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Circulating tumor DNA—From bench to bedside

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ABSTRACT

In the era of personalized medicine, tumor sampling is paramount to enable the assessment of actionable molecular aberrations to help rationalize and guide treatment decisions. Longitudinal tracking of such aberrations may also be helpful to detect emerging drug resistance and to allow for timely modifications to ongoing therapies to improve patient outcomes. Nevertheless, tumor tissue sampling involves an invasive procedure with potential risks to patients and involves logistical challenges. As such, other less invasive and safer methods such as blood sampling for molecular profiling has been gaining traction. In this article, we discuss the concept of circulating tumor DNA, the technology platforms available for its interrogation, and its current applications in the clinic. We also envision how circulating tumor DNA may be applied at multiple time points along a patient's cancer journey to guide diagnosis, prognostication, and therapeutic decisions.

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Introduction

Solid tumor sampling has been the cornerstone of cancer diagnosis for decades, and it is also the foundation upon which oncologists build treatment plans. In this era of precision medicine, armed with our improved understanding of tumor biology, including the concept of clonal evolution and intratumor heterogeneity, it has become evident that oncogenesis is a dynamic process where tumors are constantly evolving to seek compensatory escape routes under distinct selection pressures from antitumor therapeutic agents. As such, a tumor biopsy undertaken at a single time point at the time of diagnosis may not be truly representative of subsequent molecular changes that may have occurred during the course of a patient's disease because of the potential for clonal evolution.[1] A recent analysis of somatic genomic profiles with next-generation sequencing (NGS) of over 15,000 circulating tumor DNA (ctDNA) samples with matched tissue from 386 patients with advanced cancers showed concordance of more than 90% for truncal driver mutations, but only 13%-33% concordance for subclonal aberrations, such as epidermal growth factor receptor (*EGFR*) T790M mutations, likely reflecting new aberrations detected with the potential emergence of drug resistance.[2]

The acknowledgment of this phenomenon has led to growing advocation for the use of repeat tumor biopsies upon treatment progression to enable better characterization of tumors to guide treatment choices. Nonetheless, solid tumor sampling is an invasive process, which can be technically challenging and not without risks of procedural complications. Additionally, it is logistically challenging for patients to undergo frequent multiple biopsies, while issues with intratumor heterogeneity raises concerns of whether a single core biopsy is reflective of the genomic landscape of the whole tumor. The concept of a "liquid biopsy," whereby cells and other cell products from the mononuclear cellular fractions can be analyzed from a blood draw, has become an attractive alternative to solid tumor sampling. Blood sampling is a less invasive process that will allow serial sampling, thus possibly obtaining a "real time" reflection of the tumor status of a patient and potentially minimizing issues of sampling bias. As advanced cancers shed cells and other cellular fragments into the bloodstream from both primary and metastatic sites, blood sampling may also provide a global summary of tumor heterogeneity.

The Food and Drug Administration (FDA) and European Medical Agency recently approved the first blood-based companion diagnostic assay, Cobas *EGFR* mutation test v2 (Roche Molecular systems) in June 2016. This real-time polymerase chain reaction (PCR) test identifies *EGFR* exon 19 deletions and *EGFR* exon 21 L858R mutations based on the detection of ctDNA in plasma derived from EDTA anticoagulated peripheral whole blood, therefore indicating tumor sensitivity to EGFR inhibitors, such as erlotinib (Tarceva, Roche). In September 2016, this FDA approval was extended to include *EGFR* T790M testing, a common resistance mutation emerging on anti-EGFR therapy, where a positive T790M mutation indicates sensitivity to osimertinib (Targrisso, Roche).

In this article, we focus on the use of ctDNA and discuss its clinical applications as a surrogate for conventional tumor biopsies for a range of uses, including molecular profiling, monitoring of antitumor response, and emerging resistance so as to guide treatment modification.

Development and technical aspects of ctDNA assay

Although the detection of circulating free DNA (cfDNA) was first described by Mandel and Metais,[3] it was not until almost 4 decades later when Stroun et al[4] showed evidence of the presence of ctDNA in cfDNA fragments. The presence of cfDNA is largely a result of cell death, with highly fragmented, double-stranded DNA of approximately 150 bp in size being released into the bloodstream. Currently, the most successful application of cfDNA in the clinics is arguably its use in prenatal testing for fetal aneuploidy,[5] although cfDNA levels has also been linked to outcomes in patients who have suffered major trauma such as burns, septic shock, myocardial infarction, and stroke.[6] In oncology, total cfDNA levels may be elevated owing to high cell turnover, but a distinct property of cfDNA in patients with cancer is the presence of both