Serial changes in whole-genome cell-free DNA (cfDNA) identify disease progression prior to imaging in advanced NSCLC

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Highlights

- **We investigated whole-genome analysis on cfDNA from serial blood samples in 35 prospectively enrolled patients receiving treatment for advanced NSCLC.**
- **Molecular progression by cfDNA was strongly predictive of disease progression at first follow-up and shorter progression-free survival.**
- **Confirmed predictions of progression were based on blood samples taken a median of 5 weeks before imaging and clinical evaluation.**
- **Major molecular response was observed in a subset of cases and potentially provides early evidence of treatment efficacy.**

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

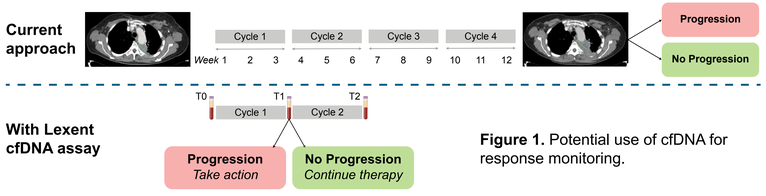


Figure 1. Potential use of cfDNA for response monitoring.

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”, 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.

Longitudinal Cohort

We prospectively enrolled and serially collected blood from 35 patients with advanced NSCLC, 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.

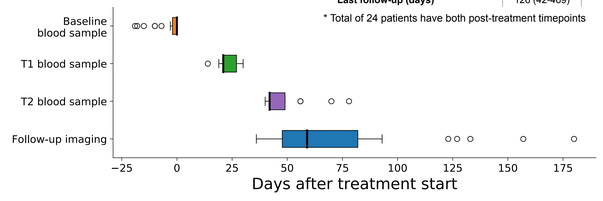
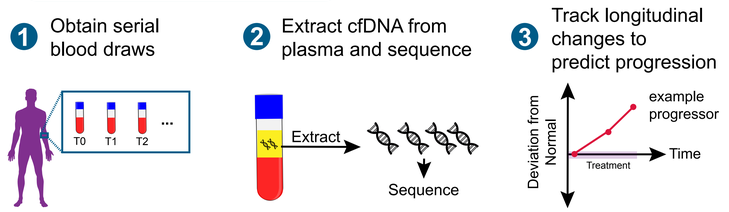


Table 1. Patient characteristics; 2017-2019.

		Median (Min-Max)	N=35 (%)
Age (years)		72 (48-87)	
Sex	Female		17 (49)
	Male		18 (51)
Immunotherapy	Yes		22 (63)
	No		13 (37)
Line of therapy	1		24 (69)
	2		7 (20)
	3+		4 (11)
T1 (days)		21 (14-30)	33 (92)
T2 (days)*		42 (40-78)	26 (72)
First follow-up (days)		59 (36-180)	
Last follow-up (days)		126 (42-469)	

* Total of 24 patients have both post-treatment timepoints

Assay Workflow



Peripheral blood was collected from patients over time (Step 1). Plasma was separated and cfDNA was extracted (Step 2). Sequencing libraries were prepared using a method optimized for whole genome sequencing (20 patients) or whole genome bisulfite sequencing (15 patients). Libraries were sequenced to a median coverage of 19X. Longitudinal changes in the fraction of ctDNA were quantified based on a patient-specific profile of whole-genome features (Step 3) [5]. Molecular progression was defined as an increase in tumor fraction from baseline.

Predictive Performance

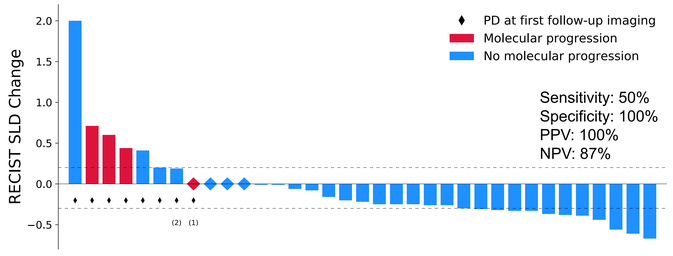


Figure 3. Molecular response assessment and imaging at first follow-up, quantified by the sum of longest diameter (SLD) in target lesions by RECIST for all patients (n=35). Footnoted cases showed clear clinical progression.

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). We compared ctDNA-based molecular response assessment to follow-up imaging and clinical assessment (Figure 3) and found that all patients with molecular progression had PD (4/4, 100% positive predictive value). For the remaining patients, 27 of 31 had nonPD (87% negative predictive value). Sensitivity to identify PD early in the treatment course was 50% based on the 4 patients with molecular progression out of 8 patients with PD at first follow-up imaging. Specificity was 100% as all 25 patients with nonPD did not have molecular progression.

Substantial changes in tumor fraction were observed shortly after treatment initiation (Figure 4A). In cases of molecular progression, the time point where it was first identified preceded the detection of PD by imaging by a median of 34 days (Figure 4B).

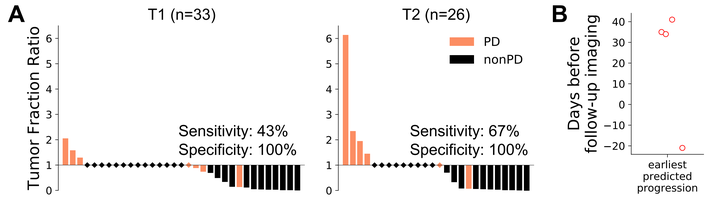


Figure 4. (A-B) Tumor fraction ratio at each time point for patients assessed as PD or nonPD at first follow-up imaging. (B) Timing of molecular progression calls relative to imaging.

Survival Analysis

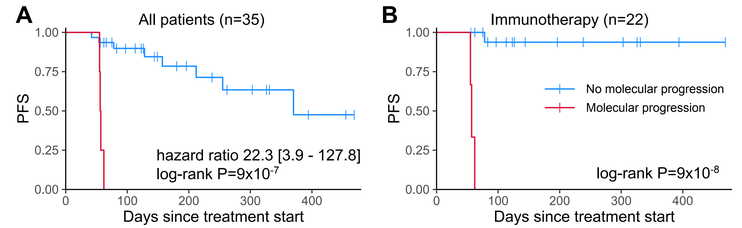


Figure 5. PFS for ctDNA-based molecular progression for (A) all patients (n=35), and (B) patients on immunotherapy (n=22).

Patients with molecular progression by ctDNA had shorter progression-free survival (PFS), a median of **56 days** versus **370 days** for others (Figure 5A, hazard ratio 22.3 [95% CI 3.9-127.8], log-rank $P=9x10^{-7}$). These results were consistent in the subset of patients on immunotherapy (Figure 5B, log-rank $P=9x10^{-8}$).

Figure 6. PFS for patients with molecular progression (n=4), MMR (n=11), or neither (n=20).

A subset of patients showed a reduction in tumor fraction by 10-fold or more (n=11), which we called a major molecular response (MMR). Such a reduction has been associated with longer PFS [5]. Integrated with imaging, such a result could potentially provide an early indication of a sustained clinical benefit. We find that while patients with MMR tended to have longer PFS (Figure 6), the additional predictive value of this result is not significant (Cox regression: molecular progression $P=5x10^{-4}$, MMR $P=0.22$).

Conclusions

- **Molecular response assessment by analyzing ctDNA 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.**
- **Blood-based signals of molecular response have potential synergy with imaging to identify patients receiving the greatest therapeutic benefit.**
- **Further studies are ongoing to develop this assay for use in clinical practice.**

Acknowledgements and References

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