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Circulating tumor DNA for diagnosis, prognosis and treatment of gastrointestinal malignancies

Kirchweiger P *et al.* ctDNA in gastrointestinal malignancies

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Abstract

Minimally invasive detection of circulating tumor DNA (ctDNA) in peripheral blood or other body fluids of patients with gastrointestinal malignancies *via* liquid biopsy has emerged as a promising biomarker. This is urgently needed, as conventional imaging and plasma protein-derived biomarkers lack sensitivity and specificity in prognosis, early detection of relapse or treatment monitoring. This review summarizes the potential role of liquid biopsy in diagnosis, prognosis and treatment monitoring of gastrointestinal malignancies, including upper gastrointestinal, liver, bile duct, pancreatic and colorectal cancer. CtDNA can now be part of the clinical routine as a promising, highly sensitive and specific biomarker with a broad range of applicability. Liquid-biopsy based postoperative relapse prediction could lead to improved survival by intensification of adjuvant treatment in patients identified to be at risk of early recurrence. Moreover, ctDNA allows monitoring of antineoplastic treatment success, with identification of potentially developed resistance or therapeutic targets during the course of treatment. It may also assist in early change of chemotherapy in metastatic gastrointestinal malignancies prior to imaging findings of relapse. Nevertheless, clinical utility is dependent on the tumor's entity and burden.

Key Words: Cell-free tumor DNA; Circulating tumor DNA; Liquid biopsy; Gastrointestinal cancer; Esophageal cancer; Gastric cancer; Liver cancer; Bile duct cancer; Pancreatic cancer; Colorectal cancer

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Core Tip: This review provides an update on the state-of-the-art circulating tumor DNA detection *via* liquid biopsy for diagnosis, prognosis and treatment in gastrointestinal malignancies and presents the strengths and limitations of this innovative method.

INTRODUCTION

Historically, tissue biopsy or plasma protein-derived tumor markers have been the fundamental pillars of cancer diagnosis, selection of treatment, monitoring of treatment effect and estimation of prognosis^[1]. As cancer is a dynamic and likely progressive disease, histological analysis of a single lesion (*i.e.*, primary tumor or metastasis) at a single time point is now being replaced by minimally invasive detection of cell-free deoxyribonucleic acid (cfDNA) *via* liquid biopsy to monitor the continual change of the disease process^[1,2,3]. Furthermore, discordance and genetic differences within the primary tumor tissue over time (temporal heterogeneity) or between the primary tumor and its metastases (spatial heterogeneity) can be observed *via* next-generation sequencing (NGS)^[2,4]. Thus, single conventional biopsies do not accurately reflect the cellular and genetic composition of malignancies^[1]. In contrast, liquid biopsies include nucleic acids or cancer cells from the entire tumor burden of the patient and can easily be conducted serially^[4].

Discovered in 1989 in patients with gastrointestinal (GI) malignancies, circulating tumor DNA (ctDNA) derives from apoptotic, necrotic, or circulating cancer cells, and

constitutes a small subset (< 0.01%) of cfDNA in the plasma of peripheral blood or other body fluids^[5]. cfDNA has become a promising tool for diagnosis, monitoring of antineoplastic treatment effect, and early detection of relapse, in addition to evaluating potential new drug targets^[6-8]. ctDNA is thought to be actively released *via* microvesicles (exons) of double-stranded DNA. Passive release of DNA fragments into the circulation from apoptotic and necrotic cells has been demonstrated^[3,9]. The amount of cfDNA is significantly higher in cancer patients than in healthy individuals, but serum levels can easily be biased by various factors; ctDNA is considered tumor-specific and more robust^[1]. Nevertheless, both values are strongly influenced by preanalytical and analytical variables. The influence of differences in type of sample collection tubes, sample storage time, performing the assay with plasma or serum, use of short or long amplification assays, or the time of blood collection have been evaluated^[10]. Plasma is considered superior to serum because of its robust DNA data with higher KRAS allele frequency. A greater absolute amount of DNA is yielded by serum samples, but is also more affected by contamination or lysis^[10]. Although the American Society of Clinical Oncology and the College of American Pathologists highly recommend plasma analysis for DNA detection, many investigators in the past used serum samples. A review by Saluja *et al*^[10,11] in 2018 claimed that 100% of gastrointestinal stromal tumor (commonly known as GIST) studies, 62% of gastric cancer studies, 29% of esophageal cancer studies, and 20% of colorectal cancer (CRC) studies used serum samples for ctDNA analysis.

Over the years, several amplification techniques, such as real-time quantitative polymerase chain reaction (referred to as qPCR)^[12], digital droplet PCR (ddPCR)^[13,14], beads, emulsion amplification, and magnetics (BEAMing)^[15] or NGS^[16] have been adopted for clinical use^[4]. The most commonly employed are digital PCR (dPCR) or ddPCR techniques using water-in-oil emulsion droplets with dispersed individual DNA strands. These fluorescently labeled samples allow a binary identification system of target mutations (*i.e.* mutant *vs* wild-type alleles), leading to a very low limit of detection (LOD) ranging from 0.1%–0.001%^[4]. One of the most used

dPCR systems for ctDNA detection is the Bio-Rad QX-200 platform^[14]. BEAMing provides a high analytical sensitivity of < 0.01% minor allele frequency (MAF) by combining emulsion PCR and flow cytometry with a focus on rare mutations in *a priori* known target mutations^[4]. NGS, on the other hand, can cover a broad range of mutations in multiple cancer-associated genes but is less sensitive than dPCR (~ 1%)^[4]. Safe-SeqS is one of the first and most commonly used NGS platforms (LOD 1%)^[17], whereas CAPP-Seq/iDES is a newer NGS technique with LODs of 0.002%-0.00025%^[18]. Depending on the entity under investigation, approaches have emerged for detection within samples with a known mutation target and those without a known mutation. In the following section we describe currently promising prospects of this new and easily harvested biomarker for diagnosis, early relapse detection, and treatment efficacy.

UPPER GI CANCER

Diagnosis

Upper GI (UGI) cancer subsumes esophageal cancer, cancer of the gastroesophageal junction, and the gastric cancer. Unfortunately, detection rates of UGI cancers are low in the early stages (approximately 20%) and reporting studies, thus, have low case numbers^[19]. However, potential targets for molecular tracking are: HOXD10 (higher methylation rates in more advanced disease); ZIC1, RUNX3, and TP53 (53%); or receptor tyrosine kinases, including KRAS (15%), FGFR2, EGFR (17%), ERBB2, PIK3CA (13%), or HER2 (17%)^[7,20,21]. NGS of metastatic UGI cancer in small case studies revealed detection rates of up to 87.5%^[21]. Detection rates greatly depend on the MAF in the site of associated metastases, with only 23.3% in the lung, 19.2% in the liver, and only 2.5% in peritoneal metastases; the primary tumor burden is represented by tumor volume^[20].

Prognosis

Relapse prediction following neoadjuvant treatment is a substantial issue in UGI cancer that affects almost all patients undergoing surgery. A study including more than 1600

patients reported postsurgical detection rates of up to about 32% and that MAF cutoff levels of $> 0.25\%$ (100% sensitivity) were associated with worse progression-free survival (PFS) (12.5 mo *vs* not reached, $P = 0.03$, $n = 22$)^[20]. A significant survival disadvantage was observed in patients undergoing treatment with checkpoint inhibitors when detecting a MAF of $> 3.5\%$ prior to treatment initiation (8.8 mo *vs* 2.5 mo, $P = 0.04$, $n = 27$)^[20]. If detectable, some mutations like PIK3CA (3.8 mo *vs* 13.6 mo, $P = 0.006$) or BRAF (5.6 mo *vs* 13.7 mo) indicate especially poor survival among stage IV patients^[20]. On the other hand, targeted therapy, when detecting HER2 or EGFR mutation, can lead to significant survival benefits (21.1 mo *vs* 14.4 mo, $P = 0.001$)^[20]. These findings need to be evaluated in larger prospective studies.

Treatment monitoring

Serial measurement of ctDNA in stage IV UGI cancer has found a significant survival benefit for patients with a $> 50\%$ decrease of the maximum MAF (13.7 mo *vs* 8.6 mo, $P = 0.02$, $n = 35$) during the course of first-line therapy^[21]. The Personalized Antibodies for Gastroesophageal Adenocarcinoma (“PANGEA”) study revealed promising results in 68 patients undergoing ctDNA-guided individualized monoclonal antibody treatment compared with historical chemotherapy controls (1-year survival of 66%, median overall survival (OS) of 15.7 mo, $P = 0.0024$, median PFS of 8.2 mo, and first-line response rate of 74% *vs* about 50%)^[22].

LIVER AND BILE DUCT CANCER

Diagnosis

CtDNA has been investigated in liver cancer patients for several years, and although it is still not in routine clinical use, liquid biopsy was shown to be superior to conventional plasma-derived biomarkers. For example, alpha-fetoprotein has a diagnostic sensitivity of 50% for hepatocellular carcinoma (HCC)^[23]. Unfortunately, HCC has a broad range of potentially mutated genes. The most common are TP53

(c.747G>T), TERT (c.1-124C>T), and CTNNB1 (c.121A>G and c.133T>C)^[24]. Generally, detection rates using liquid biopsy are expected to reach 56% in resectable HCC patients (ddPCR of 48 samples)^[24]. A study published in 2006 reported sensitivity and specificity values of 69% and 93%, respectively, for discrimination of HCC and controls using cfDNA cutoff levels^[25]. Subsequently, the presence of a combination of different methylated tumor suppressor genes, which rarely occur in the DNA of healthy tissue, had a reported sensitivity of 83.3% and specificity of 90.5% for detection of HCC^[26]. Apart from detection of malignancies, liquid biopsy and stratification following detection of methylated peroxisome proliferator-activated receptor gamma (commonly known as PPAR γ) gene promoter has also shown promise for prediction of fibrosis grade in nonalcoholic fatty liver disease^[27].

On the contrary, data on mutation detection *via* ctDNA in bile duct cancer is sparse, as cholangiocarcinoma is a rare disease. It has an estimated incidence 0.5-3.5/100.000), is often diagnosed at a metastasized stage, and the reported data is frequently pooled with liver or pancreatic cancer^[28]. Overall, about 28% of patients with bile duct cancer show TP53 mutations, followed by 17% with ARID1A mutations and 16% with KRAS mutations^[28]. However, bile duct cancer has very heterogenous mutation patterns. Using liquid biopsy in cases with a histologically verified mutation, Ettrich *et al*^[28] reported a detection rate of 92% in intrahepatic cholangiocarcinoma (IHCC) and only a 55% detection rate in extrahepatic cholangiocarcinoma (referred to as EHCC).

Prognosis

Both the untargeted (cfDNA) and targeted detection of mutation, primarily of TP53 (32%), CTNNB1 (17%), and TERT (51%), has shown prognostic potential indicating poorer disease-free survival and OS in patients with HCC, regardless of tumor stage^[23,24,29-31]. Moreover, vascular invasion, tumor mass, and level of postoperative cfDNA have emerged as independent risk factors for recurrence in patients with resectable HCC^[32].

Regarding cholangiocarcinoma, some studies reported poorer PFS when detecting mutations *via* liquid biopsy, especially in cases with ctDNA assay of TP53, KRAS, BAP1, or PBRM1 in settings of both curative and palliative intent, as compared with patients with nondetectable mutation^[33-35]. Again, the data was obtained in IHCC patients; most studies could not detect a significant correlation regarding PFS in EHCC patients^[28].

Treatment monitoring

Serial ctDNA measurement in advanced HCC has revealed progression of the disease before imaging or alpha-fetoprotein dynamics could indicate recurrence, but the studies included small case numbers^[36]. As ctDNA MAF of both IHCC and EHCC correlate with tumor load, some authors estimate a potential for treatment efficacy detection in bile duct cancer, but that needs further evaluation, as serial measurement for treatment monitoring has not yet been performed^[28,37]. In a January 2021 publication, Felden *et al*^[31] reported prospective findings of ultra-deep sequencing and ddPCR in 121 patients that supported the treatment-monitoring potential of ctDNA as a biomarker response to antineoplastic treatment.

PANCREATIC CANCER

Diagnosis

While surgical resection can improve 5-year survival by 15%-25%, fewer than 20% of patients qualify for a primarily surgical approach^[38]. In 2018, more than 50% of patients were diagnosed with distant metastases and had a 5-year survival rate of only about 3%^[39]. The mean 5-year survival of all stages of pancreatic ductal adenocarcinoma (PDAC) stages is reported to be about 6%-8%^[39]. Thus, highly sensitive and reliable biomarkers are urgently needed for earlier diagnosis. Theoretically, PDAC, which accounts for 90% of all pancreatic cancers, could be an ideal entity for agnostically driven ctDNA determination as a screening biomarker because of the high rate of histologically detectable early KRAS mutations (> 90%)^[40,41]. However, detection rates in histologically verified PDAC *via* liquid biopsy are significantly lower, controversially

reported in literature, and very much depend on the stage of the disease (43%-54% in stages I-II; 67% in stage IV, and up to 95% if a mutation had already been detected in the tissue)^[42,43,44]. Another study suggests much lower detection rates for early-stage PDAC^[45]. Using ddPCR, Berger *et al*^[46,47] reported in 2016 that mean cfDNA values (KRAS, GNAS) discriminated potentially premalignant cysts (*e.g.*, intraductal papillary mucinous neoplasms) and harmless pancreatic cysts. Nevertheless, the sensitivity and specificity were too low for applicability as a potential screening method. Thus, ctDNA offers little clinical application in diagnosis of localized pancreatic cancer, as it is inferior to the plasma-protein derived tumor marker CA 19-9, which has high sensitivity (70%-95%) and specificity (70%-90%). It also has a high vulnerability to coincident Lewis-negative blood group, acute cholangitis, obstructive jaundice, or chronic pancreatitis^[48-52]. Therefore, the gold standard for diagnosis remains imaging combined with histological verification with endoscopic ultrasound and fine needle aspiration (commonly known as EUS-FNA)^[38].

Prognosis

Although lacking usability in the initial diagnosis of PDAC, several studies demonstrated a significant correlation between both pre- and postoperative ctDNA positivity and OS [hazard ratio (HR): 2.093, $P = 0.028$] and PFS (HR: 4.543, $P = 0.006$) for both localized and metastatic PDAC (referred to as LPDAC and mPDAC, respectively)^[53-56]. In 2010, Chen *et al*^[57,58] reported a median OS of 3.9 mo *vs* 10.2 mo ($P < 0.001$) positivity in 91 patients with mPDAC, associated with mutKRAS ctDNA. In 2019, Lee *et al*^[58] reported an OS of 5.8 mo *vs* 16.3 mo in LPDAC. The same group showed 100% of patients remaining ctDNA-positive after systemic neoadjuvant treatment following an early relapse, with a median PFS of 5 mo.

Treatment monitoring

Liquid biopsy allows earlier detection of relapse compared with plasma protein-derived tumor markers (CA 19-9), with lead times of 1 mo to 2 mo, and is more sensitive

(83%) to changes in ctDNA levels^[59,43]. This could indicate a potential opportunity for monitoring treatment during palliative chemotherapy using serial liquid biopsies, and ultimately making a change of the antineoplastic agent. Data on serial measurement in advanced PDAC mostly lacks large patient numbers, although promising results have raised the hope of early response to therapy and, ultimately, relapse identification^[43]. Kruger and colleagues^[43] were the first to report the potential of ctDNAs to indicate response as early as 14 d after treatment initiation, demonstrating major superiority to plasma-protein derived tumor markers, with a specificity of 100%. Nevertheless, the clinical survival benefit by eventual change of treatment in patients with detected relapse using serial ctDNA measurements still needs to be explored. Targeted therapy could be another promising field of future research. Liquid biopsy has already found usage in PDAC patients suffering from BRCA1/2 mutations by providing PARP-inhibitors^[60].

CRC

Diagnosis

CRC is the third leading newly diagnosed malignancy worldwide^[61]. CtDNA is detectable in about 73% of stage II–III cases and 90% of patients with localized and metastatic CRC, positioning this entity as the ideal target for liquid biopsy^[62,63]. Until now, liquid biopsy has not been included in the routine screening for CRC, but samples are easily assessable. Tests are becoming more cost effective and the presence of ctDNA in early-stage CRC (46% detection rate in stage I) was reported in 2015^[64]. Acceptance of liquid biopsy in the general population appears to be high, based on a 2014 German study finding that patients not willing to undergo colonoscopy preferred blood tests over other noninvasive screening tools, like fecal occult blood tests^[65].

Prognosis

Multivariate analyses conducted in several studies have confirmed that postoperative detection of ctDNA is an independent marker of recurrence, regardless of stage and

location of the primary tumor. The 3-year PFS was 33% *vs* 87%^[66,67]. In 2019, Tie *et al*^[66] serially measured plasma ctDNA in 159 patients with locally advanced rectal carcinoma (T3/4 and/or N+) and treatment-naïve stage, post-chemoradiotherapy, of 4–10 wk after primary curative resection with adjuvant treatment. In an analysis that was blinded to ctDNA status, HRs and 3-year PFS significantly differed with positive liquid biopsy results. The 3-year PFS was 33% *vs* 87% after chemoradiotherapy (HR: 6.6, $P < 0.001$) and was 13.0 ($P < 0.001$) after resection, which allowed for stratification of patients with very high and very low risk of relapse^[66]. Based on those findings, several ongoing prospective international studies are evaluating the potential additional clinical benefit from postoperative ctDNA positivity in CRC to identify patients at high risk of recurrence^[7].

CtDNA-positive patients with stage II disease (*i.e.* with no clear recommendation for adjuvant treatment) might benefit from additional adjuvant chemotherapy. That is being tested in the DYNAMIC (ACTRN-12615000381583), COBRA (NCT04068103), and CIRCULATE AIO-KRK/PRODIGE 70 (NCT04089631/NCT04120701) trials. Whether stratifying stage III CRC patients by ctDNA results can guide decision making for intensification or de-escalation of adjuvant treatment or surveillance is being tested in the DYNAMIC-III (ACTRN-12617001566325) study^[7,68-70]. Postoperative ctDNA detection has proven to be a strong indicator of distant recurrence, with a median lead time of 10 mo compared with conventional modalities such as computed tomography (commonly known as CT) and plasma-derived biomarkers like carcinoembryonic antigen (CEA)^[71]. Regarding metastatic CRC, studies have demonstrated a significant survival benefit of wild-type carrier patients compared with patients with detectable ctDNA, dependent on the particular mutation^[72].

Treatment monitoring

At diagnosis, approximately 80% of patients with CRC present without distant metastases and undergo primary curative resection. Over 50% of patients with stage II or III cancer also show rather unspecific abnormalities, such as elevated CEA (20%), CT

abnormalities (40%), or both (13%) during the 5-year surveillance recommended by the American Society of Clinical Oncology (known as the ASCO)^[73-75]. Nevertheless, until now there is no evidence of OS improvement resulting from 5-year surveillance, including clinical and endoscopic examinations, CEA measurement, and imaging^[76,77]. Liquid biopsy could help to clarify uncertain findings. For example, ctDNA is positive in 85% of persons who experience imaging-verified relapse, whereas increased CEA levels are observed in only about 41% of radiologically verified recurrences^[78]. Moreover, lead time of liquid biopsy compared with CEA is reported to be about 8 mo^[77]. Thus, serial ctDNA measurement as a postoperative treatment monitoring method during surveillance could provide earlier detection of relapse.

The clinical benefit for OS has to be evaluated in future studies. Among other ongoing ctDNA studies investigating the benefit of adjuvant chemotherapy based on liquid biopsy findings, Danish investigators (IMPROVE-IT2; NCT04084249) have evaluated the surveillance improvement when implementing supplementary fludeoxyglucose-positron emission tomography/CT follow-up evaluation every 3 mo, based on ctDNA positivity (*i.e.* ddPCR every 4 wk) for 2 years after surgery for stage II-III CRC and early detection of relapse^[79]. Most centers use NGS prior to the start of antineoplastic treatment for identification of potential therapeutic targets, providing in advance a mutational target for liquid biopsy. Interim analysis of our own ongoing study revealed detection rates of more than 92% in metastatic CRC with ddPCR of 28.5 mL plasma samples and known mutations found in tissue samples prior to analysis. That is in line with the 8% discordance rate in a BEAMing analysis of 236 patients reported in 2018^[80].

Serial liquid biopsies allow response prediction prior to that obtained by conventional methods in metastatic-stage patients undergoing palliative chemotherapy^[81]. Furthermore, studies have demonstrated the PFS and OS benefits of repeated mutational status determination for eventual ⁶rechallenge with cetuximab/irinotecan-based regimes in initially RAS/BRAF wild-type patients or patients with acquired resistance during the course of treatment^[82,83].

CONCLUSION

CtDNA is ready to be integrated into routine clinical use in order to improve survival and relapse prediction in nonmetastatic GI cancers. It also allows for monitoring of antineoplastic treatment success for early detection of nonresponders, with potential early change of chemotherapy in metastatic GI malignancies prior to imaging findings of relapse. For some entities, especially CRC, rapid progress in liquid biopsy research could lead to fundamental changes in therapeutical strategies, accompanied by the desired survival improvement. The test is simple, cost effective, and easily assessable, although there are large differences in suitability, detection rate, progress of research (Table 1), tumor volume, and site of metastasis. Overall, lymph node metastases or peritoneal carcinosis lead to significantly lower amounts of detectable ctDNA compared with liver or lung metastases. Various techniques of target mutation detection have been established in clinical trials, and several potential preanalytical variables have to be taken into account when implementing these into routine clinical practice. Depending on the technologies in clinical use, the limits of detection range from about 1% (qPCR, Safe-SeqS) to 0.01% (ddPCR, BEAMing, CAPP-Seq/iDES)^[4]. Nevertheless, mutation detection *via* liquid biopsy has several potential pitfalls and limitations. Firstly, standardization of sample drawing and the processing methods could help avoid common mistakes leading to very heterogenous sensitivity and specificity. Secondly, measured ctDNA levels are strongly affected by the period of time between blood draw and surgery or the initiation of chemotherapy. The ideal post-interventional interval for sample assessment needs to be further explored and eventually standardized, as ctDNA levels initially increase but continuously decline over the following weeks. The same applies for systemic antineoplastic treatment, as Maron *et al*^[20] reported considerable differences in ctDNA MAF in untreated stage IV UGI patients (mean MAF: 11.6%) compared with patients receiving treatment up to 14 d prior to sample collection (mean MAF: 5%).

UGI cancer

Liquid biopsy could provide essential benefits for adjuvant and palliative treatment decision making, but low detection rates in nonmetastatic UGI cancers hinders this. Positive ctDNA after neoadjuvant treatment can identify patients with significantly increased risk of relapse (HR: 18.7), distant metastases (HR: 32.1), and cancer-associated death (HR: 23.1), but identifying how this issue should be addressed for significant survival benefit is a key question for further studies^[7]. Moreover, liquid biopsy may become integrated into treatment response prediction, especially of immunotherapy, in advanced UGI cancers^[84].

Liver cancer

Liquid biopsy offers significant prognostic potential in resectable HCC and was recently established as a promising biomarker for early response prediction of systemic therapy in advanced HCC; although, improvement regarding the LOD is necessary to implement these findings into clinical practice^[31]. Ongoing studies are attempting to lower the LOD using multifocal screening panels, which could establish ctDNA as a valuable diagnostic and predictive biomarker for HCC patients, regardless of the disease stage^[23].

Bile duct cancer

Until now, no prospective studies have investigated the benefits of liquid biopsy in bile duct cancer. Detection rates of mutations *via* liquid biopsy in histologically verified patients distinguishes between extra- and intrahepatic cholangiocarcinoma in favor of IHCC^[28]. Nevertheless, screening for certain mutations, like IDH1 or FGFR, could help to establish personalized first-line palliative antineoplastic treatment, for example with ivosidenib (IDH1) or FGFR-kinase-inhibitors in the future^[85,86].

Pancreatic cancer

For localized or locally advanced pancreatic cancer, ctDNA positivity prior to treatment is predictive of survival and relapse. This finding² could assist decision making for additional perioperative or adjuvant antineoplastic treatment of high-risk patients. Negative ctDNA, on the other hand, holds no additional informative value in those with pancreatic cancer. Since 2014, pancreatic cancer has been known to release significantly lower amounts of detectable circulating tumor cells into the bloodstream compared with most other tumors, including colorectal, gastric, lung, breast, ovarian, prostate, bladder, or renal cancer^[87]. However, some recent, small pilot studies have shown promising screening rates using specially designed detection methods. For example, hTERT promoter-regulated oncolytic herpes simplex virus-1 targeting telomerase reverse transcriptase was positive in 88.2% of 17 patients with PDAC in all stages of disease. A parallel-flow microfluidic chip detected 91.7% of 12 mPDAC patients^[4,88,89]. Methods like these need further study before they can be integrated into the clinical routine.

CRC

Significant progress has been made in ongoing trials of liquid biopsy in nonmetastatic CRC, especially on ctDNA-guided change in adjuvant therapeutic regimes, which may have a fundamental impact in future care. CRC is the ideal entity for liquid biopsy because of high rates of mutation detection and the total amount of cf/ctDNA in the plasma. This is in addition to the fact that tissue samples for Safe-SeqS/NGS are available for a sufficient proportion of patients to allow for guided mutation detection, thus resulting in very high specificity and sensitivity rates. Metastatic CRC offers even higher detection rates and could optimally benefit from the use of liquid biopsy in prognosis estimation and treatment evaluation in the future.

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