Development of a Highly-Sensitive Targeted Cell-Free DNA Epigenomic Assay for Early-Stage Multi-Cancer Screening

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Disclosure Information

Employee and Shareholder of Guardant Health, Inc
A blood-based cancer screening test should exhibit performance metrics optimized for the cancer of interest:

- Clinical diagnostic pathways must be considered.
- Required to detect early-stage disease to yield a meaningful impact on individual and net population health outcomes.

For cancers with established paradigms and proven diagnostic pathways:
- Aim to improve compliance rates with performance on par with current modalities.

For cancers without a paradigm or diagnostic pathway:
- Aim to reduce false positive rate while ensuring sensitivity is clinically meaningful.

We developed a blood-based solid tumor screening assay. Here we present feasibility data on four cancer types with differing screening clinical utility as examples.
<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>USPSTF rating</th>
<th>Population</th>
<th>Currently available screening options</th>
<th>Assessment of overall benefits and harms</th>
<th>Screening Adherence Rate</th>
<th>Target Specificity for this analysis</th>
</tr>
</thead>
</table>
| **Colorectal Cancer**¹ | A / B | Asymptomatic adults aged 45 - 75 | • Colonoscopy  
• Fecal Immunohistochemical stool test (FIT)  
• Multi-target stool DNA test  
• Methylated Sept9 blood test | Risk-to-benefit ratio supports screening | 66% | 90% |
| **Lung Cancer**² | B | Asymptomatic adults aged 50-80 with 20 pack-year history and currently smoke, or quit within last 15 years | • Low Dose CT (LDCT) | Risk-to-benefit ratio supports screening | 14% | 90% |
| **Pancreas Cancer**³ | D | Asymptomatic adults | • Endoscopic Ultrasound (EUS)  
• Magnetic resonance cholangiopancreatography (MRCP) | Risk-to-benefit ratio does NOT support screening, except in limited scenarios* | - | 95%+ |
| **Bladder Cancer** | Not Reviewed | None | No screening available | - | 95%+ |

*Individuals with a known pathogenic / likely pathogenic germline mutation in a pancreas cancer susceptibility gene or strong family history of pancreas cancer


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Conventional Methylation Technology: Low fidelity resulting in degraded performance

Conventional Bisulfite Methylation Assessment

DNA Degraded with harsh chemical treatment

Sequencing without preferential enrichment of tumor molecules

Single and degraded signal output only
Novel Epigenomic Technology: Higher Signal-to-Noise Ratio at Lower Sequencing Costs

**Novel Methylation Signal Enrichment**

- Efficient methylated molecule partitioning
- Background depletion to improve signal-to-noise ratio
- Low-cost sequencing of tumor molecules

**Conventional Bisulfite Methylation Assessment**

- DNA Degraded with harsh chemical treatment
- Sequencing without preferential enrichment of tumor molecules

**Multi-modal signal output**

**Single and degraded signal output only**
Methylation Technology Development: Higher Signal-to-Noise Ratio at Lower Sequencing Costs

Technology utilizes a broad genomic panel to capture and sequence only tumor-associated molecules enabling high molecular recovery with low sequencing costs.

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**Cohort Demographics**

<table>
<thead>
<tr>
<th>Cancer Stage</th>
<th>Cancer – free (N = 1,862)</th>
<th>Colorectal Cancer (N = 1,274)</th>
<th>Lung Cancer (N = 191)</th>
<th>Pancreas Cancer (N = 42)</th>
<th>Bladder Cancer (N = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I / II</td>
<td>54%</td>
<td>29%</td>
<td>26%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>III / IV</td>
<td>46%</td>
<td>71%</td>
<td>74%</td>
<td>73%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Median</th>
<th>Range</th>
<th>Cancer Distribution Across Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Stage</td>
<td>Median</td>
<td>Range</td>
<td>Cancer Distribution Across Cases</td>
</tr>
<tr>
<td>I / II</td>
<td>57</td>
<td>18 – 86</td>
<td>Bladder Cancer 5%</td>
</tr>
<tr>
<td>III / IV</td>
<td>65</td>
<td>19 – 93</td>
<td>Lung Cancer 3%</td>
</tr>
</tbody>
</table>

| Number of unique cohorts | 17 | 12 | 6 | 2 | 3 |

Methods: Clinical Cohorts

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Results: Cancers with Population Screening Recommendations

- **Cancer-Free (N = 1,862)**
  - Overall (N = 191): Specificity 91% (86 - 95%)
  - Stage I / II (N = 55): Specificity 87% (76 - 94%)
  - Stage III / IV (N = 136): Specificity 93% (87 - 96%)

- **Lung Cancer Sensitivity**
  - Overall (N = 1,274): Specificity 92% (90 - 93%)
  - Stage I / II (N = 692): Specificity 90% (88 - 92%)
  - Stage III / IV (N = 582): Specificity 93% (91 - 95%)

- **Colorectal Cancer Sensitivity**
  - Overall (N = 1,862): Specificity 90%

**Current Cancer Screening Methods**

- **Low Dose CT**
  - Specificity 76%
  - Sensitivity 80%

- **Colonoscopy**
  - Specificity 86%
  - Sensitivity 95%

- **Fecal Immunohistochemistry**
  - Specificity 96%
  - Sensitivity 74%

- **Multi-target stool DNA**
  - Specificity 87%
  - Sensitivity 92%

- **Methylated Sept9 blood**
  - Specificity 81%
  - Sensitivity 72%

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Results: Cancers without Population Screening Recommendations

<table>
<thead>
<tr>
<th>Cancer-Free (N = 1,862)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (N = 42)</td>
<td>81% (66 - 91%)</td>
</tr>
<tr>
<td>Stage I / II (N = 11)</td>
<td>73% (39 - 94%)</td>
</tr>
<tr>
<td>Stage III / IV (N = 31)</td>
<td>84% (66 - 95%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pancreas Cancer Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (N = 84)</td>
</tr>
<tr>
<td>Stage I / II (N = 23)</td>
</tr>
<tr>
<td>Stage III / IV (N = 61)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bladder Cancer Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (N = 42)</td>
</tr>
<tr>
<td>Stage I / II (N = 11)</td>
</tr>
<tr>
<td>Stage III / IV (N = 31)</td>
</tr>
</tbody>
</table>

Sensitivity:
- Pancreas Cancer Sensitivity: (39 - 94%)
- Bladder Cancer Sensitivity: (31 - 73%)
Results: Multi-Cancer Assay with Accurate Tissue of Origin Prediction

- Highly accurate tissue of origin (TOO) prediction is needed when more than one cancer type is evaluated as part of a single assay.

- The tissue of origin prediction evaluated at 98% specificity.

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### Accuracy Matrix*

<table>
<thead>
<tr>
<th>Predicted Cancer Type</th>
<th>Colorectal Cancer</th>
<th>Lung Cancer</th>
<th>Bladder Cancer</th>
<th>Pancreas Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Cancer Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>0.99</td>
<td>0.05</td>
<td>0.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>0.0</td>
<td>0.94</td>
<td>0.12</td>
<td>0.1</td>
</tr>
<tr>
<td>Bladder Cancer</td>
<td>0.0</td>
<td>0.01</td>
<td>0.88</td>
<td>0.0</td>
</tr>
<tr>
<td>Pancreas Cancer</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.86</td>
</tr>
</tbody>
</table>

*Accuracy matrix: Percentage of true cancer types accurately predicted

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Limitations

- Cancer cases include screen detected and symptomatically detected cases
  - Not reflective of intended use screening population
- Self-reported healthy individuals were all-comers not reflective of intended use screening population in terms of age and risk factors
Conclusions

- This multi-cancer targeted screening assay provides robust and sensitive detection of early-stage cancer at thresholds optimized for current screening paradigms with accurate tissue of origin identification.
- The assay is undergoing further data development studies in additional cancer types where screening can save lives.
- Clinical evaluation in registrational screening trials is ongoing (NCT05117840).
Thank you

- Questions?
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