INTRODUCTION

Colorectal Cancer (CRC) is the third leading cause of cancer-related death, accounting for nearly 153,020 new cases diagnosed and 52,650 deaths in 2023. Overall, the CRC mortality rate has declined by about 2% per year during the most recent decade (2010-2019), due to the effective screening techniques. Liquid biopsy-based cell free DNA (cfDNA) in blood plasma holds tremendous potential for early cancer detection. However, it is technologically challenging to achieve clinically meaningful high sensitivity and specificity in asymptomatic individuals due to significant lower tumor cfDNA fractions.

The extensive analysis of the mutations detection to encompass methylation analysis contribute to improve sensitivity and specificity for early CRC screening, diagnostic accuracy of analytical platforms, and diagnostic power to detect tumors. Herein, we developed targeted next-generation sequencing (NGS)-based assays for assessing multiple DNA methylated and mutated regions in both low-quantity (plasma cfDNA) and low-quality formalin-fixed paraffin-embedded (FFPE) tissue for CRC detection. We assessed the usefulness of integrating genomic and epigenomic signatures in cfDNA to detect CRC.

METHOD

Fig. 1: Overview of multiple OptiSeq™ CRC NGS mutation and methylation panel protocol.

RESULTS

I. Analytical Performance of OptiSeq™ CRC Methylation Panel

OptiSeq™ CRC NGS methylation detection panel showed that its analytical sensitivity was around 0.5%.

II. Analytical Performance of OptiSeq™ CRC NGS Mutation Panel

OptiSeq™ CRC mutation panel data indicated that its LOD around 0.25% VAF.

Fig. 2: Multiplex bisulfite sequencing data for OptiSeq™ Colorectal Cancer NGS methylation detection panel. (a) Heat map showing the methylation pattern in the target regions of interest between CRC (HCT116, SW480 and SW620), non-CRC (PSNH-1 and SKBr3), CRC FFPE specimens, advanced adenomas, healthy volunteers' cfDNA and matching buffy coat DNA. All analyses show extensively similar methylation differences between cancer and normal (p<0.05). (AN-Adjacent Normal, PT-Primary tumor, PR- Patient) (b) Bar graph showing the range of sequencing coverage for individual amplicons in for HCT116 (methylated DNA) and NA12878 (unmethylated DNA). (c) Left fig: analytical sensitivity of the methylation assay was assessed using methylated DNA spike-in at 0%, 0.1%, 0.25%, 0.5%, 1%, 2.5% and 5% expected levels of methylation, across 3 replicates and two independent sequencing runs. Right fig: Correlation plot between observed methylation (%) (y-axis) against expected methylation (%) (x-axis). (d) Left fig: PCR bias was assessed using methylated DNA spike-in at 0%, 10%, 25%, 50%, 75%, 100 % and 100% expected levels of methylation, across 3 replicates. Right fig: Correlation plot between observed methylation (%) (y-axis) against expected methylation (%) (x-axis) showing no PCR bias.

III. Clinical Performance

For the OptiSeq™ CRC NGS methylation panel, the sensitivity was 95% and specificity was 100%. For the OptiSeq™ CRC NGS mutation panel, the sensitivity was 98% and specificity was 100% for CRC cfDNA and FFPE samples (Table 3). However, when both panels were used for patient samples testing, the preliminary data showed that their sensitivity and specificity were 100% for CRC (Table 4). The assay needs to be tested on more CRC patient samples for validation.

Table 1: Precision of the OptiSeq™ CRC NGS mutation detection panel

<table>
<thead>
<tr>
<th>Genes</th>
<th>AML</th>
<th>HCT116</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tr>
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<td>100%</td>
<td>100%</td>
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<tr>
<td>Negative</td>
<td>Negative</td>
<td>100%</td>
<td>100%</td>
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Table 2: Clinical performance of the OptiSeq™ CRC NGS methylation detection panel

<table>
<thead>
<tr>
<th>Sample Status</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td>Positive</td>
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<td>Negative</td>
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Table 4: Integrated clinical performance of the OptiSeq™ CRC NGS mutation and methylation detection panel

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<th>Negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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CONCLUSIONS

Our targeted NGS panels evaluate DNA methylation levels of individual CpG sites across multiple regions and clinically actionable variants including single nucleotide variants (SNVs), insertions and deletions (Indels), microsatellite instability (MSI), and using low input clinical material from cfDNA and FFPE.

The OptiSeq™ CRC NGS methylation panel showed a clear and significant differences in the methylation level between normal and cancer cell lines, CRC patients and healthy volunteer cfDNA and FFPE samples demonstrating that assay provides accurate methylation data for monitoring epigenetic biomarkers in clinical samples. The post-sequencing quality control step showed that the OptiSeq™ CRC NGS methylation panel can confidently distinguish small changes in the methylation level between 0% and 5% and can be used to screen advanced adenoma cases.

The performance metrics for OptiSeq™ CRC NGS mutation panel showed the high correlation for VAFs ranged from 5% to as low as 0.25%, demonstrating the assay’s high analytical sensitivity.

The integrated genomic and epigenomic cfDNA assay provides clinically significant 100% sensitivity and specificity for CRC detection.

REFERENCE