Yield and Clinical Utility of Next-Generation Sequencing in Selected Patients With Lung Adenocarcinoma

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Abstract

Next-generation sequencing is now available for assessing genomic alterations in lung adenocarcinoma, although the performance characteristics and clinical utility is not widely known. Next-generation sequencing was performed on 49 consecutive non–small-cell lung cancer (NSCLC) specimens targeting 255 cancer-associated genes. Extended sequencing was performed successfully in 41 (83.7%) cases of NSCLC cases using a range of specimens. Clinically relevant genomic alterations were commonly found.

Background: Next-generation sequencing is available for assessing genomic alterations in non–small-cell lung cancer (NSCLC), although the performance characteristics and clinical utility has not been well characterized. This technique can be used to sequence hundreds of known cancer-associated genes. Our aim was to investigate the diagnostic success and clinically relevant results of extensive sequencing in NSCLC patients. **Patients and Methods:** A case series of 49 NSCLC patients was used to determine the success of extended next-generation sequencing, record genomic alterations, and evaluate clinical utility. Data were collected in a retrospective review. Sequencing was performed using a hybridization capture of 3320 exons from 236 cancer-related genes and 47 introns of 19 genes applied to ≥50 ng of DNA and sequenced to high, uniform coverage of 622 times. **Results:** Sequencing was successful in 29 of 32 (91%) surgical/excisional specimens, and 12 of 17 (71%) nonsurgical specimens including an endoscopic forceps biopsy, core needle biopsies, fine-needle aspirates, and effusion cytologies. All 5 transthoracic core needle biopsies failed. A total of 179 genomic alterations (average 4.37 per tumor) were found. A total of 63 were clinically relevant (average 1.54 per tumor). The most frequently mutated genes were tumor protein p53, cyclin-dependent kinase inhibitor 2A, megalencephalic leukoencephalopathy with subcortical cysts 1, rapamycin-insensitive companion of mammalian target of rapamycin, epithelial growth factor receptor, SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4, cyclin-dependent kinase inhibitor 2B, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α, Kirsten rat sarcoma viral oncogene homolog, Erb-B2 receptor tyrosine kinase 2, Serine/Threonine Kinase 11, and NK2 Homeobox 1. Sequencing results led to a change in management in 7 of 49 cases (14.3%). **Conclusion:** Extended next-generation sequencing was performed successfully in 41 (83.7%) cases of NSCLC using a range of pathology specimens. Testing had the potential to affect treatment decisions in selected patients.

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Introduction

Lung cancer remains the leading cause of cancer mortality, with the number of estimated deaths in the United States exceeding 158,000 in 2015, representing nearly 27% of all cancer deaths.[1] Approximately 85% of these malignancies will be non–small-cell lung cancer (NSCLC), with adenocarcinoma subtype accounting for < 50%.[2] Some lung adenocarcinomas have discrete genetic alterations that have potential prognostic significance and present novel therapeutic targets.[3] Epithelial growth factor receptor (*EGFR*) mutations, Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations, and translocations in anaplastic lymphoma receptor tyrosine kinase (*ALK*) were among the earliest important genomic alterations identified in lung adenocarcinoma but the list has grown significantly in the past 5 years.[4-6]

The 2013 College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guidelines endorsed by the American Society of Clinical Oncology recommend *EGFR* and *ALK* testing for all patients with advanced stage lung adenocarcinoma, and the current National Comprehensive Cancer Network guidelines also include testing for ROS proto-oncogene 1 (*ROS1*), rearranged during transfection tyrosine kinase, v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), Erb-B2 receptor tyrosine kinase 2 (*ERBB2*), and mesenchymal-epithelial transition factor receptor tyrosine kinase (*MET*) in this population.[7,8] This testing sets the stage for incorporating genotype-driven treatment for some patients with lung adenocarcinoma and has the potential to significantly affect cancer mortality.[9,10] However, it can be challenging to perform multiple single-gene molecular assays without depleting available tissue, especially in small biopsy and cytology specimens.

Next-generation DNA sequencing has been introduced as an efficient, fast, complete, and accurate method to identify targetable driver mutations that can be integrated into routine clinical practice.[11,12] This technique can be used to sequence the tumor DNA of hundreds of known cancer-associated genes, referred to as comprehensive genomic profiling (CGP). This testing modality can be used in small cytologic specimens as well as in larger surgical specimens, but the yield in these specimens remains unknown, and no standards exist for optimizing specimen acquisition and specimen handling. Efforts by surgical pathologists and cytopathologists to maximize the amount of tumor DNA for CGP is an evolving area of practice that also has yet to be standardized.[11] We describe our experience with CGP in a series of 49 cases submitted to Foundation Medicine, Inc (Cambridge, MA) for CGP from NSCLC patients at a single institution using a broad range of pathology and cytology specimens obtained using various techniques to clarify expectations for its clinical utility.

Patients and Methods

We retrospectively reviewed the records of a series of 49 patients with NSCLC at Columbia University Medical Center for whom tumor samples were submitted for CGP at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited reference laboratory (Foundation Medicine, Inc) between 2012 and 2014. Extended genetic testing was specifically requested by the treating oncologist in these cases in an attempt to identify targetable driver mutations. After the oncologist's request, existing biopsy samples were assessed for adequacy by a lung cancer pathologist at Columbia University using CGP-specific specimen preparation guidelines. Optimal tissue is either a single formalin-fixed and embedded paraffin block with a surface area of at least 25 mm^3 or 10 unstained slides plus 1 hematoxylin and eosin-stained slide. Before CGP, many of these same samples were tested internally with immunohistochemical staining and targeted molecular testing (single-gene, hotspot assays). All internal testing performed on fine needle aspiration specimens was performed on cell blocks as previously described.[13-15] Rapid on-site evaluation (ROSE) from pathology at the time of biopsy was performed for fine-needle aspirations using smears and core needle biopsies using touch imprints.

DNA was extracted at a central laboratory (Foundation Medicine, Inc) from tumor samples and was analyzed using hybridization capture of 3320 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer. At least 50 ng of DNA per specimen was isolated and sequenced to high, uniform coverage (mean 734 times) using the Illumina HiSeq2500 instrument (San Diego, CA), as previously described.[16,17] Genomic alterations (base substitutions, short insertions and deletions, focal gene amplifications, homozygous deletions, and select rearrangements) were determined and then reported for each patient sample. The test has been validated to detect base substitutions at $\geq$ 10% mutant allele frequency with $\geq$ 99% sensitivity and small insertions and deletions at $\geq$ 20% mutant allele frequency with $\geq$ 95% sensitivity, with a false discovery rate of < 1%.[16,17]

Clinically relevant genomic alterations (CRGAs) were defined broadly as those with a targeted US Food and Drug Administration (FDA)-approved anticancer drug available or those with a targeted drug in registered phase II or phase III clinical trials. The success of CGP was categorized as either yielding a full, unqualified report (full analysis); a qualified report with guideline-driven performance caveats (qualified report); the inability to sequence the tumor secondary to insufficient DNA (tissue insufficient for analysis); or the inability to sequence the tumor secondary to test failure (testing failure).

The primary outcome was the success of testing according to biopsy method. The secondary outcome measures were the genetic alterations identified, concordance with single gene hot spot testing, and the effect of genetic testing on treatment decisions.

A review of each individual patient's treatment record was done to determine the rationale for ordering CGP, and to determine if the results of CGP led to a change in CRGA-targeted treatment.

Any aspect of the work in this report that involved human patients was conducted with the ethical approval of the institutional review board at Columbia University Medical Center.

Results

Baseline characteristics of the patients with available data are provided in Table 1. There were 30 (61%) female and 19 (39%) male patients included in this study with a median age of 62.9 (range, 42-93) years, with most being lung adenocarcinoma on final pathology. Other final pathology included squamous cell carcinoma (2; 4%), sarcomatoid carcinoma of the lung (1; 2%), and unclassifiable non–small-cell carcinoma of the lung (1; 2%). Previous tissue specimens were retrieved from the pathology departmental