Cancer Detection by Molecular Testing
Re-imagining the Screening Paradigm

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Disclosures

Relevant Financial Relationship(s)

Relationship with Exact Sciences

- Mayo Clinic
  - Equity investor
  - Licensed technologies

- Dr. Ahlquist
  - Scientific Advisor
  - Inventor of licensed technology
  - Research collaborator

The DLMP Grand Rounds Planning Committee members listed below declare that they have nothing to disclose in relation to this presentation:

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Thomas Huntley           Denise Skudlarek
Learning Objectives

- Identify gaps in our current approach to cancer screening
- Describe how molecular tools might fill gaps and what performance characteristics are required
- Appraise early data on tumor detection and site prediction with methylated DNA markers

“There’s a crack in everything... that’s how the light gets in.”

Leonard Cohen
GAPS in the Current Approach to Cancer Screening

- Most cancer types excluded
  - Only 3 types screened at population level
- Integration
- Compliance & access
- Cost

Universal Cancer Screening

- Revolutionary, rational, realizable
- 3 key elements
  - Highly discriminant markers
  - Site prediction
  - Requisite analytical sensitivity
- Fills many existing gaps

<table>
<thead>
<tr>
<th>Current screening</th>
<th>Pan-Cancer test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ-centered</td>
<td>Patient-centered (multi-organ)</td>
</tr>
<tr>
<td>Exclusionary</td>
<td>Inclusive</td>
</tr>
<tr>
<td>Clunky</td>
<td>Integrated &amp; efficient</td>
</tr>
<tr>
<td>Costly</td>
<td>Cost-saving</td>
</tr>
</tbody>
</table>
Universalized & Individualized Approach to Molecular Detection of Cancer

- **Novel meth DNA markers & assay technology**
  - Highly accurate
  - Site specific

### Blood
- Blood

### Stool
- Stool

### Urine
- Urine

### Cancers
- Breast
- Lung
- Esoph
- Stom
- Pancreatic
- Liver
- Colon
- Uterine
- Ovarian
- Prostate
- Hematol
- Other

Methylated DNA Approach

**Attractive Features**

**Markers**
- Broadly informative
- Single target region per gene
- **Site specific**
- Tumor type specific

**QuARTS Assay**
- High analytical sensitivity & specificity
- Automatable, fast, low cost
Unbiased Whole Methylation Discovery (RRBS)

1. Reads mapped to in silico reference genome
2. Read depth of $\geq 10x$
3. CpG present in $\geq 50\%$ of samples
4. Define reference sample group (controls)
5. Establish background methylation threshold among controls, typically $\leq 2.5\%$
6. Measure AUC for each individual CpG, must exceed 0.65
7. Define region of interest: $\geq 5$ CpGs/100 bp
8. Define differential methylation per region (DMR): Measure sum of methylated CpGs among all CpGs in region of interest
9. Rank by variance-inflated logistic regression:
   - $P$-value $< 0.001$
   - AUC $\geq 0.9$
   - Fold-change $\geq 20$

~30,000,000 CpGs
~5,000,000 CpGs
~1,000,000 CpGs

Universal Cancer Detection with Prediction of Tumor Site

Universal cancer markers determine if cancer is present…
…reflex site-specific markers to direct diagnosis

Circulating or exfoliated DNA sampled from blood or stool
Prediction of LGI vs UGI Cancer

Regression PARtioning Tree (rPart) models

Accuracy 91%

Classification of GI Tumors by Site

By novel methylated DNA markers (rPart models)

- Validation set (266 cancers, 119 controls)
- 8-marker panel, pre-set cutoffs
- **Accuracy**
  - Universal (cancer vs control) 95%
  - LGI vs UGI cancer 94%
  - Esoph/stom vs Panc/biliary 93%
  - Overall 88%
Blood Testing

Top 10 Cancers
Investigation Plan Underway

- Whole methylome discovery & validation
- ID of universal and site-specific marker panels
- Plasma testing within and across tumor sites, optimization, and marker cut-off determinations

*Opportunities for collaboration in subsequent phase validation and extended applications.*
**Lung Cancer**
*Early Plasma Results*

- Case-control Phase I study
  - Methylated DNA (4 marker panel)
  - QuARTS assay

**Sensitivity** 96%
**Specificity** 94%

*By comparison:*
- CT Scan Screening
  - Sensitivity 90-95%
  - Specificity 75%

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**Hepatoma**
*Early Plasma Results*

- Case-control study
  - Methylated DNA (3 marker panel)
  - QuARTS assay

**Sensitivity** 95%
**Specificity** 97%

*By comparison:*
- Serum AFP
  - Sensitivity 50-60%
  - Specificity 80%
Stomach Cancer
Early Plasma Results

In plasma (3-marker panel)
Spec 100% 95%
Sens 85% 87%

Anderson et al. ACG 2016

Stool Testing
Colorectal Cancer

- Screening rate suboptimal
- Shift toward right side
  - Now ~50% R-side in US
  - Olmsted county: ~60% R-side
    CGH 2005;3:150
- Conventional screening tools
  - Biased toward L-side
- Imperative to innovate & improve
What are the critical target lesions for CRC screening?

- Curable stage cancer
- Highest risk pre-cancers
  - Large adenoma \( \text{\textit{i.e.}} \geq 2\text{cm} \)
  - Large sessile serrated polyp
  - High grade dysplasia

Stool Tests: Occult Blood Tests

Bleeding Patterns

Ahlquist et al, Cancer 1989;63:1826
Colonoscopy Screening

- Associated risks
  - 1-2% visit ER within 24 hrs*
- Requires 1-2d missed work
- Operator-dependent quality**
  - ADRs among endoscopists vary widely (7-50%)
  - Low ADRs linked to higher interval cancers
- L-sided detection bias
  - Reduction in mortality & incidence
    - L-side 70-76% (Lancet 2010; 375:1624)
    - R-side 0-24% (JNCI 2010;102:89)
    - NEJM 2013; 356:1106


Is there a better way?

Effective detection = S x C x A

S (sensitivity)
C (compliance)
A (access)

Ideal screening tool must address all 3
Molecular Stool Testing

Biological Basis

Exfoliation

↓

Fecal colonocytes

↓

DNA

Muco-cellular layer

Ahlquist et al. Hum Pathol 2000

Multi-target Stool DNA (MT-sDNA) Test

Addresses all elements of “effective detection”

- High sensitivity for CRC & greatest-risk precancers
- Unaffected by tumor site
- Operator independent (automated)
- Noninvasive
- No cathartic preparation
- No diet or medication restriction
- No missed work
- Home collection & mailing

S

C

A
MT-sDNA Test (Cologuard)  
*Optimized & Automated*

- Simple device for collection & mailing
- Preservative buffer
- Multiple markers
  - Mutant KRAS
  - Methylated BMP3 & NDRG4
  - β-actin (human DNA)
  - Hemoglobin (FIT)
- Sensitive multiplex DNA assay (QuARTS)

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**Cancer Detection by MT-sDNA**  
*Case-Control Study*

Overall sensitivity  
98%  
(91/93)

No effect of stage

No effect of site

Lidgard et al. CGH 2013:11:1313
Adenoma Detection by MT-sDNA
Case-Control Study

Sensitivity for HGD* 83%
*94% found in lesions >2cm

Lidgard et al. CGH 2013:11:1313

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Multitarget Stool DNA Testing for Colorectal-Cancer Screening

Thomas F. Imperiale, M.D., David F. Ransohoff, M.D., Steven H. Itzkowitz, M.D., Theodore R. Levin, M.D., Philip Lavin, Ph.D., Graham P. Lidgard, Ph.D., David A. Ahlquist, M.D., and Barry M. Berger, M.D.

Multicenter cross-sectional study: 10,000 patients from 90 sites

94%
Alaska Native (AN) People

- **World's highest CRC incidence**
  - AN people: \(91/100,000\)
  - US whites: \(40/100,000\)

- Many live in remote communities
- Limited access to colonoscopy
- *Prospective Mayo-Alaska study 2013-15*

MT-sDNA Detection of Colorectal Neoplasia
Alaska Native & General US Populations

<table>
<thead>
<tr>
<th>Size</th>
<th>Alaska Native People</th>
<th>U.S. Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 cm</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>≥2 cm</td>
<td>60%</td>
<td>80%</td>
</tr>
<tr>
<td>≥3 cm</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>Cancer</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

**Specificity**
- AN: 93%
- US: 90%

Detection of Sessile Serrated Polyps

MT-sDNA vs FIT

MT-sDNA is now available

- FDA & CMS approvals August 2014
  - *First ever parallel review*
- Endorsed in Screening Guidelines
  - ACS 2015
  - USPSTF 2016
  - NCCN 2016
Prix Galien Award
“Nobel in Industry”

Best Innovation in
Medical Technology
2016

Cologuard
Exact Sciences

Did you remember
to send out your
Mayo Clinic Home Colon Test?...
“Program Specificity”

- **Critical metric: average false positives/year**
  - **FIT q1yr**
    - With point false pos rate of 4-5%, average false-positives would be ~ **4-5%/yr**
  - **MT-sDNA q3yr**
    - With point false pos rate of 7-10%, average false-positives would be ~ **2-3%/yr**
    - Compared to conventional CRC screening approaches*
      - Highest benefit/harm ratio
      - Fewest lifetime colonoscopies

*2016 USPSTF Guidelines (JAMA 2016)

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**Program Sensitivity**

*Modeled Estimate (q3yr testing)*

<table>
<thead>
<tr>
<th>Large Precancer*</th>
<th>CRC (I-II)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1-2 cm</strong></td>
<td><strong>HGD</strong></td>
</tr>
</tbody>
</table>
| Screen 1         | 42-66      | 69
| Screen 2         | 71-91      | 93
| Screen 3         | 88-98      | (99) |

*Assumptions

1. Size doubling time: 6 yrs
2. Independent measurements

Ahlquist. DDS 2015; 60:623
**Knowledge of Positive MT-sDNA Improves Yield & Quality of Colonoscopy**

<table>
<thead>
<tr>
<th></th>
<th>Un-blinded</th>
<th>Blinded</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(172)</td>
<td>(72)</td>
<td></td>
</tr>
<tr>
<td>Any polyp</td>
<td>78%</td>
<td>60%</td>
<td>0.0047</td>
</tr>
<tr>
<td>Any adenoma or SSP</td>
<td>70%</td>
<td>53%</td>
<td>0.013</td>
</tr>
<tr>
<td>Advanced CRN</td>
<td>28%</td>
<td>21%</td>
<td>0.27</td>
</tr>
<tr>
<td>Flat R-sided polyp</td>
<td>40%</td>
<td>9%</td>
<td>0.0017</td>
</tr>
<tr>
<td>Med # polyps/patient</td>
<td>2</td>
<td>1</td>
<td>0.0007</td>
</tr>
<tr>
<td>Med withdrawal time</td>
<td>19 min</td>
<td>13 min</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Johnson et al. Gastrointest Endosc 2017; 85:657

**Screening Participation with MT-sDNA?**

- Survey of first 100,000 patients post-approval*
  - 42% had no prior CRC screen
- USMD Health System study on MT-sDNA ordered in prior non-compliant Medicare patients**
  - 88% (347/393) completed test
  - Of 46 test-positives, 54% had advanced CRN (4 CRC)
- National adoption rate will be influenced by multiple factors:
  - Patient/provider awareness
  - Professional acculturation
  - Ordering/reporting logistics
  - 3rd party coverage

*Berger et al. ACG 2016; **Prince et al. AACR 2016
MT-sDNA for CRC Screening

Summary

• High sensitivity for CRC and large polyps in screen setting at all anatomic sites
• Potential to prevent CRC via program detection of highest risk precancers
• User-friendly & distributable features could improve compliance and access
• FDA and CMS approved
• Included in major societal screening guidelines

Stool DNA Testing
Other Potential Applications

• Cancer surveillance in IBD
  • Case-control pilot*
    Sens at 90% spec
    CRC 100%
    Dysplasia 70%
  • Multicenter case-control**
    US - Norway

• Universal GI cancer screening?

*Cisiel et al. APT 2013;37:546  **Cisiel et al. DDW 2016

Cancer + HGD detection by 4-marker panel
Specificity 90%
Sensitivity 92%
Other Applications

Barrett’s Esophagus

Detection rates, %

<table>
<thead>
<tr>
<th>Test</th>
<th>Spec</th>
<th>Sens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trefoil pep*</td>
<td>88-94</td>
<td>73</td>
</tr>
<tr>
<td>US study</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Meth DNA**</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2-markers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Benaglia et al. Gastroenterology 2013, **Iyer et al. DDW 2016
Pancreas

Cyst fluid

<table>
<thead>
<tr>
<th>HGD/CA vs LGD/no dysp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
</tbody>
</table>

Bob Dylan

“He not busy being born …is busy dying”