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INTRODUCTION

- Liquid biopsy multi-cancer detection tests may enable earlier intervention and improve patient outcomes, especially for cancer types that currently lack standardized, population-wide screening.
- DNA methylation biomarkers have shown to be highly informative for liquid biopsy applications¹. Harbinger Health's methylation biomarkers were selected based on association with early oncogenic signals and are observed across multiple tumor types.
- Most current methylation-based multi-cancer detection tests analyze cell-free DNA (cfDNA) methylation profiles using next-generation sequencing (NGS) with machine learning classifiers.
- Alternatively, quantitative methylation specific PCR (qMSP) offers a low-cost, low-complexity, and scalable workflow that supports flexible batch sizes and decentralized testing.

OBJECTIVES

- To explore the feasibility of qMSP for multi-cancer detection, Harbinger Health developed a qMSP assay targeting six pan-cancer-informative markers and evaluated the assay using cfDNA from nine cancer types. These cancers include high-incidence and/or high-mortality tumor types, several of which lack standard population screening options.

METHODS

- Harbinger Health identified six highly informative pan-cancer 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 cfDNA². The addition of LNA blockers improved selectivity for defined methylation patterns in low abundance circulating tumor DNA (ctDNA).
- The qMSP assay 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 130 cfDNA samples obtained from the CORE-HH clinical study (NCT05435066), representing 62 non-cancer and 68 cancer samples across nine cancer types, as shown in Figure 1. The cancer samples represented a balanced stage distribution. 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) value 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 qMSP marker was positive.

METHODS

Sample Cohort

Sample Distribution by Diagnosis

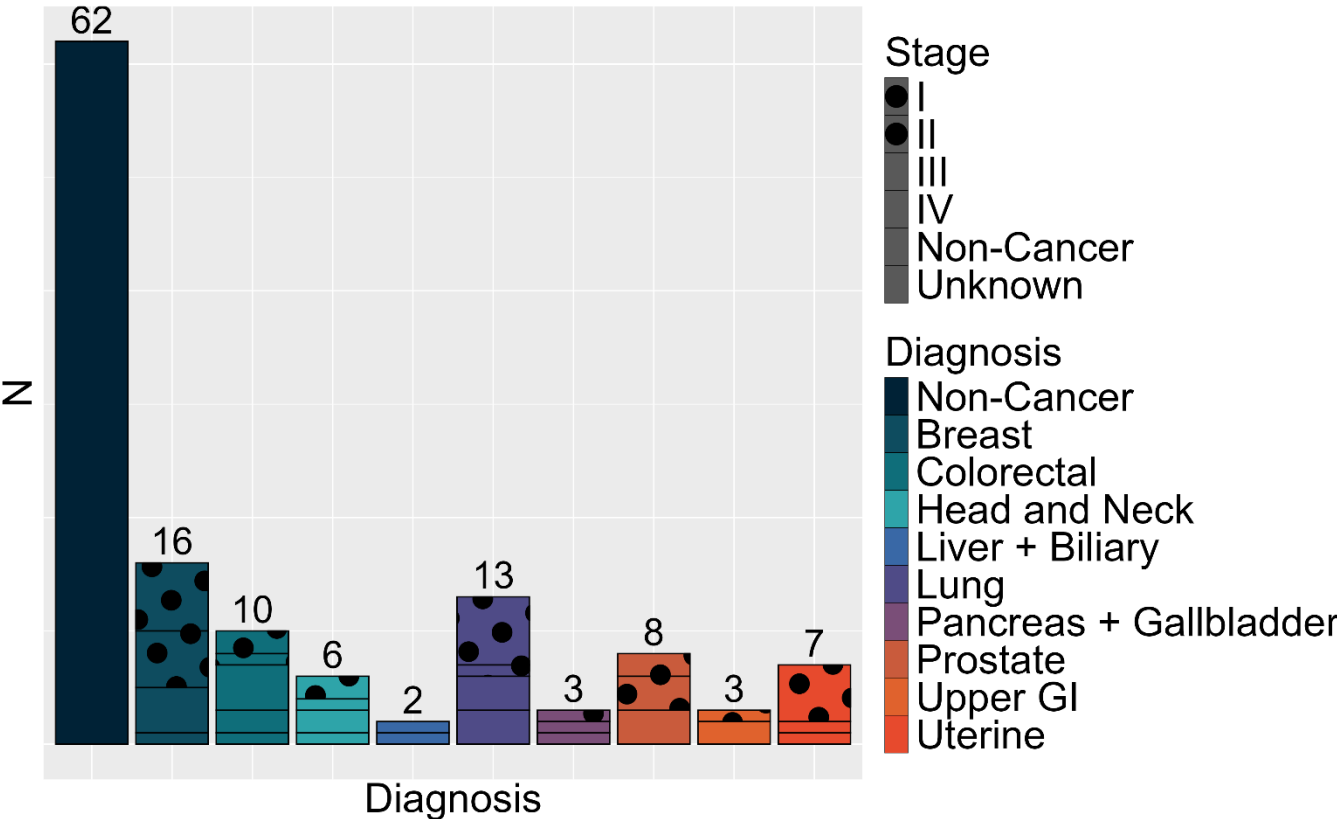


Figure 1. Sample Distribution by Cancer Type and Stage.

Metric	Category	Cancer		Non-Cancer	
		Count	Percentage	Count	Percentage
Sex	Female	40	59%	43	69%
	Male	28	41%	19	31%
	Total	68	100%	62	100%
Age	Q1	56		42	
	Q3	70		63	
	IQR	14		21	
	Median	65		51	
Race	White	54	79%	38	61%
	Black or African American	8	12%	11	18%
	Asian	2	3%	4	6%
	Native Hawaiian or OPI	1	1%	0	0%
	Other	0	0%	3	5%
	Unknown	3	4%	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.
- Using marker-specific thresholds established on 62 non-cancer samples to achieve an equivalent of 97% specificity, qMSP detected at least one positive target in 30 of 68 (44%) cancer samples.
- qMSP detected 5 of 23 (22%) stage I, 5 of 12 (42%) stage II, 7 of 17 (41%) stage III, and 12 of 13 (92%) stage IV cancer samples, as shown in Figure 3.

qMSP Target Positivity by dCt Threshold

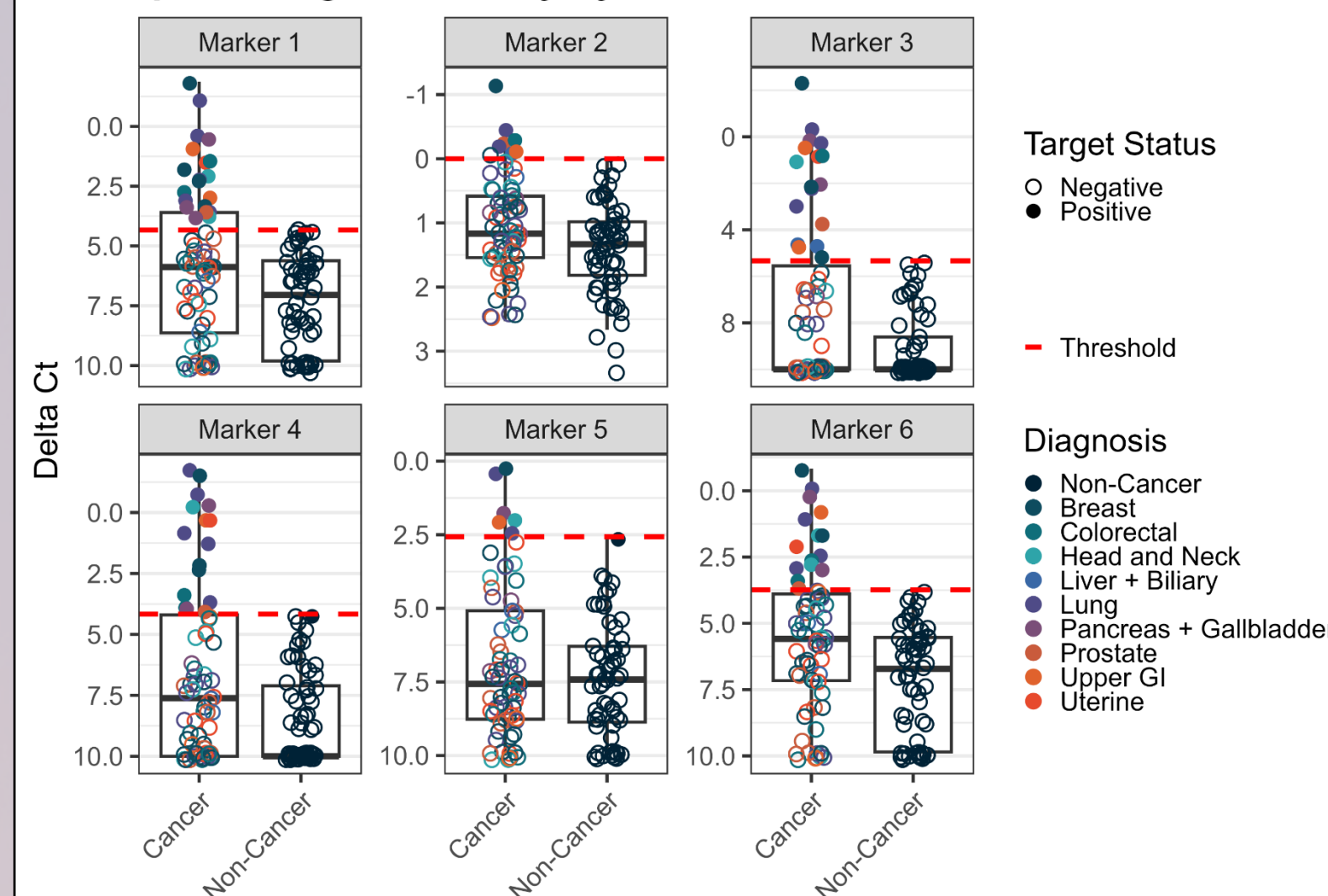


Figure 2. Delta Ct Values with Marker-Specific Thresholds.

Percent of Samples with qMSP Signal Above Defined Threshold

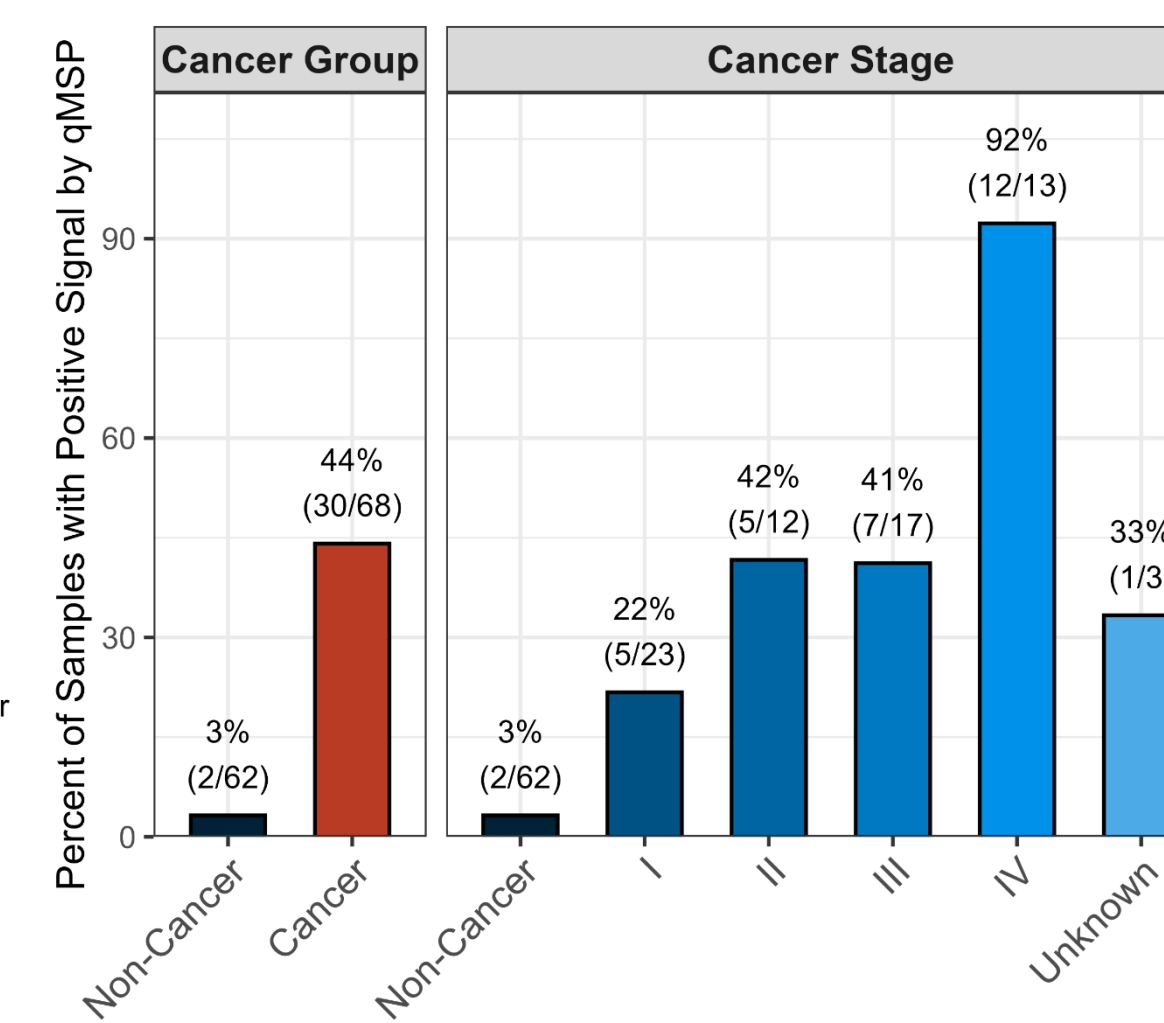


Figure 3. Percent of Samples with Positive qMSP Signal.

RESULTS

NGS Correlation

- dCt values were plotted against the relative methylation abundance of each marker by targeted sequencing. Analysis included only non-zero NGS values and valid Ct values. Marker 1 showed insufficient sequencing coverage and was excluded from analysis. Four of five markers showed strong correlation ($|r| > 0.70$) between qMSP and NGS, demonstrating technical concordance between the two methods, as shown in Figure 4.

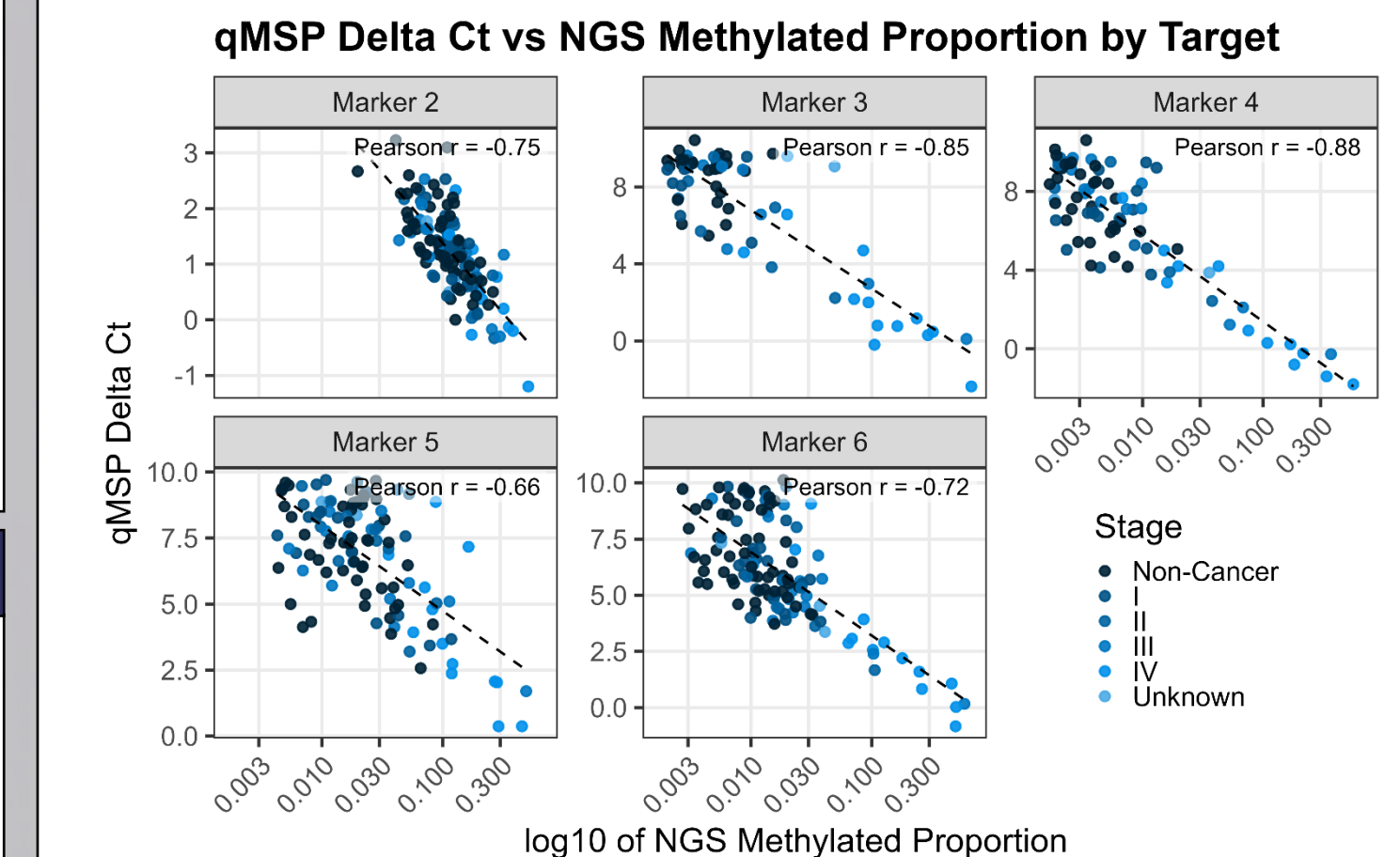


Figure 4. qMSP vs NGS Methylation Abundance.

CONCLUSIONS

- Harbinger Health demonstrates an initial proof of concept for a low-cost multi-cancer detection assay using qMSP. A qMSP assay targeting six pan-cancer-informative methylation markers differentiated non-cancer from cancer cfDNA libraries across nine cancer types in a cohort of 130 cfDNA libraries.
- qMSP data can be generated inexpensively, qPCR reactions cost less than \$5 per sample and were completed in less than five hours. Downstream analysis relied on standard data processing rather than specialized bioinformatic workflows. qMSP-based liquid biopsy approaches address key barriers to population-scale implementation such as cost, turnaround time, and batch size flexibility.
- Future improvements, including expanded biomarker selection, optimized assay design, and direct analysis from bisulfite-converted DNA, are expected to improve clinical performance.
- Through the combination of highly informative biomarkers with a low-cost, low-complexity workflow, qMSP holds potential to deliver clinical utility as a resource-efficient triage tool and broaden access to cancer detection.

REFERENCES

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DISCLOSURES

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