Integrated genomic and epigenomic cell-free DNA (cfDNA) analysis for the detection of early-stage colorectal cancer (CRC)

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Introduction

- CRC is a leading cause of cancer-related death with an estimated 100,000 new cases diagnosed in 2020.[1]
- The percentage of individuals expected to die from CRC has decreased, due in part to the increased uptake of CRC screening.[1-4]
- Despite clinical guidelines and established clinical evidence, nearly 1 in 3 American adults is not compliant with CRC screening recommendations.[2]
- Demonstrating the clinical utility of ctDNA for the detection of cancer in asymptomatic individuals has been challenged by the failure to achieve clinically meaningful sensitivity and specificity due to significantly lower tumor cell-free DNA (cfDNA) fractions and the increasing relevance of biologic confounders (e.g. clonal hematopoiesis of indeterminate potential (CHIP)).[5-7]
- The effective adoption of ctDNA for CRC screening requires improved sensitivity by expanding analysis beyond assessment of somatic genomic alterations to incorporate assessment of epigenomic signals and the ability to improve specificity by differentiating cancer related somatic alterations.

Methods

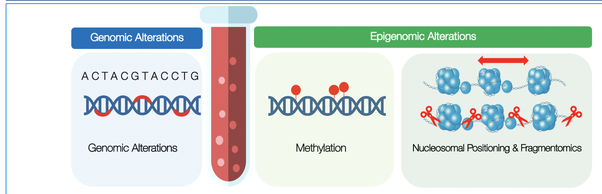


Figure 1: LUNAR-2 assay. A blood based ctDNA assay utilizing a multi-modal approach to CRC detection was developed and targets common oncogenic mutations as well as regions expected to undergo epigenomic modification in cancer (differential methylation and nucleosomal positioning changes resulting in differential cfDNA fragmentation patterns).

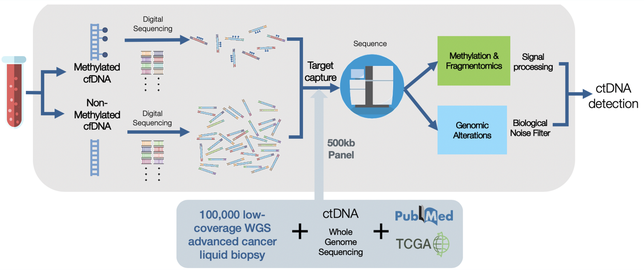
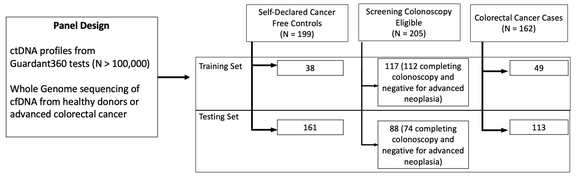


Figure 2: Laboratory and Bioinformatic workflow. Whole blood is collected and extracted cfDNA undergoes partitioning based on methylation level. Individual molecules are ligated, recombined, and processed.[8] Libraries are enriched with the 500kB panel (Figure 1), indexed, pooled, and sequenced. Three independent analyses are performed in parallel: a) detection of somatic genomic mutations and variant filtering; b) assessment of the observed distribution of cfDNA molecules across different methylation partitions; c) assessment of cfDNA fragmentation patterns in genomic regions across the panel.

Figure 3 and Table 1: Training and Test Cohorts



Training cohort was used to a) estimate the parameters of the somatic genomic alteration detection, methylation, and fragmentation analysis methods; b) train the linear classifier integrating the results of the individual callers into a single continuous predictor; c) establish a tumor presence prediction threshold over the linear classifier output for binary prediction of overall sample status targeting 95% specificity.

Table 1: Cohort Demographics		Training Cohort	Test Cohort
Colorectal Cancer Free Controls: Self-Declared Cancer Free & Colonoscopy Screen Negative	Total (N)	**155**	**244**
	Sex (N; %)		
	Male	52 (34%)	84 (34%)
	Female	103 (66%)	146 (60%)
	Unknown	0 (0%)	14 (6%)
	Median age in years (range)	61 (38-81)	63 (20-85)
Colorectal Cancer Cases	Total (N)	**49**	**113**
	Sex (N)		
	Male	26 (53%)	54 (48%)
	Female	23 (47%)	59 (52%)
	Median age in years (range)	66 (39-80)	66 (39-86)
	Colorectal Cancer Stage (N)		
	I / II	31 (63%)	87 (77%)
	III	13 (27%)	26 (23%)
	IV	5 (10%)	0 (0%)

Results

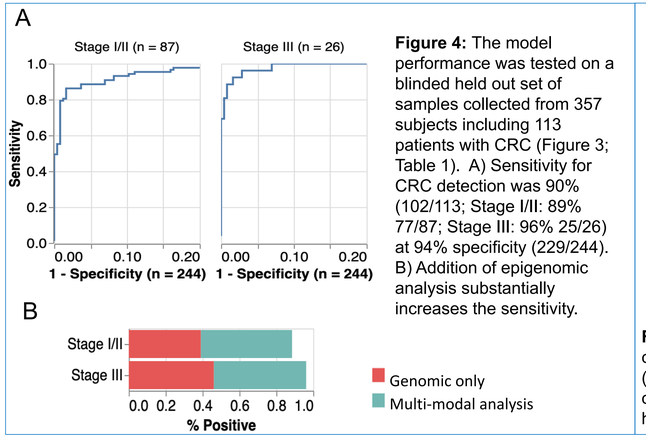


Figure 4: The model performance was tested on a blinded held out set of samples collected from 357 subjects including 113 patients with CRC (Figure 3; Table 1). A) Sensitivity for CRC detection was 90% (102/113; Stage I/II: 89% 77/87; Stage III: 96% 25/26) at 94% specificity (229/244). B) Addition of epigenomic analysis substantially increases the sensitivity.

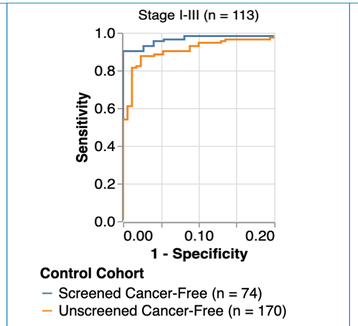


Figure 5: Applying the same models and calling thresholds result in 99% specificity (73/74) colonoscopy-screened negative controls and 92% specificity in self declared healthy donors (156/170) .

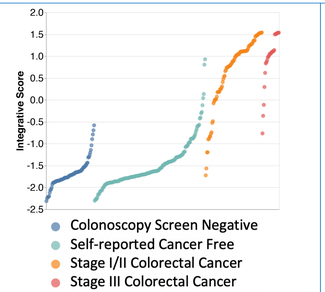


Figure 6: Integrated bioinformatic caller demonstrates clear differentiation between cases and controls. Sensitivity and specificity are robust over a wide range of thresholds supporting significant separation of the signal from variability in the control samples

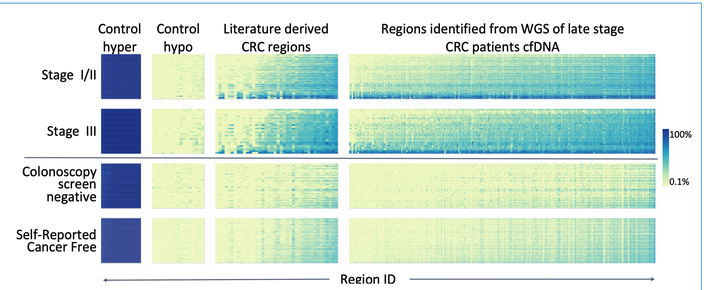


Figure 7: Strong methylation signal was observed in regions selected based on differential methylation patterns in CRC tumor tissue when compared to a set of control hypo- and hyper- methylated regions, supporting tumor origin of plasma fragments. Examining a large set of differentially methylated regions based on WGS of cfDNA from colonoscopy screened negative subjects and patients with late stage CRC, a strong and consistent methylation signal is observed that improves differentiation

Conclusions

- This blood based integrated genomic and epigenomic multi-modal ctDNA test achieved clinically significant values for the detection of CRC with 90% sensitivity and 94% specificity.
- The specificity significantly improves when tested on a set of control samples from colonoscopy screened negative subjects.
- Methylation partitioning enables simultaneous assessment of genomic and epigenomic signals using the same input material and provides substantial advantages over traditional assays that often recover less than 50% of input DNA.[9-10]
- Simultaneous assessment of genomic and epigenomic signals enables conservative genomic variant filtering thresholds in order to avoid false positive calls and provides the opportunity for multi-modal assessment from a single input source and sequencing library to quantitatively integrate information.
- The results demonstrate this multimodal ctDNA assay consistently provides sufficient sensitivity and specificity for clinical detection of early-stage CRC.
- A prospective average-risk CRC screening study is underway in a population-level observational research study.

References: 1. Siegel RL, et al. CA Cancer J Clin. 2020. 2. American Cancer Society. Colorectal Cancer Facts & Figures 2017-2019. 3. Edwards BK, et al. Cancer. 2010. 4. U.S. Cancer Statistics Working Group. Available from: www.cdc.gov/cancer/dataviz. 5. Overman M, et al. J Clin Oncol. 2017. 6. Jaiswal S, et al. N Engl J Med. 2017. 7. Steensma DP, et al. Blood. 2015. 8. Odegaard JI, et al. Clin Cancer Res. 2018. 9. Olova N, et al. Genome Biol. 2018. 9. Lister R, et al. Nature. 2009.

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