Title: Non-invasive Longitudinal Analysis of Cancer Signal to Monitor Disease Progression and Responses to Treatment

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BACKGROUND

- Currently, most non-invasive multi-cancer early detection products lack the ability to translate analytical findings to clinical outcomes. Quantitative tracking of cancer signal is the next frontier for early cancer detection and data-driven intervention.
- Harbinger Health has pioneered an NGS platform that combines novel epigenomic insights with artificial intelligence to detect cancer and monitor signal longitudinally over time to screen the population as well as track disease progression, monitor response to treatment and make predictions on prognosis.

METHODS

- We designed a comprehensive enrichment panel (17.8 Mb) which provides simultaneous methylation and mutational states for cancer and tissue-of-origin informative regions from whole genome bisulfite cfDNA libraries.
- We defined the most significant methylation motifs (MMs) that contribute to pan-cancer signal and developed an algorithm to estimate the amount of tumor-derived fragments in a cell-free DNA (cfDNA) sample. This tumor content estimate (%TC) was validated with independent, in-house generated datasets of 46 matched FFPE/cfDNA samples and 1,046 unmatched cfDNA samples.
- Using %TC, we quantified signal over time to monitor dynamic changes in the tumor. Simultaneously, we tracked orthogonal mutations across treatment.

	Subject characterization	Number of blood draws (timepoints)	Average time interval between draws
103 subjects	80 cancer	T0, T1, T2, T3	39 days
	23 healthy	T0, T1	42 days

Figure 1: Study cohort. 103 subjects were sourced; 80 cancer patients across 16 indications and 23 healthy individuals. Four blood draws (timepoints) were collected per cancer patient and two timepoints were collected per healthy individual. The average time between timepoints was ~40 days.

- To clinically assess our quantitative analysis**,** we sourced plasma from 80 cancer patients across 16 indications over the course of treatment with four timepoints, averaging 39 days apart. Timepoint 0 (T0) was pre-treatment, while T1, T2 and T3 were taken before during the course of treatment. We had endpoint RECIST data for 31 out of the 77 cancer patients, which categorizes disease progression and response by evaluating the solid tumor lesion. We also sourced 23 healthy, non-cancer subjects with two timepoints, averaging 42 days apart (**Figure 1**). All samples across these subject cohorts and timepoints were enriched with the comprehensive panel. Analysis was done to assess longitudinal tracking, predict prognosis and detect disease progression.

RESULTS

Estimating Tumor Content by Methylation

- Tumor content estimated by methylation motifs (MMs) was highly correlated (> 0.99, Pearson) to %TC by SNVs (Poster B-361).
- Methylation-estimated tumor content showed that cancer patients (T0) ranged from 0.005% to 47% with Stage IV showing the greatest tumor burden, as expected (**Figure 2**).

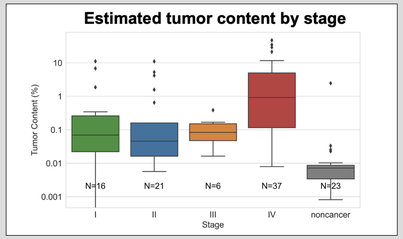

Figure 2: Estimated tumor content by stage. Significant difference ($p < 0.001$, Mann-Whitney U test) in estimated tumor content between healthy, non-cancer individuals and cancer patients at each stage (I, II, III, IV), with Bonferroni-adjusted p-value cutoff (0.05/6=0.0083). The six comparisons include stage I, II, III, IV versus non-cancer, as well as (I+II+III) versus IV and (I+II+III) versus non-cancer.

Quantitatively Tracking Disease Progression

- Average tumor content (%TC) during treatment showed separation between response groups **(Figure 2).** Partial responders (PR, n=14) had high %TC and an overall decrease from T0 to T3. Patients with progressive disease (PD, n=14) showed an overall increase in %TC between T0 and T3. Complete responders (CR, n=3) had lower initial %TC that sustained throughout treatment. Healthy, non-cancer individuals (n=23) had sustained low tumor content, as expected.
- In a small subset of three Stage IV prostate cancer patients with high initial %TC, our biomarkers were able to identify one patient that was reported PR but showed sustained high tumor content and an overall increase in %TC **(Figure 3).** This patient was deceased at last follow-up demonstrating the potential clinical advantage of molecular profiling for identifying disease progression.

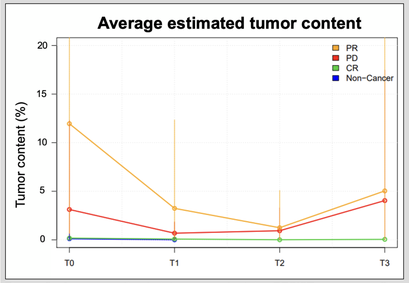

Figure 3: Average estimated tumor content by RECIST response. Clear separation between partial response (PR), progressive disease (PD) and complete response (CR). Healthy, non-cancer individuals showed low tumor content, as expected. Error bars are 95% confidence intervals.

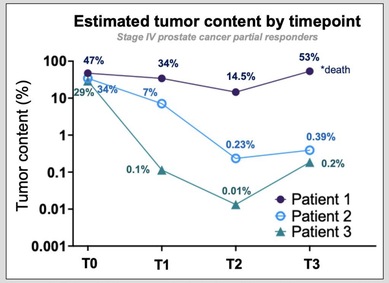

Figure 4: Estimated tumor content of stage IV prostate cancer partial responders. Patient I showed an overall increase in tumor content and subsequent death, while Patient 2 and 3 show sustained decreases in tumor content. The example of Patient 1 shows a potential advantage of molecular profiling over traditional cancer progression analyses (RECIST).

RESULTS

Predicting Prognosis through Tumor Content

- Using a longitudinal threshold (LT) derived from tumor content analysis, we observed that we could predict prognosis based on the number of serial draws (timepoints) detected that were greater than the LT (**Figure 4**).
- Patients with only one timepoint (T0) detected (n=8) had 100% survival rate, while patients with all four timepoints (n=18) detected had 39% survival rate (data not shown).
- Patients with higher initial %TC (T0) had a worse prognosis after treatment (**Table 1**).

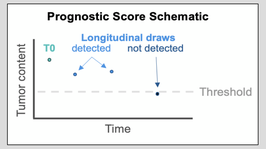

Figure 5. Prognostic Score Schematic.

Tumor content (%)	% patient deceased	Total Patients
> 0.01%	26.1	69
> 0.1%	33.3	42
> 1%	34.8	23
> 10%	50	8

Table 1. Three-year Mortality Rate Based on Initial %TC

Multi-tier Model: Screen, Confirm, Monitor

- To address low positive predictive value (PPV) in MCED, we modeled a multi-tier testing concept (**Figure 5**) that maximizes PPV by screening the population in a low-cost, low target specificity test and confirming the presence of cancer in a high specificity test. Confirmed cancers will then be longitudinally tracked using tumor content to monitor progression and predict prognosis. In this model, PPV for Tier 1 only was 18.5% and increased by over 5X using this multi-tier model, which suggests substantial improvement from current MCED tests.

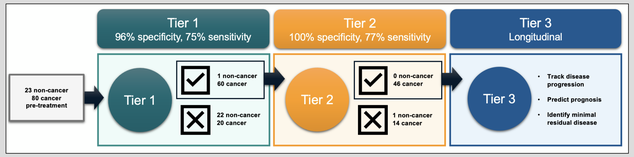

Figure 6: Multi-tier testing model. Tier 1 used estimated tumor content with a negative (non-cancer) result threshold from an independent, in-house generated dataset of 1046 samples (621 cancer, 425 non-cancer). Tier 2 used a fixed multi-layered logistic regression-based machine learning algorithm, trained with the same dataset of 1046 samples, that predicts a binary classification (yes/no). Tier 3 is a future direction of this testing that tracks %TC.

CONCLUSIONS

- We successfully showed our ability to quantitatively track patients longitudinally by tumor content estimation, which allows us to identify disease progression, monitor response and potentially predict prognosis.
- Our data suggests using a multi-tier model, similar to %TC followed by ML algorithm, can help achieve the necessary PPV (5X a single test) for MCEDs to be impactful and successful.
- As we begin to detect earlier, longitudinal profiling may allow for clinicians to intervene earlier, which would significantly improve the cancer care continuum.