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 oncocgenic 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.
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...
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 I² 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 initially identified, 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; I² = 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; I² = 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; I² = 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).

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; I² = 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; I² = 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 ctDNA 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.

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**Table 1.** Characteristics of the studies

<table>
<thead>
<tr>
<th>Type of study*</th>
<th>cfDNA (n = 17)</th>
<th>ctDNA (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>14a (82%)</td>
<td>14 (67%)</td>
</tr>
<tr>
<td>Serum</td>
<td>6b (24%)</td>
<td>9 (39%)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>9 (53%)</td>
<td>6 (26%)</td>
</tr>
<tr>
<td>Colon*</td>
<td>2 (12%)</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>2 (12%)</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>1 (6%)</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (17%)</td>
<td>9 (39%)</td>
</tr>
<tr>
<td>Type of alteration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>12 (52%)</td>
<td></td>
</tr>
<tr>
<td>Methylation</td>
<td>11 (48%)</td>
<td></td>
</tr>
<tr>
<td>Type of mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kras</td>
<td>9 (75%)</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>3 (25%)</td>
<td></td>
</tr>
<tr>
<td>Type of methylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RASSF1A</td>
<td>3 (27%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>8 (73%)</td>
<td></td>
</tr>
</tbody>
</table>

*One study reported data for both total cfDNA and ctDNA.

*One study reported data for both plasma and serum cfDNA collection.
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).

cfDNA 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...
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
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
Conception and design: A. Ocaña, A.J. Templeton, F. Vera-Badillo, E. Amir
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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
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Study supervision: A. Ocaña, F. Vera-Badillo

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References

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