Clinical Applications of Circulating Tumor DNA Profiling in GI Cancers

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ABSTRACT

Over the next few years, the analysis of circulating tumor DNA (ctDNA) through liquid biopsy is expected to enter clinical practice and revolutionize the approach to biomarker testing and treatment selection in GI cancers. In fact, growing evidence support the use of ctDNA testing as a noninvasive, effective, and highly specific tool for molecular profiling in GI cancers. Analysis of blood ctDNA has been investigated in multiple settings including early tumor detection, minimal residual disease evaluation, tumor diagnosis and evaluation of prognostic/predictive biomarkers for targeted treatment selection, longitudinal monitoring of treatment response, and identification of resistance mechanisms. Here, we review the clinical applications, advantages, and limitations of ctDNA profiling for precision oncology in GI cancers.

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INTRODUCTION

GI cancers represent the second most frequent cancer diagnosis and the leading cause of cancer-related deaths in the United States.[1] A total of about 353,800 new GI cancer cases and 174,300 estimated deaths are expected in 2024. Colorectal cancer (CRC) is the most frequent tumor type among GI malignancies, followed by pancreatic, liver, stomach, and esophageal cancers, whereas gallbladder, small intestine, anal, and other digestive organ cancers are less frequent.[1] Geographical incidence and risk factors, both environmental and genetic, are highly heterogeneous across different GI cancers, and similarly prognosis and treatment strategies, including multimodality/multidisciplinary approaches involving surgery, systemic therapies, and radiotherapy, vary significantly depending to the disease type. Despite recent advancements in the therapeutic management of GI malignancies, the prognosis for the metastatic disease remains poor and considerable efforts have been made to develop efficient tools to dissect the tumor molecular landscape leveraging modern high throughput next-generation sequencing (NGS) technologies for diagnosis, biomarker discovery, dynamic monitoring of tumor response and treatment resistance, in addition to traditional approaches focused on soluble blood markers.

Tumor tissue genetic profiling from surgical specimens or endoscopic biopsies remains the gold standard for molecular testing and diagnosis; however, growing evidence support the role of circulating tumor DNA (ctDNA) testing, commonly referred to as liquid biopsy, in GI cancers as a noninvasive and more comprehensive method to complement tumor molecular profiling, thanks to its ability to recapitulate tumor heterogeneity and to provide a real-time dynamic screening of tumor evolution under treatment pressure, disease monitoring, and patient risk stratification.[2] More recently, the role of ctDNA is expanding from its use in the metastatic setting to early tumor diagnosis and minimal residual disease (MRD) detection.

This review focuses on the clinical applications of ctDNA profiling for precision oncology in GI cancers (Table 1).

ctDNA TESTING

ctDNA, consisting of DNA fragments from small 70-200 base pairs up to large 21 kb, is released from tumor cells into the blood of patients through necrosis, apoptosis, or other active mechanisms.[97] The half-life of ctDNA in the blood ranges from approximately 15 minutes to about 2-3 hours, but the DNA material is constantly released by the tumor in the blood circulation. It has been estimated that blood ctDNA concentration directly relates to the tumor burden, and multiple studies have shown that there is high concordance between the genetic alterations found in the tumor (including copy number alterations, single nucleotide variations, point mutations, and methylation/epigenetic variants; Fig 1) and those found in the ctDNA.[100] Notably, several factors, including primary tumor location and metastatic sites, can affect the ratio between blood circulating cell-free DNA (comprising different types of DNA fragments released in the bloodstream by any cell of the body) and ctDNA, which varies from <1% to >40%,[101] hence requiring testing tools with high sensitivity and specificity.

ctDNA can be tested for known mutations or alterations in a specific tumor (tumor-informed approach) or provide uninformed results on the tumor molecular profile including

TABLE 1. Clinical Applications of ctDNA in GI Cancers

Clinical Application	Approach	Tumor Type	Disease Stage	Significance	Clinical Recommendation (available test)	Ref
Early cancer diagnosis	ctDNA mutations	CRC, gastric, esophageal, liver, and pancreatic cancers	I-III	• Cancer screening, high specificity • Correlation with tumor burden	Consider as an option for CRC screening (Epi ProColon)	3-8
	ctDNA methylation	CRC, gastric, esophageal, liver, and pancreatic cancers	I-III	• Cancer screening, high accuracy		9-18
	ctDNA mutations + methylation + fragmentomics	CRC	I-II	• Cancer screening		19-21
MRD	ctDNA mutations	CRC, gastric, esophageal, liver, and pancreatic cancers	II-III	• Perioperative and postneoadjuvant/adjuvant treatment–positive ctDNA is associated with high risk of relapse, shorter disease-free survival, and overall survival • Positive ctDNA predicted radiological recurrence 6-8 months ahead • May inform adjuvant treatment decision making and surveillance	Consider in early-stage cancers after curative intent treatment (FoundationOne Tracker)	22-46
	ctDNA mutations	CRC	IV	• Perioperative and postchemotherapy-positive ctDNA is associated with high risk of recurrence, and shorter overall survival • ctDNA predicted radiological recurrence up to 10 months in advance		47-51
	ctDNA methylation	Gastric cancer	II-III	• Strong prognostic value of postsurgical LINE-1 methylation levels in ctDNA for recurrence risk		52
	ctDNA methylation	CRC	IV	• Postsurgical detectable ctDNA in combination with baseline ctDNA mutational status is associated with recurrence-free survival and benefit from adjuvant chemotherapy		53
Molecular profiling	ctDNA mutations	All GI cancers	IV	• Molecular profiling using ctDNA is highly concordant with tumor tissue sequencing and can aid tumor diagnosis and guide targeted treatment selection	Recommended in cases of inadequate tissue collection or challenging biopsy (Guardant360 CDx, FoundationOne Liquid CDx, Caris Assure)	54-67
Treatment response	ctDNA mutations	CRC, gastric, esophageal, liver, and pancreatic cancers	IV	• Baseline ctDNA levels and/or longitudinal changes during systemic treatment hold independent prognostic/predictive value and correlate with treatment response	Consider to longitudinally monitor treatment response to ICI (FoundationOne Tracker) Not yet recommended for standard treatment monitoring	68-85
	ctDNA methylation	Gastric cancer	IV	• Gene promoter methylation is associated with decreased progression-free survival and patient overall survival		86
Treatment resistance	ctDNA mutations	CRC, gastric, esophageal, liver, and pancreatic cancers	IV	• ctDNA can detect acquired resistance mechanisms and tumor heterogeneity • Changes in ctDNA profiles can guide subsequent treatment selection and rechallenge strategies	Consider to reevaluate potential targeted treatment selection after standard treatment failure (Guardant360 CDx, FoundationOne Liquid CDx, Caris Assure)	87-96

Abbreviations: CRC, colorectal cancer; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitors; MRD, minimal residual disease.

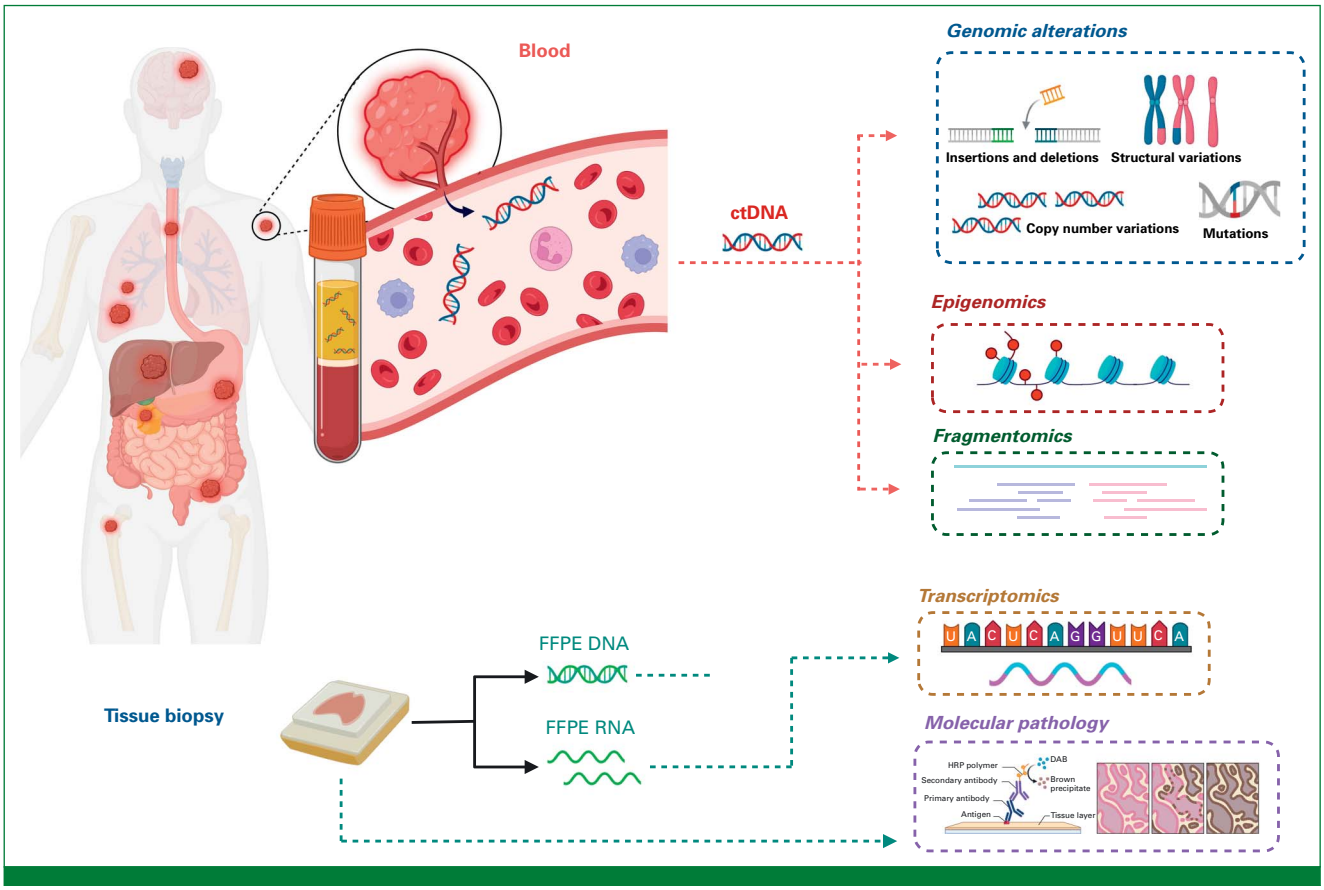

FIG 1. Molecular profiling of ctDNA in GI cancers. ctDNA is released from tumor cells (including primary tumors and metastatic sites) into the blood of patients through necrosis, apoptosis or other active mechanisms. Molecular profiling of ctDNA allows the identification of tumor genomic alterations such as structural chromosomic variations, gene copy number alterations, single nucleotide variations, point mutations, and insertion/deletions; epigenetic analysis of the methylation status of single/multiple genes or the entire genome (epigenomic), and other epigenetic variants; analysis of the cell-free DNA fragments (size, end motifs and DNA patterns[98,99]). ctDNA, circulating tumor DNA; FFPE, formalin-fixed paraffin-embedded. Adapted from Liquid & Tumor Biopsies in Genitourinary Cancers, by BioRender.com (2024). Retrieved from BioRender.com.

newly acquired tumor genetic alterations (tumor-agnostic approach). The tumor-informed approach has shown high analytical sensitivity; however, it requires initial testing of the tumor tissue for baseline profiling to identify tumor alterations that can be subsequently monitored through ctDNA.[102] The tumor-agnostic approach, independent of tumor tissue sequencing, could offer a quicker turnaround time and be informative in those cases where the tumor tissue is not available at the cost of a lower sensitivity.[102] Hence, the choice between the two should be guided by the testing goals and clinical scenario, in addition to the availability of the tumor material. Tumor-informed testing showed higher specificity and sensitivity compared with tumor-agnostic testing for the detection of MRD, monitoring of treatment response, and detection of early recurrence,[103,104] whereas a tumor-agnostic approach may better recapitulate tumor heterogeneity and allows for a broader tumor molecular profiling for targeted treatment selection or identifying the emergence of novel resistance mechanisms.

Polymerase chain reaction–based techniques and NGS multigene panels are powerful and widely used approaches in ctDNA testing when sufficient amount of ctDNA is available.[97] These approaches have high sensitivity in identifying key cancer-related alterations in genes such as *RAS*, *BRAF*, *HER2*, *BRCA*, *ALK*, *ROS1*, and *MET*, among others. However, technical challenges related to the yield of sufficient material for testing, particularly in the earlier disease stages when the ctDNA levels released by the tumor are low, can be a limitation for these analyses when used as a diagnostic tool. The analysis of differentially methylated regions on ctDNA has been proposed as a more sensitive and reliable approach for early tumor detection in these cases.[105] In fact, aberrant DNA methylation and epigenetic changes are involved in early tumor development, and detection of these alterations on different mediums, such as fecal DNA, has been leveraged for cancer screening and diagnosis.[106] Further limitations of these technologies include the lack of standards for preanalytical variables (ie, extraction methods), assay positivity cutoff criteria, and interpretation

TABLE 2. Food and Drug Administration–Approved ctDNA Platforms in GI Cancers

Assay (company)	Tumor Type	Biomarker	Method	Application
Guardant360 CDx (Guardant Health)	All solid tumors	Multigene panel	NGS	Genomic testing for targeted treatment selection
FoundationOne Liquid CDx (Foundation Medicine)	All solid tumors	Multigene panel	NGS	Genomic testing for targeted treatment selection
Caris Assure (Caris Life Sciences)[a]	All solid tumors	Plasma: cfDNA, cfRNA WBC: gDNA, mRNA	NGS (WES + WTS)	Genomic testing for targeted treatment selection
Epi ProColon (Epigenomics)	CRC	*Septin 9*	Qualitative DNA methylation by PCR	Early diagnosis
FoundationOne Tracker (Foundation Medicine)[a]	CRC	Patient-specific tumor-informed panel	NGS	MRD in early-stage cancers after curative intent treatment

Abbreviations: CRC, colorectal cancer; cfDNA, cell-free DNA; cfRNA, cell-free RNA; ctDNA, circulating tumor DNA; gDNA, genomic DNA; MRD, minimal residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction; WES, whole exome sequencing; WTS; whole transcriptome sequencing.
[a]Food and Drug Administration breakthrough device designation.

and reporting of results. Furthermore, the risk for false-positive or false-negative remains an issue and warrants careful interpretation of the analytical results.

To date, several ctDNA testing technologies have been approved by the Food and Drug Administration (FDA) for use in different malignancies including GI cancers (Table 2), and multiple studies are investigating liquid biopsy as a companion diagnostic or decision-making tool in clinical trials.

ctDNA FOR EARLY DISEASE DETECTION IN GI TUMORS

ctDNA testing has been explored as a noninvasive screening tool for GI cancer diagnosis. Limitations of this approach include the lack of information on the tumor mutational profile and risk of false-positive results.[107] On the other hand, early-stage low-volume disease may be at risk of false-negative results.

The CancerSEEK approach exploited ctDNA to test the presence of mutations in 16 genes in combination with the evaluation of eight cancer-associated proteins in patients' blood for early screening of eight common tumor types, including CRC, gastric, esophageal, liver, and pancreatic cancers.[3] The sensitivity of CancerSEEK was over 69% for esophageal, gastric, liver, and pancreatic cancer, and about 60% for CRC. Specificity, however, was over 99%, hence minimizing the risk of false-positive results.[3] Further studies are warranted to evaluate the clinical applicability of this and similar approaches.

Another approach involves testing for specific methylation patterns on plasma DNA. Liu et al[9] reported the results of a large prospective case-control study assessing an extensive methylation profile on plasma circulating free DNA and leveraging machine learning for cancer detection and tumor localization across more than 50 cancer types at different disease stages. The overall specificity of this classifier was 99.3%, with a sensitivity of 67.3% in a prespecified set of stage I-III common cancer types including CRC, esophageal, stomach, pancreas, and liver cancers.[9] Kandimalla et al[10] were also able to develop specific methylation panels integrated in a cell-free DNA methylation profiling test for early GI cancer detection, the EpiPanGI Dx. This approach leverages cancer-specific biomarkers (including CRC, esophageal, gastric, and pancreatic cancers, and hepatocellular carcinoma [HCC]), a pan-GI panel, and a multicancer biomarker panel with high prediction accuracy for GI cancer diagnosis.[10]

Another example is the FDA-approved EpiproColon test, evaluating plasma ctDNA *Septin9* gene methylation for CRC screening.[11] The EpiproColon test was noninferior to the fecal immunochemical test for early CRC detection, with a sensitivity of 72% and a specificity of 81.5%, and a negative predictive value of 99.8%.[11] Multigene methylation panels have also been tested for CRC screening, showing high detection rates with variable sensitivity and specificity, warranting further investigations.[12-15] The combination of ctDNA somatic mutations, methylation, and fragmentomic patterns has also shown extremely promising results for stage I-II CRC detection by the LUNAR-2 test (Guardant Health, Redwood City, CA).[19,20]

Several studies in other GI cancers support ctDNA as an effective tool for early cancer diagnosis. Data from a small gastric cancer series show that ctDNA levels have high reliability in early cancer detection and were able to predict tumor burden with greater sensitivity compared with traditional circulating biomarkers by comparison of pre- and postsurgical blood sampling in case-control studies.[4,5] The plasma ctDNA levels have been shown to increase with tumor stage and to correlate with vascular invasion, peritoneal relapse, and shorter survival.[108] Analysis of the cell-free DNA by MCTA-Seq in another study identified several methylation biomarkers that were able to detect gastric cancer with

high specificity (92%) and sensitivity, which increased with tumor stage (44% for stage I to 59% for stage II, 78% stage III, and 100% stage IV).[16] Results from this analysis were confirmed in CRC and HCC.[16,109]

Similarly, cell-free DNA promoter methylation testing has shown promising results as a highly sensitive and specific biomarker for early detection and diagnosis of pancreatic cancers.[17,18] Notably, the presence of *KRAS* mutations in plasma DNA has been reported as one of the first highly specific liquid biopsy–based markers for diagnosis and prognosis of pancreatic carcinoma.[6,110] In fact, *KRAS* mutations are considered driver mutations and can be found in over 90% of pancreatic cancers. The development of novel techniques and biomarkers for the diagnosis of pancreatic cancer is critical, and liquid biopsy technologies are under investigation to develop minimally invasive tools for early pancreatic cancer detection and disease monitoring (ClinicalTrials.gov identifier: NCT03334708).

Quantitative analysis of ctDNA has also been proposed as a new diagnostic biomarker for gallbladder cancer and cholangiocarcinoma.[7,8]

ctDNA FOR MRD MONITORING IN GI TUMORS

Liquid biopsy is emerging as a promising tool for evaluating MRD and early relapse after surgery in CRC, both in the adjuvant and metastatic settings. Several retrospective series have shown that postsurgery ctDNA analysis can identify patients with shorter disease-free survival,[22] and a positive postadjuvant chemotherapy ctDNA status can identify a subset of patients at high risk of recurrence.[23,24] On the basis of this initial evidence, prospective trials have confirmed the role of ctDNA testing in early-stage CRC treatment decision making.

In the randomized DYNAMIC II study, Tie et al[25] used ctDNA assessment to guide the adjuvant management in stage II CRC and, while a lower percentage of patients in the ctDNA-guided group received adjuvant treatment, the recurrence-free survival for the ctDNA-guided approach was noninferior to the standard. More recently, the same authors reported that a different pattern of recurrence could be observed between patients with undetectable and positive ctDNA levels, with local recurrence being more prevalent in the former group and distant metastases in the latter. Similar results were observed in a second study focused on locally advanced rectal cancer, where patients with ctDNA-positive results had a higher risk of recurrence, with the difference of identifying lung-only metastases as the main recurrence site in ctDNA-negative patients, hence suggesting that selected metastatic sites may pose a challenge for ctDNA detection.[111]

The prognostic role of ctDNA detection in stage II CRC and the benefit from adjuvant treatment in this high-recurrence risk subgroup have also been confirmed in the IMPROVE-IT2 and CIRCULATE trials.[26-29] The DYNAMIC III trial is currently ongoing to evaluate the use of ctDNA to tailor adjuvant treatment in stage III CRC (ACTRN12617001566325). To this point, the PEGASUS trial showed that ctDNA testing could guide the therapeutic management in stage II-III CRC, where patients with postsurgical nondetectable ctDNA underwent a de-escalated adjuvant strategy, while patients with post-adjuvant positive ctDNA received further escalated systemic treatment.[30] The observational GALAXY study from Kotani et al[31] confirmed that ctDNA positivity after surgery was the strongest prognostic factor associated with high risk of disease relapse in stage II and III CRC and identified patients who benefitted more from adjuvant treatment. Among the patients enrolled in this study, a positive MRD status by ctDNA testing could predict radiologic recurrence months before the clinical diagnosis.[32] The best approach to the treatment of MRD in these cases, however, is still to be defined. Results from the BESPOKE CRC study further showed a significant benefit from adjuvant chemotherapy in stage II-III CRC with positive MRD after surgery compared with MRD negative cases and confirmed the strong prognostic value of ctDNA testing.[33] Particularly, patients with early ctDNA clearance after adjuvant treatment had better disease-free survival compared with those who remained ctDNA positive, but worse than those who were ctDNA negative after surgery.[33]

Interestingly the INTERCEPT surveillance program, evaluating the integration of MRD testing by ctDNA after curative intent treatment into the clinical care of CRC patients, revealed that 53% of patients who tested ctDNA-positive during surveillance had concomitant radiological recurrence; hence, sensitivity and timing of MRD assessment may have to be improved for optimal detection.[112] Results from ongoing phase III trials are warranted to provide definitive evidence to promote the integration of ctDNA in the clinical decision making in early-stage CRC.

In the metastatic setting, patients with detectable perioperative ctDNA undergoing surgery (with or without chemotherapy) with curative intent for resectable CRC liver metastases showed higher risk of disease recurrence and shorter overall survival compared with those with undetectable ctDNA levels in retrospective series.[47,48] A meta-analysis of 28 studies confirmed that detectable ctDNA levels after surgical or chemotherapy treatment in metastatic CRC (mCRC) were associated with shorter progression-free and overall survival, with detection of ctDNA preceding radiological recurrence diagnosis by 10 months after curative intent treatment.[49] Similar data have been reported for selected patients with peritoneal metastases undergoing cytoreductive surgery with or without hyperthermic intraperitoneal chemotherapy.[50,51] Finally, a combination of baseline mutational profiling by ctDNA and postsurgical MRD testing by methylation-based ctDNA analysis was shown to predict adjuvant chemotherapy benefit and recurrence-free survival in patients with resectable CRC oligometastases in the prospective PRECISION study.[53] These data suggest that ctDNA testing may play a role in

clinical decision making for the optimal treatment strategy in resectable CRC metastases, warranting further evaluation in dedicated prospective trials.

The role of ctDNA in the perioperative setting in other GI tumor types is less defined and evidence is not as strong as for CRC. Nevertheless, emerging data show that this technology is promising in detecting MRD and predicting disease recurrence. Results from a prospective cohort of stage II-III gastric cancer patients undergoing surgical resection confirmed that preoperative plasma ctDNA levels independently correlated with tumor stage, and postoperative ctDNA detection was associated with reduced disease-free survival and patient overall survival, predicting recurrence 6 months ahead of radiological evidence.[34] Similar results were recently published from another prospective series investigating the role of ctDNA in patients with stage II-III gastric cancer enrolled to receive neoadjuvant chemotherapy followed by radical surgery.[35] This study also reported that longitudinal changes after neoadjuvant chemotherapy directly correlated with treatment response, suggesting that ctDNA could be a potential biomarker for early-stage gastric cancer patients undergoing treatment with curative intent. Furthermore, Ko et al[52] reported that presurgical methylation levels of LINE-1 in cell-free DNA from gastric cancer patients had strong prognostic value, with low baseline methylation levels being associated with worse survival, and postsurgical levels were an indicator of MRD with high recurrence risk.

A recent retrospective study in intrahepatic cholangiocarcinoma (ICC) investigated the role of ctDNA detection levels before surgery and found no correlation with disease recurrence or mortality rates.[36] However, the authors found a moderate-to-good correlation of ctDNA with tumor burden which may potentially be of use in contributing to inform surgical decisions or to monitor treatment response in case of neoadjuvant approaches.[36] Similarly presurgical detection of ctDNA was not associated with postsurgical outcomes in resectable esophageal adenocarcinoma (EAC), however, MRD after surgical resection or neoadjuvant treatment significantly correlated with worse disease-specific survival.[37] In this study, a tumor-informed ctDNA testing approach was superior to a tumor-agnostic approach, but it has to be noted that early-stage EAC has been reported to release low amounts of ctDNA, hence the detection power of this test alone for MRD in this setting may be limited. Nevertheless, ctDNA detection after treatment for esophageal cancer has been reported to be associated with tumor progression and patient survival in several series.[38-41]

Consistent with evidence in other disease types, ctDNA status after liver transplant with curative intent was associated with risk of disease recurrence in a small HCC series.[113] In pancreatic ductal adenocarcinoma, ctDNA detection of *KRAS* mutation in pre- or postsurgical blood testing was also independently associated with higher rates of relapses, shorter recurrence-free survival and worse overall survival, and may serve as a novel tool to inform clinical decision making, including treatment intensification strategies, in both the palliative and curative settings.[42-46] It is worth noting that the ctDNA detection rate after surgical resection with curative intent in pancreatic cancer is lower than other GI cancers, hence tumor-informed approaches can increase diagnostic sensitivity.[104]

Results of these studies across GI cancer types will have to be validated in larger cohorts to establish the role of longitudinal ctDNA evaluation during surveillance in GI cancer.

ctDNA FOR DYNAMIC BIOMARKER TESTING AND TREATMENT MONITORING IN ADVANCED GI TUMORS

In the era of precision oncology tumor molecular profiling is mandatory for several GI cancers to inform about the presence of prognostic and predictive tumor biomarkers and guide targeted treatment selection in the advanced setting. Tumor tissue testing represents the current gold standard, however, tumor spatial heterogeneity, technical difficulties in retrieving a suitable tissue sample, and dynamic tumor evolution remain an issue. Liquid biopsy, through genotyping of ctDNA can address these challenges providing a comprehensive tumor characterization, beyond the limitations of single site biopsy, including driver alterations, actionable targets and extensive biomarker testing, while allowing for a less invasive technique and longitudinal sampling to drive treatment decision making and monitor the therapeutic response and emergence of therapeutic resistance.[54,87,114]

In mCRC, current guidelines recommend extended *RAS* testing before anti-EGFR treatment to exclude patients with *RAS* mutated tumors, which are resistant to anti-EGFRs. ctDNA assessment has been shown to have high sensitivity and specificity for *RAS* mutations detection[55] and high concordance with traditional tissue-based techniques.[56,57] *KRAS*-mutant clones have been reported to emerge as mechanism of resistance to anti-EGFR treatment by liquid biopsy studies.[88,89] Notably, the mutant allele frequency rapidly declines after treatment interruption,[90] and rechallenge studies with anti-EGFRs in later treatment lines guided by ctDNA longitudinal testing have been performed showing encouraging results.[91,92] Beyond *RAS* mutations, ctDNA analysis has the ability to identify several additional mechanisms of treatment resistance and provide information on the mutational status of multiple genes aiding the identification of novel biomarkers while at the same time guiding the therapeutic choice for subsequent treatment lines when novel actionable mutations are found.[93,94] Furthermore, lower ctDNA levels before first-line treatment in stage IV CRC patients had a strong association with improved prognosis,[68] and early reduction in ctDNA levels during chemotherapy has been linked to improved treatment response and survival.[69,70] The role of ctDNA in driving the treatment of mCRC is extensively reviewed in the study by Patelli et al and Roazzi et al.[71,115]

Dynamic changes in ctDNA have been also reported to predict clinical benefit and treatment response, in other advanced GI cancers (including gastric, esophageal, pancreatic, and biliary cancers), where an early decrease of ctDNA levels during systemic treatment was associated with longer progression-free survival and patient overall survival.[72-76] Interestingly, the methylation status of tumor suppressor genes evaluated on cell-free DNA has also been linked with treatment response in advanced gastric cancer, where promoter methylation of *SOX17*, *RASSF1A*, and *Wif-1* identified patients with shorter progression-free and overall survival.[86] Furthermore, similar to CRC, ctDNA analysis can identify candidate patients and resistance mechanisms to targeted treatment such as *FGFR2* amplification.[116]

In advanced pancreatic cancer and biliary cancers, where collection of an adequate tumor specimen for tissue genetic testing may be challenging leading to delayed in diagnosis and treatment start, ctDNA can be used to support the cancer diagnosis, assess the tumor molecular profile to identify druggable alterations for treatment selection or to determine the development of resistance mechanisms and emergence of novel mutations under treatment pressure.[58-62]

In stage IV pancreatic cancers, the presence of ctDNA was associated with worse prognosis in terms of progression-free and overall survival and ctDNA levels correlated with tumor burden and liver metastatic spread.[77,78] Additionally, ctDNA concentration predicted tumor burden and treatment response in patients receiving FOLFIRINOX chemotherapy.[79,80]

Biliary cancers are highly heterogeneous and can harbor several actionable alterations such as *FGFR2* and *NTRK* fusions, *IDH1* mutations, *BRAF* mutations and *HER2* amplification. ctDNA analysis could recapitulate tumor genetic aberrations and reflected the estimated tumor volume in this setting.[63,64] Liquid biopsy has been shown to provide a high detection rate of stage III and IV cholangiocarcinoma with high concordance with tissue-based biomarkers.[65] In particular, detection of *IDH1* mutations in the ctDNA of patients with ICC has been shown to be reliable when compared with matched results on tissue biopsies,[66] and longitudinal assessment in the ClarIDHy study correlated with clinical response,[81] proving that liquid biopsy can be used as an effective tool for patient selection for targeted treatment. ctDNA testing has also been exploited for the identification of resistance mechanisms in patients with *FGFR2* fusion-positive ICC treated with FGFR inhibitors.[95] In a study from Goyal et al[95] serial ctDNA analyses showed the emergence of multiple *FGFR2* kinase domain point mutations at disease progression which were confirmed in matched tissue biopsies. It has to be noted, however, that ctDNA cannot be detected in 10%-15% of patients with advanced disease,[59,61] and the sensitivity of ctDNA detection for specific alterations, such as *FGFR2* fusions, may be lower than tissue biopsy, hence highlighting current limitations of liquid biopsy in biliary tract cancers.

Ongoing studies testing the clinical value of liquid biopsy as a supporting diagnostic tool for diagnosis and targeted treatment selection, such as the PREVAILctDNA trial (ClinicalTrials.gov identifier: NCT04566614), will provide critical evidence to support the integration of ctDNA testing in the clinical management of pancreatic and biliary tract cancers.

A novel application of ctDNA involves immunotherapy, where liquid biopsy may aid in both microsatellite instability (MSI) status detection for patient selection and evaluation of treatment response to immune checkpoint inhibitors (ICI). In fact, a high concordance has been reported between ctDNA MSI status assessment and tumor tissue testing in a series of gastric cancer patients receiving immunotherapy[67] and detection of MSI and tumor mutational burden in cell-free DNA have been shown to be feasible and to hold predictive value for treatment response in patients receiving PD-1 blockade.[82,83] Predictive models on the basis of total copy number variations in cell-free DNA were also predictive for response in advanced hepatobiliary cancer patients receiving ICI with or without levantinib.[84] The clinical utility of ctDNA-based MSI status evaluation in GI cancers has been recently confirmed by a large real-world study by Kasi et al,[117] who showed comparable MSI-H detection frequencies and ICI treatment responses in line with published evidence with the use of liquid biopsy. Furthermore, baseline ctDNA concentration and its dynamic change during treatment have been shown to correlate with tumor response and survival in patients treated with pembrolizumab.[85] Finally, longitudinal monitoring of ctDNA could contribute to identify dynamic biomarkers of ICI treatment resistance, such as alterations in WNT-signaling genes.[96]

In conclusion, liquid biopsy is emerging as an effective and highly specific tool for molecular profiling in GI cancers. Analysis of blood ctDNA can aid in early tumor detection (screening and diagnosis), MRD evaluation after treatment with curative intent to tailor the therapeutic choice, genetic evaluation for prognostic/predictive biomarkers for targeted treatment selection, dynamic treatment response monitoring and identification of resistance mechanisms. Additionally, ctDNA levels hold a prognostic value in both early-stage and advanced disease and can predict cancer recurrence ahead of radiological evidence. This technology has the advantage of being minimally invasive, which is particularly relevant for those tumors where tissue collection is challenging and for longitudinal testing, and of recapitulating tumor heterogeneity since the circulating pool of tumor DNA derives from different tumor locations and metastatic sites which may harbor subclones with different molecular makeup within the same tumor.

The applications of ctDNA are expanding and several prospective studies are integrating liquid biopsy technologies with traditional approaches to optimize the clinical decision making in GI cancer treatment. Future directions for the use of ctDNA testing in precision oncology focus on leveraging

this tool for a rapid and accurate prediction of long-term clinical outcomes, more sophisticated risk stratification, and informing personalized treatment escalation or de-escalation strategies or the early discontinuation of ineffective therapies, which could help avoid unnecessary side effects and financial burden. Limitations of ctDNA testing will need to be addressed in order to standardize analytical techniques, interpretation of results, and increase sensitivity for those tumor types with lower ctDNA release (ie, esophageal cancer or early-stage pancreatic and hepatobiliary cancers). A consensus on the optimal timing of sampling to inform the disease status in different settings for different applications will also need to be reached. Nevertheless, the role of ctDNA in CRC is supported by strong evidence, and in the next few years, ctDNA is expected to enter clinical practice and revolutionize the approach to biomarker testing and treatment selection in GI cancers.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical Applications of Circulating Tumor DNA Profiling in GI Cancers

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