Clinical Validation of a Targeted Methylation-Based Multi-Cancer Early Detection Test

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Eric A. Klein

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Stockholder in: none
Employee of: Cleveland Clinic

-and-

I will discuss the following investigational use in my presentation:
multi-cancer early detection test (Galleri™)
Benefits of Early Cancer Diagnosis

5-year cancer-specific survival (all cancers)¹

- **Localized**: 89%
- **Distant Metastases**: 21%

invasive localized tumors that have not spread beyond organ of origin

invasive cancers that have metastasized beyond the organ of origin to other parts of the body

Stage IV cancer diagnosis²

- only 18% of diagnoses lead to 45% of cancer-related deaths (within 5 years of diagnosis)

Source: SEER data


A Multi-Cancer Early Detection (MCED) Test May Decrease Cancer-Related Mortality

MCED test as a complement to existing recommended screening

Detect many lethal cancers, including unscreened cancers, using single blood sample collected at point-of-care

Identify signal origin to direct diagnostic workup

High specificity (~99%) could limit false positives and unnecessary workups

Potential for:
- More cancer cases detected through screening
- Improved patient outcomes and survival
- Decreased burden of care
Modeling the Potential Impact of GRAIL MCED Test Added to Usual Care

- **Diagnostic yield**: 31% of cancers intercepted by a MCED test
- **Tumor stage shift**: -50% reduction in late-stage (III/IV) cancer incidence (-78% in intercepted cases)
- **Mortality reductions**: -21% reduction in 5-year mortality (-40% in intercepted cases)

Modeling performance of a GRAIL MCED test in a representative population suggests it could reduce overall cancer mortality if added to usual care.

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*a* Based on annual cancer incidence (1187/100,000) in elevated risk population aged 50-79 y (data from US Surveillance, Epidemiology, and End Results [SEER] program). MCED results based upon fast tumor growth model using test version described by Liu et al.2 Usual care represents real-world cancer diagnostic processes (e.g., screening, incidental detection, symptomatic workup) as captured by SEER, which includes imperfect adherence and limited access to healthcare. MCED, multi-cancer early detection.

The Circulating Cell-Free Genome Atlas Study (CCGA Study; NCT02889978)

Prospective, multicenter, case-control, observational study

Study Goals

- Develop and validate a blood-based MCED test analyzing plasma cell-free DNA (cfDNA)
- Detect cancer signals across multiple cancer types & simultaneously predict their signal origin

Study Design

- 15,254 participants with/without cancer
- Blood samples all participants
- Tissue samples cancer only
- Follow-up for 5 years (vital status, cancer status)

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cfDNA, cell-free DNA; MCED, multi-cancer early detection test.
CCGA: Divided Into 3 Pre-Specified Substudies

**Discovery**
- Methylation Patterns
- Mutations
- Chromosome Alterations

**Assay Refinement and Classifier Development**
- Methylation Patterns
- Machine-learning classifier to differentiate cancer vs non-cancer and predict signal origin
  - Training: n=3133
  - Validation: n=1354

**Further Assay Refinement and Clinical Validation**
- Methylation Patterns
- Further refined for use as screening tool
- Clinical validation supporting product launch
  - n=5309 participants (cancer=3237; non-cancer=2069)

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Key objectives were to evaluate test performance:

| Primary | • for cancer signal detection (specificity, overall sensitivity, sensitivity by cancer class, sensitivity by clinical stage)  
|         | • for signal origin prediction (accuracy) |
| Secondary (Key) | • for cancer signal detection in a pre-specified group of 12 cancers responsible for two-thirds of cancer deaths<sup>a,1</sup>  
|         | • in cancers with and without common screening options  
|         | • by age group |

**Methods**

- cfDNA from evaluable samples was analyzed using a targeted methylation bisulfite sequencing assay and machine learning techniques
- The classifier was trained to target a specificity of 99.4% and locked before analysis

<sup>a</sup>Anus, bladder, colon/rectum, esophagus, head and neck, liver/bile duct, lung, lymphoma, ovary, pancreas, plasma cell neoplasm, and stomach.

### Demographics and Baseline Characteristics

**Demographics**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Cancer (n=2823)</th>
<th>Non-cancer (n=1254)</th>
<th>Total (N=4077)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>62.6 (11.8)</td>
<td>56.2 (12.6)</td>
<td>60.6 (12.4)</td>
</tr>
<tr>
<td>≥50, n (%)</td>
<td>2438 (86.4)</td>
<td>850 (67.8)</td>
<td>3288 (80.6)</td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1394 (49.4)</td>
<td>864 (68.9)</td>
<td>2258 (55.4)</td>
</tr>
<tr>
<td><strong>Race/Ethnicity, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whitea</td>
<td>2316 (82.0)</td>
<td>996 (79.4)</td>
<td>3312 (81.2)</td>
</tr>
<tr>
<td>Blacka</td>
<td>193 (6.8)</td>
<td>85 (6.8)</td>
<td>278 (6.8)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>192 (6.8)</td>
<td>103 (8.2)</td>
<td>295 (7.2)</td>
</tr>
<tr>
<td>Otherb</td>
<td>122 (4.3)</td>
<td>70 (5.6)</td>
<td>192 (4.7)</td>
</tr>
<tr>
<td><strong>Smoking Status, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1322 (46.8)</td>
<td>719 (57.3)</td>
<td>2041 (50.1)</td>
</tr>
<tr>
<td>Former</td>
<td>1080 (38.3)</td>
<td>402 (32.1)</td>
<td>1482 (36.4)</td>
</tr>
<tr>
<td>Current</td>
<td>378 (13.4)</td>
<td>126 (10.0)</td>
<td>504 (12.4)</td>
</tr>
<tr>
<td><strong>Family Cancer History, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2254 (79.8)</td>
<td>1014 (80.9)</td>
<td>3268 (80.2)</td>
</tr>
<tr>
<td>No</td>
<td>493 (17.5)</td>
<td>210 (16.7)</td>
<td>703 (17.2)</td>
</tr>
<tr>
<td><strong>BMI, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under/Normal</td>
<td>785 (27.8)</td>
<td>330 (26.3)</td>
<td>1115 (27.3)</td>
</tr>
<tr>
<td>Overweight</td>
<td>908 (32.2)</td>
<td>376 (30.0)</td>
<td>1284 (31.5)</td>
</tr>
<tr>
<td>Obese</td>
<td>1124 (39.8)</td>
<td>543 (43.3)</td>
<td>1667 (40.9)</td>
</tr>
</tbody>
</table>

**BMI, body mass index.**

*aNon-Hispanic.*

*bOther includes Asian, Native Hawaiian or Pacific Islander; American Indian or Alaska Native; or Other.*

*cSmoking status was missing for 50 participants.*

*dCancer history was not known in 106 participants.*

*eBMI category was missing for 11 participants.*

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**Cancer Clinical Stage Distribution**

- Stage IV: 21.9%
- Stage I: 30.1%
- Stage II: 24.9%
- Stage III: 20.0%
- Not expected to be staged: 2.4%
- Missing: 0.7%
Cancer Signal Detection: Specificity, Overall Sensitivity, and Signal Origin Prediction

Accuracy was defined as the number of correctly predicted top 1 signal origin (n=1273) divided by all cancer participants with a positive cancer signal, excluding participants with unknown primary (n=1435).

CI, two-sided 95% Wilson confidence intervals.

<table>
<thead>
<tr>
<th></th>
<th>Cancer (n=2823)</th>
<th>Non-cancer (n=1254)</th>
<th>Total (n=4077)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive</td>
<td>1453</td>
<td>6</td>
<td>1459</td>
</tr>
<tr>
<td>Test Negative</td>
<td>1370</td>
<td>1248</td>
<td>2618</td>
</tr>
</tbody>
</table>

**Specificity:** 99.5%  
(95% CI: 99.0–99.8%)  
0.5% false-positive rate

**Sensitivity:** 51.5%  
(95% CI: 49.6–53.3%)

**Signal origin prediction accuracy**: 88.7%  
(95% CI: 87.0–90.2%)

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*a Accuracy was defined as the number of correctly predicted top 1 signal origin (n=1273) divided by all cancer participants with a positive cancer signal, excluding participants with unknown primary (n=1435). CI, two-sided 95% Wilson confidence intervals.
Sensitivity of Cancer Signal Detection by Clinical Stage

Sensitivity increased with increasing clinical stage

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Sensitivity (±95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>51.5% ± 1453/2823</td>
</tr>
<tr>
<td>I</td>
<td>16.8% ± 143/849</td>
</tr>
<tr>
<td>II</td>
<td>40.4% ± 284/703</td>
</tr>
<tr>
<td>III</td>
<td>77.0% ± 436/566</td>
</tr>
<tr>
<td>IV</td>
<td>90.1% ± 557/618</td>
</tr>
<tr>
<td>I-II</td>
<td>27.5% ± 427/1552</td>
</tr>
<tr>
<td>I-III</td>
<td>40.7% ± 863/2118</td>
</tr>
</tbody>
</table>

*Sensitivity* comprises all cancer stages, including missing stage and cancer classes that do not have staging per American Joint Committee on Cancer (AJCC) staging manual.

CI, confidence interval.

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*AACR ANNUAL MEETING 2021: APRIL 10-15, 2021 AND MAY 17-21, 2021*
Sensitivity of Cancer Signal Detection in 12 Pre-Specified Cancers Responsible for Two-Thirds of Cancer Deaths

Sensitivity was higher in these 12 cancers vs overall, particularly in early-stage

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>All cancer classes</th>
<th>12 pre-specified cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>76.3%</td>
<td>51.5%</td>
</tr>
<tr>
<td>I</td>
<td>70.0%</td>
<td>37.1%</td>
</tr>
<tr>
<td>II</td>
<td>77.0%</td>
<td>40.4%</td>
</tr>
<tr>
<td>III</td>
<td>86.6%</td>
<td>90.1%</td>
</tr>
<tr>
<td>IV</td>
<td>92.8%</td>
<td>92.8%</td>
</tr>
<tr>
<td>I-II</td>
<td>53.0%</td>
<td>40.7%</td>
</tr>
<tr>
<td>I-III</td>
<td>67.6%</td>
<td>40.0%</td>
</tr>
</tbody>
</table>

*All* comprises all cancer stages, including missing stage and cancer classes that do not have staging per American Joint Committee on Cancer (AJCC) staging manual. CI, confidence interval.

12 cancers that account for 62% of US cancer deaths
- Anus
- Bladder
- Colon/rectum
- Esophagus
- Head and neck
- Liver/bile duct
- Lung
- Lymphoma
- Ovary
- Pancreas
- Plasma cell neoplasm
- Stomach

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*All* comprises all cancer stages, including missing stage and cancer classes that do not have staging per American Joint Committee on Cancer (AJCC) staging manual.

CI, confidence interval.

Cancer signals were detected across >50 AJCC cancer types, with a test positive rate across AJCC cancer types of 51.0% (95% CI: 49.1%–52.8%).
Sensitivity of Cancer Signal Detection in Cancers With and Without Common Screening Options:

With Common Screening Options: 33.7% (95% CI: 31.1–36.5%)

Without Common Screening Options: 63.8% (95% CI: 61.4–66.1%)

CI, confidence interval.
Specificity, Sensitivity, and Signal Origin Prediction by Age Group

Cancer signal detection was similar across age groups

### Specificity Across Multiple Cancer Classes

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Specificity 0%</th>
<th>Specificity 25%</th>
<th>Specificity 50%</th>
<th>Specificity 75%</th>
<th>Specificity 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1248/1254</td>
<td>845/850</td>
<td>336/338</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 y</td>
<td>403/404</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50 y</td>
<td></td>
<td>79% ± 95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65 y</td>
<td></td>
<td></td>
<td>84% ± 95% CI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Sensitivity Prediction Accuracy

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Sensitivity 0%</th>
<th>Sensitivity 25%</th>
<th>Sensitivity 50%</th>
<th>Sensitivity 75%</th>
<th>Sensitivity 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1453/2823</td>
<td>212/385</td>
<td>1241/2438</td>
<td>726/1331</td>
<td></td>
</tr>
<tr>
<td>&lt;50 y</td>
<td></td>
<td>79% ± 95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50 y</td>
<td></td>
<td></td>
<td>84% ± 95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65 y</td>
<td></td>
<td></td>
<td></td>
<td>83% ± 95% CI</td>
<td></td>
</tr>
</tbody>
</table>

### Signal Origin Prediction Accuracy

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Signal Origin Accuracy 0%</th>
<th>Signal Origin Accuracy 25%</th>
<th>Signal Origin Accuracy 50%</th>
<th>Signal Origin Accuracy 75%</th>
<th>Signal Origin Accuracy 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1273/1435</td>
<td>182/209</td>
<td>1091/1226</td>
<td>632/714</td>
<td></td>
</tr>
<tr>
<td>&lt;50 y</td>
<td>79% ± 95% CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50 y</td>
<td></td>
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<td></td>
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<td>≥65 y</td>
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</table>

*aAccuracy was defined as the number of correctly predicted top 1 signal origin divided by all cancer participants with a positive cancer signal, excluding participants with unknown primary. CI, confidence interval.*
Conclusions

In this pre-specified, large-scale, clinical validation sub-study 3 of CCGA:

- This MCED test detected cancer signals across >50 AJCC cancer types, which is critical to maximize the number of cancer cases detected in a population.

- This MCED test performed with high specificity and high accuracy of signal origin prediction, which could help direct the follow-up diagnostic work-up.

- These data support the foundation for population-scale clinical implementation of this test.