The development of genomic and proteomic technologies will aid in screening, diagnosis, treatment, and prognostication for patients with non–small-cell lung cancer.

Dorothy Fox, Morning Walk. Acrylic on canvas, 30” × 30”.

**Current Clinical Application of Genomic and Proteomic Profiling in Non–Small-Cell Lung Cancer**

Tawee T anvetyanon, MD, Benjamin C. Creelan, MD, and Alberto A. Chiappori, MD

**Background:** Genomic or proteomic profiling of cancer can be broadly defined as a systematic grouping of cancer based on its genetic or protein makeup. In the management of non–small-cell lung cancer (NSCLC), genomic and proteomic profiling applications have become useful in early disease detection, diagnosis, treatment, and prognostication.

**Methods:** We reviewed the recent literature on the applications of genomic and proteomic profiling in NSCLC. Important applications were summarized into those already adopted as standard care and those still under investigation.

**Results:** For genomic profiling, testing for EGFR mutation and ALK rearrangement has become routine for adenocarcinoma. Multiplex assay and malignancy-risk gene signature are both important applications in development. A test to predict outcome after treatment with an epidermal growth factor receptor/tyrosine kinase inhibitor and a screening blood test for lung cancer are being investigated for use in proteomic profiling.

**Conclusions:** Genomic profiling is routine in patients with NSCLC, and proteomic profiling shows promise. Additional genomic and proteomic profiling applications may also prove to be useful contributions in the care of these patients.

**Introduction**

The term *genome*, first introduced in 1920, is defined as the entirety of an organism’s genetic information. For humans, this refers to a full set of 23 chromosomes in gamete cells, consisting of DNA. Genomics is a study of genomes. If the human genome were a book, it would contain 23 chapters, and each chapter would have 50 million to 250 million letters (A, C, T, G) without spaces. At least one copy of this “book” is present in most cells of our body. The key function of the genome is to produce functional proteins. Using the same analogy, it is of interest to understand why certain “paragraphs” will be translated into action.

The term *proteome* was introduced in the 1990s, as a blend of the terms *protein* and *genome*, referring to an entire complement of proteins produced...
by a cell. Proteomics is a study of the structures and functions of the proteome. Although the measurement of cellular proteins has long been in clinical use (e.g., C-reactive protein, carcinoembryonic antigen), proteomics usually refers to a large-scale experimental analysis of proteins available in low abundance. Proteomics is more complicated than genomics because genes that encode protein may be differently expressed at varying points in time. In addition, post-translational modifications such as phosphorylation and ubiquitination can result in activity or lack of activity of the translated proteins.

Genomics and proteomics are becoming increasingly important in oncology. The last few decades have seen increasingly deeper knowledge of the pathogenesis of cancer and specific aberrations in the genome and proteome of cancer cells. For example, an aberrant gene often translates into an abnormal protein, capable of driving malignant behavior of the cells. Profiling abnormal genes may help us better classify cancers and select the optimal treatment, and profiling abnormal proteins holds even greater potential. Although cancer genomics can help predict what may happen in cancer cells, cancer proteomics can determine what actually happens because it focuses on final gene function. In this article, we summarize the clinical applications of genomic and proteomic profiling in lung cancer, briefly introducing common methodology in cancer genomics and proteomics and describing the clinical applications of these technologies.

Methodology of Cancer in Genomics and Proteomics

Methodology in cancer genomics has matured along with the completion of the Human Genome Project in 2006. Overall, approximately 23,000 genes are present in the human genome. Although every cell in the human body has the same DNA content, certain genes are turned on in a particular cell. Gene derangement has been linked to the pathogenesis of cancers. The methodology for studying the cancer genome has been extensively reviewed elsewhere. However, in brief, 2 main components are essential: microarrays and bioinformatics. A DNA microarray, or DNA chip, is a collection of microscopic DNA spots attached to a solid surface, allowing for the measurement of the simultaneous expression of thousands of genes (via messenger RNA [mRNA] expression). DNA microarray makes it possible to concurrently study multiple genes. Typically, gene expression can be visualized using a special microscope that detects fluorescence-enhanced probes. Bioinformatics is a field that specializes in biological data storage, retrieval, and analysis. Bioinformatics makes it possible to meaningfully interpret microarray experiments.

By contrast, methodology in cancer proteomics is still evolving. This is more complicated than genomics because one gene can encode more than one protein and the protein can be post-translationally modified. For these reasons, the level of mRNA expression is often poorly correlated with the level of protein expression. Moreover, protein levels are dynamic and vary in concentration in various human tissues. Nevertheless, 3 main components are essential in proteomics: (1) mass spectrometry (MS), (2) protein microarrays, and (3) nanotechnologies. MS helps to characterize proteins either by ionizing them first or by splitting them into smaller molecules first (peptide mass fingerprinting) and then identifying them based on size and charge. A protein microarray is a small piece of glass that contains thousands of molecules to grab specific proteins, thus allowing protein identification. Nanotechnology refers to the process of working with materials, devices, or structures with sizes ranging from 1 to 100 nm, which is the size of a molecule. Of several available MS techniques, the matrix-assisted laser desorption/ionization (MALDI)-MS, also known as direct tissue protein profiling, is perhaps the most widely used standard platform for studying the proteome. MALDI-MS can detect proteins of interest directly from tissue as well as localize or map the protein to areas in tissue, creating an image, and it is especially useful for tissue with heterogeneous protein expression. Currently, the type of MS most widely used with MALDI is the MALDI–time-of-flight.

Genomic Profiling

Although genomic profiling technology is still a young field, it has already become an integral part of day-to-day care for patients with lung cancer, particularly those with adenocarcinoma. Given the high annual incidence of lung cancer, even relatively small subsets of genomically distinct lung tumors represent an attractive target for drug development. Lung cancer is one of the first cancers in which the promise of predictive genomic biomarkers has been realized. For example, genomic testing for activating point mutations or deletions in the epidermal growth factor receptor (EGFR), a key cell surface receptor regulating cell proliferation (Figure), has become increasingly commonplace in clinical practice. A large number of lung adenocarcinomas are believed to harbor “actionable” targets. However, many of these putative targets for drug sensitivity remain under investigation. Herein we review some salient prognostic and predictive genomic profiling applications available for patients with non–small-cell lung cancer (NSCLC).

**EGFR**

More than a decade ago, specific alterations in the exon 19 or 21 of the **EGFR** gene were identified
as an activating event in the carcinogenesis of up to 20% of NSCLC cases. This distinct subset of NSCLC is dependent on the EGFR pathway to proliferate, a feature termed “oncogene addiction.” An inhibition of this critical pathway can result in cancer cell apoptosis regardless of the offending deletions or point mutations. In 2004, it was first observed that an EGFR mutation correlated with a clinical response to the tyrosine kinase inhibitor (TKI) gefitinib. Since then, multiple phase III clinical trials demonstrated that EGFR TKI treatment is associated with improved progression-free survival rates and, in some cases, overall survival rates when compared with platinum-based doublet chemotherapy. This effect was sustained even among elderly patients. Currently, EGFR mutation testing is recommended as part of the initial evaluation for patients with NSCLC, including those with adenocarcinoma. For patients who have cancers harboring an EGFR mutation, some oncologists prefer an initial treatment with EGFR TKI, such as erlotinib or gefitinib.

**ALK**

In 2007, it was discovered that approximately 5% of lung adenocarcinomas harbor a translocation between the EML4 gene and the ALK promoter gene. This translocation occurred most commonly at the short arm of chromosome 2, resulting in a protein kinase. In animal models, this event has been shown to cause lung tumorigenesis, suggesting that it is a critical driver of tumor cell proliferation. Therefore, EML4–ALK has become a target for drug development. In 2011, the first TKI to inhibit this inversion product, crizotinib, received an accelerated approval from the US Food and Drug Administration (FDA) for marketing in the United States based on its efficacy for cancers harboring an EML4–ALK fusion. Since then, numerous EML4–ALK genetic variants have been reported, as well as fusion products involving other genes, such as those between TFG and KIF5B. In general, testing for ALK using fluorescence in situ hybridization (FISH) is preferable to polymerase chain reaction (PCR) because of the high sensitivity of FISH in detecting multiple ALK fusion variants. Currently, the use of PCR has not been prospectively reported in clinical trials because several variants of ALK alterations could be missed. Similar to EGFR mutations, for cancers known to harbor an EML4–ALK fusion, treatment with crizotinib is recommended over chemotherapy.

**KRAS**

Although genetic testing for EGFR and ALK has already entered mainstream clinical practice, testing for other genomic alterations remains under investigation. For example, the most pervasive oncogenic mutation in lung cancer, KRAS, has been a target of recent therapeutic development. The KRAS gene encodes for a cell-signaling protein that modulates cell proliferation and apoptosis. Alterations in KRAS have been observed in up to 30% of NSCLC tumors, including mutations in codons 12 and 13. By contrast to other mutations, KRAS is typically associated with carcinogen exposure such as cigarette smoke and asbestos. KRAS is generally believed to confer a poor prognosis, although evidence to support this notion is inconsistent. However, KRAS mutations may indicate nonsquamous histology. Thus, the finding of a positive KRAS mutation could guide therapeutic choices when the histologic interpretation of a tumor is equivocal or discordant. Moreover, patients with KRAS mutations may be less likely to respond to EGFR/TKI treatment. KRAS has shown potential as a “druggable” target in NSCLC. During a phase II study, docetaxel was combined with selumetinib, an inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2, which is a signaling downstream of KRAS. The combination may result in an improvement in patient survival rates when compared with docetaxel alone. However, a phase III study will be necessary to confirm this observation. In addition to KRAS, ROS1, a member of the insulin
RTK gene family, may also implicate a chromosomal translocation in lung cancer.31

Other Emerging Applications

Squamous Cell NSCLC: Although the majority of genomic alterations identified to date have been observed in adenocarcinoma, several putative therapeutic targets in squamous cell NSCLC are emerging. A recent comprehensive analysis of the Cancer Genome Atlas utilizing multiplex testing has identified several such mutations.29 In particular, FGFR1 overexpression is present in roughly 20% of squamous cell cancers. FGFR1 overexpression may be important because it appears to be more prevalent in people with a smoking history. In addition, PIK3CA mutation, which is also prevalent among patients who smoke, may become a viable target of intervention. Although their presence has an unclear prognostic relevance, these mutations may predict sensitivity to inhibitor of mammalian target of rapamycin (mTOR) such as everolimus.32 By contrast, the DDR2 mutation seen in about 2% of squamous cell NSCLC may be more common among patients who do not smoke.33 A DDR2 mutation may also be associated with a clinical response to dasatinib.33 These newly described genetic alterations are promising therapeutic targets under investigation for squamous cell NSCLC.

Multiplex Assays: An ideal genomic assay would simultaneously test for multiple potential druggable targets. An extensively tested tool currently available is the SNaPshot (Applied Biosystems; Foster City, California), which is also being tested for use with breast, colon, and glial cancers. The assay identifies more than 50 mutation sites in 14 individual cancer genes within 15 to 20 days. In a series of 552 tumors from patients with NSCLC, 282 (51%) had at least one identifiable mutation or genetic alteration.34 The most prevalent individual mutations found were KRAS (24%), EGFR (13%), ALK (5%), PIK3CA (4%), BRAF (2%), and HER2 (1%). However, the absolute percentages from this study may be confounded by selection bias, because patients presenting to research institutions may not be precisely representative of the general population. Of note, about 40% of such patients with an identified genetic alteration went on to participate in clinical trials with an experimental therapeutic agent.

Predictive Malignancy-Risk Gene Signature: Malignancy-risk gene signature, which is a gene signature based on the mRNA expression of 94 proliferation-related genes, has recently been shown to have a predictive value for adjuvant chemotherapy in the NSCLC gene.35 The gene signature was developed based on microarray technology and identifies patients with NSCLC who will benefit from adjuvant chemotherapy. However, a validation of the malignancy-risk gene signature in a large independent dataset is still needed.

Proteomic Profiling

Proteomic profiling has been utilized to diagnose, prognosticate, and predict response to treatment. The following section summarizes the details of technologies currently available for clinical use as well as other methods in development.

Serum Tests

An EGFR/TKI, either erlotinib or gefitinib, is an effective treatment strategy for NSCLC with an activating mutation on the EGFR gene, most commonly a deletion in exon 19 and L858R point mutation in exon 21. Nevertheless, having these mutations in the EGFR gene is not a guarantee of tumor response. In a study of 197 patients with such mutations who were treated with a TKI, progressive disease quickly developed in 20 patients (10%).36 In addition, a tumor specimen is a prerequisite for performing genetic testing, making it inconvenient for some patients. It is also difficult to use tissue testing for monitoring disease progression. Therefore, the concept of a simple blood test to predict EGFR/TKI responsiveness may be attractive to some patients. Several investigational teams have introduced such technology, including genetic testing on circulating tumor cells.37 However, this requires sophisticated instruments and is not currently available for clinical use.37

A clinically validated serum test for advanced NSCLC (VeriStrat; Biodesix, Boulder, Colorado) may be a potentially viable product. The test uses proteomic technology to help predict treatment results among patients undergoing treatment with an EGFR/TKI. In 2007, Taguchi et al38 published the development of an algorithm, based on the MALDI-MS analysis of pretreatment serum, to predict responsiveness to EGFR/TKI. The authors used serum from 139 patients as a training set, identifying 8 unique protein spectra to be integrated into the algorithm used to predict treatment outcome via a binary result, either “good” or “poor.” The algorithm was tested in 163 NSCLC patients who were treated with a TKI and 158 NSCLC patients not treated with a TKI. Overall, about two-thirds of tested patients were classified as “good” and one-third as “poor” by the test. Among the cohorts treated with a TKI, the overall survival rate was higher in the “good” group than in the “poor” group. However, among the cohorts not treated with a TKI, no difference existed in the survival outcomes between the 2 groups.

Other studies have demonstrated the clinical utility of VeriStrat. In 2010, Carbone et al39 published results from 35 patients treated in a clinical trial of combination erlotinib/bevacizumab. In this study, MS was performed using pretreatment sera. Of the 35 patients, 26 (74%) were classified as “good” and 8 (26%) as “poor.” A significant difference was present in the overall survival and progression-free survival.
rates among the 2 groups. In addition, Amann et al\(^40\) published results of a single-arm, phase II study of patients with advanced NSCLC treated with first-line erlotinib. Of the 102 patients with analyzable biologic samples, 41 patients had tumor tissues adequate for gene mutation analysis, while 88 had serum interpretable for proteomic analysis using VeriStrat. Of the 41 patients with tumor genetic profiling, 3 had an \(EGFR\) mutation present and their overall survival rates appeared superior when compared with their \(EGFR\) wild-type counterparts. Of the 88 patients with serum proteomic profiling, 64 patients (73\%) were classified as “good” and 24 (27\%) were classified as “poor.” The median overall and progression-free survival rates were higher among patients classified as “good” compared with those classified as “poor.” One of the 3 patients with an \(EGFR\) mutation was classified as “poor” by the serum test. In addition, of the 6 patients with a \(KRAS\) mutation, 5 were classified as “good” by the serum test, suggesting that the proteomic test was independent of \(EGFR\) and \(KRAS\) statuses.

A subsequent study suggested the role of VeriStrat in the monitoring of TKI resistance. In 2012, Lazzari et al\(^41\) published the results of 111 patients with advanced NSCLC who were treated with gefitinib. The serum test was performed at baseline, then after 1 month of gefitinib treatment, and then every 2 months concomitantly with computed tomography. At baseline, 77 patients were classified as “good,” 31 patients as “poor,” and 3 as “indeterminate” by the serum test. A baseline VeriStrat result of “good” predicted better survival rates than a baseline result of VeriStrat “poor.” During the course of treatment, 8 patients changed from “good” to “poor,” and they also had a significantly elevated risk of progressive disease or death.

Despite evidence from the above studies, data from prospective studies to support the clinical use of VeriStrat is still lacking. Both the identity and clinical mechanism of the 8-protein spectra used in the serum test algorithm remain unknown. Many patients may not have a tumor specimen available to help predict treatment response to TKI, so VeriStrat may be a useful option. A prospective trial to evaluate VeriStrat is ongoing.\(^42\) Currently, the provisional opinion of the American Society of Clinical Oncology states that patients who are being considered for first-line therapy with an \(EGFR/TKI\) should have their tumors tested for \(EGFR\) mutations to determine whether treatment with an \(EGFR/TKI\) or chemotherapy is the appropriate choice for first-line therapy.\(^43\) At this time, VeriStrat still requires clinical validation, and trials are ongoing to confirm its clinical usefulness (NCT01652469).

### Immunobiomarker Tests

Delay in diagnosing lung cancer is a common problem in NSCLC, resulting in most cases presenting at an advanced stage. Therefore, a need exists for better approaches to enable an early diagnosis of lung cancer. Although computed tomography may be helpful in some cases, a blood test used to screen for lung cancer is an appealing idea. Proteomics technology has shown some promise for early lung cancer diagnosis using such a blood test. Tumor-associated antigens (TAAs), which are proteins exclusively expressed in cancer cells, can be detected in blood. In addition, due to the aberrant structure of TAAs, the antigen may sometimes trigger autoantibody production. Well-known antigens such as p53 and C-myc have long been found in the sera of patients with lung cancer, and their associated autoantibodies have been reported in 10\% to 30\% of cases.\(^44,45\)

In 2004, Zhong et al\(^46\) published a study aiming to comprehensively detect the production of autoantibodies to known NSCLC antigens. In a subsequent work, the authors used fluorescent microarray technology to identify phage-expressed, NSCLC-associated proteins.\(^47\) The proteins were displayed in an array fashion, which was used to efficiently measure multiple antibodies simultaneously. Using this method, the authors identified the 5 most common proteins associated with lung cancer based on 46 blood samples of patients and normal controls.

Since then, another group of investigators developed a test panel using an enzyme-linked immunosorbent technique to detect the presence of these autoantibodies in the blood.\(^48\) This technique has been further refined and, in 2010, a technical validation of an autoantibody test for lung cancer based on 6 TAAs was published.\(^49\) These antigens were produced using a specific complementary DNA recombinant technique, and the antigens were used to produce the autoantibody assay. In a clinical trial of 655 patients with NSCLC or small-cell lung cancer and 655 matched noncancer controls, the test had sensitivity and specificity rates of about 40\% and 90\%, respectively.\(^50\) A subsequent study of 574 patients confirmed these findings.\(^51\) More antigens are actively being sought to be included in the test to improve the diagnostic accuracy.\(^52\) Although the test is commercially available, it has not been submitted to the FDA for approval. A prospective trial of the test is being planned.\(^53\)

### Other Emerging Applications

#### Classifying NSCLC Subtype: MALDI-MS has been used to classify subtypes of NSCLC. In 2003, Yanagisawa et al\(^54\) used 50 pulmonary tissue samples (42 from patients with resected NSCLC and 8 from patients without cancer) to create a prediction model based on a proteomic pattern. The model differentiated normal lung tissue from NSCLC and classified a subtype of 43 blinded NSCLC samples into adenocarcinoma, large cell, or squamous cell with 100\% accuracy. Of interest,
<table>
<thead>
<tr>
<th>Genes</th>
<th>Synonyms</th>
<th>Chromosome Location</th>
<th>Mechanism of Gene Product</th>
<th>Example of Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>CD246 NBLST3</td>
<td>2</td>
<td>The gene can be oncogenic by forming a fusion with a gene such as <em>EML4</em>. The protein encoded by the gene fusion is a tyrosine kinase that drives downstream pathways involved in tumor growth and proliferation.</td>
<td>Crizotinib&lt;sup&gt;59&lt;/sup&gt;</td>
</tr>
<tr>
<td>BRAF</td>
<td>B-RAF1 BRAF1 NS7 RAF81</td>
<td>7</td>
<td>The protein BRAF regulates signal transduction serine/threonine-specific protein kinase. Alterations in the gene interfere with intracellular signaling, causing proliferation.</td>
<td>Vemurafenib&lt;sup&gt;60&lt;/sup&gt;</td>
</tr>
<tr>
<td>DDR2</td>
<td>CD167b MIG20a NTRKR3 TKT TYR10</td>
<td>1</td>
<td>The protein is a membrane-bound receptor tyrosine kinase that binds to collagen and can regulate proliferation and migration. <em>DDR2</em> gene mutation is found oncogenic in squamous histology.</td>
<td>Dasatinib&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
<tr>
<td>EGFR</td>
<td>ErbB-1 HER1</td>
<td>7</td>
<td>The gene is a cell-surface receptor for members of the epidermal growth factor family of extracellular protein ligands. Alterations in the gene interfere with intracellular signaling, causing proliferation.</td>
<td>Erlotinib&lt;sup&gt;16&lt;/sup&gt; Gefitinib&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>HER2</td>
<td>ERBB2 CD340 HER2 HER2/neu MLN 19 NEU NGL TKR1</td>
<td>17</td>
<td>The gene is a cell-surface receptor for members of the epidermal growth factor family of extracellular protein ligands. Alterations in the gene interfere with intracellular signaling, causing proliferation.</td>
<td>Lapatinib&lt;sup&gt;61&lt;/sup&gt;</td>
</tr>
<tr>
<td>KRAS</td>
<td>C-K-RAS K-RAS2A K-RAS2B K-RAS4A K-RAS4B KI-RAS KRAS1 KRAS2 NS NS3 RAS2</td>
<td>12</td>
<td>The protein product of the normal <em>KRAS</em> gene performs an essential function in normal tissue signaling. <em>KRAS</em> acts as a molecular on/off switch. It activates proteins necessary for the propagation of other receptor signals such as <em>RAF1</em> and <em>PI3KCA</em>. Mutation of the <em>KRAS</em> gene implicates many cancers.</td>
<td>Retaspimycin&lt;sup&gt;62&lt;/sup&gt; Selumetinib&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>MET</td>
<td>AUTC8 HGF RCCP2 c-Met</td>
<td>7</td>
<td>The gene contributes to tumor proliferation by either activating point mutations or gene amplification. The protein activates downstream pathways such as <em>ERBB3/PI3K/AKT</em> and is an acquired-resistance mechanism in 20% of <em>EGFR</em>-mutant lung cancer.</td>
<td>Onartuzumab&lt;sup&gt;63&lt;/sup&gt;</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>CLOVE MCAP MCM MCMTC PI3K p110-alpha</td>
<td>3</td>
<td>The gene has an enzyme-controlling cellular function. Mutation of <em>PIK3CA</em> causes defective protein, which in turn causes tumor cell proliferation.</td>
<td>XL-147&lt;sup&gt;64&lt;/sup&gt;</td>
</tr>
<tr>
<td>RET</td>
<td>CDHF12 CDHR16 HSCR1 MEN2A MEN2B MTC1 PTC RET-ELE1 RET51</td>
<td>10</td>
<td>The gene can be oncogenic by forming a fusion with genes such as <em>KIF5B</em>. The protein KIF5B/RET is a tyrosine kinase that drives downstream pathways involved in tumor growth and proliferation.</td>
<td>Cabozantinib&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td>ROS1</td>
<td>MCF3 RCD c-ros-oncogene 1</td>
<td>6</td>
<td>The gene can be oncogenic by forming a fusion with genes such as <em>HCC78</em> or <em>CD74</em>. The protein encoded by the gene fusion is a tyrosine kinase that drives downstream pathways involved in tumor growth and proliferation.</td>
<td>Crizotinib&lt;sup&gt;66&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

the authors obtained more than 1,600 protein peaks from a 1-mm frozen section specimen, and they used 15 distinct MS peaks to classify patients into those with good vs poor prognoses. In a subsequent study, the investigators revealed that protein microarrays from a formalin-fixed, paraffin-embedded tissue can be used to correctly classify the NSCLC subtype from fresh frozen tissue.55 This ability to detect and characterize tumor marker proteins from a large cohort of formalin-fixed, paraffin-embedded samples in a high-throughput manner may make the clinical application of proteomics more practical.

**Distinguishing Premalignant Lesions From Malignant Bronchial Lesions:** In a study of 53 patients with bronchial lesions, proteomic technology helped identify a premalignant lesion vs a malignant lesion.56 In this study, MALDI-MS profiling of 10 micron sections of fresh frozen tissue samples was performed. A specific proteomic profile was identified, with a predictive accuracy of more than 90% of normal, preinvasive, and invasive lung tissues. This approach characterizes the value of proteomic technology in understanding of tumorigenesis.

**Providing Prognostic Information:** Proteomic profiling has helped predict the natural course of lung cancer independent of treatment. To date, several protein biomarkers have been linked to patient outcomes. Typically, proteomics is used first to discover a specific protein of interest and other well established, widely available methods such as immunohistochemistry on tissue microarray are used to validate the prognostic value of that specific protein. Using MALDI-MS, Yanagisawa et al57 identified an MS signature associated with survival among patients with resected early-stage NSCLC. From 2,630 MS signals obtained from specimens in the training cohort, the authors derived a signature of 25 signal peaks that predicted survival rates. Other groups have found that that a high expression of myosin IIA and vimentin among specimens in the training cohort, the authors obtained more than 1,600 protein peaks from a 1-mm frozen section specimen, and they used 15 distinct MS peaks to classify patients into those with good vs poor prognoses. In a subsequent study, the investigators revealed that protein microarrays from a formalin-fixed, paraffin-embedded tissue can be used to correctly classify the NSCLC subtype from fresh frozen tissue.55 This ability to detect and characterize tumor marker proteins from a large cohort of formalin-fixed, paraffin-embedded samples in a high-throughput manner may make the clinical application of proteomics more practical.

**Distinguishing Premalignant Lesions From Malignant Bronchial Lesions:** In a study of 53 patients with bronchial lesions, proteomic technology helped identify a premalignant lesion vs a malignant lesion.56 In this study, MALDI-MS profiling of 10 micron sections of fresh frozen tissue samples was performed. A specific proteomic profile was identified, with a predictive accuracy of more than 90% of normal, preinvasive, and invasive lung tissues. This approach characterizes the value of proteomic technology in understanding of tumorigenesis.

**Providing Prognostic Information:** Proteomic profiling has helped predict the natural course of lung cancer independent of treatment. To date, several protein biomarkers have been linked to patient outcomes. Typically, proteomics is used first to discover a specific protein of interest and other well established, widely available methods such as immunohistochemistry on tissue microarray are used to validate the prognostic value of that specific protein. Using MALDI-MS, Yanagisawa et al57 identified an MS signature associated with survival among patients with resected early-stage NSCLC. From 2,630 MS signals obtained from specimens in the training cohort, the authors derived a signature of 25 signal peaks that predicted survival rates. Other groups have found that that a high expression of myosin IIA and vimentin among patients with early-stage NSCLC was associated with poor disease-free survival rates.58 This information holds the promise of refining patient selection in the adjuvant chemotherapy setting.

**Conclusions**

Genomic and proteomic profiling has now become an essential part in the care of patients with lung cancer. As outlined in the Table, numerous promising marker–drug pairings are being used or explored in both squamous cell cancer and adenocarcinoma of the lung. We are moving beyond a simple morphological and immunohistochemical diagnosis of lung cancer. As genomic and proteomic technologies continue to mature, molecular diagnoses will become increasingly useful to clinicians in all aspects, ranging from screening, diagnosis, treatment, and prognostication. To further advance knowledge with minimal delays, it is important that future studies be based on adequate and well-validated technical, statistical methodology and bioinformatics. Many challenges lie ahead, including the genetic heterogeneity within a single tumor in the same patient as well as at different time points. As these challenges are overcome, genomic and proteomic profiling will increasingly contribute to the care of patients with lung cancer.

**References**


