Whole-genome cell-free DNA (cfDNA) changes as a dynamic blood-based biomarker for early response assessment of advanced tumors

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Highlights

- **We performed whole-genome analysis of cfDNA from serial blood samples in 69 prospectively enrolled patients receiving treatment for advanced cancer.**
- **Increases in tumor-derived cfDNA were strongly predictive of disease progression at first follow-up and shorter progression-free survival.**
- **The assay had consistent predictive performance in patients on immunotherapy as well as breast and lung cancer subsets.**
- **The confirmed predictions of progression were based on blood samples taken a median of 5.5 weeks before imaging and clinical evaluation.**

Objective

Patients treated for advanced cancer face considerable uncertainty in real time regarding the effectiveness of systemic therapies while incurring a serious burden of cumulative toxicity and out-of-pocket expenses. Today, imaging (CT, PET/CT, MRI), the standard for response assessment, typically requires 3-4 months or longer on therapy before confident conclusions can be made.

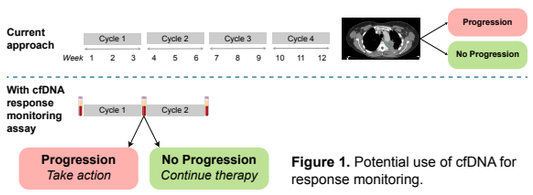
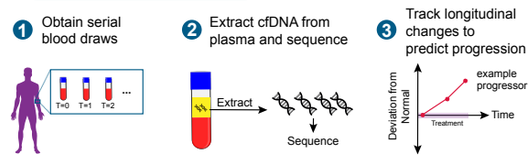


Figure 1. Potential use of cfDNA for response monitoring.

Based on the theory that radiographic progression is preceded by changes in tumor biology that are detectable in peripheral blood, what we are calling "molecular progression", we have developed a novel approach to quantitatively track changes in cfDNA to monitor response to treatment. Several distinctive features of cancer can be detected in cfDNA from plasma [1-5], which has led to the development of multiple diagnostic applications.

Assay workflow



Peripheral blood was obtained over time from patients and collected in Streck Cell-Free DNA Blood Collection tubes (Step 1). Plasma was separated from whole blood, after which cfDNA was extracted from 4 mL of plasma (Step 2). Sequencing libraries were prepared using a method optimized for whole genome sequencing (54 patients) or whole genome bisulfite sequencing (15 patients). Libraries were sequenced to a median coverage of 20X. Longitudinal changes in the fraction of tumor-derived cfDNA were quantified based on a patient-specific profile of whole-genome features. This change was used to predict progression (Step 3). Treatment response was evaluated by an independent radiologist based on RECIST 1.1 guidelines.

Longitudinal Cohort

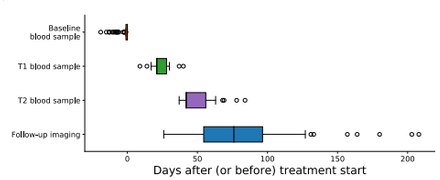
We prospectively enrolled and serially collected blood from 69 patients with advanced solid tumors, each receiving a new treatment. Blood was collected on a schedule before each cycle of treatment, and imaging was performed per standard practice.

Table 1. Patient characteristics; 2017-2018.

		Median (Min-Max)	N=69 (%)
Age (years)		70 (30-89)	
Sex	Female		41 (59)
	Male		28 (31)
Cancer type	Lung		28 (41)
	Breast		25 (36)
	GI		9 (13)
	GU		5 (7)
	Other		2 (3)
Immunotherapy	Yes		17 (25)
	No		52 (75)
Lines of therapy	1		33 (48)
	2		16 (23)
	3		11 (16)
	4+		9 (13)
T1 (days)		21 (9-40)	65 (94)
T2 (days)*		42 (37-84)	47 (68)
First follow-up (days)		76 (26-208)	
Last follow-up (days)		126 (35-469)	

* Total of 43 participants have both post-treatment timepoints

Figure 2. Sample timing. T1 blood sample was collected before the second cycle of treatment, and T2 was collected before the third cycle.



Predictive Performance

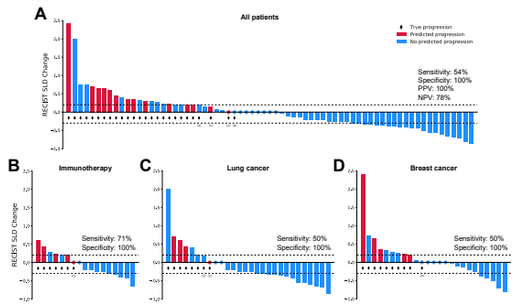


Figure 3. Waterfall plot compares cfDNA-based predictions to imaging at first follow-up, quantified by the sum of longest diameter (SLD) in target lesions by RECIST, for (A) all patients (n=69), (B) patients on immunotherapy (n=17), (C) lung cancer patients (n=28), and (D) breast cancer patients (n=25). Footnoted cases showed clear clinical progression.

The change in cancer-associated signal after the start of treatment has previously been shown to correlate with treatment response [6, 7]. Patients with an increase in cfDNA tumor fraction at either post-treatment blood collection were therefore predicted to progress. We compared cfDNA predictions to follow-up imaging (Figure 3A) and found that all patients with predicted progression did progress (14/14, 100% positive predictive value). For the remaining patients, 43 of 55 did not progress (78% negative predictive value). Sensitivity for the assay was 54% and specificity was 100%. Sensitivity was similar in patients on immunotherapy (Figure 3B, 71%), lung cancer patients (Figure 3C, 50%), and breast cancer patients (Figure 3D, 50%).

Prediction Timing

Sample timing shows potential to accelerate the clinical decision loop

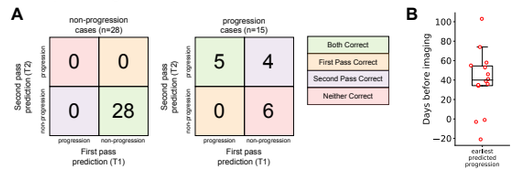


Figure 4. (A) Comparison of timepoints for patients with both post-treatment timepoints (n=43), plotted separately for progression and non-progression cases. (B) Timing of cfDNA-based predictions of progression (n=14).

Most predictions were concordant between the two cfDNA samples at T1 and T2 (Figure 4A). Out of 43 patients who had both post-treatment cfDNA samples, 4 (9%) had discordant predictions. All four of these were predicted non-progression at T1 and progression at T2. This is consistent with an improvement in sensitivity of the cfDNA test over the course of treatment, although larger studies are necessary to confirm or quantify a performance increase. For the patients who were predicted to progress, the cfDNA assay preceded clinical evaluation by a median of 40 days (Figure 4B).

Survival Analysis

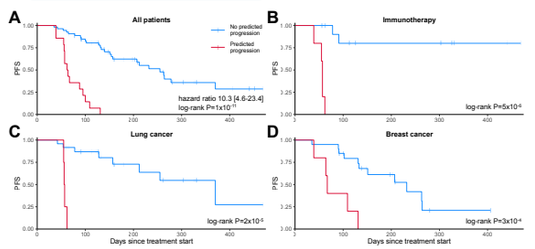


Figure 5. PFS based on imaging and clinical evaluation grouped by cfDNA prediction of progression and non-progression for (A) all patients (n=69), (B) patients on immunotherapy (n=17), (C) lung cancer patients (n=28), and (D) breast cancer patients (n=25).

For all participants in the cohort, the median PFS was 157 days. Patients with predicted progression by cfDNA had worse PFS, a median of **63 days** versus **255 days** for others (Figure 5A, hazard ratio 10.3 [95% CI 4.6-23.4], log-rank $P=1x10^{-11}$). These results were consistent in the subset of patients on immunotherapy (Figure 5B, log-rank $P=5x10^{-6}$), patients with lung cancer (Figure 5C, log-rank $P=2x10^{-5}$) and patients with breast cancer (Figure 5D, log-rank $P=3x10^{-4}$).

Conclusions

- **Analyzing cfDNA early in the course of a new therapy holds promise to identify patients with disease progression faster than traditional methods.**
- **This technology may enable early switching to other potentially effective therapies, increasing the value proposition of all delivered treatment.**
- **Predictive value of this approach appears to be independent of the underlying tumor type and therapeutic modality, which could facilitate broad clinical application.**
- **Further studies are ongoing to develop this assay for use in clinical practice.**

Acknowledgements & References

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