Plasma-Based Molecular Testing for Early Detection of Lung Cancer

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Disclosures

Eric Fung, MD, PhD is an employee of GRAIL and has stock options for GRAIL.
## Cancer Mortality Increases with Later Detection

### Five-Year Cancer-Specific Mortality (%) by AJCC Stage at Diagnosis*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Colorectal</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Ovary</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lung and Bronchus</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Stomach</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Esophagus</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Liver**</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Pancreas</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Illustration of Benefit vs Risk of a Multi-Cancer Test

FP (RISK) ≤ Low FP Cut-Off

Test Score

High

Low

Non-Cancer
Cancer Type 1 (eg, Lung)
Cancer Type 2
Cancer Types 3, 4...

Invasive cancers discovered by multicancer test (BENEFIT)

FN
Limitations of Current Screening Paradigms Represent an Opportunity for Improved Cancer Detection

Example: Early Detection of Lung Cancer is a High Unmet Medical Need

- Low-dose computed tomography (LDCT) improves lung cancer mortality in high-risk individuals\(^1,2\)
- Rate of clinical adoption remains low (1.9\%)\(^3,4\)
- Criticisms of LDCT include risk of false positives and logistical challenges\(^5\)

How cfDNA-Based Tests can Improve Cancer Detection

- Cancer genotyping using plasma cfDNA
  - Adopted for detection of specific actionable mutations
  - Currently only validated for advanced cancer
  - Uses smaller targeted gene panels
- Cancer detection using plasma cfDNA
  - Aims to identify a broader cancer “signature” rather than specific individual mutations
- Genome-wide approaches offer additional information that allow early detection
- Could address the unmet medical need

cfNA-Based Approaches to Cancer Detection

High-intensity sequencing to detect cfNAs
- Reliably detect cancer-defining cfNAs by looking broadly across the entire genome and at extraordinary depth to detect faint signals

Large-scale clinical studies
- Confirm clinical validity of tests through one of the largest clinical study programs ever conducted in genomic medicine

Powerful machine-learning approaches
- Apply the latest tools of data science to classify participants according to the presence, type, and severity of cancer
The Circulating Cell-Free Genome Atlas (CCGA) Study
CCGA is a Prospective, Longitudinal Cohort Study Designed for Cancer Detection

Blood samples (all participants)

15,000+ participants
70% with cancer
30% without
142 Sites

Tissue samples (cancer only)

Targeted sequencing cfDNA, WBCs
Whole-genome sequencing (WGS) cfDNA, WBCs
Targeted & whole-genome bisulfite sequencing (WGBS) cfDNA
Whole-genome sequencing of tumor tissue

Follow-up for 5 yrs

Cancer Participants: Data on cancer status & treatment, new cancer diagnosis, mortality
Non-Cancer Participants: Remain cancer free or develop new cancer diagnosis, data on cancer status & treatment, mortality

cfDNA, cell-free DNA; WBC, white blood cell; WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.
CCGA: Discovery, Training, and Validation for a Multi-Cancer Test

~15,000 planned participants
70% cancer : 30% non-cancer

FPI: 08/2016

12,200 reserved for future studies

2,800 participants:
Prespecified case-control substudy
1,628 cancer; 1,172 non-cancer

Training Set: 1,785
Clinically Locked*

Test Set: 1,010
Clinically Locked*

*5 participants not clinically locked were excluded.
CCGA: Prespecified Case-Control Substudy of 2,800 Participants

Training Set: 1,785 Clinically Locked

- 1,733 Clinically Evaluable
  - 984 Cancer (127 Lung Cancer)
  - 878 stage I-IV
  - 580 Non-Cancer
  - 169 Non-Cancer Assay Controls

- 1,406 Analyzable with Assay Data
  - 845 Cancer (118 Lung Cancer)
  - 561 Non-Cancer

Test Set: 1,010 Clinically Locked

- 980 Clinically Evaluable
  - 576 Cancer (47 Lung Cancer)
  - 478 stage I-IV
  - 368 Non-Cancer
  - 36 Non-Cancer Assay Controls

- 834 Analyzable with Assay Data
  - 472 Cancer (46 Lung Cancer)
  - 362 Non-Cancer

*5 participants not clinically locked were excluded.
Comparable Cancer and Non-Cancer Groups

- Clinically evaluable cancer and non-cancer groups were comparable with respect to age, sex, race/ethnicity, and BMI.
- A higher proportion of participants with lung cancer were male and were ever-smokers.

<table>
<thead>
<tr>
<th></th>
<th>Training</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Cancer</td>
<td>Cancer*</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>580</td>
<td>984</td>
</tr>
<tr>
<td>Age, Mean ± SD (years)</td>
<td>60 ± 13</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>452 (78%)</td>
<td>697 (71%)</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, Non-Hispanic</td>
<td>489 (84%)</td>
<td>846 (86%)</td>
</tr>
<tr>
<td>African American</td>
<td>47 (8%)</td>
<td>67 (7%)</td>
</tr>
<tr>
<td>Hispanic, Asian, Other</td>
<td>44 (8%)</td>
<td>71 (7%)</td>
</tr>
<tr>
<td>Smoking Status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>330 (57%)</td>
<td>484 (49%)</td>
</tr>
<tr>
<td>BMI Normal/Underweight</td>
<td>156 (27%)</td>
<td>266 (27%)</td>
</tr>
<tr>
<td>Overweight**</td>
<td>184 (32%)</td>
<td>319 (32%)</td>
</tr>
<tr>
<td>Obese***</td>
<td>240 (41%)</td>
<td>398 (40%)</td>
</tr>
</tbody>
</table>

*Cancer types by training/test: Breast (410/201), lung (127/47), prostate (74/58), colorectal (51/46), renal (29/18), uterine (28/9), pancreas (27/23), esophageal (25/22), lymphoma (25/22), head & neck (21/12), ovarian (21/7), hepatobiliary (15/16), melanoma (15/12), cervical (14/11), multiple myeloma (14/21), leukemia (13/16), thyroid (13/10), bladder (12/3), gastric (12/15), anorectal (7/3), and unknown primary/other (22/18). **BMI≥25. ***BMI≥30.
Stage Distribution and Method of Diagnosis were Consistent in Training and Test Sets

<table>
<thead>
<tr>
<th></th>
<th>Training Set</th>
<th>Test Set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer (n=984)</td>
<td>Lung Cancer (n=127)</td>
</tr>
<tr>
<td>Overall Clinical Stage (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0*</td>
<td>56 (6%)</td>
<td>1 (&lt;1%)</td>
</tr>
<tr>
<td>I</td>
<td>300 (30%)</td>
<td>23 (18%)</td>
</tr>
<tr>
<td>II</td>
<td>249 (25%)</td>
<td>14 (11%)</td>
</tr>
<tr>
<td>III</td>
<td>165 (17%)</td>
<td>39 (31%)</td>
</tr>
<tr>
<td>IV</td>
<td>164 (17%)</td>
<td>47 (37%)</td>
</tr>
<tr>
<td>Non-Informative**</td>
<td>50 (5%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Method of Dx (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosed by Screening§</td>
<td>354 (36%)</td>
<td>23 (18%)</td>
</tr>
<tr>
<td>Diagnosed by Clinical Presentation¶</td>
<td>630 (64%)</td>
<td>104 (82%)</td>
</tr>
</tbody>
</table>

*DCIS/CIS. **Staging information not available. §Percent screen-detected in training/test sets for breast cancer: 58%/58%, colorectal cancer: 29%/37%, lung cancer: 18%/15%, prostate cancer: 91%/90%, and other cancers 4%/4%. ¶Clinical presentation includes all cancers not detected by screening (ie, detected symptomatically or as incidental findings).
Prototype Sequencing Assays Used to Comprehensively Characterize Cancer-Specific cfDNA Signals

- **All major somatic and epigenetic cfDNA features characterized**

**Input**
- **WGBS**
  - cfDNA: Bisulfite sequencing, 30X depth
  - WBC gDNA: 30X depth
- **WGS**
  - cfDNA: 30X depth
  - WBC gDNA: 507 gene panel, 60,000X depth, 3,000X unique coverage
- **Targeted**
  - WBC gDNA: 507 gene panel, 60,000X depth, 3,000X unique coverage

**Interference**
- **WGBS**
  - Aging, Biological variation
- **WGS**
  - SCNA signals derived from WBCs
- **Targeted**
  - Variants derived from WBCs

**Final Features**
- **WGBS**
  - Fragment-level CpG methylation status
- **WGS**
  - cfDNA Somatic copy number
- **Targeted**
  - Non-synonymous SNVs/indels

**Classifiers**
- **WGBS Classifier**
- **WGS Classifier**
- **Targeted Classifier**

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cfDNA, cell-free deoxyribonucleic acid; WBC, white blood cell; WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.
Potential Source of False Positives: Clonal Hematopoiesis

- Previous study sequencing at ~100X: 96% of variants at >1% variant allele frequencies (VAF)\(^1\)

- Ultra-deep sequencing (this study [Training set]): <10% of WBC-matched variants at >1% VAF

- Variants at lower VAFs will require high-depth sequencing of WBCs to effectively exclude this confounding signal in cfDNA-based assays

- Early detection requires a low limit of detection, wherein low VAF CHIP is a confounding signal

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CHIP, clonal hematopoiesis of indeterminate potential; cfDNA, cell-free DNA; WBC, white blood cell; gDNA, genomic DNA.
Majority of cfDNA Variants Are WBC-Matched Clonal Hematopoiesis

- In Training set, average non-tumor WBC-matched cfDNA somatic variants (SNVs/indels) were:
  - 98% of all variants in non-cancer group
  - 71% in total cancer group
  - 54% in lung cancer group
- Number of WBC variants is positively associated with age in cancer and non-cancer groups\(^1\)

\(^1\)Swanton C et al. *J Clin Oncol.* 2018;36(suppl; abstr 12003).

cfDNA, cell-free DNA; WBC, white blood cell; SNV, single-nucleotide variant; indel, insertion or deletion.
Simulating Existing Assays¹: Not Optimized for Screening

- CCGA [Training set] used for simulation analysis:
  - 561 non-cancer; 118 participants with lung cancer
- Testing a single location (emulating ddPCR)
  - KRAS:p.G12X
  - Small number of cancer participants detected
  - Few non-cancer participants
- NGS panel reporting 813 clinically actionable variants from 30 genes²
  - More cancer participants detected
  - Many non-cancer participants detected due to WBC/CHIP variants
- CCGA targeted NGS assay with coverage of 507 genes and combined cfDNA and WBC sequencing
  - Joint cfDNA/WBC ML calling to remove WBC/CHIP variants
  - Increased detection of cancer participants
  - Reduced false-positives (specificity set at 98%)

¹Oxnard GR et al. J Clin Oncol. 2018;36(suppl; abstr LBA8501).
ddPCR, digital-droplet PCR; cfDNA, cell-free DNA; WBC, white blood cell; CHIP, clonal hematopoiesis of indeterminate potential; NGS, next generation sequencing; ML, machine learning.
Sensitivity Consistent Across Assays and at High Specificity—Training Set

- In age-matched controls, there is expected to be a latent rate of 1% of undiagnosed cancer
  - Some of those cancers will be detectable by the prototype assays and classifiers
  - The longitudinal design of the study allows us to correctly assign cancer status to individuals post-enrollment once diagnoses are reported from normal clinical practice
- We conservatively look at 98% specificity to account for these latent cases
  - Specificity will continue to evolve as follow-up is completed

WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.
High Specificity (>99%) is Feasible

5-year follow-up will enable identification of participants who are subsequently diagnosed

**Training:**
- 580 Control Participants
- 5 (<1%) Notable cancer-like signal* present
- 575 No notable cancer-like signal* (test negative or absent)
  - 3 Confirmed cancer
    - Lung IV
    - Endometrial II
    - Ovarian III
- 2 No cancer identified
  - Follow-up ongoing

**Test:**
- 368 Control Participants
- 3 (<1%) Notable cancer-like signal* present
- 365 No notable cancer-like signal* (test negative or absent)
  - 3 No cancer identified
    - Follow-up ongoing

*Notable cancer-like signal defined as ≥2 assays with significant abnormalities compared to the typical non-cancer population, or known cancer drivers present with ≥1 significant assay abnormality.
Consistent Results Across Assays and Between Training and Test Sets

- Cancers that had >40% detection in training included lung, HR-negative breast, colorectal, esophageal, head & neck, hepatobiliary, lymphoma, ovarian, and pancreatic cancers, and multiple myeloma.

Signal was also consistent in low-signal cancers (<10% in training on any of the three assays: prostate, thyroid, gastric, melanoma).

WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.
Early- and Late-Stage Cancers Detected in the Test Set

Cancer Type | N | Stage I-III | Stage IV
---|---|---|---
Lung | 27 | 19 | 0%
Esophageal | 7 | 0% | 0%
Head & Neck | 7 | 5 | 0%
Pancreas | 8 | 14 | 0%
Colorectal | 29 | 10 | 0%
Hepatobiliary | 8 | 6 | 0%
Breast (HR-) | 19 | 3 | 0%
Ovarian | 5 | 2 | 0%
Lymphoma | 13 | 5 | 0%
Multiple Myeloma | 8 | 0% | 0%

- Detection was observed in across all stages
  - Sensitivity was higher in Stage IV cancers
  - 45% cancers detected were Stages I-II
- Strong detection of cancers with high (>50%) cancer-specific mortality rates at five years¹

WGBS, whole-genome bisulfite sequencing.
Lung Cancer Detection by Smoking Status and Histologic Subtype in the Test Set

- 93% (43/46) of participants with lung cancer were ever-smokers
- Signal was detected in ever-smokers, as well as in never-smokers
  - Of 3 never-smokers, 2 were detected by the methylation assay, 1 by the WGS assay, and 3 by the targeted assay

Signal was also detected consistently across histologic subtypes (Stage I-IV WGBS assay reported):
  - 100% (5/5) of SCLC cases were detected
  - 65% (11/17) of SCC cases were detected
  - 60% (12/20) of adenocarcinoma cases were detected

WGS, whole-genome sequencing.
Summary

- Preliminary cfDNA-based blood test results detected multiple cancers, including lung, and across all stages—even early stages when treatment may be more effective
  - Test set confirmed the signal observed in the training set
  - >99% specificity is feasible
    - Targeted methods require accounting for clonal hematopoiesis
      - High detection of cancers with high mortality and that lack screening paradigms or where screening is not well-adopted
- This approach is thus promising as a multi-cancer detection test, including for early-stage cancers
- Further assay and clinical development in large-scale clinical studies, including CCGA, is ongoing

WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.
Acknowledgements

● Study participants who graciously donated their time, energy, and specimens

● CCGA investigators and collaborators for advice, enrolling participants, and collecting data and specimens
  ○ Principal Investigators from sites enrolling >35 participants in this preplanned substudy: Minetta C. Liu (Mayo Clinic, MN); David Thiel (Mayo Clinic, FL); Rosanna Lapham, MD (Spartanburg Regional Health Services, SC); Donald Richards, MD, PhD (TOPA Tyler, TX, US Oncology Network); Nicholas Lopez, MD (Baptist Health Paducah, KY); Daron G. Davis, MD (Baptist Health Lexington, KY); Mohan Tummala, MD (Mercy Springfield, MO); Peter Yu, MD (Hartford Hospital, CT); Wangjian Zhong, MD (Baptist Health, Louisville, KY); Alexander Parker, MD (Mayo Clinic Jacksonville, FL); Kristi McIntyre, MD (TOPA Dallas Presbyterian, TX, US Oncology Network); Fergus Couch (Mayo Clinic Rochester, MN); Robert Seigel (Bon Secours Greenville, SC); Allen L. Cohn, MD (Rocky Mountain Cancer Center Hale Parkway Denver, CO, US Oncology Network); Alan H. Bryce (Mayo Clinic Phoenix, AZ)

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