Value of cfDNA Concentration as a Biomarker for Cancer Prognosis and Progression

Jake Freedman[1,2]; Aseel Abdalla[2]; Jacob Till, MD, PhD[2]; Taylor A. Black[2]; Stephanie Yee[2]; Stephen Bagley, MD, MSCE[2]; Erica Carpenter, MBA, PhD[2]

[1]College of Arts and Sciences (COL 2023), University of Pennsylvania; [2]Division of Hematology and Oncology, Perelman School of Medicine

Background

Liquid Biopsy and Cell-Free DNA

- Tracking patient response to specific chemotherapy drugs during cancer treatment is crucial for outpacing tumor evolution and optimizing patient survival[1]
- Traditionally, treatment efficacy is determined by measuring changes in tumor size over the course of treatment with imaging[1], [2]
- However, imaging techniques are, in part, qualitative, and are also imperfect at distinguishing true tumor progression from pseudoprogression, i.e. apparent progression of the tumor that dissipates in time
    - Pseudoprogression interpreted as true progression may force patients to undergo unnecessary surgeries and procedures that hinder their survival
    - Necessitates quick and accurate quantitative markers for tumor burden
- Among the most promising of these biomarkers is cell-free DNA, unbound small-fragment DNA present in circulation that is released into the blood as a result of apoptosis, necrosis, as well as other factors not yet well understood[1]
- cfDNA can be extracted from several body regions; origin may depend on cancer type[1]
- Multiple studies across a variety of cancers have demonstrated associations between the concentration of cell-free DNA and prognosis and progression of the disease
    - These studies have shown that high cell-free DNA has a statistically significant association with poor overall survival (OS) and progression-free survival (PFS) when compared to low cell-free
- Concentrations of plasma cfDNA can also indicate changes in tumor size during treatment, in some instances even preceding tumor growth as assessed by imaging[2]

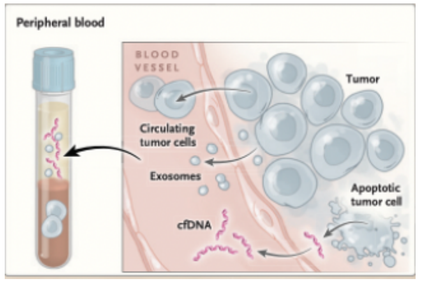
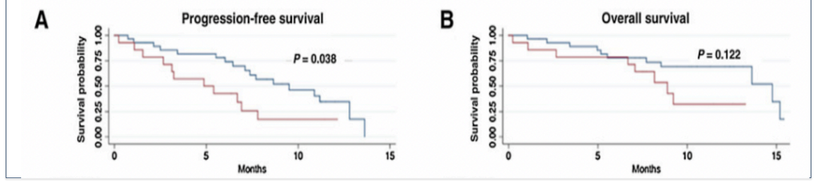


Figure 1: cfDNA and other analytes may be extracted from peripheral blood for downstream analysis[1]

Relevant Prior Work from our Lab (Bagley et al 2020)[2]

- Prospective Study: Two aims with respect to cfDNA concentration
    - Sought to assess the association between baseline cfDNA concentration and PFS and OS in a 42-member cohort of patients with glioblastoma
    - Also tracked cfDNA concentration longitudinally to compare cfDNA levels to tumor volume over time
- DNA extraction was performed with QIAamp Circulating Nucleic Acid Kit from Qiagen and quantification was performed with Power SYBR-Green PCR Master Mix from Applied Biosystems
- KM curves found a statistically significant association in PFS for patients with high vs. low cfDNA concentrations (cutoff at 13.4 ng/mL) but not for OS
- For patients with progressive disease, increases in cfDNA were often concurrent with tumor growth



Results and Figures

Author	Year	Cancer Type	Extraction Method	Quantification Method	Patient N and Cutoff	Results
Bagley[2]	2020	Glioblastoma	QIAamp Circulating Nucleic Acid Kit from Qiagen	SYBR-Green based qPCR - (Power SYBR-Green PCR Master Mix from Applied Biosystems)	N = 42 Cutoff: 13.4 ng/mL	Patients with GBM had markedly higher cfDNA concentrations than their corresponding healthy controls (mean 13.4 ng/mL vs. 6.7 ng/mL, respectively). KM curves found a statistically significant association between high baseline cfDNA and poor PFS
Thompson[7]	2016	NSCLC	Not specified	Electrophoretic separation in a massively parallel capillary array system	N = 102 Cutoff = 3 ng/uL	cfDNA concentration was an accurate prognostic indicator of OS with metastatic NSCLC
Nygaard[5]	2013	NSCLC	QIAsymphony virus/bacteria midi-kit on a QIAsymphony robot (Qiagen)	qPCR through the targeting of the peptidylprolyl isomerase A (PPIA) gene (In House)	N = 53 Cutoff = 75th percentile	Found significantly improved OS for patients below 75th percentile of cfDNA concentration; inconclusive data on correlation between tumor burden and cfDNA concentration
Li[11]	2015	NSCLC	1% sodium dodecyl sulfate containing a 50-µl aliquot of proteinase K (Life Technologies) and Wizard DNA purification resin (Promega Corp)	Quantitative PCR of the β-actin gene performed in triplicates	N = 103 Cutoff = 7 ng/mL	Weak correlation between changes in cfDNA concentration and radiologic response to treatment; did not find correlation between baseline cfDNA concentration and PFS or OS
Sirera[9]	2011	NSCLC	Extracted from 400 ul of plasma by using QIAamp-Blood Mini Kit (QIAGEN)	Quantitative real-time polymerase chain reaction targeting the gene for hTERT (human telomerase reverse transcriptase)	N = 446 Cutoff = 49.8 ng/mL	Statistically significant difference in cfDNA concentration for patients that had Cr, PR, SD, and PD. Also found a statistically significant association between both TTP and OS and cfDNA concentration.
Tissot[8]	2015	NSCLC	QIAamp Circulating Nucleic Acid Kit (Qiagen)	Creation of a standard curve through fluorometry using Pico Green dsDNA Kit (Molecular Probes)	N = 218 Cutoff = 42.12 ng/mL	PFS and OS had a statistically significant association with cfDNA concentration (cutoff at upper tertile). However, no statistical significance between baseline cfDNA concentration and disease progression was found
Goodall[6]	2017	Prostate	QIAsymphony and Circulating DNA Kit (Both Qiagen)	Quant-iT High Sensitivity Picogreen Kit (Invitrogen)	N = 50 Cutoff = 50% decrease	50% decrease in cfDNA concentration after 4 and 8 weeks of olafrinib significantly improved PFS. OS significantly improved when decrease was observed at 8 weeks but not after 4.
Valpione[3]	2017	Metastatic Melanoma	Qiagen QIAamp Circulating Nucleic Acid Kits	qPCR of RNAseP with TaqMan RNAseP Assay (Life Technologies)	N = 43 Cutoff = 89 pg/uL	Correlation between changes in cfDNA concentration and tumor burden; KM curve showed significantly better OS for patients with low baseline cfDNA
Kamat[4]	2010	Ovarian	Qiagen DNA extraction Mini kit	Real-time PCR with TaqMan Assay (Applied Biosystems) with primers directed to beta globin	N = 164 Cutoff = 78.9 ng/mL	Patients with invasive cancer had significantly higher cfDNA concentrations compared to patients with benign tumors. High preoperative cfDNA concentration was a statistically significant marker of poor OS when compared to patients with low cfDNA.
Spindler[10]	2012	Colorectal	QIAsymphony virus/bacteria midi-kit on a QIAsymphony robot (Qiagen) according to the manufacturer's instructions	Quantitative PCR (qPCR) using an in-house assay for the gene cyclophilin	N = 108 Cutoff: 75th percentile	cfDNA concentration had a statistically significant association with PFS and OS. Study had two cutoffs: quartile of cfDNA concentration and above or below 75th percentile.

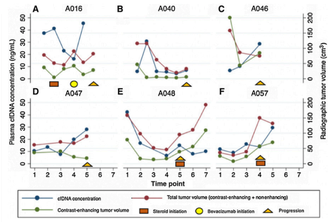


Figure 2; change in cfDNA concentration and two measures of tumor volume over time in GBM patients with progressive disease[2]

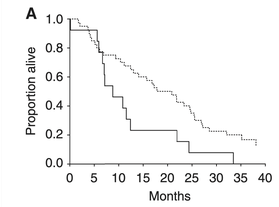


Figure 3; KM curve showing OS for NSCLC patients. Dotted line is patients above 75th percentile, solid line is patients below[5]

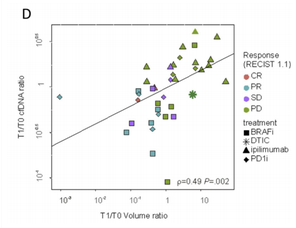


Figure 4; plot shows correlation between change in tumor volume and change in cfDNA concentration in metastatic melanoma[3]

Methods

Literature search was conducted through the PubMed database. Topics searched were: "cfDNA concentration", "plasma cfDNA", "cfDNA cancer", and "cfDNA prognosis". References were derived from Corcoran and Chabner 2018 review[1] and directly from PubMed search.

Discussion and Future Directions

All but one of the sources compiled were in consensus that cfDNA concentration is an accurate marker for prognosis in cancer patients, with high cfDNA groups displaying demonstrably poorer prognosis. It should also be noted that the only study that did not find an association between cfDNA and prognosis, Li et al 2015, used a very low cutoff for cfDNA concentration (7 ng/mL)[11]. The studies discussed in this review focused on NSCLC, with half of the studies focusing on this cancer type[5,7,8,9,11]. It would be worthwhile to conduct studies in a wider array of cancer types to better assess differences in the functionality of cfDNA for different cancers. Another potential direction may be comparing significance at different cutoffs.

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