Combined genomic and epigenomic assessment of cell-free circulating tumor DNA (ctDNA) improves assay sensitivity in early stage colorectal cancer (CRC)

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Disclosures

Employee, Director, and Shareholder of Guardant Health, Inc.
ctDNA has the potential to identify patients with early stage cancer, but accurate detection is challenging

Detection Challenges

Sensitivity
- Genomic signatures are limited to ~50% sensitivity for early cancer

Specificity
- Non-tumor sources of biological noise, such as CHIP, can compromise highly specific detection
- Using prior knowledge of tumor tissue to filter out such noise is clinically challenging
Diverse sources of signal motivate multimodal analysis of ctDNA

**Genomic Alterations**
- SNVs, InDels, Fusions, and CNVs

**Epigenomic Alterations**
- Aberrant methylation signals in tumor vs benign tissues

**Nucleosomal Positioning & Fragmentomics**
- ctDNA has differential fragment genomic position via nucleosomal positioning or epigenomic alterations at transcription factor binding sites

ctDNA fragment genomic position provides biological information

Nucleosomal (long) fragments

Sub-Nucleosomal (short) fragments

TSS: Transcription Start Site
CTCF: a DNA binding protein that binds to tens of thousands of genomic sites, some tissue-specific and others ultra-conserved


Integrated genomic and epigenomic analysis of ctDNA

- Methylation & Fragmentomics
- Genomic Alterations
- Signal processing
- Biological Noise Filter
- ctDNA detection

Diagram:
- Methylated cfDNA
- Non-Methylated cfDNA
- Digital Sequencing
- Sequence
- Target capture
- 500kb Panel
- 80,000 low-coverage WGS
- advanced cancer liquid biopsy
- ctDNA
- Whole Genome Sequencing

Reference:
Multi-modal epigenomics approach integrating methylation and fragmentomics improves signal-to-noise.

Accurate testing cohort required age-matched cases and controls

- 105 patients with a diagnosis of colorectal cancer had plasma collected prior to surgical resection
  - From three independent cohorts
- Cancer-free controls were age-matched
  - Median age was 67 years, consistent with the median age at colorectal cancer diagnosis per SEER Data
  - 8% had a diagnosis of inflammatory bowel disease

<table>
<thead>
<tr>
<th></th>
<th>Median age (in years)</th>
<th>Range (in years)</th>
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<tbody>
<tr>
<td>Cancer Free Controls</td>
<td>67</td>
<td>35 - 88</td>
</tr>
<tr>
<td>Stage I</td>
<td>65.5</td>
<td>49 - 70</td>
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<tr>
<td>Stage II</td>
<td>63</td>
<td>45 - 85</td>
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<td>Stage III</td>
<td>62</td>
<td>42 - 88</td>
</tr>
<tr>
<td>Stage IV</td>
<td>59</td>
<td>53 - 67</td>
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</tbody>
</table>

Inferred tumor level correlates between epigenomic and genomic estimate.

Promising ctDNA sensitivity and specificity for early stage CRC


<table>
<thead>
<tr>
<th>Stage</th>
<th>Genomic Sensitivity</th>
<th>Genomic Specificity</th>
<th>Integrated Sensitivity</th>
<th>Integrated Specificity</th>
<th>Genomic &amp; Epigenomic Sensitivity</th>
<th>Genomic &amp; Epigenomic Specificity</th>
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<tbody>
<tr>
<td>I</td>
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</tbody>
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Sensitivity and Specificity for Stages I to IV with target specificities of 90%, 95%, and 98%.
Summary and Next Steps

- Utilizing a **plasma-only** sequencing assay incorporating **somatic genomic and epigenomic analysis**, and a bioinformatic classifier to filter non-tumor derived variants, ctDNA detection rate in early stage CRC (I-III) can far **outperform** the detection rate of somatic sequence variant detection alone.

- The performance of the ctDNA assay needs to be further validated in larger cohorts.

- In a subgroup of patients, longitudinal ctDNA samples were collected and clinical follow-up is ongoing to evaluate post-surgery ctDNA detection rate and disease recurrence.

- These results have potentially significant implications for the clinical utility of ctDNA in early stage cancer management.

Acknowledgements

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