PATHFINDER: A Prospective Study of a Multi-Cancer Early Detection Blood Test

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On behalf of PATHFINDER study investigators
DECLARATION OF INTERESTS

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*GRAIL, LLC, is currently held separate from Illumina Inc. under the terms of the Interim Measures Order of the European Commission dated 29 October 2021
Background: Multi-Cancer Early Detection (MCED) Blood Assays

MCED testing uses a targeted methylation, next-generation sequencing (NGS)-based assay to:
• Detect and analyze cfDNA in the bloodstream
• Deploy machine learning to detect a cancer signal
• Predict the likely cancer signal origin (CSO)

Tumor sheds cfDNA fragments into bloodstream
Blood plasma isolated (contains cfDNA fragments)
Targeted methylation analysis of cfDNA (sequencing, mapping, alignment)
Machine learning classifier

Cancer signal detected
Predicted origin (eg, head and neck, lymphoid)
No cancer signal detected

cfDNA, cell-free DNA. aBisulfite treatment; targeted probes pull out fragments matching regions of interest. bFor a detected signal, the MCED test predicts 1-2 cancer signal origins (CSO) that can be either an anatomic site (eg, colorectal) or a cellular lineage (eg, lymphoid). Adapted from Liu MC, et al. Ann Oncol. 2020;31(6):745-759. PMID: 33506766

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PATHFINDER: A Prospective Cohort Study to Return the Results of MCED Tests to Participants

Adults ≥50 years enrolled from 7 US sites into 2 cohorts: *previous* history of cancer, smoking, and genetic risk; *also* collected at other time points during the study; *defined* as date when study team determines to end diagnostic evaluation triggered by a "signal detected" test result. Adapted from Nadauld LD, et al. Cancers (Basel). 2021;13(14):3501. PMID: 34298717.

MCED, multi-cancer early detection. *Previous* history of cancer, smoking, and genetic risk; *also* collected at other time points during the study; *defined* as date when study team determines to end diagnostic evaluation triggered by a "signal detected" test result. Adapted from Nadauld LD, et al. Cancers (Basel). 2021;13(14):3501. PMID: 34298717.

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Study Motivation

Understand the Clinical Experience of MCED Testing

- Understand the diagnostic evaluations precipitated by receipt of a “signal detected” MCED test
- Evaluate MCED test performance characteristics
- Understand levels of participant anxiety and distress associated with MCED testing

PATHFINDER: NCT04241796

Participants were enrolled from December 2019 to December 2020 in the ambulatory care practices at 7 US health care systems

Participants were followed for one year from the date of MCED testing (through December 2021)
PATHFINDER Study Objectives

Primary Objective
• Assess extent of diagnostic testing required to achieve diagnostic resolution following a “signal detected” test result
  o Time to diagnostic resolution
  • Diagnostic resolution was ascertained by the MD who ordered and disclosed the MCED test result
  o Number and types of diagnostic tests

Secondary Objectives
• Evaluate test performance
  o Specificity
  o Positive Predictive Value (PPV)
  o Negative Predictive Value (NPV)
  o Number Needed to Screen (NNS), Yield
  o Cancer Signal Origin\(^a\) (ie, Predicted Origin) Accuracy

• Evaluate participant reported anxiety, distress, satisfaction (presented in poster #908P)

\(^a\)For a detected signal, the MCED test predicts 1-2 cancer signal origins (CSO) that can be either an anatomic site (eg, colorectal) or a cellular lineage (eg, lymphoid).
PATHFINDER Eligibility Criteria

Inclusion:
- Adults ≥50 years who were eligible for either:
  - With Additional Risk Cohort
  - Without Additional Risk Cohort

- Eligibility for With Additional Risk Cohort:
  - Lifetime history of smoking at least 100 cigarettes
  - Hereditary cancer predisposition\(^a\)
  - A history of cancer with no treatment for >3 years\(^b\)

- Eligibility for Without Additional Risk Cohort:
  - None of the above risk factors

Exclusion:
- Clinical suspicion of malignancy
- Undergoing diagnostic evaluation for malignancy
- History of invasive or hematologic malignancy diagnosed <3 years before enrollment
- Definitive treatment for invasive or hematologic malignancy <3 years before enrollment\(^b\)

\(^a\)Genetic cancer predisposition, hereditary cancer syndrome, or meeting criteria for germline testing based on NCCN guidelines.

\(^b\)Personal history of invasive or hematologic malignancy, with definitive treatment completed >3 years prior to enrollment. Adjuvant hormone therapy for breast cancer was permissible.
# Participant Characteristics

|                                      | With Additional Risk\(^a\)  
|                                      | n = 3,681                  | Without Additional Risk \(^a\)  
<table>
<thead>
<tr>
<th></th>
<th>n = 2,940</th>
<th>Total N = 6,621</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong>(^b), in years, mean (SD)</td>
<td>64.7 (8.7)</td>
<td>61.6 (8.1)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>65%</td>
<td>62%</td>
</tr>
<tr>
<td><strong>White, Non-Hispanic</strong></td>
<td>93%</td>
<td>89%</td>
</tr>
<tr>
<td><strong>College Degree or Higher</strong></td>
<td>59%</td>
<td>71%</td>
</tr>
<tr>
<td><strong>Up to Date With Standard Cancer Screening Prior to MCED Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Colorectal Cancer</strong>(^c)</td>
<td>91%</td>
<td>92%</td>
</tr>
<tr>
<td><strong>Breast Cancer</strong>(^d)</td>
<td>78%</td>
<td>83%</td>
</tr>
</tbody>
</table>

\(^a\)Previous history of cancer, smoking, and hereditary risk.

\(^b\)Participants >85 were eligible to participate, but to protect confidentiality, 85 years was the maximum age recorded and used in calculations for participants ≥85 years of age.

\(^c\)Participants ≤75 years old, up to date with USPSTF colorectal cancer screening recommendations (n=4888 total eligible with complete information).

\(^d\)Women 50-74 years old up to date with breast cancer screening recommendations (USPSTF, MRI, or ultrasound; n=3547 total eligible with complete information).
# Cancer Signal Detection

## Study Information

- **Enrolled N=6,662**
- **Analyzeable n=6,621**

## Analysis

<table>
<thead>
<tr>
<th>Signal Detected</th>
<th>With Additional Riska n = 3,681</th>
<th>Without Additional Risk n = 2,940</th>
<th>Total N = 6,621</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal Detected</td>
<td>1.5%</td>
<td>1.2%</td>
<td>1.4%</td>
</tr>
<tr>
<td>No Signal Detected</td>
<td>98.5%</td>
<td>98.8%</td>
<td>98.6%</td>
</tr>
</tbody>
</table>

*aPrevious history of cancer, smoking, and genetic risk.*

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## Primary Objective: Time to Achieve Diagnostic Resolution

### Time to Diagnostic Resolution for Participants with MCED Signal Detected

(N=90; not calculable for 2 participants who began diagnostic evaluation before test results returned)

<table>
<thead>
<tr>
<th></th>
<th>TP 33</th>
<th>FP 57(^a)</th>
<th>Total 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median # of Days to Diagnostic Resolution (Interquartile Range)</td>
<td>57 (33, 143)</td>
<td>162 (44, 248)</td>
<td>79 (37, 219)</td>
</tr>
</tbody>
</table>

- Median time to diagnostic resolution was longer for FPs than TPs
- Most TPs reached resolution in <3 months

\(^a\)Includes 1 participant without diagnostic resolution who was conservatively assumed to be FP.

### % of Participants with Diagnostic Resolution in <3 months

- **73%**
- **42%**

TP, true positive; FP, false positive.
**Primary Objective: Extent of Diagnostic Evaluation**

### Extent and Types of Diagnostic Evaluation for Participants with MCED Signal Detected (N=90)

<table>
<thead>
<tr>
<th>% of participants</th>
<th>TP N=33&lt;sup&gt;a&lt;/sup&gt;</th>
<th>FP N=57&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total N=90&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any imaging procedure, %</td>
<td>91</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>Any advanced imaging (CT, PET, MRI)</td>
<td>88</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Any invasive procedure, %</td>
<td>82</td>
<td>30</td>
<td>49</td>
</tr>
<tr>
<td>Non-surgical invasive procedure (endoscopy, FNA, core biopsy)</td>
<td>79</td>
<td>28</td>
<td>47</td>
</tr>
<tr>
<td>Surgical invasive procedure</td>
<td>9</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

TP, true positive; FNA, fine needle aspiration; FP, false positive.

<sup>a</sup>Two participants with ‘signal detected’ MCED test result (TPs) were excluded from the diagnostic workup analysis because diagnostic testing was initiated before MCED results were returned.

<sup>b</sup>Includes 1 participant without diagnostic resolution who was conservatively assumed to be FP.
Safety

- There were no serious, study-related adverse events reported as a result of MCED testing.
- There were no serious, study-related adverse events due to diagnostic workup prompted by receipt of a “signal detected” MCED test result.
Secondary Objective: Accuracy of Predicted Cancer Origin

Test Performance: Ability to Predict Origin of Malignancy

<table>
<thead>
<tr>
<th>Predicted Origin Accuracy</th>
<th>TP</th>
<th>FP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>35</td>
<td>57</td>
<td>92</td>
</tr>
<tr>
<td>Determinate predicted origin</td>
<td>34</td>
<td>53</td>
<td>87</td>
</tr>
<tr>
<td>Indeterminate predicted origin</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>First Predicted Origin, a, n</th>
<th>29/34 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (95% CI)</td>
<td>85.3 (69.9-93.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>First or Second Predicted Origin, a, c, n</th>
<th>33/34 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (95% CI)</td>
<td>97.1 (85.1-99.8)</td>
</tr>
</tbody>
</table>

- The predicted origin helped to direct diagnostic workups

CI, confidence interval.
aFor a detected signal, the MCED test predicts cancer signal origins (CSO) that can be either an anatomic site (eg, colorectal) or a cellular lineage (eg, lymphoid).
bExcludes 1 participant with indeterminate origin prediction from the true positive per study protocol.
cProportion of first or second origin correctly predicted among true positive participants.
Cancers Diagnosed After a True Positive MCED Signal

19 Solid Tumors

- Oropharyngeal (n=2)
- Breast ♀ (n=5)
- Liver (n=1)
- Intrahepatic Bile Ducts (n=1)
- Colon/Rectum (n=2)
- Prostate ♂ (n=2)
- Lung (n=1)
- Pancreas (n=1)
- Small Intestine (n=1)
- Uterus ♀ (n=1)
- Ovary ♀ (n=1)
- Bone (n=1)

35 people were diagnosed with 36 cancers

17 Hematologic Cancers

- Waldenstrom Macroglobulinemia (n=2)
- Plasma Cell Myeloma/Disorders (n=1)
- Lymphoid Leukemia (n=2)
- Waldenstrom Macroglobulinemia (n=2)
- Lymphoma (n=12)

- 24 in high-risk cohort
- 11 in not-high-risk cohort
- 7 recurrent cancers
- 14 early-stage cancers
- 26 cancers lacking standard screening

AJCC Staging: ▲ Stage I □ Stage II ○ Stage III/IV/No Stage/Recurrent
Available Screening: USPSTF cancer screening or No standard screening

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Cancers Identified Within One Year of MCED Testing

Participants with Cancers Detected by Either Screening or Clinical Findings

121 participants had a cancer diagnosis within 1 year

- 35/121 (29%) had cancer diagnosed and positive MCED
  - 2/35 had cancer detected by the MCED test but work-up began before results were disclosed

### MCED Detection

<table>
<thead>
<tr>
<th>No. of Participants</th>
<th>Screening Detection</th>
<th>Clinical Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCED</td>
<td>Signs/Symptoms</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

### Additional Findings

- Based on participants with cancer status assessment at the end of the study.
- 3 thyroid and 6 melanoma.
- Breast, cervical, colorectal, lung, and prostate cancer.
- 1 incidental radiology finding, 1 incidental finding on routine physical exam, 2 changed lab values, 1 surveillance of prior cancer, 1 follow-up after MGUS diagnosis.

MCED, multi-cancer early detection.

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### Secondary Objective: MCED Test Performance

<table>
<thead>
<tr>
<th>Test Performance Metric</th>
<th>Result</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Signal Detected</td>
<td>1.4%</td>
<td>92/6621</td>
</tr>
<tr>
<td>Specificity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.1%</td>
<td>6253/6290</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV)</td>
<td>38.0%</td>
<td>35/92</td>
</tr>
<tr>
<td>Negative Predictive Value (NPV)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.6%</td>
<td>6235/6321</td>
</tr>
<tr>
<td>Number needed to screen to detect one cancer</td>
<td>189</td>
<td>6621/35</td>
</tr>
<tr>
<td>Yield</td>
<td>0.5%</td>
<td>35/6621</td>
</tr>
</tbody>
</table>

McED, multi-cancer early detection.

<sup>a</sup>Based on participants with cancer status assessment at the end of the study.
Secondary Objective: Performance of a Refined MCED Test

- The banked specimens were re-analyzed using a refined version of the MCED assay.
- These results were not returned to participants or their physicians.
- The refined MCED assay was intended to:
  - Reduce false positives with hematologic predictions.
  - Improve prediction of the tumor origin.
### Secondary Objective: Performance of a Refined MCED Test

<table>
<thead>
<tr>
<th>Test Performance Metric</th>
<th>Refined Version of MCED Test (Results Not Returned to Participants or Providers)</th>
<th>MCED Test Results Returned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Signal Detected</td>
<td>0.9% 58/6578</td>
<td>1.4% 1.4%</td>
</tr>
<tr>
<td>Specificity(^a)</td>
<td>99.5% 6216/6249</td>
<td>99.1%</td>
</tr>
<tr>
<td>Positive Predictive Value PPV</td>
<td>43.1% 25/58</td>
<td>38.0%</td>
</tr>
<tr>
<td>Negative Predictive Value NPV(^a)</td>
<td>98.5% 6216/6311</td>
<td>98.6%</td>
</tr>
<tr>
<td>Number needed to screen, No.</td>
<td>263 6578/25</td>
<td>189</td>
</tr>
<tr>
<td>Yield</td>
<td>0.38% 25/6578</td>
<td>0.53%</td>
</tr>
<tr>
<td>First Predicted Origin</td>
<td>84.0% 21/25</td>
<td>85.3%</td>
</tr>
<tr>
<td>First or Second Predicted Origin(^b)</td>
<td>88.0% 22/25</td>
<td>97.1%</td>
</tr>
</tbody>
</table>

\(^a\)Based on participants with cancer status assessment at the end of the study.

\(^b\)Proportion of first or second origins correctly predicted among true positive participants.
Conclusions

- MCED screening was safely implemented for adults with and without additional cancer risk
- 1.4% of participants had a cancer signal detected
- 0.5% of participants were diagnosed with cancer due to MCED signal detection
- Median time to diagnostic resolution was 79 days
- High accuracy of predicted origin enabled targeted diagnostic evaluations
- Most diagnostic evaluations involved imaging, few required invasive procedures
- This study shows that it is feasible to detect cancers early using blood tests
Limitations and Caveats

- It will be important for future clinical utility studies to evaluate whether MCED testing reduces mortality, the primary goal of all cancer screening.
- Study cohort had limited diversity.
- 12 month follow-up was incomplete for 3% of the cohort.
- Further work is needed to understand the reasons underlying both false positive and false negative MCED test results.
- Participation in current cancer screenings with established effectiveness must continue as MCED technologies are refined.
Future/Ongoing Work

• Evaluation of MCED screening in cohorts with greater socioeconomic, ethnic and racial diversity

• Optimization of test performance characteristics

• Ongoing PATHFINDER2 study is screening 20,000 individuals using the refined version of the MCED test

• NHS-Galleri (ISRCTN 91431511) will assess clinical utility of the refined MCED test in a randomized trial of 140,000 adults 50-77 in the UK’s NHS. This pragmatic trial compares the incidence of advanced cancer diagnoses among participants who have annual MCED screening for 3 years to those who have usual care only
Thank you to study participants who were recruited from:

- Dana Farber Cancer Institute
- Sutter Health
- OHSU Knight Cancer Institute
- US Oncology
- Intermountain Healthcare
- Cleveland Clinic
- Mayo Clinic

PATHFINDER Secondary Objective:
Patient Reported Outcomes
Presented in poster #908P