Comprehensive genomic analysis of circulating tumor DNA for patients with advanced non-small cell lung cancer

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The advent of therapies targeted to specific gene driver alterations has necessitated the development of predictive biomarkers of response that can be interrogated prior to the start of treatment. In patients with advanced non-small cell lung cancer (NSCLC), the National Comprehensive Cancer Network (NCCN) Guidelines recommend broad molecular profiling of molecular alterations, including alterations in driver genes (*EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *ERBB2*, and *RET*) for which effective drugs are available, as well as *KRAS*, which may identify patients not likely to benefit from them (1). The gold standard for testing of molecular alterations as companion diagnostics is tumor tissue. However, in advanced NSCLC cases where it is not feasible to obtain an adequate sample of tumor tissue, blood-based biopsies are gaining acceptance as an alternative source of tumor DNA (2). Currently, the US Food and Drug Administration (FDA)-approval for molecular profiling of tumor DNA derived from circulation is limited to detection of common *EGFR* mutations.

Usually referred to as liquid biopsies, blood-based biopsies rely on isolation and genotyping of circulating cell-free tumor DNA (ctDNA) from plasma, and are fast becoming part of routine oncology practice. Beyond informing the management of patients with advanced disease with respect to targeted therapies, the analysis of genomic alterations in plasma ctDNA shows promise for early diagnosis, risk stratification, detection of minimal residual disease, and tumor surveillance. In contrast to tissue biopsy, blood sampling is minimally invasive and can easily be repeated throughout the course of therapy, to enable real-time monitoring for treatment response and emergence of acquired resistance (3). Tumors continually shed DNA into the blood, due to cell necrosis or apoptosis. The ctDNA half-life is short (~2 hours), as it is rapidly cleared from the blood by the liver and kidneys. Its level in the bloodstream varies across different tumor types and disease stages (4). Moreover, as ctDNA is shed from both primary and metastatic tumor sites, it can provide insights into the intra- and inter-tumor heterogeneity, with potentially less bias than tissue biopsies. Since NSCLC is characterized by a high clonal heterogeneity between primary tumor site and distant metastases (5), and therapy imposes further selection pressures, longitudinal ctDNA analysis would potentially enable the dynamic monitoring of genomic alterations to inform treatment decisions over time. While the first liquid biopsy-based tests have been focused on single genes, comprehensive gene panels, ranging from a few dozen to several hundred genes, are increasingly being developed. Much of this recent trend is explained by the increasing availability of large-scale genomic data and the development of highly sensitive sequencing technologies.

In a recent issue of the *Journal of Thoracic Oncology*, Schrock and colleagues reported plasma DNA genomic profiling in a large retrospective cohort of 1,552 patients with advanced NSCLC (6). Using hybrid capture-based next-generation sequencing (NGS), they analyzed genomic alterations in 62 genes, including substitutions, short insertions and deletions, rearrangements and copy number variations (CNVs). For each sample, the fraction of ctDNA present in blood was first estimated by calculating

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the maximum somatic allele frequency (MSAF), which measures the AF of all somatic alterations. Evidence for ctDNA in blood (MSAF >0) was found in 80% of cases, and 86% of these presented a reportable genomic alteration (known/likely functional variants) with the most frequently altered genes being *TP53*, *EGFR*, *KRAS*, *NF1*, and *PIK3CA*. Clinically actionable genomic alterations referred to by NSCLC NCCN Guidelines were reported in 32% of cases with evidence of ctDNA, and *EGFR* was the most frequent among those. To evaluate the accuracy of the test, the authors compared the abundance of genomic alterations in plasma with that previously described in tissues from two large databases that closely match the intended-use population (The Cancer Genome Atlas and FoundationCORE). Frequencies of short variants (single nucleotide substitutions and short insertions/deletions) were similar for most of the genes, with the exception of *EGFR* and *KRAS* which were more often mutated in ctDNA and tissues, respectively. However, CNVs were far less frequently identified in ctDNA, suggesting the need of higher quantities of ctDNA to perform this type of analysis. Finally, the concordance of genomic alterations between ctDNA and tumor tissues was compared in 33 patients with detectable ctDNA, for which matching tissue was available and had previously been molecularly characterized. Among the subset of genes commonly interrogated in these paired samples, the overall number of alterations reported in ctDNA was lower than in tissue. In total, 64% of alterations present in tissue were detected in ctDNA. This large proportion of nonoverlapping alterations can be in part explained by the low sensitivity of the ctDNA assay, particularly to detect gene amplifications, or the limited release of ctDNA by so-called non-shedding tumors. This result emphasizes the necessity of complementary tissue analysis to accurately characterize the genetic complexity of NSCLC. Conversely, 81% of alterations in ctDNA were also observed in tissue, suggesting the diagnostic accuracy of ctDNA analysis and its clinical utility when tissue biopsies are not clinically feasible. This result also highlights the potential of ctDNA to capture tumor heterogeneity from multiple metastatic sites.

Ultimately, the clinical utility of comprehensive molecular characterization of ctDNA by this NGS-based gene panel will be established if the test improves patient management (i.e., reduction in the costs, complications and delays associated with tissue biopsies) and survival. The study by Schrock and colleagues lacked information of treatment context and outcomes, thus the potential impact of this test on treatment decisions and its correlation with patient outcomes remains to be determined. Moreover, although the concordance analysis between ctDNA and matched tumor tissue is consistent with prior reports (7,8), the diagnostic accuracy of the test would have been determined more precisely and meaningfully if a larger set of matched samples had been used. Finally, the study highlights the challenge of detection sensitivity, as evidenced by the reduced frequencies of CNVs observed in ctDNA and the large fraction of nonoverlapping genomic alterations identified between the two biopsy types. Despite these limitations, results of this study were consistent with those reported with a different ctDNA testing platform, in terms of profile and frequency of tumor-specific alterations, and the concordance with matched tissue samples (7,8). The expanded version of this test, covering more than 70 genes and genomic biomarkers for microsatellite instability and blood tumor mutational burden, has recently been granted breakthrough device designation by the FDA, potentially making it the first broad NGS liquid biopsy test to achieve regulatory approval to inform the use of targeted therapies, including immunotherapies (9).

A growing number of NGS-based liquid biopsy panels indicated to guide personalized therapies are entering the market, and they are rapidly gaining clinical adoption. Assay sensitivity can be variable in the detection of certain genomic alterations, and the potential for biased assessment and support for different therapy decisions based on the ctDNA platform used is worrisome (10,11). Hence, robust analytical standards will need to be established and independent comparison of NGS panels will be necessary to clarify the accuracy of this approach to inform patient treatment decisions. The increased sensitivity of NGS-based liquid biopsy panels also comes with a potential drawback. Unexpected genomic alterations have been found at low allele frequencies in ctDNA that are not likely derived from the tumor but instead related to clonal hematopoiesis, an aging related gain of somatic mutations in blood cells that can cause false-positive results if not carefully screened (12).

Comprehensive genomic profiling of ctDNA has potential to improve the clinical management of patients with advanced NSCLC. However, detailed and carefully conducted retrospective analyses of tissues for which the genomic profiling has proven clinical utility, or prospective clinical trials, will be needed to evaluate whether a test actually improves patient outcomes.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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