

# Use of Circulating Tumor DNA for Cancer Immunotherapy

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## Abstract

Liquid biopsy offers a versatile, noninvasive opportunity to diagnose, characterize, and monitor disease in patients with cancer. There are particularly promising applications with which to use liquid biopsies to predict and evaluate response to immunotherapy. Circulating tumor DNA (ctDNA) can reflect the genomic state of a patient's overall disease and, thus, might identify prognostic and predictive biomarkers for immune checkpoint inhibitor therapy. ctDNA might also be a proxy for a patient's overall disease burden, which could be used for early diagnosis and monitoring treatment response.

These applications can enable novel trial designs, such as enrollment of early-stage patients with a high risk for relapse, and the evaluation of response patterns unique to immunotherapies. However, barriers to the widespread adoption of ctDNA assessment remain, including the absence of standardized procedures for collecting and processing ctDNA samples and relatively limited data on clinical utility. Identifying and solving these challenges could allow ctDNA to become a powerful clinical and research tool in the era of personalized immunotherapy.

## Introduction

Liquid biopsy might enable minimally invasive approaches for disease progression monitoring, minimal residual disease (MRD) detection, and genomic profiling of cancers (1, 2). Circulating tumor cells, nucleic acids, proteins, and metabolites from tumors can be measured in bodily fluids, such as blood, urine, and cerebrospinal fluid, and used to identify genetic mutational hallmarks of the cancer from which they arose (1). This review will focus on characterizing circulating tumor DNA (ctDNA) in plasma and its application in clinical immunotherapy.

In healthy individuals, the majority of cell-free DNA (cfDNA) arises from hematopoietic cells undergoing cell death (3), whereas, in patients with cancer, DNA from tumor cells can also be present in the plasma as ctDNA (1, 2). ctDNA differs from host cfDNA through characteristic somatic genomic alterations (4) and positional characteristics of DNA fragments that suggest nonhematopoietic tissue of origin (5). ctDNA assays can identify tumor-specific genetic alterations (e.g., somatic point mutations, loss of heterozygosity, gene fusions, gene copy number variations, and DNA methylation changes) without performing invasive tumor biopsy (6) and are already used for identifying populations likely to respond to targeted therapy (7, 8).

## Opportunities and Challenges of Liquid Biopsies

The minimally invasive nature of liquid biopsy sampling compared with the usually more invasive collection of a tumor tissue biopsy offers a clear opportunity in immunotherapy (Table 1). Beyond the potential risk and discomfort to patients who undergo invasive biopsy, the location of the tumor is sometimes challenging to reach safely by needle biopsy; furthermore, tissue may be exhausted by standard assays used in clinical care or for patient selection [e.g., testing for programmed death ligand 1 (PD-L1) expression and alterations in *EGFR*, *ALK*, *BRAF*, or *ROS1* in lung adenocarcinoma]. Tumor biopsy specimens are typically obtained infrequently: once at diagnosis and occasionally during the clinical course, such as times of recurrence or more rarely, resistance. In contrast, the ease and safety of liquid biopsy provides a provocative opportunity for more frequent, serial monitoring of disease and therapeutic response (2). The very short (~2 hours) half-life of ctDNA (9) also permits rapid assessment of tumor-related changes to the point where real-time monitoring is possible for molecular biomarkers of response or relapse (6, 8, 10).

There are additional opportunities for the use of liquid biopsy in both early and metastatic cancers. ctDNA may facilitate the detection and diagnosis of early-stage disease or posttreatment recurrence/residual disease (i.e., before the emergence of symptoms or before tumor lesions are visible by imaging; refs. 1, 11–15). In metastatic cancer, liquid biopsy can capture a systemic synthesis of intra- and intertumoral heterogeneity, potentially permitting a more comprehensive assessment of the molecular architecture of the tumor (16). Liquid biopsy might simultaneously identify potential mechanisms of resistance in different metastatic lesions, targetable molecular alterations, or mutations that predict therapeutic effectiveness (15).

The success of the liquid biopsy depends on the tumor shedding quantifiable amounts of ctDNA, which is not universal, even in cases of metastatic disease (15, 17). Furthermore, it is difficult to identify the origin of ctDNA (primary tumor or metastasis) and

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**Table 1.** Challenges and opportunities of tumor and liquid biopsy sampling in immuno-oncology

	Challenges/Disadvantages	Opportunities/Benefits
Tumor biopsy	<ul style="list-style-type: none"> <li>Invasive; snapshot of disease status</li> <li>Does not account for disease heterogeneity across sites (tumor/metastatic)</li> <li>Location or size of tumor might render sampling infeasible</li> <li>Serial monitoring infeasible because of necessity for multiple risky procedures</li> <li>Confirmation of PD requires lead time of about 4 weeks</li> <li>Inadvertent misclassification of SD/PD due to individual tumor kinetics</li> </ul>	<ul style="list-style-type: none"> <li>Certainty regarding location of sample (primary, metastatic)</li> <li>Established procedures for sample collection and processing</li> <li>Accessibility of processing tools</li> </ul>
Liquid biopsy	<ul style="list-style-type: none"> <li>Potential heterogeneity between quantities and genetic characteristics of ctDNA shed from different metastatic sites</li> <li>Relevance of tumor heterogeneity to immuno-oncology and targeted treatments still under study</li> <li>Differential DNA shedding between tumor types and patients</li> <li>Insufficient data regarding temporal ctDNA analyte in liquid biopsy samples</li> <li>Dearth of information about influence of patient physiological characteristics on ctDNA levels</li> <li>Technology is undergoing development; no standardized procedures/workflow for processing ctDNA samples; preferred vendors and methods not yet clear</li> <li>Difficult to capture structural variations using current technologies</li> <li>Specific but not sensitive (positive ctDNA finding shows disease but negative finding does not guarantee lack thereof)</li> </ul>	<ul style="list-style-type: none"> <li>No FFPE artefacts</li> <li>Non- (urine, saliva) or minimally (blood, CSF) invasive</li> <li>Tumor location irrelevant; even small tumors can release ctDNA</li> <li>Serial and longitudinal monitoring feasible, with ability to track genomic changes over time without repeat biopsy, including resistance mechanisms</li> <li>Tumor response can be monitored frequently; potential to expedite confirmation of PD</li> <li>Potential to identify mechanisms of resistance in different metastatic lesions</li> <li>Frequent assessment of tumor response possible</li> <li>Might enable more accurate identification of tumor response to ICIs irrespective of tumor growth kinetics</li> <li>Real-time assessment of tumor dynamics and response</li> <li>Might help distinguish between distinct tumor response patterns with ICIs</li> <li>Diagnosis of early-stage disease and early detection of postsurgery recurrence/residual disease in patients NED by imaging (before symptom emergence), yielding potential to design trials in high-risk adjuvant setting</li> <li>Improved judgement of stable disease</li> <li>Identification of targetable molecular alterations in patients with no alternative treatment options</li> <li>Fast processing of sample</li> </ul>

Abbreviations: CSF, cerebrospinal fluid; FFPE, formalin-fixed paraffin-embedded; NED, no evidence of disease; PD, disease progression; SD, stable disease.

attribute heterogeneity to particular tumor sites (18). The impact of clonal hematopoietic (CH) mutations (i.e., somatic changes in blood cells unrelated to the cancer being assessed) on the interpretation of ctDNA mutations also remains a particular challenge (19). The prevalence of cancer-associated mutations in leukocytes increases with age, even in people in whom cancer never develops (1, 20). CH has a prevalence in the general population of 10%, increasing to >20% in persons 60 to 69 years of age (21). CH mutations cannot necessarily be distinguished from somatic tumor mutations based on gene alone, because similar genes (e.g., *TP53*) can be altered in both CH and tumors (Table 2). One solution is the sequencing of cellular DNA derived from circulating leukocytes to avoid the misinterpretation of CH mutations as ctDNA mutations (4, 13, 19).

Technologies supporting the use of liquid biopsy are rapidly developing. Procedures for collecting and processing ctDNA samples still require standardization, and panels for assessing specific cancer-relevant mutations and broader genomic metrics, such as tumor mutational burden (TMB) and microsatellite instability (MSI) status, vary markedly across assays. In addition, sensitivity to detect small amounts of ctDNA in the plasma varies across assay technologies. The dynamic nature of the field offers both opportunities for innovation and challenges for assessing clinical utility across heterogeneous assays.

The relative importance of tissue-plasma concordance compared with the direct correlation of ctDNA metrics to clinical outcomes remains to be determined (22), as neither tumor tissue nor ctDNA is a gold standard; concordance can be highly variable

(Table 2; refs. 1, 2, 11, 23). Potential sources of discordance include varied sensitivity of ctDNA assays relative to tissue assays, tumor heterogeneity (intra- and intertumoral), temporal changes in disease state subsequent to archival tissue biopsies, and differential ctDNA shedding (24).

There are also few data on natural variability and the influence of physiologic characteristics of patients with cancer on the dynamics of ctDNA in the clinical setting, issues that several governmental, nongovernmental, academic, and industry groups are now tackling (25–27).

## Using ctDNA to Select Patients for Immunotherapy Treatment

Somatic alterations detectable in ctDNA may facilitate selection of patients for immunotherapy treatment. Immune checkpoint inhibitors (ICI), particularly anti-programmed death 1 (PD-1)/anti-PD-L1 agents, have shown clinical efficacy across many solid tumors (10, 15). Mismatch repair deficiency (MMR-D), also known as MSI, is an inherited or somatic condition leading to tumors characterized by a very high number of small genomic alterations (single-nucleotide variants and indels). Tumors with MMR-D/MSI became the first pan-tumor indication for checkpoint blockade. Such alterations can be detected using tumor tissue or ctDNA (28, 29), but tissue availability may limit tissue testing for MMR/MSI. A survey of 151 U.S. oncologists who treat patients with colorectal cancer concluded that the most common reason for not performing MMR-D/MSI testing was insufficient

**Table 2.** Ongoing challenges and potential resolutions for broader application of ctDNA genomic analysis in immuno-oncology

Ongoing challenges	Potential resolution
Tumor-plasma concordance	<ul style="list-style-type: none"> <li>Longitudinal studies to establish normal temporal variation in tumor-plasma concordance               <ul style="list-style-type: none"> <li>- During oncologic evolution</li> <li>- Across sample collection times</li> </ul> </li> <li>Focus on driver mutations</li> <li>Development of analysis platforms that are more sensitive</li> </ul>
Clonal hematopoiesis	<ul style="list-style-type: none"> <li>Paired ctDNA and PBC genotyping               <ul style="list-style-type: none"> <li>- Only mutations detected in ctDNA considered derived from tumor</li> </ul> </li> <li>DNA sequencing of original tumor to inform mutations for ctDNA assessment</li> </ul>
Postsurgery ctDNA fluctuations	<ul style="list-style-type: none"> <li>Establish in timeline studies in large populations with different types of cancer</li> </ul>
Influence of patient physiological state on ctDNA	<ul style="list-style-type: none"> <li>Prospective studies in large cohorts to determine               <ul style="list-style-type: none"> <li>- Kinetics of ctDNA activity</li> <li>- Implications of ctDNA peaks for response</li> <li>- Clinical meaning of persistent posttreatment clonal populations</li> </ul> </li> <li>Histologic studies to identify the factors modulating ctDNA release</li> </ul>
Sensitivity/specificity	<ul style="list-style-type: none"> <li>Balance sensitivity and specificity of analysis platforms</li> </ul>
Interpatient and interstudy comparison	<ul style="list-style-type: none"> <li>Establish standardized methods of extraction, isolation, and quantification of analytes of ctDNA</li> </ul>

Abbreviation: PBC, peripheral blood cell.

tissue sampling (stated by 48.3% of physicians), a challenge that ctDNA-based MMR-D testing could address.

Elevated TMB might represent another biomarker of response to ICI blockade (30–34), although there is no approved diagnostic indication at this time. Blood-based diagnostic tests to measure TMB are in development (35–37). TMB, measured in ctDNA, was assessed retrospectively and showed an association with clinical outcomes in patients with non-small cell lung cancer (NSCLC) treated with immunotherapy (38, 39). In a retrospective analysis of the POPLAR study of atezolizumab versus chemotherapy in second-line NSCLC, increased blood TMB was associated with improved benefit [progression-free survival and overall survival (OS)] to atezolizumab relative to chemotherapy at cutoffs of 10, 16, and 20 mutations per megabase (mut/Mb). The investigators chose to pursue the cutoff point of 16, for which the median OS was 13.0 months with atezolizumab and 7.4 months with docetaxel. When applied to a second study in NSCLC (OAK), patients with sufficient ctDNA and TMB >16 mut/Mb demonstrated a median OS of 13.5 months if treated with atezolizumab versus 6.8 months with docetaxel, with a HR of 0.62 [95% confidence interval (CI), 0.43–0.9].

In addition, the association between TMB measured with ctDNA and response to immunotherapy was highlighted in the MYSTIC study (ClinicalTrials.gov, NCT02453282) of durvalumab with or without tremelimumab as first-line treatment of NSCLC. MYSTIC missed its primary endpoint of OS in PD-L1–selected patients. However, a retrospective exploratory analysis was performed on tumor [41% of intent-to-treat (ITT) population] and plasma (72.4% of ITT population); several TMB cutoffs were applied. A cutoff  $\geq 10$  mut/Mb in tumor demonstrated a median OS of 16.6 months

(range, 9.7–27.3) for patients treated with durvalumab plus tremelimumab, 18.6 months (9.3–22.0) with durvalumab, and 11.9 months (9.1–16.0) with chemotherapy. In patients whose tumors harbored <10 mut/Mb, there was no benefit to immunotherapy compared with chemotherapy. When applying stepwise increasing blood TMB (bTMB) cutoffs (range, 4–20 mut/Mb), the authors found improved benefit for durvalumab plus tremelimumab relative to chemotherapy at higher cutoffs. For example, in patients with bTMB  $\geq 20$  mut/Mb, the median OS was 21.9 months (11.4–32.8) for patients treated with durvalumab plus tremelimumab, 12.6 (7.6–18.6) with durvalumab alone, and 10.0 (8.1–11.7) with chemotherapy. Again, there were no such differences in patients with bTMB <20 mut/Mb (40, 41). Although exploratory, these data suggest that elevated TMB, as measured by ctDNA, may correlate with improved OS upon treatment with checkpoint blockade compared with chemotherapy. These concepts are being explored prospectively in several studies, including NEPTUNE (ClinicalTrials.gov, NCT02542293). The relationship between elevated TMB measured in blood and tissue and overall response is also being tested in CheckMate-848, a study of nivolumab alone or in combination with ipilimumab to treat patients with multiple solid tumors (excluding NSCLC, melanoma, and renal cell carcinoma; ClinicalTrials.gov, NCT03668119).

In bTMB studies in NSCLC, the correlation between bTMB and tTMB was moderate: Spearman correlation coefficient was 0.64 (95% CI, 0.56–0.71) in POPLAR/OAK and 0.6 (no CI given) in MYSTIC. Despite the imperfect correlation, clinical results suggest that increased bTMB and increased tTMB are both similarly associated with improved benefit of immunotherapy. This pattern was also seen in a small study of pembrolizumab in gastric cancer (22), suggesting that bTMB may provide modestly different, but nevertheless independently important information compared with tTMB. Further efforts are needed to design studies to compare the predictive value of tTMB and bTMB (22).

It is also important to note that ctDNA detectability itself may be prognostic and possibly predictive in patients treated with ICIs. In an analysis by Socinski and colleagues (42) of B-FIRST, a phase II study of patients with NSCLC treated with atezolizumab, those patients with insufficient ctDNA to assess TMB experienced significantly improved response rates [ORR 34.5% in patients with maximum somatic allelic fraction (MSAF) <1% vs. ORR 10.1% in those with MSAF  $\geq 1\%$ ], comparable with selecting by elevated TMB. This study also demonstrated that ctDNA detectability correlated with tumor burden (number of target lesions and sum of largest diameters), a negative prognostic factor in some studies (42). Altogether, these studies suggest that both ctDNA burden and TMB need to be taken into account when correlating ctDNA measures to outcomes to immunotherapy, where undetectable ctDNA or elevated bTMB is associated with improved response while detectable ctDNA with low TMB is associated with worse outcomes. Delineating the prognostic versus predictive components of this relationship, along with defining the lower limit of reliable detectability, will be important to assess with prospective studies before clinical application of these metrics.

## ctDNA to Guide Clinical Development in the Adjuvant Setting

Immunotherapy is used in the adjuvant setting for unselected patients with stage III NSCLC treated with chemoradiotherapy and patients with stage III (nonocular/mucosal) melanoma,

demonstrating the proof of principle that immunotherapy can prevent or delay relapse for some patients (43, 44). But optimal use of immunotherapy in the adjuvant setting requires confident identification of patients with residual, microscopic disease after definitive therapy to prioritize adjuvant interventions and maximize the opportunity for cure (45, 46). ctDNA is poised to help solve this challenge, to permit allocation of patients to treatment with checkpoint blockade based on MRD detection status (45, 46). The ability of ctDNA to predict disease recurrence was previously shown in studies in lung and colon cancers through detection of MRD, shortly after completing local therapy (46, 47). Next-generation sequencing (NGS)-based ctDNA profiling was also used in patients with early-stage NSCLC and breast cancer to assess MRD (48, 49). Although these studies have demonstrated the prognostic importance of ctDNA in solid tumors after definitive treatment, it is unknown whether selection of high-risk patients will also effectively identify those with greater likelihood of cure from immunotherapy. To address this question, at least one prospective approach to integrating ctDNA and immunotherapy in the adjuvant setting has been announced already (48), with several others likely to follow.

It is important to note that the technologies in this space are evolving rapidly; the sensitivity or limit of detection of the test used in a given clinical trial is likely to impact the association between ctDNA MRD and clinical outcomes. For localized tumors, analysis platforms with greater sensitivity are necessary because early-stage tumors may be associated with levels of ctDNA that are below the limit of detection with some assays (2, 50). However, ultrasensitive detection must be balanced against an increasing rate of false positives. In addition, the issue of CH is particularly relevant in the adjuvant setting: >25% of patients with NSCLC have CH, with relevant *JAK2*, *KRAS*, and *TP53* mutations being identified (along with the more common leukemia-associated genes), and CH is strongly associated with smoking and prior radiotherapy (51); therefore, identifying these genes by liquid biopsy after curative therapy might incorrectly lead to suspicion of recurrence (19, 52). As mentioned, this issue may be resolved with the aid of paired ctDNA/cfDNA and peripheral blood cell genotyping (19). Another approach is to perform DNA sequencing on the original tumor to inform the generation of a more focused list of mutations for subsequent ctDNA/cfDNA assessment, thus potentially allowing for both greater sensitivity (through deeper focused sequencing) and specificity (as fewer false-positive results are expected from a focused list than from broad genomic characterization; refs. 52, 53).

### ctDNA and Response Assessment in Clinical Trials

ctDNA, in addition to its role as a selection tool, has the potential to be used as a surrogate end point in clinical trials (45), and as a possible adjunct to radiographic assessments. In hematologic malignancies, the association between MRD (i.e., loss of measurable cancer in the blood or bone marrow by NGS) and clinical outcomes is substantial and robust in patients with acute lymphoblastic leukemia (ALL) across therapies, assessment methods, cut-off levels, time points, disease, and study types (54). In particular, MRD detection is an early response indicator in ALL, leading to approval by the FDA in 2018 for MRD monitoring in ALL and multiple myeloma (55).

Achieving MRD negativity might even qualify as an endpoint for drug registration (54, 56).

Response assessment poses a particular conundrum for immunotherapies, for which responses may be delayed and/or deepen over time. In one study of nivolumab and ipilimumab in melanoma, the complete response rate increased from 11.5% to 19% with two additional years of follow-up, suggesting initial radiographic assessments did not capture the ultimate potential for tumor shrinkage in patients (57, 58). In a study of neoadjuvant PD-1 blockade, the rate of radiologic response was only 10% compared with the rate of major pathologic response of 45% in the same patients (59). In addition, modest changes in radiologically assessed response in patients treated with ICIs can be associated with substantial differences in OS (60, 61). Overall, these experiences demonstrate that radiologic assessment of response is incompletely capturing the patients and the degree to which benefit from immunotherapy is achieved. The benefit of ICIs lies in the potential durability of their effect more so than in their effect on tumor size. The possibility that changes in ctDNA can distinguish the depth and/or likely durability of systemic anticancer response requires evaluation. Furthermore, earlier and more accurate identification of ICI nonresponse by ctDNA could render clinical practice and trials more efficient, theoretically leading to significant cost savings and prevention of unnecessary adverse events, while also allowing nonresponding patients to receive other, potentially efficacious, therapies.

In between these applications of ctDNA to determine response or nonresponse is the significant ongoing challenge of determining the meaning of "stable" radiologic disease. Patients may be classified as having radiologically stable disease (SD) because they simply have innately indolent disease or because of ongoing disease control from active antitumor immunity (62). Although CT cannot be used to distinguish between these scenarios, ctDNA might be. A carefully designed study with a statistically powered focus on patients with SD could enable investigators to determine whether ctDNA can help parse which patients experience progression or true treatment response. Therefore, beyond correlating with radiologic response, ctDNA could provide distinct insight about *in vivo* response dynamics that are not effectively captured by routine radiology. The assessment of ctDNA in large, randomized studies is necessary to determine whether ctDNA correlates with or can act as a valid proxy for radiologic assessment of efficacy and clinical outcomes using accepted surrogate end points in solid tumor clinical studies (63).

ctDNA could also be complementary to radiologic assessment as a tool to dissect the distinct tumor response patterns that can occasionally occur with immunotherapies, such as pseudoprogression and mixed response (64, 65). In melanoma, ctDNA has correlated with radiologic outcome and might be useful in distinguishing regression, progression, and pseudoprogression in this setting (66).

### Conclusions

In the era of personalized therapy, ctDNA measurement from liquid biopsy might evolve into a powerful tool, with the potential to select populations for specific immunotherapy or combination treatments, predict response, facilitate adjudication of benefit, and provide earlier insight about response or resistance, all while minimizing the need for tumor tissue biopsy (Table 3). If ctDNA levels are shown to correlate with long-term outcomes

**Table 3.** Proposed or potential applications of ctDNA in immuno-oncology clinical development

	Localized disease	Metastatic disease
<b>Potential for selection or stratification at enrollment</b>		
Detection of disease at high risk of recurrence	X	
Molecular characterization to allocate to treatment (e.g., MSI, TMB, HRD)	X	X
<b>Potential end points</b>		
Detection of recurrence in the absence of radiographic disease	X	X (early detection of acquired resistance)
Resolution of detectable ctDNA	X	X
<b>Other</b>		
Translational research of disease biology, including understanding tumor clonal dynamics, response and resistance	X	X
Therapy deescalation in patients who achieve undetectable ctDNA		X

Abbreviations: ctDNA, circulating tumor DNA; HRD, homologous recombination deficiency.

such as OS in patients treated with ICI alone or in combination with other agents, ctDNA might also have a role as an end point in clinical studies to accelerate study execution. However, the resolution of the many issues discussed herein will be necessary to drive the implementation of this minimally or noninvasive alternative in routine clinical practice. Of particular importance is methodological and workflow standardization, without which it will be difficult to draw meaningful conclusions or compare results across studies. Moreover, the natural temporal evolution of

a patient's disease, as measured in ctDNA, must be established as the backdrop against which changes therein can be measured. Cost considerations and access will also be important. Optimistically, the relative ease and minimal infrastructure necessary for blood collection highlights how ctDNA has the powerful potential to facilitate access and reduce disparities globally, but this goal can be achieved only if cost is considered feasible. As with any new technology, practical and commercial considerations will likely lead to the adoption of dominant platforms with which newcomers to the space will align and against which they will be measured. Single- and multi-gene mutation assays on tumor tissue-derived DNA are already in routine use. Ongoing studies of focused and broad-based mutational profiling in ctDNA promise to further define the clinical utility of this minimally invasive approach, broadly in oncology practice and also in the unique clinical context of immune-oncology therapies.

### Disclosure of Potential Conflicts of Interest

A. Snyder holds ownership interest in Merck. M.P. Morrissey holds ownership interest in Merck and Novartis. M.D. Hellmann holds ownership interest in Shattuck Labs and Immunai and is a consultant/advisory board member for Merck, AstraZeneca, Bristol-Myers Squibb, Genentech, Mirati, Syndax, Nektar, Shattuck Labs, and Immunai. No other potential conflicts of interest were disclosed.

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