Dynamic changes in whole-genome cell-free DNA (cfDNA) identify disease progression prior to imaging in advanced solid tumors

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Highlights

- **We performed whole-genome analysis of cfDNA from serial blood samples in 93 prospectively enrolled patients receiving treatment for advanced cancer.**
- **Increases in tumor-derived cfDNA (ctDNA) 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 across both breast and lung cancer subsets.**
- **ctDNA molecular progression precedes imaging and clinical progression by a median of 40 days.**

Background

Patients treated for advanced-stage cancers endure a considerable burden of cumulative toxicity, out of pocket expenses and the opportunity cost of being treated with ineffective therapies. Today, imaging (CT, PET/CT, MRI), the standard for response assessment, typically requires 10-16 weeks or longer on therapy before confident conclusions can be drawn. Therefore, faster and more reliable feedback from tumor biology could significantly improve net medical benefits for this heavily treated cohort.

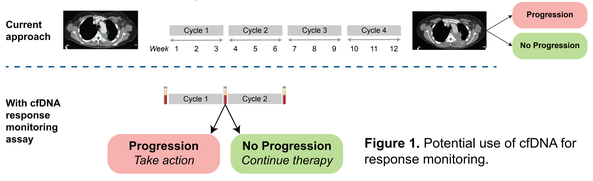


Figure 1. Potential use of cfDNA for response monitoring.

Given that radiographic progression is preceded by upstream changes in tumor biology that can be detected in peripheral blood, what we are calling "Molecular Progression", we have developed a novel approach to quantitatively track changes in ctDNA to monitor response to treatment as a complement to imaging-based assessment. Several distinctive features of cancer can be detected in ctDNA from plasma[1-5], which has led to the development of multiple diagnostic applications.

Conflict of Interest Disclosure
This study was sponsored by Lexent Bio. AAD reports travel support (Lexent Bio, Menarini Silicon Biosystems). NP, AR, AS, RS, NL, TW, PG, BW, JC, HW, AT, JCP and HT are current or previous employees of Lexent Bio. WTI reports advisory board membership (Genentech), honoraria (Outcomes Insights), and attended investigator meeting (EMD Serono). YKC has received research grants (Abbvie, BMS, Biodesix, Lexent Bio, Freenome) and honoraria/advisory board membership (Roche/Genentech, AstraZeneca, Foundation Medicine, Counsyl, Neogenomics, Guardant Health, Boehringer Ingelheim, Biodesix, Immuneoncia, Lilly Oncology, Merck, Takeda). DC, RWL and MSO have no conflicts of interest.

Methods

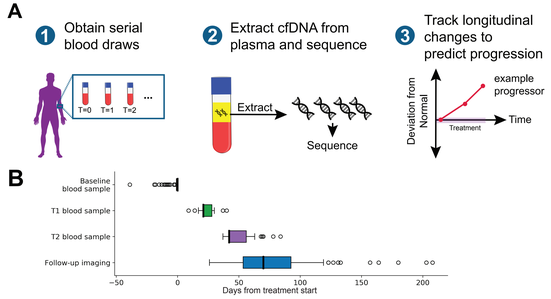


Figure 2. A) Peripheral blood was obtained over time from patients and collected in Streck Cell-Free DNA Blood Collection tubes (Step 1). Plasma was separated from whole blood, after which cfDNA was extracted from 4 mL of plasma (Step 2). Sequencing libraries were prepared using a method optimized for whole genome sequencing (54 patients) or whole genome bisulfite sequencing (39 patients). Libraries were sequenced to a median coverage of 20X. Longitudinal changes in the fraction of ctDNA 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. **B)** Sample timing. T1 blood sample was collected before the second cycle of treatment (median of 3 weeks after treatment start), and T2 was collected before the third cycle (median of 6 weeks after treatment start). All baseline blood samples were required to be prior to treatment start with the majority being collected on the same day as treatment start. The median first follow-up assessment was 70 days after treatment start.

Patients

	Median (Min-Max)	N= 93 (%)
Age	70 (30-89)	
Sex		
Female		52 (55.9)
Male		41 (44.1)
Cancer type		
Lung		40 (43.0)
Breast		25 (26.9)
GI		16 (17.2)
GU		5 (5.4)
Other		7 (7.5)
Treatment types		
Chemotherapy		33 (35.5)
Chemotherapy, Antibody		10 (10.8)
Immunotherapy		25 (26.9)
Immunotherapy, Chemotherapy		9 (9.7)
Endocrine		4 (4.3)
Endocrine, CDK4/6i		6 (6.5)
Targeted alone		6 (6.5)
Other		1 (1.1)
Lines of therapy		
1		48 (51.6)
2		24 (25.8)
3+		21 (22.6)
T1 (days)	21 (9-40)	87 (93.5)
T2 (days)*	42 (37-84)	67 (72.0)
First follow-up (days)	81 (35-208)	
Last follow-up (days)	157 (35-645)	

Table 1. Participant Characteristics
Between May 2017 and June 2019, we prospectively enrolled and serially collected blood from 93 patients with advanced solid tumors, each receiving a new treatment after either having failed on a previous treatment or after a new diagnosis of cancer. Breast and lung cancers are the most commonly represented in the cohort with 40 and 25 patients respectively. A variety of treatment combinations were employed with the most common class being chemotherapy alone.

Predictive Performance

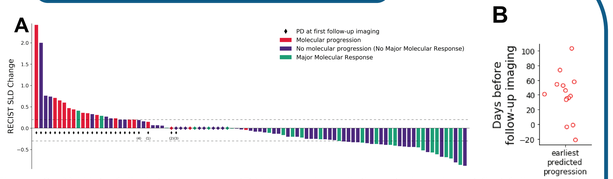
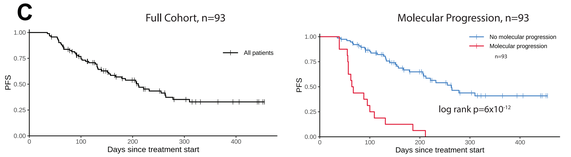
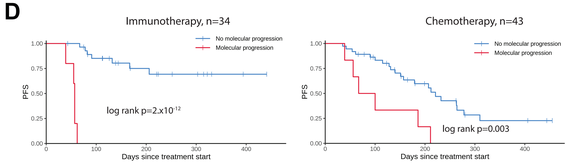


Figure 3. A) Waterfall plot compares ctDNA-based predictions to imaging at first follow-up, quantified by the sum of longest diameter (SLD) in target lesions by RECIST, for all patients (n=93). Predictions of molecular progression are indicated by bar color (n=16 with molecular progression, n=53 no molecular progression, n=24 major molecular response). Based on these predictions sensitivity for the assay was 54% and specificity was 97%. Sensitivity was observed to be higher at T2 (61%) than T1 (38%). Footnoted patients showed clear clinical progression. **B)** Distribution of how long the collection date of the first detection of molecular progression preceded the detection by imaging (median of 40 days).



C) Kaplan Meier plots of progression-free survival times (PFS) are shown for the full cohort (median PFS of 211 days) and subcohorts based on the ctDNA assay. Patients with molecular progression (16) at either follow up time point, had shorter PFS (median 64 days) compared to patients without an increase (77; median 263 days), with a hazard ratio of 7.2 (95% confidence interval 3.8-13.8).



D) The predictive performance was consistent in the subsets of patients on immunotherapy (either alone or in combination with another therapy) and chemotherapy either alone or in combination with a therapy other than immunotherapy. For patients on immunotherapy the sensitivity was 71%, specificity was 100%, the median PFS for patients with molecular progression was 57 days and median PFS was not reached for with no molecular progression. For patients on chemotherapy alone the sensitivity was 36%, specificity was 94%, the median PFS for patients with molecular progression was 67 days and median PFS was 221 days for patients with no molecular progression.

Major Molecular Response

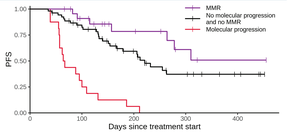


Figure 4. To determine if there is potential long term predictive power for patients who respond exceptionally well to a new line of therapy, we defined a major molecular response (MMR) as a less than 0.1 tumor fraction ratio between any follow up time point and baseline. Patients with a MMR showed longer PFS (n = 24; median not reached) relative to the remainder of the cohort (n = 53; median of 221 days).

Conclusions

- **Analyzing ctDNA early in the course of a new line of therapy holds promise to identify patients with disease progression faster than traditional methods.**
- **MMR has the potential to identify patients with an exceptional response early in the course of treatment with better clinical outcome, giving reassurance that there is a strong benefit.**
- **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 would facilitate broad clinical application.**
- **Further studies are ongoing to develop this assay system for use in clinical practice and therapeutic clinical development.**

References & Acknowledgments

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