Profiling Non–Small Cell Lung Cancer: From Tumor to Blood

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Running title: Expanded Profiling of ctDNA in Lung Cancer

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Summary
Circulating cell-free tumor DNA has shown great promise for non-invasive genomic profiling to guide the administration of targeted therapies in non-small cell lung cancer. With advancements in molecular technology, it is now possible to interrogate multiple clinically actionable genetic drivers in the blood with a single assay.
In this issue of *Clinical Cancer Research*, Paweletz and colleagues describe a next-generation sequencing (NGS) assay to detect multiple actionable genetic changes in cell-free plasma DNA of non-small cell lung cancer (NSCLC) patients.

Over the past decade, the development of genotype-directed therapeutic strategies has transformed the treatment of NSCLC. Large-scale genomic analysis has led to the characterization of multiple key targetable molecular aberrations in NSCLC, including somatic mutations in *EGFR*, *BRAF* and *HER2*, and rearrangements involving *ALK*, *ROS1* and *RET*. These molecular targets each confer differential sensitivity to various therapeutic agents such as EGFR tyrosine kinase inhibitors (TKIs), ALK-directed TKIs, and others. Matching the therapies with the genetic profiles of individual tumors has led to improved clinical benefit compared to non-targeted therapies.

One of the major hurdles in the practical implementation of targeted therapies in NSCLC is the limited availability of tumor biopsy materials for molecular profiling at the time of disease diagnosis. Even when tissue biopsy is available, it may occasionally fail to detect driver mutations due to spatial heterogeneity or sub-optimal tissue quality. In the relapse setting, the growing availability of new therapeutic agents that effectively target resistance-conferring mutation calls for the need to characterize the tumor genome repeatedly for timely clinical intervention. In this regard, the analysis of cell-free DNA shed from the tumor to the circulation, so-called circulating tumor DNA, presents a new opportunity for noninvasive, serial molecular profiling. This DNA can be collected via a simple blood draw and may better encapsulate the full genomic heterogeneity of a patient’s disease compared to a single biopsy. Exploratory analyses from multiple large-scale clinical trials have demonstrated the high accuracy of detecting *EGFR* mutations in plasma DNA of NSCLC patients. Following these reports, the European Medicines Agency and the China Food and Drug Administration have recently extended the drug label of gefitinib (IRESSA, one type of EGFR-TKI) to include the detection of *EGFR* mutations in the blood of NSCLC patients to guide the administration of the drug when tumor materials are not available. Among all solid tumors, this represents one of the first cancer types for which the blood is officially recognized as an alternative specimen to determine mutation status for targeted therapies.

In NSCLC, most of the reported blood-based assays have utilized mutant allele-specific amplification or probe-based detection targeting one or a few
variants at a time. These approaches are usually restricted to hotspot mutations, and hence in the case of negative results, would need to be supplemented by multiple additional assays to screen for other possible oncogenic drivers. Further, these approaches are incapable of profiling the full spectrum of mutations that may emerge in the setting of acquired resistance. An assay that could interrogate multiple types of genetic aberrations in parallel is needed.

In this issue of *Clinical Cancer Research*, Paweletz and colleagues (1) describe a NGS assay that utilizes a modified hybridization capture approach, coupled with molecular barcoding, to target mutations and structural rearrangements in 11 oncogenic drivers in NSCLC (*EGFR, KRAS, ALK, HER2, BRAF, NRAS, PIK3CA, MET, MEK1, ROS1* and *RET*). These genes, altogether, compose approximately 50% of molecular subsets of lung adenocarcinoma (2). The assay was validated in defined mixtures of normal DNA spiked-in with serially diluted cell line DNA, and the authors reported a sensitivity and specificity of 88% and 100% for detecting previously characterized mutations present at 0.1% allele frequency or higher. The assay was evaluated blindly in the plasma samples from 48 NSCLC patients and demonstrated an overall sensitivity of 77% (48/62) for 62 known driver and resistance mutations. In two patients, the assay was able to detect oncogenic drivers in plasma that were missed in tumor biopsies.

This study adds another example to demonstrate the potential of using plasma DNA as a noninvasive tool for clinical genotyping in cancer. Importantly, this study, together with other NGS-based NSCLC assays reported to date (6-8), illustrates the strength of using NGS approach to screen multiple types of oncogenic drivers in parallel for comprehensive genomic plasma profiling in NSCLC. The advantages of a comprehensive approach are two-fold: it increases the chance of detecting at least one actionable oncogenic driver in a single attempt, such that potentially minimal time and clinical materials are required; it also reveals the presence of multiple concurrent drivers and tracks their relative abundance over time, such that the most dominant actionable drivers can be identified timely for clinical intervention (Figure 1). In NSCLC in particular, the expanding understanding of resistance mechanisms in the settings of first- and third-generation TKIs has led to the proposal of novel treatment strategies, including the optimization of dosage schedule (9) and the administration of sequential or combination therapies (10). These new possible regimens urge for a tool that can accurately profile the tumor genome safely and repeatedly.
There are multiple challenges in translating NGS-based plasma profiling assays to clinical implementation. The analytical workflow needs to be standardized such that it could be completed within a clinically compatible timeframe at a reasonable cost. In this regard, the fact that the assay reported by Paweletz et al is capable of a turnaround time of 6 business days from blood-draw to result is encouraging, yet leaving room for improvement. Technical challenges specific to the handling of plasma samples also need to be addressed, including the effects of pre-analytical factors, such as potential delays in samples processing, on the quality and integrity of the samples (11). The analytical performance of the assay, including sensitivity, specificity and reproducibility, needs to be thoroughly validated in a clinically certified laboratory. To date, there are at least two commercially available blood-based *EGFR* mutation assays that have obtained CE marking, an European conformity standard for in-vitro diagnostic medical device, to screen for *EGFR* mutations in the blood of NSCLC patients to guide TKI therapies. These assays include the Therascreen® *EGFR* RGQ Plasma PCR kit (Qiagen) and the Cobas® *EGFR* Mutation Test v2 (Roche), both of which target multiple reported mutations in *EGFR*. They represent the first regulatory registration of liquid biopsy companion diagnostic tests for TKI in NSCLC. It is expected that the number of plasma DNA profiling assays involving other molecular markers in NSCLC will continue to grow across the world.

As cancer care develops towards precision medicine, tumor profiling using NGS-based, multiple-gene assays has demonstrated remarkable throughput and robustness in a clinical setting to guide treatment decision (12). We envision that longitudinal blood-based profiling will supplement tumor genotyping to track the heterogeneous, evolving genomic landscape to inform treatment strategies and achieve safer, more precise cancer management.
References


Figure 1: Longitudinal profiling of cell-free DNA to detect driver mutations in non-small cell lung cancer before, during, and after treatment. Relevant driver mutations recurrently observed at diagnosis and at resistance are shown in the two pie charts. Changes in the relative representation of different clonal populations of cells (blue, red, green) are reflected in the levels of cell-free DNA in the plasma.
Figure 1:

Molecular profiling at diagnosis

- EGFR
- KRAS
- PIK3CA
- NRAS
- HER2
- BRAF
- MEK1
- Other

Longitudinal follow-up

- ctDNA levels
- Time from start of treatment
- Treatment 1
- Treatment 2

Identifying actionable resistance mechanisms

- EGFR T790M
- MET amp
- ALK amp
- ROS1
- BRAF
- HER2
- Other EGFR mut (e.g., C797S)

Plasma

Tumor

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