Sample Features Associated with Success Rates in Population-Based *EGFR* Mutation Testing

Carolyn J. Shiau, MD, FRCPC,† Jesse P. Babwah, MD, FRCPC,*
Gilda da Cunha Santos, MD, PhD, FRCPC, Jenna R. Sykes, MMath,‡ Scott L. Boerner, MD, FRCPC,*
William R. Geddie, MD, FRCPC, Natasha B. Leighl, MD, MMedSc, FRCPC,§ Cuihong Wei, PhD,*
Suzanne Kamel-Reid, PhD, David M. Hwang, MD, PhD, FRCPC,* and Ming-Sound Tsao, MD, FRCPC**

Introduction: Epidermal growth factor receptor (*EGFR*) mutation testing has become critical in the treatment of patients with advanced non–small-cell lung cancer. This study involves a large cohort and epidemiologically unselected series of *EGFR* mutation testing for patients with nonsquamous non–small-cell lung cancer in a North American population to determine sample-related factors that influence success in clinical *EGFR* testing.

Methods: Data from consecutive cases of Canadian province-wide testing at a centralized diagnostic laboratory for a 24-month period were reviewed. Samples were tested for exon-19 deletion and exon-21 L858R mutations using a validated polymerase chain reaction method with 1% to 5% detection sensitivity.

Results: From 2651 samples submitted, 2404 samples were tested with 2293 samples eligible for analysis (1780 histology and 513 cytology specimens). The overall test-failure rate was 5.4% with overall mutation rate of 20.6%. No significant differences in the failure rate, mutation rate, or mutation type were found between histology and cytology samples. Although tumor cellularity was significantly associated with test-success or mutation rates in histology and cytology specimens, respectively, mutations could be detected in all specimen types. Significant rates of *EGFR* mutation were detected in cases with thyroid transcription factor (TTF)-1–negative immunohistochemistry (6.7%) and mucinous component (9.0%).

Conclusions: *EGFR* mutation testing should be attempted in any specimen, whether histologic or cytologic. Samples should not be excluded from testing based on TTF-1 status or histologic features. Pathologists should report the amount of available tumor for testing. However, suboptimal samples with a negative *EGFR* mutation result should be considered for repeat testing with an alternate sample.

Key Words: *Epidermal growth factor receptor*, Lung cancer, Fragment analysis, Cytology, TTF-1.

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The discoveries of genetic aberrations including mutations involving epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase[1,2] as drivers of tumorigenesis in non–small-cell lung cancer (NSCLC) has established the basis for personalized medicine for patients with lung cancer. The availability of agents for effective targeted therapies has led to a growing interest in the molecular classification of lung cancer. Specific mutations in the tyrosine kinase domain of *EGFR* (exons 18 to 21) sensitize patients to tyrosine kinase inhibitors with high specificity against *EGFR*, such as erlotinib and gefitinib.[3,4] Exon-19 deletions and exon-21 L858R substitution[5,6] represent approximately 90% of *EGFR* mutations that are sensitive to *EGFR* tyrosine kinase inhibitors. Mutations occur most often in nonsquamous NSCLC at rates of 10% to 50%, depending on patient characteristics, including smoking status, ethnicity, and tumor histology.[1,7–10] Attempts to streamline mutation testing by clinicopathological characteristics to predict mutation status have not been accepted as sufficiently discriminating to apply at the clinical level.[11–13]

More than 70% of patients with lung cancer present at advanced stage and are unresectable.[11,14] Therefore, the most common approach to acquire tissue to establish a diagnosis is histologic core-needle or cytologic fine-needle biopsy (FNB).[15] Many testing centers use polymerase chain reaction (PCR) followed by direct nucleic acid sequence analysis (Sanger sequencing) to establish mutation status. A recognized pitfall of this method is the significant false-negative rate (up to 30%) due to the requirement for high tumor cellularity.[16–18] Thus, many techniques to increase sensitivity for detecting mutant sequences have been increasingly used in clinical settings, including allele-specific PCR or matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry approaches.[11,17–23]

Although *EGFR* mutation testing may be routinely conducted on patients seen in large academic institutions,

*Department of Pathology, University Health Network, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; †Department of Pathology, Royal Columbian Hospital, New Westminster, British Columbia, Canada; ‡Department of Biostatistics, University Health Network, and Princess Margaret Cancer Centre, Toronto, Ontario, Canada; and §Division of Hematology and Medical Oncology, Princess Margaret Cancer Centre, and Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

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Address for correspondence: Ming-Sound Tsao, Department of Pathology, University Health Network, 200 Elizabeth Street, Toronto, ON, Canada M5G 2C5. E-mail: ming.tsao@uhn.ca

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tissue material for testing is often available from nonacademic centers with a primary focus on establishing diagnosis. A centralized testing model for specimens from various institutions provides a cost-effective and patient-centered approach to avoid a second procedure to obtain additional tissue for molecular tests. In addition, examining tumor samples from eligible patients with lung cancer in a defined geographic area offers an opportunity to collect large-scale North American population-based data on yield of testing and mutation rates in various sample types, currently not available in the literature. In 2010, a Pan-Canadian coordinated effort was initiated to make *EGFR* mutation testing available for patients being considered for first-line gefitinib treatment, separate from anaplastic lymphoma kinase testing. To ensure uniformity in testing of high case volumes, five centralized testing centers were chosen, including University Health Network (UHN) Molecular Diagnostics Laboratory for patients in Ontario.

This study examines retrospectively the sample-related characteristics that correlate with test success and allelic mutation frequency rates specifically for *EGFR* in an epidemiologically unselected patient population, with the goal of defining parameters that may help streamline and set current benchmarks for *EGFR* mutation testing.

PATIENTS AND METHODS

Patients and Samples

The results used in this analysis are from consecutive *EGFR* mutation testing conducted at the UHN through the EGFR Canada program for 24 months (March 16, 2010 to March 14, 2012), according to data analysis protocol approved by the UHN Research Ethics Board (Figure 1S, Supplementary Digital Content 1, http://links.lww.com/JTO/A602). The testing program was established for all patients with locally advanced or metastatic nonsquamous NSCLC in the province of Ontario (population of approximately 13 million and approximately 19% Asian), who may be eligible for first-line gefitinib treatment in Canada (Figure 2S, Supplementary Digital Content 2, http://links.lww.com/JTO/A603). Data were collected from the UHN pathology CoPath database and correlated with patient characteristics from the *EGFR* Canada database when available (Table 1S, Supplementary Digital Content 3, http://links.lww.com/JTO/A604).

Preanalytical Data Available

Standard protocol for *EGFR* mutation (Figure 1S, Supplementary Digital Content 1, http://links.lww.com/JTO/A602) testing included an initial review of the hematoxylin-eosin (HE)–stained section, prepared at the same time as unstained sections for DNA isolation, from the submitted tumor block. The slides and reports were reviewed by a pulmonary pathologist (DMH and M-ST) or cytopathologist (GdCS, SLB, and WRG). Sample-related parameters available in original reports or as assessed by pathologists were recorded (see Supplementary Materials, Supplementary Digital Content 3, http://links.lww.com/JTO/A604). For histology samples, pathologists marked the tumor areas on the HE section to guide macrodissection by the molecular laboratory technologists (Fig. 1). Cases with inadequate remaining tumor (no tumor cells remaining on HE section), incorrect tumor type (e.g., squamous), or duplicate specimen were excluded from further testing.

EGFR Mutation Testing

Mutation testing was conducted using fragment analysis (exon-19 deletions) and restriction fragment length polymorphism (exon-21 L858R) methods (see Supplementary Materials, Supplementary Digital Content 3, http://links.lww.com/JTO/A604).[24,25] The detection limit has been established at 1% to 5% by serial dilutions of relevant cell line DNA.[25] A reagent control, negative control, and two positive controls were included with each run.[20,24–26] All test results were reviewed by a molecular geneticist (SK-R and CW). Final test results were reported as (1) positive for exon-19 deletion, (2) positive for exon-21 L858R mutation, (3) negative for exon-19 deletion or exon-21 L858R mutation, or (4) inconclusive. With the latter result, the originating pathologist or medical oncologist was encouraged to submit an alternate tumor sample for further testing.

Statistical Analysis

Variables examined for significance (see Supplementary Materials, Supplementary Digital Content 3, http://links.lww.com/JTO/A604) included sex, age, Asian ethnicity, smoking status, clinical stage, specimen type, anatomical site, diagnosis, mucinous component, TTF-1 immunohistochemistry (IHC), tumor cellularity, and laboratory of origin. Additional variables were recorded for histology (histologic subtype, differentiation, and macrodissection area) and cytology samples (cell content, fixative, and necrosis). Factors that predicted for *EGFR* mutation and test success were analyzed using multivariate logistic regression with a backward selection algorithm, using odds ratios calculated for test success versus test failure or mutation positive versus wild type. A Wilcoxon signed rank test was used to compare tumor cellularity in matched samples. Optimal cutpoints for tumor cellularity and macrodissection area were chosen by identifying the smallest p value for test failure, with the condition of a minimum of 10% of data per subgroup.

Because of the absence of complete data for every case, a multiple imputation procedure was used to generate values for missing data.[27,28] A separate multivariate analysis was performed on cases with complete data sets ($n = 1808$) for comparison, showing the same selection of significant variables for test success and *EGFR* mutation. Thus, the multiple imputation data are presented to include the larger sample size. A two-sided p value of 0.05 was used to assess statistical significance. All analyses were performed using R software.[29]

Excluding samples representing multiple tests on the same lesion due to a first failed attempt potentially adds bias to the test-success rate. However, with the first failed sample included in the test-success model, the overall conclusions found were identical. Thus, these samples and all other duplicate samples were excluded from analysis (Fig. 2).

RESULTS

Altogether 2651 consecutive samples were submitted for *EGFR* testing during the 24-month period (Fig. 2, and