Validation of a multi-modal blood-based test for the detection of colorectal cancer with sub single molecule sensitivity

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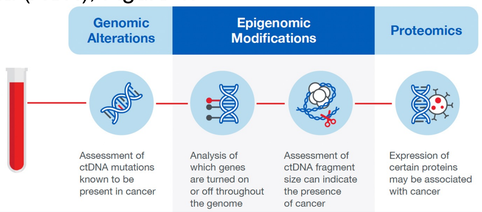
Introduction

- Blood-based colorectal cancer (CRC) screening tests can improve adherence to screening guidelines.
- Yet, current commercially available options have poor sensitivity and specificity preventing effective implementation into routine clinical care.
- Here we report the analytical and clinical validation of a blood-based test, Shield™, for the detection of CRC and advanced colorectal neoplasia (ACN); Figure 1.

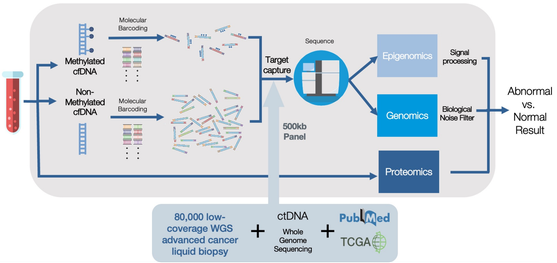
Figure 1: The blood-based test, Shield, aims to detect ACN by identifying tumor-associated biomarkers including genomic and epigenomic (methylation and fragmentomics) signatures in cell-free DNA (cfDNA) and protein expression.



Methods

Assay workflow: Plasma-derived cfDNA was profiled using a custom assay that enriches fragments with dense CpG methylation and further depletes uninformative background molecules containing unmethylated CpGs (Figure 2). cfDNA and protein results are integrated into a binary "abnormal" vs "normal" result using a proprietary bioinformatic pipeline (Guardant Health, USA)

Figure 2: A targeted assay was designed that detects genomic, epigenomic, and proteomic modifications associated with CRC. Total cfDNA was extracted, partitioned based on methylation level, and analyzed. Data were filtered using a variant classifier to differentiate tumor derived from non-tumor derived alterations without *a priori* knowledge of tissue or germline sequencing results. cfDNA and protein results are integrated into a binary "abnormal" versus "normal" results



Assay Development: The assay was trained on samples from > 6,000 unique individuals

- Training: 2,685 ACN-negative and 1,698 with ACN
- Threshold Setting: 1,072 ACN-negative and 551 with ACN
- Calling thresholds were frozen prior to validation, targeting a 91.5% specificity

Analytical and Clinical Validation: Each aspect of the analytical and clinical validation study followed Nex-StoCT CLIA working group and CLSI guidelines.

- Assay was validated using samples collected from multiple cohorts, including prospective screening collections designed to capture intended use population and retrospective case-control cohorts designed to enrich for subjects with CRC.
  - CRC subjects recruited across 6 unique cohorts in US, Canada, and EU
  - Advanced adenoma (AA) and ACN negative subjects were collected in the US
- Cases and controls were balanced by age and gender

Results

Analytical Validation:

- Limit of detection (LoD) was established across six dilutions; 0.5%, 0.3%, 0.1%, 0.05%, 0.02%, and 0.01% with at least 20 replicates per dilution for CRC cases and normal controls
  - Even for low cfDNA mass inputs of less than 4ng, the 95% LoD was determined to be less than 1 tumor-derived genomic equivalent (0.5), indicating over at least 10-fold increase in assay sensitivity compared to best-in-class assays for somatic mutation detection
- Precision studies in 60 positive and 60 negative clinical sample replicates yielded > 95% average positive and negative percent agreement both within and between batches
- Endogenous interference studies in clinical positive and negative samples and minimally manipulated samples yielded > 90% positive and negative percent agreement between reference control and common endogenous substances:
  - albumin, bilirubin, hemoglobin, triglycerides, and genomic DNA

Clinical Validation:

	CRC	AA	ACN-Negative
Number of Unique Subjects	92	51	166
Median age in years (range)	64 (23 – 84)	66 (58 – 79)	64 (46 – 84)
Percent Female	46%	42%	47%

92% Overall ACN Specificity N = 166 (95% CI: 86 – 95%)

94% Screen-Negative Specificity N = 112 (95% CI: (88 – 95%)

91% Overall Sensitivity N = 92 (95% CI: 84 – 95%)

90% Stage I Sensitivity N = 29 (95% CI: 73 – 96%)

97% Stage II Sensitivity N = 35 (95% CI: 85 – 99%)

86% Stage III Sensitivity N = 28 (95% CI: 69 – 94%)

20% AA Sensitivity N = 51 (95% CI: 11 – 32%)

CI: Confidence interval

Conclusions

- Here we present the analytical and clinical validation of a multi-modal blood-based test for the detection of colorectal cancer
- Analytical validation yielded a 95% LoD at less than 1 tumor derived genomic equivalent, indicating at least a 10-fold increase in sensitivity over somatic assays
- Clinical validation demonstrated an overall sensitivity of 91% for CRC, 20% for AA, and 92% specificity for ACN with greater than 90% sensitivity for Stage I and Stage II CRC
- This test is currently being evaluated in a registrational study: **E**valuation of the **c**tDNA **L**UNAR Test **i**n an Average **P**atient **S**creening **E**pisode (ECLIPSE), NCT04136002
- Interventional clinical studies are in development