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Review

Circulating tumor DNA testing in advanced non-small cell lung cancer

Everett J. Moding[a,b,c], Maximilian Diehn[a,b,c,*], Heather A. Wakelee[a,d,*]

[a] Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA 94305, USA
[b] Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305, USA
[c] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
[d] Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA

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ABSTRACT

Circulating tumor DNA (ctDNA) shed from cancer cells into the peripheral blood can be non-invasively collected and tested for the presence of tumor-specific mutations. Mutations identified in ctDNA can predict responses to targeted therapies and emerging evidence suggests that changes in ctDNA levels over time can be used to monitor response to therapy and detect disease recurrence. Given the emergence of targeted therapies in advanced non-small cell lung cancer (NSCLC), liquid biopsies utilizing ctDNA testing represent a powerful approach to genotype tumors and monitor for the development of resistance. Here, we review current and potential future clinical applications of ctDNA testing for patients with advanced NSCLC.

1. Introduction

Tumors continually shed DNA into peripheral blood due to cell turnover both when not being treated and in response to therapy [1]. Circulating tumor DNA (ctDNA), which can be non-invasively detected in the plasma samples of patients through simple blood draws, delivers a unique window into an individual patient's tumor biology [2]. Studies in multiple tumor types have demonstrated that ctDNA testing can effectively predict the response of patients to targeted therapies [3–5]. Furthermore, emerging data suggests that ctDNA levels can be used to monitor response to local and systemic therapies [6].

Personalized and adaptive therapy continues to emerge and revolutionize the treatment of non-small cell lung cancer (NSCLC) [7]. Accurate information regarding the mutational status of a patient's tumor is critical to guide treatment decisions. However, easily accessible tumor tissue is not always available, and a small sample of one lesion may not demonstrate the full mutational picture within a given patient [8]. In these clinical situations, ctDNA testing can provide valuable and unique insights that can help guide therapy. Here, we review ctDNA testing approaches and present an extended clinical vignette of a patient with metastatic epidermal growth factor receptor (EGFR)-mutant lung cancer who undergoes targeted therapy but ultimately develops treatment resistance. After each step in his clinical course, we review the clinical applications and interpretation of ctDNA testing and highlight promising approaches that may become available in the future.

2. ctDNA testing approaches

Several approaches have been developed to analyze the presence and quantity of ctDNA ranging from single-locus amplification to whole genome sequencing (Table 1) [9]. Initial studies utilized polymerase chain reaction (PCR)-based amplification of specific cancer-associated mutations [10,11]. Allele-specific PCR provides reliable amplification of hot-spot mutations, but low sensitivity limits the application of this approach to early stage lung cancers or patients with very low burden of disease. Digital PCR improves upon conventional PCR by partitioning samples into multiple, smaller reactions allowing for absolute quantification and increased sensitivity [12,13]. Both conventional and digital PCR-based approaches test for a limited number of well-defined mutations. As a result, their applicability is limited to patients with common driver mutations.

The advent of next generation sequencing (NGS) has led to the development of several additional ctDNA testing approaches. One broad approach utilizes a combination of multiplexed PCR assays to amplify a small number of regions of interest followed by NGS to identify gene mutations and quantify the fraction of mutant alleles [14–16]. This approach allows for high sensitivity but interrogates a small number of genes and cannot detect copy number variants or structural variants if the breakpoint sequence has not been previously characterized. A second approach involving hybrid capture followed by NGS maintains an extremely high level of sensitivity while allowing larger panels of variants to be identified and quantified in each sample [17,18]. Furthermore, this approach enables detection of copy number

* Corresponding authors at: 875 Blake Wilbur Dr, Stanford, CA 94305, USA.
E-mail addresses: diehn@stanford.edu (M. Diehn), hwakelee@stanford.edu (H.A. Wakelee).

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Table 1
Comparison of ctDNA testing approaches.

Test	Description	Detection Limit	Variants Detected	Advantages	Disadvantages	Cost
Allele-specific PCR	Amplification and quantification of pre-selected variants	0.1–1% [10,11]	Well-defined SNVs and Indels	– Lowest cost	– Small number of variants tested per sample – Lower sensitivity	$
Digital PCR	Amplication of pre-selected variants after partitioning into multiple reactions to increase sensitivity	0.01–0.1% [12,13]	Well-defined SNVs and Indels	– High sensitivity – Lower cost	– Small number of variants tested per sample	$
Amplicon-based NGS	Deep sequencing of PCR amplicons	0.01–2% [14,15]	SNVs and Indels	– High sensitivity – Less expensive than other NGS-based methods	– Fewer variants tested per sample than other NGS-based methods	$$
Capture-based NGS	Deep sequencing of hybrid captured DNA molecules	0.00025–0.01% [17,18]	SNVs, Indels, SCNAs, and recurrent SVs	– Highest sensitivity – Broadly applicable	– Less comprehensive than whole exome and genome NGS	$$-$$$
Whole Exome NGS	Deep sequencing the exome	5–10% [19]	SNVs, Indels, SCNAs, and SVs	– Entire exome analyzed – Broadly applicable	– Expensive – Low sensitivity	$$$$
Whole Genome NGS	Deep sequencing of the genome	1–10% [20,21]	SNVs, Indels, SCNAs, and SVs	– Entire genome analyzed – Broadly applicable	– Expensive – Low sensitivity	$$-$$$

Abbreviations: PCR = polymerase chain reaction, NGS = next generation sequencing, SNVs = single nucleotide variations, Indels = insertions or deletions, SCNAs = somatic copy number alterations, SVs = structural variants.

variants and recurrent structural variants. Finally, several groups have demonstrated deep sequencing of the whole exome [19] or genome [20,21] can provide comprehensive profiling of ctDNA. Although feasible, these approaches are limited to application in patients with advanced disease due to the high costs per sample and relatively low sensitivity.

Currently, the cobas EGFR Mutation Test v2 (Roche Molecular Systems, Inc) is the only liquid biopsy approved by the FDA, and we review the application of this test below. Multiple commercial laboratories offer laboratory developed tests (LDTs) that are regulated under the Clinical Laboratory Improvement Amendments (CLIA) program, including both PCR-based and NGS-based approaches. A detailed description of the many available LDTs is beyond the scope of this review.

3. Testing for EGFR mutations

3.1. Sample case: part 1

A 54-year-old never-smoking man presents with chronic cough and worsening shortness of breath and is found to have a right lower lobe mass along with multiple small bilateral pulmonary nodules on CT and three subcentimeter enhancing brain lesions on MRI. He undergoes two CT-guided biopsies with a non-diagnostic first sample and the second sample showing sparse malignant cells consistent with lung adenocarcinoma. There was insufficient tissue for molecular testing.

3.2. ctDNA testing for the diagnosis of EGFR mutations

EGFR gene mutations have been reported in 43% of lung adenocarcinomas in never smokers and 11% of lung adenocarcinomas in smokers in a population of patients from the United States [22]. The rates of EGFR mutations vary by ethnicity and location, with the highest reported rates of EGFR mutations occurring in Asian populations [23]. Multiple randomized controlled trials have demonstrated improved progression-free survival with the EGFR tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib, and afatinib compared with chemotherapy for EGFR-mutant metastatic NSCLC [24–28]. As a result, EGFR TKIs are currently recommended as first-line treatment for EGFR-mutant metastatic NSCLC.

Tissue sampling remains the gold standard for molecular testing of tumors, but clinical situations such as the example above often arise when inadequate tissue is available for testing or the risk of tumor biopsy is too high. In these situations, non-invasive genotyping using ctDNA can provide valuable information about the mutational status of a patient's tumor. Several approaches have been demonstrated to effectively detect activating mutations of the EGFR gene from plasma samples.

The cobas EGFR Mutation Test v2 is a real-time PCR based assay that was originally approved by the FDA to test for EGFR mutations in formalin-fixed paraffin-embedded specimens [29]. The application to plasma samples was validated as part of the ENSURE clinical trial, which compared first line erlotinib versus gemcitabine and cisplatin [26]. The FDA noted in their approval that the test may benefit patients who are unable to provide a tumor specimen for EGFR testing. Although the cobas EGFR Mutation Test v2 can detect multiple mutations in exons 18–21, including L861Q, G719X, and S768I, it is currently only approved as an indication for erlotinib therapy when exon 19 deletions and L858R substitution mutations are detected. In 76.7% of patients with exon 19 deletion or L858R mutations detected from tissue samples, the same mutation was detected in the plasma, suggesting that plasma samples can substitute for tissue biopsy in the majority but not all patients. Thus, the FDA recommends that negative plasma samples should prompt additional tissue sampling.

Several additional randomized trials comparing EGFR TKIs to chemotherapy have demonstrated the ability of allele-specific PCR ctDNA testing to predict outcomes to treatment. The IPASS study compared first-line gefitinib versus carboplatin and paclitaxel in a Japanese cohort of patients with advanced lung adenocarcinoma. Plasma ctDNA testing led to a high rate of false negatives (56.9%) when using tissue as a reference, but progression-free survival was significantly longer in patients with positive ctDNA testing who received gefitinib compared with chemotherapy [30]. Similarly, in European patients with EGFR mutations detected with ctDNA testing on the EURTAC trial, erlotinib treatment was associated with a longer progression-free survival compared with combination chemotherapy [31]. Afatinib improved progression-free survival compared with platinum doublet chemotherapy on the LUX-Lung 3/6 trials for patients with EGFR-mutant ctDNA [32]. Finally, the FASTACT-2 study compared erlotinib versus placebo after 6 cycle of gemcitabine and platinum chemotherapy and demonstrated a significant PFS benefit for erlotinib in patients positive for EGFR mutations but not in patients negative for EGFR mutations by ctDNA testing [33]. Several additional clinical studies have demonstrated the concordance of ctDNA testing for EGFR mutations with tumor biopsies and the ability of ctDNA testing to predict responses to EGFR TKIs [5,34–39].

3.3. Sample case: part 2

The patient's plasma is collected and sent for ctDNA EGFR testing