Circulating tumour DNA-Based molecular residual disease detection in resectable cancers: a systematic review and meta-analysis

Jiachun Zheng,[a] Chuling Qin,[b] Qianxi Wang,[b] Dongbo Tian,[a,] and Zisheng Chen[a,**]*

[a]Department of Respiratory and Critical Care Medicine, Affiliated Qingyuan Hospital of Guangzhou Medical University, Qingyuan People's Hospital, Qingyuan, 511518, China
[b]Guangzhou Medical University, Guangzhou, 511436, China

Summary

Background Circulating tumour DNA (ctDNA)-based molecular residual disease (MRD) detection technology has been widely used for recurrence evaluation, but there is no agreement on the efficacy of assessing recurrence and overall survival (OS) prognosis, as well as the sensitivity and specificity of landmark detection and longitudinal detection.

Methods We systematically searched Pubmed, Embase, Cochrane, and Scopus for prospective studies or randomized controlled trials that collected blood samples prospectively. The search period was from Jan 1, 2013, to Sept 10, 2023. We excluded retrospective studies. The primary endpoint was to assess the hazard ratio (HR) between circulating tumour DNA positive (ctDNA+) and negative (ctDNA-) for recurrence-free survival incidence (RFS), disease-free survival (DFS), progression-free survival (PFS), event-free survival (EFS), time to recurrence (TTR), distant metastasis-free survival (DMFS) or OS in patients with resectable cancers. We calculated the pooled HR of recurrence and OS and 95% confidence interval (CI) in patients with resected cancers using a random-effects model. Pooled sensitivity and specificity were estimated using the bivariate random effects model.

Findings This systematic review and meta-analysis returned 7578 records, yielding 80 included studies after exclusion. We found that the HR of recurrence across all included cancers between patients with ctDNA+ and ctDNA- was 7.48 (95% CI 6.39–8.77), and the OS was 5.58 (95% CI 4.17–7.48). We also found that the sensitivity, area under the summary receiver operating characteristic curve (AUSROC) and diagnostic odds ratio (DOR) of longitudinal tests were higher than that of landmark tests between patients with ctDNA+ and ctDNA- (0.74, 95% CI 0.68–0.80 vs. 0.50, 95% CI 0.46–0.55; 0.88 vs. 0.80; 25.70, 95% CI 13.20–45.40 vs. 9.90, 95% CI 7.77–12.40).

Interpretation Postoperative ctDNA testing was a significant prognosis factor for recurrence and OS in patients with resectable cancers. However, the overall sensitivity of ctDNA-MRD detection could be better. Longitudinal monitoring can improve the sensitivity, AUSROC, and DOR.

Funding Special fund project for clinical research of Qingyuan People's Hospital (QYRYCRC2023006), plan on enhancing scientific research in GMU (GZMU-SH-301).

Keywords: Molecular residual disease (MRD); Circulating tumour DNA; Surgery; Adjuvant therapy; Meta-analysis

Introduction

Cancer remains a major public health problem worldwide. According to Cancer Statistics, 2023,[1] until 2020, in America, colorectal cancer (CRC), lung cancer, pancreatic adenocarcinoma (PAAD), bladder cancer (BLCA), melanoma, breast cancer (BC), gastric cancer (GC), hepatocellular carcinoma (HCC), ovarian cancer (OV) and esophageal carcinoma (ESCA) predicted for 7.81%, 12.17%, 3.27%, 4.20%, 4.98%, 15.35%, 1.35%, 2.10%, 1.01%, 1.10% of new cases in 2023, and the mortality rates were 8.62%, 20.84%, 8.29%, 2.74%, 1.31%, 7.17%, 1.83%, 4.82%, 2.18%, 2.64%,

Research in context

Evidence before this study

More and more studies have demonstrated that circulating tumour DNA (ctDNA)-MRD (minimal residual disease) analysis can detect recurrence earlier than imaging, and we hypothesized that patients with ctDNA+ are at greater risk of recurrence than patients with ctDNA-. We systematically searched Pubmed, Embase, Cochrane and Scopus, supplemented by ASCO, ESMO, and Google, for prospective studies or randomized controlled trials that collected blood samples prospectively. The search period was from Jan 1, 2013, to Sept 10, 2023. Keywords used include "Molecular Residual Disease (MRD)," "circulating tumour DNA," "surgery," "Drug Therapy, Adjuvant," "Chemotherapy," "Adjuvants, Immunologic," "Radiotherapy, Adjuvant," and "Targeted therapy" and their MeSH terms. We excluded retrospective studies and considered only papers published in English.

Added value of this study

This meta-analysis provided more comprehensive and standardized evidence of the hazard ratio (HR) of recurrence and OS, and summarized the primary data of patients with different cancers. It indicated that ctDNA was a risk factor for recurrence in resectable cancers, and the sensitivity of longitudinal detection was higher than landmark detection. In

addition, the subgroup analysis of detection time and technology determined which time point and which detection technology was the most appropriate for different cancers. At the same time, the benefit of postoperative adjuvant therapy for patients with ctDNA+ in CRC may be a hint that ctDNA detection can be used as clinical evidence of ctDNA-based adjuvant therapy.

Implications of all the available evidence

Our findings had several potential implications for clinical practice and future research. First of all, our result showed that ctDNA was a prognostic factor in resectable cancers, and it can be considered an indicator of postoperative recurrence detection, but the detection time and detection technology should be considered simultaneously. Secondly, patients with ctDNA+ in post-adjuvant therapy also had a recurrence risk, and this indicates that further study of the value of ctDNA+ should focus on analyzing the difference and relationship between the ctDNA-based adjuvant therapy group and the conventional management therapy group. What's more, longitudinal detection showed a high sensitivity for detecting recurrence, which made it interesting to think about when to test.

respectively. In China, the morbidity rates in the above cancers at last count were 10.04%, 20.38%, 2.47%, 2.03%, 0.17%, 7.53%, 9.76%, 9.57%, 1.41%, 6.21%, and the mortality rates were 8.10%, 27.22%, 3.64%, 1.40%, 0.16%, 2.97%, 11.95%, 13.94%, 1.13%, 8.03%.[2]

With the large-scale development of low-dose chest computed tomography (LDCT), gastrointestinal endoscope, mammography screening[3] and other measures, early cancers were more likely to be detected. An increase in treatment methods, such as neoadjuvant single/double immunotherapy, targeting, chemoradiotherapy plus immunotherapy, and combined surgical resection, has potentially cured more people. However, there is still a proportion of patients with relapse after surgery, and traditional methods of monitoring for recurrence post-operation, including imaging, may have difficulty detecting microscopic recurrence.

Circulating tumour DNA (ctDNA) is cancer-related free DNA fragments released into the blood by apoptotic or necrotic tumour cells, which do not exist in normal people. With the development of cancer monitoring technology, ctDNA-MRD was superior to imaging and could detect recurrence 5.01 months before imaging,[4] indicating the existence of micro-residual and assessing the overall survival (OS). Furthermore, adjuvant therapy based on ctDNA positive (ctDNA+) seems to be beneficial, but ctDNA negative (ctDNA-) adjuvant

therapy has not shown benefits.[5] Based on this, the current National Comprehensive Cancer Network (NCCN) treatment guidelines[6] and Chinese expert consensus[7] recommend ctDNA-MRD for CRC and NSCLC detection. However, methods and strategies for detecting ctDNA-MRD in patients vary from study to study and from cancer to cancer, which presents a challenge for clinicians to interpret MRD results. For example, the current detection methods, including Droplet Digital PCR (ddPCR),[8] multiplex PCR next generation sequencing (mPCR-NGS),[9] whole genome sequencing (WGS),[10] Fast Aneuploidy Screening Test-sequencing System (FAST-SeqS),[11] hybridization capture-based NGS,[12] circulating single-molecule amplification and resequencing technology (cSMART),[13] Guardant Reveal,[14] ctDNA methylation assays,[15] single-cell universal poly (A)-independent RNA sequencing (SUPeR-seq),[16] which makes result diversity.

To investigate the role of ctDNA-MRD in survival benefits and landmark/longitudinal detection for resectable cancers, we conducted a systematic meta-analysis to analyze survival benefits between patients with ctDNA+ and ctDNA- and determine the overall sensitivity and specificity of ctDNA-MRD as a prognostic biomarker. Subgroup analyses were conducted based on ctDNA testing time and technique and whether adjuvant therapy was performed.

Methods

Search strategy and selection criteria

We systematically searched Pubmed, Embase, Cochrane, and Scopus, supplemented by ASCO, ESMO, and Google, for prospective studies or randomized controlled trials that collected blood samples prospectively. The search period was from Jan 1, 2013, to Sept 10, 2023. Keywords used include "Molecular Residual Disease (MRD)," "circulating tumour DNA," "surgery," "Drug Therapy, Adjuvant," "Chemotherapy," "Adjuvants, Immunologic," "Radiotherapy, Adjuvant," and "Targeted therapy" and their MeSH terms.

The inclusion criteria: (1) prospective studies or randomized controlled trials which collected blood samples prospectively; (2) patients with confirmed resectable cancers (I-IV stage); (3) The analysis group was classified as patients with ctDNA+ and ctDNA-; (4) data available on outcome indicators: disease-free survival (DFS), progression-free survival (PFS), relapse-free survival (RFS), event-free survival (EFS), time to recurrence (TTR), distant metastasis-free survival (DMFS) or OS was reported as HR; (5) ctDNA testing in postoperative or post-adjuvant therapy after surgery; (6) ctDNA-MRD landmark detection or longitudinal detection in postoperative; (7) only papers published in English. The exclusion criteria: (1) unresectable cancer; (2) Individual cases, non-availability of data (HR of DFS, PFS, RFS, EFS, TTR, DMFS and OS or the number of patients with ctDNA+ and ctDNA-); (3) No pre-designed blood samples were collected or it is not clear if there was a pre-design blood collection; (4) ctDNA present in urine or other body fluids; (5) The time of ctDNA blood collection and analysis was non-postoperation; (6) articles with inconsistent titles and abstracts, reviews, animal tests, and systematic reviews. Three researchers (J.Z., C.Q., Q.W.) screened each record, and each report was retrieved independently, and a consensus was reached between 3 reviewers in disputes between them.

Study quality

Three researchers (J.Z., C.Q., Q.W.) evaluate the quality of the article, and if there is a dispute, all researchers discuss and reach a consensus decision. All studies were analyzed using the Newcastle-Ottawa Scale (NOS)[17] as a measure of bias. All studies with a performance score of at least 7 were considered high-quality studies.

Data collection

Data was extracted independently by 3 investigators (J.Z., C.Q., Q.W.), and a consensus was reached between 3 reviewers in disputes between them. The senior investigators reviewed the results (D.T., Z.C.). Information regarding target outcomes was obtained and contained in Microsoft Office software when available. General data was recorded from each study: the first author, year of publication, journal, the age of the subjects, sex, intervention, the population's country, sample extraction time, the number of patients with ctDNA+ and ctDNA-, the number of patients with recurrence and non-recurrence, tumour type and stage, adjuvant treatment, ctDNA detection method, follow-up time and measurement indicators of the study subjects, etc. The HR of RFS, DFS, PFS, EFS, TTR and DMFS for detecting recurrence and OS was obtained to compare patients with ctDNA+ and ctDNA- groups.

Furthermore, we compared the sensitivity and specificity of each cancer landmark and longitudinal detection analysis. Landmark detection is defined as a single test at a pre-specified time point after surgery, and patients with positive detection are classified as ctDNA+. Longitudinal detection is defined as multiple detections within the follow-up time after surgery, and patients with positive detection are also classified as ctDNA+. At the same time, we also included the sensitivity and specificity of ctDNA detection technology.

Data analysis

Based on each study, we estimated or calculated the HR for each risk factor and outcomes of interest with 95% CI. RFS, DFS, PFS, EFS, TTR and DMFS were measured from the date of surgery to the verified first radiologic recurrence (local or distant) or death as a result of cancer/any cause while OS was detecting death.[13,18–20] Pooled HR of RFS, DFS, PFS, EFS, TTR and DMFS for detecting recurrence and OS and their 95% CI were generated using a random-effects model due to the prevalence of heterogeneity. Pooled sensitivity and specificity and diagnostic odds ratio (DOR) were estimated using the bivariate random effects model.[21] The diagnostic performance of each subgroup was compared by drawing sROC curves using a bivariate random effects model.[21] The AUSROC was estimated using a univariate model[22] and the bivariate random effects model.[21] To test for heterogeneity between studies, we use Q-test,[23] Higgins,[24] Chi-square,[25] and Zhou and Dendukuri's approach.[26] The heterogeneity was defined as $I^2 > 50\%$,[27] and the P-value was testing performance. Furthermore, subgroup analyses were conducted based on ctDNA testing time, technique and whether adjuvant therapy was performed. The reporting bias was assessed using a funnel plot and Egger's and Begg's tests. We used the Trim-and-fill method to estimate and adjust the number and outcomes of missing studies when the reporting bias existed. We performed a regression analysis to monitor time and technique, the quality of included studies, and the proportion of patients with ctDNA- and ctDNA+. We performed a sensitivity analysis by removing each study in turn. The meta (version 6.5-0)[28] and mada (version 0.5.11)[25] packages in R (version 4.2.3) were used to conduct all statistical analyses. The forestploter (version 3.1.3)[29] package in R was used to conduct to form forest map of the data generated by meta package. The adjuvant treatment group was defined as patients all receiving adjuvant treatment,

and the non-adjuvant treatment group was defined as no patient in the group receiving adjuvant treatment. Pan cancer was defined as those cancers we included.

In all cases, P-values were 2-tailed; statistical significance was defined as P-value <0.05. It was registered on Prospero before starting the searches (registration number CRD42023438133); an updated search was done on Sept 10, 2023. And until Oct 10, we systematically searched for updates that were relevant to the included abstracts. All information sources were taken from the included studies and their Supplementary Materials.

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Ethics statement
No ethical approvals were required for this study.

Results
Search results
A total of 7578 articles were retrieved, including 1363 from Pubmed, 3416 from Embase, 454 from Cochrane, 826 from Scopus and 1519 from other databases. One thousand eight hundred forty-one duplicated articles were screened, and 5329 articles with inconsistent titles and abstracts, reviews, animal tests, and systematic reviews were excluded. Three hundred and twenty-eight articles did not meet the inclusion criteria, and as a result, 80 articles were included. Until Oct 10, we have updated one study relevant to the included abstract. In the end, we included 18 abstracts[30–47] and 62 studies,[4,13,15,20,48–104] 73 of which were prospective studies, and 7 were randomized controlled trials (Table 1). The flow chart is as follows: Fig. 1.

Twenty studies were for CRC, 14 for non-small lung cancer (NSCLC), 10 for colorectal liver metastases (CRLM), 7 for PAAD, 6 for ESCA, 6 for BC, 5 for GC, 5 for BLCA, 3 for OV, 3 for melanoma and 1 for HCC (Table 1).

The quality evaluation table of all studies was in Table S1.

Description of eligible studies
This study included 54 studies with only recurrence, 24 with recurrence and OS, and 2 with only OS. From the perspective of various national studies, China had the most studies, which may be related to the incidence of NSCLC (Table S2). The data and features of the included articles are shown in Table 1 and Tables S2–S6.

Recurrence and OS detection and analysis
For each cancer, pooled HR of recurrence and OS were shown in Figure S1 and Figure S2. Two thousand five hundred sixty-five patients with ctDNA+ and 10,763 patients with ctDNA- were in recurrence detection, 766 patients with ctDNA+ and 2482 patients with ctDNA- were in OS detection (Figs. 2 and 3). We presented a

Report type	
Clinical trial	62 (78%)
Conference abstract	18 (22%)
Trial design	
Prospective studies	73 (91%)
Randomized controlled trial	7 (9%)
Participant	
Mid-age	
~50	3 (4%)
51~60	18 (22%)
61~70	33 (41%)
71~	4 (5%)
Unknown	22 (28%)
ctDNA detection technology	
Hybridization capture-based NGS	34 (42%)
mPCR-NGS	25 (31%)
ddPCR	13 (16%)
Guardant Reveal	4 (5%)
cSMART	2 (3%)
ctDNA methylation	2 (3%)
Cancer	
CRC	20 (25%)
NSCLC	14 (17%)
CRLM	10 (12%)
PAAD	7 (9%)
ESCA	6 (8%)
BC	6 (8%)
GC	5 (6%)
BLCA	5 (6%)
OV	3 (4%)
Melanoma	3 (4%)
HCC	1 (1%)
Mid-follow up(month)	
1~10	6 (8%)
11~20	21 (26%)
21~30	18 (22%)
31~40	15 (19%)
>40	7 (9%)
Unknown	13 (16%)
Outcome	
Only recurrence	54 (67%)
OS and recurrence	24 (30%)
Only OS	2 (3%)
Detection time	
Landmark	52 (65%)
Landmark and Longitudinal	10 (12%)
Landmark and Adjuvant therapy	5 (6%)
Landmark, Longitudinal and Adjuvant therapy	7 (9%)
Longitudinal	6 (8%)

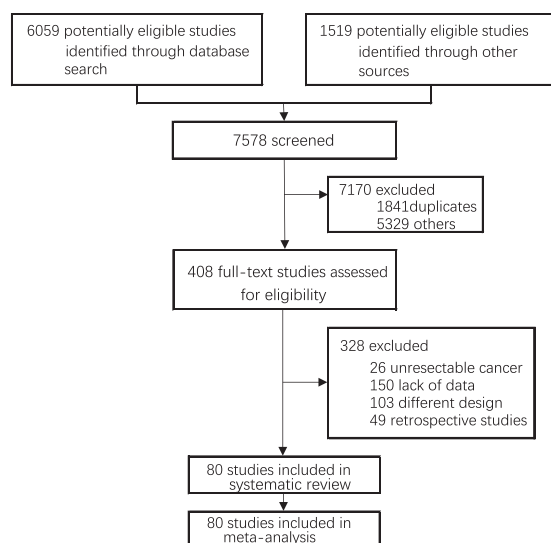*Table 1*: Characteristics of the eligible trials.

Fig. 1: Study selection PRISMA (preferred reporting items for systematic reviews and meta-analyses) 2020 flow diagram for systematic review of HRs of recurrence and survival for resectable cancer in CRC, NSCLC, CRLM, PAAD, BLCA, melanoma, BC, GC, HCC, OV and ESCA.

random effect model-based pan-cancer meta-analysis. By univariate analysis, excluding only single studies (HCC), the highest pooled HR of recurrence was BLCA (25.86, 95% CI 3.29–202.94, p (Q-test) = 0.02, I^2 (Higgins) = 74%), and the lowest pooled HR was PAAD (3.46, 95% CI 2.01–5.95, p (Q-test) = 0.26, I^2 (Higgins) = 24%). Excluding only single studies (BC, OV and BLCA), the highest pooled HR of OS was ESCA (8.69, 95% CI 2.12–35.62, p (Q-test) = 0.19, I^2 (Higgins) = 42%), and the lowest pooled HR was melanoma (3.55, 95% CI 0.58–21.72, p (Q-test) = 0.16, I^2 (Higgins) = 49%). Summarizing the HR of recurrence and OS in pan-cancer, the

pooled HR of recurrence was 7.48 (95% CI 6.39–8.77, p (Q-test) <0.001; I^2 (Higgins) = 73%) and the OS was 5.58 (95% CI 4.17–7.48, p (Q-test) <0.001; I^2 (Higgins) = 55%), respectively. Overall, there was no significant heterogeneity among cancers except for NSCLC, CRC, and BLCA for recurrence detection. For OS, there was no significant heterogeneity among cancers except for CRC (Figs. 2 and 3). The multivariable analysis also showed the ctDNA was a prognosis factor for recurrence and OS (pooled HR of recurrence: 7.07 (95% CI 5.66–8.83, p (Q-test)<0.001, I^2 (Higgins) = 79%); pooled HR of OS: 2.76 (95% CI 1.85–4.12, p (Q-test) = 0.06, I^2 (Higgins) = 43%) in all cancers (Figures S3 and S4). Most of the studies were adjusted variables for age, sex, and pathological stage (Table S7).

We conducted a subgroup analysis to observe whether there was any difference in HR of recurrence with different detection times. The result showed that the recurrence survival benefit of longitudinal detection was higher than that at the landmark detection in patients with ctDNA- in NSCLC, CRC and BC except ESCA (Figures S5–S8). Although CRLM, GC, and BLCA were the only single studies in longitudinal detection, we can still observe the same phenomenon as many longitudinal combined studies (Figures S9–S11). Patients with ctDNA- in post-adjuvant therapy also have recurrence survival benefits. We also summarized these studies in Figures S12 and S13.

Furthermore, subgroup analysis was also performed in recurrence according to with or without adjuvant therapy in landmark detection in CRC. It showed a significantly reduced risk of recurrence after adjuvant therapy in landmark detection for patients with ctDNA+ compared to those with no treatment (6.63, 95% CI 4.24–10.35, p (Q-test) = 0.57, I^2 (Higgins) = 0% vs 17.67, 95% CI 9.47–32.96, p (Q-test) = 0.93, I^2 (Higgins) = 0%, Figure S14).

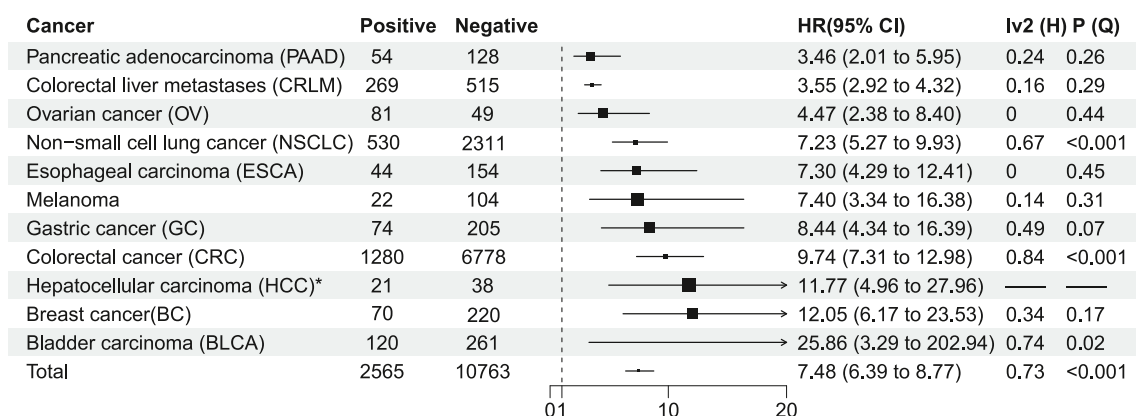| Cancer	Positive	Negative	HR(95% CI)	Iv2 (H)	P (Q)
Pancreatic adenocarcinoma (PAAD)	54	128	3.46 (2.01 to 5.95)	0.24	0.26
Colorectal liver metastases (CRLM)	269	515	3.55 (2.92 to 4.32)	0.16	0.29
Ovarian cancer (OV)	81	49	4.47 (2.38 to 8.40)	0	0.44
Non−small cell lung cancer (NSCLC)	530	2311	7.23 (5.27 to 9.93)	0.67	<0.001
Esophageal carcinoma (ESCA)	44	154	7.30 (4.29 to 12.41)	0	0.45
Melanoma	22	104	7.40 (3.34 to 16.38)	0.14	0.31
Gastric cancer (GC)	74	205	8.44 (4.34 to 16.39)	0.49	0.07
Colorectal cancer (CRC)	1280	6778	9.74 (7.31 to 12.98)	0.84	<0.001
Hepatocellular carcinoma (HCC)*	21	38	11.77 (4.96 to 27.96)	——	——
Breast cancer(BC)	70	220	12.05 (6.17 to 23.53)	0.34	0.17
Bladder carcinoma (BLCA)	120	261	25.86 (3.29 to 202.94)	0.74	0.02
Total	2565	10763	7.48 (6.39 to 8.77)	0.73	<0.001

01   10   20

Fig. 2: Summary collection of HRs of univariate analysis of recurrence of CRC, NSCLC, CRLM, PAAD, BLCA, melanoma, BC, GC, HCC, OV and ESCA; * = single study; Negative = ctDNA-; Positive = ctDNA+. Vertical dashed line is invalid line, and 95% confidence interval crossing is not statistically significant; H = Higgins' approach; Q = Q-test.
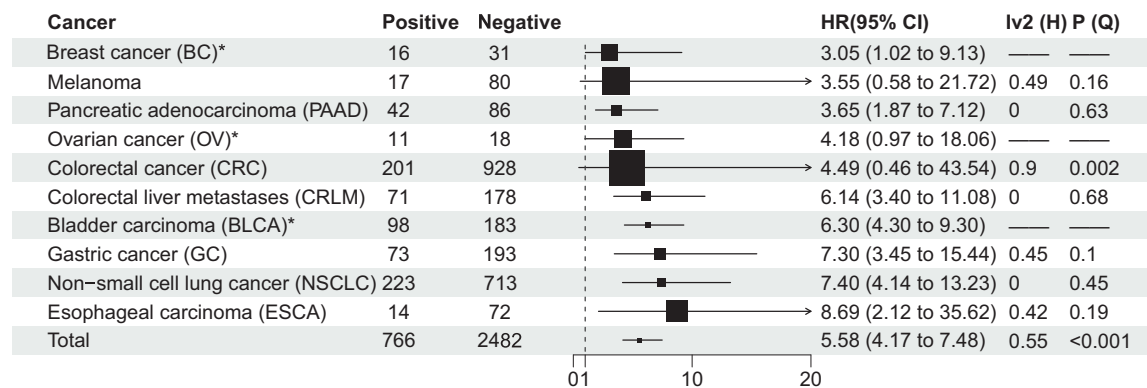
Cancer	Positive	Negative	HR(95% CI)	Iv2 (H)	P (Q)
Breast cancer (BC)*	16	31	3.05 (1.02 to 9.13)	——	——
Melanoma	17	80	3.55 (0.58 to 21.72)	0.49	0.16
Pancreatic adenocarcinoma (PAAD)	42	86	3.65 (1.87 to 7.12)	0	0.63
Ovarian cancer (OV)*	11	18	4.18 (0.97 to 18.06)	——	——
Colorectal cancer (CRC)	201	928	4.49 (0.46 to 43.54)	0.9	0.002
Colorectal liver metastases (CRLM)	71	178	6.14 (3.40 to 11.08)	0	0.68
Bladder carcinoma (BLCA)*	98	183	6.30 (4.30 to 9.30)	——	——
Gastric cancer (GC)	73	193	7.30 (3.45 to 15.44)	0.45	0.1
Non−small cell lung cancer (NSCLC)	223	713	7.40 (4.14 to 13.23)	0	0.45
Esophageal carcinoma (ESCA)	14	72	8.69 (2.12 to 35.62)	0.42	0.19
Total	766	2482	5.58 (4.17 to 7.48)	0.55	<0.001

Fig. 3: Summary collection of HRs of univariate analysis of OS of CRC, NSCLC, CRLM, PAAD, BLCA, melanoma, BC, GC, OV and ESCA; * = single study; Negative = ctDNA-; Positive = ctDNA+; Vertical dashed line is invalid line, and 95% confidence interval crossing is not statistically significant; H = Higgins' approach; Q = Q-test.

Sensitivity and specificity

Pool analysis

We extracted data with calculable sensitivity and specificity from the included studies. For each cancer, pooled sensitivity and specificity were shown in Table 2. Summarizing the sensitivity and specificity of pan-cancer, we found that pooled sensitivity and specificity of cancer recurrence were 0.58 (95% CI 0.53–0.62, I^2 (Zhou and Dendukuri's approach) = 2.40%) and 0.90 (95% CI 0.88–0.92, I^2 (Zhou and Dendukuri's approach) = 2.40%), respectively.

The meta-regression analysis showed that the heterogeneity of sensitivity was due to the monitoring time (p (z-test) <0.001) and the proportion of patients with positive (p (z-test) <0.001) in the landmark. Detection technology (A = mPCR-NGS, B = ddPCR, C = hybridization capture-based NGS) was a significant difference in landmark (p (z-test) = 0.021, Table S8).

Subgroup analysis

Next, we performed a subgroup analysis of the detection time. One thousand five-hundred four patients with relapse were 770 ctDNA+, and 3629 patients without relapse were 288 ctDNA+ at the landmark time. Four hundred ninety-eight patients with relapse were 373 ctDNA+, and 1315 patients without relapse were 139 ctDNA+ at the longitudinal time. Due to insufficient cancer studies, only CRC, NSCLC, CRLM, ESCA, PAAD, BC, BLCA and GC were analyzed via a bivariate model (Table 2). All cancer sensitivity was greater than 0.5 except CRLM and ESCA. In a landmark, the lowest sensitivity of the merge was CRLM, NSCLC and GC, and the highest was PAAD (Table 2).

CRLM detected the AUSROC of landmark detection with the lowest value and CRC with the highest value by univariate analysis, while NSCLC and CRLM had the lowest value and CRC with the highest value by bivariate analysis (Table 3). GC detected the DOR of landmark detection with the lowest value and with the PAAD highest value (Table 2). AUSROC is a comprehensive indicator that is more reliable than a single indicator such as DOR, so AUSROC results were adopted. In SROC meta-regression models, longitudinal detection was the better method to detect recurrence than landmark detection with AUSROC of 0.88 vs 0.80 and with DOR of 25.7 (95% CI 13.20–45.40) vs 9.90 (95% CI 7.77–12.40) (Table 2) by bivariate analysis. The ROC curve is shown in Fig. 4.

The proportion of patients with ctDNA- and ctDNA+ caused heterogeneity. We stratified the proportions and calculated the AUSROC using univariate and bivariate models. AUSROC decreased with the increase in the proportion of patients who were negative in the univariate model except for the ratio hierarchy of [9,13] (Table 3).

Total sensitivity and specificity of landmark time points were 0.50 (95% CI 0.46–0.55, I^2 (Zhou and Dendukuri's approach) = 1.70%) and 0.91 (95% CI 0.89–0.92, I^2 (Zhou and Dendukuri's approach) = 1.70%), respectively, while longitudinal time points were 0.74 (95% CI 0.68–0.80, I^2 (Zhou and Dendukuri's approach) = 6.90%) and 0.89 (95% CI 0.85–0.93, I^2 (Zhou and Dendukuri's approach) = 6.90%, Table 2). The pooled sensitivity and specificity of the detection time of all cancers were summarized in Table 2.

Subgroup analysis was also performed using monitoring techniques. Due to insufficient studies, this subgroup only analyzed landmark detection. The mPCR-NGS showed the highest sensitivity compared to the other two technologies (ddPCR and hybridization capture-based NGS). Table S9 summarizes the pooled

Subgroups	Number of data	Sens	LL	UL	Fpr	LL	UL	Iv2 (Z and D)	DOR	LL	UL	AUC
Cancer												
CRLM	9	0.49	0.40	0.57	0.11	0.07	0.17	11.30%	7.86	4.31	13.20	0.85
1	8	0.45	0.39	0.52	0.11	0.07	0.17	0%	6.70	3.91	10.70	0.71
2	1	–	–	–	–	–	–	–	–	–	–	–
ESCA	5	0.50	0.35	0.66	0.09	0.04	0.18	0%	11.80	3.95	27.70	0.88
1	3	–	–	–	–	–	–	–	–	–	–	–
2	2	–	–	–	–	–	–	–	–	–	–	–
GC	6	0.55	0.38	0.72	0.14	0.09	0.21	0%	8.31	2.91	18.90	0.85
1	5	0.45	0.34	0.57	0.16	0.11	0.23	5.70%	4.69	2.27	8.63	0.77
2	1	–	–	–	–	–	–	–	–	–	–	–
CRC	18	0.58	0.48	0.68	0.08	0.06	0.11	12.90%	16.10	9.14	26.20	0.89
1	14	0.51	0.41	0.61	0.09	0.07	0.11	9.90%	11.40	6.99	17.50	0.89
2	4	0.84	0.73	0.91	0.05	0.01	0.20	0%	154.00	14.80	638.00	0.89
PAAD	4	0.58	0.44	0.71	0.13	0.04	0.32	0%	11.90	2.87	33.40	0.66
1	4	0.58	0.44	0.71	0.13	0.04	0.32	0%	11.90	2.87	33.40	0.66
NSCLC	15	0.61	0.51	0.70	0.11	0.07	0.16	15.90%	13.60	7.73	22.20	0.83
1	7	0.45	0.35	0.55	0.07	0.03	0.13	0%	11.80	6.41	20.10	0.71
2	8	0.75	0.65	0.82	0.14	0.09	0.22	0%	19.50	7.14	43.20	0.86
BC	5	0.65	0.42	0.82	0.04	0.02	0.09	0%	58.40	11.40	182.00	0.96
1	3	–	–	–	–	–	–	–	–	–	–	–
2	2	–	–	–	–	–	–	–	–	–	–	–
HCC	1	–	–	–	–	–	–	–	–	–	–	–
BLCA	4	0.59	0.51	0.66	0.11	0.08	0.16	1.30%	11.40	6.58	18.50	0.75
1	3	–	–	–	–	–	–	–	–	–	–	–
2	1	–	–	–	–	–	–	–	–	–	–	–
OV	2	–	–	–	–	–	–	–	–	–	–	–
1	2	–	–	–	–	–	–	–	–	–	–	–
melanoma	1	–	–	–	–	–	–	–	–	–	–	–
Time	70	0.58	0.53	0.62	0.10	0.08	0.12	2.40%	12.90	9.94	16.50	0.85
1	50	0.50	0.46	0.55	0.09	0.08	0.11	1.70%	9.90	7.77	12.40	0.80
2	20	0.74	0.68	0.80	0.11	0.07	0.15	6.90%	25.70	13.20	45.40	0.88

The Iv2 estimated by Zhou and Dendukuri approach (Z and D); 1 = landmark detection; 2 = longitudinal detection; Sens: sensitivity; Fpr: false-positive rate. DOR: diagnostic odds ratio; AUSROC (AUC): area under the summary receiver operating characteristic curve.

Table 2: The Sens, Fpr, AUSROC (AUC) and DOR of each cancer by bivariate random effects model.

sensitivity and specificity of detection techniques for all cancers. The AUSROC is shown in Tables S9 and S10.

Reporting bias analysis
The reporting bias was assessed using a funnel plot and Egger's and Begg's tests (Figure S15). The Egger's and Begg's tests results for HR of Univariate analysis of NSCLC and CRC showed that in this meta-analysis their reporting bias is non-significant (NSCLC: Univariate (u)-egger: t = 0.34, df = 23, p (t-test) = 0.7392, begg: z = 0.91, p (z-test) = 0.3624; CRC: u-egger: t (t-test) = 1.74, df = 33, p (t-test) = 0.0910, begg: z = 0.37, p (z-test) = 0.7120). However, CRLM had to report bias (egger: t = 2.58, df = 11, p (t-test) = 0.0254, begg: z = 2.50, p (z-test) = 0.0124). For multivariable analysis, the Egger's and Begg's tests results of NSCLC showed that its reporting bias is non-significant (multivariable(m)-egger: t = 0.75, df = 9, p (t-test) = 0.4711, begg: z = 0.31, p (z-test) = 0.7555) while CRC showed reporting bias (m-egger: t = 2.33, df = 22, p (t-test) = 0.0293, begg: z = 0.82, p (z-test) = 0.4130). Then, we used the Trim-and-fill method for estimating and adjusting for the number and outcomes of missing studies in CRLM-u and NSCLC-m. It was shown that pooled HR after adjusting still makes sense (CRLM-u: 3.33, 95% CI 2.61–4.23, p (z-test) < 0.001; NSCLC-m: 8.29, 95% CI 5.65–12.19, p (z-test) < 0.001). The meta-regression analysis showed that the heterogeneity was not due to the quality of studies (Tables S8 and S11).

Sensitivity analysis
We performed a sensitivity analysis (which is used to describe supporting analyses to check the findings' robustness to the model assumptions in this paragraph) by removing each study. The sensitivity analysis results of recurrence and OS are shown in

Cancer	AUSROC-u	Low	High	P (Chi-square)	AUSROC-b
CRC					
1	0.79	0.75	0.84	0.23	0.89
NSCLC					
1	0.78	0.71	0.87	0.82	0.71
2	0.87	0.82	0.93	0.22	0.86
CRLM					
1	0.73	0.70	0.80	0.50	0.71
GC					
1	0.78	0.70	0.90	0.26	0.77
All					
1	0.80	0.77	0.82	0.44	0.80
2	0.91	0.88	0.94	0.11	0.88
Ratio					
0~1	0.87	0.77	0.99	0.25	0.91
1~5	0.84	0.81	0.86	0.48	0.85
5~9	0.78	0.73	0.84	0.25	0.68
9~13	0.77	0.70	0.85	0.26	0.71

1 = landmark detection; 2 = longitudinal detection; The ratio hierarchy is (0~1), [1, 5), [5, 9), [9, 13); Ratio = the number of patients with ctDNA-/the number of patients with ctDNA+.

Table 3: AUSROC values of each cancer by univariate (u) model and bivariate model (b).

Figures S16–S20, indicating that the pooled estimates were not materially altered by using the leave-one-out sensitivity analysis.

Discussion

The results of this study showed that ctDNA was a risk factor for resectable cancer recurrence, and its HR ranges from 3.46 to 25.86. In line with this, ctDNA is considered a risk factor for OS, HR from 3.05 to 8.69. BLCA had the highest survival benefit in the pooled HR of univariate analysis in recurrence, while ESCA had the highest survival benefit in the pooled HR of multivariable analysis, indicating that clinicopathological variables had a possible impact on the results of those studies, especially sex, age and pathological stage. Although the survival benefit of PAAD was lower than other cancers, the diagnostic sensitivity of PAAD in landmark detection was higher than that of other cancers (CRLM, GC, CRC and NSCLC). It may correlated with the low five-year survival rate in PAAD. The survival benefit of CRLM was lower than that of other cancers except PAAD, and the diagnostic sensitivity was lower than that of other cancers (PAAD and CRC) in landmark detection.

We also found an interesting phenomenon: longitudinal detection can significantly improve diagnostic efficiency. The sensitivity of longitudinal detection and AUSROC both indicate that longitudinal detection has a higher diagnostic value. From the data extracted in the included study, the time point of longitudinal detection was mostly 3–6 months after surgery, which may also be consistent with the follow-up time of surgery. However, whether this time point was the most appropriate needs to be verified; it is worth researching to find the time when negative turns to positive and the association between potentially positive patients and risk factors.

At present, ctDNA detection mainly focuses on CRC, NSCLC, GC, BC, PAAD, ESCA, CRLM, OV, BLCA, melanoma and HCC; these cancers account for about 65% of all tumor-related deaths in the world,[105] of which CRC, NSCLC and BC were diagnosed in the early state accounted for 39%, 16% and 62%,[106] respectively. These patients preferred radical surgical resection treatment, but the 5-year OS rate varies from cancer to cancer, OS in BC up to 99%, NSCLC only 56%.[106] The main reason for this result may be postoperative minimal residual cancers and occult metastasis. Previous meta-analyses of single cancer all showed that the presence of ctDNA was associated with cancer recurrence (for CRC,[107] BC,[108] NSCLC,[109] and ESCA,[110] respectively). Our meta-analysis defined the time point as postoperative. The objective was to clarify the prognostic significance of the presence of ctDNA after cancer resection and to explore the significance of single and follow-up time point detection after surgery. Our meta-analysis via coincident inclusion and exclusion criteria makes comparing the results of various cancers more possible and summarizes the value of prognosis assessment and diagnostic accuracy of ctDNA-MRD.

Regarding diagnostic accuracy, the pooled AUSROC and DOR results showed higher longitudinal analysis accuracy than landmark analysis. The results of the sensitivity and specificity analysis can also be confirmed.

Multiple cancer studies have found that longitudinal detection is more sensitive than landmark detection,[63,69,75,77,92] and our subgroup analysis result was highly consistent with this rule. In addition, the pooled HR of recurrence showed that regardless of landmark detection or post-adjuvant treatment detection, the survival benefit of longitudinal detection was the highest for BC, NSCLC, and CRC, except for ESCA.

It is not only the detection time that affects the results but also the detection technique. Previous studies have found that the detection efficiency of ddPCR and NGS was different. Loupakis F et al.[71] and Zhang H et al.[111] compared ddPCR with NGS technology, and it was found that NGS was more sensitive to detecting positive results. Our subgroup analysis of ctDNA detection technology also found that ddPCR was less sensitive than NGS. At the same time, we also observed that the overall sensitivity could be better, and increasing the sequencing depth and finding other ways to improve the sensitivity of NGS detection are necessary problems to be solved. In addition, the sensitivity and survival benefit of longitudinal detection of ESCA[70] was down, and it may be associated with using a non-esophageal adenocarcinoma (EAC) cancer-specific gene
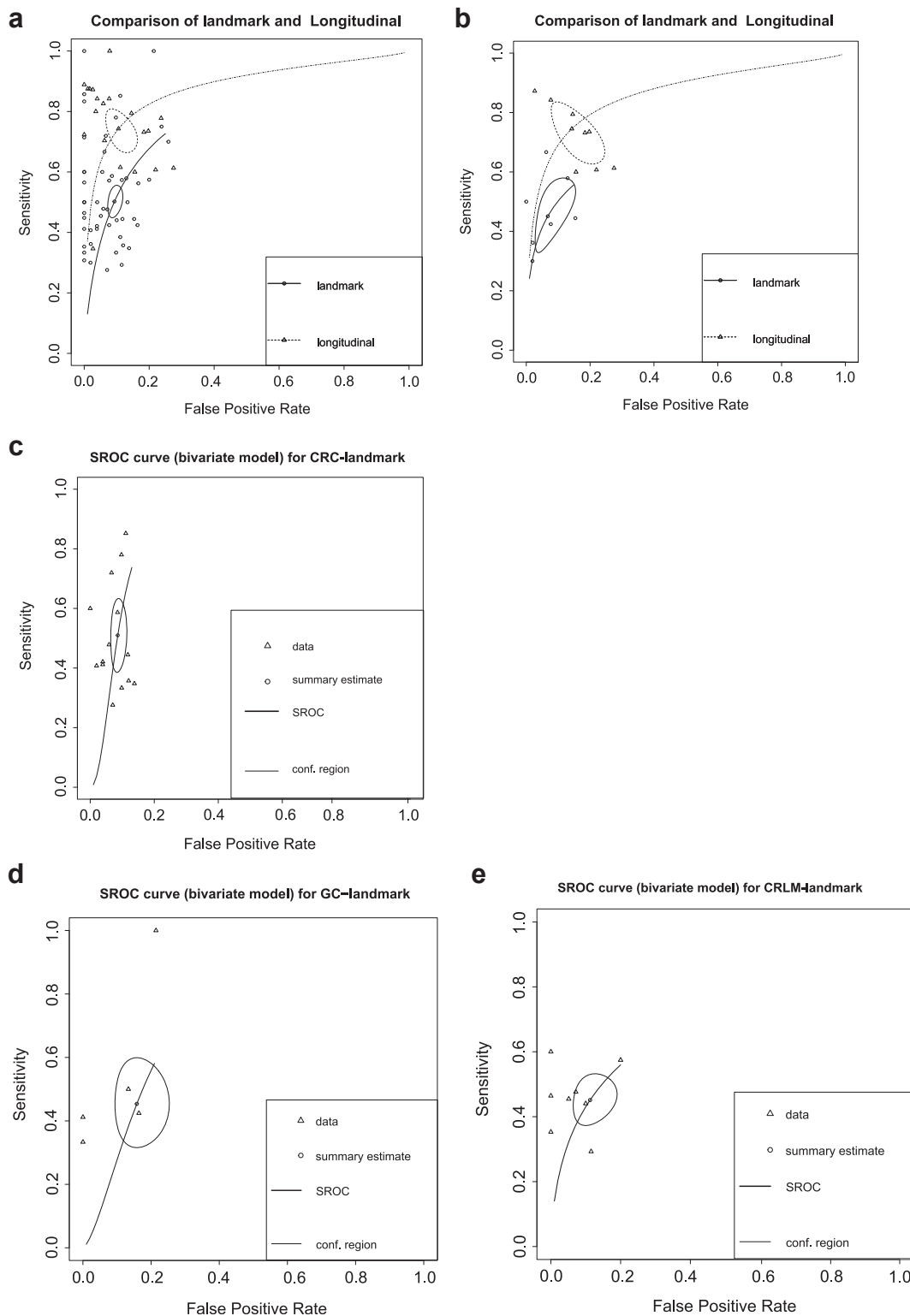
Fig. 4: SROC curve (bivariate model) of each cancer: a: sROC curve for all cancers combinations of landmark detection and longitudinal detection; b: sROC curve for NSCLC combinations of landmark detection and longitudinal detection; c: sROC curve for CRC combinations of landmark detection; d: sROC curve for GC combinations of landmark detection; e: sROC curve for CRLM combinations of landmark detection. conf. region: estimate of sensitivity and specificity to show the region containing likely combinations of the mean value of sensitivity and specificity.

panel, which was tumor-naïve method while others were tumor-informed method in ESCA. It means there is a need for specific cfDNA panels.

In addition, we found that studies with more patients with ctDNA- were less sensitive, which may understate the test's sensitivity. This means that there could be a discrepancy in the number of patients with ctDNA+ and ctDNA-. Despite the low relapse population in the ctDNA- group, they may still represent a significant portion of the relapse population.

Postoperative adjuvant therapy was a common means for patients with resectable cancer to reduce the risk of recurrence and improve the OS. Related studies[71,80,90] showed that the survival benefit of the ctDNA+ population group would be improved after adjuvant therapy. We analyzed whether adjuvant therapy should be used. The result showed that the survival benefit of patients with ctDNA+ after adjuvant therapy increased in CRC. It suggested whether it would be correlated with the benefit of adjuvant therapy in the future, but it still needs more well-designed clinical studies.

Overall, ctDNA is very valuable for prognostic assessment and shows different prognostic values among different cancers. In addition, postoperative adjuvant therapy significantly improved the prognosis of patients with ctDNA+ in CRC. All these suggest the possibility of detecting ctDNA after surgery. Further study of the value of ctDNA+ should focus on analyzing the difference and relationship between the ctDNA-based adjuvant therapy group and the conventional management therapy group. Besides, verifying the specific longitudinal detection time point is also a problem that needs to be solved. The longitudinal detection has many complicated factors. Firstly, the relationship between the duration time of ctDNA+ and recurrence should be clarified; secondly, the different strengths of association with recurrence risk between ctDNA single positive and multiple positive should be clarified. What's more, it is necessary to explore the association between potentially positive patients and risk factors.

Limitations of our meta-analysis study: there were a few related studies on some cancers in this study, such as melanoma, HCC, and BLCA, which need to be more convincing to confirm the results. Therefore, further exploration and study in this direction are needed. In addition, there needs to be a clear definition of ctDNA-MRD and a uniform standard for NGS detection panel and sequencing depth, which may also lead to differences in study results. Lastly, there was no clear definition of ctDNA+ and ctDNA-, which may affect the false positive rate to some extent. What's more, the ratio of patients with ctDNA- and ctDNA+ affected the sensitivity estimate, which may have influenced the results of the study.

References
1 Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17–48.
2 Zheng R, Zhang S, Zeng H, et al. Cancer incidence and mortality in China, 2016. *J Natl Cancer Cent*. 2022;2(1):1–9.
3 Schiffman JD, Fisher PG, Gibbs P. Early detection of cancer: past, present, and future. *Am Soc Clin Oncol Educ Book*. 2015;(35):57–65.
4 Chen G, Peng J, Xiao Q, et al. Postoperative circulating tumor DNA as markers of recurrence risk in stages II to III colorectal cancer. *J Hematol Oncol*. 2021;14(1):80.
5 Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021;595(7867):432–437.
6 Benson AB, Venook AP, Al-Hawary MM, et al. Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw*. 2021;19(3):329–359.
7 WU Yi-long LS, Ying C, Qing-hua Z, et al. Expert consensus of molecular residual disease for non-small. *Cell Lung Cancer*. 2021;21(3):129–135.
8 O'Leary B, Hrebien S, Beaney M, et al. Comparison of BEAMing and droplet digital PCR for circulating tumor DNA analysis. *Clin Chem*. 2019;65(11):1405–1413.
9 Jamal-Hanjani M, Wilson GA, Horswell S, et al. Detection of ubiquitous and heterogeneous mutations in cell-free DNA from patients with early-stage non-small-cell lung cancer. *Ann Oncol*. 2016;27(5):862–867.
10 Imperial R, Nazer M, Ahmed Z, et al. Matched whole-genome sequencing (WGS) and whole-exome sequencing (WES) of tumor tissue with circulating tumor DNA (ctDNA) analysis: complementary modalities in clinical practice. *Cancers*. 2019;11(9):1399.
11 Belic J, Koch M, Ulz P, et al. Rapid identification of plasma DNA samples with increased ctDNA levels by a modified FAST-SeqS approach. *Clin Chem*. 2015;61(6):838–849.
12 Moding EJ, Nabet BY, Alizadeh AA, et al. Detecting liquid remnants of solid tumors: circulating tumor DNA minimal residual disease. *Cancer Discov*. 2021;11(12):2968–2986.
13 Chen K, Zhao H, Shi Y, et al. Perioperative dynamic changes in circulating tumor DNA in patients with lung cancer (DYNAMIC). *Clin Cancer Res*. 2019;25(23):7058–7067.
14 Parikh AR, Van Seventer EE, Siravegna G, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. *Clin Cancer Res*. 2021;27(20):5586–5594.

15 Mo S, Ye L, Wang D, et al. Early detection of molecular residual disease and risk stratification for stage I to III colorectal cancer via circulating tumor DNA methylation. *JAMA Oncol.* 2023;9(6):770–778.

16 Gu J, Lei F, Wang X, et al. 458P Circulating tumor DNA analysis predicting recurrence risk in patients with stage I-III colorectal cancer. *Ann Oncol.* 2021;32(suppl 5):S561.

17 Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Hospital Research Institute. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed September 10, 2023.

18 Delgado A, Guddati AK. Clinical endpoints in oncology - a primer. *Am J Cancer Res.* 2021;11(4):1121–1131.

19 Rasola C, Laurent-Puig P, André T, et al. Time to recurrence and its relation to survival after recurrence in patients resected for stage III colon cancer. *Eur J Cancer.* 2023;194:113321.

20 Eroglu Z, Krinshpun S, Kalashnikova E, et al. Circulating tumor DNA-based molecular residual disease detection for treatment monitoring in advanced melanoma patients. *Cancer.* 2023;129(11):1723–1734.

21 Reitsma J, Glas A, Rutjes A, Scholten R, Bossuyt P, Zwinderman A. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol.* 2005;58:982–990.

22 Holling H, Boehning W, Boehning D. Meta-analysis of diagnostic studies based upon SROC-curves: a mixed model approach using a proportional hazards model. *Stat Model Int J.* 2012;12:347–375.

23 Viechtbauer W. Confidence intervals for the amount of heterogeneity in meta-analysis. *Stat Med.* 2007;26:37–52.

24 Higgins J, Thompson S, Deeks J, Altman D. Measuring inconsistency in meta-analyses. *Br Med J.* 2003;327:557–560.

25 Doebler P. *mada: Meta-Analysis of Diagnostic Accuracy.* 2022.

26 Zhou Y, Dendukuri N. Statistics for quantifying heterogeneity in univariate and bivariate meta-analyses of binary data: the case of meta-analyses of diagnostic accuracy. *Stat Med.* 2014;33:2701–2717. 24. https://doi.org/10.1002/sim.6115.

27 Pasceri V, Patti G, Pelliccia F, et al. Complete revascularization during primary percutaneous coronary intervention reduces death and myocardial infarction in patients with multivessel disease: meta-analysis and meta-regression of randomized trials. *JACC Cardiovasc Interv.* 2018;11(9):833–843. https://doi.org/10.1016/j.jcin.2018.02.028.

28 Schwarzer G. *General package for meta-analysis. Evidence based mental health CRAN - package meta (r-project.org).* 2023.

29 Gordon M, Lumley T. Advanced forest plot using 'grid' graphics. https://cran.r-project.org/web/packages/forestplot/index.html. Accessed September 10, 2023.

30 Xue P, Shao Y, Zhou X, et al. Abstract 1037: circulating tumor DNA-based molecular residual disease predicts relapse in patients with resectable gastric cancer. *Cancer Res.* 2023;83(suppl 7):1037.

31 Watanabe J, Oki E, Kotani D, et al. Postoperative circulating tumor DNA-based molecular residual disease in patients with BRAF V600E and MSI-H colorectal cancer: updated results from GALAXY study in the CIRCULATE-Japan. *JCO Glob Oncol.* 2023;9(suppl 1):32.

32 Wang X, Wang H, Chen X, et al. Patient-specific tumor-informed circulating tumor DNA (ctDNA) assay to predict cancer recurrence in patients with resected pancreatic cancer. *J Clin Oncol.* 2023;41(suppl 4):744.

33 Takei S, Kotani D, Sato K, et al. Longitudinal circulating tumor DNA monitoring in patients with esophageal squamous cell carcinoma. *J Clin Oncol.* 2023;41(suppl 16):3041.

34 Gerlinger M, Gordon A, Barber LJ, et al. Abstract 5591: circulating tumor DNA for recurrence prediction and efficacy analysis in the ICONIC trial of peri-operative FLOT and avelumab (PD-L1) in localized esophago-gastric adenocarcinoma. *Cancer Res.* 2023;83(suppl 7):5591.

35 Ananda S, Wang Y, Hong W, et al. Circulating tumor DNA (ctDNA) as a marker of residual disease and recurrence in resected stage I-IV epithelial ovarian cancer (EOC). *J Clin Oncol.* 2023;41(suppl 16):5553.

36 Zhou J, Yang J, Zhang Z, et al. Abstract 5917: patient-specific tumor-informed circulating tumor DNA (ctDNA) analysis for postoperative monitoring of patients with stages I-III colorectal cancer (CRC). *Cancer Res.* 2022;82(suppl 12):5917.

37 Overman MJ, Vauthey J-N, Aloia TA, et al. Circulating tumor DNA (ctDNA) utilizing a high-sensitivity panel to detect minimal residual disease post liver hepatectomy and predict disease recurrence. 2017;35(suppl 15):3522.

38 Sharma P, Stecklein SR, Kimler BF, et al. Impact of post-treatment ctDNA and residual cancer burden (RCB) on outcomes in patients with triple-negative breast cancer (TNBC) and residual disease. *Cancer Res.* 2022;82(suppl 4):P2.

39 McNamara S, Patel RD, Adams H-P, et al. Detection of minimal residual disease (MRD) in colorectal cancer (CRC) patients UICC stage II/III by ultra-deep sequencing of cfDNA from post-surgery plasma. *J Clin Oncol.* 2022;40(suppl 4):26.

40 Han SW, Heo S, Lim Y, et al. 381P A tumor-informed, hybrid-capture based ctDNA assay for minimal residual disease (MRD) detection in colorectal cancer (CRC) patients after curative surgery. *Ann Oncol.* 2022;33(suppl 7):S710–S711.

41 Genta S, Araujo DV, Keshavarzi S, et al. Leveraging personalized circulating tumor DNA (ctDNA) for detection and monitoring of molecular residual disease in high-risk melanoma. *J Clin Oncol.* 2022;40(suppl 16):9579.

42 Tan A, Lai G, Saw S, et al. MA07.06 circulating tumor DNA for monitoring minimal residual disease and early detection of recurrence in early stage lung cancer. *J Thorac Oncol.* 2021;16(suppl 10):S907.

43 Chee B, Ibrahim F, Esquivel M, et al. Circulating tumor derived cell-free DNA (ctDNA) to predict recurrence of metastatic colorectal cancer (mCRC) following curative intent surgery or radiation. 2021;39(suppl 15):3565.

44 Chapman JS, Pierson WE, Smith-McCune K, et al. Abstract 552: circulating tumor DNA predicts disease recurrence in ovarian cancer patients. *Cancer Res.* 2021;81(suppl 13):552.

45 Anandappa G, Starling N, Begum R, et al. Minimal residual disease (MRD) detection with circulating tumor DNA (ctDNA) from personalized assays in stage II-III colorectal cancer patients in a U.K. multicenter prospective study (TRACC). *J Clin Oncol.* 2021;39(suppl 3):102.

46 Popova A, Fedyanin M, Pokataev I, et al. P-279 Clinical utility of circulating tumor DNA in resectable and advanced pancreatic cancer. *Ann Oncol.* 2020;31(suppl 3):S180–S181.

47 Fedyanin M, Ignatova E, Boyarskikh U, et al. 137P Clinical utility of circulating tumour DNA (ctDNA) in resectable gastric cancer (GC). *Ann Oncol.* 2020;31(suppl 6):S1295.

48 Morimoto Y, Matsuda S, Kawakubo H, et al. Tumor burden monitoring with circulating tumor DNA during treatment in patients with esophageal squamous cell carcinoma. *Ann Surg Oncol.* 2023;30(6):3747–3756.

49 Liu W, Jin KM, Zhang MH, et al. Recurrence prediction by circulating tumor DNA in the patient with colorectal liver metastases after hepatectomy: a prospective biomarker study. *Ann Surg Oncol.* 2023;30(8):4916–4926.

50 Kotani D, Oki E, Nakamura Y, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. *Nat Med.* 2023;29(1):127–134.

51 Jiang H, Huang F, Yang Y, et al. Postoperative circulating tumor DNA testing based on tumor naive strategy after liver metastasis surgery in colorectal cancer patients. *Front Oncol.* 2023;13:1153685.

52 Hofste LSM, Geerlings MJ, von Rhein D, et al. Circulating tumor DNA detection after neoadjuvant treatment and surgery predicts recurrence in patients with early-stage and locally advanced rectal cancer. *Eur J Surg Oncol.* 2023;49(7):1283–1290.

53 Fu R, Huang J, Tian X, et al. Postoperative circulating tumor DNA can refine risk stratification in resectable lung cancer: results from a multicenter study. *Mol Oncol.* 2023;17(5):825–838.

54 Chen K, Kang G, Zhang Z, et al. Individualized dynamic methylation-based analysis of cell-free DNA in postoperative monitoring of lung cancer. *BMC Med.* 2023;21(1):255.

55 Xia L, Mei J, Kang R, et al. Perioperative ctDNA-based molecular residual disease detection for non-small cell lung cancer: a prospective multicenter cohort study (LUNGCA-1). *Clin Cancer Res.* 2022;28(15):3308–3317.

56 Wang S, Li M, Zhang J, et al. Circulating tumor DNA integrating tissue clonality detects minimal residual disease in resectable non-small-cell lung cancer. *J Hematol Oncol.* 2022;15(1):137.

57 Szabados B, Kockx M, Assaf ZJ, et al. Final results of neoadjuvant atezolizumab in cisplatin-ineligible patients with muscle-invasive urothelial cancer of the bladder. *Eur Urol.* 2022;82(2):212–222.

58 Reinert T, Petersen LMS, Henriksen TV, et al. Circulating tumor DNA for prognosis assessment and postoperative management after curative-intent resection of colorectal liver metastases. *Int J Cancer.* 2022;150(9):1537–1548.

59 Nishioka Y, Chun YS, Overman MJ, et al. Effect of Co-mutation of RAS and TP53 on postoperative ctDNA detection and early recurrence after hepatectomy for colorectal liver metastases. *J Am Coll Surg*. 2022;234(4):474–483.

60 Newhook TE, Overman MJ, Chun YS, et al. Prospective study of perioperative circulating tumor DNA dynamics in patients undergoing hepatectomy for colorectal liver metastases. *Ann Surg*. 2023 May 1;277(5):813–820.

61 Li Y, Mo S, Zhang L, et al. Postoperative circulating tumor DNA combined with consensus molecular subtypes can better predict outcomes in stage III colon cancers: a prospective cohort study. *Eur J Cancer*. 2022;169:198–209.

62 Li N, Wang BX, Li J, et al. Perioperative circulating tumor DNA as a potential prognostic marker for operable stage I to IIIA non-small cell lung cancer. *Cancer*. 2022;128(4):708–718.

63 Henriksen TV, Tarazona N, Frydendahl A, et al. Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, toward assessment of adjuvant therapy efficacy and clinical behavior of recurrences. *Clin Cancer Res*. 2022;28(3):507–517.

64 Hata T, Mizuma M, Motoi F, et al. Prognostic impact of postoperative circulating tumor DNA as a molecular minimal residual disease marker in patients with pancreatic cancer undergoing surgical resection. *J Hepatobiliary Pancreat Sci*. 2022;30(6):815–824.

65 Chao A, Chen SJ, Chen HC, et al. Mutations in circulating tumor DNA detected in the postoperative period predict poor survival in patients with ovarian cancer. *Biomed J*. 2022;46(5):100563.

66 Carrasco R, Ingelmo-Torres M, Gomez A, et al. Cell-free DNA as a prognostic biomarker for monitoring muscle-invasive bladder cancer. *Int J Mol Sci*. 2022;23(19):11732.

67 Zhou Y, Xu Y, Wang C, et al. Serial circulating tumor DNA identification associated with the efficacy and prognosis of neoadjuvant chemotherapy in breast cancer. *Breast Cancer Res Treat*. 2021;188(3):661–673.

68 Tie J, Wang Y, Cohen J, et al. Circulating tumor DNA dynamics and recurrence risk in patients undergoing curative intent resection of colorectal cancer liver metastases: a prospective cohort study. *PLoS Med*. 2021;18(5):e1003620.

69 Qiu B, Guo W, Zhang F, et al. Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC. *Nat Commun*. 2021;12(1):6770.

70 Ococks E, Frankell AM, Masque SN, et al. Longitudinal tracking of 97 esophageal adenocarcinomas using liquid biopsy sampling. *Ann Oncol*. 2021;32(4):522–532.

71 Loupakis F, Sharma S, Derouazi M, et al. Detection of molecular residual disease using personalized circulating tumor DNA assay in patients with colorectal cancer undergoing resection of metastases. *JCO Precis Oncol*. 2021;5(PO.21):00101.

72 Liu T, Yao Q, Jin H. Plasma circulating tumor DNA sequencing predicts minimal residual disease in resectable esophageal squamous cell carcinoma. *Front Oncol*. 2021;11:616209.

73 Benhaim L, Bouché O, Normand C, et al. Circulating tumor DNA is a prognostic marker of tumor recurrence in stage II and III colorectal cancer: multicentric, prospective cohort study (ALGECOLS). *Eur J Cancer*. 2021;159:24–33.

74 Yang W, You N, Jia M, et al. Undetectable circulating tumor DNA levels correlate with low risk of recurrence/metastasis in postoperative pathologic stage I lung adenocarcinoma patients. *Lung Cancer*. 2020;146:327–334.

75 Yang J, Gong Y, Lam VK, et al. Deep sequencing of circulating tumor DNA detects molecular residual disease and predicts recurrence in gastric cancer. *Cell Death Dis*. 2020;11(5):346.

76 Peng M, Huang Q, Yin W, et al. Circulating tumor DNA as a prognostic biomarker in localized non-small cell lung cancer. *Front Oncol*. 2020;10:561598.

77 Openshaw MR, Mohamed AA, Ottolini B, et al. Longitudinal monitoring of circulating tumour DNA improves prognostication and relapse detection in gastroesophageal adenocarcinoma. *Br J Cancer*. 2020;123(8):1271–1279.

78 Kuang PP, Li N, Liu Z, et al. Circulating tumor DNA analyses as a potential marker of recurrence and effectiveness of adjuvant chemotherapy for resected non-small-cell lung cancer. *Front Oncol*. 2020;10:595650.

79 Jiang J, Ye S, Xu Y, et al. Circulating tumor DNA as a potential marker to detect minimal residual disease and predict recurrence in pancreatic cancer. *Front Oncol*. 2020;10:1220.

80 Tie J, Cohen JD, Wang Y, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. *Gut*. 2019;68(4):663–671.

81 Kitahata Y, Kawai M, Hirono S, et al. Circulating tumor DNA as a potential prognostic marker in patients with borderline-resectable pancreatic cancer undergoing neoadjuvant chemotherapy followed by pancreatectomy. *Ann Surg Oncol*. 2022;29(3):1596–1605.

82 Tarazona N, Gimeno-Valiente F, Gambardella V, et al. Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. *Ann Oncol*. 2019;30(11):1804–1812.

83 Tan L, Sandhu S, Lee RJ, et al. Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA. *Ann Oncol*. 2019;30(5):804–814.

84 Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124–1131.

85 Lee B, Lipton L, Cohen J, et al. Circulating tumor DNA as a potential marker of adjuvant chemotherapy benefit following surgery for localized pancreatic cancer. *Ann Oncol*. 2019;30(9):1472–1478.

86 Coombes RC, Page K, Salari R, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. *Clin Cancer Res*. 2019;25(14):4255–4263.

87 Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019;37(18):1547–1557.

88 Schøler LV, Reinert T, Ørntoft MW, et al. Clinical implications of monitoring circulating tumor DNA in patients with colorectal cancer. *Clin Cancer Res*. 2017;23(18):5437–5445.

89 Chen YH, Hancock BA, Solzak JP, et al. Next-generation sequencing of circulating tumor DNA to predict recurrence in triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy. *NPJ Breast Cancer*. 2017;3:24.

90 Tie J, Wang Y, Tomasetti C, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci Transl Med*. 2016;8(346):346ra92.

91 Yamaguchi T, Uemura K, Murakami Y, et al. Clinical implications of pre- and postoperative circulating tumor DNA in patients with resected pancreatic ductal adenocarcinoma. *Ann Surg Oncol*. 2021;28(6):3135–3144.

92 Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med*. 2015;7(302):302ra133.

93 Powles T, Assaf ZJ, Degaonkar V, et al. Updated overall survival by circulating tumor DNA status from the phase 3 IMvigor010 trial: adjuvant atezolizumab versus observation in muscle-invasive urothelial carcinoma. *Eur Urol*. 2023;S0302-2838(23):02902.

94 Chen K, Yang F, Shen H, et al. Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. *Cancer Cell*. 2023;S1535-6108(23):286.

95 Zhang JT, Liu SY, Gao W, et al. Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer. *Cancer Discov*. 2022;12(7):1690–1701.

96 Waldeck S, Mitschke J, Wiesemann S, et al. Early assessment of circulating tumor DNA after curative-intent resection predicts tumor recurrence in early-stage and locally advanced non-small-cell lung cancer. *Mol Oncol*. 2022;16(2):527–537.

97 Wang DS, Yang H, Liu XY, et al. Dynamic monitoring of circulating tumor DNA to predict prognosis and efficacy of adjuvant chemotherapy after resection of colorectal liver metastases. *Theranostics*. 2021;11(14):7018–7028.

98 Tie J, Cohen JD, Lahouel K, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. *N Engl J Med*. 2022;386(24):2261–2272.

99 Schneider BP, Jiang G, Ballinger TJ, et al. BRE12-158: a post-neoadjuvant, randomized phase II trial of personalized therapy versus treatment of physician's choice for patients with residual triple-negative breast cancer. *J Clin Oncol*. 2022;40(4):345–355.

100 Taieb J, Taly V, Henriques J, et al. Prognostic value and relation with adjuvant treatment duration of ctDNA in stage III colon cancer: a post hoc analysis of the PRODIGE-GERCOR IDEA-France trial. *Clin Cancer Res*. 2021;27(20):5638–5646.

101 Bolhuis K, van 't Erve I, Mijnals C, et al. Postoperative circulating tumour DNA is associated with pathologic response and

recurrence-free survival after resection of colorectal cancer liver metastases. *eBioMedicine*. 2021;70:103498.

102 Leal A, van Grieken NCT, Palsgrove DN, et al. White blood cell and cell-free DNA analyses for detection of residual disease in gastric cancer. *Nat Commun*. 2020;11(1):525.

103 Zhao L, Jiang L, Liu Y, et al. Integrated analysis of circulating tumour cells and circulating tumour DNA to detect minimal residual disease in hepatocellular carcinoma. *Clin Transl Med*. 2022;12(4):e793.

104 Yuan S-Q, Huang Y-S, Nie R-C, et al. Circulating tumor DNA and recurrence risk in stage II-III gastric cancer. *Cancer Commun*. 2023;43(12):1312–1325.

105 Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249.

106 Wender RC, Brawley OW, Fedewa SA, et al. A blueprint for cancer screening and early detection: advancing screening's contribution to cancer control. *CA A Cancer J Clin*. 2018;69(1):50–79.

107 Mi J, Han X, Wang R, et al. Circulation tumour DNA in predicting recurrence and prognosis in operable colorectal cancer patients: a meta-analysis. *Eur J Clin Invest*. 2022;52(12):e13842.

108 Cullinane C, Fleming C, O'Leary DP, et al. Association of circulating tumor DNA with disease-free survival in breast cancer: a systematic review and meta-analysis. *JAMA Netw Open*. 2020;3(11):e2026921.

109 Guo RQ, Peng JZ, Sun J, Li YM. Clinical significance of circulating tumor DNA in localized non-small cell lung cancer: a systematic review and meta-analysis. *Clin Exp Med*. 2023;23(5):1621–1631.

110 Chidambaram S, Markar SR. Clinical utility and applicability of circulating tumor DNA testing in esophageal cancer: a systematic review and meta-analysis. *Dis Esophagus*. 2022;35(2).

111 Zhang H, Liu R, Yan C, et al. Advantage of next-generation sequencing in dynamic monitoring of circulating tumor DNA over droplet digital PCR in cetuximab treated colorectal cancer patients. *Transl Oncol*. 2019;12(3):426–431.