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Development and Clinical Utility of a Blood-Based Test Service for the Rapid Identification of Actionable Mutations in Non–Small Cell Lung Carcinoma

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Nearly 80% of cancer patients do not have genetic mutation results available at initial oncology consultation; up to 25% of patients begin treatment before receiving their results. These factors hinder the ability to pursue optimal treatment strategies. This study validates a blood-based genome-testing service that provides accurate results within 72 hours. We focused on targetable variants in advanced non–small cell lung carcinoma—epidermal growth factor receptor gene (*EGFR*) variant L858R, exon 19 deletion (ΔE746–A750), and T790M; GTPase Kirsten ras gene (*KRAS*) variants G12C/D/V; and echinoderm microtubule associated protein like and 4 anaplastic lymphoma receptor tyrosine kinase fusion (*EML4-ALK*) transcripts 1/2/3. Test development included method and clinical validation using samples from donors with ($n = 219$) or without ($n = 30$) cancer. Clinical sensitivity and specificity for each variant ranged from 78.6% to 100% and 94.2% to 100%, respectively. We also report on 1643 non–small cell lung carcinoma samples processed in our CLIA-certified laboratory. Mutation results were available within 72 hours for 94% of the tests evaluated. We detected 10.5% mutations for EGFR sensitizing ($n = 2801$ samples tested), 13.8% mutations for EGFR resistance ($n = 1055$), 13.2% mutations in *KRAS* ($n = 3477$), and 2% mutations for *EML4-ALK* fusion ($n = 304$). This rapid, highly sensitive, and actionable blood-based assay service expands testing options and supports faster treatment decisions. *(J Mol Diagn 2017, 19: 404–416; http://dx.doi.org/10.1016/j.jmoldx.2016.11.004)*

Somatic variants, including rearrangements, point mutations, and indels, are critical genetic alterations that influence malignant transformation and ultimately may result in disease progression. The clinical significance and importance of aberrations in epidermal growth factor receptor (EGFR), Kirsten ras (KRas), and anaplastic lymphoma receptor tyrosine kinase (ALK) have been previously reported.[1–5] The identification of the driver genomic alterations (oncogenic drivers) and targeting those specific alterations with therapy are critical aspects of today's approach to the management of cancer.[6,7]

The standard approach to the identification of actionable variants in patients with non–small cell lung carcinoma (NSCLC) is analysis of a tissue biopsy sample. The US Food and Drug Administration has approved formalin-fixed, paraffin-embedded–based tests for the detection of *EGFR* mutations [the Cobas (Roche, Basel, Switzerland) and Therascreen (Qiagen, Valencia, CA) kits], *KRAS* mutations (Therascreen), and *ALK* rearrangements (Vysis ALK Break Apart FISH Probe Kit; Abbott Diagnostics, Lake Forest, IL), and for ALK immunohistochemistry testing (clone D5F3; Ventana/Roche, Tucson, AZ). However, there are

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limitations of tissue-based mutation testing. For instance, approximately one fourth of patients with NSCLC are either not candidates for biopsy or have insufficient tissue samples recovered from the initial biopsy.[8,9] This can limit the treating physicians' ability to fully diagnose the cancer genotype. Additionally, tissue biopsies may be accompanied by discomfort in the patient and inherent clinical risks, including bleeding in the lungs, infection, and pneumothorax. A tissue biopsy sample may also not be representative of the total load and spectrum of mutated cells, especially in patients with advanced disease stages and metastases. Genetic changes can occur in the interval between removal of the initial biopsy sample and the start of the specific targeted treatment, especially in patients who are first treated with conventional chemotherapy or radiotherapy; however, surgical complications and economic considerations make multiple or serial tissue biopsies impractical in most cases.[1,10] Importantly as well, results from tissue-based testing can take weeks to obtain and can delay time to treatment.[7,11]

Prior studies have demonstrated that circulating nucleic acids of cellular origin from normal and cancer cells can be isolated from blood.[11–14] Importantly, genomic information from a patient's tumor can be obtained within days of a blood draw. Thus, tracking tumor-associated genetic aberrations in the blood can be used for quickly and noninvasively determining whether targeted therapies are a treatment option; assessing the presence of residual disease, recurrence, or relapse; and detecting the emergence of therapy-resistant cancer cells more quickly than conventional tissue-based methods.[14–16]

The EGFR gene (*EGFR*) is mutated in an estimated 10% to 40% of patients with NSCLC.[17] Approximately 90% of these *EGFR* mutations occur in either exon 19 from E746-A750 or as an amino acid substitution in exon 21 at codon 858 (L858R),[18] both of which confer sensitivity to the EGFR tyrosine kinase inhibitors gefitinib, erlotinib, and afatinib. The *EGFR* T790M mutation is the most commonly recognized mechanism of drug resistance to these first-line EGFR tyrosine kinase inhibitors, accounting for nearly 50% of the acquired resistance.[19,20] T790M, initially considered as relevant only as a resistance marker, is also now actionable with the regulatory approvals of osimertinib for use in *EGFR* T790M mutation–positive NSCLC cases.

The ALK gene (*ALK*) encodes a kinase that promotes cellular proliferation; genomic rearrangements in *ALK* can drive aberrant proliferation and tumorigenesis in a number of cancers. The most prevalent rearrangement involving the *ALK* locus in NSCLC is a chromosome-2 inversion that leads to juxtaposition of the associated echinoderm microtubule associated protein like 4 gene (*EML4*), resulting in the *EML4-ALK* transcript variant 1 (E13:A20), variant 2 (E20:A20), and variant 3 (E6:A20).[21,22] Patients with *ALK* rearrangements have generally mutually exclusive mutations in *EGFR* and *KRAS* and do not respond to EGFR tyrosine kinase inhibitors.[23] In this event though, there are targeted therapies for ALK-positive tumors, including crizotinib, ceritinib, and alectinib.

KRAS variants account for approximately 15% to 25% of the driver mutations found in patients with NSCLC.[17,24] The *KRAS* G12C, G12V, and G12D mutations, for which we developed assays in this study, are the most prevalent and together account for approximately 76% of all *KRAS* mutations.[24] Importantly, *KRAS* encodes a GTPase protein that functions downstream of EGFR; thus, tumors that contain *KRAS* driver mutations are nonresponsive to EGFR tyrosine kinase inhibitors.[6,25] Additionally, the detection of a *KRAS* mutation in a liquid biopsy sample can serve as an indicator that the quantity and quality of collected tumor circulating cell-free (cf) DNA are sufficient for identifying a cancer-specific driver mutation. Since oncogenic drivers are typically mutually exclusive,[26] the detection of a *KRAS* mutation can increase confidence that another mutation has not been missed. For these reasons, *KRAS* mutation testing is commonly performed even in the absence of a currently approved targeted agent directed at specific *KRAS* variants in NSCLC.

We used Droplet Digital (dd) PCR (Bio-Rad, Pleasanton, CA), which was optimized for rare mutation detection from blood. ddPCR is a highly sensitive gene-mutation detection method that is based on the partitioning of DNA into droplets.[27] There are now several ddPCR assays that have been developed for various common and rare cancer mutations; these assays have demonstrated utility in the clinical setting.[11,14,28,29] Our assays were developed using several specimen types and included synthetic DNA oligonucleotides, cell line (tumor)-derived RNA and DNA, cell line materials spiked into normal plasma, as well as circulating nucleic acids isolated from whole-blood samples from donors with or without cancer. In the *EGFR* and *KRAS* rare mutation–detection tests, we measured the presence of DNA somatic variants and the relevant wild-type (WT) sequences in dual-detection assays. In the case of the *EML4-ALK* gene expression test, cDNA copied from the plasma circulating RNA was measured. Here, the fusion transcript assays and reference glucuronidase-ß (control) gene (*GUSB*) assay were multiplexed to perform *EML4-ALK* testing. We report on the results from technical feasibility and verification and validation studies using the aforementioned reference and donor specimens. A schematic outline of these studies is shown in Supplemental Figure S1. We further detail analyses of >1600 clinical cases tested using these assays after the assays were made available through our CLIA-certified laboratory.

We have developed a highly sensitive and robust blood-based assay to identify EGFR-sensitizing mutations (L858R and ΔE746–A750), the EGFR-resistance mutation (T790M), *KRAS* G12C/D/V in circulating DNA, and *EML4-ALK* variants 1/2/3 in circulating RNA. Our results indicate that, in clinical practice, circulating nucleic acids can be used as a rapid and reliable indicator of positive mutation status of a tumor in the absence of tissue.