Journal of the National Cancer Center 5 (2025) 156–166

Contents lists available at ScienceDirect

Journal of the National Cancer Center

journal homepage: www.elsevier.com/locate/jncc

Full Length Article

The global progress and quality assessment of research on the association between circulating tumor DNA and clinical prognosis: a systematic review

Meng Zhang [1,2,†], Xiaowei Chen [1,2,†], Qingxin Zhou [3], Nana Guo [4], Baoshan Cao [5], Hongmei Zeng [6], Wanqing Chen [7], Feng Sun [1,2,8,*]

[1] *Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China*
[2] *Key Laboratory of Epidemiology of Major Diseases (Peking University), Ministry of Education, Beijing, China*
[3] *Tianjin Centers for Disease Control and Prevention, Tianjin, China*
[4] *Hebei Centers for Disease Control and Prevention, Shijiazhuang, China*
[5] *Department of Medical Oncology and Radiation Sickness, Peking University Third Hospital, Beijing, China*
[6] *National Central Cancer Registry, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*
[7] *Office of Cancer Screening, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*
[8] *Xinjiang Medical University, Urumqi, China*

ARTICLE INFO

Keywords:
Circulating tumor DNA
Solid tumor
Prognosis
Overall survival

ABSTRACT

Objective: Circulating tumor DNA (ctDNA) has shown potential as a prognostic biomarker in patients with solid tumors. This study aimed to systematically summarize the global application of ctDNA in the prognostic management of solid tumor patients and to evaluate the quality of the current studies.

Methods: PubMed, Web of Science, Embase, Cochrane Library, Scopus, and clinical trials.gov databases were searched to collect cohort studies on ctDNA in the prognosis of solid tumor patients from January 2016 to May 2022. The language was limited to English. Information including general information, participants and cancer characteristics, ctDNA and outcome information were extracted. The quality of the studies was assessed using the Newcastle–Ottawa Scale checklist.

Results: A total of 214 studies were included in the final analysis, encompassing 21,076 patients. The number of studies has increased annually from 2016 to 2022. The most common types of solid tumors studied were colorectal cancer (27.10 %), lung cancer (20.09 %), pancreatic cancer (16.82 %), and breast cancer (14.02 %). The top three journals by number of publications had an impact factor in 2023 greater than 10. Of the studies, the median sample size was 69 (interquartile range: 41–111), 69.81 % had a sample size <100, 68.92 % had a median/mean age ≥60 years, and 74.05 % were from developed countries. Multi-center studies accounted for 40.36 %. Additionally, 29.82 % of the studies had a bias risk score ≤6. Only 16.67 % of studies on liver cancer had a bias risk score >6. The primary criteria not met by the studies included “Adequacy of follow-up of cohorts” (33.33 %), “Assessment of outcome” (32.16 %) and “Representativeness of the exposed cohort” (27.49 %).

Conclusions: The prognostic value of ctDNA in patients with solid tumors is gaining increasing attention, leading to a steady rise in the number of studies. However, many studies still suffer from small sample sizes and a lack of representativeness. Furthermore, details regarding ctDNA detection methods and results reporting are often insufficiently described. There is an urgent need to improve the quality of such research.

1. Introduction

The global burden of cancer remains a significant public health challenge, with millions of new cases diagnosed annually and a substantial proportion of these resulting in mortality.[1] Tracking disease prognosis in cancer patients enables physicians to customize treatment, leading to better outcomes. Circulating tumor DNA (ctDNA) refers to short DNA sequences shed by tumor cells into the systemic circulation[2] and contains tumor-specific genetic and epigenetic abnormalities,[3] which can be obtained through liquid biopsy. Over the past years, ctDNA has demon-

* Corresponding author.
E-mail address: sunfeng@bjmu.edu.cn (F. Sun).
† These authors contributed equally to this work.

https://doi.org/10.1016/j.jncc.2024.10.002
Received 28 July 2024; Received in revised form 24 September 2024; Accepted 30 October 2024
2667-0054/© 2024 Chinese National Cancer Center. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

strated great potential as a noninvasive biomarker in early diagnosis, prognostic stratification, disease monitoring, and treatment response assessment among patients with cancer, leading to better clinical decision-making.[4,5]

Especially in terms of prognosis, patients with tumors who have detectable ctDNA exhibit lower survival rates compared to those without detectable ctDNA.[6,7] Increased ctDNA concentrations are also associated with poorer clinical and radiological outcomes, which suggests prognostic utility for ctDNA.[8,9] Additionally, the long-term management of cancer prognosis often imposes a significant burden of frequent medical visits, along with the corresponding transportation, time and financial costs, which may affect patient compliance. Utilizing liquid biopsy to obtain ctDNA for monitoring prognosis has the potential to increase the convenience and improve the management of patients.[10] Compared to repeated imaging or other more complex methods, regular blood draws at a nearby site are more likely to enhance compliance, pose relatively lower risks, and be more cost-effective. It also allows for repeated sampling over time, providing a deeper understanding of progression and enabling real-time monitoring of cancer burden after treatment,[11] helping clinicians provide the best possible care. Furthermore, not all cancer patients have equal access to new medical technologies and optimal prognostic care, with disparities existing along racial and income lines. Since liquid biopsy may be easily integrated into routine healthcare, ctDNA analysis may help to alleviate many of the access barriers faced by patients throughout the cancer care continuum, contributing to mitigating health disparities.[10]

Due to the above reasons, ctDNA has been proven a promising prognostic marker of predicting survival and relapse across a range of solid tumors such as colorectal cancer,[12] breast cancer,[13] and pancreatic cancer.[14] A meta-analysis included 23 studies with evaluable ctDNA in stage I–IV colorectal cancer patients and results showed that ctDNA status after curative-intent surgery was a significant and independent predictor of recurrence-free survival (RFS).[15] Nader-Marta et al. found that ctDNA detection was significantly associated with worse disease-free survival (DFS) and overall survival (OS) in patients with operable breast cancer.[16] Through early prognostic risk assessment, ctDNA testing can facilitate personalized treatment, allowing cancer patients with a low risk of relapse to avoid adverse treatment effects and unnecessary costs.

Although evidence on the association between ctDNA and prognostic outcomes in patients with solid tumors is rapidly increasing, there is a disparity in the number of studies across different types of cancers. Previous studies have focused on its potential in early-stage cancers and specific tumor types,[3] often without a comprehensive synthesis of the global application and publication status of these studies in different solid tumor types. Additionally, a systematic evaluation and comparison of the study populations and quality across different cancers have not been conducted. Therefore, it is necessary to comprehensively review and assess the current research of ctDNA for prognosis.

This study conducted a systematic review of global research on ctDNA used for predicting prognosis of patients with all kinds of solid tumors. Through this study, we analyzed the publication status, basic characteristics, study populations, exposure and outcome information, and assessed the quality of these studies. Furthermore, we compared studies across different types of cancer. Our aim was to identify areas for improvement in ctDNA prognostic research for various cancers, providing reference and direction for enhancing the quality of future studies. The findings of this review could contribute to enhancing the relevance and impact of ctDNA research in oncology, ultimately leading to better clinical outcomes for cancer patients.

2. Materials and methods

2.1. Protocol and registration

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines[17] to identify studies that assessed the association of ctDNA and clinical prognostic outcomes in patients with solid tumors. The study protocol had been prospectively registered on PROSPERO (CRD42022323474, CRD 42022331326, and CRD42022335695).

2.2. Search strategy

PubMed, Web of Science, Embase, Cochrane Library, Scopus, and the clinical trials.gov database were searched from January 2016 to May 2022. The search strategy involved a combination of relevant keywords and medical subject headings related to ctDNA (e.g., “ctDNA”, “circulating tumor DNA”, and “minimal residual disease”) alongside specific solid tumor types (e.g., “breast cancer”, “lung cancer”, “colorectal cancer”, etc.). Detailed search strategies for each platform are shown in Supplementary material A. The search results were imported into EndNote X8 and duplicates were removed automatically by the software. Subsequently, the titles and abstracts were carefully examined based on the predefined inclusion and exclusion criteria. Finally, potential eligible full-text researches were thoroughly reviewed.

2.3. Study selection

We included only studies where ctDNA was considered a binary variable (positive or negative). The inclusion criteria were: (i) original articles and conference abstracts about cohort studies (prospective or retrospective); (ii) studies that reported patients with solid tumors; (iii) documented collection and measurement of ctDNA; (iv) clinical prognostic outcomes data were reported; and (v) studies written in English.

The exclusion criteria were: (i) studies with no available result (reviews, editorials, comments, or studies with a sample size ≤5) or ongoing studies without results; (ii) studies which only analyzed the association between elevated or reduced ctDNA quantitative levels or cell-free DNA and prognostic outcomes; (iii) studies focusing on diagnosis or screening outcomes; (iv) conference abstracts published before 2020. Other details have been previously described in previous studies.[18,19]

2.4. Data extraction

The following information was extracted from the selected studies: (i) general information: title, first author, publication year, type of study, and journal; (ii) participants and cancer characteristics: country, number of centers, sample size, cancer stage, gender, median or mean age, race, and follow-up duration; (iii) ctDNA information: ctDNA sequencing methods and time points of ctDNA collecting; (iv) study outcomes: OS (defined as the time from surgery or treatment to death),[20] RFS (defined as the time from surgery or treatment to the time of recurrence or death),[21] progression free survival (PFS, defined as the time from surgery or treatment until first evidence of disease progression or death),[20] DFS (defined as the time from surgery or treatment until evidence of disease recurrence).[22] When different outcomes had varying follow-up durations, the longest follow-up duration was selected.

Both the study selection and data extraction were performed independently and concurrently by two researchers. Any discrepancies were resolved through discussion.

2.5. Quality assessment (Risk of bias)

The quality of the included cohort studies was assessed using the Newcastle–Ottawa Scale (NOS) checklist,[23] which consists of eight sections and rates studies on a scale of 0 to 9, indicating poor to high quality, respectively.

2.6. Statistical analysis

We described the categorical variables by count (percentage, excluding the number missing in the denominator). Continuous variables

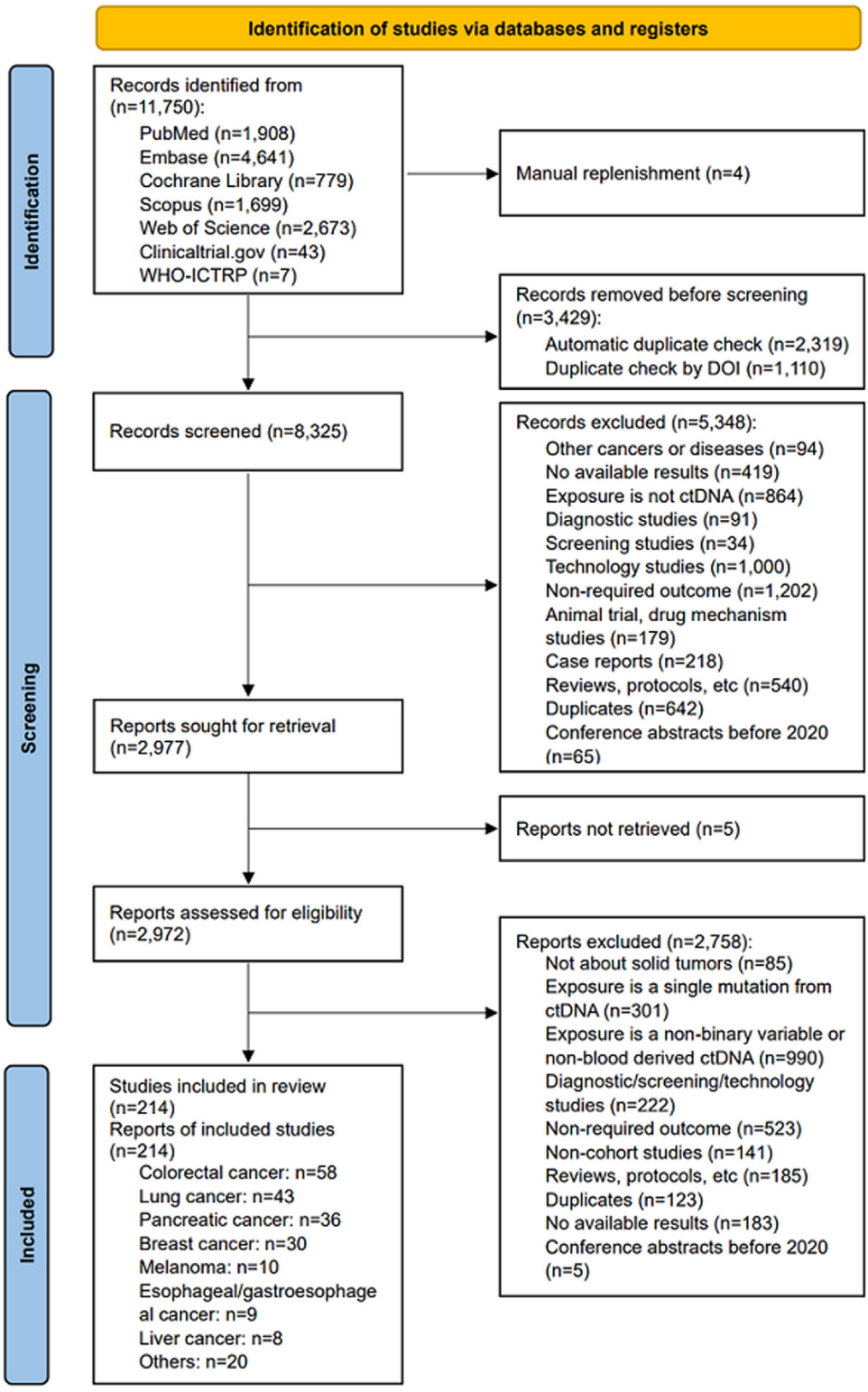

Fig. 1. Flow chart of records inclusion and exclusion. ctDNA, circulating tumor DNA.

were described by mean (standard deviation) or median (interquartile range, IQR). χ^2 test or Kruskal-Wallis H test were used to compare the proportions in different cancer subgroups. A two-tailed significance level of 0.05 was used. All analyses were performed using SPSS 26.0 software. GraphPad Prism 8.4.2 was used for creating graphs.

3. Results

3.1. Literature search results

A total of 11,750 studies were searched, and 214 studies were included in the final analysis (Fig. 1 and Supplementary material Table

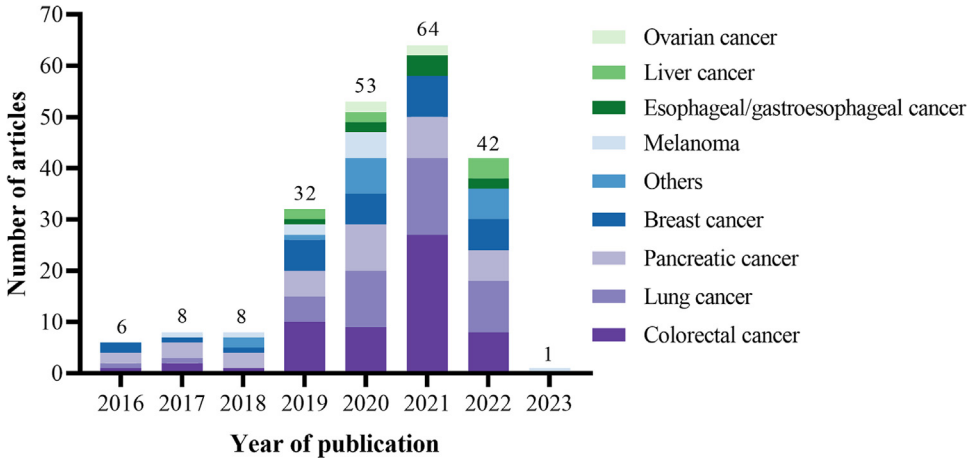

Fig. 2. Number of publications on different cancers from 2016 to 2023.

Table 1
The top 10 journals according to the number of publications included.

Rank	Journal	Count (%)	Impact factor
1	*Journal of Clinical Oncology*	21 (9.81)	45.4
2	*Clinical Cancer Research*	20 (9.35)	11.5
3	*Annals of Oncology*	19 (8.88)	50.5
4	*Cancer Research*	8 (3.74)	11.2
4	*Frontiers in Oncology*	8 (3.74)	4.7
6	*British Journal of Cancer*	7 (3.27)	8.8
7	*International Journal of Cancer*	6 (2.80)	6.4
7	*Molecular Oncology*	6 (2.80)	6.6
7	*Cancers (Basel)*	6 (2.80)	5.2
10	*JAMA Oncology*	5 (2.34)	28.4
–	Others	108 (50.47)	–

B.1). Among these, 27.10 % studies were related to colorectal cancer (including studies on colorectal liver metastases), 20.09 % to lung cancer, 16.82 % to pancreatic cancer, 14.02 % to breast cancer, 4.67 % to melanoma, 4.21 % to esophageal/gastroesophageal cancer, 3.74 % to liver cancer, and 9.35 % of other cancer types (including ovarian cancer, renal cell carcinoma, bladder cancer, gastric cancer, anal squamous cell carcinoma, neuroendocrine neoplasms, oropharyngeal squamous cell carcinoma, osteosarcoma, etc.).

3.2. General information

79.91 % of the studies were original research, and 20.09 % were conference abstracts. The included studies were published between 2016 and 2023 (Fig. 2), with a notable increase in the number of studies from 2019 onwards. Early publications were primarily focused on colorectal cancer, lung cancer, pancreatic cancer, and breast cancer. Table 1 listed the top 10 journals by the number of included studies, with *Journal of Clinical Oncology*, *Clinical Cancer Research*, and *Annals of Oncology* ranking first, second, and third, respectively, all with impact factors in 2023 exceeding 10.

3.3. Participants and cancer characteristics

Table 2 presents the distribution of countries among the total studies and the studies grouping according to different cancer types. The most common countries of origin for the study samples were China (23.78 %), the United States of America (USA) (14.59 %), Japan (10.81 %), France (9.19 %), Australia (5.95 %), and Denmark (5.95 %). Additionally, 40.00 % of the studies on melanoma involved populations from Australia. The proportion of studies from different continents was 38.38 % in Europe, 37.84 % in Asia, 15.68 % in Northern America, and 6.49 % in Oceania, respectively. The difference in distribution of continents among various cancer types was statistically significant (P = 0.009). Studies with population from developing countries only accounted for 25.41 %.

The total sample size across studies was 21,076 individuals (range: 8–1127) and the median sample size was 69 (IQR: 41–111). 14.15 % of the studies had a sample size of <30, 55.66 % of the studies had a sample size of 30–99, and 30.19 % had a sample size of ≥100 (Table 3). There was no statistically significant difference in sample size distribution across different cancer types (P = 0.137). 68.92 % of the studies had a median/mean age of ≥60 years, though over 65.00 % of studies on breast cancer and liver cancer had a median/mean age of <60 years old. Except for studies on breast cancer, lung cancer, and ovarian cancer, male participants were more prevalent in other common cancer types (Supplementary material Table B.2).

Multi-center studies accounted for 40.36 % of the research, while studies on lung cancer, pancreatic cancer, esophageal/gastroesophageal cancer, and liver cancer were predominantly single-center, with single-center studies accounting for 56.25 %, 82.76 %, 100 %, and 100 %, respectively. Only 21.62 % of the studies from China were multi-center, which was lower than that of countries other than China (P = 0.011). Compared to the other stages, the number of studies containing cancer patients with stage IV was the lowest, with only 43.51 %. Median/mean follow-up duration was reported in 138 studies, ranging from 3.7 months to 127.2 months, with 65.94 % of studies having a follow-up time between 12 and 36 months.

3.4. ctDNA information and outcomes

As shown in Table 4, methods for ctDNA sequencing mainly included next generation sequencing (58.54 %) and digital polymerase chain reaction (25.85 %). 9.27 % of the studies used both methods. A detailed analysis of exposure and outcome information showed that 20.28 % of studies collected only one blood sample during the study period to analyze the presence of ctDNA (Table 4). ctDNA collected from baseline (before any treatment) and any time after surgery were most frequently used for association analysis of prognostic outcomes, with 59.05 % and 51.90 % of studies using these time points, respectively. By reported outcomes, 49.53 % of the studies used OS, with a higher proportion in pancreatic cancer and melanoma studies, at 86.11 % and 70.00 %, respectively. DFS/RFS was used in 51.87 % of the studies, with colorectal cancer and liver cancer having higher usage rates at 75.86 % and 75.00 %, respectively. Additionally, 16.82 % of the studies reported both OS and PFS, and 20.56 % reported both OS and DFS/RFS.

3.5. Quality assessment

We also evaluated the completeness of survival analysis presented in 171 full-text studies and found that only 59.76 % reported the respective OS/RFS/DFS/PFS in the ctDNA-positive and ctDNA-negative

Table 2
Country distribution of included studies.

Characteristics	Cancer type, n (%)								*P* value
	Total	Colorectal cancer	Lung cancer	Pancreatic cancer	Breast cancer	Melanoma	Esophageal/gastroesophageal cancer	Liver cancer	
Country ($N = 185$)[a]									0.086
China	44 (23.78)	11 (20.37)	13 (34.21)	6 (19.35)	6 (24.00)	–	2 (33.33)	5 (83.33)	
USA	27 (14.59)	6 (11.11)	7 (18.42)	5 (16.13)	5 (20.00)	1 (10.00)	1 (16.67)	–	
Japan	20 (10.81)	4 (7.41)	–	8 (25.81)	2 (8.00)	–	–	–	
France	17 (9.19)	4 (7.41)	3 (7.89)	3 (9.68)	4 (16.00)	1 (10.00)	–	1 (16.67)	
Australia	11 (5.95)	6 (11.11)	–	–	–	4 (40.00)	1 (16.67)	–	
Denmark	11 (5.95)	8 (14.81)	–	1 (3.23)	–	1 (10.00)	–	–	
Other countries[b]	50 (27.03)	13 (24.07)	14 (36.84)	7 (22.58)	7 (28.00)	3 (30.00)	2 (33.33)	–	
≥ 2 countries	5 (2.70)	2 (3.70)	1 (2.63)	1 (3.23)	1 (4.00)	–	–	–	
Continent ($N = 185$)[a,c]									0.009
Europe	71 (38.38)	24 (44.44)	14 (36.84)	10 (32.26)	11 (44.00)	5 (50.00)	2 (33.33)	1 (16.67)	
Asia	70 (37.84)	17 (31.48)	16 (42.11)	15 (48.39)	8 (32.00)	–	2 (33.33)	5 (83.33)	
Northern America	29 (15.68)	6 (11.11)	7 (18.42)	5 (16.13)	6 (24.00)	1 (10.00)	1 (16.67)	–	
South America	1 (0.54)	–	–	–	–	–	–	–	
Oceania	12 (6.49)	7 (12.96)	–	–	–	4 (40.00)	1 (16.67)	–	
≥ 2 continents	2 (1.08)	–	1 (2.63)	1 (3.23)	–	–	–	–	
Type of country ($N = 185$)[a,d]									0.051
Developing	47 (25.41)	11 (20.37)	14 (36.84)	7 (22.58)	6 (24.00)	–	2 (33.33)	5 (83.33)	
Developed	137 (74.05)	43 (79.63)	23 (60.53)	24 (77.42)	19 (76.00)	10 (100)	4 (66.67)	1 (16.67)	
Both covered	1 (0.54)	–	1 (2.63)	–	–	–	-	–	

[a] Only 185 studies reported the country from which the study population came.
[b] Other countries include UK, Netherlands, Germany, Spain, Italy, South Korea, Belgium, Austria, Sweden, Russia, Germany, Czech Republic, Norway, Singapore, Greece, Canada, Brazil, Israel, a total of 18 countries.
[c] One study of oropharyngeal squamous cell carcinoma was from South America. Two studies were conducted with populations from more than two continents, one of which was conducted with populations from Oceania and Asia, and the other was from Asia, Europe, and Northern America.
[d] Among the countries involved in this study, China, Russia and Brazil were considered as developing countries. USA, Japan, France, Denmark, Australia, UK, Netherlands, Germany, Spain, Italy, South Korea, Belgium, Austria, Sweden, Germany, Czech Republic, Norway, Singapore, Greece, Canada, and Israel were considered as developed countries. "-" indicates no study published.
Abbreviations: UK, the United Kingdom; USA, the United States of America.

Table 3
Basic characteristics of included studies.

Characteristics	Cancer type, n (%)								*P* value
	Total	Colorectal cancer	Lung cancer	Pancreatic cancer	Breast cancer	Melanoma	Esophageal/gastroesophageal cancer	Liver cancer	
Sample size (N = 212)[a]									0.137
< 30	30 (14.15)	4 (6.90)	9 (20.93)	7 (20.00)	4 (13.79 %)	1 (10.00)	1 (11.11)	1 (12.50)	
30–49	46 (21.70)	13 (22.41)	9 (20.93)	5 (14.29)	9 (31.03 %)	–	3 (33.33)	4 (50.00)	
50–99	72 (33.96)	18 (31.03)	13 (30.23)	9 (25.71)	9 (34.48 %)	6 (60.00)	5 (55.56)	3 (37.50)	
100–199	49 (23.11)	17 (29.31)	8 (18.60)	12 (34.29)	7 (20.69 %)	3 (30.00)	–	–	
≥200	15 (7.08)	6 (10.34)	4 (9.30)	2 (5.71)	–	–	–	–	
Age, years (N = 148)[a]									<0.001
< 50	8 (5.41)	–	–	–	7 (38.89)	–	–	–	
50–59	38 (25.68)	10 (21.74)	6 (20.69)	1 (4.17)	10 (55.56)	3 (37.50)	–	4 (66.67)	
60–69	87 (58.78)	32 (69.57)	21 (72.41)	19 (79.17)	1 (5.56)	4 (50.00)	6 (85.71)	1 (16.67)	
≥70	15 (10.14)	4 (8.70)	2 (6.90)	4 (16.67)	–	1 (12.50)	1 (14.29)	1 (16.67)	
Number of centers (N = 166)[a]									<0.001
Multi	67 (40.36)	27 (60.00)	14 (43.75)	5 (17.24)	13 (54.17)	4 (50.00)	–	–	
Single	99 (59.64)	18 (40.00)	18 (56.25)	24 (82.76)	11 (45.83)	4 (50.00)	7 (100)	7 (100)	
Cancer stages (N = 131)[a]									
Stage I	73 (55.73)	14 (43.75)	20 (58.82)	9 (64.29)	8 (50.00)	–	5 (83.33)	7 (100)	0.002
Stage II	96 (73.28)	25 (78.13)	23 (67.65)	10 (71.43)	15 (93.75)	1 (11.11)	5 (83.33)	6 (85.71)	0.001
Stage III	111 (84.73)	29 (90.63)	28 (82.35)	12 (85.71)	13 (81.25)	7 (77.78)	6 (100)	4 (57.14)	0.384
Stage IV	57 (43.51)	10 (31.25)	13 (38.24)	11 (78.57)	3 (18.75)	6 (66.67)	2 (33.33)	1 (14.29)	0.007
Follow-up time, months (N = 138)[a]									0.156
< 12	15 (10.87)	2 (5.41)	2 (6.45)	5 (22.73)	4 (18.18)	–	–	–	
12–23	52 (37.68)	12 (32.43)	14 (45.16)	11 (50.00)	5 (22.73)	3 (37.50)	1 (16.67)	2 (100)	
24–35	39 (28.26)	14 (37.84)	7 (22.58)	3 (13.64)	4 (18.18)	4 (50.00)	4 (66.67)	–	
≥ 36	32 (23.19)	9 (24.32)	8 (25.81)	3 (13.64)	9 (40.91)	1 (12.50)	1 (16.67)	–	

[a] Only 212 studies reported sample size, 148 reported average/median age, 166 reported the number of centers included in the study, 131 reported cancer stages, and 138 reported average/median follow-up time.
"-" indicates no study published.

Table 4
ctDNA-related outcome information and bias risk score of included studies.

Characteristics	Cancer type, n (%)								*P* value
	Total	Colorectal cancer	Lung cancer	Pancreatic cancer	Breast cancer	Melanoma	Esophageal/gastroesophageal cancer	Liver cancer	
ctDNA sequencing method (N = 205)									<0.001
NGS only	120 (58.54)	33 (58.93)	32 (82.05)	11 (32.35)	20 (68.97)	1 (10.00)	6 (66.67)	6 (75.00)	
dPCR only	53 (25.85)	14 (25.00)	2 (5.13)	15 (44.12)	4 (13.79)	9 (90.00)	–	2 (25.00)	
NGS and dPCR	19 (9.27)	4 (7.14)	2 (5.13)	6 (17.65)	3 (10.34)	–	2 (22.22)	–	
Others	13 (6.34)	5 (8.93)	3 (7.69)	2 (5.88)	2 (6.90)	–	1 (11.11)	–	
The number of times ctDNA was collected (N = 212)									0.070
1	43 (20.28)	10 (17.24)	3 (7.14)	13 (37.14)	6 (20.00)	3 (30.00)	2 (22.22)	2 (25.00)	
≥ 2	169 (79.72)	48 (82.76)	39 (92.86)	22 (62.86)	24 (80.00)	7 (70.00)	7 (77.78)	6 (75.00)	
Time point of collecting ctDNA for association analysis (N = 210)									
Baseline	124 (59.05)	26 (44.83)	21 (50.00)	28 (82.35)	22 (75.86)	6 (60.00)	5 (55.56)	3 (37.5)	0.005
During or after NAT and before surgery	26 (12.38)	10 (17.24)	1 (2.38)	2 (5.88)	10 (34.48)	–	2 (22.22)	–	0.001
Any time after surgery	109 (51.90)	45 (77.59)	18 (42.86)	11 (32.35)	11 (37.93)	4 (40.00)	4 (44.44)	5 (62.5)	<0.001
During or after CRT and without surgery	17 (8.10)	2 (3.45)	7 (16.67)	3 (8.82)	2 (6.90)	–	1 (11.11)	–	0.255
Outcome (N = 214)									
OS	106 (49.53)	15 (25.86)	20 (46.51)	31 (86.11)	11 (36.67)	7 (70.00)	5 (55.56)	5 (62.50)	<0.001
PFS	63 (29.44)	4 (6.90)	19 (44.19)	13 (36.11)	8 (26.67)	6 (60.00)	4 (44.44)	2 (25.00)	<0.001
DFS/RFS	111 (51.87)	44 (75.86)	17 (39.53)	17 (47.22)	12 (40.00)	2 (20.00)	4 (44.44)	6 (75.00)	<0.001
Association analysis									
Median OS/RFS/DFS/PFS (N = 164)	98 (59.76)	15 (33.33)	23 (69.70)	23 (82.14)	13 (59.09)	10 (100)	5 (83.33)	4 (66.67)	<0.001
Events (N = 171)	138 (80.70)	48 (100)	26 (78.79)	14 (48.28)	23 (92.00)	10 (100)	4 (66.67)	5 (83.33)	<0.001
HR/OR value (N = 171)	147 (85.96)	43 (89.58)	26 (78.79)	26 (89.66)	20 (80.00)	10 (100)	5 (83.33)	5 (83.33)	0.583
Confidence interval of HR/OR (N = 171)	140 (81.87)	42 (87.50)	24 (72.73)	25 (86.21)	20 (80.00)	10 (100)	4 (66.67)	3 (50.00)	0.109
Bias score (N = 171)									0.099
1–6	51 (29.82)	15 (31.25)	8 (24.24)	10 (34.48)	5 (20.00)	3 (30.00)	1 (16.67)	5 (83.33)	
7–9	120 (70.18)	33 (68.75)	25 (75.76)	19 (65.52)	20 (80.00)	7 (70.00)	5 (83.33)	1 (16.67)	

Abbreviations: ctDNA, circulating tumor DNA; NGS, next generation sequencing; dPCR, digital polymerase chain reaction; NAT, neoadjuvant therapy; CRT, chemoradiotherapy; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; RFS, recurrence-free survival; HR, hazard ratio; OR, odds ratio; "-", no study published.

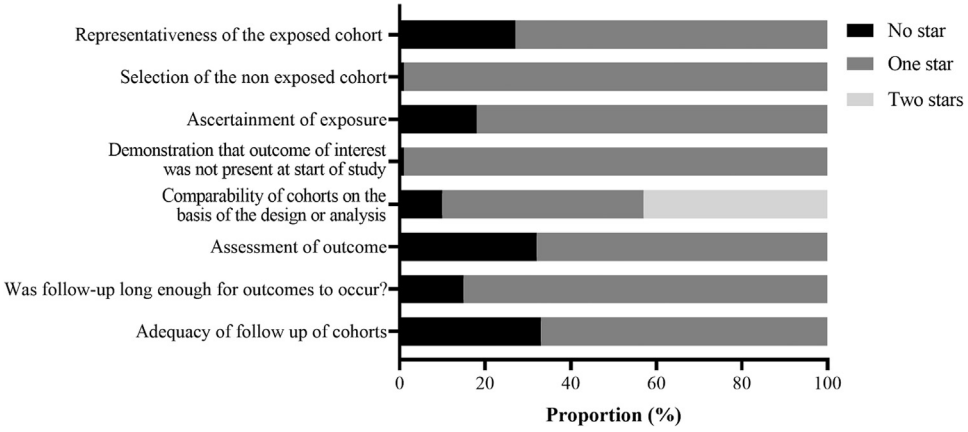

Fig. 3. Risk of bias of included studies.

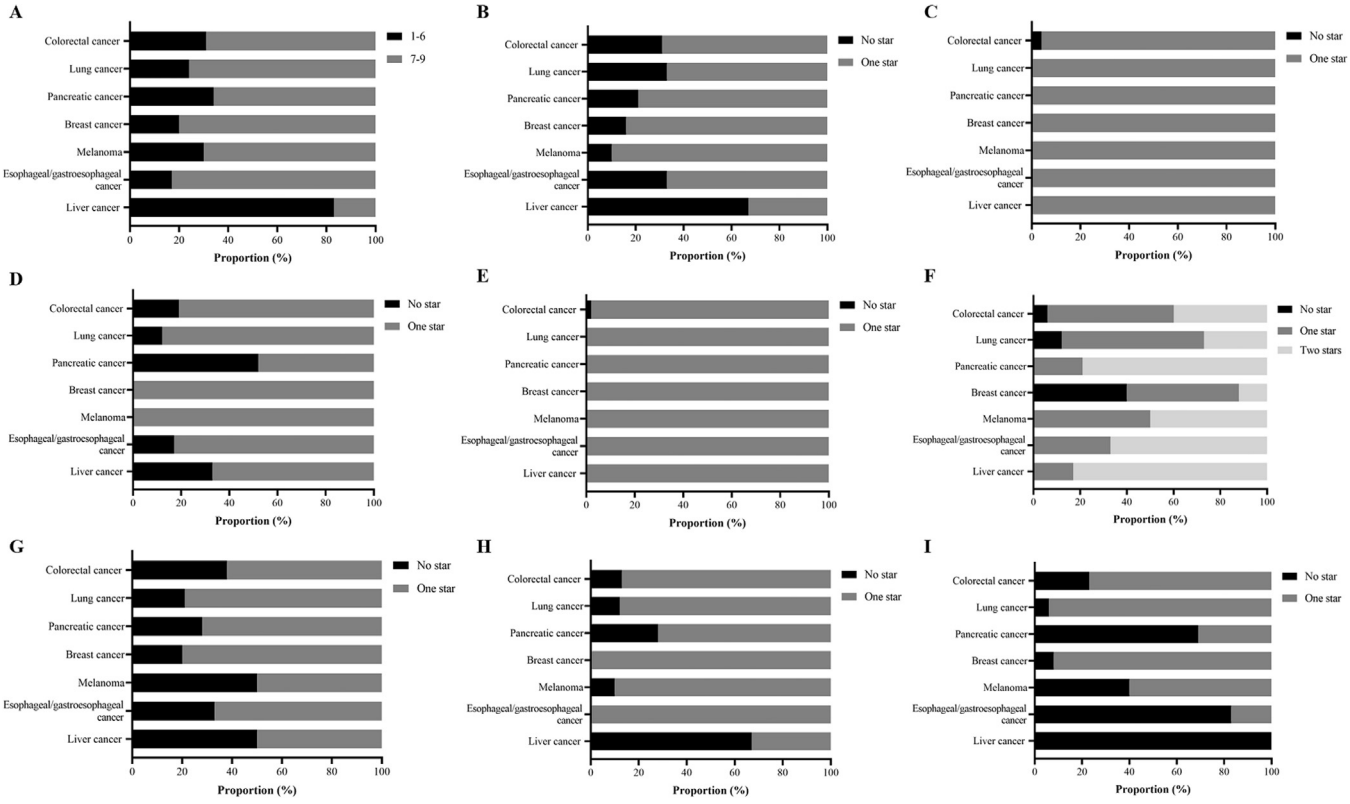

Fig. 4. Detailed risk of bias of different solid tumors. (A) Total risk score. (B) Representativeness of the exposed cohort. (C) Selection of the non-exposed cohort. (D) Ascertainment of exposure. (E) Demonstration that outcome of interest was not present at start of study. (F) Comparability of cohorts on the basis of the design or analysis. (G) Assessment of outcome. (H) Was follow-up long enough for outcomes to occur? (I) Adequacy of follow up of cohorts.

groups (Table 4). Quality assessment of the full-text studies indicated that 29.82 % had a bias risk score of ≤6, with 1.17 % scoring 1–3 and 28.65 % studies scoring 4–6. Only 16.67 % of studies on liver cancer had a risk score >6 (Table 4). As shown in Fig. 3, the main items with "no star" ratings in the bias assessment were "Representativeness of the exposed cohort" (27.49 %), "Assessment of outcome" (32.16 %), and "Adequacy of follow-up of cohorts" (33.33 %). The differences in scores for the items "Ascertainment of exposure" ($P < 0.001$), "Comparability of cohorts on the basis of the design or analysis" ($P < 0.001$), "Was follow-up long enough for outcomes to occur?" ($P = 0.001$), and "Adequacy of follow-up of cohorts" ($P = 0.001$), were statistically significant among different types of solid tumors (Fig. 4). Compared to other solid tumors, studies related to pancreatic cancer had a higher proportion (51.72 %) of "no star" ratings for the item "Ascertainment of exposure". Breast cancer studies had a lower proportion (12.00 %) of "two stars" ratings for the item "Comparability of cohorts on the basis of the design or analysis". Liver cancer studies had a higher proportion of "no star" ratings for the items "Was follow-up long enough for outcomes to occur?" (66.67 %) and "Adequacy of follow-up of cohorts" (100.00 %).

4. Discussion

This systematic review included a total of 214 studies on the prognostic value of ctDNA, with the majority focusing on colorectal cancer, lung cancer, pancreatic cancer, and breast cancer. The number of studies has increased rapidly since 2019, and the journals in which these studies were published generally have relatively high impact factors, highlighting the significant clinical importance of this type of research. How-

ever, most of the current studies have small sample sizes and lack representativeness, often focusing on older adults, males, and early-stage patients, mainly from developed countries, and single-center cohorts, with few long-term follow-ups. Additionally, some studies have insufficiently standardized reporting, particularly in definitions of exposures and outcomes. Research on liver cancer, in particular, urgently needs to improve its study quality.

This study found that the most extensively researched solid tumors were colorectal cancer, lung cancer, pancreatic cancer, breast cancer, and melanoma. Previous ctDNA reviews which did not restrict their focus to specific cancers have also primarily concentrated on these tumors.[24,25] The number of studies on different cancers is closely related to the incidence of these cancers. Global cancer statistics for the year 2022 showed that lung cancer was the most frequently diagnosed cancer (12.4 % of all cancers), followed by breast cancer (11.6 %) and colorectal cancer (9.6 %).[26] The incidence of different cancer types varies significantly across countries, influenced by factors such as environmental conditions, genetic predispositions, and lifestyle factors.[27] The incidence of various cancers shows significant regional variation, with the highest rates of melanoma reported in regions such as Australasia due to factors like higher exposure to ultraviolet radiation and many fair-skinned populations of European descent, while lung cancer incidence is disproportionately higher in Eastern Asia, influenced by factors such as smoking prevalence and air pollution.[26,28,29] Our review found that a large proportion of melanoma studies were conducted in Australia. This geographic variation in cancer prevalence is critical to understand the global application of ctDNA, as it suggests that certain cancers are more extensively studied in regions where they are more common, leading to a concentration of research efforts in these areas.

Although the incidence of pancreatic cancer ranks only the 13th, the number of related ctDNA prognostic studies ranks the third. This may be due to the high frequency of gene mutations such as *KRAS* in pancreatic cancer and the presence of a vast vasculature around the vicinity of the primary tumor and the liver metastases.[14,30] With *KRAS* mutations found in nearly all pancreatic ductal adenocarcinoma patients, this cancer type is considered the most *RAS*-addicted of all cancers.[31] Additionally, compared to other tumors, many pancreatic cancer patients exhibit no obvious symptoms in the early stages and are often diagnosed at an unresectable stage, which makes tumor tissue difficult to obtain,[32] while ctDNA can be conveniently acquired through non-invasive liquid biopsy. These factors make pancreatic cancer a frequent focus for ctDNA prognostic research.

The differences in outcomes and gender ratio of different tumors are also related to the characteristics of the cancer. For instance, we found that studies on pancreatic cancer primarily focused on OS, whereas research related to colorectal cancer predominantly centered on DFS/RFS. This is likely due to pancreatic cancer being one of the cancers with the poorest prognosis, with a short survival time after diagnosis. Only 15 %–20 % of patients are eligible for surgical resection[33,34] and only about 12 % of patients are predicted to survive beyond 5 years.[35] Therefore, predicting OS for pancreatic cancer patients may be more appropriate than assessing the risk of recurrence. In contrast, the 5-year relative survival rate for colorectal cancer patients is approximately 65 %,[36] and about 15 % and 30 % of stage II and III patients experience recurrence after treatment, respectively.[37] Early ctDNA detection could identify patients who may benefit from subsequent treatment, and help better allocate monitoring resources to these high-risk patients for recurrence.[38] The ability to prognosticate cancer using ctDNA offers significant clinical benefits, enabling early identification and screening of high-risk patients for more aggressive treatments.[39] This personalized approach improves survival outcomes and reduces the burden of overtreatment.

This study found that the median/mean age of participants in most cancers was predominantly over 60 years old. Jonathan et al. found that individuals aged 60–69 years were more willing to participate in cancer surveys compared to other age groups,[40] which is consistent with our findings. Previous research has highlighted issues of underrepresentation of elderly patients in cancer-related clinical trials.[41] However, since this systematic review only included cohort studies and ctDNA analysis as a non-invasive test, there may not have been strict upper age limits in the inclusion criteria. Nevertheless, with the increasing incidence rates of early-onset cancer (cancers diagnosed in people <50 years) from 2010 to 2019,[42] it is also important to understand the efficacy of ctDNA in prognosis of non-elderly patients. Therefore, future research should aim to enhance participation among this demographic to supplement evidence pertaining to younger cancer patients. Furthermore, many included studies have the problem of small sample sizes, lower proportion of stage IV patients, a predominant focus on single-center settings, and mainly from developed countries, leading to inadequate representativeness and affecting the generalizability, equity, and reliability of study outcomes. This is also one of the directions for improvement.

This study also found that many articles lack reporting standards. It is essential to provide detailed information on baseline characteristics of participants, follow-up duration, the specific methods used for ctDNA detection, limits of detection, the types of gene panels used, clear definitions of outcomes, and complete results. Different methods can lead to variations in ctDNA detection rates. Additionally, various studies have different definitions of whether ctDNA is detectable (positive or negative), but many studies do not clearly specify this. These gaps in reporting can affect the reproducibility of results and their clinical application.

This study was the first to systematically summarize the global application status of ctDNA in the prognosis of all solid tumors. We not only extracted research details but also conducted quality assessment for all original studies and compared the application of ctDNA and quality on different cancers. This helps in targeting improvements to research quality.

However, this study has some limitations. First, to control the number of studies and heterogeneity, only cohort studies were included, and the focus was solely on ctDNA obtained from blood, and studies where exposure was a binary variable. The conversion of ctDNA from positive to negative has been recognized as a significant prognostic indicator.[43,44] However, as the transition between ctDNA positivity and negativity is difficult to standardize across different studies, and the number of included studies was already substantial, we didn't further include the conversion in our analysis. Secondly, this study is a qualitative analysis and did not examine the association between ctDNA and various prognostic outcomes, but our studies of specific meta-analyses for breast cancer have been published[18,19] and the results for other cancer types will be detailed in future articles. Additionally, there was considerable heterogeneity among the included studies due to differences in ctDNA classifications, such as the varying limits of detection among different assays, which presents challenges in comparing study results, particularly in late-stage cancers where the majority of patients will be ctDNA-positive.[45] Besides prognosis, the applications of ctDNA in early diagnosis and treatment response are other exciting areas, but they are beyond the scope of this review. Finally, we limited this review to studies published after 2016, following the US Food and Drug Administration's first approval of liquid biopsy tests that year,[46] and excluded earlier studies. However, another review found that the majority of studies were published after 2018, suggesting a small potential impact on our findings.[47]

5. Conclusions

In summary, studies on using ctDNA to predict the prognosis of patients with solid tumors hold significant research value and application prospects, with increasingly gaining attention. However, its application in less common solid tumors requires further exploration. Future studies should focus on increasing sample sizes and representativeness, incorporating more evidence from non-elderly patients and developing countries, as well as providing detailed descriptions of ctDNA detection methods and results in their reports. These steps will help improve the quality of research in this area.

Declaration of competing interest

The authors declare that they have no conflict of interests that may have influenced the results of this work.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (grant numbers: 72474008, 72074011), the Capital's Funds for Health Improvement and Research (grant number: 2024–1G-4023), and the Special Project for Director, China Center for Evidence Based Traditional Chinese Medicine (grant number: 2020YJSZX-2).

Author contributions

M.Z. and X.C. conducted the data curation and writing original draft. M.Z. and Q.Z. performed the formal analysis and investigation. N.G., B.C., H.Z., W.C, and F.S. reviewed and edited the manuscript. F.S. conducted the supervision and validation. F.S. was responsible for the conceptualization, funding acquisition and project administration.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jncc.2024.10.002.

References

1. Deo SVS, Sharma J, Kumar S. GLOBOCAN 2020 report on global cancer burden: challenges and opportunities for surgical oncologists. *Ann Surg Oncol*. 2022;29(11):6497–6500. doi:10.1245/s10434-022-12151-6.
2. Chae YK, Oh MS. Detection of minimal residual disease using ctDNA in lung cancer: current evidence and future directions. *J Thorac Oncol*. 2019;14(1):16–24. doi:10.1016/j.jtho.2018.09.022.
3. Peng Y, Mei W, Ma K, Zeng C. Circulating tumor DNA and minimal residual disease (MRD) in solid tumors: current horizons and future perspectives. *Front Oncol*. 2021;11:763790. doi:10.3389/fonc.2021.763790.
4. Campos-Carrillo A, Weitzel JN, Sahoo P, et al. Circulating tumor DNA as an early cancer detection tool. *Pharmacol Ther*. 2020;207:107458. doi:10.1016/j.pharmthera.2019.107458.
5. Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol*. 2021;32(2):229–239. doi:10.1016/j.annonc.2020.11.007.
6. Nygaard AD, Garm Spindler K-L, Pallisgaard N, Andersen RF, Jakobsen A. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. *Lung Cancer*. 2013;79(3):312–317. doi:10.1016/j.lungcan.2012.11.016.
7. Gray ES, Rizos H, Reid AL, et al. Circulating tumor DNA to monitor treatment response and detect acquired resistance in patients with metastatic melanoma. *Oncotarget*. 2015;6(39):42008–42018. doi:10.18632/oncotarget.5788.
8. Dawson S-J, Tsui DWY, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med*. 2013;368(13):1199–1209. doi:10.1056/NEJMoa1213261.
9. Parkinson CA, Gale D, Piskorz AM, et al. Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: a retrospective study. *PLoS Med*. 2016;13(12):e1002198. doi:10.1371/journal.pmed.1002198.
10. Febbo PG, Allo M, Alme EB, et al. Recommendations for the equitable and widespread implementation of liquid biopsy for cancer care. *JCO Precis Oncol*. 2024;8:e2300382. doi:10.1200/PO.23.00382.
11. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer*. 2017;17(4):223–238. doi:10.1038/nrc.2017.7.
12. Mi J, Han X, Wang R, Ma R, Zhao D. Circulation tumour DNA in predicting recurrence and prognosis in operable colorectal cancer patients: a meta-analysis. *Eur J Clin Invest*. 2022;52(12):e13842. doi:10.1111/eci.13842.
13. Papakonstantinou A, Gonzalez NS, Pimentel I, et al. Prognostic value of ctDNA detection in patients with early breast cancer undergoing neoadjuvant therapy: a systematic review and meta-analysis. *Cancer Treat Rev*. 2022;104:102362. doi:10.1016/j.ctrv.2022.102362.
14. Guven DC, Sahin TK, Yildirim HC, Aktepe OH, Dizdar O, Yalcin S. A systematic review and meta-analysis of the association between circulating tumor DNA (ctDNA) and prognosis in pancreatic cancer. *Crit Rev Oncol Hematol*. 2021;168:103528. doi:10.1016/j.critrevonc.2021.103528.
15. Chidharla A, Rapoport E, Agarwal K, et al. Circulating tumor DNA as a minimal residual disease assessment and recurrence risk in patients undergoing curative-intent resection with or without adjuvant chemotherapy in colorectal cancer: a systematic review and meta-analysis. *Int J Mol Sci*. 2023;24(12):10230. doi:10.3390/ijms241210230.
16. Nader-Marta G, Monteforte M, Agostinetto E, et al. Circulating tumor DNA for predicting recurrence in patients with operable breast cancer: a systematic review and meta-analysis. *ESMO Open*. 2024;9(3):102390. doi:10.1016/j.esmoop.2024.102390.
17. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097.
18. Guo N, Zhou Q, Zhang M, et al. The prognostic role of circulating tumor DNA across breast cancer molecular subtypes: a systematic review and meta-analysis. *J Natl Cancer Cent*. 2024;4(2):153–161. doi:10.1016/j.jncc.2024.04.005.
19. Guo N, Zhou Q, Chen X, et al. Circulating tumor DNA as prognostic markers of relapsed breast cancer: a systematic review and meta-analysis. *J Natl Cancer Cent*. 2024;4(1):63–73. doi:10.1016/j.jncc.2024.01.003.
20. Lebwohl D, Kay A, Berg W, Baladi JF, Zheng J. Progression-free survival: gaining on overall survival as a gold standard and accelerating drug development. *Cancer J*. 2009;15(5):386–394. doi:10.1097/PPO.0b013e3181b9c5ec.
21. Liu Y, Yang T, Wei Y-W. What is the difference between overall survival, recurrence-free survival and time-to-recurrence? *Br J Surg*. 2020;107(12):e634. doi:10.1002/bjs.11945.
22. Kilickap S, Demirci U, Karadurmus N, Dogan M, Akinci B, Sendur MAN. Endpoints in oncology clinical trials. *J BUON*. 2018;23(7):1–6.
23. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. 2010;25(9):603–605. doi:10.1007/s10654-010-9491-z.
24. Markou A, Tzanikou E, Lianidou E. The potential of liquid biopsy in the management of cancer patients. *Semin Cancer Biol*. 2022;84:69–79. doi:10.1016/j.semcancer.2022.03.013.
25. Reichert ZR, Morgan TM, Li G, et al. Prognostic value of plasma circulating tumor DNA fraction across four common cancer types: a real-world outcomes study. *Ann Oncol*. 2023;34(1):111–120. doi:10.1016/j.annonc.2022.09.163.
26. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. doi:10.3322/caac.21834.
27. Sobti RC, Thakur M, Kaur T. Cancer: epidemiology, racial, and geographical disparities. In: Sobti RC, Sugimura H, Sobti A, eds. *Molecular Biomarkers for Cancer Diagnosis and Therapy*. Singapore: Springer Nature Singapore; 2024:31–52. doi:10.1007/978-981-99-3746-2_3.
28. Rundle CW, Militello M, Barber C, Presley CL, Rietcheck HR, Dellavalle RP. Epidemiologic burden of skin cancer in the US and worldwide. *Curr Dermatol Rep*. 2020;9(4):309–322. doi:10.1007/s13671-020-00311-4.
29. Arnold M, Singh D, Laversanne M, et al. Global burden of cutaneous melanoma in 2020 and projections to 2040. *JAMA Dermatol*. 2022;158(5):495–503. doi:10.1001/jamadermatol.2022.0160.
30. Uesato Y, Sasahira N, Ozaka M, Sasaki T, Takatsuki M, Zembutsu H. Evaluation of circulating tumor DNA as a biomarker in pancreatic cancer with liver metastasis. *PLoS One*. 2020;15(7):e0235623. doi:10.1371/journal.pone.0235623.
31. Waters AM, Der CJ. KRAS: the critical driver and therapeutic target for pancreatic cancer. *Cold Spring Harb Perspect Med*. 2018;8(9):a031435. doi:10.1101/cshperspect.a031435.
32. Pietrasz D, Pécuchet N, Garlan F, et al. Plasma circulating tumor DNA in pancreatic cancer patients is a prognostic marker. *Clin Cancer Res*. 2017;23(1):116–123. doi:10.1158/1078-0432.Ccr-16-0806.
33. Blackford AL, Canto MI, Klein AP, Hruban RH, Goggins M. Recent trends in the incidence and survival of stage 1A pancreatic cancer: a surveillance, epidemiology, and end results analysis. *J Natl Cancer Inst*. 2020;112(11):1162–1169. doi:10.1093/jnci/djaa004.
34. Stoffel EM, Brand RE, Goggins M. Pancreatic cancer: changing epidemiology and new approaches to risk assessment, early detection, and prevention. *Gastroenterology*. 2023;164(5):752–765. doi:10.1053/j.gastro.2023.02.012.
35. Halbrook CJ, Lyssiotis CA, Pasca di Magliano M, Maitra A. Pancreatic cancer: advances and challenges. *Cell*. 2023;186(8):1729–1754. doi:10.1016/j.cell.2023.02.014.
36. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(3):233–254. doi:10.3322/caac.21772.
37. Osterman E, Glimelius B. Recurrence risk after up-to-date colon cancer staging, surgery, and pathology: analysis of the entire swedish population. *Dis Colon Rectum*. 2018;61(9):1016–1025. doi:10.1097/dcr.0000000000001158.
38. Malla M, Loree JM, Kasi PM, Parikh AR. Using circulating tumor DNA in colorectal cancer: current and evolving practices. *J Clin Oncol*. 2022;40(24):2846–2857. doi:10.1200/jco.21.02615.
39. Panet F, Papakonstantinou A, Borrell M, Vivancos J, Vivancos A, Oliveira M. Use of ctDNA in early breast cancer: analytical validity and clinical potential. *NPJ Breast Cancer*. 2024;10(1):50. doi:10.1038/s41523-024-00653-3.
40. Banks J, Hollinghurst S, Bigwood L, Peters TJ, Walter FM, Hamilton W. Preferences for cancer investigation: a vignette-based study of primary-care attendees. *Lancet Oncol*. 2014;15(2):232–240. doi:10.1016/s1470-2045(13)70588-6.
41. Sedrak MS, Freedman RA, Cohen HJ, et al. Older adult participation in cancer clinical trials: a systematic review of barriers and interventions. *CA Cancer J Clin*. 2021;71(1):78–92. doi:10.3322/caac.21638.
42. Koh B, Tan DJH, Ng CH, et al. Patterns in cancer incidence among people younger than 50 years in the US, 2010 to 2019. *JAMA Netw Open*. 2023;6(8):e2328171. doi:10.1001/jamanetworkopen.2023.28171.
43. Tolmeijer SH, Boerrigter E, Sumiyoshi T, et al. Early on-treatment changes in circulating tumor DNA fraction and response to enzalutamide or abiraterone in metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2023;29(15):2835–2844. doi:10.1158/1078-0432.Ccr-22-2998.

44. 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. doi:10.1038/s41591-022-02115-4.
45. Cohen SA, Liu MC, Aleshin A. Practical recommendations for using ctDNA in clinical decision making. *Nature*. 2023;619(7969):259–268. doi:10.1038/s41586-023-06225-y.
46. US Food & Drug Administration. Cobas EGFR mutation test v2. 2016. Accessed June 18, 2024. https://www.fda.gov/drugs/resources-information-approved-drugs/cobas-egfr-mutation-test-v2#:í:text=On%20June%201%2C%202016%2C%20the%20U.%20S.%20Food,cancer%20%28NSCLC%29%20eligible%20for%20treatment%20with%20Tarceva%C2%AE%20%28erlotinib%29.
47. Jones RP, Pugh SA, Graham J, Primrose JN, Barriuso J. Circulating tumour DNA as a biomarker in resectable and irresectable stage IV colorectal cancer; a systematic review and meta-analysis. *Eur J Cancer*. 2021;144:368–381. doi:10.1016/j.ejca.2020.11.025.