Longitudinal changes in whole-genome cell-free DNA and methylation as a blood-based biomarker to identify early disease progression in advanced breast cancer

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Highlights

• We investigated whole-genome analysis of cell-free DNA (cfDNA) from serial blood samples in 24 prospectively enrolled patients being treated for advanced breast cancer.

• Molecular Progression by cfDNA was strongly predictive of radiographic progression at first follow-up, as well as shorter progression-free survival (PFS) and overall survival (OS).

• Liquid biopsy indicated earlier signs of progression, as Molecular Progression was called from blood samples between 2 to 15 weeks before imaging showed progression.

• We explored genome-wide methylation for 11 of the patients and found an additional 2 cases with molecular evidence of progression from cancer-associated methylation changes, showing a potential to increase predictive performance.

Background

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 2-4 months or longer on therapy before confident conclusions can be made.

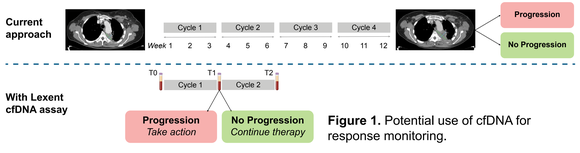


Figure 1. Potential use of cfDNA for response monitoring.

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 (MP), we have developed a novel approach to quantitatively track changes in circulating tumor DNA (ctDNA) to monitor response to treatment. Several distinctive features of cancer can be detected in ctDNA from plasma [1-4], 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, RS, AS, NL, AT and HT are current or previous employees of Lexent Bio. 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 to disclose.

Assay Workflow

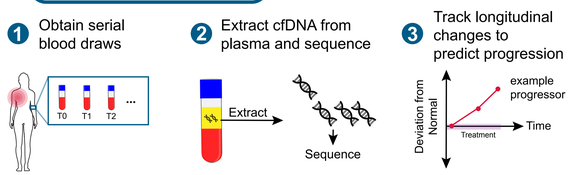


Figure 2. Schematic of the assay workflow.

Peripheral blood was collected from patients over time (Fig. 2, step 1). Plasma was separated and cfDNA was extracted (Fig. 2, step 2). Sequencing libraries were prepared using a method optimized for whole-genome sequencing (WGS, 13 patients), whole-genome bisulfite sequencing (WGBS, 5 patients), or both protocols separately (6 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 (Fig. 2, step 3) [5]. Molecular Progression was defined as an increase in tumor fraction from baseline.

Patients

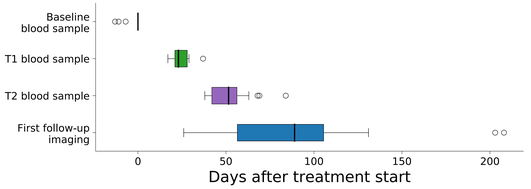
We prospectively enrolled and serially collected blood from 24 patients with advanced breast cancer, each receiving a new treatment (Table 1). Blood was collected on a schedule before each cycle of treatment, and imaging was performed per standard practice (Fig. 3). Treatment response was evaluated by an independent radiologist based on RECIST 1.1 guidelines to determine an outcome of progressive disease (PD) or non-progressive disease (nonPD, including stable disease or partial response).

Table 1. Patient characteristics, 2017 - 2019.

		Median (Min-Max)	N=24 (%)
Age (years)		65 (30-83)	
Sex	Female		24 (100)
Subtype	HR+, HER2-		14 (58)
	HR+, HER2+		5 (21)
	Triple Negative		5 (21)
Treatment	Chemotherapy		8 (33)
	Endocrine, CDK4/6i		6 (25)
	Chemotherapy, Antibody*		5 (21)
	Endocrine		3 (12)
	Immunotherapy ± HDACi		2 (8)
Lines of therapy	1		5 (21)
	2		7 (29)
	3+		12 (50)
Timing (days since treatment start)	T1	23 (17-37)	23 (96)
	T2	49 (38-84)	20 (83)**
	First follow-up	89 (26-208)	
	Last follow-up	453 (139-708)	

* HER2-directed antibody, trastuzumab and/or pertuzumab
** 19 patients had both post-treatment samples

Figure 3. Sample timing. T1 blood sample was collected before the second cycle of treatment, and T2 was collected before the third cycle.



Predictive Performance

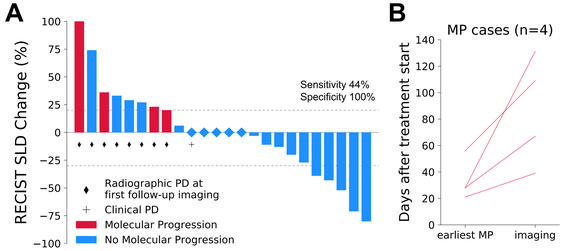


Figure 4. A. Waterfall plot compares ctDNA-based Molecular Progression calls at either T1 or T2 to first follow-up imaging quantified by the sum of longest diameters (SLD) in target regions by RECIST for all patients (n=24). **B.** Timing of Molecular Progression and radiographic identification of PD. For the 4 patients with Molecular Progression, the calls were from samples that preceded imaging by 18, 39, 53, and 103 days.

We compared ctDNA-based calls of Molecular Progression at either post-treatment timepoint to first-followup imaging (Fig. 4A) and found that all patients with MP also had PD at first follow-up imaging (4/4). For the remaining patients, 15 of 20 had nonPD at first follow-up imaging. Sensitivity for the assay was **44%** and specificity was **100%**. In the 4 MP cases, calls were from samples that preceded standard of care imaging by between **2 to 15 weeks** (Fig. 4B).

Survival Analysis

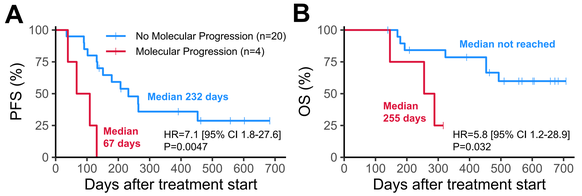


Figure 5. (A) Progression-free survival (PFS) and **(B)** overall survival (OS) plotted for all patients grouped by Molecular Progression. Patients with Molecular Progression had significantly shorter PFS (P=0.0047) and OS (P=0.032).

For all participants in the cohort (n=24), the median PFS was 179 days and median OS was not reached. We carried out survival analysis with the Cox proportional hazards model, and tested significance of the association between Molecular Progression and survival using Wald's test. Patients with MP had shorter PFS, a median of **67 days** versus **232 days** for others (Fig. 5A, HR=7.1 [95% CI 1.8-27.6], P=0.0047). Patients with MP also had shorter OS, with a median of **255 days** versus **median not reached** for others (Fig. 5B, HR=5.8 [95% CI 1.2-28.9], P=0.032).

Methylation Results

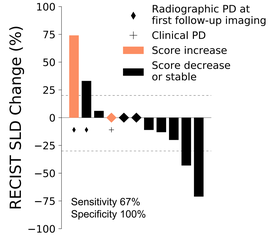


Figure 6. Waterfall plot as in Fig. 4 compares radiographic assessment by RECIST to methylation changes in cfDNA for the subset of patients analyzed with WGBS (n=11).

For patients assessed with WGBS (n=11), we computed a methylation score that quantifies the deviation of each from normal samples from a cohort of 21 healthy subjects [6]. When there was an increase in methylation score from baseline beyond the background variation observed in healthy samples we predicted progression (Fig. 6).

2 of 3 cases with clinical or radiographic PD were predicted to progress based on the cancer-associated methylation score. Neither of these cases showed Molecular Progression based on whole-genome features not including methylation (as in Fig. 4). **All 8 patients** with radiographic nonPD were not predicted to progress by the same measure. This result indicates the potential to incorporate whole-genome methylation in order to increase the clinical sensitivity of the assay while maintaining high specificity.

Conclusions

• Analyzing whole-genome 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.

• Lexent Bio is developing this assay for use in clinical practice.

Acknowledgements & References

We thank all participants and their families, as well as all clinicians and their research staff. We also thank Dr. Brian McNamee for review of all clinical images.

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