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Review

The potential role of minimal/molecular residual disease in colorectal cancer: curative surgery, radiotherapy and beyond

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ABSTRACT

Detection of minimal/molecular residual disease (MRD) based on ctDNA assay develops from hematological malignancies to solid tumors. Generally, there are two mainstream assays in MRD testing technology: tumor-informed and tumor-agnostic. For colorectal cancer (CRC), MRD is used not only to monitor recurrence and predict prognosis, but also to help in clinical decision making and assessment of clinical efficacy in the settings of curative surgery, radiotherapy, chemotherapy and surveillance. Accumulated clinical trials are exploring roles of MRD in early or advanced stages of CRC. Here, we give an overview of how MRD is and will be used in CRC.

1. Introduction

Colorectal cancer (CRC) ranks third in terms of incidence, but second in terms of mortality worldwide, with more than 1.9 million new cases and 935,000 deaths estimated in 2020.[1] In China, the incidence and mortality rate of CRC has been increasing in recent years.[2] Therefore, this review summarizes the recent technical development and the potential roles of minimal or molecular residual disease (MRD) in CRC during the overall management including curative surgery, radiotherapy, chemotherapy and surveillance.

1.1. The concept of ctDNA and MRD

All healthy individuals harbor cell-free DNA (cfDNA, 180–200 bp) in plasma, which usually originates from apoptosis, necrosis, exosomes, etc. It can be found in various body fluids, mainly from hematopoietic cells, and its half-life is 16 min-2.5 h. The increase in human cfDNA is mainly due to certain physiological activities and clinical diseases (such as tumor, inflammation, cerebral infarction, acute injury, transplantation, etc.).[3] After cfDNA is released into circulation, the kidneys, liver and spleen rapidly remove cfDNA. In patients with cancer, a fraction of the cfDNA originates from cancer cells, i.e., circulating tumor DNA (ctDNA). Different ctDNA levels are associated with clinical and pathological features of the cancer, including staging, tumor burden, localization, angiogenesis, and treatment response.[3–7] In addition, ctDNA levels vary depending on tumor grade (e.g., slow versus rapid progression), shedding rate, and other biological factors.[8] High levels of ctDNA are usually found in patients with metastases, and detectable levels after curative surgery or radiotherapy indicate the presence of MRD. MRD refers to the state in which cancer patients have not achieved complete remission (CR) after receiving radical treatment, and there are still tumor cells in the body.[9] MRD is often used in hematological malignancies, and is now widely used in solid tumors. For solid tumors, due to the limited availability of materials, there are no routine molecular detection methods in clinical practice. Imaging and tumor serology are often used to detect tumors. In general, MRD-positive patients compared with MRD-negative patients have a worse prognosis, earlier relapse, and earlier death. MRD is also an important cause of clinical relapse.[10]

"The father of liquid biopsy", Klaus Pantel, pointed out the detection of MRD in blood samples can indicate tumor recurrence earlier than imaging; it may translate into better outcome for the patients with timely treatment of MRD.[11] Clinical detection of MRD can be applied to the early or advanced stage of cancers: non-metastatic local cancers with or without neoadjuvant therapy, adjuvant or post-adjuvant therapy for latent recurrence, and metastatic ones with systemic therapy for dominant recurrence. Following radical surgery or treatment with curative intent, ctDNA is often used to detect MRD even in the absence of clinical or radiological recurrence,[12] and the short half-life of ctDNA makes it ideal for real-time monitoring. CtDNA is only a small part of cfDNA.[3] According to some studies [4,13,14] in the early stages of cancer, the total ctDNA may be less than 1% of the total cfDNA concentration. Therefore, the depth of ctDNA sequencing must be much higher than

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Table 1
Comparison of tumor-informed and tumor-agnostic assays for detecting MRD.

Technical classification	Tumor-informed assays	Tumor-agnostic assays
Specimens for sequencing and panel designing	1. Rely on tissue sampling 2. Mainly for personalized customized sequencing 3. There are also fixed panels	1. Based on plasma only 2. Fixed panel 3. One size for all
Panel coverage and depth	1. Relatively less coverage sites (small panel) 2. High to ultra-high depth (max. 100,000 ×)	1. Relatively more coverage sites (large panel) 2. Medium to high depth (30,000 × to 40,000 ×)
Sensitivity and accuracy	1. Higher sensitivity 2. Higher accuracy	1. Lower sensitivity (higher missed detection rate) 2. Lower accuracy
Process and cost	1. Need to obtain tumor tissue 2. The technical process is complicated 3. Relatively high cost	1. No need to obtain tumor tissue 2. The technical process is simple 3. Relatively low cost
Usage	1. Postoperative MRD detection 2. Recurrence monitoring 3. Curative effect monitoring	1. Molecular typing 2. Detection of emerging drug resistance mutations
Brand examples	1. Customized panel: Signatera, PCM, RaDaR, ASTRA, Safe-SeqS 2. Fixed panel: MRDetect	Fixed panel: CAPP-seq, Guardant Reveal

Abbreviations: CAPP-seq, cancer personalized profiling by deep sequencing; MRD, minimal residual disease.

that of tissue sequencing to detect extremely small amounts of genetic mutations.[15–17]

The timing of ctDNA assessment is critical to provide reliable information and requires careful landmark series to determine appropriate timing. CtDNA levels may be falsely elevated immediately after surgery or the start of treatment. According to the recommendations of the National Clinical Research Institute Colon and Rectum Working Group of America, it is advisable to perform ctDNA assessment 4 to 8 weeks after surgery and 2 weeks after the start or end of treatment,[18] and this working group provides a minimum standard time frame and time points for ctDNA testing in the perioperative period or at relapse.[19]

1.2. Introduction to MRD mainstream detection technologies

Recently, new advances in MRD detection technologies have been continuously emerging. Generally, there are two mainstream assays in MRD testing technology as shown in Table 1. As a representative of tumor-informed approaches, the Signatera test, a Clinical Laboratory Improvement Amendments (CLIA)-certified ctDNA assessment assay, has received U.S. Food and Drug Administration (FDA) breakthrough device designation for MRD detecting since 8 May 2019. To date, a serial of prospective studies such as Dynamic II/III, CIRCULATE and COBRA are ongoing in the MRD setting to further explore what ctDNA may be used to help clinical decision making based on both tumor-informed and tumor-agnostic platforms.[20–23]

2. MRD clinical research progress for CRC

2.1. The main application of MRD detection in CRC

MRD detection is mainly used in guiding postoperative adjuvant treatment (<6 months after radical surgery) and monitoring recurrence (>6 months after radical surgery) in CRC. Postoperative adjuvant therapy is applied to high risk stage II and III CRC. The necessity of postoperative adjuvant chemotherapy (ACT) can be assessed through postoperative MRD detection, and upgradation or downgradation of treatment can be carried out accordingly.[24–26] Recurrence monitoring by testing ctDNA is mainly used in stage I-III CRC. Compared with imaging and tumor biomarkers, MRD detection can predict tumor recurrence in advance, making earlier treatment possible. The accumulated literature on roles of ctDNA across all stages of patients with CRC was shown in Table 2.

2.1.1. Role of ctDNA-based MRD in monitoring recurrence

The National Comprehensive Cancer Network (NCCN) clinical practice guideline version 2.2021 for colon cancer cited an article [27] using the Signatera approach to enroll patients with stage I-III colon cancer who have undergone radical surgery ($n = 130$), with randomized grouping of both arms. The blood sampling landmarks include 30 days before and after the surgery, and the monitoring points were followed up every 3 months to 3 years. In the preoperative blood test, 88.5% of patients were positive for ctDNA. On the 30th day postoperatively, the recurrence risk of positive patients was 7.2 times that of ctDNA-negative ones. At the first point after ACT, the risk of recurrence in ctDNA-positive patients is 17.5 times higher than that of negative ones. CtDNA status is independently related to recurrence. CtDNA predicted recurrence 8.7 months earlier than imaging on average, and the maximum was 16.5 months earlier. Similar results have been documented in other studies about CRC.[28–30] A similar trial (on postoperative ctDNA as markers of recurrence risk in stages II to III CRC) conducted by our team also found that serial ctDNA detections after surgery or adjuvant treatment can definitely suggest tumor recurrence, and the hazard ratio (HR) of patients with positive ctDNA is 10–12 times as high as that of patients with negative ctDNA. If ctDNA is detected positive for three times, the HR will be even higher at 32.0. In addition, ctDNA testing will predict relapse 5.0 months earlier than CT imaging.[31] In another cohort of patients with resected stages I-III CRC, the Safe-SeqS ctDNA plasma test found a 0% recurrence rate in ctDNA-negative patients, suggesting that ctDNA has the potential to serve as an exclusionary test, which is sufficient for patients who have infrequent computed tomography (CT) scans/magnetic resonance imaging (MRI) or follow-ups.[29]

2.1.2. Role of ctDNA-based MRD in monitoring prognosis

The four studies[12,17,30,32] cited in the NCCN version 2.2021 guidelines (all were tumor-informed routes) pointed out that postoperative ctDNA negativity or positivity can be an independent predictor for patients' prognosis. Moreover, the effect of postoperative ctDNA status on recurrence-free survival (RFS) was greater than any individual clinicopathological risk factor or any combination of clinicopathological factors. Patients with postoperative positive ctDNA (detectable) have significantly lower overall survival (OS) and RFS than negative patients. Positive ctDNA after surgery indicates more relapse and poor prognosis; positive ctDNA before surgery cannot be used as a prognostic indicator, but it can be used as a reference indicator for the sensitivity of ctDNA products as well. Therefore, we should measure the performance of MRD testing products in clinical research, such as the positive rate of preoperative blood, the positive and negative predictive value of recurrence monitoring.

An Australian study by Tie and colleagues on 230 patients with stage II colon cancer found that among patients in the stage II cohort who did not receive adjuvant chemotherapy ($n = 178$), 14 showed positive ctDNA at 4 to 10 weeks postoperatively, and 11 of these 14 (79%) patients relapsed at a median follow-up time of 27 months (HR, 18 [95% CI, 7.9–40]; $P < 0.001$). In contrast, among 164 negative ctDNA pa-

tients, only 16 (9.8%) suffered from cancer recurrence.[12] This indicated an extremely high risk of radiological recurrence in stage II CRC patients with positive postoperative ctDNA in the absence of chemotherapy. This risk is greater than in patients with stage III colon cancer, who routinely receive adjuvant therapy. Notably, stage II CRC patients with negative postoperative ctDNA have a lower risk of radiological recurrence and thus a higher 3-year RFS (90%), which is not different from patients with stage I CRC.[33] In another report from the Australian group, which studied 96 patients with stage III colon cancer, ctDNA was found to be detectable in 21% of patients postoperatively. The 3-year recurrence-free interval (RFI) for positive ctDNA in circulation was 47% compared with 76% for those with negative ctDNA. In addition, the 3-year RFI for those with positive ctDNA in samples collected after ACT was 30%, compared with 77% for those with undetectable levels of ctDNA (HR, 6.8).[32] Recently, a post hoc analysis of the PRODIGE-GERCOR IDEA-France trial also testified that postoperative ctDNA was found in 13.8% (140/993) of stage III colon cancer patients. The 3-year disease-free survival (DFS) was 66.4% for ctDNA-positive patents as compared with 76.7% for ctDNA-negative ones after a median follow-up of 6.6 years.[34]

As for locally advanced rectal cancer (LARC), in a Danish study of 123 patients from two biomarker trials, both baseline and longitudinal points of samples showed that elevated cfDNA levels were associated with an increased risk of local and distant recurrence and reduced DFS.[35] Tie's Australian research group showed a strong correlation between the presence of ctDNA in blood samples after chemoradiotherapy (CRT) and surgery and the risk of relapse and relapse-free survival. CtDNA was detected in pretreatment, postradiotherapy and postoperative plasma specimens in 77%, 8.3% and 12% of patients. CtDNA was positive after radiotherapy (HR, 6.6; $P < 0.001$) or positive ctDNA after surgery (HR, 13.0; $P < 0.001$), with a significantly lower relapse-free survival rate. The 3-year RFS rate was estimated to be 33% for postoperative ctDNA-positive patients in contrast to 87% for postoperative ctDNA-negative patients. Postoperative ctDNA testing could predict recurrence for patients with or without receiving ACT (chemotherapy: HR, 10.0; $P < 0.001$; no chemotherapy: HR, 22.0; $P < 0.001$).[36] In a separate prospective multicenter study, plasma specimens were collected before CRT, and 4–6 weeks after the end of CRT, 4–10 weeks after surgery before ACT (postoperative). The use of ACT was left to the discretion of clinicians who were blinded to the ctDNA results. CtDNA was detected postoperatively in 12% of cases and strongly associated with recurrence (HR, 11; $P < 0.001$), independent of use of ACT. Pathologic complete response (pCR) was obtained in 21% of patients, and 27% had pathologically node-positive (pN+) disease. PCR (HR, 0.32; $P = 0.10$) had a trend toward a lower risk of recurrence compared with non-pCR (HR, 4.3; $P < 0.001$).[37] Several other groups have shown that ctDNA status after surgery or chemotherapy appears to be the most important predictive index for treatment outcome in LARC, as detected ctDNA both at baseline or during chemotherapy was shown to be significantly correlated with any parameter that traditionally reflects tumor response.[17,38,39]

Prognostic value of ctDNA in metastatic disease was also documented. The PREDATOR trial found that postoperative ctDNA status in patients with stage IV CRC predicted disease progression with a sensitivity of 72%, a specificity of 93.3% and a positive predictive value of 96.7%. Positive MRD was associated with decreased DFS (HR, 5.80 [95% CI, 3.5–9.7]; $P < 0.001$) and OS (HR, 16.0 [95% CI, 3.9–68.0]; $P < 0.001$). In addition, in a subgroup analysis, OS was 100% in ctDNA-negative patients who did not receive postoperative chemotherapy with a median follow-up of 50 months.[40] Another study suggested that patients with 4-week ctDNA change (decreasing ≥30%) had a median progression free survival (PFS) of 175 days versus 59.5 days (HR, 3.29 [95% CI, 1.55–7.00]; $P < 0.0001$).[41] Meanwhile, it has been shown that metastatic CRC (mCRC) patients receiving second-line chemotherapy have better objective response rate (ORR), PFS and OS after 2 and 8 weeks of initiation of chemotherapy with a decrease in ctDNA levels up to ≤50%.[42]

2.1.3. Role of ctDNA-based MRD in clinical decision making

According to previous clinical trials, 80% of stage II patients can be cured by surgery alone, while 16% of patients have chemical resistance, and only 4% of patients are chemically sensitive, and thus benefit.[47–49] A study showed that adjuvant treatment reduced the recurrence rate by 30% in patients with stage III colon cancer, while the use of postoperative chemotherapy in patients with stage II colon cancer was still controversial.[50]

Table 2
Existing literature on roles of ctDNA across all stages of patients with CRC.

References	CRC setting	Sample size	Results
Reinert et al., [27] 2019	Stages I, II, III	125	CtDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5 [95% CI, 9.8–193.5]; $P < 0.001$).
Wang et al., [29] 2019	Stages I, II, III	58	The recurrence rate among patients with positive ctDNA levels was 77% (10 of 13 patients). Meanwhile, among the 45 patients with negative ctDNA throughout follow-up, none (0%; 95% CI, 0–7.9%) experienced a relapse, with a median follow-up of 49 months.
Tarazona et al., [30] 2020	Stages I, II, III	193 (125 from Reinert et al., [27])	Longitudinal ctDNA assessment detected MRD with 99% specificity and significantly associated with relapse-free survival (HR, 53 [95% CI, 19–149]; $P < 0.001$).
Chen et al., [31] 2021	Stages II,III	240	During surveillance after surgery, ctDNA positivity was also associated with extremely high recurrence risk (HR, 32.02 [95% CI, 10.79–95.08]; $P < 0.001$).
Tie et al., [32] 2019	Stage III	96	Postsurgical ctDNA status remained independently associated with recurrence-free interval (HR, 7.5 [95% CI, 3.5–16.1]; $P < 0.001$).
Henriksen et al., [43] 2021	Stages I, II, III	265 (125 from Reinert et al., [27])	Positive postopevative MRD markedly reduced relapse-free survival (HR, 7.1 [95% CI, 3.4–15]; $P < 0.001$) compared with tumor marker CEA.
Shirasu et al., [44] 2021	Stages I, II, III (prospective study)	808	Positive preoperative ctDNA was associated with advanced pathologic stage. Nodal positivity is a high postoperative predictor for ctDNA positivity ($P < 0.001$).
Anandappa et al., [45] 2021	Stages II, III	122	ctDNA-guided MRD assessment facilitates detection of patients with CRC at high risk of recurrence (HR: 28.8 [95% CI, 3.5–234.1]; $P < 0.001$).
Parikh et al., [46] 2021	Stages I-IV	103	Plasma-only MRD detection approach showed favorable sensitivity and specificity for predicting recurrence. Positive predictive value: 100%; HR, 11.28 ($P < 0.0001$). Single time point sensitivity 55.6%.

Abbreviations: CEA, carcinoembryonic antigen; CRC, colorectal cancer; ctDNA, circulating tumor DNA; MRD, minimal residual disease; HR, hazard ratio.

Table 3
Ongoing prospective studies on ctDNA-guided management of CRC.

Name of the study	Clinical trial No.	Sample size	Study population	Arms of study	Objective
DYNAMIC-II (RCT)	ACTRN1261500381583	450	Stage II colon cancer	Control arm: standard of care; Experimental arm: ctDNA guided treatment	Investigate ctDNA-guided adjuvant management of stage II colon cancer
COBRA (RCT-II/III)	NCT04068103	1408	Stage IIA colon cancer (low risk)	Comparator arm: surveillance; Detectable ctDNA: chemotheray for up to eight cycles; Undetectable ctDNA: surveillance	Investigate ctDNA-guided management of stage II colon cancer
IMPROVE-IT2 (RCT)	NCT04084249	254	High-risk stage II and III CRC	Experimental arm: ctDNA testing every 4 months for 2 years, PET-CT if ctDNA becomes positive; Control arm: surveillance	Investigate utility of ctDNA during surveillance postoperatively; study the percentage of patients who recurred, who received intended curative or local metastasis-directed treatment
DYNAMIC-III (RCT-II/III)	ACTRN1261701566325	1000	Stage III colon cancer	Control arm: standard of care; Experimental arm: ctDNA-guided treatment	Investigate ctDNA-guided adjuvant management of stage III colon cancer
ALTAIR (RCT - Japan)	NCT04457297	240	Stage III CRC after completion of 3 months of CAPOX	Experimental arm: II line adjuvant trifluridine/ tipiracil; Control arm: surveillance	Investigate ctDNA-guided second-line adjuvant therapy management
CIRCULATE-IDEA (RCT-III)	NCT05174169	1912	Resected high-risk stage II or low-risk stage III colon cancer	Standard-of-care arm: CAPOX; ctDNA-guided arm: no ACT	Estimate 3-year DFS
ACT3 Escalation (RCT in MSS cohort)	NCT03803553	500	Stage III CRC: test for ctDNA 3–6 weeks after standard adjuvant chemotherapy	Patients who are ctDNA-positive after completion of 3 -6 months of adjuvant FOLFOX/CAPOX are randomised to: Experimental arm:additional FOLFIRI for 6 months Control arm:(a)surveillance with ctDNA monitoring,(b)additional 6 months of Encorafenib/ Binimetinib/Cetuximab (BRAF mutant),(c)additional 6 months of Nivolumab (MSI-H)	Investigate ctDNA-guided management in stage III CRC

Abbreviations: ACT, adjuvant chemotherapy; CAPOX, capecitabine and oxaliplatin; CRC, colorectal cancer; ctDNA, circulating tumor DNA; DFS, disease-free survival; FOLFIRI, fluorouracil, leucovorin, and irinotecan; MSI, microsatellite instability; MSS, microsatellite stable; PET-CT, positron emission tomography-computed tomography; RCT, randomized controlled trial.

There are ongoing studies on ctDNA-guided management of CRC (Table 3). The COBRA study (NCT-04068103) was a phase II/III study of ctDNA as a predictive biomarker for ACT in patients with stage IIA colon cancer; this study would determine who would and would not benefit from chemotherapy in post-surgical colon cancer patients.[22] The IMPROVE-IT trial (NCT03748680) was open to surgically resected patients with adenocarcinoma of the colon or rectum, pathologically stage I or II disease and detectable ctDNA in plasma samples two weeks after radical resection, although there was no indication for these patients to accept ACT according to the Danish Colorectal Cancer Group (DCCG) guidelines. The main objective of the study was to investigate whether the use of standard ACT could improve DFS in patients with positive MRD detected by ctDNA.[51] The randomized DYNAMIC II trial aimed to explore whether patients with stage II colon cancer could be given or exempted from ACT without compromising survival in the presence or absence of postoperative ctDNA.[20] The primary end point RFS at 2 years was 93.5% in ctDNA-guided management group, similar to 92.4% in standard management group (HR, 0.96 [95% CI, 0.51–1.82]). CtDNA-guided approach (treating only patients with positive postoperative ctDNA) could reduce ACT use without compromising RFS. The tumor-informed personalized approach was used for ctDNA analysis. For stage III colon cancer, the phase II/III DYNAMIC-III study (ACTRN1261500381583) was still under enrollment to assess the strategy of chemotherapy escalation or de-escalation as guided by ctDNA positivity or negativity.[23] The Dutch trial MEDOCC–CREATE (NL6281/NTR6455) investigated whether ACT reduced the risk of recurrence in patients with stage II colon cancer with detectable ctDNA after surgery.[52]

Similarly, a new type of adaptive platform trial named CIRCULATE-Japan was initiated to evaluate therapeutic response guided by ctDNA analysis for MRD detection based on the tumor-informed assay Signatera^{TM} and to refine precision adjuvant therapy for clinical stages II to IV or recurrent CRC. This project was composed of one observational study GALAXY and two randomized phase III trials VEGA (CAPOX or observation for 3 months in patients with high--risk stage II or low-risk stage III colon cancer with negative MRD in the GALAXY study) and ALTAIR (CAPOX for 4 cycles followed by trifluridine/tipiracil or placebo for 6 cycles in patients with resected CRC with positive MRD in the GALAXY study). Therefore, CIRCULATE--Japan included both “de-escalation” and “escalation” trials for MRD- negative and -positive patients respectively, and defined the prognostic and/or predictive value of postoperative MRD after radical surgery.[53] The PEGASUS trial uniquely investigated a step-down strategy from initial ACT based on ctDNA clearance, and escalation to a full 6-month systemic treatment with 5-fluorouracil (5-FU) and irinotecan (FOLFIRI) if ctDNA positivity persists or reappears after the initial clearance period. These ctDNA-guided adaptive platform trials would accelerate clinical development and further enabled precision oncology in the field of adjuvant therapy. In addition, it had been shown that rapid ctDNA growth was associated with poorer overall survival, which had important implications for informing clinicians the urgency of intervention.[43]

2.2. MRD helps assessment of clinical efficacy

CtDNA testing for MRD detection can be applied to neoadjuvant therapy, postoperative ACT, targeted drugs and immunotherapy for assess-

ing clinical efficacy.[24,28] A study showed that ctDNA in chemotherapy-sensitive patients continued to decline during postoperative chemotherapy, while in chemotherapy-insensitive patients it declined at the beginning and then increased during chemotherapy. For patients with LACR receiving neoadjuvant CRT, clinical complete response criteria should include negative ctDNA except for non-evidence of tumor from the pelvic MRI, colonoscopy and digital rectal examination, and this study showed an increased risk of recurrence in 150 patients with LACR who had ctDNA detected after neoadjuvant chemotherapy or surgery, regardless of pathological risk level or adjuvant therapy.[36] Recently, four landmarks of ctDNA (at baseline, during neoCRT, pre-surgery and post-surgery) could assess the response of treatment in LARC patients receiving neoadjuvant CRC. CtDNA was positive in 75.0%, 15.6%, 10.5%, and 6.7% of cases at the four time points, respectively. No preoperative ctDNA was detected in 29 patients with pathological complete response (ypCR). Preoperative ctDNA positive rate was negatively associated with pathological tumor regression grade ($P < 0.001$) and pathologic T stage ($P = 0.002$). During a median 18.8-month follow-up, ctDNA positivity at all four time points was correlated to a shorter metastasis-free survival (MFS) ($P < 0.05$).[17] These studies demonstrated that ctDNA is a real-time surveillance biomarker which can accurately monitor tumor load and predict ypCR, OS, DFS, and MFS.[54,55] Tie et al. also demonstrated in patients with stage II colon cancer that positive ctDNA at the end of ACT predicted a very high risk of radiological recurrence. If ctDNA was detectable after chemotherapy, 100% of patients relapsed.[56] In contrast, in stage III patients, Tie et al. reported that ctDNA status in post-chemotherapy samples was strongly associated with RFI (HR: 6.8 [95% CI, 11.0–157.0]; $P < 0.001$). Three-year RFI was 30% (95% CI, 9%–55%) in cases with detectable ctDNA after chemotherapy and 77% (95% CI, 60%–87%) in negative cases.[32]

CtDNA testing for MRD can also define the patient population who will benefit from ACT for a 3-month or 6-month duration. The IDEA study testified that the non-inferiority endpoint of 3-month vs 6-month chemotherapy for the 3-year DFS rate was not reached for patients with stage III colon cancer, but the incidence of grade 3–5 adverse events in the 3-month group was significantly lower than that of the 6-month group ($P < 0.0001$).[57] IDEA France showed that DFS for stage III CRC patients with positive ctDNA and no chemotherapy was the shortest, andthat for the ones with negative ctDNA and chemotherapy was the longest. Regarding the value of ctDNA in helping determine the optimum duration of adjuvant therapy, the post hoc analysis of the PRODIGE-GERCOR IDEA-France trial suggested a poorer prognosis for the ctDNA-positive patients receiving three months of ACT than the group receiving six months of ACT, in particular for patients with high-risk stage III colon cancer.[58]

Whether ACT induces ctDNA clearance is unclear. It was reported that ctDNA became undetectable after finishing all cycles of ACT in 9 out of 18 patients with resected stage III colon cancer who was observed to have better RFS as compared with those who retained detected ctDNA (HR, 5.1; $P = 0.02$).[32,59] In another study of 13 of the postoperative ctDNA-positive patients in whom plasma samples were collected during and after ACT for up to 3 years, only 3 of the 13 patients (23% [95% CI, 8.2%–50%]) showed complete and permanent clearance of plasma ctDNA at the end of ACT and at further follow-up. These three patients did not relapse at 36 months of follow-up. However, the remaining 10 patients had temporary clearance of plasma ctDNA or no clearance at all and all relapsed.[43] It surely needed further validation on the clearance of detectable ctDNA following radical surgery during ACT. If all ctDNA for MRD detection could be captured, and then cleared by ACT, clinical trials could use ctDNA clearance as a surrogate for DFS, which would be helpful for time- and input-saving.

During systemic therapy in mCRC, decreases in ctDNA levels were associated with tumor response.[4,60–63] Garlan et al. showed that mCRC patients who were observed a ≥80% reduction in ctDNA concentration after first- or second-line chemotherapy had significantly higher ORRs (47.1% versus 0%; $P = 0.003$) and longer median PFS (8.5 versus 2.4 months; HR, 0.19 [95% CI, 0.09–0.40]; $P < 0.0001$) and OS (27.1 versus 11.2 months; HR, 0.25 [95% CI, 0.11–0.57]; $P < 0.001$), suggesting early change in ctDNA levels (after the first or second cycle) was a marker of outcome.[61] Tie et al. assessed ctDNA levels in 53 mCRC patients receiving standard first-line chemotherapy. Tumor tissues were sequenced using 15 genomes frequently mutated in mCRC to identify candidate mutations for ctDNA analysis. For each patient, one tumor mutation was selected and the presence and levels of ctDNA in plasma samples were assessed using Safe-SeqS. The results showed that patients with reduced ctDNA before the second cycle also had radiologically confirmed responses after 8–10 weeks.[60] The above findings demonstrated the role of ctDNA as a biomarker in detecting MRD, tracking treatment response, and monitoring disease.

2.3. Application of MRD in the perioperative period of liver metastases from colorectal cancer

In all CRCs presented with liver metastases, more than 96% of patients were ctDNA positive.[26] In addition, patients with positive ctDNA before liver metastases resection had half survival time of patients with negative ctDNA. Therefore, we could use ctDNA to detect MRD to predict the recurrence of CRC liver metastasis (CRLM), and patients with negative ctDNA before surgery should be given the priority to undergo surgery.[9] In fact, ctDNA was a prognostic index for patients receiving both hepatectomy and no surgery. For ctDNA positive patients, the survival benefit of major liver section resection might be challenged; therefore, less invasive surgery or intervention was applied to receive no evidence of disease (NED) based on Response Evaluation Criteria in Solid Tumors (RECISIT) criteria. In the perioperative surveillance study of patients with operable CRLM, 54 patients were analyzed with a median follow-up of 51 months by using the tumor-informed personalized tracking approach (Safe-Seqs). The results showed that the five-year RFS after adjuvant therapy was 66.7% vs 0% in preoperative positive ctDNA clearance patients vs persistent positive ctDNA patients. After the overall adjuvant therapy was completed, the 5-year RFS of ctDNA positive vs negative patients was 16.7% vs 69.3%; and the 5-year OS was 17.3% vs 82.0%. For the neoadjuvant chemotherapy group, there was a median 40.93-fold (19.10 to 87.73; $P < 0.001$) decrease in ctDNA mutant allele fractions, but ctDNA clearance during neoadjuvant chemotherapy was not correlated to a better RFS.[64] Preoperative ctDNA status showed to be predictive of the risk of recurrence in patients with CRLMs undergoing surgical resection.[65–68] Several research groups similarly showed an association between plasma ctDNA levels and tumor load, reduced ctDNA during preoperative chemotherapy and improved tumor response, and postoperative or postchemotherapy positive ctDNA and shorter RFS after resection of CRLMs.[69–71] Other studies demonstrated the feasibility of plasma ctDNA testing for MRD in mCRC patients receiving neoadjuvant chemotherapy and a potential correlation between MRD and pCR with undetectable ctDNA in neoadjuvant chemotherapy.[72,73] Besides, in four other studies involving ctDNA and resection of liver metastases from CRC, all supported a strong negative prognostic impact of ctDNA.[25,71,74,75]

3. The technical bottleneck of MRD

At present, one of the bottlenecks of MRD detection is that the amount of ctDNA is very low and the false negative rate is high. Studies found that the frequency of detection of ctDNA mutations was about 0.1% when the average diameter of lung adenocarcinoma tumors was 2.6 cm. In multi-cancer studies, about 53% of patients had on average one mutation detected in plasma at the first relapse and the variant allele frequency was 0.01%–0.1%. In every 10 ml of whole blood, there are about 4 ml of plasma and about 12,000 ctDNA molecules. Theoretically, the mutation frequency is at the level of one in 10,000, which requires extremely high detection sensitivity. In other words, when the whole

blood sample volume is 20 ml, it is possible to ensure more stable detection of the 10,000-level variation.[27,76] According to clinical settings, the volume of blood collected should be optimized (for instance, higher volumes of plasma [up to 60 ml] might be required for the detection of MRD) in order to assess therapy responses.

Another detection bottleneck of MRD is the need for ultra-high-sensitivity detection methods. The amount of ctDNA into the blood varies depending on the type of cancers. Different cancers has different abilities to release ctDNA. For example, CRC has relatively more ctDNA, and more ctDNA in the advanced stage. Additionally, tumors close to blood vessels release more ctDNA, but the total amount was still very small. The sensitivity thresholds of MRD in different cancers also vary. For examples, to detect MRD in multiple myeloma, the sensitivity threshold was up to 10^(−5) and 10^(−6).[77] However, for lung cancers, the Chinese expert panel consensus mentioned the minimum limit detection (LOD) should be at least 0.02%. The detection threshold of MRD in CRC patients following surgery has not yet been identified.

Finally, due to high background noise, MRD detection has high requirements for biometric analysis. There are two main reasons for false positivity in ctDNA sequencing. One is the interference of clonal hematopoiesis (CH): More than 50% of circulating cfDNA mutations are associated with clonal hematopoiesis rather than tumor-derived mutations. The load is positively correlated with age. In addition, more than 50% of the mutations in the blood of cancer patients come from white blood cells, and the common ones are *TP53, DNMT3A, TET2*, etc. Therefore, when ctDNA detection is used to evaluate MRD of lung cancer, the evaluation criteria could not be simply one-size-fits-all, and it is necessary to comprehensively consider driving molecular events, clinical treatment factors, and analytical screening strategies. Tissue prior strategies could ensure the specificity of somatic variant tumor origin, reduce the influence of clonal hematopoiesis, and ensure the accuracy of ctDNA detection. Another reason is germline mutations. We could effectively filter a patient's clonal hematopoietic mutations and germline mutations through isodepth sequencing of the patient's white blood cells. Therefore, the accuracy required by MRD testing places extremely high requirements for the biometric analysis process, and lowering the filtering standards would cause false positive results.[78]

4. Warnings of ctDNA detection and analysis

According to the specified guidelines, we should use a large-bore needle (≤21 G) to take blood, and extract ctDNA from plasma for analysis to exclude contamination during coagulation.[79–81] Blood was typically drawn into K2 EDTA tubes, ideally within 4–6 h for plasma separation (up to 24 h, temporary storage at 4 °C is possible).[79–84] Detection of MRD usually requires at least 20 ml through at most 60 ml of plasma, which is sequentially centrifuged at 800 *g* to 1600 *g* at 4 °C.[80,81] At present, DNA could be extracted and purified from plasma by off-the-shelf commercial kits, but the specific purification method needed to be customized according to the upstream and downstream pre-analysis methods.[81] Current ctDNA analysis techniques could be classified according to whether polymerase chain reaction (PCR) or next-generation sequencing (NGS) is involved. PCR-based detection techniques have low cost and high sensitivity, and are suitable for detecting trace amounts of DNA in blood, but it also has inevitable drawbacks: low throughput and inability to detect unknown mutations.[85] NGS-based techniques could theoretically sequence entire genomes. We could actually adjust for genomic aberrations in ctDNA based on sequencing of tumor tissue to increase sensitivity and reduce the risk of false positive results. However, this adjustment would further increase the cost and delay analysis of results, which are significant obstacles for for timely treatment.[86] Perhaps the combined analysis of ctDNA and other tumor markers could improve the accuracy of the analysis.

5. The main controversies about the use of MRD in clinical surveillance

MRD is used in clinical surveillance to obtain more accurate patient classification, earlier and shorter treatment, and less injury.[54,28] However, MRD testing also has limitations in clinical practices: ctDNA might not be detected in patients with some metastatic sites such as peritoneum and brain; similarly, a small portion of patients with intact primary tumors might have undetectable ctDNA [11]; there are many ctDNA products with uneven sensitivity in aspects such as analytical LOD, sampling volume, sampling time, quantity of input molecules and tumor burden; the experimental operation process lacks standardization; the result evaluation lacks standardization; most products have insufficient clinical verification data; different cutoffs of mutated ctDNA numbers are defined as positive MRD. Combining ctDNA and imaging evaluation optimally for detecting disease relapse would help in developing evidence-based management guidelines. The main controversy in front of us is: CtDNA could only predict recurrence and could not change the current treatment; would it increase patients' anxiety if the current recurrence is displayed prematurely without treatment? If ctDNA suggests the possibility of recurrence, should patients need to start treatment immediately? Is there a cut-off value? What should we do for patients who receive all adjuvant therapies but are still ctDNA positive? What new therapies could we employ in this area to potentially cure them? What is the best surveillance strategy for the patients? What is the point of spending more money to test ctDNA?

6. Future perspective and conclusions

To summarize, the application of MRD/immune score (IS) in the adjuvant treatment of early bowel cancer could help to monitor the recurrence of early disease after surgery, and to guide the development of individualized ACT after surgery (based on the "positivity and negativity" of MRD/IS results, to select chemotherapy/no chemotherapy, to decide single agent/combination, and to give for 3 months/6 months). In addition, the technology currently has a series of problems, such as: what size panel to use, how to better design the experiment, which genes to detect, timing of detection, and how to intervene if MRD is positive but imaging is negative? Should the NED criteria include MRD in the postsurgical period of resected mCRC? Although the prognostic significance of MRD has been established, further validated trials are needed to confirm the predictive value of MRD in the coming future.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Author contributions

W.H., M.X. and T.S. wrote this manuscript and assisted with formating and language editing. R.X. made a substantial contribution to discussions of content. G.C. reviewed and edited the manuscript prior to submission. All authors read and approved the final manuscript.

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