Reflex testing of resected stage I through III lung adenocarcinomas for *EGFR* and *KRAS* mutation: Report on initial experience and clinical utility at a single center

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Objective: The genes *KRAS* and *EGFR* have emerged as potential targets for therapy in lung adenocarcinoma; mutations in these genes can be found in almost half of patients. In anticipation of the clinical importance of molecularly defined adenocarcinoma subgroups for the treatment of patients with resected stages I through III lung adenocarcinoma, the Memorial Sloan–Kettering Cancer Center (MSKCC) Departments of Surgery and Pathology have collaborated since 2006 to conduct reflex testing of tumor specimens for *EGFR* and *KRAS* mutations.

Methods: Using established methods, the identification of *EGFR* exon 19 deletions and exon 21 L858R mutations was performed. In samples lacking these 2 sensitizing *EGFR* mutations, *KRAS* analysis was done.

Results: We studied a total of 1831 patients who had stage I through IV lung adenocarcinomas and detected 448 *KRAS* and 364 *EGFR* mutations. Of these patients, a subset of 855 (78%) patients with stages I through III adenocarcinoma of the lung who underwent curative surgical resection at MSKCC were tested. In patients with early stage disease, 158 *EGFR* mutations and 207 *KRAS* mutations were detected.

Conclusions: The results of the first 3 years of reflex testing at MSKCC reported here demonstrate the feasibility, clinical utility, and potential of this approach. This information allowed for enrollment of patients into clinical trials to explore mutation-specific, directed therapy and led to retrospective studies related to patient outcome. In addition, it may inform selection of chemotherapy for recurrent disease and may help to distinguish multiple primary tumors from metastatic disease. (J Thorac Cardiovasc Surg 2011;141:476-80)

Despite successful surgery, half of patients with resected non–small-cell lung cancer (NSCLC) recur and die within 5 years.[1] In patients with resected stage II through III NSCLC, 4 months of cisplatin-based chemotherapy improves survival rates by nearly 20% (relative risk reduction), with an absolute risk reduction of 10% to 13%.[2] Pathologic stage is the only prospectively validated clinical factor used to select patients for adjuvant chemotherapy.[3] More effective adjuvant therapies are sorely needed. The most promising molecular markers for the selection of treatment are somatic mutations in lung adenocarcinomas.

Epidermal growth factor receptor (*EGFR*) is a signaling protein attached to the cell membrane. Ligand binding to the extracellular portion of the *EGFR* protein leads to increases in cellular proliferation, motility, adhesion, invasion, blocking of apoptosis, and resistance to chemotherapy.[4] Erlotinib is an *EGFR* tyrosine kinase inhibitor currently approved for patients with metastatic NSCLC. Of all clinically relevant *EGFR* mutations, 90% are either missense mutations in exon 21 or deletions in exon 19.[5] Patients with deletions in exon 19 have better prognoses than patients with missense mutations in exon 21 when treated with erlotinib and gefinitib.[6] *EGFR* mutations are more common in people who have never smoked, in Asians, and in women with adenocarcinoma.[7] Patients with advanced NSCLC and *EGFR* exon 19 or 21 mutations have been noted to have high rates of radiologic response to gefitinib.[8] A recent trial in East Asian nonsmokers or former light smokers with stage IV adenocarcinoma evaluated gefitinib against carboplatin and paclitaxel as first-line chemotherapy. In the subgroup of patients with *EGFR* mutations, patients treated with gefitinib had higher objective responses—71% as opposed to 1%—and longer progression-free survival.[9] These trials exemplify the importance of *EGFR* mutations as predictors of response and clinical benefit from tyrosine kinase inhibitors in patients with stage IV lung adenocarcinoma.

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Abbreviations and Acronyms

ACCP = American College of Chest Physicians
NSCLC = non–small-cell lung cancer
MSKCC = Memorial Sloan–Kettering Cancer Center
PCR = polymerase chain reaction

KRAS genes encode guanosine triphosphate-binding proteins that regulate cell growth, differentiation, and apoptosis.[10] *KRAS* mutations occur in 20% to 30% of lung adenocarcinomas.[11] More than 80% occur in exon 2 of *KRAS* in codon 12, with the remaining mutations occurring in codons 13 and 61. Although detected in some who have never smoked, they are found more often in current and former cigarette smokers and have been associated with poor prognoses in several studies.[12,13] In stage IV patients, *KRAS* mutations predict lack of response to *EGFR* tyrosine kinase inhibitors and lower efficacy of chemotherapy when combined with erlotinib.[14] The results of a prospective clinical trial of adjuvant chemotherapy for resected stage IB-II NSCLC did not demonstrate a benefit from adjuvant chemotherapy in the *KRAS* mutant subgroup (HR, 0.95; 95% CI; $P = .87$).[15] At MSKCC, routine reflex testing for *EGFR* and *KRAS* mutations was instituted for all patients with resected lung adenocarcinomas in 2006. Justification for this reflex testing is outlined in Table 1. Although it does not guide standard practice, the molecular information has been used to enroll patients in clinical trials of novel adjuvant therapy specifically targeting *EGFR* and *KRAS*.[3] Knowing that as much as 50% of patients with resected NSCLC would experience recurrence, we believed that the results of the molecular tests could inform the selection of chemotherapy for recurrent disease. *EGFR/KRAS* testing has been used to assist in distinguishing multiple primary tumors from metastatic tumors.[16]

Resected specimens routinely provide excess tissue for molecular profiling. In contrast, patients with advanced NSCLC are usually diagnosed with a fine needle aspirate, limiting available tissue for analysis. Universal testing of all resected lung adenocarcinomas for *EGFR* and *KRAS* mutations has allowed for unique research efforts, including retrospective studies related to outcome and prospective studies of novel agents. Here we report the results of the first 3 years of reflex testing conducted by the pathology department of our institution.

MATERIALS AND METHODS

We tested all patients who underwent lung resection because of adenocarcinoma. *EGFR/KRAS* mutations are extremely uncommon in large cell carcinomas, small cell carcinomas, and squamous cell carcinomas.[17] At the time of gross prosection, a slice of tumor was frozen and retained along with a corresponding section for paraffin embedding and hematoxylin and eosin (H&E) staining. Initially, DNA was extracted from frozen tissue. After observing that several cases lacked sufficient tumoral DNA when the frozen tissues were used, we modified the process and instead extracted DNA from formalin-fixed paraffin-embedded tissue to ensure that a sufficient percentage of tumor cells (target >50%) was present in the tissue from which the DNA was prepared. After microscopic examination confirmed the diagnosis of adenocarcinoma, tissue was sent to our CLIA-certified molecular diagnostic laboratory in the Department of Pathology for extraction of DNA and identification of *EGFR* exon 19 deletions and exon 21 L858R mutations by polymerase chain reaction (PCR) assay. Nonsequencing-based PCR assays are used to detect these mutations.[18] In samples lacking these 2 sensitizing *EGFR* mutations, *KRAS* analysis is done by direct sequencing of exon 2 using PCR products. Molecular profiling for *EGFR/KRAS* has been approved by the New York State Department of Health. A molecular diagnostic report is created and forwarded to the patient's medical record for inspection by the patient's surgeon and medical oncologist (Figure 1).

RESULTS

Between 2006 and April 2009, tumors of 1831 patients with stage I through IV adenocarcinoma of the lung underwent reflex testing, and 448 *KRAS* and 364 *EGFR* mutations were detected. Of those patients, 1097 with stages I through III adenocarcinoma of the lung underwent curative surgical resection at MSKCC. A subset of 855 patients (78%) were tested. In the patients in the early stages, 158 *EGFR* mutations and 207 *KRAS* mutations were detected (Table 2). The group included 291 males and 564 females; 594 patients were in stage I; 111 were in stage II; and 147 were in stage III.

In 2006, 124 patients underwent mutation testing; 19% had *EGFR* mutations, and 23% had *KRAS* mutations (Table 3). In 2007, 324 patients underwent mutation testing; 14% had *EGFR* mutations, and 20% had *KRAS* mutations. In 2008, 293 patients underwent mutation testing; 25% had *EGFR* mutations, and 30% had *KRAS* mutations. As of April 2009, 114 patients have undergone mutation testing; 25% have *EGFR* mutations and 30% have *KRAS* mutations (Table 3). Already, 15% of these patients have experienced recurrences, and genetic information from their resected specimens has been used to select therapy.

DISCUSSION

Reflex testing of adenocarcinomas for *EGFR* and *KRAS* mutations at MSKCC over the past 3 years reveals some interesting observations. Nearly 78% of patients with early-stage disease have undergone mutation testing.

The majority of patients with resected adenocarcinoma are females, at a nearly 2 to 1 ratio. It is unclear whether this trend is the result of demographics unique to our patient population, is related to the preponderance of adenocarcinoma in females versus males, or is related to differing smoking habits in females and males. This sex distribution is not seen in our advanced-stage patients; in a database of 1081 cases of stage IV adenocarcinomas, 59% were women. It is interesting that in a recent report concerning a large group of patients that was screened for lung cancer, it was found that the prevalence of screen-detected early