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Low-cost detection of advanced adenomas in cfDNA using quantitative methylation-specific PCR

Harbinger Health

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INTRODUCTION

- Removal of advanced adenomas (AA) during colonoscopy has been shown to substantially reduce colorectal cancer incidence and mortality[1]. However, many screen-eligible patients remain unscreened due to the invasive nature of colonoscopy.
- Non-invasive liquid biopsy tests hold significant promise to broaden screening access and enable earlier intervention through advanced adenoma detection.
- Harbinger Health's biomarkers, which were selected based on association with early oncogenic signals and are observed across multiple tumor types, were down-selected to a small panel of highly informative pan-cancer markers to enable more accessible detection technologies such as qMSP.
- Quantitative methylation-specific PCR (qMSP) provides a rapid, low-cost, and scalable approach for detecting advanced adenomas from cell-free DNA (cfDNA).

OBJECTIVES

- To explore the feasibility of qMSP for advanced adenoma detection, Harbinger Health developed a qMSP assay targeting six pan-cancer-informative methylation markers and evaluated the assay using cfDNA from non-cancer and advanced adenoma samples.

METHODS

- Harbinger Health identified six highly pan-cancer-informative methylation markers from a broader set of biomarker regions of interest.
- Three multiplex qMSP assays were developed for six methylation markers. Each methylation marker spans multiple contiguously methylated CpG sites. Primer and hydrolysis probes were designed to span methylated CpG sites to drive methylation-specificity. Additionally, each multiplex qMSP included a methylation-independent reference assay to estimate total amplifiable cfDNA input per reaction for sample normalization.
- Despite the informative nature of these biomarkers, biological processes such as aging can introduce stochastic methylation in non-cancer cfDNA, which increases background methylation signal and attenuates disease detection. To mitigate this confounding background, locked nucleic acid (LNA) blockers were designed to span non-methylated CpG sites to minimize probe cross-hybridization to stochastically methylated somatic cfDNA, as shown in Figure 1[2]. The addition of LNA blockers improved selectivity for defined methylation patterns in low abundance circulating tumor DNA (ctDNA).

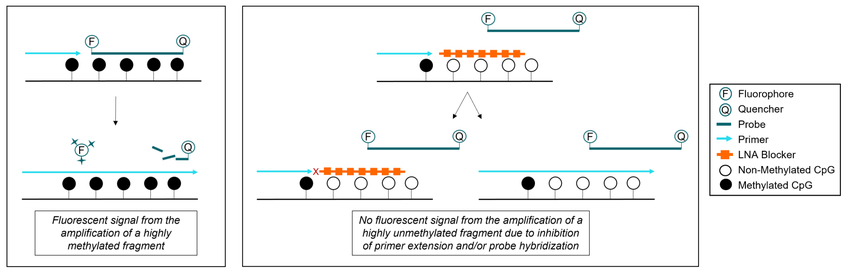

Figure 1. qMSP Design Methodology.

METHODS

- The qMSP panel underwent technical validation during development and demonstrated high analytical sensitivity, specificity, and reproducibility for detecting defined methylation patterns using synthetic controls.
- To evaluate clinical performance, the qMSP assay was applied to 100 cfDNA libraries, including 62 cfDNA samples from patients with no reported cancer in the CORE-HH clinical study (NCT05435066) and 38 commercially sourced cfDNA samples collected from patients with confirmed advanced colorectal adenomas. A summary of sample characteristics is described in Table 1.
- cfDNA samples were processed into bisulfite libraries and analyzed by qMSP using 5 ng of library per reaction. Libraries were utilized as the available in-house material for the initial assessment of qMSP as a low-cost readout.
- For each marker, the cycle threshold (Ct) was normalized to the internal reference Ct to calculate the delta Ct (dCt) and assess the relative abundance of methylated copies. A target-specific dCt threshold was applied to each marker, and a marker was called positive if the dCt was lower than the specified threshold. A sample was called positive if at least one marker was positive.

Metric	Category	Advanced Adenoma		Non-Cancer	
		Count	Percentage	Count	Percentage
Sex	Female	24	63%	43	69%
	Male	14	37%	19	31%
	Total	38	100%	62	100%
Metric	Category	Years		Years	
Age	Q1	52		42	
	Q3	70		63	
	IQR	18		21	
	Median	65		51	
Metric	Category	Count	Percentage	Count	Percentage
Race	White	36	94%	38	61%
	Black or African American	1	3%	11	18%
	Asian	0	0%	4	6%
	Native Hawaiian or OPI	0	0%	0	0%
	Other	0	0%	3	5%
	Unknown	1	3%	6	10%

Table 1. Sample Characteristics.

RESULTS

- The dCt values for each qMSP assay were calculated, and the non-cancer samples (N = 62) were used to set targeted specificity thresholds, as shown in Figure 2.

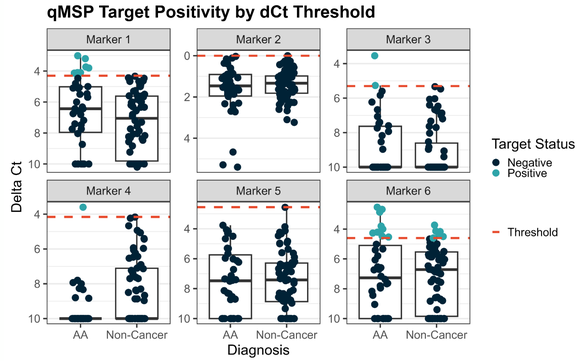

Figure 2. Delta Ct Values with Marker-Specific Thresholds.

RESULTS

- Using marker-specific detection thresholds established on 62 non-cancer samples to achieve an equivalent of 90% specificity, qMSP detected at least one positive target in 16 of 38 (42%) advanced adenoma samples, as shown in Figure 3.

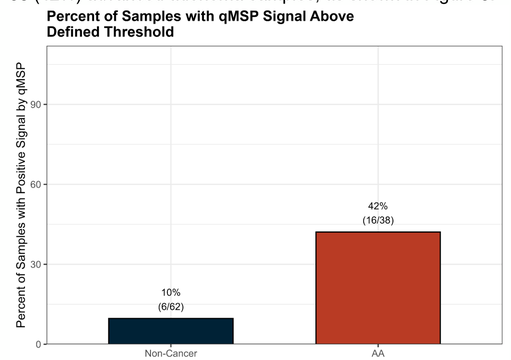

Figure 3. Percent of Samples with Positive qMSP Signal.
Note: Correction to abstract – sensitivity was misreported, correct value shown here.

CONCLUSIONS

- Harbinger Health demonstrates the feasibility of qMSP for advanced colorectal adenoma detection from cfDNA. A qMSP assay targeting six pan-cancer-associated methylation markers effectively distinguished advanced adenoma samples from non-cancer samples in a cohort of 100 cfDNA libraries.
- AA detection by liquid biopsy is limited by low shed of tumor-derived DNA into plasma from small, precancerous lesions[3].
- Harbinger Health's biomarkers are associated with early oncogenic signals and are observed across multiple tumor types, including AA tissue. Notably, the current six-marker selection was not optimized for AA detection. The development of an expanded qMSP panel which incorporates AA-specific biomarkers is expected to improve clinical performance.
- qMSP data can be generated inexpensively, qMSP reactions cost less than $5 per sample and were completed in less than 5 hours. Downstream analysis relied on standard data processing rather than specialized bioinformatic workflows. qMSP addresses key barriers to population-scale implementation such as cost, turnaround time, and batch size flexibility.
- qMSP is operationally tractable for time-constrained and resource-constrained use cases within cancer care. For example, qMSP may have potential utility as a resource-efficient triage tool to inform colonoscopy referral.

REFERENCES

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DISCLOSURES

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