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

Kirchweger 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; Gastrointestinal cancer; Liquid biopsy; 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[1-3]. Furthermore, disconcordance 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. Two reviews[10,11] published 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 PPARg) 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%, which is also due to the early systemic spread of the disease[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)[42-44]. Another study suggests much lower detection rates for early-stage PDAC[45]. Using ddPCR, Berger *et al*[46] 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[47-51]. 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)[52-55]. In 2010, Chen *et al*[56] 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*[57] 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[43,58]. 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[59].

**CRC**

***Diagnosis***

CRC is the third leading newly diagnosed malignancy worldwide[60]. 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[61,62]. 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[63]. 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[64].

***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%[65,66]. In 2019, Tie *et al*[65] 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[65]. 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[7,67-69]. 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)[70]. 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[71].

***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)[72-74]. Nevertheless, until now there is no evidence of OS improvement resulting from 5-year surveillance, including clinical and endoscopic examinations, CEA measurement, and imaging[75,76]. 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[77]. Moreover, lead time of liquid biopsy compared with CEA is reported to be about 8 mo[76]. 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[78]. 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% disconcordance rate in a BEAMing analysis of 236 patients reported in 2018[79].

Serial liquid biopsies allow response prediction prior to that obtained by conventional methods in metastatic-stage patients undergoing palliative chemotherapy[80]. Furthermore, studies have demonstrated the PFS and OS benefits of repeated mutational status determination for eventual rechallenge with cetuximab/irinotecan-based regimes in initially RAS/BRAF wild-type patients or patients with acquired resistance during the course of treatment[81,82].

**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[83].

***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[84,85].

***Pancreatic cancer***

For localized or locally advanced pancreatic cancer, ctDNA positivity prior to treatment is predictive of survival and relapse. This finding 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[86]. 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[4,87,88]. 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.

**REFERENCES**

1 **Galarza Fortuna GM**, Dvir K. Circulating tumor DNA: Where are we now? A mini review of the literature. *World J Clin Oncol* 2020; **11**: 723-731 [PMID: 33033694 DOI: 10.5306/wjco.v11.i9.723]

2 **McGranahan N**, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* 2017; **168**: 613-628 [PMID: 28187284 DOI: 10.1016/j.cell.2017.01.018]

3 **Schwarzenbach H**, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011; **11**: 426-437 [PMID: 21562580 DOI: 10.1038/nrc3066]

4 **Lee JS**, Park SS, Lee YK, Norton JA, Jeffrey SS. Liquid biopsy in pancreatic ductal adenocarcinoma: current status of circulating tumor cells and circulating tumor DNA. *Mol Oncol* 2019; **13**: 1623-1650 [PMID: 31243883 DOI: 10.1002/1878-0261.12537]

5 **Stroun M**, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. *Oncology* 1989; **46**: 318-322 [PMID: 2779946 DOI: 10.1159/000226740]

6 **Wan JCM**, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, Pacey S, Baird R, Rosenfeld N. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* 2017; **17**: 223-238 [PMID: 28233803 DOI: 10.1038/nrc.2017.7]

7 **To YH**, Lee B, Wong HL, Gibbs P, Tie J. Circulating Tumour DNA to Guide Treatment of Gastrointestinal Malignancies. *Visc Med* 2020; **36**: 388-396 [PMID: 33178736 DOI: 10.1159/000509657]

8 **Ferreira MM**, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. *Mol Oncol* 2016; **10**: 374-394 [PMID: 26897752 DOI: 10.1016/j.molonc.2016.01.007]

9 **Alix-Panabières C**, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. *Cancer Discov* 2016; **6**: 479-491 [PMID: 26969689 DOI: 10.1158/2159-8290.CD-15-1483]

10 **Saluja H**, Karapetis CS, Pedersen SK, Young GP, Symonds EL. The Use of Circulating Tumor DNA for Prognosis of Gastrointestinal Cancers. *Front Oncol* 2018; **8**: 275 [PMID: 30087854 DOI: 10.3389/fonc.2018.00275]

11 **Merker JD**, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, Lindeman N, Lockwood CM, Rai AJ, Schilsky RL, Tsimberidou AM, Vasalos P, Billman BL, Oliver TK, Bruinooge SS, Hayes DF, Turner NC. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J Clin Oncol* 2018; **36**: 1631-1641 [PMID: 29504847 DOI: 10.1200/JCO.2017.76.8671]

12 **Brown P**. The Cobas® EGFR Mutation Test v2 assay. *Future Oncol* 2016; **12**: 451-452 [PMID: 26838018 DOI: 10.2217/fon.15.311]

13 **Vogelstein B**, Kinzler KW. Digital PCR. *Proc Natl Acad Sci USA* 1999; **96**: 9236-9241 [PMID: 10430926 DOI: 10.1073/pnas.96.16.9236]

14 **Gorgannezhad L**, Umer M, Islam MN, Nguyen NT, Shiddiky MJA. Circulating tumor DNA and liquid biopsy: opportunities, challenges, and recent advances in detection technologies. *Lab Chip* 2018; **18**: 1174-1196 [PMID: 29569666 DOI: 10.1039/C8LC00100F]

15 **Chen WW**, Balaj L, Liau LM, Samuels ML, Kotsopoulos SK, Maguire CA, Loguidice L, Soto H, Garrett M, Zhu LD, Sivaraman S, Chen C, Wong ET, Carter BS, Hochberg FH, Breakefield XO, Skog J. BEAMing and Droplet Digital PCR Analysis of Mutant IDH1 mRNA in Glioma Patient Serum and Cerebrospinal Fluid Extracellular Vesicles. *Mol Ther Nucleic Acids* 2013; **2**: e109 [PMID: 23881452 DOI: 10.1038/mtna.2013.28]

16 **Zill OA**, Greene C, Sebisanovic D, Siew LM, Leng J, Vu M, Hendifar AE, Wang Z, Atreya CE, Kelley RK, Van Loon K, Ko AH, Tempero MA, Bivona TG, Munster PN, Talasaz A, Collisson EA. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. *Cancer Discov* 2015; **5**: 1040-1048 [PMID: 26109333 DOI: 10.1158/2159-8290.CD-15-0274]

17 **Kinde I**, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. *Proc Natl Acad Sci USA* 2011; **108**: 9530-9535 [PMID: 21586637 DOI: 10.1073/pnas.1105422108]

18 **Newman AM**, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, Stehr H, Liu CL, Bratman SV, Say C, Zhou L, Carter JN, West RB, Sledge GW, Shrager JB, Loo BW Jr, Neal JW, Wakelee HA, Diehn M, Alizadeh AA. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat Biotechnol* 2016; **34**: 547-555 [PMID: 27018799 DOI: 10.1038/nbt.3520]

19 **Cabel L**, Decraene C, Bieche I, Pierga JY, Bennamoun M, Fuks D, Ferraz JM, Lefevre M, Baulande S, Bernard V, Vacher S, Mariani P, Proudhon C, Bidard FC, Louvet C. Limited Sensitivity of Circulating Tumor DNA Detection by Droplet Digital PCR in Non-Metastatic Operable Gastric Cancer Patients. *Cancers (Basel)* 2019; **11** [PMID: 30901876 DOI: 10.3390/cancers11030396]

20 **Maron SB**, Chase LM, Lomnicki S, Kochanny S, Moore KL, Joshi SS, Landron S, Johnson J, Kiedrowski LA, Nagy RJ, Lanman RB, Kim ST, Lee J, Catenacci DVT. Circulating Tumor DNA Sequencing Analysis of Gastroesophageal Adenocarcinoma. *Clin Cancer Res* 2019; **25**: 7098-7112 [PMID: 31427281 DOI: 10.1158/1078-0432.CCR-19-1704]

21 **Pectasides E**, Stachler MD, Derks S, Liu Y, Maron S, Islam M, Alpert L, Kwak H, Kindler H, Polite B, Sharma MR, Allen K, O'Day E, Lomnicki S, Maranto M, Kanteti R, Fitzpatrick C, Weber C, Setia N, Xiao SY, Hart J, Nagy RJ, Kim KM, Choi MG, Min BH, Nason KS, O'Keefe L, Watanabe M, Baba H, Lanman R, Agoston AT, Oh DJ, Dunford A, Thorner AR, Ducar MD, Wollison BM, Coleman HA, Ji Y, Posner MC, Roggin K, Turaga K, Chang P, Hogarth K, Siddiqui U, Gelrud A, Ha G, Freeman SS, Rhoades J, Reed S, Gydush G, Rotem D, Davison J, Imamura Y, Adalsteinsson V, Lee J, Bass AJ, Catenacci DV. Genomic Heterogeneity as a Barrier to Precision Medicine in Gastroesophageal Adenocarcinoma. *Cancer Discov* 2018; **8**: 37-48 [PMID: 28978556 DOI: 10.1158/2159-8290.CD-17-0395]

22 **Catenacci DVT**, Moya S, Lomnicki S, Chase LM, Peterson BF, Reizine N, Alpert L, Setia N, Xiao SY, Hart J, Siddiqui UD, Hogarth DK, Eng OS, Turaga K, Roggin K, Posner MC, Chang P, Narula S, Rampurwala M, Ji Y, Karrison T, Liao CY, Polite BN, Kindler HL. Personalized Antibodies for Gastroesophageal Adenocarcinoma (PANGEA): A Phase II Study Evaluating an Individualized Treatment Strategy for Metastatic Disease. *Cancer Discov* 2021; **11**: 308-325 [PMID: 33234578 DOI: 10.1158/2159-8290.CD-20-1408]

23 **Ye Q**, Ling S, Zheng S, Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. *Mol Cancer* 2019; **18**: 114 [PMID: 31269959 DOI: 10.1186/s12943-019-1043-x]

24 **Huang A**, Zhang X, Zhou SL, Cao Y, Huang XW, Fan J, Yang XR, Zhou J. Detecting Circulating Tumor DNA in Hepatocellular Carcinoma Patients Using Droplet Digital PCR Is Feasible and Reflects Intratumoral Heterogeneity. *J Cancer* 2016; **7**: 1907-1914 [PMID: 27698932 DOI: 10.7150/jca.15823]

25 **Tokuhisa Y**, Iizuka N, Sakaida I, Moribe T, Fujita N, Miura T, Tamatsukuri S, Ishitsuka H, Uchida K, Terai S, Sakamoto K, Tamesa T, Oka M. Circulating cell-free DNA as a predictive marker for distant metastasis of hepatitis C virus-related hepatocellular carcinoma. *Br J Cancer* 2007; **97**: 1399-1403 [PMID: 17940509 DOI: 10.1038/sj.bjc.6604034]

26 **Xu RH**, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, Yi S, Shi W, Quan Q, Li K, Zheng L, Zhang H, Caughey BA, Zhao Q, Hou J, Zhang R, Xu Y, Cai H, Li G, Hou R, Zhong Z, Lin D, Fu X, Zhu J, Duan Y, Yu M, Ying B, Zhang W, Wang J, Zhang E, Zhang C, Li O, Guo R, Carter H, Zhu JK, Hao X, Zhang K. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017; **16**: 1155-1161 [PMID: 29035356 DOI: 10.1038/nmat4997]

27 **Hardy T**, Zeybel M, Day CP, Dipper C, Masson S, McPherson S, Henderson E, Tiniakos D, White S, French J, Mann DA, Anstee QM, Mann J. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. *Gut* 2017; **66**: 1321-1328 [PMID: 27002005 DOI: 10.1136/gutjnl-2016-311526]

28 **Ettrich TJ**, Schwerdel D, Dolnik A, Beuter F, Blätte TJ, Schmidt SA, Stanescu-Siegmund N, Steinacker J, Marienfeld R, Kleger A, Bullinger L, Seufferlein T, Berger AW. Genotyping of circulating tumor DNA in cholangiocarcinoma reveals diagnostic and prognostic information. *Sci Rep* 2019; **9**: 13261 [PMID: 31519967 DOI: 10.1038/s41598-019-49860-0]

29 **Ren N**, Ye QH, Qin LX, Zhang BH, Liu YK, Tang ZY. Circulating DNA level is negatively associated with the long-term survival of hepatocellular carcinoma patients. *World J Gastroenterol* 2006; **12**: 3911-3914 [PMID: 16804981 DOI: 10.3748/wjg.v12.i24.3911]

30 **Liao W**, Yang H, Xu H, Wang Y, Ge P, Ren J, Xu W, Lu X, Sang X, Zhong S, Zhang H, Mao Y. Noninvasive detection of tumor-associated mutations from circulating cell-free DNA in hepatocellular carcinoma patients by targeted deep sequencing. *Oncotarget* 2016; **7**: 40481-40490 [PMID: 27248174 DOI: 10.18632/oncotarget.9629]

31 **von Felden J**, Craig AJ, Garcia-Lezana T, Labgaa I, Haber PK, D'Avola D, Asgharpour A, Dieterich D, Bonaccorso A, Torres-Martin M, Sia D, Sung MW, Tabrizian P, Schwartz M, Llovet JM, Villanueva A. Mutations in circulating tumor DNA predict primary resistance to systemic therapies in advanced hepatocellular carcinoma. *Oncogene* 2021; **40**: 140-151 [PMID: 33097857 DOI: 10.1038/s41388-020-01519-1]

32 **Long G**, Fang T, Su W, Mi X, Zhou L. The prognostic value of postoperative circulating cell-free DNA in operable hepatocellular carcinoma. *Scand J Gastroenterol* 2020; **55**: 1441-1446 [PMID: 33119422 DOI: 10.1080/00365521.2020.1839127]

33 **Baumgartner JM**, Raymond VM, Lanman RB, Tran L, Kelly KJ, Lowy AM, Kurzrock R. Preoperative Circulating Tumor DNA in Patients with Peritoneal Carcinomatosis is an Independent Predictor of Progression-Free Survival. *Ann Surg Oncol* 2018; **25**: 2400-2408 [PMID: 29948422 DOI: 10.1245/s10434-018-6561-z]

34 **Nakamura H**, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. *Nat Genet* 2015; **47**: 1003-1010 [PMID: 26258846 DOI: 10.1038/ng.3375]

35 **Zou S**, Li J, Zhou H, Frech C, Jiang X, Chu JS, Zhao X, Li Y, Li Q, Wang H, Hu J, Kong G, Wu M, Ding C, Chen N, Hu H. Mutational landscape of intrahepatic cholangiocarcinoma. *Nat Commun* 2014; **5**: 5696 [PMID: 25526346 DOI: 10.1038/ncomms6696]

36 **Cai ZX**, Chen G, Zeng YY, Dong XQ, Lin MJ, Huang XH, Zhang D, Liu XL, Liu JF. Circulating tumor DNA profiling reveals clonal evolution and real-time disease progression in advanced hepatocellular carcinoma. *Int J Cancer* 2017; **141**: 977-985 [PMID: 28543104 DOI: 10.1002/ijc.30798]

37 **Ahn DH**, Javle M, Ahn CW, Jain A, Mikhail S, Noonan AM, Ciombor K, Wu C, Shroff RT, Chen JL, Bekaii-Saab T. Next-generation sequencing survey of biliary tract cancer reveals the association between tumor somatic variants and chemotherapy resistance. *Cancer* 2016; **122**: 3657-3666 [PMID: 27495988 DOI: 10.1002/cncr.30247]

38 **Strobel O**, Neoptolemos J, Jäger D, Büchler MW. Optimizing the outcomes of pancreatic cancer surgery. *Nat Rev Clin Oncol* 2019; **16**: 11-26 [PMID: 30341417 DOI: 10.1038/s41571-018-0112-1]

39 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]

40 **Kanda M**, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012; **142**: 730-733.e9 [PMID: 22226782 DOI: 10.1053/j.gastro.2011.12.042]

41 **Goel G**, Sun W. Novel approaches in the management of pancreatic ductal adenocarcinoma: potential promises for the future. *J Hematol Oncol* 2015; **8**: 44 [PMID: 25935754 DOI: 10.1186/s13045-015-0141-5]

42 **Lee B,** Cohen J, Lipton LR, Tie J, Javed AA, Li L, Goldstein D, Cooray P, Nagrial A, Burge ME, Tebbutt NC, Nikfarjam M, Harris M, O'Broin-Lennon AM, Wolfgang CL, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Potential role of circulating tumor DNA (ctDNA) in the early diagnosis and post-operative management of localised pancreatic cancer. *J Clin Oncol* 2017; **35**: 4101-4101 [DOI: 10.1200/jco.2017.35.15\_suppl.4101]

43 **Kruger S**, Heinemann V, Ross C, Diehl F, Nagel D, Ormanns S, Liebmann S, Prinz-Bravin I, Westphalen CB, Haas M, Jung A, Kirchner T, von Bergwelt-Baildon M, Boeck S, Holdenrieder S. Repeated mutKRAS ctDNA measurements represent a novel and promising tool for early response prediction and therapy monitoring in advanced pancreatic cancer. *Ann Oncol* 2018; **29**: 2348-2355 [PMID: 30346475 DOI: 10.1093/annonc/mdy417]

44 **Berger AW**, Schwerdel D, Ettrich TJ, Hann A, Schmidt SA, Kleger A, Marienfeld R, Seufferlein T. Targeted deep sequencing of circulating tumor DNA in metastatic pancreatic cancer. *Oncotarget* 2018; **9**: 2076-2085 [PMID: 29416754 DOI: 10.18632/oncotarget.23330]

45 **Groot VP**, Mosier S, Javed AA, Teinor JA, Gemenetzis G, Ding D, Haley LM, Yu J, Burkhart RA, Hasanain A, Debeljak M, Kamiyama H, Narang A, Laheru DA, Zheng L, Lin MT, Gocke CD, Fishman EK, Hruban RH, Goggins MG, Molenaar IQ, Cameron JL, Weiss MJ, Velculescu VE, He J, Wolfgang CL, Eshleman JR. Circulating Tumor DNA as a Clinical Test in Resected Pancreatic Cancer. *Clin Cancer Res* 2019; **25**: 4973-4984 [PMID: 31142500 DOI: 10.1158/1078-0432.CCR-19-0197]

46 **Berger AW**, Schwerdel D, Costa IG, Hackert T, Strobel O, Lam S, Barth TF, Schröppel B, Meining A, Büchler MW, Zenke M, Hermann PC, Seufferlein T, Kleger A. Detection of Hot-Spot Mutations in Circulating Cell-Free DNA From Patients With Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Gastroenterology* 2016; **151**: 267-270 [PMID: 27343369 DOI: 10.1053/j.gastro.2016.04.034]

47 **Cohen JD**, Javed AA, Thoburn C, Wong F, Tie J, Gibbs P, Schmidt CM, Yip-Schneider MT, Allen PJ, Schattner M, Brand RE, Singhi AD, Petersen GM, Hong SM, Kim SC, Falconi M, Doglioni C, Weiss MJ, Ahuja N, He J, Makary MA, Maitra A, Hanash SM, Dal Molin M, Wang Y, Li L, Ptak J, Dobbyn L, Schaefer J, Silliman N, Popoli M, Goggins MG, Hruban RH, Wolfgang CL, Klein AP, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Lennon AM. Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. *Proc Natl Acad Sci USA* 2017; **114**: 10202-10207 [PMID: 28874546 DOI: 10.1073/pnas.1704961114]

48 **Ballehaninna UK**, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. *J Gastrointest Oncol* 2012; **3**: 105-119 [PMID: 22811878 DOI: 10.3978/j.issn.2078-6891.2011.021]

49 **Scarà S**, Bottoni P, Scatena R. CA 19-9: Biochemical and Clinical Aspects. *Adv Exp Med Biol* 2015; **867**: 247-260 [PMID: 26530370 DOI: 10.1007/978-94-017-7215-0\_15]

50 **Tanaka N**, Okada S, Ueno H, Okusaka T, Ikeda M. The usefulness of serial changes in serum CA19-9 levels in the diagnosis of pancreatic cancer. *Pancreas* 2000; **20**: 378-381 [PMID: 10824692 DOI: 10.1097/00006676-200005000-00007]

51 **Passerini R**, Cassatella MC, Boveri S, Salvatici M, Radice D, Zorzino L, Galli C, Sandri MT. The pitfalls of CA19-9: routine testing and comparison of two automated immunoassays in a reference oncology center. *Am J Clin Pathol* 2012; **138**: 281-287 [PMID: 22904141 DOI: 10.1309/AJCPOPNPLLCYR07H]

52 **Effenberger KE**, Schroeder C, Hanssen A, Wolter S, Eulenburg C, Tachezy M, Gebauer F, Izbicki JR, Pantel K, Bockhorn M. Improved Risk Stratification by Circulating Tumor Cell Counts in Pancreatic Cancer. *Clin Cancer Res* 2018; **24**: 2844-2850 [PMID: 29559560 DOI: 10.1158/1078-0432.CCR-18-0120]

53 **Bernard V**, Kim DU, San Lucas FA, Castillo J, Allenson K, Mulu FC, Stephens BM, Huang J, Semaan A, Guerrero PA, Kamyabi N, Zhao J, Hurd MW, Koay EJ, Taniguchi CM, Herman JM, Javle M, Wolff R, Katz M, Varadhachary G, Maitra A, Alvarez HA. Circulating Nucleic Acids Are Associated With Outcomes of Patients With Pancreatic Cancer. *Gastroenterology* 2019; **156**: 108-118.e4 [PMID: 30240661 DOI: 10.1053/j.gastro.2018.09.022]

54 **Kim MK**, Woo SM, Park B, Yoon KA, Kim YH, Joo J, Lee WJ, Han SS, Park SJ, Kong SY. Prognostic Implications of Multiplex Detection of *KRAS* Mutations in Cell-Free DNA from Patients with Pancreatic Ductal Adenocarcinoma. *Clin Chem* 2018; **64**: 726-734 [PMID: 29352043 DOI: 10.1373/clinchem.2017.283721]

55 **Pietrasz D**, Pécuchet N, Garlan F, Didelot A, Dubreuil O, Doat S, Imbert-Bismut F, Karoui M, Vaillant JC, Taly V, Laurent-Puig P, Bachet JB. Plasma Circulating Tumor DNA in Pancreatic Cancer Patients Is a Prognostic Marker. *Clin Cancer Res* 2017; **23**: 116-123 [PMID: 27993964 DOI: 10.1158/1078-0432.CCR-16-0806]

56 **Chen H**, Tu H, Meng ZQ, Chen Z, Wang P, Liu LM. K-ras mutational status predicts poor prognosis in unresectable pancreatic cancer. *Eur J Surg Oncol* 2010; **36**: 657-662 [PMID: 20542658 DOI: 10.1016/j.ejso.2010.05.014]

57 **Lee B**, Lipton L, Cohen J, Tie J, Javed AA, Li L, Goldstein D, Burge M, Cooray P, Nagrial A, Tebbutt NC, Thomson B, Nikfarjam M, Harris M, Haydon A, Lawrence B, Tai DWM, Simons K, Lennon AM, Wolfgang CL, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA as a potential marker of adjuvant chemotherapy benefit following surgery for localized pancreatic cancer. *Ann Oncol* 2019; **30**: 1472-1478 [PMID: 31250894 DOI: 10.1093/annonc/mdz200]

58 **Park G**, Park JK, Son DS, Shin SH, Kim YJ, Jeon HJ, Lee J, Park WY, Lee KH, Park D. Utility of targeted deep sequencing for detecting circulating tumor DNA in pancreatic cancer patients. *Sci Rep* 2018; **8**: 11631 [PMID: 30072705 DOI: 10.1038/s41598-018-30100-w]

59 **Golan T,** Varadhachary GR, Sela T, Fogelman DR, Halperin N, Shroff RT, Halparin S, Xiao L, Aderka D, Maitra A, Ackerstein A, Wolff RA, Shacham-Shmueli E, Javie MM. Phase II study of olaparib for BRCAness phenotype in pancreatic cancer. J. Clin. Oncol. 2018 [DOI: 10.1200/jco.2018.36.4\_suppl.297]

60 **Arnold M**, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; **66**: 683-691 [PMID: 26818619 DOI: 10.1136/gutjnl-2015-310912]

61 **Tie J**, Kinde I, Wang Y, Wong HL, Roebert J, Christie M, Tacey M, Wong R, Singh M, Karapetis CS, Desai J, Tran B, Strausberg RL, Diaz LA Jr, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol* 2015; **26**: 1715-1722 [PMID: 25851626 DOI: 10.1093/annonc/mdv177]

62 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA Jr. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

63 **Tie J,** Wang Y, Kinde I, Steel M, Elsaleh H, Singh MS, Turner NH, Tran B, Strausberg R, Diaz LA, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA (ctDNA) in nonmetastatic colorectal cancer (CRC): Potential role as a screening tool. *J Clin Oncol* 2015; **33**: 518-518 [DOI: 10.1200/jco.2015.33.3\_suppl.518]

64 **Adler A**, Geiger S, Keil A, Bias H, Schatz P, deVos T, Dhein J, Zimmermann M, Tauber R, Wiedenmann B. Improving compliance to colorectal cancer screening using blood and stool based tests in patients refusing screening colonoscopy in Germany. *BMC Gastroenterol* 2014; **14**: 183 [PMID: 25326034 DOI: 10.1186/1471-230X-14-183]

65 **Tie J**, Cohen JD, Wang Y, Li L, Christie M, Simons K, Elsaleh H, Kosmider S, Wong R, Yip D, Lee M, Tran B, Rangiah D, Burge M, Goldstein D, Singh M, Skinner I, Faragher I, Croxford M, Bampton C, Haydon A, Jones IT, S Karapetis C, Price T, Schaefer MJ, Ptak J, Dobbyn L, Silliman N, Kinde I, Tomasetti C, Papadopoulos N, Kinzler K, Volgestein B, Gibbs P. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. *Gut* 2019; **68**: 663-671 [PMID: 29420226 DOI: 10.1136/gutjnl-2017-315852]

66 **Reinert T**, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, Knudsen M, Nordentoft I, Wu HT, Tin AS, Heilskov Rasmussen M, Vang S, Shchegrova S, Frydendahl Boll Johansen A, Srinivasan R, Assaf Z, Balcioglu M, Olson A, Dashner S, Hafez D, Navarro S, Goel S, Rabinowitz M, Billings P, Sigurjonsson S, Dyrskjøt L, Swenerton R, Aleshin A, Laurberg S, Husted Madsen A, Kannerup AS, Stribolt K, Palmelund Krag S, Iversen LH, Gotschalck Sunesen K, Lin CJ, Zimmermann BG, Lindbjerg Andersen C. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. *JAMA Oncol* 2019; **5**: 1124-1131 [PMID: 31070691 DOI: 10.1001/jamaoncol.2019.0528]

67 **Morris VK,** Yothers G, Kopetz S, Jacobs SA, Lucas PC, Iqbal A, Boland PM, Deming DA, Scott AJ, Lim HJ, Wolmark N, George TJ. NRG-GI005 (COBRA): Phase II/III study of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer. *J Clin Oncol* 2020 [DOI: 10.1200/jco.2020.38.4\_suppl.tps261]

68 **Folprecht G**, Reinacher-Schick, A, Tannapfel A, Weitz J, Kossler T, Weiss L, Aust DE, von Bubnoff N, Kramer M, Thiede C. Circulating tumor DNA-based decision for adjuvant treatment in colon cancer stage II evaluation: (CIRCULATE-trial) AIO-KRK-0217. *J Clin Oncol* 2020 [DOI: 10.1200/jco.2020.38.4\_suppl.tps273]

69 **Taïeb J**, Benhaim L, Laurent Puig P, Le Malicot K, Emile JF, Geillon F, Tougeron D, Manfredi S, Chauvenet M, Taly V, Lepage C, André T. "Decision for adjuvant treatment in stage II colon cancer based on circulating tumor DNA:The CIRCULATE-PRODIGE 70 trial". *Dig Liver Dis* 2020; **52**: 730-733 [PMID: 32482534 DOI: 10.1016/j.dld.2020.04.010]

70 **Reinert T**, Schøler LV, Thomsen R, Tobiasen H, Vang S, Nordentoft I, Lamy P, Kannerup AS, Mortensen FV, Stribolt K, Hamilton-Dutoit S, Nielsen HJ, Laurberg S, Pallisgaard N, Pedersen JS, Ørntoft TF, Andersen CL. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. *Gut* 2016; **65**: 625-634 [PMID: 25654990 DOI: 10.1136/gutjnl-2014-308859]

71 **Formica V**, Lucchetti J, Doldo E, Riondino S, Morelli C, Argirò R, Renzi N, Nitti D, Nardecchia A, Dell'Aquila E, Ferroni P, Guadagni F, Palmieri G, Orlandi A, Roselli M. Clinical Utility of Plasma KRAS, NRAS and BRAF Mutational Analysis with Real Time PCR in Metastatic Colorectal Cancer Patients-The Importance of Tissue/Plasma Discordant Cases. *J Clin Med* 2020; **10** [PMID: 33383664 DOI: 10.3390/jcm10010087]

72 **Wong R**, Tie J, Lee M, Cohen J, Wang Y, Li L, Ma S, Christie M, Kosmider S, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. The potential role of circulating tumor DNA (ctDNA) in the further investigation of colorectal cancer patients with nonspecific findings on standard investigations. *Int J Cancer* 2019; **145**: 540-547 [PMID: 30628066 DOI: 10.1002/ijc.32117]

73 **Chao M**, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. *J Clin Oncol* 2009; **27**: e279-80; author reply e281 [PMID: 19901127 DOI: 10.1200/JCO.2009.25.6156]

74 **Meyerhardt JA**, Mangu PB, Flynn PJ, Korde L, Loprinzi CL, Minsky BD, Petrelli NJ, Ryan K, Schrag DH, Wong SL, Benson AB 3rd; American Society of Clinical Oncology. Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. *J Clin Oncol* 2013; **31**: 4465-4470 [PMID: 24220554 DOI: 10.1200/JCO.2013.50.7442]

75 **Lepage C**, Pheliip JM, Cany L, Barbier E, Manfredi S, Deguiral P, Faroux R, Baconnier M, Pezet D, Duchmann J, Terrebonne E, Adenis A, Benabdelghani M, Ain J, Breysacher G, Boillot-Benedetto I, Pelaquier A, Prost P, Lievre A, Bouche O. 398O Effect of 5 years of imaging and CEA follow-up to detect recurrence of colorectal cancer (CRC) - PRODIGE 13 a FFCD phase III trial. *Ann Oncol* 2020; **31:** S410 [DOI: 10.1016/j.annonc.2020.08.509]

76 **Naidoo M**, Gibbs P, Tie J. ctDNA and Adjuvant Therapy for Colorectal Cancer: Time to Re-Invent Our Treatment Paradigm. *Cancers (Basel)* 2021; **13** [PMID: 33477814 DOI: 10.3390/cancers13020346]

77 **Tie J**, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, Kosmider S, Skinner I, Wong R, Steel M, Tran B, Desai J, Jones I, Haydon A, Hayes T, Price TJ, Strausberg RL, Diaz LA Jr, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci Transl Med* 2016; **8**: 346ra92 [PMID: 27384348 DOI: 10.1126/scitranslmed.aaf6219]

78 **Nors J**, Henriksen TV, Gotschalck KA, Juul T, Søgaard J, Iversen LH, Andersen CL. IMPROVE-IT2: implementing noninvasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer - intervention trial 2. Study protocol. *Acta Oncol* 2020; **59**: 336-341 [PMID: 31920137 DOI: 10.1080/0284186X.2019.1711170]

79 **García-Foncillas J**, Tabernero J, Élez E, Aranda E, Benavides M, Camps C, Jantus-Lewintre E, López R, Muinelo-Romay L, Montagut C, Antón A, López G, Díaz-Rubio E, Rojo F, Vivancos A. Prospective multicenter real-world RAS mutation comparison between OncoBEAM-based liquid biopsy and tissue analysis in metastatic colorectal cancer. *Br J Cancer* 2018; **119**: 1464-1470 [PMID: 30467411 DOI: 10.1038/s41416-018-0293-5]

80 **Holm M**, Andersson E, Osterlund E, Ovissi A, Soveri LM, Anttonen AK, Kytölä S, Aittomäki K, Osterlund P, Ristimäki A. Detection of KRAS mutations in liquid biopsies from metastatic colorectal cancer patients using droplet digital PCR, Idylla, and next generation sequencing. *PLoS One* 2020; **15**: e0239819 [PMID: 33237900 DOI: 10.1371/journal.pone.0239819]

81 **Khan KH**, Cunningham D, Werner B, Vlachogiannis G, Spiteri I, Heide T, Mateos JF, Vatsiou A, Lampis A, Damavandi MD, Lote H, Huntingford IS, Hedayat S, Chau I, Tunariu N, Mentrasti G, Trevisani F, Rao S, Anandappa G, Watkins D, Starling N, Thomas J, Peckitt C, Khan N, Rugge M, Begum R, Hezelova B, Bryant A, Jones T, Proszek P, Fassan M, Hahne JC, Hubank M, Braconi C, Sottoriva A, Valeri N. Longitudinal Liquid Biopsy and Mathematical Modeling of Clonal Evolution Forecast Time to Treatment Failure in the PROSPECT-C Phase II Colorectal Cancer Clinical Trial. *Cancer Discov* 2018; **8**: 1270-1285 [PMID: 30166348 DOI: 10.1158/2159-8290.CD-17-0891]

82 **Cremolini C**, Rossini D, Dell'Aquila E, Lonardi S, Conca E, Del Re M, Busico A, Pietrantonio F, Danesi R, Aprile G, Tamburini E, Barone C, Masi G, Pantano F, Pucci F, Corsi DC, Pella N, Bergamo F, Rofi E, Barbara C, Falcone A, Santini D. Rechallenge for Patients With RAS and BRAF Wild-Type Metastatic Colorectal Cancer With Acquired Resistance to First-line Cetuximab and Irinotecan: A Phase 2 Single-Arm Clinical Trial. *JAMA Oncol* 2019; **5**: 343-350 [PMID: 30476968 DOI: 10.1001/jamaoncol.2018.5080]

83 **Jin Y**, Chen DL, Wang F, Yang CP, Chen XX, You JQ, Huang JS, Shao Y, Zhu DQ, Ouyang YM, Luo HY, Wang ZQ, Wang FH, Li YH, Xu RH, Zhang DS. The predicting role of circulating tumor DNA landscape in gastric cancer patients treated with immune checkpoint inhibitors. *Mol Cancer* 2020; **19**: 154 [PMID: 33126883 DOI: 10.1186/s12943-020-01274-7]

84 **Javle M**, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, Kang HC, Catenacci D, Ali S, Krishnan S, Ahn D, Bocobo AG, Zuo M, Kaseb A, Miller V, Stephens PJ, Meric-Bernstam F, Shroff R, Ross J. Biliary cancer: Utility of next-generation sequencing for clinical management. *Cancer* 2016; **122**: 3838-3847 [PMID: 27622582 DOI: 10.1002/cncr.30254]

85 **Javle M**, Lowery M, Shroff RT, Weiss KH, Springfeld C, Borad MJ, Ramanathan RK, Goyal L, Sadeghi S, Macarulla T, El-Khoueiry A, Kelley RK, Borbath I, Choo SP, Oh DY, Philip PA, Chen LT, Reungwetwattana T, Van Cutsem E, Yeh KH, Ciombor K, Finn RS, Patel A, Sen S, Porter D, Isaacs R, Zhu AX, Abou-Alfa GK, Bekaii-Saab T. Phase II Study of BGJ398 in Patients With FGFR-Altered Advanced Cholangiocarcinoma. *J Clin Oncol* 2018; **36**: 276-282 [PMID: 29182496 DOI: 10.1200/JCO.2017.75.5009]

86 **Allard WJ**, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW, Terstappen LW. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004; **10**: 6897-6904 [PMID: 15501967 DOI: 10.1158/1078-0432.CCR-04-0378]

87 **Zhang W**, Bao L, Yang S, Qian Z, Dong M, Yin L, Zhao Q, Ge K, Deng Z, Zhang J, Qi F, An Z, Yu Y, Wang Q, Wu R, Fan F, Zhang L, Chen X, Na Y, Feng L, Liu L, Zhu Y, Qin T, Zhang S, Zhang Y, Zhang X, Wang J, Yi X, Zou L, Xin HW, Ditzel HJ, Gao H, Zhang K, Liu B, Cheng S. Tumor-selective replication herpes simplex virus-based technology significantly improves clinical detection and prognostication of viable circulating tumor cells. *Oncotarget* 2016; **7**: 39768-39783 [PMID: 27206795 DOI: 10.18632/oncotarget.9465]

88 **Chang CL**, Huang W, Jalal SI, Chan BD, Mahmood A, Shahda S, O'Neil BH, Matei DE, Savran CA. Circulating tumor cell detection using a parallel flow micro-aperture chip system. *Lab Chip* 2015; **15**: 1677-1688 [PMID: 25687986 DOI: 10.1039/c5lc00100e]

89 **Strijker M**, Soer EC, de Pastena M, Creemers A, Balduzzi A, Beagan JJ, Busch OR, van Delden OM, Halfwerk H, van Hooft JE, van Lienden KP, Marchegiani G, Meijer SL, van Noesel CJ, Reinten RJ, Roos E, Schokker S, Verheij J, van de Vijver MJ, Waasdorp C, Wilmink JW, Ylstra B, Besselink MG, Bijlsma MF, Dijk F, van Laarhoven HW. Circulating tumor DNA quantity is related to tumor volume and both predict survival in metastatic pancreatic ductal adenocarcinoma. *Int J Cancer* 2020; **146**: 1445-1456 [PMID: 31340061 DOI: 10.1002/ijc.32586]

90 **Manca P**, Corallo S, Busico A, Lonardi S, Corti F, Antoniotti C, Procaccio L, Clavarezza M, Smiroldo V, Tomasello G, Murialdo R, Sartore-Bianchi A, Racca P, Pagani F, Randon G, Martinetti A, Sottotetti E, Palermo F, Perrone F, Tamborini E, Prisciandaro M, Raimondi A, Di Bartolomeo M, Morano F, Pietrantonio F. The Added Value of Baseline Circulating Tumor DNA Profiling in Patients with Molecularly Hyperselected, Left-sided Metastatic Colorectal Cancer. *Clin Cancer Res* 2021; **27**: 2505-2514 [PMID: 33547199 DOI: 10.1158/1078-0432.CCR-20-4699]

91 **Lamarca A**, Kapacee Z, Breeze M, Bell C, Belcher D, Staiger H, Taylor C, McNamara MG, Hubner RA, Valle JW. Molecular Profiling in Daily Clinical Practice: Practicalities in Advanced Cholangiocarcinoma and Other Biliary Tract Cancers. *J Clin Med* 2020; **9** [PMID: 32899345 DOI: 10.3390/jcm9092854]

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**Table 1 Detection rates and impact on outcome of circulating tumor DNA in gastrointestinal cancer**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Entity** | **Detection rate** | **Common target**  | **OS ctDNA −/ +** | **PFS ctDNA −/ +** |
| mPDAC  | 67%-75%[43] | > 90% KRAS, but also TP53, SMAD4  | 8.4 *vs* 3.2[89]  | 5 *vs* 3.9[43]  |
| lPDAC  | 21%-69%[42] | 16.3 *vs* 5.8[57]  | 19 *vs* 8[57] |
| mCRC  | > 90%[79] | KRAS, NRAS, BRAF, PIK3CA, NRAS, APC, TP53, EGFR, ERBB3/4  | 36.5 *vs* 17.1[90]  | RAS 8.3 *vs* BRAF 4.5 *vs* wild-type 22.9[72]  |
| lCRC  | 73%(43%-80%)[62,63] | - | 87% *vs* 33%[65] |
| 3-yr PFS |
| mUGIC  | 87.5%[21] | TP53, HER2, MET, EGFR, KRAS  | 13.7 *vs* 8.6[20]  | 7.4 *vs* 4.9[83]  |
| lUGIC  | 20%[19] | 66.9 *vs* 37.7[10]  | 12.5 *vs* not reached[20]  |
| HCC  | 56.3%[24] | TP53, CTNNB1, TERT  | 61% *vs* 24%[29] | 47% *vs* 22%[29] |
| 3-yr OS  | 3-yr PFS  |
| mIHCC  | 92%[28] | TP53, KRAS, ARID1A | 16.4 *vs* 7.4[91] | 8.2 *vs* 4.6[91]  |
| mEHCC  | 55%[28] | NS[28] | NS[28] |

−/+: ctDNA negative/positive; ctDNA: Circulating tumor DNA; HCC: Hepatocellular carcinoma; lCRC: Localized colorectal carcinoma; lPDAC: Localized pancreatic ductal adenocarcinoma; lUGIC: Localized upper gastrointestinal carcinoma; mCRC: Metastatic colorectal carcinoma; mEHCC: Metastatic extrahepatic cholangiocarcinoma; mIHCC: Metastatic intrahepatic cholangiocarcinoma; mPDAC: Metastatic pancreatic ductal adenocarcinoma; mUGIC: Metastatic upper gastrointestinal carcinoma; NS: Not significant; OS: Overall survival in months; PFS: Progressive-free survival in months.