



# The Clinical Landscape of Circulating Tumor DNA in Gastrointestinal Malignancies

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Technologies for genomic analyses have revealed more details in cancer biology and have changed standard treatments for cancer, including the introduction of targeted gene-specific therapy. Currently, liquid biopsies are increasingly being utilized in clinical trials and research settings to analyze circulating tumor DNA (ctDNA) from peripheral blood. Several studies have shown the potential of ctDNA in the screening, prognostication, molecular profiling, and monitoring of gastrointestinal malignancies. Although limitations continue to exist in the use of ctDNA, such as method standardization, the sensitivity, concordance with tumor tissue, and regulatory issues, this field offers promising benefits for cancer treatment. A deeper understanding of tumor biology *via* ctDNA analyses and ctDNA-guided clinical trials will lead to the increasing use of ctDNA in clinical practice in the near future; this development will result in the improvement of outcomes among patients with gastrointestinal malignancies.

**Keywords:** circulating tumor DNA, colorectal cancer, gastric cancer, esophageal cancer, gastrointestinal malignancies

## INTRODUCTION

Over several decades, the gold standard in the diagnosis and screening of tumors has been tissue biopsy (1). However, conventional tissue biopsies are invasive, painful, and carry a risk of complications such as bleeding, local infection, and damage to neighboring tissues. Moreover, a tissue biopsy cannot always reflect tumor dynamics or response to treatment. The recent era of precision medicine, which represents a paradigm shift in cancer, has challenged the gold standard in diagnosis by introducing a transition from tissue biopsy to liquid biopsy. Compared with tissue biopsies, liquid biopsies carry minimal potential risk and can be repeatedly performed in routine practice during cancer treatment by using peripheral blood. Furthermore, liquid biopsies have the potential to provide more complete information regarding the biology of whole tumors despite tumor heterogeneity. Liquid biopsies include the testing of soluble factors, such as circulating tumor DNA (ctDNA) and circulating cell-free DNA (cfDNA), as well as proteins and tumor markers (2). cfDNA is highly fragmented DNA that is released from necrotic or apoptotic cells into the bloodstream (3–5). cfDNA consists of DNA from healthy cells and tumor cells, whereas ctDNA is defined as DNA that is derived only from primary or metastatic tumor cells.

Since Mandel and Metais (6) reported fragmented DNA in blood for the first time in 1948, technologies for cfDNA quantification have changed over 70 years from quantitative polymerase chain reaction to complex BEAMing and deep next-generation sequencing (NGS), thus achieving improvements in the sensitivity and specificity of cfDNA detection. With the development of sensitive techniques that can detect rare mutations, the heterogeneous landscape of tumors can be determined using blood samples. In fact, National Comprehensive Cancer Network guideline for non-small cell lung cancer (version 4. 2018) states that plasma biopsy should be considered if repeat biopsy is not feasible (7).

Here, we review ctDNA in gastrointestinal malignancies by focusing on clinical utility and future perspectives.

## ctDNA AND RELATED TECHNOLOGIES

The presence of cfDNA in the blood is a well-established fact, and DNA fragments are released from dying cells because of cellular turnover or other types of cell death (2). In cancer patients, a fraction of cfDNA is tumor derived and is termed ctDNA. ctDNA originates from primary tumors, metastatic tumor cells, or circulating tumor cells. ctDNA molecules are shorter than non-mutant cfDNA molecules in plasma, as demonstrated by PCR (8, 9) and sequencing (10, 11).

Representative approaches for analyzing ctDNA are summarized in **Table 1** (12). Mutation-specific real-time or endpoint PCR has been used for the detection of point mutations in ctDNA (13–17). More recently, digital PCR methods such as BEAMing and droplet digital PCR have been developed to improve the identification of genomic alterations in ctDNA (18–20). The recent implementation of NGS has allowed the direct sequence-based detection of chromosomal alterations in plasma DNA (21–23); however, it is necessary to distinguish the relatively few somatic alterations in ctDNA from the larger numbers of structural variants present in the germline cells of all individuals. Bioinformatics-based filters that enrich high-confidence somatic structural alterations while eliminating germline and artifactual changes have been developed (12). In addition, importantly, amplification in ctDNA can be depend on both the amount of ctDNA in the plasma due to high tumor burden and high copy number of specific gene. Commercially available kits for the NGS assays of ctDNA are summarized in **Table 2**.

## EARLY DETECTION OF CANCER

The early detection of cancer is one of the most important issues in reducing cancer-related deaths. In many cases, gastrointestinal cancer is detected *via* endoscopy or CT scans conducted for symptoms such as anorexia, abdominal pain, or constipation. ctDNA may have a potential role in the noninvasive early diagnosis and screening of gastrointestinal cancer. Even localized cancers shed DNA into circulation; therefore, ctDNA can be detected in patients with localized cancers, in addition to patients with advanced or metastatic cancers.

In a study across several early and late-stage cancers, ctDNA was detected in 73, 57, and 48% of patients with colorectal cancer (CRC), gastroesophageal cancer, and pancreatic cancer, respectively (25). The use of several biomarkers in ctDNA including the levels of overall ctDNA, *ALU247* fragment concentration (26), *KRAS* mutations (27, 28), *TP53* mutations (29, 30), *BRAF* mutations (28), and *septin 9* (*SEPT9*) methylation (31–34) have been demonstrated for the diagnosis of CRC. Also, detection of methylated *SEPT9* DNA in plasma is US FDA approved as a blood test for CRC screening. Compared with biomarkers for CRC, biomarkers for the diagnosis of gastric cancer (GC) and esophageal cancer have been assessed in a relatively small number of cohorts (25, 35). For the early detection or screening of cancers including CRC, GC, and esophageal cancer, the sensitivity of ctDNA analysis needs to be improved. Analysis that can be performed using a few milliliters of blood would be suitable for cancer screening; however, increasing the analytical sensitivity beyond 0.1% may not provide clinical benefits because it also leads to difficulties in distinguishing oncological mutations and sampling noise. In fact, cancer-associated genomic alterations have been found in plasma from healthy individuals (36). In addition, because many cancers share common gene mutations such as *TP53* mutations and *KRAS* mutations, ctDNA presents challenges in the detection of the specific organ sites of malignancies. To overcome these issues in ctDNA, the methylation profiling of cfDNA has been investigated in cancer diagnosis. Methylation haplotyping in plasma is a promising strategy for the early detection of a tumor and its primary growth site (37). Studies have reported the utility of methylation scores from over 9,000 CpG sites in cfDNA for cancer detection, with 76.3% accuracy for the prediction of cancer type (38).

Despite the above hurdles to the use of ctDNA in cancer screening, it is expected that the clinical use of ctDNA is less than a decade away because of its utility and convenience in cancer screening (**Figure 1**).

## PROGNOSIS AND DETECTION OF RESIDUAL DISEASE

Following curative therapy for gastrointestinal malignancies, ctDNA may be a potential biomarker for minimal residual disease. The detection of ctDNA even in the absence of any other clinical

**TABLE 1** | Available assays of detection of circulating tumor DNA (12, 24).

Characteristic	PCR assays		Next-generation sequencing (NGS) assays	
	Allele-specific PCR	Emulsion PCR	Amplicon-based targeted NGS	Capture-based targeted NGS
Variants potentially detected	Known recurring mutations	Known recurring mutations	Any exonic mutations, copy number gains	Exonic mutations, intronic gene fusions, copy number gains
Quantitation	Semiquantitative	Absolute or relative quantitation, wide dynamic range	Quantitation of relative AF, but vulnerable to PCR amplification bias	Quantitation of relative AF
Speed	Rapid	Rapid	Slower	Slower
Examples	Cobas (Roche), theascreen (Qiagen)	Droplet digital PCR (Biorad), BEAMing (Sysmex Inostics)	Tam-seq (Invitae)	Guardant360 (Guardant), cancerselect (personal genome diagnostics)

evidence of disease may mean that the patient has higher risk of relapse. In a cohort of 230 patients with stage II CRC, the assessment of ctDNA using the Safe-SeqS NGS method at the first visit after surgery indicated that recurrence-free survival at 3 years was 0% in a ctDNA-positive group and 90% in a ctDNA-negative group (39). Other studies have also demonstrated that the persistent detection of ctDNA after local therapy (surgery or radical radiotherapy) predicts a high risk of relapse in patients with colon cancer (40, 41). In addition, methylated *BCAT1/IKZF1* have been evaluated as biomarkers for CRC (42, 43). Of 397 patients with CRC who underwent primary tumor resection, odds ratio of a positive CEA test for recurrence was 6.9 (95% CI 2–22) compared to 14.4 (5–23, 25–40) for *BCAT1/IKZF1*.

In a meta-analysis of 16 studies including 1,193 patients with GC, the presence of ctDNA was significantly associated with the shorter disease-free survival (HR 4.36, 95% CI 3.08–6.16,  $p < 0.001$ ) and overall survival (HR 1.77, 95% CI 1.38–2.28,  $p < 0.001$ ) of GC patients, with high specificity (0.95, 95% CI

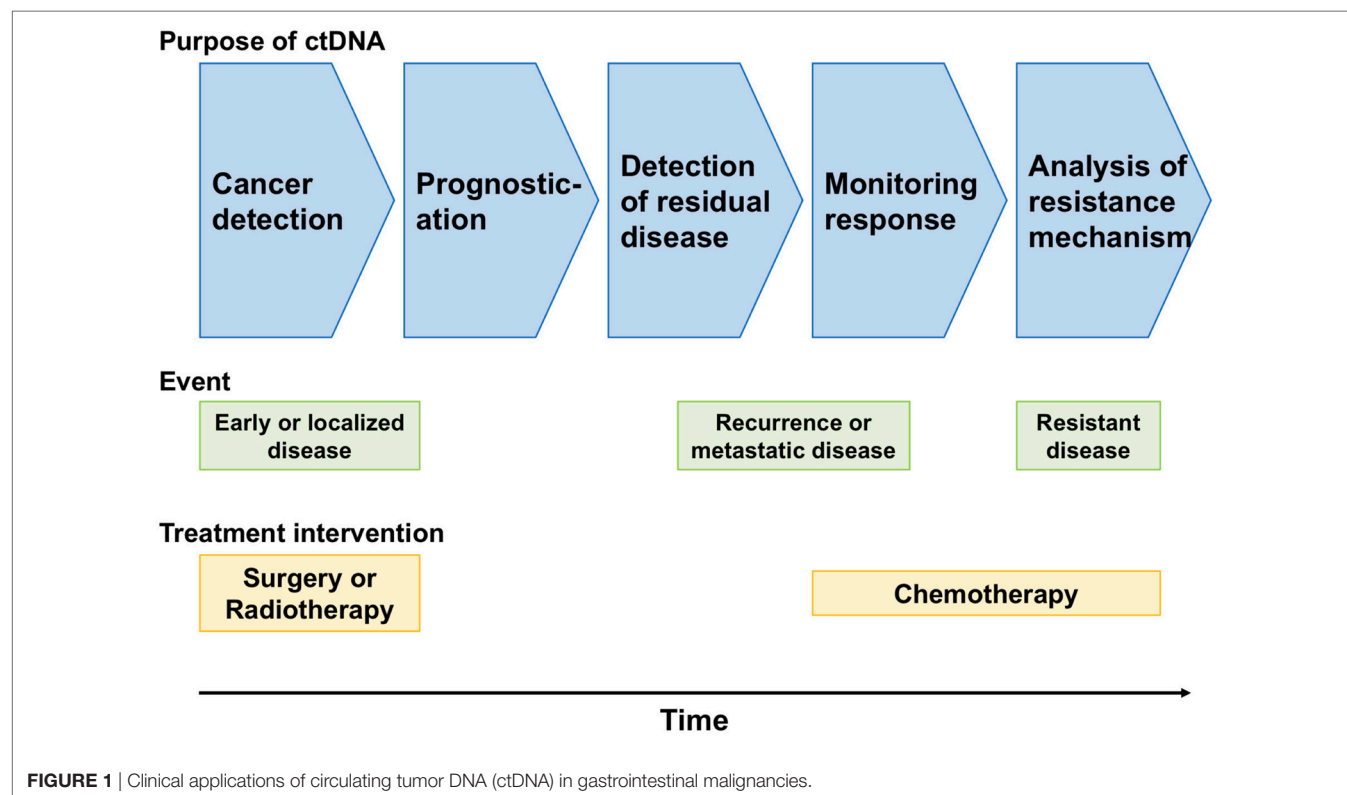
0.93–0.96) and relatively moderate sensitivity (0.62, 95% CI 0.59–0.65) (44). Another study demonstrated that the level of ctDNA was associated with tumor recurrence in patients who underwent curative surgery for GC (45). Similarly, several studies have reported the tumor-associated mutations in ctDNA and the prognosis of patients with esophageal cancer (46, 47); however, these studies included a limited number of patients, and further investigations are warranted. Almost all of these studies followed a retrospective design and provided limited validation for clinical use in gastrointestinal malignancies. One of the ideal applications of ctDNA is in the early detection of residual disease or recurrence compared with CT imaging and tumor markers. A more attractive idea is patient-specific ctDNA panels in patients who have undergone curative surgery (41, 48). Individual surgical tumor samples may provide a great opportunity to obtain tumor DNA from each patient to guide the design of patient-specific ctDNA panels from peripheral blood samples. Despite hurdles such as tumor heterogeneity, validation, regulatory issues, and quality control of individual panels, patient-specific ctDNA panels are potential biomarkers for postoperative monitoring.

**TABLE 2** | Commercially available circulating tumor DNA next-generation sequencing assays.

Panel	Company	Gene number	Assays
Guardant360	Guardant Health	73	Capture-based
PlasmaSELECT-R64	Personal Genome Diagnostics	64 + MSI	Capture-based
FoundationACT	Foundation Medicine	62	Capture-based
Oncomine Colon ctDNA Assay	Thermo Fisher Scientific	14	Amplicon-based

## BIOMARKERS OF CHEMOTHERAPY RESPONSE AND RESISTANCE IN METASTATIC DISEASE

Another clinical potential of ctDNA is in the determination of systemic chemotherapy regimens, the prediction of response to chemotherapy, and the identification of resistance mechanisms. The short half-life of ctDNA enables the real-time monitoring



of cancer burden, in contrast to radiological imaging or tumor markers. Indeed, a prospective study of 53 patients with metastatic CRC demonstrated that early changes in ctDNA during first-line chemotherapy predicted later radiologic response. Significant reductions in ctDNA levels were observed before cycle 2 and were correlated with CT response at 8–10 weeks (odds ratio 5.25 with a 10-fold ctDNA reduction;  $p = 0.016$ ) (49). Blood-based monitoring is an ideal strategy during cancer treatment because of its minimal invasiveness and avoidance of radiation exposure.

## RAS Mutations in Metastatic CRC

The assessment of *RAS* status has been mandatory in patients with metastatic CRC to predict the response of cetuximab and panitumumab to anti-EGFR antibodies (50–55). A double-blinded prospective study of 106 patients with mCRC has been performed to compare the *KRAS* mutation status assessed using tumor tissue *via* routine gold-standard methods to that assessed using plasma DNA *via* qPCR-based methods; the resultant specificity and sensitivity for the detection of *KRAS* point mutations were 98 and 92%, respectively, resulting in 96% concordance (28). In addition, the retrospective exploratory analysis in a biomarker subgroup of the CORRECT trial, which was a phase III trial investigating the efficacy and safety of regorafenib in patients with mCRC, confirmed the utility of detecting *KRAS*, *BRAF*, and *PIK3CA* mutations in ctDNA. Plasma DNA detected with BEAMing in 503 patients demonstrated that mutation status in ctDNA changed dynamically during chemotherapy and differed from that in pretreatment archival tissue (56). Currently, the OncoBEAM *RAS* CRC assay is the only European committee *in vitro* diagnostic test for *RAS* mutations in ctDNA. This assay is a qualitative PCR-based test and allows for the detection of 34 mutations within exons 2, 3, and 4 of *KRAS* and *NRAS* genes from a single blood sample. Four large cohort studies have been reported to achieve high concordance of approximately >90% (range, 89.7–93.3%) between OncoBEAM using plasma ctDNA and tumor tissue analysis in patients with CRC (57–60).

Acquired resistance to anti-EGFR antibody therapy has also been found by using ctDNA analyses. ctDNA from 28 patients receiving panitumumab monotherapy was assessed using qPCR, and 9 out of 24 (38%) patients whose tumors were initially *KRAS* wild-type showed *KRAS* mutations in ctDNA after panitumumab treatment (61). This study suggested that the emergence of *KRAS* mutations is a mechanism of resistance to anti-EGFR therapy and that these mutations may be detected in ctDNA as a more sensitive monitoring tool than radiological imaging. More recently, other studies have also demonstrated mutations associated with the resistance and decline of mutant *KRAS* clones after the withdrawal of anti-EGFR therapy (62, 63).

## BRAF Mutations in Metastatic CRC

*BRAF* mutations have been confirmed to be associated with poor prognosis in patients with metastatic CRC; moreover, the limited efficacy of anti-EGFR therapy in patients with *BRAF*-mutant metastatic CRC has been shown in several studies (64–66). The analysis of *BRAF* mutations in ctDNA by using qPCR-based methods has shown specificity and sensitivity of 100% (28). Based on preclinical studies (67, 68), the clinical trials of dual

EGFR and MAPK signaling pathway inhibition in patients with *BRAF*-mutant metastatic CRC is ongoing. In a phase Ib study of a combination therapy of dabrafenib, trametinib, and panitumumab, *BRAF* V600E mutant burden in ctDNA was more markedly reduced in responders than in nonresponders, and the emergence of *RAS* mutations was seen with disease progression in 9 of 22 patients (41%) (69). This exploratory analysis suggested that the monitoring of *BRAF* V600E mutant fraction in ctDNA could effectively predict response to combination therapy including a *BRAF* inhibitor and that overcoming the emergence of *RAS*-mutant subclones is important in combating resistance to this combination therapy.

## Other Alterations in Metastatic CRC

*HER2* or *MET* amplification is also known as a mechanism of resistance to anti-EGFR therapy in patients with metastatic CRC. The patient-derived xenograft models of *HER2*-amplified CRC showed resistance to anti-EGFR therapy (70, 71). In addition, the frequency of *HER2* amplifications increased from approximately 3% in treatment-naïve patients to over 10% in patients who were administered anti-EGFR therapy (72). Although there are few studies regarding the concordance of *HER2* status between ctDNA and tissue samples, 4 of 18 (22%) patients exhibited *HER2* amplification in ctDNA by digital PCR after cetuximab therapy despite being negative for *HER2* amplification prior to anti-EGFR therapy (73). The promising results of trastuzumab and T-DM1 combination therapy in the HERACLES trial (71) have encouraged clinical trials in patients with *HER2*-positive metastatic CRC such as the MyPathway trial (74) and the TRIUMPH trial (75); notably, the TRIUMPH trial includes patients with *HER2* amplification detected using not only tissue samples but also ctDNA analysis using an NGS-based method.

Another important alteration that causes resistance to anti-EGFR therapy is *MET* amplification. A preclinical model of *MET*-amplified CRC also showed resistance to anti-EGFR therapy (76). In fact, *MET* amplification in ctDNA was detected using NGS in 12 of 53 (22.6%) patients who showed disease progression with anti-EGFR therapy; no such amplification was detected in patients before cetuximab therapy. Furthermore, *MET* amplification in ctDNA was not detected in patients with *RAS* mutations after cetuximab therapy, thus suggesting that *MET* amplification is one of the mechanisms (other than *RAS* mutations) that cause resistance to anti-EGFR therapy (77). In a phase Ib trial of cabozantinib and panitumumab combination therapy, the preliminary evidence of efficacy in patients with *MET*-amplified metastatic CRC was reported (78).

## HER2 Amplification in GC

The amplification of the *HER2* gene or overexpression of the *HER2* protein, which contributes to cancer progression, has been reported in approximately 20% of patients with advanced GC (79, 80). According to the results of the ToGA trial, *HER2* is a key biomarker of *HER2*-targeted therapy using trastuzumab for advanced GC (79). The gold-standard diagnostic method for detecting *HER2* positivity and suitability for trastuzumab therapy is an immunohistochemistry score of 3+ or 2+ with a positive

result in fluorescence *in situ* hybridization. A retrospective study of 52 patients with advanced GC and 40 healthy volunteers demonstrated that the plasma *HER2-RPPH1* ratio (with *RPPH1* as a reference gene) was significantly higher in patients with *HER2*-positive tumors than those with *HER2*-negative tumors (81). More recently, the droplet digital PCR of *HER2* copy number in ctDNA has been reported. In a study of 60 patients with GC, including 17 patients who developed recurrence and 30 healthy volunteers, preoperative plasma *HER2* ratio correlated with tumor *HER2* status; postoperative plasma *HER2* ratios were high during the recurrence of tumors, which were diagnosed as *HER2*-negative tumors in surgery samples (82). Considering that *HER2* status may be altered after recurrence, the *HER2* copy number analysis in ctDNA enables the real-time evaluation of *HER2* status and leads to more effective treatment choices with *HER2*-targeted agents.

## FUTURE PERSPECTIVES

Overall, the data generated in all studies discussed above support the potential role of ctDNA in the diagnosis and treatment of patients with gastrointestinal malignancies. Despite a few limitations, including the standardization of detection and ctDNA quantification, the sensitivity, and concordance between ctDNA and tissue biopsies that currently hinder the routine use of ctDNA in clinical trials and clinical practice, its use would allow a deeper understanding of cancer biology and enable better cancer treatment, thus leading to improvements in patient survival.

In the context of clinical trials for metastatic disease treatment, several studies are ongoing or have been conducted using eligibility criteria based on gene alterations in ctDNA. A prospective study on the comprehensive ctDNA-guided treatment of advanced GC and lung cancers is ongoing in Korea (83). Another trial called the Targeted Agent and Profiling Utilization Registry, which is a large basket/umbrella trial sponsored by the American Society of Clinical Oncology, is accepting patient selection on the basis of ctDNA analysis (NCT02693535). In addition, an umbrella trial in

patients with mCRC based on the molecular profiling of ctDNA, including the status of *HER2*, *BRAF* V600E, *BRAF* non-V600E, *MET*, or high tumor mutation burden, is ongoing in Japan. If promising results are obtained in these clinical trials, ctDNA will be used in routine clinical practice and in clinical trials in the near future.

Economic and regulatory issues still hinder the practical use of ctDNA. Although most guidelines recommend that comprehensive molecular profiling should be conducted, the substantial costs of NGS assays lead many community oncologists to rely on PCR tissue tests and do not understand the added benefit of a comprehensive genomic test. In addition, emerging ctDNA-guided clinical trials are essential to obtain approval for the use of ctDNA in clinical practice. These barriers need to be challenged, perhaps initially in patients with CRC, which is one of the most prevalent gastrointestinal malignancies worldwide. Simultaneously, further studies are needed on other gastrointestinal malignancies such as esophageal cancer and GC to identify the best gene biomarkers that are detectable in ctDNA for the diagnosis, prognosis, and prediction of therapy response.

## CONCLUDING REMARKS

The potential role of ctDNA in gastrointestinal malignancies has been shown in basic studies, retrospective studies, and limited prospective studies. A paradigm shift in cancer diagnosis and treatment in ctDNA-based clinical trials and clinical practice will occur in the near future, thus leading to the availability of more DNA sequence information compared with that in the past decade. Although some limitations continue to exist on the use of ctDNA in clinical practice and clinical trials, ctDNA-based personalized therapy promises to improve patient outcomes and quality of life.

## AUTHOR CONTRIBUTIONS

This review was drafted by KS and DK and was revised by HB.

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