Multi-institutional Oncogenic Driver Mutation Analysis in Lung Adenocarcinoma

The Lung Cancer Mutation Consortium Experience

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Introduction: Molecular genetic analyses of lung adenocarcinoma have recently become standard of care for treatment selection. The Lung Cancer Mutation Consortium was formed to enable collaborative multi-institutional analyses of 10 potential oncogenic driver mutations. Technical aspects of testing and clinicopathologic correlations are presented.

Methods: Mutation testing in at least one of the eight genes (*epidermal growth factor receptor* [*EGFR*], *KRAS, ERBB2*, *AKT1, BRAF, MEK1, NRAS,* and *PIK3CA)* using SNaPshot, mass spectrometry, Sanger sequencing+/− peptide nucleic acid and/or sizing assays, along with *anaplastic lymphoma kinase* (*ALK*) and/or *MET* fluorescence in situ hybridization, were performed in six labs on 1007 patients from 14 institutions.

Results: In all, 1007 specimens had mutation analysis performed, and 733 specimens had all 10 genes analyzed. Mutation identification rates did not vary by analytic method. Biopsy and cytology specimens were inadequate for testing in 26% and 35% of cases compared with 5% of surgical specimens. Among the 1007 cases with mutation analysis performed, *EGFR*, *KRAS, ALK*, and *ERBB2* alterations were detected in 22%, 25%, 8.5%, and 2.4% of cases, respectively. *EGFR* mutations were highly associated with female sex, Asian race, and never-smoking status; and less strongly associated with stage IV disease, presence of bone metastases, and absence of adrenal metastases. *ALK* rearrangements were strongly associated with never-smoking status and more weakly associated with presence of liver metastases. *ERBB2* mutations were strongly associated with Asian race and never-smoking status. Two mutations were seen in 2.7% of samples, all but one of which involved one or more of *PIK3CA, ALK*, or *MET*.

Conclusion: Multi-institutional molecular analysis across multiple platforms, sample types, and institutions can yield consistent results and novel clinicopathological observations.

Key Words: Lung adenocarcinoma, Mutation, FISH, Genotyping, LCMC.

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Molecular genetic testing is a central component of pathological analysis for several types of cancers. Although results formally reported in the medical record must be generated

in laboratories subject to Clinical Laboratory Improvement Amendments (CLIA) certification, the degree of interlaboratory variation with regard to molecular pathology methods and results remains poorly characterized outside of laboratory proficiency testing and studies designed specifically to evaluate concordance. Molecular testing in patients with advanced lung cancer and other solid tumors presents unique challenges. The use of minimally invasive procedures to obtain tissue for diagnosis often limits available tumor material for molecular testing. Variable preanalytic methodology introduces the potential for poor nucleic acid preservation in formalin-fixed, paraffin-embedded tumor samples. Furthermore, the continuing identification of new driver mutations can lead to repetitive testing of the same sample exhausting the material available.

Molecular genetic testing became central to the clinical management of advanced lung adenocarcinoma (ACA) after the discovery of a strong association between activating *epidermal growth factor receptor* (*EGFR*) mutations and clinical response to targeted EGFR tyrosine kinase inhibitors in 2004.[1–3] This was followed by the identification of rearrangements of the anaplastic lymphoma kinase (*ALK*) gene in lung ACA in 2007, which are in turn uniquely sensitive to treatment with ALK kinase inhibitors.[4,5] *EGFR* mutation analysis and *ALK* fluorescence in situ hybridization (FISH) are now guideline-recommended standard-of-care at the time of diagnosis for advanced lung ACA to inform the initial systemic treatment.[6] Ongoing recognition of potentially targetable oncogenic drivers in lung ACA[7] indicates a need for efficient multiplexed analyses. Indeed, many institutions in the United States and worldwide have implemented routine analyses of multiple genes in lung ACA.[8–10] A growing number of commercial and academic institutions are implementing next generation sequencing of large gene panels as a more efficient approach to molecular testing across multiple cancer types.[11–13]

The Lung Cancer Mutation Consortium (LCMC) was established in 2008 as a multi-institutional program investigating the frequency of selected oncogenic drivers in lung ACA and using the results to treat the enrolled subjects with targeted therapies, either as part of standard clinical care or on investigational protocols. Fourteen institutions participated in the LCMC and either performed testing locally or utilized another LCMC site. Analytical methods at testing sites were left up to each institution, as long as they met CLIA standards.

The primary results of the LCMC study have recently been reported.[14] Here, we provide additional information on methods used at the different institutions, results of blinded proficiency testing, effects of sample type and testing platform on assay success and mutation detection rates, and validation of mutations identified in lung cancer specimens with more than one putative driver alteration. Further, we examine sample failure rates and present a correlation between the presence of oncogenic driver mutations and clinicopathologic findings.

MATERIALS AND METHODS

Patient Recruitment and Enrollment

Fourteen clinical sites participated in the LCMC (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A814). All participating sites obtained local Institutional Review Board approval for participation in this study. Patients with stage IV or recurrent lung ACA; Southwest Oncology Group performance status of 0, 1, or 2; expected survival of more than 6 months; and adequate tissue for molecular analyses were eligible for entry on this study. One thousand five hundred and forty-two patients were enrolled, and 1102 were deemed eligible. The most common reason for ineligibility was inadequate pathologic material to complete the multiplexed testing (n = 286 of 440 ineligible; 65%). Epidemiologic and clinicopathologic data were collected on these subjects, including age, sex, race, smoking history, stage at diagnosis, metastatic sites, and survival.[14]

Pathology Evaluation

Anatomic pathologists at each institution confirmed a diagnosis of lung adenocarcinoma, assessed tumor content, and determined specimen adequacy based on analytic sensitivity of their testing platform (Table 1). Samples were enriched for tumor content using manual microdissection. Central confirmation of lung ACA diagnosis was based on review of an hematoxylin and eosin-stained histology slide or a scanned image (Aperio, Vista, CA), by I.I.W., J.F., or W.A.F. At the time of central review, expert pathologists enumerated percentage of each histologic pattern, including lepidic, acinar, papillary, micropapillary, solid, and variants (mucinous, colloid, fetal, and enteric, as appropriate), according to the current criteria.[15]

Among the 1102 eligible patients, 1015 were confirmed as ACA histology and 2 as adenosquamous carcinoma by central pathology review. In 85 cases, slides were not provided for central review. Among cases with confirmed histology, at least one molecular assay was performed in 1007 cases. Small mutations were defined as single nucleotide variants and small insertion/deletion (indel) mutations. Testing for at least one small-mutation gene (eight genes, see below) was performed for 989 cases, *ALK* FISH testing was performed in 926 cases, and *MET* FISH testing in 833. The 10-marker panel including small mutation and FISH testing was completed in its entirety for 733 patients.

Mutational Analyses

The vast majority of the mutation analyses were performed in six diagnostic laboratories, using methods summarized in Table 1. The complete panel of small mutations consisted of four small indels and 93 point mutations occurring in eight genes (*AKT1*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MAP2K1*, *NRAS*, and *PIK3CA*; Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/JTO/A814). Because of variability in testing platforms, not all mutations were evaluated at all sites, but every site tested at least half of the complete set of mutations.

Three different methodologies were used for genotyping, and the analytic sensitivities for the major testing laboratories (defined as those testing ≥ 4% of the total cases) are shown in Table 1. The methods for all mutational analyses have been previously published.[15–17] Briefly, SNaPshot (Life Technologies, Grand Island, NY) is a multiplex polymerase

