (KRAS), excision repair cross-complementation group 1 (ERCC1), ribonucleotide reductase 1 (RRM1), and echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase (ELM4-ALK) will be discussed. For each marker, we will address normal function in the cancer cell; impact on function when it is present, absent, or mutated; testing for the marker; incidence or frequency of the marker as well as characteristics (if known) of patients more likely to be positive for the marker; and function of the marker as prognostic, predictive, or both. We will also address implications for the advanced practitioner.

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According to the American Cancer Society's recently published global cancer statistics for 2008 (ACS, 2010), lung cancer was the most commonly diagnosed cancer and the leading cause of cancer death for males, and the fourth most commonly diagnosed cancer and second leading cause of cancer death for females. In the United States, lung cancer is the leading cause of cancer death for both males and females (Jemal et al., 2011). Despite the impact of this disease on the economies of nations and on the individuals affected, the pace of improvement in lung cancer survival has been slow and frustrating. The 5-year survival for non–small cell lung cancer (NSCLC)—taking into account adenocarcinoma, squamous cell, and large cell—was 13% in 1977. In 2005, more than 30 years later, 5-year survival is only 16%, a gain of a mere 3% (ACS, 2010). Since some

of this progress actually reflects improvement in supportive care, it is abundantly clear that better therapy has made little impact on lung cancer outcomes. It is especially frustrating because lung cancer lags behind other cancers, such as breast cancer and non-Hodgkin lymphoma, where improvement in survival rates over those same 25 years has been 15% and 21%, respectively (ACS, 2010).

Improvement in lung cancer survival has been slow for a number of reasons, the first of which is the lack of an effective method for early detection. That may be changing. Just recently, the National Cancer Institute (NCI) issued a preliminary report of the National Lung Screening Trial in which 53,000 heavy or former smokers were randomized to yearly low-dose spiral CT vs. yearly chest x-ray. They found 20% fewer lung cancer deaths among patients screened with CT (NCI, 2011). However, for patients who already have lung cancer or who will be diagnosed in the near future, we have to depend on developing more effective therapies in order to extend their lives.

Fortunately, we now have the capability to begin to analyze tumors at the molecular level, and there is a great deal of excitement in the lung cancer community about these developments. Using biomarkers to help us understand the disease and design therapies to treat it is providing hope for the future. In this article we will discuss the biomarkers that are currently being used in clinical practice, as well as some of those in clinical trials. We will also discuss the implications of these developments for the oncology advanced practitioner (AP).

The Function of Biomarkers

The function of biomarkers was very well described by Grande, Viale, and Yamamoto (2010) in a previous article in this series, "Biomarkers in colorectal cancer: Implications for nursing practice." They describe biomarkers according to function as diagnostic, prognostic, predictive, staging, and monitoring. The diagnostic function would be especially useful in lung cancer, since the symptoms that eventually lead to diagnosis (cough, shortness of breath, weight loss, hemoptysis, and pain) are seen when the disease is advanced or metastatic. There is a clear need in lung cancer for a means to screen for early disease so that patients can be identified while they can still be cured. One method in development is an "electronic nose," which can analyze patterns of volatile organic compounds, and which is able to distinguish between the exhaled breath of patients with non-small cell lung cancer and that of patients with chronic obstructive pulmonary disease, as well as that of healthy controls (Dragonieri et al., 2009).

In most solid tumors, the TNM staging system has long been in use as the way that we determine the stage of a lung cancer. A recent update to that staging system has been accomplished in an effort to refine the method by which we place patients into groups to help determine appropriate therapy. Even with revisions, this is an imperfect tool, as within each stage some patients do better than others. Finding a biomarker that would further refine the staging system would be helpful, but to date no biomarker has been identified that is either qualitatively or quantitatively useful in the assignment of lung cancer stage.

Finally, biomarkers can be prognostic and/or predictive. Prognostic markers forecast which tumors are likely to recur or lead to death, independent of therapy. Predictive markers are useful in selecting patients for a particular therapy (Aggarwal, Somaiah, & Simon, 2010; Grande et al., 2010). In lung cancer, most of the current utility of biomarkers has been in their predictive value. Currently there is only one US Food and Drug Administration (FDA)-approved therapy (erlotinib) that is indicated, according to the National Comprehensive Cancer Network (NCCN) guidelines (NCCN, 2011), for patients who test positive for a particular biomarker: the epidermal growth factor receptor (EGFR).

Knowledge of potential biomarkers for small cell lung cancer is at a very early stage of development and will not be discussed in this article. The remainder of this article will address lung cancer biomarkers for non–small cell lung cancer, with an emphasis on those that are either already used in clinical practice or those being studied in current clinical trials. For each marker, we will address normal function in the cancer cell; impact on function when it is present, absent, or mutated; testing for the marker; incidence or frequency of the marker as well as characteristics (if known) of patients more likely to be positive for the marker; and function of the marker as prognostic, predictive, or both. (See Table 1 for an overview of the

biomarkers discussed in this article.) We will also address implications for the oncology AP.

Epidermal Growth Factor Receptor

EGFR, otherwise known as HER1, is a member of the HER family of receptors. It is a transmembrane, ligand-binding receptor found on normal cells that plays a major role in cellular proliferation and differentiation (Yano et al., 2003). When its ligand binds, it dimerizes and autophosphorylates, causing a cascade of downstream signals that results in cell growth, differention, and apoptosis. EGFR is often overexpressed or mutated in non–small cell lung cancer cells, causing dysregulation of this signaling pathway with resulting uncontrolled proliferation, invasion, and inhibition of apoptosis. EGFR has been described and evaluated in three different formats: EGFR protein expression, *EGFR* gene copy number, and functional somatic mutations in the *EGFR* gene (Hirsch & Witta, 2005).

There are different ways to test for and interpret abnormalities in EGFR in cancer cells. EGFR immunohistochemistry (IHC) staining tests for protein overexpression on the cell; fluorescence in situ hybridization (FISH) determines the *EGFR* gene copy number; and molecular mutational analysis identifies the presence of a mutation within the tyrosine kinase (TK) domain in the DNA (Hirsch, Varella-Garcia, & Capuzzo, 2009). Testing by IHC and FISH can be performed on small cytology specimens; however, molecular analysis for *EGFR* mutation requires larger amounts of DNA, usually best obtained with core needle biopsies or surgical pathology specimens. Bone specimens are rarely useful for mutation analysis due to the decalcification process performed to obtain the original pathologic diagnosis.

Testing for EGFR IHC is a staining procedure that can be done reliably by any pathology laboratory. Gene copy number via FISH is also a resonably simple test that is relatively widely performed. *EGFR* mutation testing, however, must be performed by a molecular pathology team. The original patent for testing of *EGFR* mutation was developed by Genzyme Corporation, Cambridge, Massachusetts in 2006 (Medical News Today, 2006). Many hospitals and private molecular pathology testing companies have purchased the *EGFR* mutation test from Genzyme, and it is now being implemented at many institutions.

This test uses cells from tumor-rich areas that are microdissected, and DNA is extracted and amplified via polymerase chain reaction (PCR), followed by bidirectional sequencing of exons 18 through 21 in the tyrosine kinase domain of the *EGFR* gene (Waknine, 2005). *EGFR* mutations are identified as being present in the first four exons (18–21) of the tyrosine kinase domain of *EGFR*. Three types of *EGFR* mutations have been identified. The most common *EGFR* mutation is the exon 19 deletion mutation, accounting for 60% of all *EGFR* mutations. The exon 21 L858R missense

Table 1. Overview of Biomarkers in Non–Small Cell Lung Cancer

Marker	Use	Clinical significance	Agent
EGFR	To determine response to EGFR inhibitors	Patients with activation mutation for EGFR are more likely to benefit from TKI therapy	Erlotinib, gefitinib
ERCC1	To determine potential resistance to platinum compounds	Patients with low ERCC 1 are more likely to respond to platinum compounds	Cisplatin, carboplatin
RRM1	To determine potential resistance to gemcitabine	Patients with low RRM 1 are more likely to respond to gemcitabine	Gemcitabine
KRAS	To determine resistance to TKI therapy	Clinical utility not definitively established. *KRAS* mutant patients may have higher risk of recurrence postoperatively	None identified
ELM4-ALK	To determine response to agents that target the ALK fusion protein	Selection of patients for referral for clinical trials with ALK inhibitors	Crizotinib

Note. EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor. Adapted from Grande, Viale, & Yamamoto (2010).

mutation accounts for about 25%. The rare point mutations exons 18, 20, and 21 and insertion/duplication in exon 20 are much less common (Johnson, Jackman, & Jänne, 2006).

EGFR mutations have been reported to be present in approximately 13% of patients (Rosell et al., 2010). They are generally exclusive to patients with adenocarcinoma, though there have been case reports of some cases of NSCLC with squamous or other histology. *EGFR* mutations are most commonly found in patients who are never-smokers, are of Asian ethnicity, and have adenocarcinoma histology (Lynch et al., 2004). Significantly fewer *EGFR* mutations are found in the African American population when compared to Caucasian counterparts (Leidner et al., 2009).

Gefitinib (Iressa) was the first EGFR tyrosine kinase inhibitor (TKI) to be studied and approved based on phase II trials showing positive response rates (Fukuoka et al., 2003) However, a phase III trial of gefitinib vs. placebo in previously treated patients with NSCLC failed to show an improvement in overall survival (OS) (Thatcher et al., 2005). Subsequently, the drug was pulled from the United States market. However, a recent clinical trial showed a significant progression-free survival (PFS) benefit with first-line gefinitib over carboplatin/paclitaxel chemotherapy for patients with NSCLC who harbor an *EGFR* mutation (Mok et al., 2009). Another trial showed similar results in the second-line setting, suggesting an improvement in PFS for patients with an *EGFR* mutation or high gene copy number who receive gefitinib vs. those who receive docetaxel chemotherapy (Douillard et al., 2010). Based on these results, gefitinib is approved in some countries for NSCLC patients who have documented *EGFR* mutations.

Erlotinib is another EGFR-TKI that is approved in the United States for the second-line treatment of NSCLC, regardless of *EGFR* mutation status, based on phase III data showing an improvement in PFS and OS (Shepherd et al., 2005). It also has an indication for maintenance therapy after chemotherapy with stable disease (Cappuzzo et al., 2010). In the NCCN guidelines (NCCN, 2011), erlotinib is indicated for first-line therapy in patients with an established *EGFR* mutation.

Cetuximab (Erbitux) is a monoclonal antibody EGFR inhibitor that binds to the receptor on the cell surface, thus blocking the ligand from binding. It does not have approval for use in the United States in NSCLC; however, it does have a Medicare compendia listing and is in the NCCN guidelines (NCCN, 2011) for use with vinorelbine/cisplatin (VC) as front-line therapy for metastatic NSCLC. Cetuximab, when added to VC chemotherapy in the first-line setting, had an improvement in OS over VC chemotherapy alone (Pirker et al., 2009).

A papulopustular rash is the most common side effect, seen as a class effect of all three of these EGFR-TKIs. The rash is often manageable with dermatologic treatments, though at times dose reductions or discontinuation of therapy is necessary. An array of other dermatologic side effects can occur, including hair, skin, and nail changes. Diarrhea, the second most common side effect of erlotinib, is usually controlled with loperamide. Cetuximab is also associated with hypersensitivity reactions and magnesium wasting. Gefinitib and erlotinib can cause interstitial lung disease, which in rare cases can be fatal.

EGFR gene copy number and, to a lesser extent, protein expression, may be predictive biomarkers for treatment of NSCLC (Carlson, Garrison, Ramsey, & Veenstra, 2009). *EGFR* mutation has been predictive for treatment response in clinical trials with EGFR inhibitors (Mok et al., 2009; Douillard et al., 2010). Whether *EGFR* mutation is a prognostic indicator of survival has yet to be determined, because it is not yet known whether it is the excellent response to EGFR inhibitor therapy or the natural course of the disease that predicts for longer survival. Within *EGFR* mutations, the exon 19 deletion mutation has been associated with a twofold longer survival than the exon 21 missense mutation, while the rare exon 20 insertion mutation has been associated with resistance to EGFR inhibitors.

KRAS

KRAS is one of the more common mutations associated with NSCLC. It is one of the family of rat sarcoma (RAS) proteins. Found widely in mammalian cells, RAS proteins are felt to be crucial to many aspects of normal cellular physiology, including proliferation, survival, and differentiation. RAS impacts these diverse processes by regulating the activation of at least 10 downstream effector pathways, the best studied of which is the MAP (mitogen-activated protein) kinase prolifer-

ation pathway. This is also referred to as the RAF (serine/threonine protein-specific kinases)-MEK (or MAP2K), ERK (extracellular signal-regulated kinase) cascade, which are specific kinases that are activated by RAS. In normal cells, RAS proteins are located on the inner surface of the cell membrane in an inactive state until a cell-surface receptor is activated by the binding of its specific ligand. Receptor activation leads to conversion of RAS to its active state, which may then trigger a number of intracellular signaling cascades (Westra et al., 1993, Rodenhuis, 1992; Vakiani & Solit, 2011).

RAS was first identified as a proto-oncogene by Harvey in 1964 and further studied by Kirsten in 1967 (Malumbres & Barbacid, 2003) to produce sarcoma in rats. Since then, three human *RAS* proto-oncogenes have been identified: *HRAS* (homologous to the oncogene of the Harvey rat sarcoma virus), the *KRAS/RAS2* gene (homologous to the oncogene of the Kirsten rat sarcoma virus), and the *NRAS* gene (first isolated from a human neuroblastoma) (Mascaux et al., 2005). Loss of function mutations of these *RAS* proto-oncogenes are believed to contribute to the transformation of normal cells into cancer cells by producing proteins that remain in their "active" states, thereby allowing for unregulated proliferative cell signaling to occur (Rodenhuis, 1992).

RAS oncogenes have been identified in a host of human cancers. *KRAS* mutations in particular are most commonly seen in pancreatic, colorectal, and lung carcinomas (Rodenhuis & Slebos, 1990). In lung cancer, *KRAS* is most frequently associated with NSCLC. Mutations in *KRAS* appear in roughly 20% to 30% of adenocarcinomas and less frequently in squamous cell carcinomas. It is unclear why *KRAS* mutations are extremely rare in small cell lung cancer (Rodenhuis et al., 1988) and why 90% of lung cancer–associated *RAS* mutations are located on the *KRAS/RAS2* gene rather than on *NRAS* or *HRAS*.

Historically, *KRAS* mutation in NSCLC has been strongly associated with cigarette smoking (Ahrendt et al., 2001; Rodenhuis & Slebos, 1992; Westra et al., 1993). In 1987, Barbacid showed that chemical carcinogens caused *RAS* gene mutations, demonstrating the first link between chemical carcinogenesis and oncogenes (Barbacid, 1987; Rodenhuis & Slebos, 1992). However, recent data have called this into question. One paper by Riely and colleagues in 2008 suggested that smoking status was not associated with the frequency of *KRAS* mutation. Of 482 tumors, 81 were *KRAS* mutation–positive. Of these, 18% were never-smokers, 22% were former smokers, and 25% were current smokers (Riely et al., 2008). The authors postulated that the higher rate of *KRAS* mutation in never-smokers in their study contradicted prior studies because never-smokers were underrepresented in the older studies. They also found a difference between smokers and never-smokers in the type of *KRAS* mutation. Transversion mutations (G-to-C or G-to-T) were associated with smoking history, whereas transition mutations (G-to-A) were associated with never-smokers.

Race may also be a factor in the incidence of *KRAS* mutation. In patients of Western/European descent, the incidence of being *KRAS*-positive is 25%, which is somewhat higher than the incidence in African Americans. Interestingly, *KRAS* is less common in Asian populations. *KRAS* mutations have not been associated with age or sex (Riely et al., 2008; Roberts, Stinchcombe, Der, & Socinski, 2010).

There are currently no FDA-approved tests for *KRAS* mutations, but many hospital-based molecular laboratories and reference labs (e.g., Genpath, Response Diagnostics, Clarient) use assays that have been independently validated. Detection of *KRAS* mutation is generally accomplished with PCR or direct DNA-sequencing techniques.

The association of *KRAS* mutations with lung cancer and the availability of a reliable test to detect the mutation suggest that *KRAS* would be an ideal target for therapy. Unfortunately, considerable effort has been expended but to date no *KRAS*-targeted therapies have proven effective. However, an area of promising research has been the use of *KRAS* status as a predictive biomarker for guiding therapeutic intervention. Unlike other oncogenic biomarkers that are predictive of favorable therapeutic responses, *KRAS* mutations can be used to predict lack of response to therapy, in particular, EGFR-directed therapies. This may be because activation of RAS protein allows for tumor cells to grow independently of EGFR signaling, rendering them resistant to EGFR-TKIs (Riely et al., 2008). In colorectal cancer, mutation of *KRAS* predicts resistance to anti-EGFR mono-

clonal antibodies (Allegra et al., 2009).

TRIBUTE, a phase III randomized trial in which patients received first-line treatment for NSCLC, compared platinum doublet therapy with or without erlotinib. In a subset analysis of patients on this study, 21% of patients tested positive for *KRAS* mutation. Of the *KRAS*-mutated group, those on the erlotinib treatment arm had a lower overall survival rate (Eberhard et al., 2005). The FLEX trial and BMS099 are two randomized phase III clinical trials that combined cetuximab with platinum-based therapy in chemotherapy-naive NSCLC patients (Lynch et al., 2010; Pirker et al., 2009). Both studies looked at *KRAS* status as a biomarker for predicting response to therapy. Roberts and colleagues (2010) note that in the FLEX trial, "The response rates observed in the cetuximab-containing arm in patients with *KRAS* wild-type and *KRAS* mutant tumors were 37.3% and 36.8%, respectively (p = .96). Thus, the benefit of cetuximab was observed regardless of *KRAS* mutational status."

The same was true of the BMS099 trial in which a trend toward improved OS was seen in those patients who received cetuximab in addition to chemotherapy. There was no difference in progression-free survival or response in *KRAS* wild-type vs. mutant-positive patients. The researchers conclude that the small sample size did not permit a more definitive recommendation for the use of cetuximab and the role of *KRAS* as a predictor in NSCLC (Roberts et al., 2010; Langer & Socinski, 2011).

In addition to the multiple studies looking at *KRAS* in lung cancer as a predictive marker, it is also being investigated as a prognostic indicator. Wild-type *KRAS* status showed a positive correlation with survival in the BR.21 trial (Zhu et al., 2008), a placebo-controlled trial evaluating erlotinib as second- or third-line therapy in NSCLC patients (Shepherd et al., 2008). Other studies have also looked at *KRAS* as a marker of recurrence after surgery and as a marker for selecting those patients who would benefit from adjuvant chemotherapy in NSCLC. It has been suggested that *KRAS*-positive patients with completely resected stage IB or II disease had an increased risk of recurrence (Rodenhuis & Slebos, 1992).

The impact of *KRAS* status on adjuvant therapy has been tested. The JBR.10 trial investigated adjuvant vinorelbine and cisplatin vs. observation in patients with resected stage IB or II NSCLC. The effect of mutation status on overall survival was also explored. Patients with wild-type *KRAS* had a survival advantage with adjuvant chemotherapy while those with *KRAS* mutations did not (Winton et al., 2005). While interesting, the results were not clinically significant; further study is needed to determine relevance and impact on progression and overall survival for the patient with the *KRAS* mutation after surgical resection.

Overall, one weakness of many studies investigating *KRAS* has been that they are generally retrospective or meta-analyses. In order to determine the true prognostic and predictive value of the *KRAS* mutation, prospective clinical trials are needed in order to better elucidate its role in the treatment of lung cancer patients.

ERCC1

One theory for the association between smoking and lung cancer is that smoking induces damage to DNA, creating mutant cancerous cells that are able to escape normal DNA repair pathways (Neumann, Sturgis, & Wei, 2005). One of the most important DNA repair pathways is the nuclear excision repair (NER) pathway, which recognizes and repairs platinum-DNA adducts. DNA adducts are formed when cisplatin binds to DNA, leading to strand breaks that then inhibit replication, eventually leading to cell death (Neumann et al., 2005; Aggarwal et al., 2010). Excision repair cross-complementation group 1 (ERCC1) is an enzyme protein involved in the final step of the NER pathway. When high levels of ERCC1 are present, the damaged cell can repair itself and thereby is platinum-resistant; low levels of ERCC1 suggest platinum sensitivity (Aggarwal et al., 2010; Simon et al., 2007). Since cisplatinum is a standard agent used to treat lung cancer in both the adjuvant and advanced disease settings (Scagliotti et al., 2003; Schiller, Harrington, & Belani, 2002), the ability to identify those patients with tumors resistant to platinum would spare them the significant side effects associated with this agent.

As is the case with each of the biomarkers for lung cancer discussed in this article, the optimal method for determining the presence or absence of ERCC1 has not been established. Testing for ERCC1 can be done by IHC (Olaussen et al., 2006), by tissue microarray automated quantitative analysis (AQUA) (Zheng, Chen, Li, & Haddad,

2007), or by reverse transcriptase PCR (RT-PCR) (Cobo et al., 2007; Simon, Sharma, Cantor, Smith, & Bepler, 2005). Further research is ongoing to determine the optimal method of testing and also to develop reliable and affordable tests that will be available outside of a clinical trial setting. To date, no particular profile of patient characteristics has been identified to help to select patients who are likely to be either high or low on ERCC1.

The potential for ERCC1 as a biomarker in lung cancer has been illustrated by Olaussen et al. (2006), who looked at samples from the tumors of patients in the International Adjuvant Lung Trial (IALT), a multinational study in which resected patients were randomized to either cisplatin-based adjuvant chemotherapy or no chemotherapy postoperatively. In this IALT biology study Olaussen and colleagues were able to use IHC to identify ERCC1-positive and ERCC1-negative tumors. They determined that those with ERCC1-negative tumors who were in the chemotherapy group had a higher 5-year overall survival than patients with ERCC1-negative tumors in the control group (47% vs. 39%, respectively). Conversely, those with ERCC1-positive tumors who received chemotherapy did not have improved survival compared to the control group (adjusted hazard ratio for death, 1.14; 95% confidence interval (CI), 0.84 to 1.55; p = .40). These findings illustrate the theory that platinum-based chemotherapy benefits only those whose tumors cannot repair the cisplatin-induced DNA adducts. This establishes ERCC1 as a predictive biomarker.

In addition to its predictive function, ERCC1 is also potentially useful as a prognostic marker. In the IALT analysis referenced above, Olaussen and his colleagues (2006) also suggested that ERCC1 status may be an independent prognostic feature, regardless of therapy, with patients in the control group (no chemotherapy), with ERCC1-positive tumors faring better (hazard ratio for death 0.66, p = .009) than those in the control group with ERCC1-negative tumors.

Simon et al. (2005) also looked at tumor specimens from patients with NSCLC who had undergone resection, analyzing 51 specimens. Using a technique that quantified ERCC1 expression, and dichotomizing the cohort into < 50 and > 50, they found that patients with high ERCC1-expressing tumors had a better survival than those with low ERCC1 expression. They theorized that those with an intact DNA repair mechanism (high ERCC1) had a greater ability to repair genetic aberrations and thus reduce the tumor's malignant potential and its ability to recur after resection. If we can identify patients at a low risk of recurrence, they may be spared the toxicity of adjuvant postoperative treatment.

RRM1

Ribonucleotide reductase is an enzyme composed of two subunits, RRM1 and RRM2, that are required for DNA synthesis and repair. RRM1 is the main target of the chemotherapy drug gemcitabine, which acts by inhibiting ribonucleotide reductase, thereby blocking the pathway for DNA synthesis. When RRM1 is overexpressed, it can overcome the cytotoxic effects of gemcitabine, thereby decreasing the efficacy of the drug. Identification of RRM1 levels is assessed using RT-PCR. As with ERCC1, it is not yet apparent which patients are likely to be low or high for this marker.

A number of studies have detected a relationship between RRM1 levels and therapeutic response to gemcitabine. The Spanish Lung Cancer Group (Rosell et al., 2004) conducted a trial with 557 patients with lung cancer who had malignant effusions or metastatic disease, randomizing them to three different regimens. When tumor samples were analyzed from patients in the cisplatin/gemcitabine arm, patients with low RRM1 expression had significantly longer median survival than those with high levels (13.7 vs. 3.6 months; 95% CI, 9.6–17.8 months; p = .009). Similarly, a group of South Korean researchers retrospectively analyzed tissue from 40 patients who received gemcitabine-based chemotherapy from March 2004 to December 2008 at three university medical centers (Lee et al., 2010). Overall survival for the RRM1-positive group was significantly shorter than for the RRM1-negative group (p = .022) Disease control rate (partial response plus stable disease) was also less in the RRM1-positive group than in the RRM1-negative group (23% vs. 56%, respectively; p = .053). In this situation, selecting patients for gemcitabine therapy based on their RRM1 status appears to have a predictive function.

The overexpression of RRM1 has been linked to metastasis suppression (Gautam, Li, & Bepler, 2003) and to better survival in early-stage resected NSCLC patients (Zheng et al., 2007). The

ability to select patients who are less likely to have disease recurrence, and who therefore can be spared adjuvant chemotherapy, would be very useful. In this situation, high levels of expression of RRM1 have a prognostic function.

Combining ERCC1 and RRM1 to Direct Therapy

Non–small cell lung cancer is a complex disease; it would be exceedingly simplistic to assume that any one isolated characteristic could account for the wide variability in patient response to treatment. Teasing out these intricacies will take many years, but some researchers are making a start. In an effort to demonstrate the feasibility and efficacy of individualizing therapy based on the predictive function of ERCC1 and RRM1, Simon et al. (2007) conducted a prospective phase II clinical trial in treatment-naive patients with advanced NSCLC. On the basis of ERCC1 and RRM1 expression, patients were assigned to one of four chemotherapy doublet regimens, as per Table 2.

Analysis of the results revealed a response rate of 44%, a 1-year survival rate of 59%, and a median OS of 13.3 months, which Simon and colleagues said compared favorably with their prior experience with similar patients in phase II trials. A multi-institutional phase III trial MADeIT (Molecular Analyses Directed Individualized Therapy), which is based on these same molecular markers, randomizes patients to either the personalized therapy described in Table 1 or a standard doublet of carboplatin/gemcitabine. The trial was closed to accrual in late 2010, and the results are eagerly anticipated (George Simon, personal communication, February 28, 2011).

Table 2. Chemotherapy Doublet Regimens Assigned on the Basis of ERCC1 and RRM1 Expression

	Low RRM1	High RRM1
Low ERCC1	Carboplatin/ gemcitabine	Carboplatin/ docetaxel
High ERCC1	Docetaxel/ gemcitabine	Docetaxel/ vinorelbine

Note. Adapted from Simon et al. (2007).

ELM4-ALK

The *EML4-ALK* fusion oncogene represents a novel molecular target in NSCLC. In 2007, Mano and his team of investigators in Japan were the first to identify the unique fusion gene in a surgical specimen from a patient with lung adenocarcinoma (Sasaki, Rodig, Chirieac, & Jänne, 2010). When the tissue was examined they found that the *EML4* gene and the *ALK* gene had become fused together. These genes are normally separate but are both located on chromosome 2p. The fusion results from a small inversion within chromosome 2p, which leads to expression of a chimeric tyrosine kinase (Soda, 2007). The inversion on chromosome 2p is most commonly found in lung cancer cell lines but has also been identified in breast and colorectal cancers (Lin, 2009). At least 11 variants of *EML4-ALK* fusions have been reported (V1, V2, V3a, V3b, V3a/b, V4, V5a, V5b, V5a/b, V6, and V7). Variants 1 and 3a/b are the most common variants. The clinical significance of these variants has not been determined (Sasaki et al., 2010).

There is currently no established method for detecting *EML4-ALK* in NSCLC. Numerous methods have been validated as sensitive and specific for identifying the genetic lesions; these include RT-PCR, IHC, and FISH. A poster presented at the 2011 United States and Canadian Academy of Pathology annual meeting compared IHC, FISH, and RT-PCR for the detection of *EML4-ALK* translocation variants in NSCLC. It found that RT-PCR was the most sensitive and least subjective methodology for *EML4-ALK* variant 1 detection. While it did not test all 11 known variants, it was concluded that this approach would likely yield the greatest assay sensitivity (Wallander, Geiersbach, Tripp, & Layfield, 2011).

An abstract from the 2010 American Society of Clinical Oncology (ASCO) annual meeting described a RT-PCR diagnostic method for identifying all 11 known *EML4-ALK* variants (Danenberg et al., 2010). This method potentially offers a quantitative, reproducible result with a turnaround time of approximately 5 to 7 days. This technique also has the capability of amplifying the gene, thereby making it potentially more useful for those patients from whom only a small tissue specimen can be obtained.

EML4-ALK occurs in a distinctive clini-

cal subgroup of NSCLC patients. These patients have many of the same clinical features as NSCLC patients who harbor *EGFR* mutations; however, apart from unique exceptions, *EML4-ALK* and *EGFR* mutations are mutually exclusive. A study by Shaw et al. (2009) demonstrated that the fusion protein is most often found in patients with the histologic subtype adenocarcinoma, including adenocarcinoma with bronchoaveolar carcinoma features. Her study also identified a mixed adenosquamous carcinoma that was *EML4-ALK*–positive. *EML4-ALK* patients were significantly younger than those who did not have the mutation (average age, 52 vs. 64 years). In terms of gender, studies conflict as to whether more males or females harbor the translocation. Most studies have identified a male predominance. The gene was originally identified in a smoker with lung cancer but research has shown that it is much more common in never/former/light smokers (defined as < 10 pack-years and quit >1 year ago) (Sasaki et al., 2010). Shaw's study (2009) suggests that in patients with NSCLC who have clinical characteristics associated with *EGFR* mutation, but who have tested negative for EGFR, as many as one in three patients may harbor the *EML4-ALK* fusion protein.

Various studies have reported on the incidence of the fusion protein in lung tumor tissue: Soda et al. (2007) 6.7%; Sasaki et al. (2010) 3%–13%; Shaw et al. (2009) 1%–4.9%; Horn & Pao (2009) 3%–7%; Garber (2010) 3%–5%. The variability found is likely a result of the different methods of detection used in the numerous studies (Horn & Pao, 2009). In general, it is thought that approximately 5% of all NSCLC cases contain an *ELM4-ALK* translocation (Sasaki, 2010). Given this percentage, of the 160,000 new cases of NSCLC diagnosed each year in the United States, approximately 8,000 patients are *EML4-ALK*–positive.

Cancer cell lines that harbor the *ALK* gene are sensitive to *ALK* inhibitors (Garber, 2010). *EML4-ALK* exerts an oncogenicity both in vitro and in vivo (Mano, 2008). *ALK* inhibitors lead to apoptosis in vitro and tumor shrinkage in vivo (Sasaki, 2010). PF-02341066 (crizotinib),is the only orally bioavailable inhibitor that is currently under clinical development. Phase I studies of this drug started in May 2006, and data presented at the 2009 ASCO annual meeting showed an impressive 53% response rate and a disease control rate of 79% (Kwak, Camidge, & Clark, 2009).

Given these striking results, two more trials were mounted: a phase II trial (PROFILE 1005) of single-agent crizotinib in *EML4-ALK*–positive NSCLC and a randomized phase III trial (PROFILE 1007) of crizotinib compared with standard second-line chemotherapy (pemetrexed or docetaxel) in second-line treatment for *EML4-ALK*–positive NSCLC. The phase II study was intended for patients not eligible for the phase III trial or for patients randomized to chemotherapy who subsequently developed progressive disease. Clinical trials with crizotinib are currently offered at 227 locations (see www.clinicaltrials.gov). Advanced practitioners should direct their *EML4-ALK*–positive patients to one of these centers to help facilitate prompt enrollment into a trial.

Crizotinib appears to be well-tolerated taken twice daily. The most commonly reported side effects were nausea, vomiting, and visual disturbances, which were mild (grade 1) and subsided with time. Less common side effects include diarrhea and fatigue (10%–29% of patients). Less than 10% of patients may experience constipation, fever, upper respiratory infection, loss of appetite, dehydration, muscle spasms, cough, shortness of breath, numbness and tingling in the hands and feet, abdominal swelling, anemia, dizziness, headache, edema, elevated transaminases, disorders of the skin or tissue beneath the skin, inflammation of the esophagus, and indigestion (Pfizer, crizotinib investigators' brochure, December 2010). Unfortunately, crizotinib is not curative. Patients eventually develop resistance and relapse (Garber, 2010).

New *ALK* inhibitor drug compounds are emerging, with the goal of developing a drug that will overcome the drug resistance, which appears to develop over time. A drug from Ariad Pharmaceuticals, AP-26113, has shown 10-fold greater potency and specificity than crizotinib. Other companies such as Cephalon, GlaxoSmithKline, and X-276 are all in the preclinical stages of testing compounds designed to defeat resistant mutations. In addition to its role as a predictor of *ALK* inhibitor sensitivity, *EML4-ALK* positivity was associated with resistance to EGFR tyrosine kinase inhibitors (Shaw et al., 2010).

ELM4-ALK may also have a prognostic function. A study by Shaw et al. (2010) demonstrated

that patients with *EML4-ALK*–positive NSCLC have superior outcomes. This retrospective study compared 477 metastatic NSCLC patients with and without *EML4-ALK*. Results showed that patients with both *EML4-ALK* and *EGFR* mutations demonstrated a longer overall survival than those with wild-type *ALK* and *EGFR,* with a 1-year survival of 66%.

Implications for the Advanced Practitioner

As the era of biomarkers in lung cancer care becomes a reality, the AP can help to ensure patients have access to appropriate therapy. Although current NCCN guidelines only mandate testing for *EGFR*, tests for *KRAS*, ERCC1, RRM1, and *ELM4-ALK* are available, both in many clinical facilities as well as at commercial laboratories, which will help patients navigate treatment decisions informed by molecular results. Recognition of the characteristics of patients more likely to harbor the *EGFR* mutation or the ALK fusion protein should lead to routine testing of patients who fit the profile. When possible, patients should be treated with targeted agents according to the genetic makeup of their tumors, and not empirically with chemotherapy (Horn, 2010), potentially avoiding unnecessary and toxic therapy.

Advanced practitioners should advocate for biomarker testing at diagnosis, not only because tissue is most readily available at that time, but also so that educated therapeutic decisions can be made at the outset. Considering targeted therapies only after the patient has progressed on one or more chemotherapy regimens may preclude their inclusion in clinical trials for targeted agents.

The AP will play a role in identifying patients for testing and ordering the appropriate tests. Certainly any patient with an adenocarcinoma histology should be considered a candidate for *EGFR* testing, particularly if that person is female, a never or light smoker, and of Asian ethnicity. In some practices, all adenocarcinomas are tested for *EGFR* and for *KRAS*. If both of these are negative, and the patient is young and a never or light smoker, the *ELM4-ALK* test should be done; patients with the translocation should be referred for a clinical trial with an *ALK* inhibitor.

The AP will also be ordering biopsies to secure tumor specimens that are sufficient and suitable for testing. Core biopsies should be done, when possible, as fine-needle aspiration (FNA) biopsies often do not yield enough tissue for molecular analysis. Because a core biopsy vs. FNA of the lung carries a somewhat higher risk of pneumothorax, in appropriate situations it may be more prudent to biopsy a metastatic site, thereby securing tissue and establishing that the patient has stage IV disease with one procedure. There is no full agreement on which site of disease or which method of securing the biopsy is most reliable in defining the true nature of the mutation. One study found that bronchoscopy specimens were not as reliable as surgical specimens to define the mutation. Work is ongoing to develop tests that can be done on less tissue or even on cytological specimens. And even though there is no unanimity on which method of testing is most appropriate for each of these markers (e.g., *EGFR* testing by FISH vs. IHC vs. DNA activation mutation), the AP must know which test she/he is ordering and be familiar with the literature which validates the test.

Once patients have been identified as appropriate for a particular therapy, the AP will need to assist the patient to get access to the appropriate agent. Because the EGFR-TKIs are orally available, the patient must obtain them through their prescription plan. No generic equivalents are available for these medications and they are very expensive as compared to many other noncancer oral medications. The respective pharmaceutical companies that make the EGFR-TKIs have copay assistance programs as well as free drug programs for indigent patients. Specialty pharmacies often offer the easiest access to obtaining the oral EGFR-TKIs, as many regular commercial pharmacies do not stock the medications due to cost. Advanced practitioners who are aware of these barriers can help patients obtain their medication more quickly and at the lowest possible cost.

The AP will play a primary role in the management of the side effects associated with these targeted agents. While the side-effect profiles are often less onerous than those associated with traditional chemotherapy regimens, certain side effects, such as skin rash and diarrhea, can be severe and dose-limiting. Advanced practitioners need to provide appropriate supportive care so that patients can remain on therapy. And of course, patients and families need to be educated regarding

the specific side effects expected, which are often quite different from those they may have experienced if treated previously with more traditional chemotherapy regimens.

As we gain more experience with these targeted agents, and as the data from ongoing studies continue to accumulate and mature, we will become more knowledgeable and adept in the management of these patients. Advanced practitioners will play a pivotal role in the treatment of lung cancer according to individual biomarkers, as we move forward into an era of truly personalized treatment for lung cancer.

DISCLOSURES

Kristen Kreamer has served on advisory boards for Genentech; Beth Eaby-Sandy has served on speakers' bureaus for Genentech, Eli Lilly & Co., Merck & Co., and has acted as a consultant for Amgen.

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