Research Article

Cancer Epidemiology, Biomarkers & Prevention

Circulating DNA and Survival in Solid Tumors

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Abstract

Background: The ability to undertake molecular analysis to inform on prognosis and predictors of response to therapy is limited by accessibility of tissue. Measurement of total circulating free DNA (cfDNA) or circulating tumor DNA (ctDNA) in peripheral blood may allow easier access to tumor material and help to predict clinical outcomes.

Methods: A systematic review of electronic databases identified publications exploring the association between cfDNA or ctDNA and overall survival (OS) in solid tumors. HRs for OS were extracted from multivariable analyses and included in a meta-analysis. Pooled HRs were computed and weighted using generic inverse variance and random-effect modeling. For studies not reporting multivariable analyses, univariable ORs were estimated from Kaplan–Meier curves for OS at 1 and 3 years.

Results: Thirty-nine studies comprising 4,052 patients were included in the analysis. Detection of ctDNA was associated with a significantly worse OS in multivariable analyses [HR, 2.70; 95% confidence interval (CI), 2.02–3.61; $P < 0.001$). Similar results were observed in the univariable analyses at 3 and 1 year (OR, 4.83; 95% CI, 3.20–7.28; $P < 0.001$).There was also a statistically significant association between high total cfDNA and worse OS for studies reporting multivariable and univariate data at 3 years (HR, 1.91; 95% CI, 1.59–2.29; $P < 0.001$ and OR, 2.82; 95% CI, 1.93–4.13; $P < 0.001$, respectively).

Conclusions: High levels of total cfDNA and presence of ctDNA are associated with worse survival in solid tumors.

Impact: Circulating DNA is associated with worse outcome in solid tumors. *Cancer Epidemiol Biomarkers Prev; 25(2); 399–406. ©2015 AACR.*

Introduction

Identification of molecular mechanisms associated with cancer prognosis and response to therapy has seen substantial advances in recent years (1, 2). Typically, such identification involves molecular techniques that require the availability of tumor material from either a primary or metastatic site. Availability of such specimens is often limited as additional biopsies are cumbersome and not always feasible, and this restricts the evaluation of molecular markers in many studies and in daily practice (3, 4). Furthermore, molecular studies are undertaken typically on archival tumor material, which may not be representative of the current burden of disease. Consequently, evaluation of tumor material from more accessible sites such as peripheral blood has been an area of interest. In this setting, the analyses of both circulating tumor cells (CTC) and circulating DNA have been undertaken (5, 6).

The presence of circulating DNA is currently under evaluation in many different areas of biomedical research including prenatal diagnosis, renal failure, brain injury, and cancer biomarker research, among others (5, 7–9). The basis of detection of circulating DNA in peripheral blood relates to the release of this material from normal and tumor cells that have increased turnover, apoptosis, and necrosis usually in response to cellular stress (5, 7). Cancer patients have a much higher level of total circulating DNA (from both normal and malignant sources) compared with healthy individuals (5, 10, 11). The presence of total circulating free DNA (cfDNA) in addition to circulating tumor DNA (ctDNA) in peripheral blood could potentially be used as a surrogate tumor biomarker (5). ctDNA is identified typically by specific genetic alterations such as methylation or mutations in DNA that are characteristic of oncogenic transformation (12, 13). The identification of these molecular alterations is performed typically by the amplification of the genome region by polymerase chain reaction (PCR) followed by sequencing analyses or by methylation-specific PCR (12, 13). Other techniques including digital PCR or assessment of major chromosomal abnormalities such as translocations, inversions, and deletions are also in use (14, 15).

A number of studies have evaluated both cfDNA and ctDNA as prognostic factors and explored their role as a marker of response to therapy (16, 17). Although published data are abundant, results differ among studies; therefore, a comprehensive evaluation of the current knowledge is warranted.

The relative contribution of tumor-derived DNA to cfDNA is variable and is influenced by burden of disease. In patients with a low burden of disease, the majority of cfDNA in peripheral blood can arise from nontransformed cells rather than from the tumor.

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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With this consideration, we aimed to analyze studies reporting quantification of total cfDNA and detection of ctDNA in blood and their association with survival in patients with solid tumors. As release of DNA into the bloodstream is associated with tumor burden, a greater systemic response, and a more aggressive phenotype (5), we hypothesized that presence of both forms of DNA are linked with worse outcome.

Materials and Methods

This analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (18).

Data sources and study selection

MEDLINE (Host: PubMed) was searched for studies published between February 1999 and April 2015, which evaluated the association between cfDNA and survival in patients with solid tumors. We used the MeSH terms "cell-free DNA or circulating DNA" and "plasma or serum" and "cancer" and "human" and "survival". Eligible studies reported HRs and 95% confidence interval (CI) and/or *P* value for overall survival (OS) from multivariable analyses, or provided Kaplan–Meier curves for OS at 1 and 3 years based on the presence of measurable cfDNA and ctDNA from univariable analyses. Identification of subgroups of high and low levels of total cfDNA was based on the cutoff selected in individual studies (see Supplementary Table S1 online). For ctDNA, subgroups were defined on the basis of the presence or absence of a genetic alteration. Studies that quantified circulating viral DNA, those in which no control group was available and studies reporting outcome of patients who had received biologic therapies against the molecular alteration identified by ctDNA were excluded. In addition, only studies with either form of DNA were evaluated before treatment was included.

Data extraction

Two reviewers (L. Díez-González and A. Ocaña) independently evaluated all titles identified by the search strategy. The results were then pooled and all potentially relevant publications were retrieved in full and assessed for eligibility. Disagreement was resolved by consensus with a third author (D.C. García-Olmo). The following information was captured using electronic abstraction forms: first author, year of publication, tumor type, number of patients in each arm including disease site and stage group, cutoff used to define presence of cfDNA, DNA measurement method, mutation or methylation evaluated in ctDNA, and treatment type.

HRs for OS were extracted from multivariable analyses where available. If HRs were not reported, we extracted the odds of

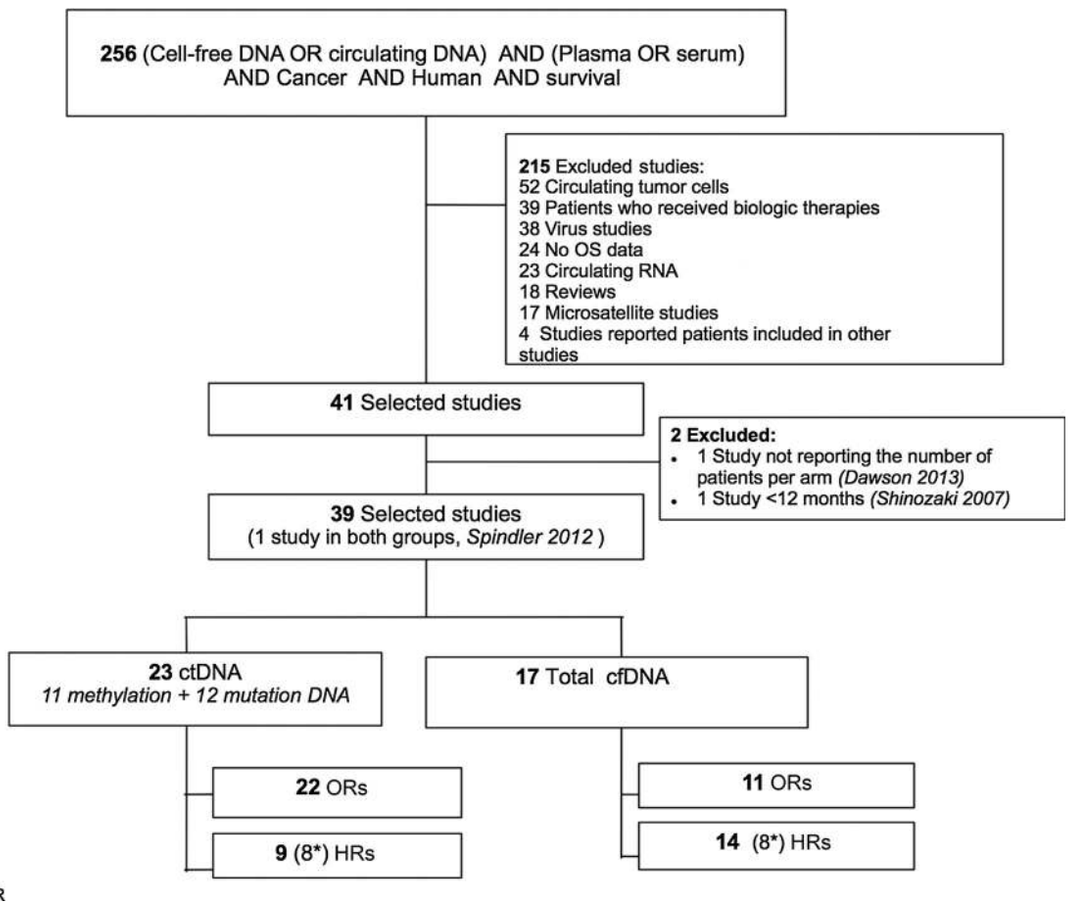

Figure 1.
Flow chart of the selected studies.

survival at 1 and 3 years from Kaplan–Meier curves and calculated ORs with 95% CIs. For studies reporting both HR and Kaplan–Meier curves, we preferentially used the multivariable HR.

Data synthesis and statistical analysis

The primary analysis comprised the comparison of the hazards of death (OS) for high levels compared with those with low levels for studies reporting cfDNA and presence or absence of molecular aberrations for studies reporting ctDNA. Secondary analysis comprised the comparison of odds of death at 1 and 3 year. Study characteristics were reported descriptively using means and proportions. Estimates of HRs and their respective 95% CIs were weighted and pooled using the generic inverse variance and random-effect model. Because of the variability in event probabilities and inclusion of some studies with rare events, pooling of ORs was conducted using the Mantel–Haenszel random-effect model. All meta-analyses were conducted using RevMan 5.3 analysis software (Cochrane Collaboration). Statistical heterogeneity was assessed using the Cochran Q and I^2 statistics. Subgroup analyses were conducted as described by Deeks and colleagues (19). All statistical tests were two-sided, and statistical significance was defined as $P < 0.05$. No corrections were made for multiple testing.

Results

Selection and characteristics of studies

Of the 256 abstracts identified initially, 217 were excluded and 39 studies were included in the analysis (refs. 20–58; see Fig. 1). Included studies comprised a total of 4,052 patients with non–small cell lung cancer (NSCLC), colon, ovarian, pancreatic, gastric, hepatocellular, breast, and prostate carcinomas as well as melanoma and neuroblastoma. Characteristics of included studies are shown in Table 1. Supplementary Table S1 shows an in-depth description of each of the included studies.

Association of ctDNA with OS

Pooled analyses of nine studies reporting multivariable HRs showed that the presence of ctDNA was associated with worse OS (HR, 2.70; 95% CI, 2.02–3.61; $P < 0.001$; Fig. 2A). There was no evidence of inter-study heterogeneity (Cochran Q, $P = 0.74$; $I^2 = 0\%$).

Sixteen studies reported univariable data for OS at 3 years. Pooled results showed that expression of ctDNA was associated with a significantly worse OS (OR, 4.83; 95% CI, 3.20–7.28; $P < 0.001$; Fig. 2B). There was significant inter-study heterogeneity (Cochran Q, $P = 0.07$; $I^2 = 37\%$). Heterogeneity was driven by one study in NSCLC, which failed to show a prognostic effect of Kras-mutated ctDNA compared with wild-type ctDNA. Removal of this study led to a higher magnitude of association with worse OS (OR, 5.33; 95% CI, 3.74–7.59) and no evidence of heterogeneity (Cochran Q, $P = 0.28$; $I^2 = 16\%$). Results were confirmed when evaluating 21 studies reporting data for one-year OS (OR, 4.37; 95% CI, 2.59–7.38; $P < 0.001$; Supplementary Fig. S1).

Table 1. Characteristics of the studies

Type of study[a]	cfDNA ($n = 17$)	ctDNA ($n = 23$)
Blood sample		
Plasma	14[b] (82%)	14 (61%)
Serum	4[b] (24%)	9 (39%)
Tumor type		
NSCLC	9 (53%)	6 (26%)
Colon[a]	2 (12%)	4 (17%)
Ovarian	2 (12%)	2 (9%)
Pancreatic	1 (6%)	2 (9%)
Others	3 (17%)	9 (39%)
Type of alteration		
Mutation		12 (52%)
Methylation		11 (48%)
Type of mutation		
Kras		9 (75%)
p53		3 (25%)
Type of methylation		
RASSF1A		3 (27%)
Others		8 (73%)

[a]One study reported data for both total cfDNA and ctDNA.
[b]One study reported data for both plasma and serum cfDNA collection.

Association of cfDNA with OS

Analyses of fourteen studies reporting multivariable HR showed that high levels of cfDNA were associated with worse survival (HR, 1.91; 95% CI, 1.59–2.29; $P < 0.001$; Fig. 3A). There was significant inter-study heterogeneity (Cochran Q, $P = 0.04$; $I^2 = 45\%$). This was driven by general heterogeneity, with exclusion of studies with outlying data not leading to changes in heterogeneity metrics.

Similarly, pooled analyses of eight studies reporting univariable data for OS at three years showed that high levels of cfDNA were associated with a significantly worse OS (OR, 2.82; 95% CI, 1.93–4.13; $P < 0.001$; Fig. 3B). There was no evidence inter-study heterogeneity (Cochran Q, $P = 0.34$; $I^2 = 11\%$). An evaluation of ten studies with OS data at one year confirmed the association with worse outcome (OR, 3.51; 95% CI, 1.91–6.46; $P < 0.001$; Supplementary Fig. S2).

Association with survival by tumor subtype and molecular alteration

There was no apparent difference in the magnitude of effect of ctDNA on OS based on tumor site. For NSCLC, the HR was 2.48 (95% CI, 1.68–3.68), for colorectal cancer it was 5.56 (95% CI, 2.42–12.82), and for other tumor sites it was 2.39 (95% CI, 1.46–3.94). These differences were not statistically significant (subgroup difference $P = 0.19$, see Fig. 4). Pooled analyses of studies evaluating cfDNA by tumor subtypes confirmed the association with poor outcome. For NSCLC, colorectal, ovarian, and other unselected tumors, the HRs were 1.93 (95% CI, 1.39–2.68), 1.65 (95% CI, 1.42–1.92), 2.39 (95% CI, 1.28–4.45), and 3.02 (95% CI, 1.62–4.72), respectively. These differences approached, but did not meet statistical significance (subgroup difference $P = 0.06$).

There were no apparent differences between analysis of ctDNA or cfDNA in plasma or in serum (subgroup difference $P = 0.32$ and $P = 0.08$, respectively; Supplementary Fig. S3). Finally, for ctDNA, there was no difference between identification of tumor DNA source by methylation or mutation (HR, 2.58; 95% CI, 1.76–3.76 vs. HR, 3.07; 95% CI, 1.75–5.39, subgroup difference $P = 0.61$).

Discussion

In the current study, we describe an independent association between the presence of peripheral blood DNA including either total cfDNA or tumor-specific DNA and worse outcome in various solid tumors.

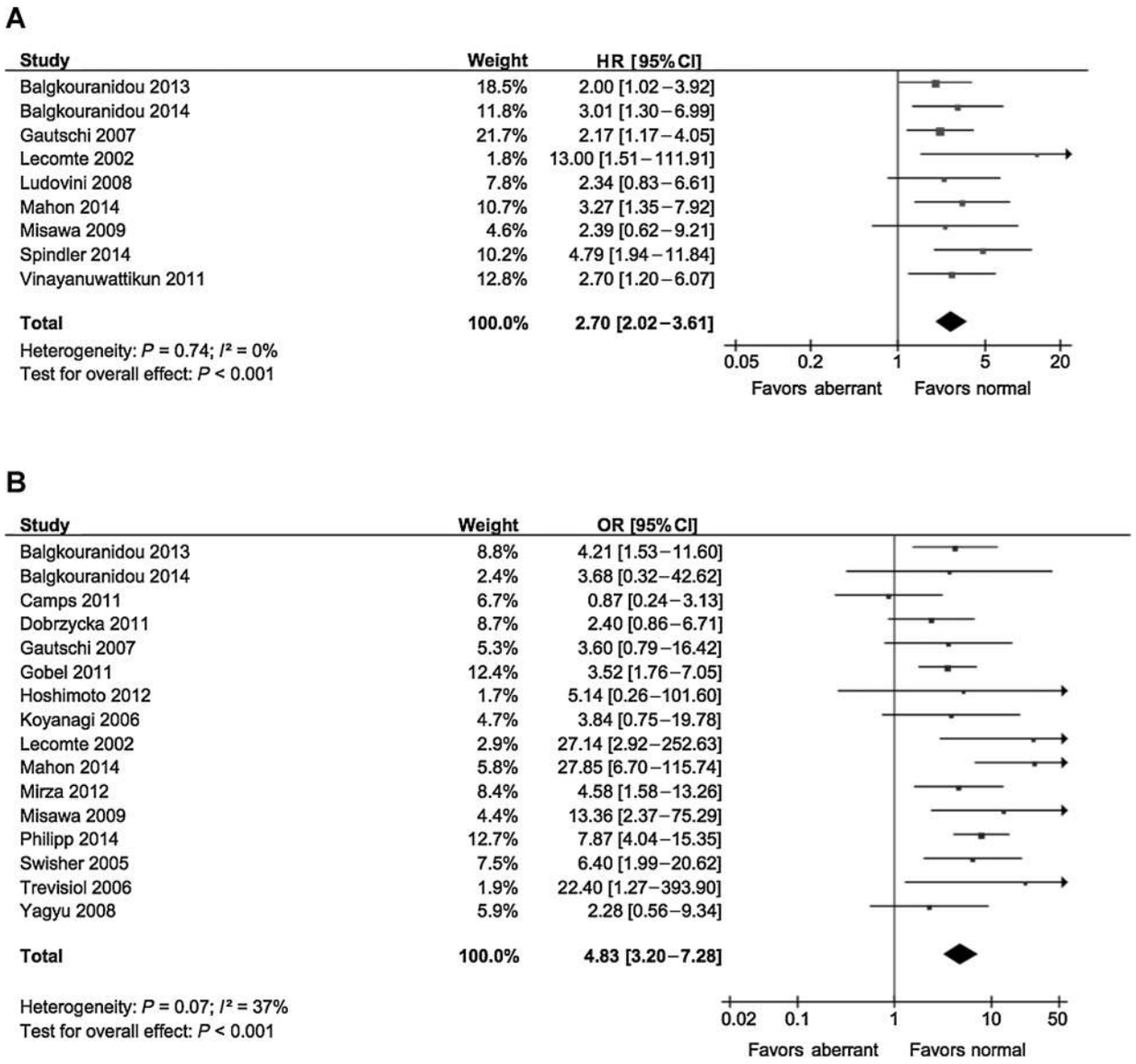

Figure 2.
Forest plot showing pooled HR for OS for ctDNA (A) and pooled OR for OS at 3 years for ctDNA (B).

Our results suggest that peripheral blood DNA can be used as an indirect measure of tumor biology. From a biologic perspective, as DNA is delivered to the blood stream from necrotic cells that are not otherwise removed (11), tumors with high tumor volume or those with rapid proliferation could potentially release greater quantities of DNA to the circulation. However, it should be mentioned that a high proportion of cfDNA comes from non-transformed cells likely due to a systemic inflammatory response (27). Therefore, evaluation of total cfDNA in blood likely represents both adverse tumor-specific and host response characteristics. In the case of ctDNA, specific genetic aberrations such as mutations or methylations can identify tumor-specific DNA fragments.

Evaluation of DNA in patients' blood has been suggested as a biomarker to identify patients with worse outcome and to monitor response to treatment (51). This approach offers several advantages including minimally invasive access and a rapid evaluation compared with tumor biopsy. Beyond commonly used tumor markers in ovarian and prostate cancers such as CA-125 and prostate-specific antigen, few serum biomarkers have been validated for monitoring of tumor volume and response to treatment. The assessment of cfDNA depends on consensus in relation to the specific cutoff to consider the expression as positive. The additional value of cfDNA relative to CTCs which have also been shown to be associated with poor outcome warrants further research (12, 13).

ctDNA assessment depends on the molecular alterations (methylation or mutation) evaluated. In our analysis, the magnitude of effect of ctDNA was similar to that of total cfDNA questioning the added prognostic value of evaluating tumor-specific DNA as prognostic marker. As detailed above, total cfDNA

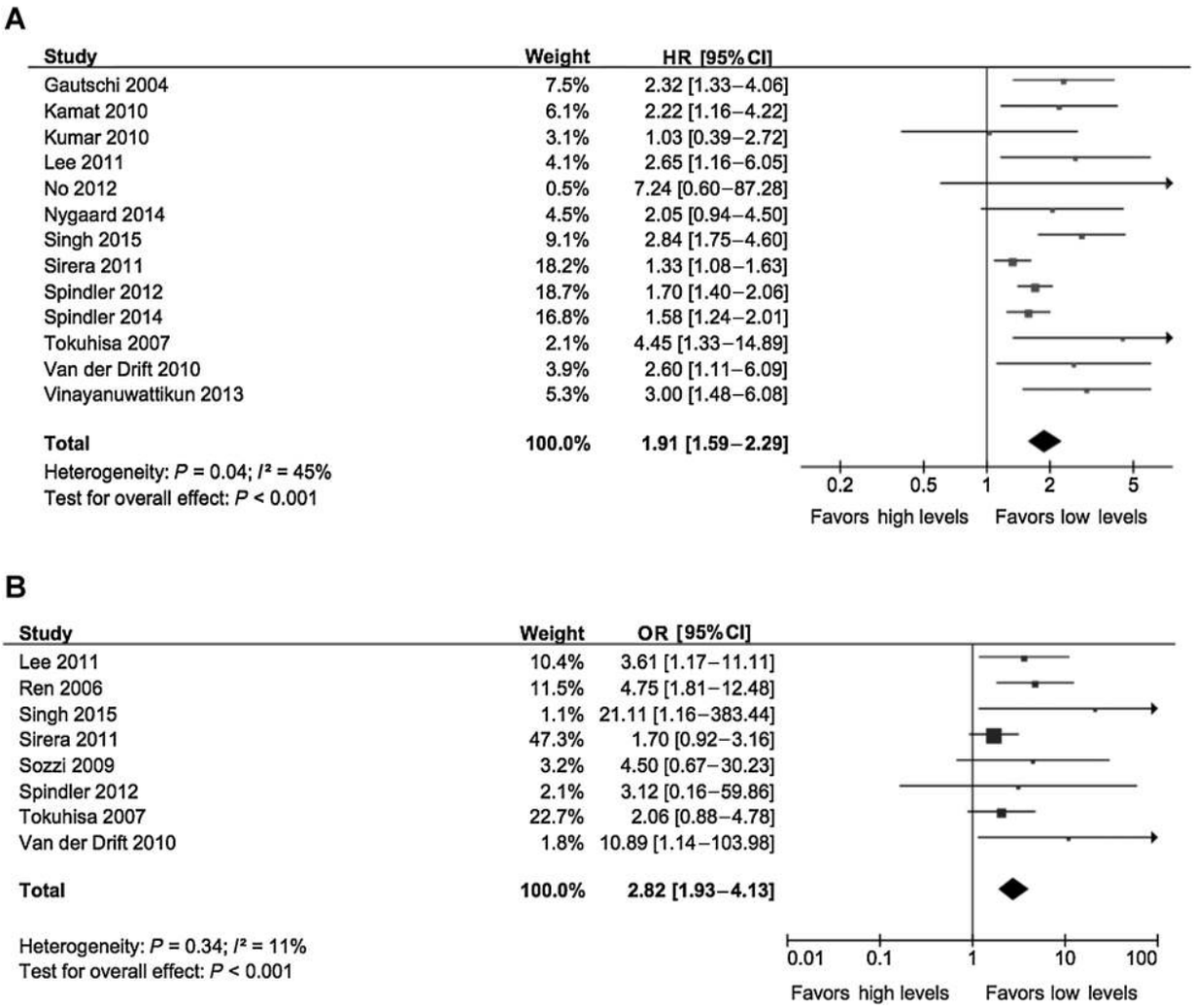

Figure 3.
Forest plot showing pooled HR for OS for total ctDNA (A) and pooled OR for overall survival at 3 years for ctDNA (B).

likely represents not only a measure of tumor volume and biology, but also host response and this may be of utility in the assessment of prognosis. In addition, it is possible that among studies exploring ctDNA using mutational analysis, the chosen mutation may not have been representative of the total tumor burden or perhaps was not a measure of more aggressive clones. In this context, it is also known that molecular alterations such as mutations or amplifications also exist in premalignant lesions (59). The use of ctDNA to monitor response to therapies or evaluating novel mechanisms of resistance has been suggested (12, 13), but it remains unclear whether total cfDNA may be able to provide similar information.

Our study has limitations. This is a meta-analysis of the literature and is therefore more likely to be compromised by selection bias with enrichment for studies reporting positive results. Furthermore, HRs were not reported in some studies so we performed a combined analysis including studies reporting odds of death at 1 and 3 years. The magnitude of effect of our analysis of odds of death at 1 and 3 years was greater than the hazards of death suggesting that some of the effect may be confounded by other prognostic factors. An additional limitation resulted from different cutoffs used for the determination of high expression of cfDNA in patients' blood, and when evaluating ctDNA. The potential for selection bias in relation to the molecular alteration identified cannot be excluded. A further concern is the inter-study variability in a number of our analyses. Finally, the vast majority of included studies were in advanced/metastatic malignancy. It is possible that the lack of difference observed in the prognostic influence of ctDNA and cfDNA may relate to the inclusion predominantly of patients with a high burden of disease. Consequently, it is unclear whether these results can be generalized to early-stage cancer where the burden of disease is substantially lower.

In conclusion, DNA in peripheral blood is associated with worse outcome for both total cfDNA and tumor-specific ctDNA. Validation studies exploring the additional benefit of ctDNA compared with total cfDNA are warranted, and international guidelines aimed at reducing heterogeneity of methods will

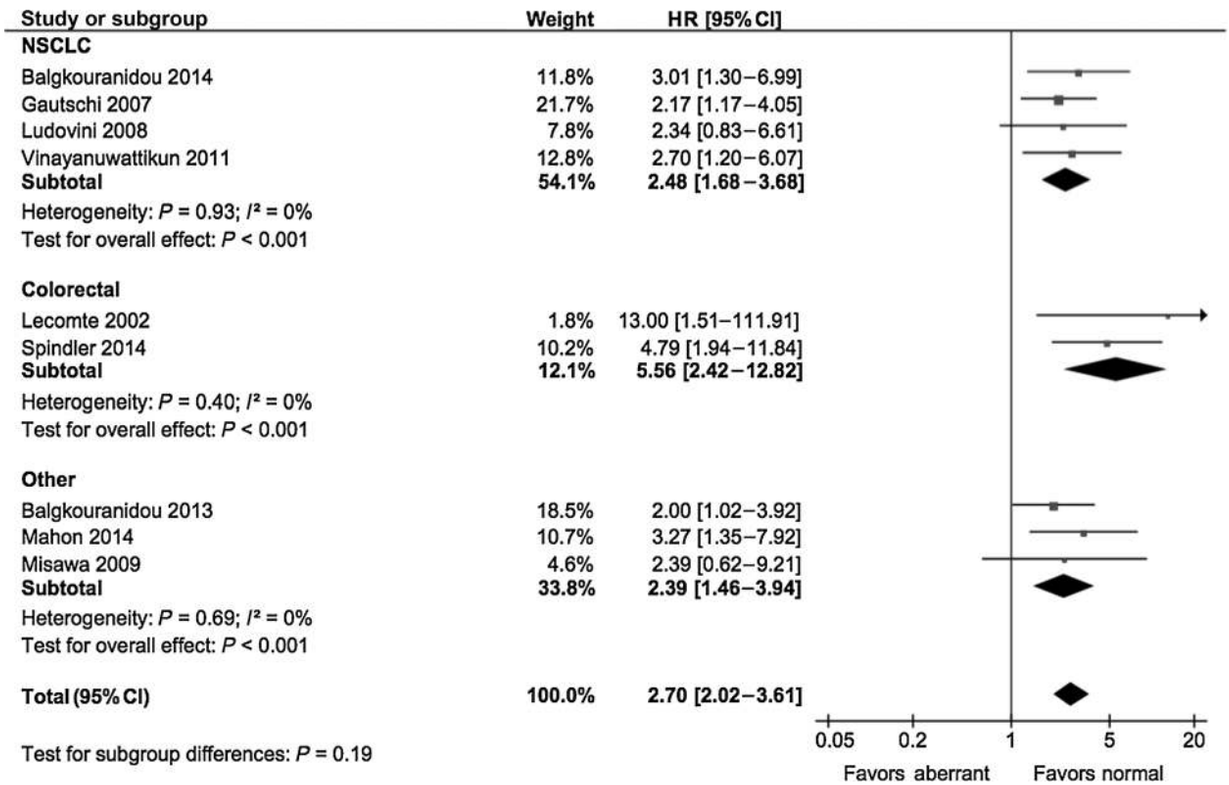

Figure 4.
Forest plot showing pooled HR for OS for ctDNA based on disease site.

improve the accuracy of the clinical impact for the assessment of both forms of DNA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Ocaña, L. Díez-González, D.C. García-Olmo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.J. Templeton, F. Vera-Badillo, M.J. Escribano, V. Corrales-Sánchez, F. Andrés-Pretel, E. Amir
Writing, review, and/or revision of the manuscript: A. Ocaña, L. Díez-González, D.C. García-Olmo, A.J. Templeton, F. Vera-Badillo, G. Serrano-Heras, B. Seruga, A. Pandiella, E. Amir
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Díez-González
Study supervision: A. Ocaña, F. Vera-Badillo

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References

1. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–58.
2. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108–13.
3. Sawyers CL. The cancer biomarker problem. Nature 2008;452:548–52.
4. Ocana A, Pandiella A, Siu LL, Tannock IF. Preclinical development of molecular-targeted agents for cancer. Nat Rev Clin Oncol 2011;8:200–9.
5. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579–86.
6. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–91.
7. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985–90.
8. Papageorgiou EA, Karagrigoriou A, Tsaliki E, Velissariou V, Carter NP, Patsalis PC. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. Nat Med 2011;17:510–3.
9. Lo YM, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998;339:1734–8.
10. Beck J, Urnovitz HB, Mitchell WM, Schutz E. Next generation sequencing of serum circulating nucleic acids from patients with invasive ductal breast cancer reveals differences to healthy and nonmalignant controls. Mol Cancer Res 2010;8:335–42.

11. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61:1659–65.
12. Dawson SJ, Rosenfeld N, Caldas C. Circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;369:93–4.
13. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
14. Leary RJ, Kinde I, Diehl F, Schmidt K, Clouser C, Duncan C, et al. Development of personalized tumor biomarkers using massively parallel sequencing. Sci Transl Med 2010;2:20ra14.
15. Olsson E, Winter C, George A, Chen Y, Howlin J, Tang MH, et al. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med 2015;7:1034–47.
16. Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res 2012;18:3462–9.
17. Kimura H, Kasahara K, Kawaishi M, Kunitoh H, Tamura T, Holloway B, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res 2006;12:3915–21.
18. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. Open Med 2009;3:e123–30.
19. Deeks JJ. Systematic reviews in health care: Systematic reviews of evaluations of diagnostic and screening tests. BMJ 2001;323:157–62.
20. Balgkouranidou I, Karayiannakis A, Matthaios D, Bolanaki H, Tripsianis G, Tentes AA, et al. Assessment of SOX17 DNA methylation in cell free DNA from patients with operable gastric cancer. Association with prognostic variables and survival. Clin Chem Lab Med 2013;51:1505–10.
21. Balgkouranidou I, Chimonidou M, Milaki G, Tsarouxa EG, Kakolyris S, Welch DR, et al. Breast cancer metastasis suppressor-1 promoter methylation in cell-free DNA provides prognostic information in non-small cell lung cancer. Br J Cancer 2014;110:2054–62.
22. Gautschi O, Huegli B, Ziegler A, Gugger M, Heighway J, Ratschiller D, et al. Origin and prognostic value of circulating KRAS mutations in lung cancer patients. Cancer Lett 2007;254:265–73.
23. Lecomte T, Berger A, Zinzindohoue F, Micard S, Landi B, Blons H, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer 2002;100:542–8.
24. Ludovini V, Pistola L, Gregorc V, Floriani I, Rulli E, Piattoni S, et al. Plasma DNA, microsatellite alterations, and p53 tumor mutations are associated with disease-free survival in radically resected non-small cell lung cancer patients: a study of the perugia multidisciplinary team for thoracic oncology. J Thorac Oncol 2008;3:365–73.
25. Mahon KL, Qu W, Devaney J, Paul C, Castillo L, Wykes RJ, et al. Methylated Glutathione S-transferase 1 (mGSTP1) is a potential plasma free DNA epigenetic marker of prognosis and response to chemotherapy in castrate-resistant prostate cancer. Br J Cancer 2014;111:1802–9.
26. Misawa A, Tanaka S, Yagyu S, Tsuchiya K, Iehara T, Sugimoto T, et al. RASSF1A hypermethylation in pretreatment serum DNA of neuroblastoma patients: a prognostic marker. Br J Cancer 2009;100:399–404.
27. Spindler KL, Appelt AL, Pallisgaard N, Andersen RF, Brandslund I, Jakobsen A. Cell-free DNA in healthy individuals, noncancerous disease and strong prognostic value in colorectal cancer. Int J Cancer 2014;135:2984–91.
28. Vinayanuwattikun C, Sriuranpong V, Tanasanvimon S, Chantranuwat P, Mutirangura A. Epithelial-specific methylation marker: a potential plasma biomarker in advanced non-small cell lung cancer. J Thorac Oncol 2011;6:1818–25.
29. Camps C, Sirera R, Bremnes R, Blasco A, Sancho E, Bayo P, et al. Is there a prognostic role of K-ras point mutations in the serum of patients with advanced non-small cell lung cancer? Lung Cancer 2005;50:339–46.
30. Camps C, Jantus-Lewintre E, Cabrera A, Blasco A, Sanmartin E, Gallach S, et al. The identification of KRAS mutations at codon 12 in plasma DNA is not a prognostic factor in advanced non-small cell lung cancer patients. Lung Cancer 2011;72:365–9.
31. Dobrzycka B, Terlikowski SJ, Kinalski M, Kowalczuk O, Niklinska W, Chyczewski L. Circulating free DNA and p53 antibodies in plasma of patients with ovarian epithelial cancers. Ann Oncol 2011;22:1133–40.
32. Gobel G, Auer D, Gaugg I, Schneitter A, Lesche R, Muller-Holzner E, et al. Prognostic significance of methylated RASSF1A and PITX2 genes in blood- and bone marrow plasma of breast cancer patients. Breast Cancer Res Treat 2011;130:109–17.
33. Hoshimoto S, Kuo CT, Chong KK, Takeshima TL, Takei Y, Li MW, et al. AIM1 and LINE-1 epigenetic aberrations in tumor and serum relate to melanoma progression and disease outcome. J Invest Dermatol 2012;132:1689–97.
34. Koyanagi K, Mori T, O'Day SJ, Martinez SR, Wang HJ, Hoon DS. Association of circulating tumor cells with serum tumor-related methylated DNA in peripheral blood of melanoma patients. Cancer Res 2006;66:6111–7.
35. Mirza S, Sharma G, Parshad R, Srivastava A, Gupta SD, Ralhan R. Clinical significance of promoter hypermethylation of ERbeta and RARbeta2 in tumor and serum DNA in Indian breast cancer patients. Ann Surg Oncol 2012;19:3107–15.
36. Philipp AB, Nagel D, Stieber P, Lamerz R, Thalhammer I, Herbst A, et al. Circulating cell-free methylated DNA and lactate dehydrogenase release in colorectal cancer. BMC Cancer 2014;14:245.
37. Swisher EM, Wollan M, Mahtani SM, Willner JB, Garcia R, Goff BA, et al. Tumor-specific p53 sequences in blood and peritoneal fluid of women with epithelial ovarian cancer. Am J Obstet Gynecol 2005;193:662–7.
38. Trevisiol C, Di Fabio F, Nascimbeni R, Peloso L, Salbe C, Ferruzzi E, et al. Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastases. Int J Biol Markers 2006;21:223–8.
39. Yagyu S, Gotoh T, Iehara T, Miyachi M, Katsumi Y, Tsubai-Shimizu S, et al. Circulating methylated-DCR2 gene in serum as an indicator of prognosis and therapeutic efficacy in patients with MYCN nonamplified neuroblastoma. Clin Cancer Res 2008;14:7011–9.
40. Gautschi O, Bigosch C, Huegli B, Jermann M, Marx A, Chasse E, et al. Circulating deoxyribonucleic Acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy. J Clin Oncol 2004;22:4157–64.
41. Kamat AA, Baldwin M, Urbauer D, Dang D, Han LY, Godwin A, et al. Plasma cell-free DNA in ovarian cancer: an independent prognostic biomarker. Cancer 2010;116:1918–25.
42. Kumar S, Guleria R, Singh V, Bharti AC, Mohan A, Das BC. Efficacy of circulating plasma DNA as a diagnostic tool for advanced non-small cell lung cancer and its predictive utility for survival and response to chemotherapy. Lung Cancer 2010;70:211–7.
43. Lee YJ, Yoon KA, Han JY, Kim HT, Yun T, Lee GK, et al. Circulating cell-free DNA in plasma of never smokers with advanced lung adenocarcinoma receiving gefitinib or standard chemotherapy as first-line therapy. Clin Cancer Res 2011;17:5179–87.
44. No JH, Kim K, Park KH, Kim YB. Cell-free DNA level as a prognostic biomarker for epithelial ovarian cancer. Anticancer Res 2012;32:3467–71.
45. Nygaard AD, Holdgaard PC, Spindler KL, Pallisgaard N, Jakobsen A. The correlation between cell-free DNA and tumour burden was estimated by PET/CT in patients with advanced NSCLC. Br J Cancer 2014;110:363–8.
46. Singh N, Gupta S, Pandey RM, Chauhan SS, Saraya A. High levels of cell-free circulating nucleic acids in pancreatic cancer are associated with vascular encasement, metastasis and poor survival. Cancer Invest 2015;33:78–85.
47. Sirera R, Bremnes RM, Cabrera A, Jantus-Lewintre E, Sanmartin E, Blasco A, et al. Circulating DNA is a useful prognostic factor in patients with advanced non-small cell lung cancer. J Thorac Oncol 2011;6:286–90.
48. Spindler KL, Pallisgaard N, Vogelius I, Jakobsen A. Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. Clin Cancer Res 2012;18:1177–85.
49. Tokuhisa Y, Iizuka N, Sakaida I, Moribe T, Fujita N, Miura T, et al. Circulating cell-free DNA as a predictive marker for distant metastasis of hepatitis C virus-related hepatocellular carcinoma. Br J Cancer 2007;97:1399–403.
50. van der Drift MA, Hol BE, Klaassen CH, Prinsen CF, van Aarssen YA, Donders R, et al. Circulating DNA is a non-invasive prognostic factor for survival in non-small cell lung cancer. Lung Cancer 2010;68:283–7.
51. Vinayanuwattikun C, Winayanuwattikun P, Chantranuwat P, Mutirangura A, Sriuranpong V. The impact of non-tumor-derived circulating nucleic

acids implicates the prognosis of non-small cell lung cancer. J Cancer Res Clin Oncol 2013;139:67–76.
52. Ren N, Ye QH, Qin LX, Zhang BH, Liu YK, Tang ZY. Circulating DNA level is negatively associated with the long-term survival of hepatocellular carcinoma patients. World J Gastroenterol 2006;12:3911–4.
53. Sozzi G, Roz L, Conte D, Mariani L, Andriani F, Lo Vullo S, et al. Plasma DNA quantification in lung cancer computed tomography screening: five-year results of a prospective study. Am J Respir Crit Care Med 2009;179:69–74.
54. Bidard FC, Madic J, Mariani P, Piperno-Neumann S, Rampanou A, Servois V, et al. Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. Int J Cancer 2014;134:1207–13.
55. Camps C, Sirera R, Bremnes RM, Rodenas V, Blasco A, Safont MJ, et al. Quantification in the serum of the catalytic fraction of reverse telomerase: a useful prognostic factor in advanced non-small cell lung cancer. Anticancer Res 2006;26:4905–9.
56. Castells A, Puig P, Mora J, Boadas J, Boix L, Urgell E, et al. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. J Clin Oncol 1999;17:578–84.
57. Chen H, Tu H, Meng ZQ, Chen Z, Wang P, Liu LM. K-ras mutational status predicts poor prognosis in unresectable pancreatic cancer. Eur J Surg Oncol 2010;36:657–62.
58. Madic J, Kiialainen A, Bidard FC, Birzele F, Ramey G, Leroy Q, et al. Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients. Int J Cancer 2015;136:2158–65.
59. Ocana A, Pandiella A. Personalized therapies in the cancer "omics" era. Mol Cancer 2010;9:202.