Serial changes in tumor-derived whole-genome cell-free DNA (cfDNA) fraction to identify early disease progression prior to imaging

Andrew A. Davis[1], Wade T. Iams[2], David Chan[3], Michael S. Oh[1], Robert W. Lentz[1], Neil Peterman[4], Alex Robertson[4], Abhik Shah[4], Rohith Srivas[4], Nicole Lambert[4], Timothy Wilson[4], Peter George[4], Becky Wong[4], Ayse Tezcan[4], Ram Yalamanchili[4], Ken Nesmith[4], John C. Spinosa[4], Haluk Tezcan[4], Young Kwang Chae[1]

1. Northwestern University Feinberg School of Medicine, Chicago, IL; 2. Vanderbilt University Medical Center, Nashville, TN; 3. Cancer Care Associates TMPN, Redondo Beach, CA; 4. Lexent Bio, Inc., San Francisco, CA

Highlights

- **We investigated whole genome sequencing (WGS) of cfDNA from serial blood samples in 54 prospectively enrolled patients receiving treatment for metastatic cancer.**
- **Increases in tumor-derived cfDNA were strongly predictive of disease progression at first follow-up and shorter progression-free survival.**
- **Prediction of progression was based on blood samples taken a median of 5.5 weeks before imaging and clinical evaluation.**

Objective

Patients treated for metastatic cancer face considerable uncertainty about the effectiveness of systemic therapies that carry serious side effects, risks, and cost. Today, imaging (CT, PET/CT, MRI), the standard for response assessment, requires 3-4 months or longer on therapy before confident conclusions can be made.

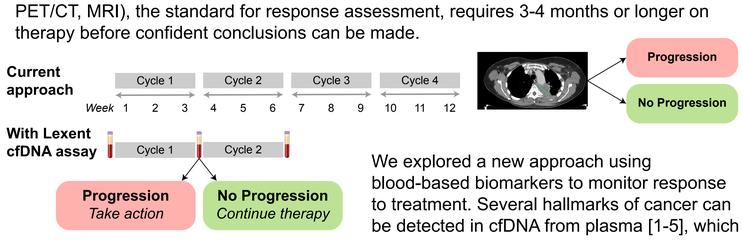

Figure 1. Potential use of cfDNA for response monitoring.

We explored a new approach using blood-based biomarkers to monitor response to treatment. Several hallmarks of cancer can be detected in cfDNA from plasma [1-5], which has led to the development of multiple diagnostic applications.

Longitudinal Cohort

We prospectively enrolled and serially collected blood from 54 patients with metastatic 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.

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

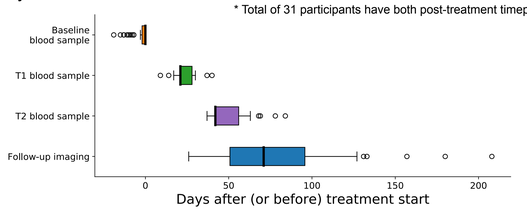

Table 1. Patient characteristics; 2017 - 2018.

		Median (Min-Max)	N=54 (%)
Age (years)		70 (31-89)	
Sex	Female		35 (65)
	Male		19 (35)
Cancer type	Lung		21 (39)
	Breast		20 (37)
	GI		6 (11)
	GU		5 (9)
	Other		2 (4)
Immunotherapy	Yes		14 (26)
	No		40 (74)
Lines of therapy	1		23 (43)
	2		13 (24)
	3		12 (22)
	4+		6 (11)
T1 (days)		21 (9-40)	51 (94)
T2 (days)*		42 (37-84)	34 (63)
First follow-up (days)		71 (26-208)	
Last follow-up (days)		111 (35-469)	

* Total of 31 participants have both post-treatment timepoints

Methods

- Peripheral blood collected in Streck Cell-Free DNA Blood Collection Tubes®
- Separated 4mL plasma from each blood sample and isolated cfDNA
- Libraries prepared with a method optimized for whole genome sequencing (WGS)
- Sequenced libraries to a median coverage of 20X
- Quantified longitudinal changes in the fraction of tumor-derived cfDNA based on a patient-specific profile of whole genome features
- Treatment response evaluated based on RECIST 1.1 guidelines, confirmed by an independent radiologist

Prediction Performance

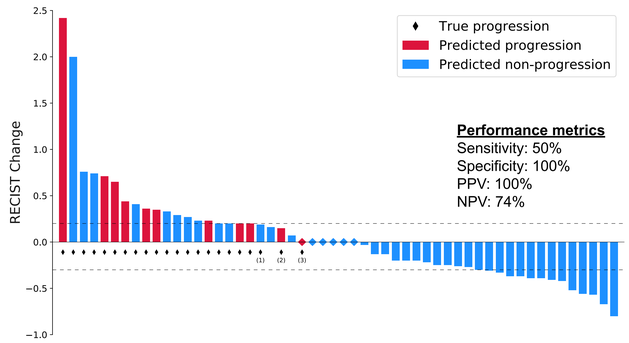

Figure 3. Waterfall plot compares cfDNA-based predictions to imaging at first follow-up (n=54). Footnoted cases showed clear clinical progression.

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. All patients with predicted progression did progress at the first follow-up evaluation (11/11, 100% positive predictive value). For the remaining patients, 32 of 43 did not progress (74% negative predictive value). Sensitivity for the assay was 50% and specificity was 100%.

Sample timing shows potential to accelerate the clinical decision loop

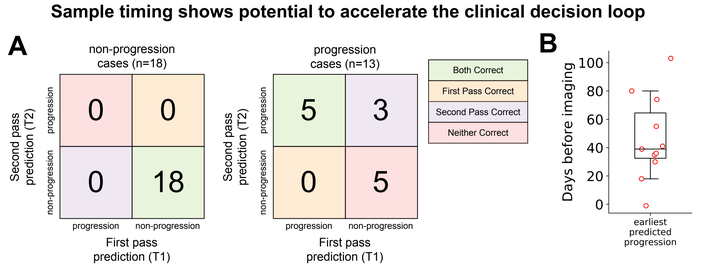

Figure 4. (A) Comparison of timepoints for patients with both post-treatment timepoints (n=31), plotted separately for progression and non-progression cases. (B) Timing of cfDNA-based predictions of progression (n=11).

Most predictions were concordant between the two cfDNA samples at T1 and T2 (Figure 4A). Out of 31 patients who had both post-treatment cfDNA samples, 3 (10%) had discordant predictions. All three 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 39 days (Figure 4B).

Survival Analysis

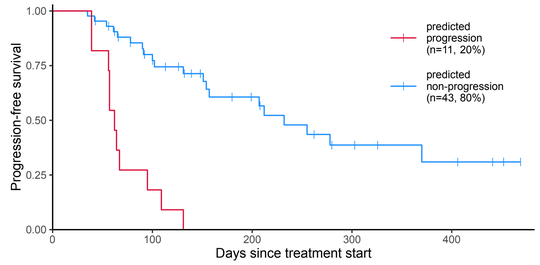

Figure 5. PFS based on imaging and clinical evaluation grouped by cfDNA prediction of progression and non-progression.

For all participants in the cohort, the median progression-free survival (PFS) was 154 days. Patients with predicted progression by cfDNA, indicated by an increase in tumor fraction at either post-treatment blood collection, had worse PFS compared to patients that did not show an increase (hazard ratio 8.0, [95% CI 3.4-19.2], log-rank $p=4.5\times10^{-8}$). Median PFS was **62 days** for patients with predicted progression versus **232 days** for others (Figure 5).

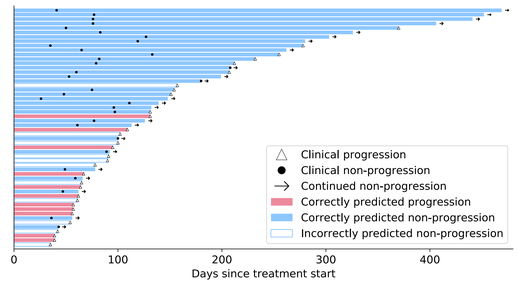

Figure 6. Swimmer plot shows progression-free follow-up for each participant (n=54).

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

Acknowledgements & References

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