

# Molecular biologic substaging of non-small cell lung cancer

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The staging system for non-small cell lung cancer (NSCLC) provides a framework for the assessment of prognosis and the assignment of therapy for all patients with a new diagnosis of lung cancer, estimated to be 169,500 in the United States in 2001.<sup>1</sup> The most recent revision of the lung cancer staging system, which considers the size and location of the primary tumor (T), the involvement of regional lymph nodes (N), and the presence of distant metastases (M), is based on the analysis of a collected database representing all clinical, surgical-pathologic, and follow-up information for 5319 patients treated for primary lung cancer.<sup>2</sup> Similar results have been reported for a population of 6670 patients treated in Japan.<sup>3</sup>

The power of these large databases in predicting prognosis is self-evident. Nevertheless, the inherent inaccuracy of the staging process should be brought to attention. According to the TNM system, the predicted 5-year survival after complete resection for T1 N0 M0 NSCLC (stage IA) is only 67%.<sup>1</sup> Therefore 33% of patients with putative stage IA NSCLC have incorrectly staged disease and will die of it, predominantly from the development of metastatic disease not detected at the time of diagnosis and initial therapy.

Molecular biologic staging is the assessment of tumor markers associated with various oncogenic mechanisms to improve the risk stratification provided by conventional TNM staging. Biologic staging may target oncogenes, oncogenic protein products, growth factors, or receptors. The biologic techniques used include antibody-directed assessment of markers (immunohistochemical assay) and direct genetic sequencing with single-strand conformational polymorphism or reverse transcriptase-polymerase chain reaction. Molecular biologic staging may be applied to the primary tumor, lymph nodes, bone marrow, or serum and used to establish the diagnosis of malignancy at earlier stage, to assess prognosis, to detect occult metastases, and to predict chemotherapy resistance.

The purpose of the assessment of prognostic markers in the primary tumor is to discriminate patients, or groups of patients, with early-stage disease whose risk of recurrence is sufficiently high to justify adjuvant therapy. The use of a panel of markers may improve the effectiveness of this approach, because expression of individual oncogenic markers is low in NSCLC: p53 and epidermal growth factor receptor are expressed in approximately 43% and 52% of tumors, respectively.<sup>4</sup> Studies that evaluate molecular prognostic variables must be limited to early-stage disease; the inclusion of patients with advanced-stage disease dilutes the potential prognostic value of the markers, because that subgroup of patients would have a dismal prognosis regardless of marker status.<sup>4,5</sup> In a study of patients with stage I disease, molecular substaging discriminated groups of patients with 5-year survivals ranging from 37% (5 negative prognostic markers) to 80% (1 negative prognostic marker).

Molecular markers that are assessed to detect the presence of occult metastases in lymph nodes, bone marrow, or serum should have a high prevalence, and the techniques to measure these marker must have high sensitivity and specificity. The intrinsic prognostic value of the markers as related to the primary tumor is irrelevant; the

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diagnostic or prognostic value of these markers is determined by their presence in distant sites, suggesting occult metastatic disease. Currently the most effective markers to detect occult metastases are epithelial markers, such as cytokeratins, which are present in 70% to 80% of tumors but are not normally present in lymph nodes, bone marrow, or serum.<sup>6-8</sup>

The ability of molecular biologic markers to predict results of chemotherapy would enable the clinician to design therapy for the individual tumor. In addition, identifying and understanding the mechanisms of treatment resistance offers another pathway to intervene, by blocking or reversing the mechanism of resistance. Furthermore, the understanding of the molecular mechanism of receptor activity and DNA repair enables the study of pharmacologic targeting with chemotherapy or biologic agents, such as epidermal growth factor receptor antibodies or tyrosine kinase inhibitors.<sup>9</sup>

The study by Ahrendt and colleagues<sup>10</sup> in this issue of the *Journal* uses molecular techniques to detect occult micrometastases in the lymph nodes of patients with completely resected stage I NSCLC and attempts to correlate the presence of micrometastatic disease with prognosis.<sup>10</sup> Contrary to most similar studies, this report did not find that the presence of occult lymph node metastases altered survival. Ahrendt and colleagues chose two gene markers, *K-ras* and *TP53*, which have prevalences of 44% and 52%, respectively; the ability to detect micrometastatic disease with markers present in only half of the patients limits the utility of this technique. The ideal marker for detecting occult metastatic disease would be present in nearly all tumors and would be undetectable in the lymph nodes, bone marrow, and serum of control subjects.

Molecular biologic substaging of patients with stage I NSCLC may have the potential to alter therapy, in addition to improving risk stratification. In a recent study the prognostic value of the molecular markers was more powerful than that of lymph node involvement.<sup>11</sup> The effectiveness of chemotherapy for patients with stage IB NSCLC is being evaluated in an ongoing Cancer and Leukemia Group B (CALGB) protocol, CALGB 9633. In this study, patients with completely resected stage IB NSCLC are randomly assigned to receive postoperative chemotherapy (carboplatin and paclitaxel) or observation; all patients in the study will have tumors analyzed for a panel of molecular markers to determine the prognostic significance of the markers with respect to chemotherapy.

The ultimate power of molecular biologic substaging depends on the ability to alter therapy and improve outcome, which has not yet been demonstrated. With current technology, however, it would be possible to perform a

biopsy on a patient with clinical stage I NSCLC and determine the relative prognosis on the basis of the molecular substaging. Patients with strong negative prognostic markers and patients with occult metastases in the bone marrow or serum might be treated with induction biologic therapy or chemotherapy; furthermore, the choice of agents would be determined by the biologic characteristics of the tumor. Other applications of biologic staging include sentinel lymph node assessment, which would allow pathologists to focus more intensely on a limited amount of tissue to detect occult metastatic disease.<sup>12</sup> This strategy will become even more accurate with the development of real-time reverse transcriptase-polymerase chain reaction, enabling the intraoperative analysis of genetic mutations. In the near future it is possible that patients with NSCLC will have their disease staged and treated according to a TNMB staging system: tumor, nodes, metastases, and biology.

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