Biomarkers in Early-Stage Non–Small-Cell Lung Cancer

Current Concepts and Future Directions

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Abstract: Advances in molecular biology and bioinformatics have resulted in the identification of a number of potential biomarkers that could be relevant in the management of patients with non–small-cell lung cancer (NSCLC). Although there is an increasing amount of literature related to these biomarkers, major issues need to be resolved including validity and reproducibility of results. Additionally, in order to interpret the existing literature accurately, a clear distinction must be made between the prognostic and predictive value of biomarkers. The practical applicability of biomarker discovery for patients with lung cancer includes the identification of patients with early-stage NSCLC who are most likely to benefit from adjuvant therapy. Information gleaned from biomarkers has the potential to help in evaluating the role of targeted therapies including immunotherapy in the neoadjuvant and adjuvant setting. The role of gene signatures and the use of newer platforms such as RNA, methylation, and protein signatures is being explored in patients with early-stage NSCLC. This review focuses on the applications of biomarker discovery in patients with early-stage NSCLC.

Key Words: Lung cancer, Biomarkers, Early-stage lung cancer, Predictive, Prognostic.

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Lung cancer is the leading cause of cancer-related mortality in the United States and the worldwide. Non–small-cell lung cancer (NSCLC) is the most common form of lung cancer. Early-stage NSCLC (ES-NSCLC; stages I and II) accounts for approximately 18% of the cases.[1] Most of these patients are treated with curative intent and often require multimodality therapy.[2,3] Despite these aggressive measures, the survival associated with ES-NSCLC is less than optimal with a 5-year overall survival (OS) ranging from 50% for stage IA disease to 15% for stage IIIA NSCLC.[4]

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ES-NSCLC has assumed particular significance in recent years for two main reasons. First, the incidence of ES-NSCLC is expected to rise due to the use of computed tomography screening of high-risk patients which has demonstrated a survival benefit.[5] Second, the outcomes of ES-NSCLC may potentially benefit from an improved understanding of the molecular and immunologic basis of NSCLC which has already led to improved outcomes in advanced NSCLC.

Several clinical trials have demonstrated improved survival with postoperative chemotherapy in selected patients who undergo complete surgical resection.[6,7] Available evidence supports the use of adjuvant chemotherapy for stage II and stage IIIA, but not for stage IA NSCLC.[8]

There are several shortfalls to the current approach of selecting patients for adjuvant therapy based on the surgical stage alone. Given the marginal benefits and potential toxicities associated with chemotherapy, perhaps the greatest challenge lies in the identification of patients at the greatest risk of recurrence. One approach to identifying high-risk patients focuses on the biology of ES-NSCLC in an effort to predict the risk of recurrence and the potential for response to treatment by using biomarkers.

A biomarker is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." A prognostic biomarker is a factor that is associated with an outcome that is independent of treatment, whereas a predictive biomarker interacts with the treatment to influence outcome.[9] There is good clinical evidence for a limited number of biomarkers that are used in clinical practice. Examples include the use of hormone receptor status in breast cancer. These biomarkers are prognostic of improved survival independent of cancer treatment and also predict the benefit of hormonal therapy with drugs such as tamoxifen.[10] Despite a concerted effort, there is a lack of biomarkers with potential application in the management of ES-NSCLC.

The search for a prognostic and predictive biomarker has to take into consideration two key points: the strength of evidence to support its use and the depth of information provided by a biomarker that adds to what is already known about the disease based on the clinical parameters. Although a plethora of potential prognostic biomarkers have been proposed in the past couple of decades, very few have been validated. In this review, we have focused on a very small number of these biomarkers, including immune markers and molecular signatures relevant to ES-NSCLC because of the potential promise associated with them.

PROGNOSTIC BIOMARKERS

P53

The tumor suppressor gene, *p53* is frequently altered in NSCLC.[11] Although it is a well-established poor prognostic factor in many tumors,[12,13] in ES-NSCLC its prognostic role is controversial. A subgroup analysis of *CALGB 9633*, a phase III trial that randomized patients with stage IB NSCLC to observation or adjuvant chemotherapy, showed that *p53* expression by immunohistochemistry (IHC) was detectable in 47% of the tumors, and correlated with shorter disease-free survival (hazard ratio [HR], 1.95; $p = 0.003$) and OS (HR, 2.30; $p = 0.0005$) in multivariate analyses.[14] A meta-analysis of pooled patient data from 43 studies which included patients with ES-NSCLC who underwent potentially curative resection showed that *p53* mutation or overexpression was an indicator of poor prognosis, especially in patients with adenocarcinoma (ADC). Compared with patients with no alterations, patients with ADC and *p53* overexpression or mutations had a 21.8% ($p = 0.0000039$) and 48% ($p = 0.000031$) reduction in 5-year OS, respectively.[15]

KRAS

RAS belongs to the family of small guanosine triphosphatase (GTPase) proteins. Rodenhuis et al. first reported an association between *KRAS* mutations and NSCLC. They studied 39 NSCLC samples for the presence of *NRAS*, *KRAS,* and *HRAS* mutations or amplifications and concluded that mutational *KRAS* activation may be an important early event in the pathogenesis of ADC of the lung.[16] They also showed that *KRAS* mutations were present in more than 30% of the ADCs and was more frequent in smokers.[17] Studies in ES-NSCLC report that *KRAS* mutations, especially at codon 12, are associated with worse progression-free survival (PFS) and OS.[18,19] Slebos et al.[20] were the first to show that differences in PFS and OS in patients with ES-NSCLC with and without *KRAS* mutations were significant ($p = 0.038$ and $p = 0.001$). The prognostic significance of *KRAS* in NSCLC was evaluated in a combined analysis of eight studies with a total of 881 patients. *KRAS* mutations were detected in 25% of the cases and involved codons 12, 13, and 61 of the *KRAS* gene. For the *KRAS* mutant group, the relative risk for mortality was 2.35 (95% CI, 1.61–3.22), compared with patients with wild type *KRAS*. However, these studies were heterogeneous and there were no adjustments for other clinical variables.[21] In recent studies, the relevance of different amino acid substitutions in *KRAS* has been analyzed. Preclinical and retrospective data point out the importance of specific *KRAS* mutations on the prognosis of NSCLC, such as G12C or G12V in contrast to other substitutions.[22] Despite the data presented above, the prognostic significance of *KRAS* remains controversial. In an analysis involving 300 patients with ES-NSCLC with tumors harboring *KRAS* mutations enrolled in four adjuvant trials, the presence or absence of mutations in *KRAS* codon 12 did not confer a survival disadvantage in the observation arms of these trials.[23]

PROGNOSTIC AND PREDICTIVE BIOMARKERS

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase cell surface receptor family. Mutations in the gene encoding this protein results in constitutive activation and amplification of intracellular signals which lead to proliferation, invasion, and migration of the cancer cells.[24] The overall implications of the presence of *EGFR* mutations or amplification in ES-NSLC are not well defined. Rusch et al.[25] detected *EGFR* overexpression by IHC in 74 (71%) of 96 ES-NSCLC tumor samples. However, there was no association with OS. In a separate study, 53 ES-NSCLC tumor samples (79% ADC) were analyzed for the presence of *EGFR* mutations in exon 19 and 21 by polymerase chain reaction (PCR), and 32% samples were found to be harbor mutations. Presence of an *EGFR* mutation was identified as a favorable prognostic factor, with 5-year OS of 92% for *EGFR*-mutated versus 57% for EGFR wild-type tumors ($p = 0.037$).[26] The same group reported a retrospective analysis of 180 patients with either *KRAS* codon 12 mutation or *EGFR* mutation (exons 18–21). This study showed that the presence of an *EGFR* mutation was associated with longer OS ($p = 0.048$). However, there was no impact on PFS.[27] Liu et al.[28] examined 130 ES-ADC samples for *EGFR* mutations in exon 19 and 21 by nested PCR, and detected mutations in 44.3% samples. Presence of an *EGFR* mutation did not have an impact on median PFS (36.6 months for *EGFR*-mutated versus 25.7 months for *EGFR* wild-type tumors; $p = 0.56$). A large retrospective study reported the outcome of 1118 patients with resected ES-NSCLC of whom 20% had an *EGFR* mutation. The presence of an *EGFR* mutation correlated with longer OS (HR, 0.51; $p < 0.001$). A subgroup analysis was conducted in from a different dataset of 286 resected ES-NSCLC ADCs harboring EGFR mutations to determine the effect of adjuvant EGFR tyrosine kinase inhibitor (TKI) therapy. Among 286 patients receiving adjuvant TKI Cox regression analysis demonstrated a significant improvement in PFS (HR, 0.43; $p = 0.001$), but no significant differences in OS.[29]

Although, as illustrated above multiple retrospective analyses demonstrate improved survival in patients with completely resected *EGFR*-mutated ES-NSCLC, definitive conclusions can only be drawn by conducting large prospective clinical trials in this patient population.

Her2

Her2, a receptor tyrosine kinase and a member of the EGFR family is overexpressed in 20% of the advanced NSCLC and mutated in less than 2%.[30,31] In ES-NSCLC, studies suggest that overexpression of *Her2* mRNA or protein is associated with an unfavorable prognosis.[32,33] In a retrospective study, 239 tumor samples of patients with ES-NSCLC were evaluated for *Her2* overexpression, which was defined as an IHC score of 2+/3+ (scoring based on staining intensity and the number of cells stained). *Her2* overexpression was detected in 18% of the tumors. The relapse rate for *Her2*-positive versus *Her2*-negative tumors was 60% versus 33%, respectively ($p = 0.03$) in the absence of adjuvant treatment.[34]