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Original Article

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Original Article

Prognostic value of circulating tumor DNA in different cancer types detected by ultra-low-pass whole-genome sequencing: a systematic review and patient-level survival data meta-analysis

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Abstract

Ultra-low-pass whole-genome sequencing (ULP-WGS) (≤0.5 × coverage) of plasma cell-free DNA (cfDNA) has emerged as a low-cost, promising tool to assess the circulating tumor DNA (ctDNA) fraction. This meta-analysis aims to summarize the current findings and comprehensively investigate the prognostic value of baseline ctDNA detected by ULP-WGS in solid tumors. A systematic review was carried out by searching PubMed/MEDLINE and Scopus databases to identify eligible studies conducted between January 2014 and January 2024. Inclusion criteria comprised studies with reported overall survival and progression-free survival outcomes across therapy-naïve patients with different solid tumors. All patients underwent baseline ULP-WGS of plasma cfDNA and were categorized as ctDNA positive (tumor fraction ≥10%) or negative (tumor fraction <10%). A one-stage meta-analysis was performed using patient-level survival data reconstructed from published articles. A Cox proportional hazards model with shared frailty was used to assess the difference in survival between arms. A total of six studies, comprising 620 patients (367 negative ctDNA and 253 positive ctDNA), were included in the overall survival analysis, while five studies, involving 349 patients (212 negative ctDNA and 137 positive ctDNA), were included in the progression-free survival analysis. The meta-analysis showed that patients with baseline positive ctDNA had a significantly higher risk of death (HR = 2.60, 95% CI: 2.01–3.36) and disease progression (HR = 2.28, 95% CI: 1.71–3.05) compared to those with negative ctDNA. The presence of a positive ctDNA at baseline is associated with increased risk of death and progression in patients with same-stage cancer.

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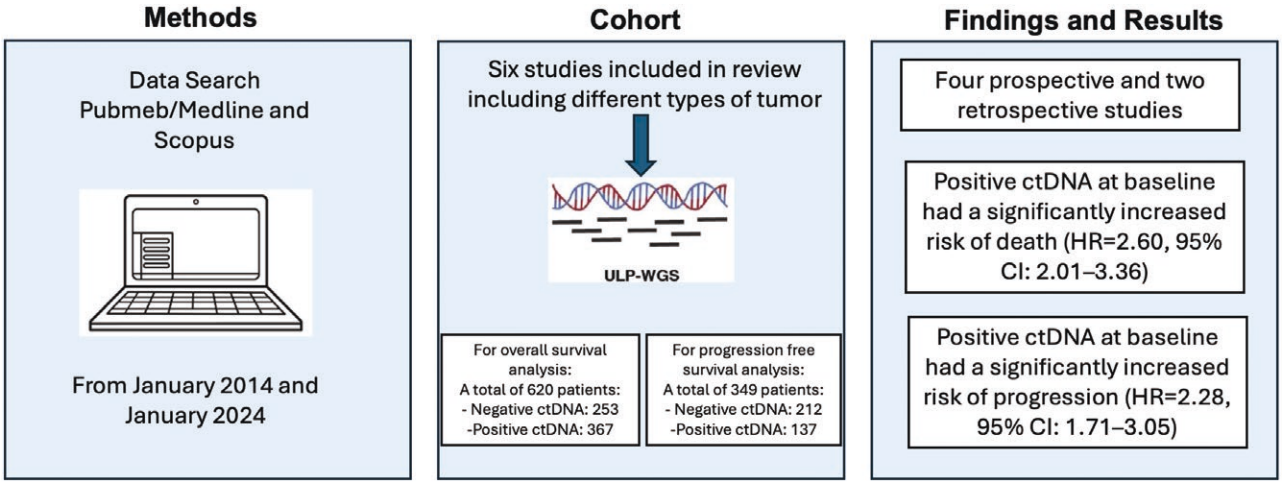
Graphical Abstract



Keywords: circulating tumor DNA; progression-free survival; overall survival; patient-level meta-analysis; whole-genome sequencing

Introduction

Precision medicine attempts to evaluate the specific characteristics of each patient's disease, often employing next-generation sequencing (NGS) of tumor tissue obtained through standard biopsy procedures. However, the conventional method of sampling and sequencing tumor tissue faces significant challenges, especially for patients with metastatic cancer. Repeated tumor biopsies are often impractical in these cases, and as a result, they fail to capture the dynamic evolution of the disease over time (1,2). Thus, there is a clear need for novel noninvasive biomarkers that may help predict prognosis and monitor treatment response, thereby guiding a more personalized therapy.

The genomic analysis of cell-free DNA (cfDNA) extracted from plasma is considered a noninvasive method for prognostication and evaluation of treatment response. This is due to the composition of cfDNA, which includes short fragments of DNA originating from both normal and tumor cells, including circulating tumor DNA (ctDNA) or tumor fraction (3). Unlike a single tumor tissue biopsy, ctDNA provides a more precise depiction of the comprehensive mutational profile and heterogeneity observed across various lesions within an individual patient. ctDNA analysis can be repeated over time, allowing dynamic monitoring of the disease progression and treatment response (3–5), and offers valuable insights into prognostic differences between patients in the same tumor stage (6,7).

Short-read sequencing NGS panel assays are commonly used to detect single nucleotide variations (SNVs) or small indels in tumors or ctDNA. These NGS panels have high sequencing depth (ranging from 5000X to 12000X) so that somatic SNVs in low abundance clones can be confidently identified (8). Instead, the lower depth and broader genomic coverage provided by WGS is preferred over targeted panels for assessing large structural variations, inferring Copy Number Alterations (CNAs), or calculating the ctDNA fraction (6). For this reason, WGS has emerged as a valuable tool for detecting ctDNA and CNAs. The latter represents a crucial subset of somatic mutations, encompassing amplifications or deletions of chromosomal regions. These alterations often lead to the overexpression of oncogenes or the loss of tumor suppressor genes (TSGs), thereby contributing to the process of carcinogenesis (9). For example, cfDNA WGS with an approximate sequencing depth equivalent to five times the entire genome (5x) has demonstrated potential in detecting ctDNA and clinically relevant genomic alterations in tumors (10–13). Nevertheless, performing WGS at such depth incurs significant sequencing expenses that limit standard clinical use. Some studies have explored the use of low-pass whole-genome sequencing (LP-WGS) with reduced depth coverage. Even with a coverage as low as 1.5x, the expenses associated with this approach remain prohibitive for routine clinical implementation (14).

To address this challenge, ultra-low-pass whole-genome sequencing (ULP-WGS) (with coverage ≤0.5x) has emerged as a cost-effective and promising alternative to estimate the amount of ctDNA and to detect CNAs (14). The application of ULP-WGS of plasma cfDNA to detect ctDNA has gained traction across cancer types (3). However, detection of ctDNA using ULP-WGS may be challenging due to the often-low tumor fractions in a high background of nontumoral cfDNA in patients with minimal tumor burden (1,3). The objective of this systematic review is to elucidate the prognostic value of detecting ctDNA using ULP-WGS of plasma cfDNA across various solid tumors. By synthesizing existing literature, we aim to provide insights into the clinical utility of this approach and its implications for personalized cancer care.

Methods

Search strategy and study selection

A systematic literature review was conducted in accordance with Cochrane recommendations (15). Two electronic databases, namely PubMed/MEDLINE and Scopus, were systematically searched to retrieve English-language studies published between January 2014 and January 2024. Additionally, a manual search and review of reference lists were conducted.

Study retrieval was performed by using "ultra-low-pass whole-genome sequencing," "low-pass whole-genome sequencing," and "shallow whole-genome sequencing" as free text words combined with "tumor," as well as a manual

search and review of the reference list to identify relevant studies. Various combinations of these keywords were utilized. The study protocol was registered and periodically updated on the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42024524793).

Three authors (M.S., D.A., and J.A.) independently screened article titles and abstracts, followed by blind and duplicate full-text reviews to identify eligible studies. For articles with disagreements, a decision was reached by group consensus.

Inclusion criteria and data extraction

The analysis included both prospective and retrospective studies involving patients with solid tumors. These studies compare survival outcomes (overall survival (OS) and/or progression-free survival (PFS)) between groups with positive ctDNA or negative ctDNA. In instances with three arms, we included the groups of patients with the highest and lowest tertiles of ctDNA, classifying them as positive or negative ctDNA. A positive ctDNA status was defined as a tumor fraction of ≥10%. This threshold is based on ichorCNA's validated performance, which requires a tumor fraction of ≥10% in cfDNA for accurate genomic profiling. At a 3% tumor fraction threshold, ichorCNA achieves 95% sensitivity for detecting tumor presence (based on 1288 positive cases) and 91% specificity for predicting tumor absence (based on 22 healthy donors). When the tumor fraction threshold is raised to 10%, the analysis demonstrated 91% sensitivity for tumor detection and 100% specificity for confirming tumor absence (3). The studies should include Kaplan–Meier curves and either log-rank tests or hazard ratios (HR). In our study, the baseline timepoint was defined as the period following diagnosis but prior to any treatment initiation (naïve patients).

Only analyses using ULP-WGS of plasma cfDNA with an average genome-wide fold coverage of ≤0.5x were considered eligible. We compared costs across three sequencing platforms based on market costs in Spain: Nextseq, Novaseq, and MGI-400. At coverage ≤0.5x, ULP-WGS offers a significantly more cost-effective option in each platform for routine clinical practice (Supplementary Table S1, Supplementary Fig. S1).

Exclusion criteria included studies that utilized cfDNA from sources other than plasma, patients without solid tumors or with hematological cancers, and those lacking survival data. Metaanalyses, review articles, case reports, and study protocols were also excluded.

Several key aspects were extracted from each study, including the first author's name, year of publication, cancer type, cfDNA input, library preparation method, sequencing technique, median depth coverage of WGS, percentage of patients with detectable ctDNA, prevalent CNAs, *P*-values from log-rank tests of OS and PFS, and HRs with corresponding 95% confidence intervals.

Risk of bias assessment

The risk of bias for the selected studies was assessed using the Newcastle-Ottawa Scale (NOS) (16). Two reviewers (M.S. and D.A.) performed the assessments independently in duplicate, and any disagreements were resolved by consensus. Three areas (i.e. selection, comparability, and outcome) were reviewed and evaluated in eight domains, according to the NOS. The studies were categorized as low, moderate, and high quality according to the score obtained: <4 points, 4–6 points, and ≥7 points, respectively. Disagreements were resolved by group consensus (Supplementary Table S2).

Statistical analysis

A meta-analysis of OS and PFS based on reconstructed patient-level survival data was performed. Survival data were initially extracted from published Kaplan–Meier plots using DigitizeIt software. The iterative algorithm described by Guyot *et al.* was then applied to transform patient-level survival data, estimating time-to-event parameters while maintaining the monotonicity constraint (17–19). A one-stage meta-analysis using patient-level survival data was conducted. Cox proportional hazards models were used to assess the difference in survival between arms. A gamma shared frailty model was used for the primary analysis to account for between-study heterogeneity (20). A stratified model and a two-stage meta-analysis using an inverse variance fixed-effects model were used as part of the sensitivity analyses. The marginal model was also conducted. Additionally, as a nonparametric method, the difference in restricted median survival time (RMST) was estimated using the naive Kaplan–Meier method (21). As an additional sensitivity analysis, the survival analysis was repeated, excluding studies that only reported results from patients in early stages, focusing solely on those with advanced stages.

Statistical heterogeneity was evaluated using the Cochrane Q test and the I^2 statistic, which quantifies the proportion of total variability across studies due to heterogeneity. I^2 values of 25%, 50%, and 75% were considered to indicate low, moderate, and high heterogeneity, respectively (22). Publication bias was assessed visually using funnel plots.

To assess the proportionality assumption, visual plots of predicted versus observed survival functions and scaled Schoenfeld residuals were examined, and the Grambsch–Therneau test was performed (23).

P-values less than 0.05 were considered statistically significant. Statistical analysis was conducted using STATA version 16 (StataCorp, College Station, Texas, USA).

Results

Data retrieval

The search strategy initially yielded 323 articles. Following the removal of duplicates and screening of abstracts, 52 articles remained for full-text review (Fig. 1). Detailed information regarding studies that did not meet the inclusion criteria and the reasons for their exclusion are provided in the Supplementary Table S3. The data from the study by Sogbe *et al.* was extracted directly from the original dataset rather than from the manuscript.

As shown in Table 1, six studies were included in the analysis (6,24–28), all of which were available for OS analysis, with five also contributing to PFS analysis. For OS, a total of 620 patients were analyzed, with 367 in the negative ctDNA group and 253 in the positive ctDNA group. For PFS, the studies involved 349 patients, with 212 in the negative ctDNA group and 137 in the positive ctDNA group. The patients had a range of solid tumors, including metastatic nonsmall cell lung cancer (24,27,28), localized osteosarcoma (25), metastatic castration-resistant prostate cancer (26), and advanced

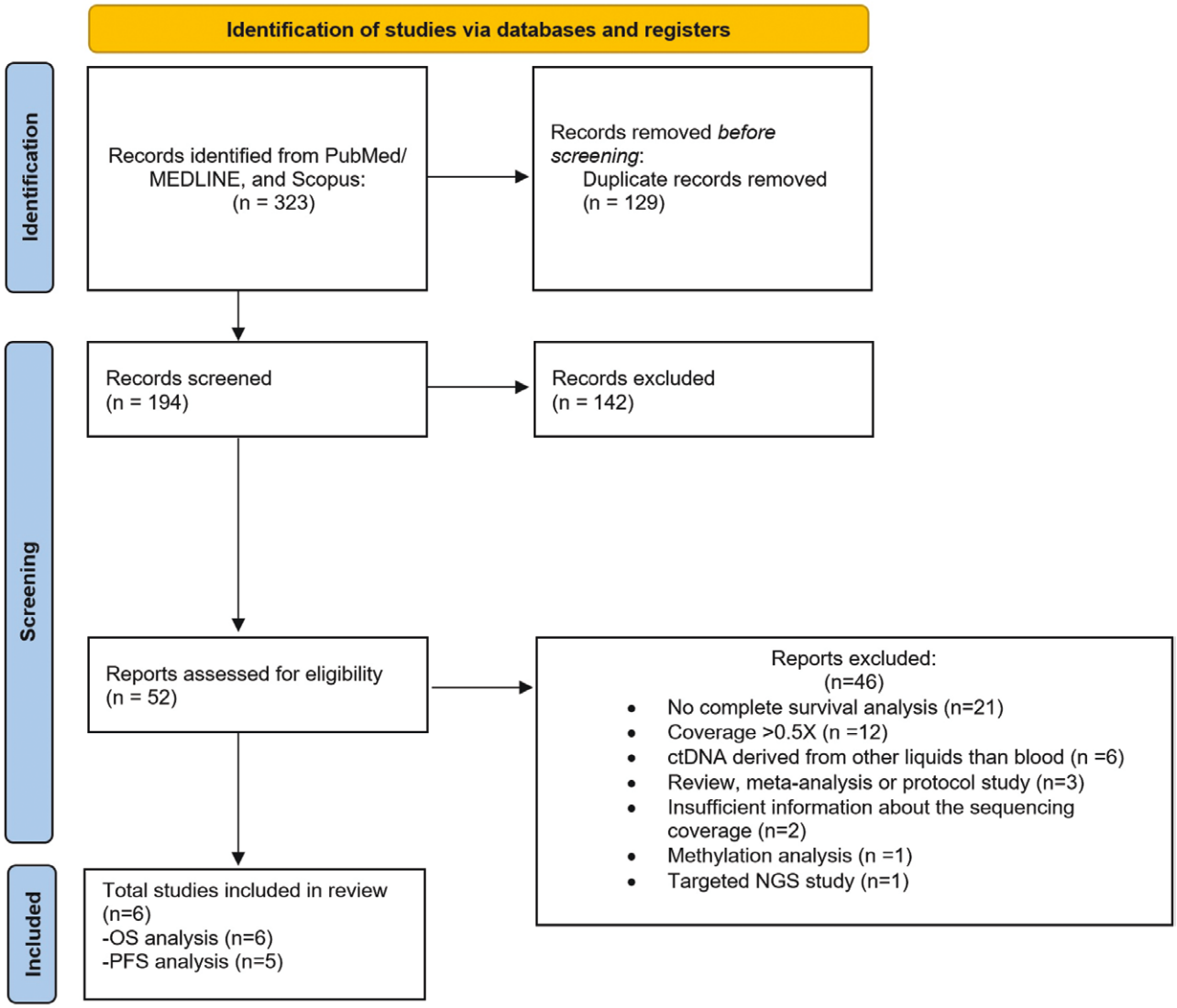


Figure 1. Flowchart of the selection of publications included in the feasibility analysis and meta-analysis

hepatocellular carcinoma (6). The studies were conducted in Western countries, namely Spain, Germany, the USA, and Denmark.

Feasibility of ULP-WGS of cfDNA

To assess the technical aspects of ULP-WGS of plasma cfDNA, we reviewed the preanalytical and analytical parameters from all studies. ULP-WGS was conducted on plasma samples, achieving a median coverage of 0.3X (range: 0.2–0.5X) utilizing cfDNA inputs ranging from 1 to 50 ng of total nucleic acids. Extraction of cfDNA was consistently performed from plasma collected in EDTA tubes across all studies. The detection rate of ctDNA varied from 28% to 61% across the studies. Four out of the five studies focused on patients in advanced stages of cancer, with only one study involving patients at an early stage of the disease.

Copy number alteration analysis

In addition to detecting ctDNA, ULP-WGS of plasma cfDNA also facilitated the analysis of CNAs. The most frequently reported CNAs were gains in 3q, 8q, 1q, 7q, and 5p, while losses encompassed 13q, 8p, 4q, 13q, 16q, and 5q. Two studies failed to report CNAs (27,28). Among the four studies that reported CNAs, only three explored their association with OS (6,25,26). In patients receiving systemic treatment for advanced hepatocellular carcinoma, the loss of the long arm of chromosomes 5 and 16 was associated with shorter median OS ($P < 0.05$) (6). Moreover, in patients diagnosed with metastatic castration-resistant prostate cancer, the loss of 8p and the gain of 9q were significantly associated with worse OS ($P < 0.05$) (26). Similarly, in patients with localized osteosarcoma, the presence of a gain in 8q exhibited a trend towards worse OS ($P < 0.10$) (25).

Survival analysis

Upon reconstructing the survival data, the extracted patient-level survival data matched the originally published values. Three published analyses did not satisfy the proportional hazards assumption (26–28) (S2.1, S2.7, and S2.16 in the Supplementary Material). OS and PFS analyses are summarized in Table 2.

Table 1. Preanalytical and analytical parameters of all studies which performed ULP-WGS of cfDNA.

First author	Type of tumor	cfDNA input	Library Prep	Sequencing method	% of patients with detectable ctDNA	Median depth coverage of WGS	Most common CNAs	Clinicopathologic features in the multivariable Cox proportional hazards model	Main results
Chen et al. (24)	Metastatic NSCLC	4–50 ng	KAPA library quantification kit (Kapa Biosystems)	Illumina NextSeq	32.71% (35/107)	0.5x	3q gain	NA	Patients with positive ctDNA had significantly increased hazard of death ($P = 0.026$, HR = 1.97; 95% CI:1.08–3.57) and increased hazard of progression ($P = 0.23$, HR = 1.33; 95% CI: 0.82–2.15).
Shulman et al. (25)	Localized osteosarcoma	40 ng	Kapa hyper prep kit (Kapa Biosystems)	HiSeq 2500	56.94% (41/72)	0.2x	8q gain	Age <14 versus age ≥14 years and sex	Patients with positive ctDNA had increased hazard of death ($P = 0.075$, HR = 3.96; 95% CI: 0.86–18.10) and increased hazard of progression ($P = 0.172$, HR = 1.94; 95% CI: 0.74–5.07).
Nørgaard et al. (26)	Metastatic castration-resistant prostate cancer	7–50 ng	Kapa hyper prep kit (Kapa Biosystems)	Illumina Novaseq	36.36% (36/99)	0.5x	8p loss, 8q gain, and 13q loss	PSA (ng/ml), alkaline phosphatase (U/L), metastases (bone only, lymph node, bone and lymph node, visceral)	Patients with positive ctDNA had significantly increased hazard of death ($P = 0.000$, HR = 3.75; 95% CI: 2.09–6.76) and significantly increased hazard of progression ($P = 0.000$, HR = 4.12: 95% CI, 2.50–6.79).
Sogbe et al. (6)	Advanced HCC	2.5 ng	NEBNext Ultra II DNA library prep kit	NextSeq2000	58.06% (18/31)	0.3x	Gains: 1q, 8q, 7q, and 5p Losses: 8p, 4q, 13q, 16q, and 5q	Extrahepatic spread, macrovascular invasion, tumor size ≥ 5 cm, AFP ≥20 ng/ml, and type of treatment (sorafenib versus immunotherapy).	Patients with positive ctDNA had significantly increased hazard of death ($P = 0.016$, HR = 3.49; 95% CI: 1.26–9.61) and increased hazard of progression ($P = 0.094$, HR = 2.03; 95% CI: 0.88–4.65).
Carbonell et al. (27)	Metastatic NSCLC	4–20 ng	NEBNext UltraTM DNA library prep kit	HiSeq2500	17.5% (7/40)	0.2x	NA	Histology (adenocarcinoma, squamous cell carcinoma), smoking, sex, immune checkpoint inhibitor treatment line (first versus second)	Patients with positive ctDNA had increased hazard of death ($P = 0.111$, HR = 2.05; 95% CI: 0.84–4.99) and significantly increased hazard of progression ($P = 0.001$, HR = 4.73; 95% CI, 1.89–11.81).
Dietz et al. (28)	Metastatic ALK + NSCLC	1–2.5 ng	Kapa Hyper Prep kit (Kapa Biosystems)	HiSeq4000q	42.9% (116/271)	0.5x	NA	NA	Patients with positive ctDNA had significantly increased hazard of death ($P < 0.001$, HR = 2.7; 95% CI: 1.8–4.0). This study has no PFS analysis.

CNA, copy number alteration; NA, not available.

Table 2. Survival analysis using reconstructed patient-level data survival information.

Survival analysis			
Overall survival	One-stage meta-analysis	Effect size (95% CI)	*P*-value
	Marginal HR	1.857 (1.453–2.372)	**<0.0001**
	Stratified HR	2.656 (2.047–3.445)	**<0.0001**
	Shared frailty HR*	2.603 (2.012–3.368)	**<0.0001**
	"Two stage" meta-analysis		
	HR (random-effects)	2.64 (2.03–3.45)	**<0.0001**
	HR (random-effects) (excluding nonproportional hazards assumption)	2.87 (1.96–4.19)	
	Nonparametric models		
	RMST difference (up to 36 months)	−6.3 months (−8.56 to −4.12)	**<0.001**
Progression-free survival	One-stage meta-analysis	Effect size (95% CI)	*P*-value
	Marginal HR	1.360 (1.043–1.774)	**0.0246**
	Stratified HR	2.336 (1.736–3.143)	**<0.0001**
	Shared frailty HR[a]	2.281 (1.708–3.047)	**<0.0001**
	"Two stage" meta-analysis		
	HR (fixed-effects)	2.39 (1.80–2.19)	**<0.0001**
	HR (fixed-effects) (excluding nonproportional hazards assumption)	2.22 (1.64–3.01)	
	Nonparametric models		
	RMST difference (up to 36 months)	−3.7 months (−6.80 to −0.61)	**0.019**

HR, hazard ratio; RMST, restricted median survival time.
[a]Primary analysis.

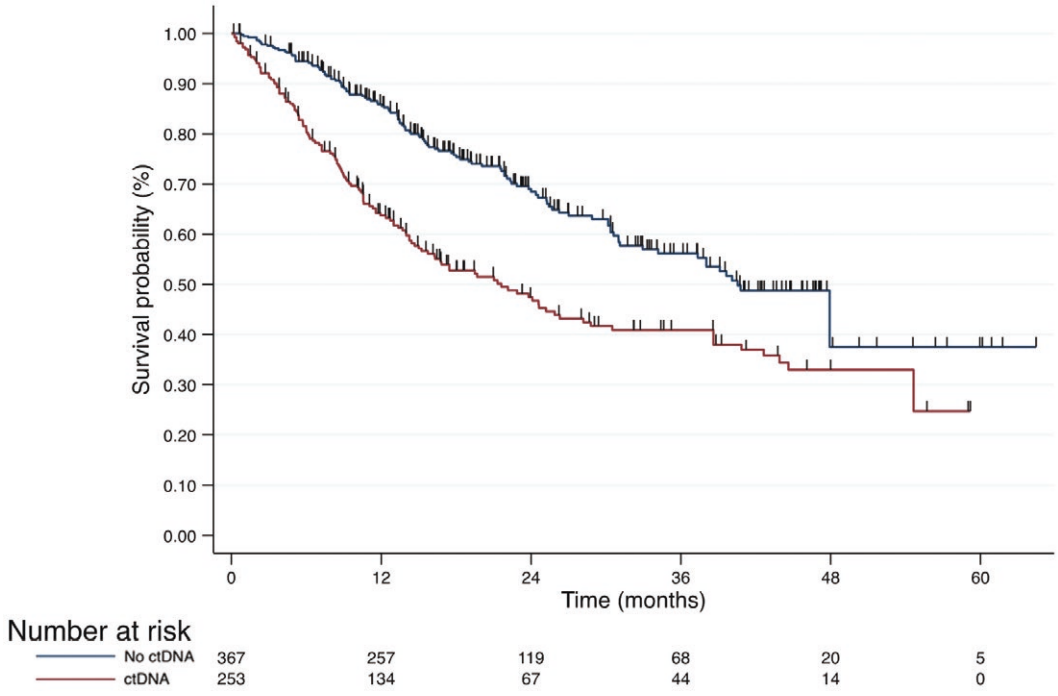


Figure 2. Overall survival based on the presence of circulating tumor DNA (ctDNA). The figure presents reconstructed survival curves accompanied by number-at-risk tables

The Kaplan–Meier-estimated OS rates at 1- and 3-years were 63.7% (95% CI, 57.2–69.5) and 40.9% (95% CI, 33.7–48.0) respectively in the negative ctDNA group, and 85.9% (95% CI, 81.7–89.2) and 56.2% (95% CI, 49.1–62.7) in the positive ctDNA group (log-rank *P*-value < 0.0001) (Fig. 2).

Shared frailty Cox regression analysis showed that having a positive ctDNA was significantly associated with an increase in the risk of death compared to having a negative ctDNA (HR = 2.60; 95% CI, 2.01–3.36; $P < 0.0001$). The positive ctDNA group was also associated with a significant decrease of 6.3 months of RMST at 3 years ($P < 0.001$), which translates into a relative decrease in life expectancy of 22.9%. In the two-stage meta-analysis, the pooled HR was 2.64 (95% CI, 2.03–3.45; $P < 0.0001$) (Supplementary Fig. S2).

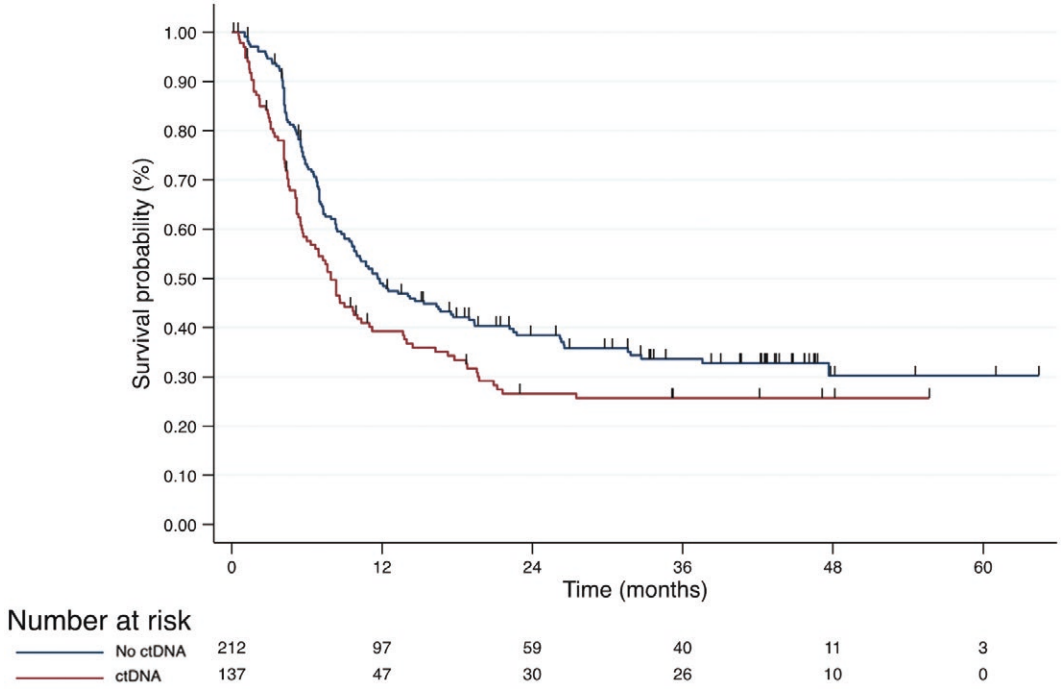


Figure 3. Progression-free survival based on the presence of circulating tumor DNA (ctDNA). The figure presents reconstructed survival curves accompanied by number-at-risk tables

For the 212 patients in the negative ctDNA group, the 1- and 3-PFS rates were 48.9% (95% CI, 41.8–55.7) and 33.6% (95% CI, 26.8–40.5), respectively. For the positive ctDNA group, the corresponding PFS rates at 1- and 3-years were 39.3% (95% CI, 30.8–47.6) and 25.7% (95% CI, 18.3–33.7) (log-rank *P*-value = 0.0224) (Fig. 3).

Shared frailty Cox proportional hazards model demonstrated a significant increase in the risk of progression associated with positive ctDNA (HR = 2.28; 95% CI, 1.71–3.05; $P < 0.0001$). This result was consistent with that obtained in the two-stage meta-analysis, which found a similar increased hazard associated with positive ctDNA (HR = 2.39; 95% CI, 1.80–3.19; $P < 0.0001$) (Supplementary Fig S3). The RMST between both arms at 3 years was 3.7 months lower in the positive ctDNA group ($P < 0.019$), which translates into a relative decrease in progression-free expectancy of 25.2%.

In the sensitivity analysis that omitted patients in early stages, one study focusing on localized osteosarcoma was excluded (25). This analysis revealed that the presence of ctDNA in advanced stages correlated with a statistically elevated hazard of death and progression, along with a widened gap in OS and PFS rates at 1 and 3 years between patients with and without ctDNA (Supplementary Figs S4 and S5). Furthermore, in the RMST analysis, the presence of ctDNA was associated with a notably reduced 3-year RMST exceeding 8 months for both OS and PFS (Supplementary Table S4).

Discussion

To our knowledge, this is the first meta-analysis to comprehensively investigate the prognostic role of ctDNA detected by ULP-WGS in patients with different solid tumors. Patients with positive ctDNA status at baseline have significantly worse prognosis (both PFS and OS) than those with negative ctDNA status.

Tumor staging with clinical and imaging features allows allocating patients in groups with different prognosis. Based on our results, detectable ctDNA may serve as a noninvasive biomarker of worse prognosis in patients with advanced stages of different cancers. The identification of ctDNA using ULP-WGS could provide an additional signal of tumor aggressiveness and may allow more accurate risk stratification, treatment planning, and follow-up.

ULP-WGS presents several advantages over other options for routine clinical practice. Easy processing, low cost, and rapid readout stand out as the most relevant ones. The scope of ULP-WGS is not to detect specific somatic variants, which are covered by high-depth targeted NGS panels, but instead to identify copy number gains or losses using genomic inference. This may yield a precise map of large genomic aberrations and the interesting possibility of precisely calculating the tumor fraction. Notably, detecting ctDNA and CNAs with this approach possesses a new and distinctive capacity to gather comprehensive somatic information in any type of cancer from a circulating analyte that can be repeatedly measured along the patient's journey. The pool of ctDNA analyzed by ULP-WGS could also capture tumor heterogeneity.

Limitations of ULP-WGS include its lower sensitivity and the need of a relatively high tumor burden for effective detection of ctDNA and CNAs, as shown by the poor performance in previous studies. Studies in prostate cancer have shown that ctDNA was not detected in patients with local versus metastatic disease (26,29). A lower sensitivity in earlier stages has also been reported in hepatocellular carcinoma (6). Reduced necrosis and vascularization of localized small tumors with diminished proliferative rate may account for these observations (29,30). More sensitive methods would be needed in earlier stages or for the detection of minimal residual disease, such as the detection of tumor-derived SNV using droplet digital PCR or

deep-targeted sequencing (14). Understanding the strengths and limitations of ULP-WGS underscores the importance of tailoring the approach to the specific clinical context and disease stage for optimal utility.

CNAs are large somatic alterations found in cancer cells, including amplifications or deletions commonly associated with overexpressed oncogenes or the loss of TSGs. CNAs are believed to contribute to carcinogenesis, tumor progression, and the development of therapy resistance (31,32). In patients with metastatic prostate and breast cancer, tumor-derived CNAs were detected in ctDNA using ULP-WGS, and these were found to be concordant with those observed in the corresponding tumor tissue (3).

In the present meta-analysis, we summarize data from a variety of cancers demonstrating distinct patterns of CNAs, leading to changes in the expression of well-known oncogenes, or TSGs. For instance, in metastatic nonsmall cell lung cancer, the gain of 3q was frequent that encompass oncogenes such as *PIK3CA* and *SOX2* (24). Osteosarcoma frequently exhibits a gain in 8q (25), indicating an amplification in MYC, which is associated with a poorer prognosis (33). Regarding metastatic castration-resistant prostate cancer, it commonly presents with the loss of 8p and 13q harboring TSGs such as *CSMD1* and *ITM2B*, respectively. Besides *MYC*, the gain of 8q encompasses also *AEG1*, presumably promoting tumor progression (26,34). In hepatocellular carcinoma, prevalent gains involve chromosomes 1q, 8q, 7q, and 5p (6), which encompass well-established key driver oncogenes like *MCL1*, *MET*, *MYC*, and *TERT*, respectively. Conversely, frequent losses were observed in regions such as 8p, 4q, 13q, 16q, and 5q (6), housing TSGs including *GTF2H2*, *NAIP*, *OCLN* (all in 5q13.2 chromosomal band) (35), and E-cadherin (located in 16q22.1) (9,36,37). Notably, chromosome 8p harbors several oncogenes, three of them clustered in 8p21 (*CCDC25*, *ELP3*, *SH2D4A*), and others spread along 8p22 (*DLC1*, *SORBS3*) and 8p11 (*PLPBP*) (35), while 4q loss could be linked to the TSG *ING2* (9,38), and 13q loss to *RB1* (39).

This study is subject to several limitations that warrant consideration. Firstly, notable disparities were observed among studies regarding preanalytical conditions, as detailed in Table 1. These variations contributed to discrepancies in result comparability. Discrepancies in technical sequencing aspects, such as sequencing coverage and the quantity of cfDNA input, were evident across studies.

Another limitation is the diverse efficacy of the treatments received in each clinical scenario. Additionally, not all studies have demonstrated survival analyses incorporating CNAs. Conducting sequential analysis is essential for promptly evaluating treatment responses and informing decisions about whether to maintain or modify therapies (27). These limitations underscore the need for further research to address these inconsistencies and enhance the robustness of conclusions drawn from ctDNA analysis in cancer management.

In summary, our study suggests that the detection of ctDNA using ULP-WGS of plasma cfDNA deserves to be further explored as a prognostic marker for advanced stage patients with various cancer types.

Supplementary data

Supplementary data is available at *Carcinogenesis* online.

Acknowledgments

None declared.

Author contributions

M.S., D.A., and J.A. equally contributed to the conception and design of the research. M.S., J.A., and D.A. contributed to the acquisition and analysis of the data. M.S. and D.A. contributed to the interpretation of the data. M.S., B.S., D.A., and J.A. drafted the manuscript. All authors have read and approved the final manuscript.

Ethics statement

Not applicable.

Conflict of interest

B.S. reports consultancy fees from Adaptimmune, Astra Zeneca, Bayer, BMS, Boston Scientific, Eisai, Incyte, Ipsen, MSD, Roche, Sanofi, and Sirtex Medical; speaker fees from Astra Zeneca, Bayer, BMS, Eisai, Incyte, Roche, and Sirtex Medical. M.D.T.-A.: travel grants from ESAI, Bayern, Roche, and Pfizer. J.A. is member of a scientific steering committee of Roche Spain and has received fees for talks by Pfizer and Roche. The rest of the authors have nothing to declare.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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