Type of Article: Meta-analysis

Title: Prognostic Significance of Postoperative and Post-Adjuvant Circulating Tumor DNA in Resected Colon Cancer: A Systematic Review and Meta-Analysis

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Clinical Question Box

Does postoperative or post-adjuvant chemotherapy ctDNA positivity predict recurrence and survival in patients with resected colon cancer?

Postoperative ctDNA positivity is associated with more than a fivefold increased risk of recurrence and an approximately fourfold increased risk of mortality. Persistent ctDNA detection after adjuvant chemotherapy identifies an even higher-risk subgroup, with roughly an elevenfold increased risk of relapse, suggesting ongoing minimal residual disease. These findings support ctDNA positivity as a robust biomarker for postoperative risk stratification and provide potential treatment guidance.

Abstract

Background: Circulating tumor DNA (ctDNA) is a promising biomarker for detecting minimal residual disease in colon cancer. We performed a systematic review and meta-analysis to assess the prognostic value of postoperative and post-adjuvant chemotherapy (ACT) ctDNA positivity in resected colon cancer.

Methods: We systematically searched PubMed, Embase, Web of Science, and the Cochrane Library for studies evaluating ctDNA after curative-intent surgery and/or ACT. Hazard ratios (HRs) for recurrence-free survival (RFS), disease-free survival (DFS), and overall survival (OS) were pooled using random-effects models.

Results: Twenty-three studies comprising 10,217 patients were included. Postoperative ctDNA positivity was significantly associated with worse RFS (HR: 5.46, 95% CI: 3.79–7.85, $p < 0.01$, $I^2 = 88\%$). In stage III disease, the pooled HR was 4.52 (95% CI: 2.90–7.05), while in stage II disease, the pooled HR was 6.67 (95% CI: 0.94–46.01). Post-ACT ctDNA positivity was associated with a marked increase in risk of recurrence (HR: 11.21, 95% CI: 6.92–18.15, $p < 0.01$, $I^2 = 58\%$). Among stage III patients, the pooled HR was 10.83 (95% CI: 5.38–21.82), with sensitivity analysis yielding a stable estimate (HR: 6.84, 95% CI: 4.58–10.21, $I^2 = 8\%$). Postoperative ctDNA positivity was also associated with inferior OS (HR: 3.99, 95% CI: 2.43–6.55, $p < 0.01$, $I^2 = 90\%$) and worse DFS (HR: 4.92, 95% CI: 2.60–9.32, $p < 0.01$, $I^2 = 83.5\%$).

Conclusions: Postoperative and post-ACT ctDNA positivity are strong predictors of recurrence and mortality in resected colon cancer. ctDNA represents a robust biomarker of minimal residual disease that can inform risk-adapted treatment strategies. Prospective randomized trials are needed to determine whether ctDNA-guided management improves survival outcomes.

Keywords: Colon cancer; circulating tumor DNA; minimal residual disease; recurrence-free survival; disease-free survival; overall survival

Introduction

Colon cancer remains one of the most common malignancies worldwide and a leading cause of cancer-related mortality.[1] In 2022, approximately 1.9–2.2 million new cases of colorectal cancer were reported globally, with nearly 900,000 deaths attributed to the disease, making it one of the most impactful cancers in terms of both incidence and mortality.[2] Surgical resection is the primary curative treatment for localized colon cancer. However, despite complete macroscopic tumor removal, approximately 20–40% of patients with stage II–III disease experience recurrence, largely due to undetected minimal residual disease (MRD) present at the time of surgery.[3] Current postoperative risk stratification relies largely on clinicopathologic features such as tumor stage, lymph node involvement, lymphovascular invasion, perineural invasion, and tumor differentiation.[4] Although these factors provide population-level prognostic information, they lack precision to accurately predict recurrence risk at the individual patient level, leading to overtreatment in some and undertreatment in others.

Circulating tumor DNA (ctDNA), a tumor-derived fraction of cell-free DNA released into the bloodstream through apoptosis, necrosis, and active secretion, has emerged as a promising biomarker for MRD detection.[5] Advances in highly sensitive molecular techniques, including digital PCR and next-generation sequencing–based assays, now allow for the detection of low-frequency tumor-specific mutations in plasma following curative-intent surgery.[6] In the postoperative setting, detectable ctDNA is hypothesized to reflect persistent microscopic disease, whereas undetectable ctDNA may indicate effective surgical clearance. Over the past decade, multiple prospective and retrospective studies in resected colon cancer have investigated the prognostic significance of postoperative ctDNA detection.[7,8] Accumulating evidence suggests that

patients with detectable ctDNA after surgery have a substantially higher risk of recurrence compared to ctDNA-negative patients, with hazard ratios often markedly elevated.

Despite these promising findings, considerable heterogeneity exists among studies with regard to assay platforms, timing of blood collection, thresholds for ctDNA positivity, adjuvant treatment strategies, and reported clinical endpoints—including recurrence-free survival (RFS), disease-free survival (DFS), and overall survival (OS)—across different stages of colon cancer. Moreover, ctDNA detection rates and their prognostic implications differ by stage, potentially influencing risk stratification and adjuvant treatment decision-making. Therefore, this meta-analysis systematically synthesized current evidence on the stage-specific prognostic and predictive value of postoperative ctDNA detection following curative-intent surgery in colon cancer patients.

Methods

Study Design

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[9] The study protocol was prospectively registered with the University Hospital Medical Information Network (UMIN; registration ID: UMIN000060788) to ensure methodological transparency and minimize reporting bias.[10] Because this study was based exclusively on published data, institutional review board approval was waived.

Literature Search Strategy

A systematic literature search was conducted in PubMed, Embase, Web of Science, and the Cochrane Library, covering records from database inception through January 31, 2026. The search strategy incorporated both controlled vocabulary and free-text keywords, including "colon cancer," "circulating tumor DNA," "ctDNA," "minimal residual disease," "surgery," and "recurrence." The detailed search strategies for each database, along with the corresponding numbers of retrieved records, are presented in Figure S1. In addition, the reference lists of relevant reviews and eligible articles were manually screened to identify any further potentially eligible studies.

Eligibility Criteria

Eligibility criteria were as follows: Studies were included if they met all of the following conditions: (1) patients with colon cancer who underwent curative-intent surgery; (2) postoperative ctDNA tested using plasma samples only; and (3) observational (prospective or retrospective cohort) or randomized controlled trial design. Studies were excluded if: (1) they reported updated results of published cohorts without providing independent data; (2) outcome data were not

extractable or were insufficient to estimate effect sizes; or (3) the primary focus was cost-effectiveness rather than prognostic outcomes.

Data Extraction and Outcome Measures

Data extraction was independently performed by two investigators (E.W. and M.S.)using a standardized data collection form. The extracted information included study characteristics (first author, publication year, country, and study design), sample size, patient demographics, tumor stage, timing and methodology of ctDNA assessment, proportion of ctDNA-positive patients, details of adjuvant treatment, follow-up duration, and reported survival outcomes. Discrepancies between reviewers were resolved through discussion; if consensus could not be reached, a third reviewer (D.N.)was consulted for adjudication. Hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) for RFS, DFS, and OS were preferentially extracted from multivariable analyses when available. When HRs were not directly reported, they were estimated from the available survival data whenever feasible. Additionally, postoperative ctDNA status following adjuvant chemotherapy (ACT) was assessed when such data were available.

Quality Assessment

The methodological quality of included observational studies was assessed using the Newcastle–Ottawa Scale (NOS), which evaluates the domains of selection, comparability, and outcome.[11] Studies with higher NOS scores were considered to have a lower risk of bias. For randomized controlled trials, risk of bias was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool.[12]

Statistical Analysis

All statistical analyses were conducted using Review Manager (RevMan 5.4). Pooled HRs with corresponding 95% CIs were calculated to evaluate the association between postoperative ctDNA positivity and survival outcomes. Statistical heterogeneity was assessed using the Chi-square (Q) test and quantified with the I^2 statistic. A random-effects model was applied when significant heterogeneity was detected ($I^2 > 50\%$); otherwise, a fixed-effects model was used. Sensitivity analyses were performed to evaluate the stability of pooled estimates. A two-sided *p*-value < 0.05 was considered statistically significant.

Results

Study Selection and Characteristics

The study selection process is summarized in Figure S1. A total of 901 records were identified through database searches. After the removal of 79 duplicates, 822 records remained for title and abstract screening, of which 752 were excluded. The full texts of the remaining articles were then assessed for eligibility, and 47 studies were further excluded. Ultimately, 23 studies met the inclusion criteria and were included in the final analysis. The baseline characteristics of the included studies are summarized in Table 1.[13-35] A total of 10,217 patients with resected colon cancer were included, from studies published between 2016 and 2026. Of these, twelve studies focused on stage III disease, while two studies each specifically investigated stage II and stage IV disease, respectively. The remaining studies included mixed populations comprising stage I–III or stage II–III patients. Sample sizes ranged from 24 to 2,260 patients. The median or mean age ranged from 56 to 72 years, and the proportion of male patients varied between 49% and 69%. The proportion of postoperative ctDNA-positive patients showed substantial variability, ranging from 6.1% to 64.2%, reflecting differences in stage distribution, timing of sampling, and assay platforms. The timing of postoperative ctDNA assessment varied widely, ranging from 3–7 days to 3–12 weeks after surgery, with some studies not reporting the exact timing. Follow-up duration ranged from 16.7 months to over 6 years. Most studies used next-generation sequencing–based (NGS-based) platforms, including Safe-SeqS, Signatera, and mPCR-NGS.

Post-Surgical ctDNA Status and RFS

Seventeen studies assessed the association between postoperative ctDNA status and RFS (Figure 1). The pooled analysis showed a significantly increased risk of recurrence in ctDNA-positive

patients (HR: 5.46, 95% CI: 3.79–7.85, $p < 0.01$), with substantial heterogeneity ($I^2 = 88\%$). Two studies focusing on stage II disease reported a pooled HR of 6.67 (95% CI: 0.94–46.01, $p = 0.05$, $I^2 = 92\%$). Ten studies focusing on stage III reported a pooled HR of 4.52 (95% CI: 2.90–7.05, $p < 0.01$, $I^2 = 90\%$). Four studies including stage I–III patients demonstrated a pooled HR of 7.48 (95% CI: 3.55–15.76, $p < 0.001$, $I^2 = 83\%$). Due to substantial heterogeneity, a sensitivity analysis was conducted in stage III patients. The pooled result remained statistically significant, with an HR of 4.86 (95% CI: 4.26–5.54, $p < 0.001$), and heterogeneity was eliminated ($I^2 = 0\%$) (Figure S2).

Post-ACT ctDNA Status and RFS

Nine studies evaluated post-ACT ctDNA status and its association with RFS in stage I–III patients (Figure 2). The pooled analysis demonstrated a significantly increased risk of recurrence in ctDNA-positive patients (HR: 11.21, 95% CI: 6.92–18.15, $p < 0.01$, $I^2 = 58\%$). The pooled HR of six studies focusing on stage III disease was 10.83 (95% CI: 5.38–21.82, $p < 0.01$, $I^2 = 72\%$). Three studies conducted on stage I–III patients reported a pooled HR of 13.04 (95% CI: 7.36–23.10, $p < 0.001$, $I^2 = 0\%$). Due to substantial heterogeneity in the stage III subgroup, a sensitivity analysis was performed. The association remained statistically significant (HR: 6.84, 95% CI: 4.58–10.21, $p < 0.001$), and heterogeneity was markedly reduced ($I^2 = 8\%$) (Figure S3).

Post-Surgical ctDNA Status and OS

Nine studies evaluated post-ACT ctDNA status and its association with OS in stage II–IV patients (Figure 3). The pooled analysis demonstrated a significantly increased risk of mortality in ctDNA-positive patients (HR: 3.99, 95% CI: 2.43–6.55, $p < 0.01$), with substantial heterogeneity ($I^2 = 90\%$). Two studies focusing on stage IV disease yielded a pooled HR of 15.19 (95% CI: 3.41–

67.72, $p < 0.01$, $I^2 = 0\%$), while two studies conducted on stage II–III patients reported a pooled HR of 6.33 (95% CI: 2.26–17.75, $p < 0.001$, $I^2 = 71\%$). Due to substantial heterogeneity in the stage III subgroup, a sensitivity analysis was performed. The association remained statistically significant (HR: 5.91, 95% CI: 4.72–7.40, $p < 0.001$), and heterogeneity was eliminated ($I^2 = 0\%$) (Figure S4).

Post-Surgical ctDNA Status and DFS

Seven studies evaluated post-ACT ctDNA status and its association with DFS in stage I–IV patients (Figure 4). The pooled analysis demonstrated a significantly increased risk of recurrence in ctDNA-positive patients (HR: 4.92, 95% CI: 2.60–9.32, $p < 0.01$, $I^2 = 83.5\%$). Due to substantial heterogeneity, a sensitivity analysis was performed. The association remained statistically significant (HR: 6.12, 95% CI: 4.78–7.82, $p < 0.001$), and heterogeneity was markedly reduced ($I^2 = 8\%$) (Figure S5).

Subgroup analysis

Subgroup analysis

Subgroup analysis was performed to explore potential sources of heterogeneity according to assay methodology among studies including only stage III disease (Figure S6). For RFS, two PCR-based studies were included, showing a pooled HR of 5.97 (95% CI: 4.50–7.92; $I^2 = 0\%$). In comparison, NGS-based studies showed a pooled HR of 4.59 (95% CI: 3.97–5.32; $I^2 = 0\%$). The overall pooled HR was 4.86 (95% CI: 4.25–5.54; $I^2 = 0\%$). Although heterogeneity within each subgroup was absent, moderate heterogeneity was observed between subgroups ($I^2 = 61.6\%$). Subgroup analysis according to postoperative ctDNA testing timing was not feasible because six studies did not report sampling time, while the remaining seven studies used different postoperative time points.

Risk of Bias Assessment

Funnel plots for RFS, DFS, and OS showed no significant asymmetry, suggesting a low likelihood of substantial publication bias (Figure S7 to S10). Methodological quality was assessed using the Newcastle–Ottawa Scale (Table S2). Overall, the included observational studies were of high quality, with adequate cohort selection, comparability, and outcome assessment. The overall risk of bias was considered acceptable for inclusion in the quantitative synthesis.

Discussion

In this comprehensive meta-analysis of patients with resected colon cancer, ctDNA positivity after curative-intent surgery and ACT was strongly and consistently associated with inferior survival outcomes. Postoperative ctDNA positivity was significantly associated with worse RFS, DFS, and OS. Similarly, persistent ctDNA positivity following ACT was associated with a marked increase in risk of recurrence. Collectively, these findings support ctDNA as a robust biomarker of MRD in colon cancer.[36,37] Despite complete macroscopic tumor resection, a substantial proportion of patients with stage II–III colon cancer experience recurrence, likely reflecting undetected residual disease. Traditional clinicopathologic risk factors offer limited individualized risk discrimination, leading to potential overtreatment of low-risk patients and undertreatment of high-risk individuals.[38] Our pooled analysis demonstrated more than a fivefold increased risk of recurrence among postoperative ctDNA-positive patients, underscoring the strong prognostic stratification afforded by molecular detection of residual disease.

ctDNA is released into the circulation through apoptosis, necrosis, and active tumor cell shedding. Following complete tumor resection, circulating ctDNA levels are expected to decline rapidly; therefore, persistent detection likely reflects residual viable tumor clones with proliferative potential and risk of clinical relapse.[39] In this setting, ctDNA provides a real-time molecular measure of tumor presence that complements pathologic staging and radiologic surveillance.[40] Although ctDNA detection rates varied across studies, likely due to differences in stage distribution, sampling timing, and assay platforms, most employed NGS-based methods, including tumor-informed approaches such as Signatera and Safe-SeqS, as well as multiplex PCR–NGS assays. Despite methodological heterogeneity, the prognostic effect was consistent, supporting ctDNA as a biologically valid marker of MRD rather than a platform-specific artifact.

Postoperative ctDNA positivity was consistently associated with adverse outcomes across disease stages. The association with recurrence remained significant in both stage II and stage III disease. In stage III, the pooled HR remained robust after sensitivity analyses addressing heterogeneity, supporting the stability of the finding. Although the stage II subgroup included fewer studies and showed wider CIs, the magnitude of the effect was similarly elevated, indicating that ctDNA positivity is a biologically significant marker of persistent tumor presence irrespective of stage. Moreover, postoperative ctDNA positivity was associated with a nearly fourfold increased risk of mortality, with particularly pronounced effects observed in stage IV disease.

In contrast, the prognostic impact of post-ACT ctDNA positivity was even more marked. Across nine studies, detectable ctDNA after chemotherapy was associated with an approximately elevenfold increased risk of recurrence. This strong association indicates that post-ACT ctDNA positivity is a robust marker of poor prognosis.[41] However, interpretation of this finding requires caution because adjuvant chemotherapy duration and intensity varied across studies, and reporting of treatment completion, adherence, and the timing of ctDNA assessment was inconsistent. These limitations precluded stratified analyses and limited our ability to distinguish prognostic significance from potential predictive implications.

Several limitations merit consideration. Substantial heterogeneity was observed in multiple pooled analyses, likely due to differences in stage distribution, assay methodologies, and timing of blood collection. Although sensitivity analyses reduced heterogeneity in key subgroups, residual variability remained. Moreover, most included studies were observational, introducing potential confounding despite multivariable adjustment. Variation in postoperative sampling time points may also have influenced detection rates due to perioperative ctDNA release. Additionally, the absence of individual patient-level data precluded more granular analyses based on molecular

subtypes (e.g., RAS or BRAF status) or specific treatment regimens. Finally, although ctDNA positivity demonstrates strong prognostic value, optimal management of ctDNA-positive patients remains undefined. Ongoing prospective randomized trials are needed to determine if early therapeutic intervention based solely on molecular detection can improve long-term survival.

Conclusion

This meta-analysis demonstrates that ctDNA positivity following curative-intent surgery and adjuvant chemotherapy is strongly associated with inferior survival outcomes in resected colon cancer. Postoperative and post-ACT ctDNA detection identifies patients at markedly increased risk of recurrence and mortality, supporting ctDNA as a robust biomarker of minimal residual disease. Despite methodological heterogeneity, the consistency of findings highlights the clinical relevance of ctDNA-guided risk stratification. Prospective randomized trials are needed to determine whether ctDNA-directed treatment strategies can improve survival while avoiding overtreatment.

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Table 1: Characteristics of enrolled studies

Author	Stage	Cases	Age	Male	ct-DNA Positive	Follow-up	ct-DNA Timing	ct-DNA Method	Outcomes
Tie et al. 2016	II	230	66 (10.4)	131 (57%)	14 (6.1%)	27 m	4–10 weeks	NGS (Safe-SeqS)	RFS
Tie et al. 2025(1)	II	291	65 (12.5)	154 (52%)	45 (15.5%)	59.7 m	4–7 weeks	NGS (Safe-SeqS)	RFS, OS
Chen et al. 2021	II–III	240	58 (11.2)	136 (57%)	154 (64.2%)	27.4 m	3–7 days	NGS	RFS
Nakamura et al. 2024	II–III	2240	65 (10)	1091 (49%)	336 (15.0%)	16.7 m	N.S.	NGS (Signatera)	DFS, OS
Wullaert et al. 2025	II–III	188	67 (9.6)	130 (69%)	117 (62.2%)	37 m	3 weeks	NGS	RFS
Diergaarde et al. 2025	III	124	65 (10.8)	66 (53%)	46 (41.1%)	4.8 y	3–12 weeks	NGS	RFS, OS
Franken et al. 2026	III	206	63 (9.3)	110 (53%)	26 (12.6%)	43.9 m	N.S.	NGS	RFS
Frydendahl et al. 2024	III	144	66 (9.6)	87 (60%)	19 (17.0%)	36 m	N.S.	NGS	RFS
Henriksen et al. 2022	III	160	N.S.	95 (59%)	20 (14.3%)	35 m	2–4 weeks	mPCR NGS	RFS
Li et al. 2022	III	151	59 (11.1)	90 (60%)	24 (15.9%)	33.5 m	N.S.	NGS	RFS
Rubio-Alarcón et al. 2025	III	209	60 (11.7)	112 (54%)	28 (13.4%)	40 m	1–3 weeks	NGS	RFS
Sinicrope et al. 2026	III	2260	58 (11.1)	1202 (53%)	461 (20.4%)	6.1 y	N.S.	NGS	RFS, DFS, OS
Taieb et al. 2021	III	1017	63 (9.6)	576 (57%)	140 (13.8%)	6.6 y	42 days	ddPCR (Methylation)	DFS, OS
Taieb et al. 2025	III	554	64 (9.4)	322 (58%)	109 (19.7%)	6.7 y	39 days	NGS	RFS, OS
Tie et al. 2019	III	96	59 (13.5)	49 (51%)	20 (20.8%)	28.9 m	4–10 weeks	NGS (Safe-SeqS)	RFS
Tie et al. 2025 (2)	III	482	60 (14.8)	262 (54%)	129 (26.8%)	47 m	N.S.	NGS (Safe-SeqS)	RFS
Zhang et al. 2025	III	940	61 (10.8)	515 (55%)	173 (18.4%)	6 y	N.S.	NGS (Signatera)	DFS, OS
Gao et al. 2023	I–III	108	57 (9.1)	81 (65%)	17 (15.7%)	36.7 m	7–10 days	NGS	RFS
Mo et al. 2023	I–III	296	60 (10.3)	186 (62%)	59 (23.1%)	21 m	2–12 weeks	ddPCR (Methylation)	RFS
Slater et al. 2024	I–III	214	62 (13)	128 (60%)	21 (9.8%)	30.3 m	N.S.	NGS	RFS
Tarazona et al. 2019	I–III	94	71 (9.1)	61 (65%)	60 (63.8%)	24.7 m	N.S.	ddPCR (Tumor-informed)	DFS
Lonardi et al. 2022	IV	69	56 (14)	46 (67%)	31 (44.9%)	N.S.	N.S.	NGS	DFS, OS
Sanz-Garcia et al. 2025	IV	24	66 (11.1)	16 (67%)	7 (29.2%)	20.7 m	4 weeks	NGS	DFS, OS

ct-DNA, circulating tumor DNA; NGS, next-generation sequencing; Safe-SeqS, Safe-Sequencing System; mPCR, multiplex polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction; RFS, recurrence-free survival; DFS, disease-free survival; OS, overall survival; N.S., not specified; m, months; y, years.

Figure 1. Meta-analysis of hazard ratios for post-surgical ctDNA status and RFS

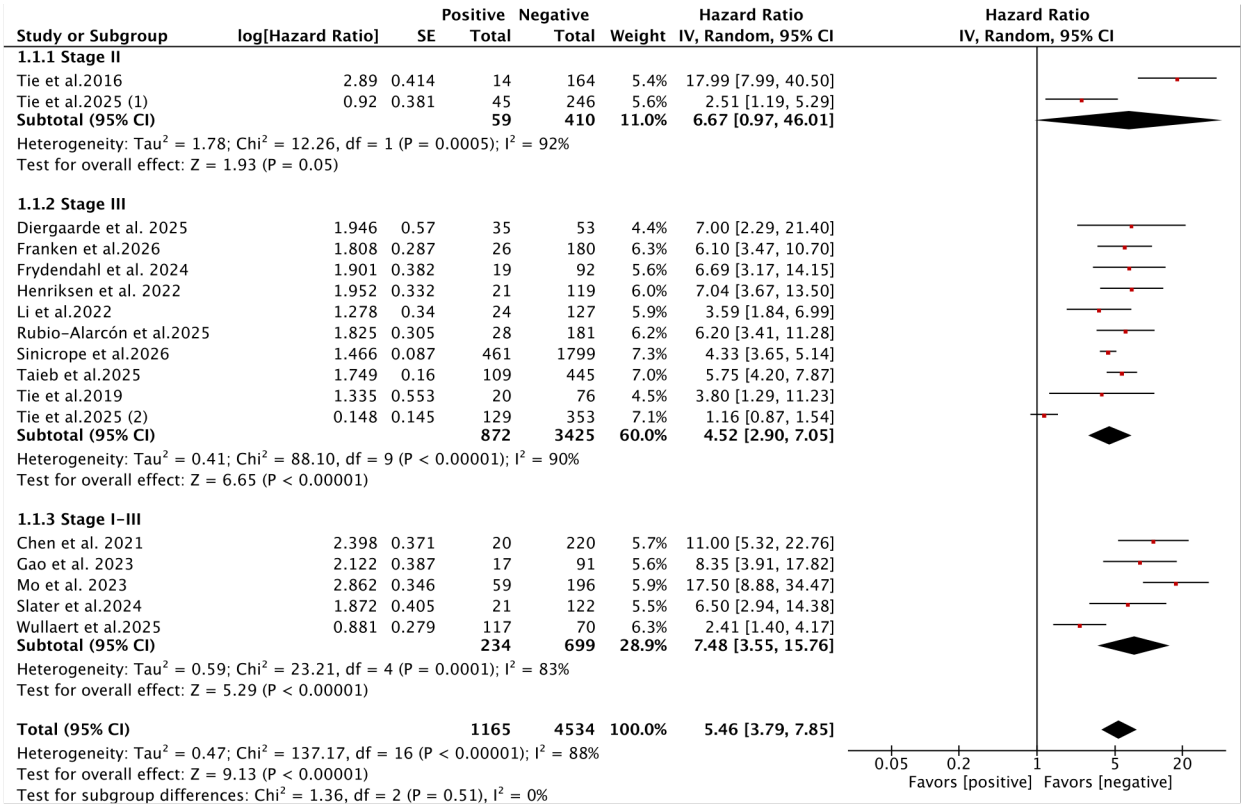

Figure 2. Meta-analysis of hazard ratios for post-chemotherapy ctDNA status and RFS

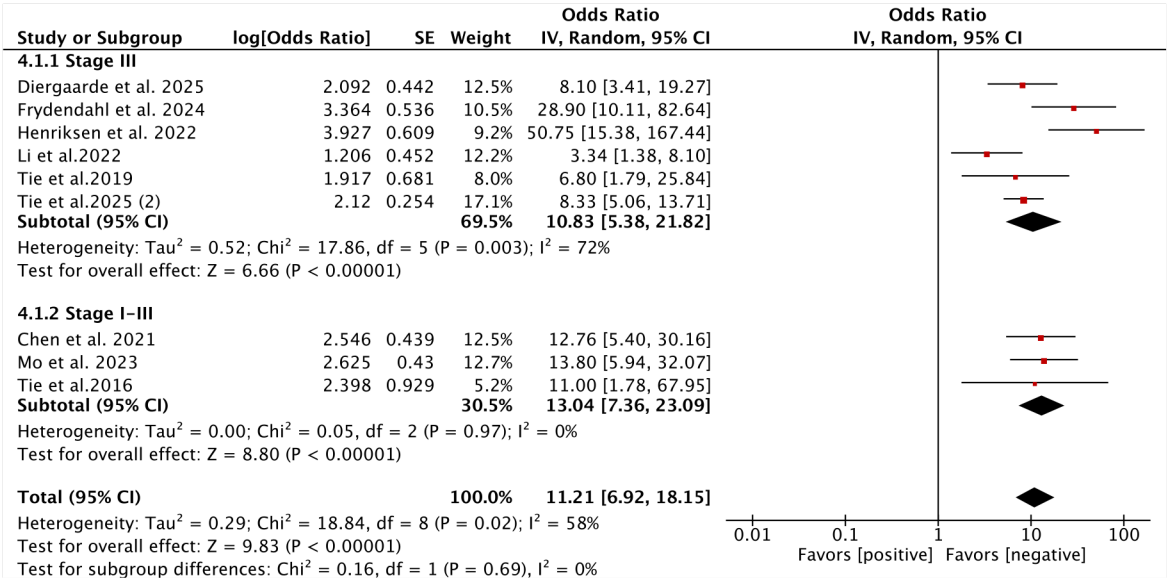

Figure 3. Meta-analysis of hazard ratios for post-surgical ctDNA status and OS

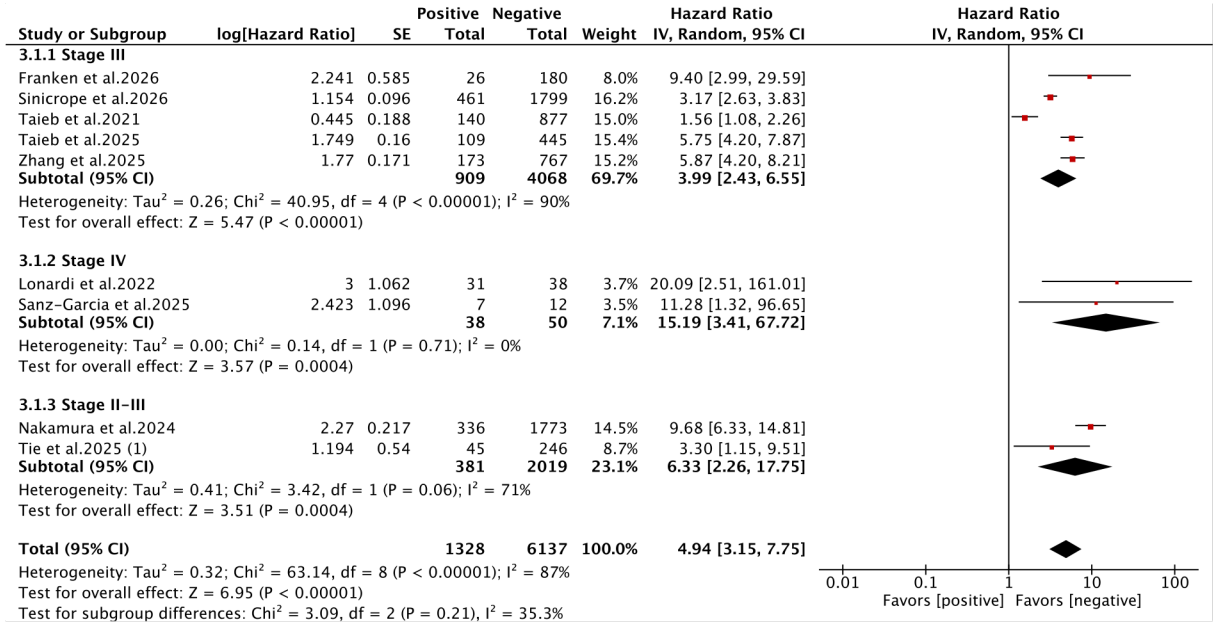

Figure 4. Meta-analysis of hazard ratios for post-surgical ctDNA status and DFS