**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.²
- Despite clinical guidelines and established evidence, nearly 1 in 3 American adults is not compliant with CRC screening recommendations.³
- Demonstrating the clinical utility of cfDNA 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 (CHIPs)).⁴⁻⁵
- The effective adoption of cfDNA for CRC screening requires improved sensitivity by expanding beyond assessment of somatic genomic alterations to incorporate assessment of epigenetic signals and the ability to improve specificity by differentiating cancer-related somatic alterations.

**Methods**

- **Figure 2:** Laboratory and Bioinformatic workflow. Whole blood is collected and extracted cfDNA undergoes partitioning based on methylation level. Indicators of hypomethylation, recombinant, and processed. Libraries are enriched with the 500bp panel (Figure 1, indexed, pooled, and sequenced. Three different 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 methylation patterns in genomic regions across the panel.

**Results**

- **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 hypermethylated regions, supporting the origin of clonal fragments. Examining a large set of differentially methylated regions based on WGS of cfDNA from cfDNA 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 cfDNA 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.²⁻⁴
- 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 cfDNA 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.

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