

Efficacy of circulating tumour DNA in guiding postoperative adjuvant chemotherapy for resectable colorectal cancer: A meta-analysis based on cohort studies

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Abstract. The value of circulating tumour DNA (ctDNA) in resectable colorectal cancer (CRC) remains unclear. The present study evaluated the efficacy of ctDNA-guided adjuvant chemotherapy (ACT) in patients with resectable CRC after surgery. A systematic search was conducted in PubMed, Web of Science, EMBASE and the Cochrane Library databases for relevant studies published from January 2010 to July 2026. The inclusion criteria were studies that: i) Included patients with resectable CRC who underwent postoperative ctDNA testing before ACT initiation; ii) stratified the assessment of ACT efficacy based on ctDNA test results; and iii) reported recurrence-free survival (RFS) and/or overall survival (OS). The hazard ratio (HR) and 95% confidence interval (CI) were used to evaluate the results. The meta-analysis included 4 cohort studies and 1 randomized controlled trial, involving 2,081 evaluable patients with resectable CRC (441 cases in the ctDNA-positive group and 1,640 cases in the ctDNA negative group). Among the patients positive for ctDNA, the RFS rate (HR, 0.34; 95% CI, 0.16-0.73; P=0.006) and OS rate (HR,

0.40; 95% CI, 0.20-0.81; P=0.01) of the group that received ACT were significantly improved compared with those in patients who did not receive ACT. Among the patients negative for ctDNA, there was no statistically significant difference in the RFS rate (HR, 1.05; 95% CI, 0.70-1.60; P=0.802) and OS rate (HR, 0.66; 95% CI, 0.28-1.56; P=0.348) between the ACT and non-ACT groups. Therefore, the present study revealed that ctDNA testing may serve as a marker for guiding ACT in patients with resectable CRC after surgery.

Introduction

Colorectal cancer (CRC) currently ranks as the third most common cancer type worldwide, with ~1.9 million new cases estimated to have occurred in 2022. In terms of mortality, CRC is the second leading cause of cancer-related death globally (1). Curative-intent radical surgery aimed at a complete cure is the preferred treatment regimen for the vast majority of patients with resectable CRC (2). However, ~30% of patients with stage I-III CRC experience recurrence of the disease (3).

In an effort to further augment clinical efficacy, adjuvant chemotherapy (ACT) is widely adopted as a standard post-operative treatment strategy. Evidence regarding the survival benefits of ACT for stage II colon cancer remains controversial. The MOSAIC trial revealed that the FOLFOX regimen (oxaliplatin combined with fluorouracil and leucovorin) improved disease-free survival, yet the clinical benefit was modest (4). The XELOXA trial demonstrated that XELOX (capecitabine combined with oxaliplatin) markedly improved the 5-year disease-free survival rate of patients with stage III colon cancer (5). Rectal cancer differs markedly from colon cancer in terms of tumour biological features and therapeutic strategies. Neoadjuvant chemoradiotherapy followed by total mesorectal excision serves as the cornerstone of treatment for locally advanced rectal cancer, whereas the value of ACT remains unclear. The ADORE trial indicated that certain patients with locally advanced rectal cancer might benefit from postoperative FOLFOX after neoadjuvant therapy (6). By contrast, the EORTC 22921 trial demonstrated that adjuvant

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Abbreviations: CRC, colorectal cancer; ACT, adjuvant chemotherapy; ctDNA, circulating tumour DNA; MRD, minimal residual disease; CRLM, colorectal liver metastases; RFS, recurrence-free survival; OS, overall survival; RCT, randomized controlled trial; HR, hazard ratio; CI, confidence interval; NGS, next-generation sequencing; PCR, polymerase chain reaction; dPCR, digital PCR; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin

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fluorouracil-based chemotherapy after neoadjuvant chemoradiotherapy did not affect disease-free survival or overall survival (OS) in patients with clinical stage T3 or T4 resectable rectal cancer (7). Perioperative chemotherapy has been evaluated in multiple clinical trials for patients with resected colorectal liver metastases (CRLM; stage IV disease). For instance, the EORTC trial validated that the perioperative FOLFOX regimen improved progression-free survival among patients with resectable CRLM. However, the implications of the perioperative FOLFOX regimen for ACT alone remain controversial (8).

Although ACT is widely administered in clinical practice, the current clinical evaluation system for guiding postoperative ACT has prominent flaws and unresolved clinical challenges. At present, clinicians rely mainly on routine pathological features (9), imaging examinations (10) and serum tumour markers, including carcinoembryonic antigen and carbohydrate antigen 19-9, to determine the necessity of ACT (10-13), which represents empirical stratification with limited accuracy. This technical limitation creates a critical clinical dilemma. On the one hand, a study investigating circulating tumour DNA (ctDNA)-guided management for stage II colon cancer demonstrated that >80% of patients exhibited undetectable postoperative ctDNA. Such patients achieved a 3-year recurrence-free survival (RFS) rate as high as 92.5% without ACT (14); nevertheless, in routine clinical practice, some patients still routinely receive ACT, potentially resulting in overtreatment (15) that yields no meaningful survival benefits yet exposes them to chemotherapy-related adverse events such as myelosuppression (16) and gastrointestinal toxicity (17), impairing postoperative quality of life and wasting medical resources. On the other hand, patients with minimal residual disease (MRD) and high recurrence risk cannot be identified early via traditional assessments (18), causing them to miss the optimal window for adjuvant therapy and face markedly elevated long-term recurrence risks. In summary, the existing clinical stratification framework lacks precise, individualized predictors of therapeutic response, which acts as a major bottleneck restricting the precise and standardized delivery of postoperative ACT for CRC. Novel high-sensitivity biomarkers are urgently required to refine postoperative treatment decision-making.

In recent years, ctDNA has emerged as a promising non-invasive biomarker. ctDNA demonstrates notable potential in detecting MRD in patients with CRC following curative treatment, thereby providing valuable references for subsequent treatment decision-making (19). Based on this, some researchers have proposed a hypothesis, which can be stratified according to the ctDNA detection results: A positive ctDNA status indicates MRD and these patients are associated with an elevated risk of tumour recurrence; therefore, postoperative ACT may confer survival benefits. By contrast, for patients that show no detectable ctDNA under the current assay sensitivity, routine chemotherapy constitutes overtreatment that yields no survival gain and triggers chemotherapy-related adverse effects (20,21). Nonetheless, false-negative results remain possible due to tumour heterogeneity and the limited technical sensitivity of detection platforms. However, there is a lack of unified evidence-based consensus in this field and, to the best of our knowledge, no systematic meta-analysis has

pooled the existing research evidence to verify the clinical efficacy of ctDNA-guided ACT for CRC.

Considering the limitations of conventional risk assessment tools and the current gap in clinical evidence, a meta-analysis was performed in the present study to comprehensively evaluate the clinical efficacy of ctDNA-directed postoperative ACT among patients with resectable CRC. The present study aimed to provide evidence to facilitate the development of precise, individualized postoperative adjuvant treatment strategies for CRC.

Materials and methods

Protocol and guidance. The protocol for the present systematic review was registered with the International Prospective Register of Systematic Reviews (<https://www.crd.york.ac.uk/prospero/>) in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (22). The registration number is CRD420251131202.

Literature retrieval. The present study systematically searched the PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science (<https://www.webofscience.com/>), EMBASE (<https://www.embase.com/>) and Cochrane Library databases (<https://www.cochranelibrary.com/>) for articles published between January 2010 and July 2026. January 2010 was selected as the starting time point, as published studies indicated that high sensitivity ctDNA detection assays were underdeveloped and seldom used for postoperative risk stratification of CRC before 2010, with the earliest relevant literature emerging in 2011 (23,24). No language restrictions were applied during the search and the retrieval focused on articles exploring the value of ctDNA-guided postoperative ACT in patients with resectable CRC. Detailed search strategies are presented in Table SI. The aforementioned literature retrieval was independently conducted by two researchers. Any discrepancies arising during this process were resolved through consultation with a third author. All retrieved article data were imported into EndNote software (version 21; Clarivate), followed by the removal of duplicate records. To avoid missing potential articles, a secondary search was also conducted on the reference lists of the retrieved literature.

Inclusion and exclusion criteria. In accordance with the pre-established inclusion criteria, studies that met the following criteria were included in the present study: i) Study population comprised patients diagnosed with resectable CRC (stages I-IV), with stage IV patients limited to those with resectable CRLM; ii) ctDNA testing was performed in patients after surgery and before ACT; iii) patients were stratified into ctDNA-positive and ctDNA-negative groups according to ctDNA testing results. Within each ctDNA subgroup, survival outcomes were directly compared between patients receiving ACT and those managed with surveillance alone to assess the efficacy of ACT; and iv) the study reported RFS and/or OS [notably, RFS was uniformly defined as the time interval from the date of definitive surgery to the first radiologically confirmed disease recurrence or death, and recurrence was strictly verified via standardized radiological examinations according to the Japanese Society for Cancer of the Colon

and Rectum 2019 colorectal cancer treatment guidelines (25)]. Regarding study design, both interventional and non-interventional studies were eligible, including randomized controlled trials (RCTs) and cohort studies (prospective or retrospective).

Exclusion criteria were as follows: i) Studies involving patients with unresectable CRC (where patients fail to achieve a 'no evidence of disease' status after surgery); ii) studies with unavailable or insufficient data for statistical analysis; and iii) unpublished studies or studies for which the full text could not be obtained. Furthermore, for studies with multiple published updated versions, only the most comprehensive or the latest articles were incorporated.

Data extraction. In accordance with the pre-defined data extraction criteria, relevant data were extracted from the included studies, covering the following four categories: i) Study basic information (publication year, authors and country); ii) patient baseline characteristics (tumour stage and median follow-up duration); iii) study protocols (ctDNA detection methods, ctDNA positivity definition criteria, ACT regimens and timing of post-surgery ctDNA detection); and iv) outcomes (RFS and OS).

Quality assessment. In the present study, the Newcastle-Ottawa Quality Assessment Scale (26) was utilized to assess the quality of included cohort studies, while Cochrane Collaboration's tool (27) for assessing risk of bias was adopted for bias evaluation of RCTs. The Newcastle-Ottawa Quality Assessment Scale evaluated the included studies through three major domains (encompassing eight items), specifically: i) Selection of study population; ii) comparability; and iii) exposure or outcome (26). Only studies with a score ≥ 6 were included in the analysis. The Cochrane Collaboration's tool for assessing risk of bias was used to primarily evaluate the risk of bias across six domains: i) Selection bias; ii) performance bias; iii) detection bias; iv) attrition bias; v) reporting bias; and vi) other bias. Each area is classified as 'low risk', 'unclear risk' or 'high risk' (27).

Statistical analysis. The present study was statistically analysed using Stata (version 12.0; StataCorp LP). Hazard ratio (HR) with 95% confidence interval (CI) was used to assess the association between postoperative ACT and prognosis in patients with CRC. A $HR < 1$ indicated that postoperative ACT could improve the prognosis of patients. Conversely, a $HR > 1$ indicated that postoperative ACT was associated with a poorer prognosis. Heterogeneity was evaluated using the χ^2 test and its magnitude was quantified using the I^2 statistic. A significant level of heterogeneity was considered present when $I^2 \geq 50\%$, whereas low heterogeneity was indicated when $I^2 < 50\%$. Given the inherent heterogeneity (for example tumour stage, chemotherapy regimens, ctDNA detection methods and criteria for defining ctDNA positivity) across the included studies, a random-effects model was employed to improve the reliability of the results. When ≥ 5 studies were included, Egger's test was adopted to assess publication bias, and a sensitivity analysis was performed by excluding each study one by one. All statistical analyses were performed using two-tailed tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Study selection. In the present study, a literature search was performed in the PubMed, Web of Science, EMBASE and Cochrane Library databases, which initially retrieved a total of 17,870 entries. After the removal of duplicates, 10,582 entries remained and no additional search sources were utilized. Following title and abstract screening, 10,456 entries were initially excluded, leaving 126 studies for full-text assessment. After reviewing these 126 full texts, 5 studies were retained, with 121 excluded. Of the 121 excluded studies, 111 were excluded due to the study design not meeting the criteria, 6 due to lack of relevant data and 4 because the included patients did not meet the criteria. Details are presented in Fig. 1.

Study characteristics. A total of 4 cohort studies and 1 RCT were included, with 1 published in 2024, 2 in 2025 and the remaining 2 in 2026 (28-32). These studies were primarily conducted in Japan and China, and the patient recruitment periods of all studies fell within the timeframe 2014-2025. In total, 1 study enrolled patients with high-risk pathological stage II-III colon cancer and resected CRLM, 1 study included patients with resected stage I-IV CRC, 1 study enrolled patients with stage II colon cancer and the remaining 2 studies focused on patients with resectable CRLM. Among these studies, all 5 reported RFS, while 4 of them additionally reported OS. A total of 441 patients were included in the ctDNA-positive group, while 1,640 patients were included in the ctDNA-negative group. Detailed information is presented in Table I.

The ACT regimens were predominantly based on the combination of oxaliplatin, leucovorin and 5-fluorouracil. The main detection methods for ctDNA were next-generation sequencing (NGS)-based assays and polymerase chain reaction (PCR)-based assays. A strategy that took into account both sensitivity and specificity was commonly adopted to define ctDNA-positive patients: ctDNA was determined to be positive if at least two tumour-specific variants were detected; if only one variant was identified, confirmation of a ctDNA-positive status required the variant allele frequency of this variant to reach a pre-defined threshold (typically $\geq 0.1\%$). Postoperative ctDNA sampling mostly occurred 1 month after resection and the median follow-up duration ranged from 1.2 to 43.2 months. Detailed information is provided in Tables SII and SIII.

Quality assessment. The quality of the 1 RCT and 4 cohort studies included was assessed using the Cochrane Collaboration's tool and Newcastle-Ottawa Quality Assessment Scale, respectively. Full evaluation indicators are presented in Tables SIV and SV. The total score for each of the 4 cohort studies ranged from 7 to 9. The included RCT had low-bias risks in sequence generation, allocation concealment and selective outcome reporting. High-bias risks existed for blinding and free of other bias sources, and the risk of incomplete outcome data was unclear.

ACT survival in patients positive or negative for ctDNA. Among the patients positive for ctDNA, those who received ACT had a significantly improved RFS rate compared with those who did not ($HR, 0.34; 95\% CI, 0.16-0.73; P=0.006$; Fig. 2). Heterogeneity analysis revealed moderate heterogeneity

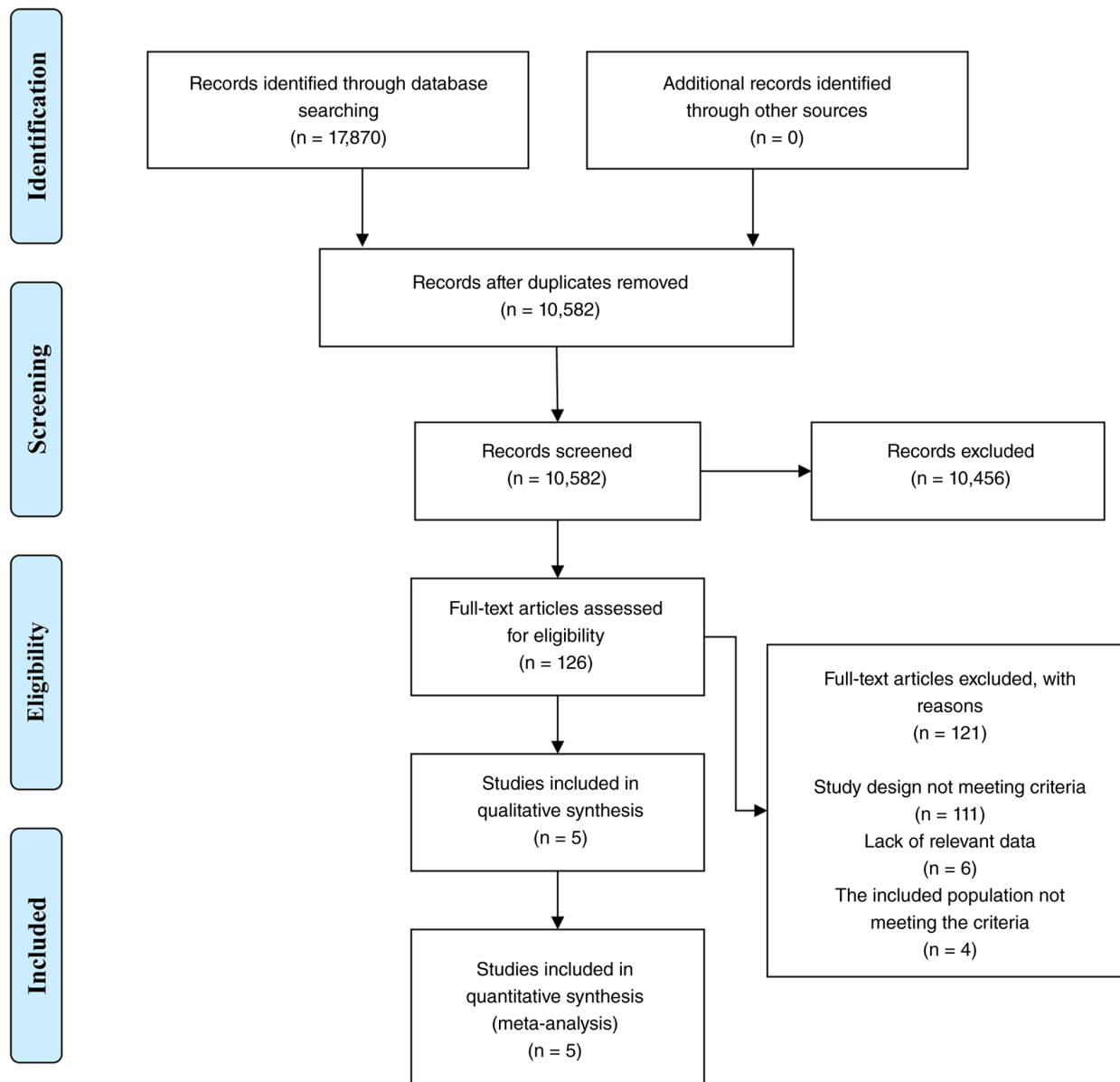


Figure 1. Flow diagram of the selection process.

($I^2=60.3\%$; $P=0.039$). Further analysis of the OS rate indicated that, among the patients positive for ctDNA, the group that received ACT also demonstrated an improved survival outcome (HR, 0.40; 95% CI, 0.20-0.81; $P=0.01$; Fig. 3). Heterogeneity analysis indicated no significant heterogeneity ($I^2=0.00\%$; $P=0.873$).

Among the patients negative for ctDNA, no statistically significant differences in RFS rate and OS rate were observed between the ACT group and the non-ACT group. The pooled results were HR=1.05 (95% CI, 0.70-1.60; $P=0.802$; Fig. 4) for the RFS rate and HR=0.66 (95% CI, 0.28-1.56; $P=0.348$; Fig. 5) for the OS rate. The heterogeneity test revealed no notable heterogeneity for both outcomes with $I^2=0.00\%$ (RFS: $P=0.476$; OS: $P=0.846$).

Publication bias and sensitivity analysis. Publication bias and sensitivity analyses were performed on the pooled effect sizes of RFS in patients positive for ctDNA. The Egger's test yielded

a P-value of 0.927, indicating no significant publication bias (Fig. S1). Sensitivity analysis via sequential exclusion of individual studies revealed no notable shifts in the pooled HRs, verifying the robustness of the study conclusions (Fig. S2).

Discussion

The present study systematically reviewed 4 cohort studies and 1 RCT and demonstrated that ACT in patients positive for ctDNA significantly improved their clinical prognosis (including RFS and OS). However, in patients negative for ctDNA, ACT failed to yield a significant improvement in prognosis (including RFS and OS). Notably, this finding carries clinical implications: ACT can be omitted for patients negative for ctDNA at low risk, which not only reduces chemotherapy-related toxicities but also alleviates the economic burden on healthcare systems and patients. Based on the aforementioned findings, ctDNA-guided ACT decision-making

Table I. Characteristics of studies included in the meta-analysis.

First author/s, year	Country	Recruitment period	Participants	Outcome	Patients with ctDNA-positive, n		Patients with ctDNA-negative, n		(Refs.)
					Exposure	Control	Exposure	Control	
Kataoka <i>et al</i> , 2026	Japan and China	2020-2024	High-risk pathological stage II or stage III colon cancer and resected CRLM	RFS, OS	173	131	628	713	(31)
Fan <i>et al</i> , 2024	China	2017-2021	Resected stages I-IV CRC	RFS	35	8	147	110	(28)
Kawashima <i>et al</i> , 2025	Japan	2014-2021	Resected CRLM	RFS, OS	5	6	25	17	(29)
Xu <i>et al</i> , 2025	China	2019-2023	Resected CRLM	RFS, OS	29	18	NA	NA	(30)
Folprecht <i>et al</i> , 2026	Germany and Austria	2020-2025	Stage II colon cancer	RFS, OS	21	15	NA	NA	(32)

NA, not available; CRC, colorectal cancer; CRLM, colorectal liver metastases; RFS, recurrence-free survival; OS, overall survival; ctDNA, circulating tumour DNA.

exhibits considerable potential for optimizing the treatment of patients with resectable CRC.

Researchers first reported the presence of ctDNA in the serum of individuals with cancer in 1977 (33). In 2014, ctDNA testing for epidermal growth factor receptor mutation detection was granted its first approval to concurrently aid in Erizar diagnosis, thereby officially initiating the clinical application of ctDNA (34). With advancements in technologies such as reverse transcription-quantitative PCR, digital droplet PCR and NGS, ctDNA detection has become increasingly sophisticated and has been extensively explored in clinical applications, including drug target detection, drug resistance mechanism analysis and early tumour screening (35-37).

When tumours progress to the advanced stage, tumour cells undergo metabolic and apoptotic alterations, and the released ctDNA can enter the peripheral blood circulation (38). Currently, it is widely recognized that ctDNA levels are associated with tumour burden, with higher ctDNA concentrations typically indicating a greater tumour burden (36,39). Multiple cohort studies on CRC have confirmed a marked correlation between ctDNA levels and the volumes of primary and metastatic lesions (40,41). For primary tumours, the mutant copy number and concentration of plasma ctDNA correlate positively with tumour size, T stage and depth of invasion (40). Patients with advanced or metastatic CRC present with markedly higher ctDNA abundance than those with early-stage disease, which validates that primary tumour burden serves as a key determinant of ctDNA concentration (42). Additionally, another study revealed that the plasma ctDNA concentration in patients with solid tumours is moderately positively correlated with the total tumour burden measured by computed tomography. This suggests a strong association between plasma ctDNA concentration and disease status, with the association being most prominent in patients with disease progression (43). This close linkage between ctDNA concentration and underlying tumour load provides the biological basis for postoperative MRD detection: The absence of detectable ctDNA typically reflects complete resection or curative treatment; conversely, persistently detectable ctDNA indicates the presence of residual micrometastatic disease, which underpins the core rationale of the present study of stratifying patients by post-surgical ctDNA status to guide ACT.

Over a decade ago, Diehl *et al* (44) first demonstrated that in patients with stage II-IV CRC who underwent curative-intent surgery, the median ctDNA level decreased by 97% within <1 day and by 99% within 10 days. By contrast, if a curative resection was not achieved, the ctDNA levels demonstrated no marked decrease or increase. This finding reflects that tumour-derived cell-free DNA persists in the circulation following incomplete resection and this biomarker for minimal residual lesions predicts an elevated subsequent risk of tumour recurrence in patients. From this finding, it can be inferred that ACT is needed to eliminate these micrometastatic lesions, which are referred to as MRD in the present study.

Based on the aforementioned findings, some scholars propose that ctDNA can serve as a tool for evaluating prognosis, an assumption that has been validated by multiple studies to date. In 2016, Tie *et al* (45) evaluated the predictive value of ctDNA for radiological recurrence in patients who did not receive ACT by analysing ctDNA levels at

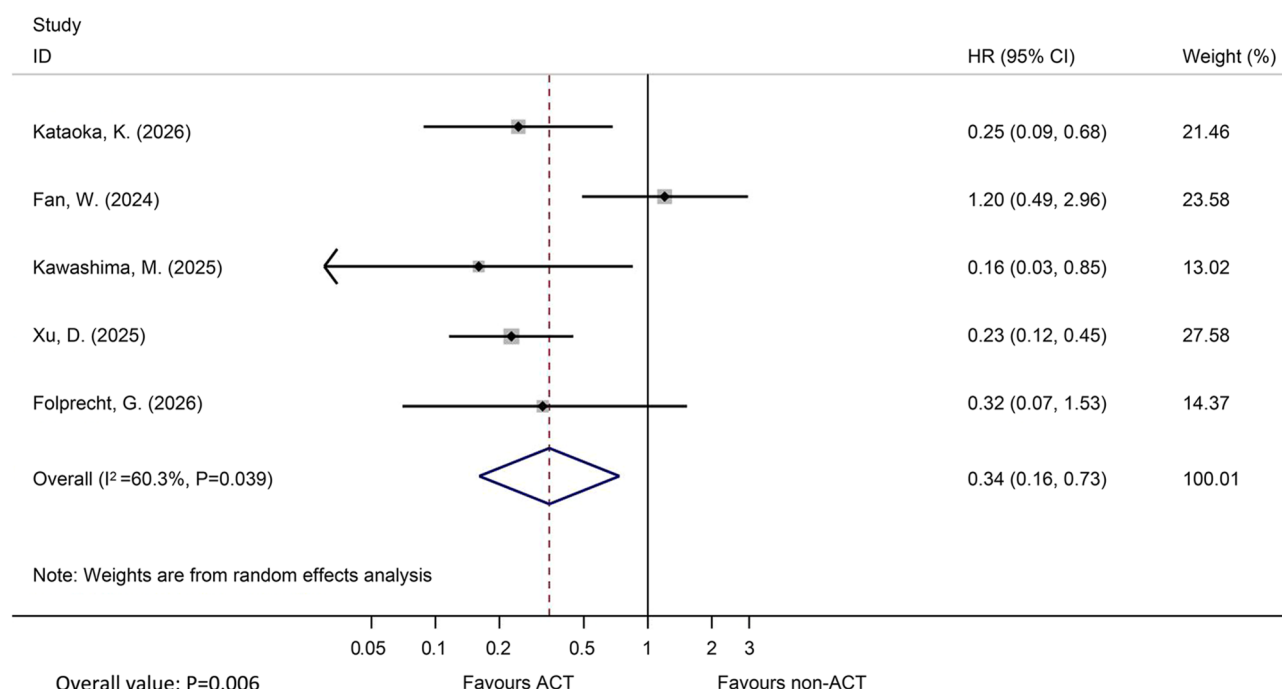


Figure 2. Forest plot of the association between ACT and recurrence-free survival in the circulating tumour DNA-positive group of patients with resectable colorectal cancer. $P=0.006$. ACT, adjuvant chemotherapy; HR, hazard ratio; 95% CI, 95% confidence interval.

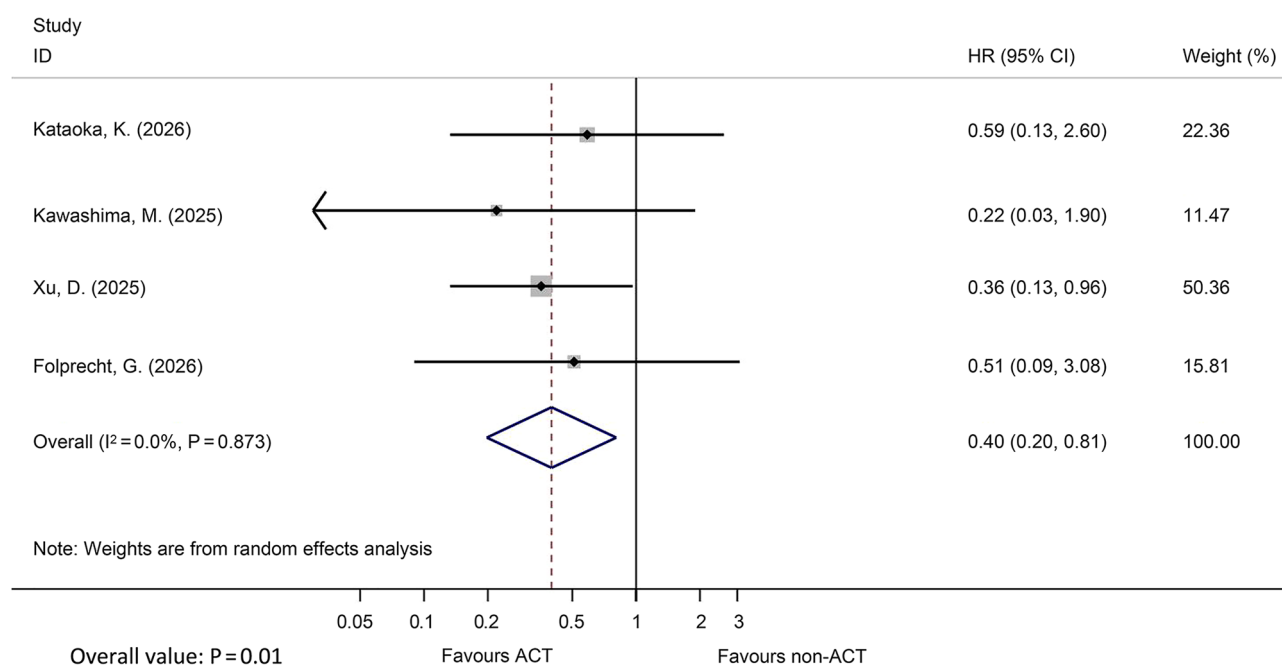


Figure 3. Forest plot of the association between ACT and overall survival in the circulating tumour DNA-positive group of patients with resectable colorectal cancer. $P=0.01$. ACT, adjuvant chemotherapy; HR, hazard ratio; 95% CI, 95% confidence interval.

different postoperative time points. The results indicated that, compared with that in patients negative for ctDNA after surgery, the RFS time of patients positive for ctDNA was markedly shortened. Compared with traditional imaging studies and serological tests, ctDNA testing exhibits higher specificity and has emerged as a valuable tool for the dynamic assessment of tumour burden in patients (46,47). Furthermore, numerous studies have confirmed that ctDNA-based MRD monitoring can identify signs of tumour persistence or

recurrence up to 8-12 months earlier than conventional detection methods (48-50).

Currently, the implementation of ACT for patients with CRC is based on clinicopathological risk factors, including pathological TNM staging, lymphovascular invasion, clinical obstruction and perforation (2). However, it has been reported that, in patients with stage II CRC who have clinical and pathological risk factors, ACT does not notably improve OS and is accompanied by numerous adverse events, including

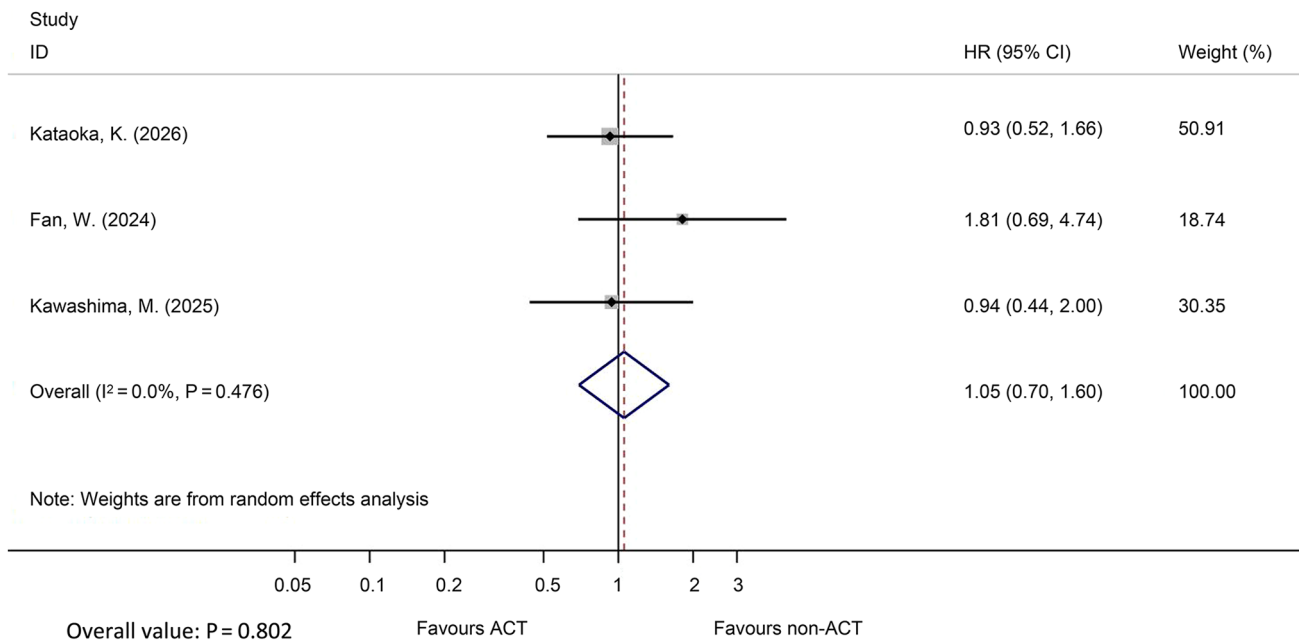


Figure 4. Forest plots of the association between ACT and recurrence-free survival in the circulating tumour DNA-negative group of patients with resectable colorectal cancer. $P=0.802$. ACT, adjuvant chemotherapy; HR, hazard ratio; 95% CI, 95% confidence interval.

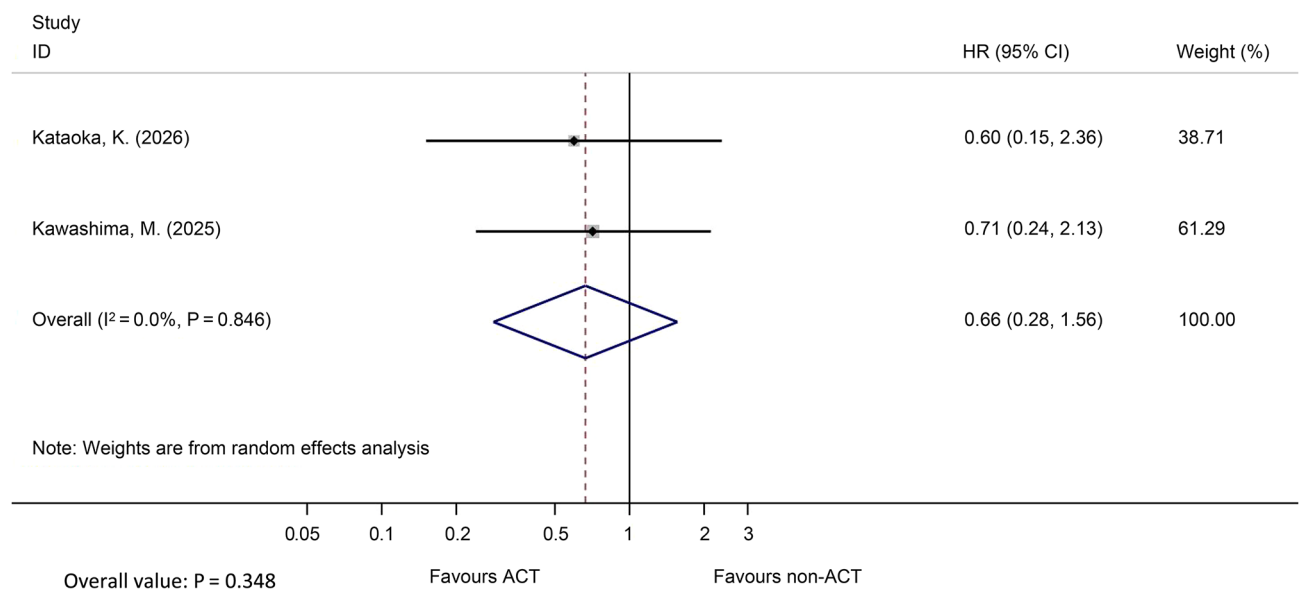


Figure 5. Forest plots of the association between ACT and overall survival in the circulating tumour DNA-negative group of patients with resectable colorectal cancer. $P=0.348$. ACT, adjuvant chemotherapy; HR, hazard ratio; 95% CI, 95% confidence interval.

gastrointestinal toxicity and myelosuppression (51). By contrast, based on the characteristics of ctDNA, researchers have conducted a series of explorations to apply ctDNA in the identification of high-risk populations. Kotani *et al* (52) demonstrated that, regardless of pathological stage, patients with a high risk of recurrence (ctDNA-positive) were likely to derive benefit from ACT. In the DYNAMIC trial, among patients in the ctDNA-guided arm, the subgroup of patients with ctDNA-positive stage II colon cancer derived a marked benefit from ACT. Untreated patients negative for ctDNA had an extremely low risk of recurrence and could avoid ACT (14). Consistent with previous studies, the present study

demonstrated that the postoperative ctDNA status could guide the implementation of ACT.

In the present study, positive ctDNA in patients indicated the presence of MRD and identified patients at a higher risk of postoperative tumor recurrence, thereby supporting the use of ACT to consolidate surgical efficacy and reduce recurrence risk. By contrast, ctDNA-negative patients carried a relatively low residual tumour cell burden, and ACT did not further improve clinical outcomes in this subgroup. This may be due to the residual tumour cells in patients negative for ctDNA being eliminated or undetectable. Therefore, ACT failed to improve the clinical efficacy of this group of patients. Nevertheless,

other underlying mechanisms cannot be excluded. First, the DYNAMIC-III trial demonstrated that 13.5% of postoperative ctDNA-negative patients ultimately relapsed, with lung-only and peritoneal recurrences accounting for 39 and 34% of these ctDNA-negative relapses, respectively (53). Consistently, a large nationwide Danish cohort study showed that peritoneal and pulmonary recurrences yielded the lowest postoperative ctDNA detection rates (~20 and 19%, respectively) (54). These findings indicate that blood-based ctDNA testing fails to capture non-hematogenous metastatic pathways (such as peritoneal or isolated pulmonary dissemination), because ctDNA is released primarily into the peripheral blood, and anatomical compartmental barriers (such as the mesothelial plasma-peritoneal interface) limit DNA entry into the circulation (53,54). Second, although some patients negative for ctDNA might benefit from ACT to some extent, the improvement in efficacy was limited and did not reach statistical significance in the present study. Furthermore, given the limited sample size of the present cohort, these findings require validation through large-scale, long-term follow-up clinical trials. In short, for patients with resectable CRC after surgery, regardless of their pathological status, ACT is recommended for patients positive for ctDNA to achieve radical eradication of MRD and thus obtain substantial clinical benefits based on the present study. Conversely, for patients negative for ctDNA, close surveillance alone might be sufficient to achieve a favourable prognosis.

In the present study, moderate inter-study heterogeneity was observed for RFS in patients positive for ctDNA, which was mainly attributable to mixed disease stages, inconsistent ctDNA detection protocols and other relevant factors. Due to the limited number of included studies, stratified subgroup analyses could not be performed to identify differences in chemotherapy benefits across various patient subgroups. Nevertheless, sensitivity analyses confirmed the robustness of the core conclusions of the present meta-analysis despite the moderate heterogeneity.

The outcomes of one landmark clinical trial, DYNAMIC III, published in late 2025, appear to contradict the core findings of the present study at first glance (53). The clinical trial revealed that treatment de-escalation failed to meet the non-inferiority standard, while intensified adjuvant regimens brought no RFS benefits to patients positive for ctDNA. However, the seemingly conflicting results between the large DYNAMIC III RCT and the present pooled meta-analysis data could be reasonably explained by fundamental differences in trial design. First, there were different primary research questions: The present meta-analysis directly compared survival outcomes between patients who received ACT and those under surveillance alone, stratified by ctDNA status. By contrast, DYNAMIC III only tested high vs. low intensity chemotherapy against standard regimens and contained a no-treatment observation arm. Second, there were distinct intervention strategies: All participants positive for ctDNA in the DYNAMIC III trial received chemotherapy of varying intensities, with the study only comparing high intensity vs. standard chemotherapy regimens. The DYNAMIC III trial finding that intensified chemotherapy yielded no RFS benefits did not refute the core conclusion of the present study that patients positive for ctDNA achieved improved survival when receiving

chemotherapy rather than undergoing surveillance alone. Third, there are divergent implications for populations negative for ctDNA: Non-inferiority was not achieved with reduced oxaliplatin dosing among patients negative for ctDNA in DYNAMIC III, which did not conflict with the finding of the present study showing that complete omission of ACT yielded no survival disadvantage.

The ctDNA detection methods vary among different institutions, and currently, the mainstream detection technologies are mainly concentrated in categories such as PCR-related technologies and NGS. The ctDNA detection methods can be further divided into tumour-informed and tumour-agnostic detection strategies according to whether preoperative tumour tissue sequencing is required. Digital PCR (dPCR) is an emerging detection technology combined with PCR, which can be used for detecting low-level mutations in samples (55). A key limitation across all dPCR platforms is that each detection assay can only analyse a limited number of mutations. However, the dPCR readouts do not require any bioinformatics analyses, which markedly shortens the turnaround time (56). By contrast, NGS enables simultaneous sequencing of millions of DNA fragments, generating extensive datasets that provide a more in-depth and comprehensive genetic analysis approach to identifying genetic alterations (57). However, the concentration of ctDNA in peripheral blood is relatively low and conventional NGS platforms have a restricted lower limit of detection, making it hard to accurately identify mutations with low variant allele frequencies (58). This technical limitation carries clear clinical risks: A high limit of detection may fail to capture trace ctDNA, leading to false-negative classification for some patients positive for ctDNA. Such misclassification deprives these patients of intensified ACT and substantially impairs the accuracy of recurrence risk stratification. Single-molecule barcoding technology assigns unique molecular tags to individual DNA fragments, which theoretically notably improves the detection sensitivity of NGS for low-abundance ctDNA mutations and holds promising developmental prospects (23). Nevertheless, this technique is currently hampered by high testing costs and cumbersome experimental workflows, creating substantial barriers to large-scale clinical adoption. Tumour-informed assays rely on prior sequencing of resected tumour tissue to identify patient-specific somatic mutations for subsequent ctDNA tracking. This approach delivers high sensitivity for MRD detection yet entails a lengthy workflow that may delay the initiation of ACT. By contrast, tumour-agnostic assays adopt a fixed universal gene panel without tumour tissue matching and feature shorter turnaround time yet exhibit relatively lower MRD detection sensitivity (21).

At present, there is no consensus among various studies regarding the optimal method for detecting ctDNA. Among the 5 studies included in the present research, although the detection technologies all involved NGS, the specific protocols exhibited notable differences: Kataoka *et al* (31), Kawashima *et al* (29) and Folprecht *et al* (32) employed amplicon-based NGS, whereas Fan *et al* (28) and Xu *et al* (30) adopted hybrid capture-based NGS. Further analysis revealed that there were still differences in the technical details among the studies: Kataoka *et al* (31) adopted a detection

protocol based on personalized tumour-customized 16-plex PCR combined with NGS; Kawashima *et al* (29) employed a protocol of targeted amplicon NGS combined with digital droplet PCR; and Xu *et al* (30) used tumour-customized target sequence capture NGS technology, with the core being targeted capture sequencing based on the J25 customized gene panel. In summary, there is heterogeneity in the methods of ctDNA detection among different studies. At present, the best standards need to be further verified.

In addition to discrepancies in ctDNA detection technologies and analytical sensitivity, the timing of blood collection constitutes another critical confounding factor. To eliminate heterogeneity stemming from inconsistent sampling windows across studies, the present study specified that ctDNA samples had to be collected after curative resection and prior to ACT initiation for all included studies. Standardized pre-chemotherapy baseline sampling prevents false-negative ctDNA results caused by chemotherapy-induced clearance of ctDNA, accurately reflects the postsurgical residual tumour burden in patients and improves the comparability of ACT efficacy across different studies. The ALTAIR trial delivered early systemic therapy to patients with newly detectable ctDNA after chemotherapy and the trials subgroup analysis demonstrated marked survival improvements in participants with high tumour molecular load (elevated ctDNA levels) (59). However, this trial was designed as a post-chemotherapy salvage intervention study, which indirectly verifies that pre-chemotherapy baseline ctDNA testing is indispensable for identifying patients who would benefit from first-line postoperative ACT. In addition, the ALTAIR trial adopted centralized rapid testing to enable timely intervention, indicating that sample turnaround time directly restricts the clinical application of ctDNA-guided early chemotherapy.

The present meta-analysis primarily focused on risk stratification for postoperative ACT, with only a subset of enrolled patients receiving preoperative neoadjuvant therapy. A review by Martini *et al* (21) demonstrated that longitudinal dynamic monitoring of ctDNA enabled real-time assessment of tumour response during total neoadjuvant therapy for locally advanced rectal cancer (clinically staged as cT3-T4 and/or cN+). Data from the NOMINATE trial illustrated that all patients who achieved a clinical complete response attained ctDNA clearance and sustained ctDNA negativity throughout total neoadjuvant therapy, whereas the ctDNA clearance rate among patients who failed to reach a clinical complete response was 51% (60). Persistent ctDNA positivity at restaging predicted pathological residual disease and was associated with significantly shorter disease-free survival time (HR, 6.7; $P=0.005$), indicating that sustained ctDNA positivity is associated with poor neoadjuvant treatment response and elevated recurrence risk. However, the primary studies pooled in the present analysis lacked consistent collection of baseline blood samples prior to neoadjuvant therapy, so the pooled data could not be used to quantitatively analyse the predictive value of ctDNA for neoadjuvant therapeutic efficacy. Prospective trials with standardized blood collection protocols are warranted in the future to further clarify the guiding role of ctDNA in modifying neoadjuvant regimens.

To the best of our knowledge, the present study is the first meta-analysis to explore the efficacy of ACT guided by ctDNA

in patients with resectable CRC. Nevertheless, several potential limitations of the present meta-analysis should be acknowledged. First, the small number of included RCTs yielded a low certainty of evidence and the conclusions require further validation. Second, the limited number of studies and overall sample size precluded more detailed stratified subgroup analyses. Third, moderate between-study heterogeneity was observed. The pooled population was mixed, including patients with non-metastatic primary CRC and those with resectable CRLM. Tumour biological behaviour, recurrence risk and chemotherapy response varied substantially across disease stages and metastatic status. Furthermore, postoperative sampling windows were inconsistent and ctDNA assay platforms, lower limits of detection and positivity cut-offs lacked standardization. These confounding factors collectively introduced heterogeneity, indicating that the findings of the present study remain exploratory and require verification in additional high-quality trials. Fourth, multivariable regression analyses could not be performed. Although original studies adjusted for multiple covariates, relevant raw data were unavailable, prohibiting meta-analytic multivariable adjustment to account for potential confounders.

In conclusion, in the present study, among patients with resectable CRC, postoperative ctDNA positivity was associated with survival gains linked to ACT, whereas no relevant survival association with ACT was observed in individuals negative for ctDNA. These pooled associations suggest ctDNA testing may act as a potential biomarker for risk stratification and provide reference information for postoperative ACT decision-making. Given that most included evidence originates from observational cohort studies with residual confounding by indication, further large-scale multicentre randomized trials are required to validate these findings.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

JR, WS, WWS, XZ, YC, XF and JS contributed to the conception and development of the present study. JS designed the research process. JR and WWS searched the database for corresponding articles. YC and XF extracted useful information from the articles. XZ used statistical software for analysis. WS drafted the meta-analysis. JR and WS confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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