

# Circulating Tumor DNA Provides a Sneak Peek into Treatment Responses in Non-Small Cell Lung Cancer

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Circulating tumor DNA (ctDNA) holds great promise as a noninvasive diagnostic tool to guide treatment for patients with lung cancer. Two studies by Phallen and colleagues and Anagnostou and colleagues correlated sensitive measures of ctDNA with clinical responses to tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors, respectively, in

patients with non-small cell lung cancer (NSCLC). Together, these studies further highlight the potential clinical utility of serial ctDNA monitoring in patients with NSCLC undergoing treatment with both targeted therapies and immunotherapies.

See related articles by Phallen et al., p. 1204, and Anagnostou et al., p. 1214

Lung cancer emerged as a leading cause of cancer-related death in the 20th century, due, in part, to the paucity of effective treatments for advanced stage disease. Although lung cancer remains the leading cause of cancer-related mortality worldwide, breakthroughs in basic and translational research have led to the development of novel therapies for advanced stages of this disease.

The discovery that the presence of activating mutations in *EGFR* underlies the selective sensitivity of a subset of non-small cell lung cancer (NSCLC) to *EGFR* tyrosine kinase inhibitors (TKI) ushered in the era of precision medicine in lung cancer care. Numerous studies have categorized the spectrum of activating mutations in *EGFR*, as well as in numerous other oncogenic driver genes in NSCLC, enabling clinicians to employ genomic sequencing of tumors to predict responses to specific targeted therapies. Although prior studies have established the ability to identify a limited number of activating and resistance mutations in *EGFR* through sequencing of ctDNA (1), the capacity to monitor a wider spectrum of activating oncogenic mutations in ctDNA is currently under active investigation.

More recently, the development of immune checkpoint inhibitors that reinvigorate the host antitumor immune response have improved clinical outcomes for a substantial fraction of patients whose lung cancers do not harbor any targetable genomic alterations (2). Because of the complex interactions between the tumor and host immune system, the identification of precise predictive biomarkers for these immunotherapies has proved challenging. For immune checkpoint inhibitors that target the programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1)

pathway, tumor PD-L1 expression and tumor mutational burden have emerged as the most widely utilized predictors of treatment response (2, 3). However, intense efforts to identify more reliable predictive and prognostic biomarkers are ongoing.

Modern sequencing technologies and advances in computational biology have enabled the detection of circulating tumor DNA (ctDNA) in patients with both hematologic and solid malignancies. A substantial body of literature has correlated ctDNA levels to clinical responses to targeted therapies (1) and immunotherapies (4, 5) in several malignancies, nominating dynamic changes in ctDNA as a versatile biomarker of treatment response. Given the ease of obtaining ctDNA and the ability of this tool to capture both intra- and intertumoral clonal heterogeneity, "liquid biopsies" have the potential to supplement standard tumor biopsies to help guide clinical decision-making.

In this issue of *Cancer Research*, Phallen and colleagues (6) correlated ctDNA levels with clinical response to TKIs in patients with *EGFR*- or *ERBB2*-mutant NSCLC. To capture clonal heterogeneity within these patients with advanced NSCLC, the authors utilized a recently developed targeted error correction sequencing (TEC-seq) approach to monitor dynamic changes in ctDNA levels during TKI therapy. From these TEC-seq data, they devised a metric termed "cell-free tumor-load" (cfTL), which reflects either the abundance of mutant allele fractions or a measure of somatic chromosomal copy number alterations found in ctDNA. The authors categorized patients whose cfTL decreased >98.4% after initiation of TKIs as "molecular responders," whereas patients whose cfTL decreased by <98.4% comprised "molecular nonresponders." Notably, the degree to which cfTL decreased provided an indication of clinical response to TKI therapy on average 4 weeks earlier than routine CT imaging. Moreover, patients in the "molecular responder" group experienced significantly improved progression-free survival compared with "molecular nonresponders." This study provides further support for the development of ctDNA monitoring assays as routine clinical tools to guide treatment decisions for NSCLC patients receiving targeted therapies (1).

In addition to evaluating target gene sequences, Phallen and colleagues analyzed off-target reads from their targeted sequencing panel as an orthogonal measure of ctDNA load. Similar to methods used to obtain sequence information on mitochondrial

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and microbial DNA from exome sequencing, this approach allowed the authors to infer somatic chromosomal copy number alterations across the genome to generate a plasma aneuploidy score for each ctDNA sample. Of note, plasma aneuploidy scores also decreased in patients who responded to TKI therapy. This technique illustrates the potential value of low-coverage, off-target sequencing reads, which can provide an independent assessment of ctDNA load.

In a second study in this issue of *Cancer Research*, Anagnostou and colleagues (7) employed TEC-seq to correlate ctDNA levels with clinical response to immune checkpoint inhibitors in patients with metastatic NSCLC. The authors uncovered three patterns of ctDNA kinetics in response to immunotherapy: (i) "molecular response," characterized by clearance of ctDNA, (ii) "molecular resistance," characterized by stable or rising ctDNA levels, or (iii) "molecular acquired resistance," characterized by initial clearance of mutant alleles, followed by subsequent reemergence of those same alleles. In agreement with prior studies (4, 5), "molecular responders" experienced prolonged progression-free survival and overall survival compared with patients without such "molecular responses." Similar to the study by Phallen and colleagues, changes in ctDNA levels predicted clinical response to immunotherapy both earlier and more accurately than standard CT imaging.

The complexity of the interactions between cancers and the host immune system has proved to be a major barrier to the identification of biomarkers to measure the robustness of the antitumor immune response after immunotherapy. In contrast, in patients with HIV/AIDS, clinicians can track the HIV viral load and the patient CD4 T-cell count to obtain dynamic assessments of viral replication and the robustness of the host immune system, respectively. However, individual tumors likely harbor unique sets of neoantigens derived from nonsynonymous somatic mutations, thus complicating efforts to characterize tumor-specific T-cell responses. Expanding upon prior studies that have analyzed shifts in the T-cell receptor (TCR) repertoire (8) and polyclonal T-cell expansion (9) after immunotherapy, Anagnostou and colleagues assessed changes in the frequency of productive TCR rearrangements in peripheral blood lymphocytes as a surrogate measure of clonal T-cell expansion in NSCLC patients receiving immunotherapy. In an effort to track only the tumor-specific T-cell clones, the authors restricted their analyses of productive TCR rearrangements to only those TCRs that were present in both matched pretreatment tumor biopsies and pre- and posttreatment peripheral blood lymphocytes. They found that dynamic changes in productive TCR frequencies in peripheral blood lymphocytes correlated with changes in ctDNA and responses to immunotherapy. Further advances in techniques to interrogate clonal T-cell responses to immune checkpoint blockade will deepen our understanding of antitumor T-cell immunity and may contribute

to the development of therapeutic tumor vaccines and/or genetically modified T-cell therapies.

The studies by Phallen and colleagues and Anagnostou and colleagues demonstrate that serial ctDNA monitoring can facilitate the early detection of both primary and acquired resistance to targeted therapy and immunotherapy, respectively. This capability for early detection of therapeutic resistance provides an opportunity to interrogate the genomic mechanisms of resistance through whole-exome sequencing of ctDNA. An advantage of using ctDNA rather than tissue obtained from a single tumor biopsy for whole-exome sequencing is that ctDNA sequencing may capture genomic resistance mechanisms that may be missed because of intratumoral or intertumoral clonal heterogeneity. Importantly, the genomic alterations uncovered through whole-exome sequencing of ctDNA appear to be representative of those found in tumor biopsies (10). Therefore, whole-exome sequencing of ctDNA may uncover novel mechanisms of resistance to TKIs and immunotherapies.

Whether the early detection of therapeutic resistance in ctDNA can serve as an actionable biomarker is unknown. Future studies will need to examine whether serial ctDNA monitoring can be leveraged to tailor therapeutic strategies for individual patients with NSCLC. For example, it will be important to examine whether switching treatment regimens at the earliest detection of "molecular resistance" will improve patient survival. Moreover, serial ctDNA monitoring may help determine whether patients with NSCLC who achieve complete or near complete responses with immunotherapy-based regimens should discontinue their treatment regimens. Finally, as the options for TKI- or immunotherapy-based combination therapies continue to expand, one can utilize serial ctDNA monitoring to design clinical trials that investigate whether early treatment escalation from monotherapies to combination therapies can improve clinical outcomes.

Together, the studies by Phallen and colleagues and Anagnostou and colleagues illustrate the promise of serial ctDNA monitoring as an emergent diagnostic tool with the potential to guide therapeutic decision-making in patients with NSCLC. Although these studies show a significant predictive power for ctDNA "molecular response," each study represents only a small patient cohort. Prospective controlled studies with larger patient cohorts would allow for statistically robust analyses of the potential diagnostic benefit of serial ctDNA monitoring.

#### Disclosure of Potential Conflicts of Interest

M. Meyerson is an inventor on a patent for the use of EGFR mutants in lung cancer diagnosis, licensed to LabCorp. No potential conflicts of interest were disclosed by the other author.

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#### References

- Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, Messineo MM, et al. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. *Clin Cancer Res* 2014;20:1698–705.
- Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Czoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375:1823–33.
- Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med* 2017;376:2415–26.
- Goldberg SB, Narayan A, Kole AJ, Decker RH, Teyssir J, Carriero NJ, et al. Early assessment of lung cancer immunotherapy response via circulating tumor DNA. *Clin Cancer Res* 2018;24:1872–80.
- Raja R, Kuziora M, Brohawn PZ, Higgs BW, Gupta A, Dennis PA, et al. Early reduction in ctDNA predicts survival in patients with lung and bladder cancer treated with durvalumab. *Clin Cancer Res* 2018;24:6212–22.
- Phallen J, Leal A, Woodward BD, Forde PM, Naidoo J, Marrone KA, et al. Early noninvasive detection of response to targeted therapy in non-small cell lung cancer. *Cancer Res* 2019;79:1204–13.

7. Anagnostou V, Forde PM, White JR, Niknafs N, Hruban C, Naidoo J, et al. Dynamics of tumor and immune responses during immune checkpoint blockade in non-small cell lung cancer. *Cancer Res* 2019; 79:1214–25.
8. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 2017;171:934–49.
9. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, Wieland A, et al. Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. *Proc Natl Acad Sci U S A* 2017; 114:4993–8.
10. Manier S, Park J, Capelletti M, Bustoros M, Freeman SS, Ha G, et al. Whole-exome sequencing of cell-free DNA and circulating tumor cells in multiple myeloma. *Nat Commun* 2018;9:1691.

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