# Blood Worth Bottling: Circulating Tumor DNA as a Cancer Biomarker

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*See related article by Jahr et al., Cancer Res 2001;61:1659–65.*

Circulating cell-free DNA (cfDNA) was discovered in 1948 (1); however, it was not until 1994 that it was used to identify the presence of tumor-specific mutations, such as *KRAS* in the plasma of patients with cancer (2). These observations, in combination with the finding that levels of cfDNA are higher in cancer patients than healthy individuals (3, 4), sparked widespread interest in the potential of circulating tumor DNA (ctDNA) as a noninvasive biomarker. Jahr and colleagues sought to answer a number of important questions regarding the origin and nature of cfDNA, including what proportion of cfDNA is derived from ctDNA and the cell of origin for nontumor cfDNA (4). One of their key findings was that cfDNA from apoptotic and necrotic cells can be distinguished on the basis of the size of the DNA fragments (4). Understanding the origin and nature of ctDNA was necessary for optimizing its isolation, for the design of diagnostic assays, and for identifying the clinical scenarios that are most amenable to interrogating ctDNA. Using methylation-specific PCR of the *CDKN2A* promoter, Jahr and colleagues found that the proportion of ctDNA varied between patients, ranging from <10% to >90% of total cfDNA (4). With the subsequent development of more sensitive detection methods, recent studies have found that the fraction of ctDNA can be as low as approximately 0.01% (5–7).

The last 5 years have seen a rapid expansion in the analysis of ctDNA, enabled largely by the development of massively parallel, deep DNA sequencing. Digital PCR, BEAMing, and personalized analysis of rearranged ends offer high levels of sensitivity for ctDNA detection but require prior knowledge of somatic mutations present in the subject's tumor and the development of mutation-specific assays (6–8). By comparison, next-generation sequencing (NGS)–based approaches allow a more comprehensive analysis of ctDNA without prior knowledge of the genomic landscape of a subject's tumor. Targeted sequencing of PCR-amplified (e.g., TAm-Seq; ref. 8) or hybridization-captured (e.g., CAPP-seq; ref. 5) DNA fragments have been used to detect mutations in commonly mutated genes. More recently, exome- and whole-genome sequencing have also been applied to ctDNA (9, 10). The full spectrum of mutation types have been identified in ctDNA, including point mutations (5, 8–13), small insertions and deletions (5, 9, 13), chromosomal rearrangements, including copy number aberrations (5, 7, 10, 11, 14), and epigenetic mutations (4, 15), depending on the type of methodology utilized. Improvements in NGS technologies and an ongoing reduction in cost suggest that unbiased approaches will likely play an increasing role in analysis of ctDNA in the future.

ctDNA has been identified in patients with various cancer types and at different stages of disease progression (16), indicating its suitability as a biomarker in a range of clinical settings. ctDNA is more frequently detected in patients with advanced, metastatic disease than patients with localized disease (16), suggesting that it may have limited utility as an early-detection biomarker until assay sensitivity can be improved. In patients with advanced malignancies, the use of ctDNA analysis for blood-based tumor genotyping has seen the application of this strategy across several cancer types to guide the selection of targeted therapies. The application of ctDNA analysis in this context is providing a unique opportunity to facilitate personalized treatment decisions by allowing patient-specific genomic changes to be monitored in real time using a minimally invasive technique.

Serial sampling of ctDNA has been used to monitor tumor dynamics after surgery and during treatment, with ctDNA levels correlating with tumor burden assessed by imaging, across multiple cancer types (5, 6, 11, 13). In breast cancer patients, ctDNA levels have been shown to predict relapse, response to treatment, and disease progression earlier than standard imaging approaches (8, 11, 13). In colorectal cancer, a recent analysis of ctDNA has also suggested utility for selecting patients for adjuvant chemotherapy in the treatment of stage II disease. Patients with detectable postoperative ctDNA levels were at a higher risk of recurrence than ctDNA-negative patients, identifying a potential group of patients most likely to benefit from adjuvant chemotherapy (17). The application of ctDNA analysis has the potential to allow risk stratification across a range of solid malignancies to guide the selection of adjuvant therapy and to provide an opportunity for minimal residual disease monitoring following treatment.

ctDNA has also shown a role in monitoring treatment resistance by identifying the emergence or increasing levels of mutations associated with treatment failure (9). In breast cancer, *ESR1* mutations have been identified as an important mechanism of resistance to aromatase inhibitor therapy, and the emergence of these mutations can be identified through serial ctDNA analysis in patients on treatment (18). In non–small cell lung cancer patients, activating *EGFR* mutations that cause erlotinib or gefitinib resistance can also be detected in ctDNA (5, 9). Similarly, in colorectal cancer patients undergoing EGFR blockade, treatment resistance can be conferred by activating *KRAS* mutations, *MET* amplification, or mutations in other *EGFR* pathway genes, all of which have been identified in ctDNA samples collected prior to treatment or at relapse (12, 14, 16). Detection of mutations that lead to treatment resistance using ctDNA suggests that there is clinical utility in sampling ctDNA for guiding therapy selection during recurrence or progression. Moreover, the application of serial ctDNA analysis using whole-exome sequencing of plasma DNA

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has been shown to provide a comprehensive and unbiased assessment of genomic changes during the acquisition of treatment resistance, providing new opportunities to study resistance mechanisms in patients receiving novel therapies (9).

Utilizing ctDNA as a liquid biopsy has several advantages over standard biopsy or surgical collection of tumor tissue. As it is noninvasive, samples can be collected serially with ease, facilitating the temporal analysis of genomic changes throughout treatment and disease progression (8, 9, 12). Cancer shows both intrapatient and intratumor heterogeneity; therefore, biopsy of a single site is limited in its ability to allow detection of subclonal or metastasis-specific mutations that lead to chemoresistance. In contrast, monitoring ctDNA can provide a more accurate global representation of tumor mutational load from all disease sites throughout the patient. Chan and colleagues examined a patient with synchronous breast and ovarian cancer, performing whole-genome sequencing of multiple regions of the tumors in addition to ctDNA, and observed genetic aberrations that were unique to each region/cancer type, as well as mutations that were shared between sites detectable in plasma (10). Furthermore, Murtaza and colleagues examined a patient with metastatic breast cancer through multiregional tumor biopsies and serial plasma analysis, revealing the ability of ctDNA to provide dynamic sampling of somatic alterations across multiple disease sites, reflecting the size and activity of distinct tumor subclones in response to treatment (19). Together, these studies have shown the potential of ctDNA analysis as a liquid biopsy alternative to tissue biopsies to overcome many of the limitations associated with tumor tissue sampling.

In combination with earlier detection of recurrence, detection of mutations that lead to treatment resistance, and subsequent guiding of therapy selection, monitoring ctDNA has significant potential to improve patient outcomes in the future. A major challenge involves improvements in sensitivity to allow the detection of mutations that are private to some metastatic sites and that may drive resistance and ultimately overwhelm the patient. Future improvements and standardization of sequencing approaches for ctDNA analysis, as well as validation of the clinical utility of ctDNA testing in various clinical settings, will allow the potential of this biomarker to be realized. The work by Jahr and colleagues, 15 years ago, anticipated the recent rapid growth of studies on ctDNA and provided a valuable step toward the development of this significant clinical tool to facilitate precision cancer medicine.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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