

# OMEC COLORECTAL CANCER SCREENING COMMITTEE MEETING

Saturday, May 30, DDW Chicago, 2009

Presenter: D. Ahlquist



## MOLECULAR DETECTION OF PRECANCERS

Technical Requirements

David Ahlquist

June 30, 2009

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## Precursor Lesion Detection in CRC Screening

- Essential for **CRC prevention**
- Assay of *occult bleeding* misses most
- Stool assay of *exfoliated* markers offers candidate approach
- Technical requisites?

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## Precursor Lesion Detection in CRC Screening

- |                                 |                              |
|---------------------------------|------------------------------|
| • <b>Correct target lesions</b> | • Full colorectal coverage   |
| • Broadly informative markers   | • Right medium               |
| • Marker stability              | • Appropriate test frequency |
| • High analytical sensitivity   | • Effective test penetration |

3

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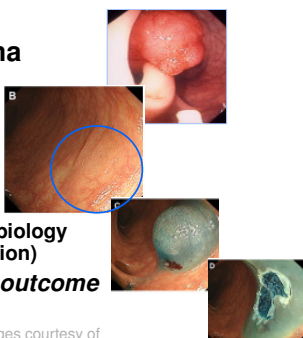
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MAYO CLINIC

## Correct Target Lesions

- **Advanced adenoma**
- **Serrated polyps\***
  - Precursor for >20% of CRCs
  - Elderly, R>L colon
  - Sinister natural hx
  - Different molecular biology (mut*BRAF*, methylation)
  - *Often ignored as outcome*



\*Sessile serrated adenoma images courtesy of Dr. Won Kee Song, Mayo Clinic

4

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## Precursor Lesion Detection in CRC Screening

- **Correct target lesions**
- **Broadly informative markers**
- **Marker stability**
- **High analytical sensitivity**
- **Full colorectal coverage**
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5

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## Broadly Informative Markers

- **Well-established genetic and epigenetic alterations in precursor lesion tissue**
  - **Advanced adenoma**
    - *APC* 70-80%, *KRAS* 40-50%, meth genes >70%
  - **Serrated polyps**
    - *BRAF* ~70%, *KRAS* ~15-30%, meth genes >90%
- **Several marker combinations detect ~100% of adv adenomas on tissue specimens, e.g *APC*, *KRAS*, meth vimentin** (Ann Intern Med, 2008;149:441)

6

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- Correct target lesions
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7

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**MAYO CLINIC**

## Marker Stability in Stool

- Markers variably metabolized in stool
- DNA instability accounted for sub-optimal neoplasm detection rates in multicenter with 1<sup>st</sup> generation tests
- **Stabilization buffer preserves marker yield**

Zou et al. CEBP 2006; 15:1115

Days at Room Temperature	100 mM EDTA	10 mM EDTA
Day 0	100	100
Day 1	110	80
Day 3	110	40
Day 5	100	20

8

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9

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## High Analytical Sensitivity

- For stool DNA markers with adv adenomas, median M:WT ~0.5%
- Assay M:WT detection limit:
  - 1<sup>st</sup> gen stool DNA >1%
  - Next gen <0.1% (e.g digital melt curve, BEAMING)
- Higher analytical sens yields higher clinical sens for adv adenomas

Zou et al. Gastroenterology 2009;136: 459

Assay	Analytical Sensitivity (%)
DMC (16/27)	~60
Hemocour (2/27)	~5
Hemocour-BEAM (6/27)	~15
EXACT (7/27)	~25

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## Detection of Adv Adenomas by Next Generation Stool DNA Assay

Zou et al, DDW 2009

**Subjects:** 100 colonoscopic normals, 27 patients with adenomas >1cm

**Marker panel:** Alu, KRAS, APC, mBMP3

**Detection at 90% specificity**

	<u>Sensitivity, %</u>
Ad $\geq$ 1cm	63
Ad $\geq$ 2cm	78
Ad $\geq$ 3cm	90

11

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**MAYO CLINIC**

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12

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 **Full Anatomic Coverage of Colorectum**

- **Structural screening approaches biased toward left-sided CRC mortality reduction**
  - **Flex sig** (Selby. NEJM 1992;326:653)
  - **Colonoscopy** (Baxter. Ann Intern Med 2009;150:1)
- **Ideal stool test as complement or alternative to structural screening would detect both R and L lesions**

13

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 **Stool Test Detection of CRN (CA + Adv Ad) in Screen Setting: Effect of Tumor Site**

	%		p-value
	Prox	Dist	
Morikawa (n=21,805)*			
<b>FIT</b>	<b>16</b>	<b>31</b>	<b>&lt;0.0001</b>
Ahlquist (n=4,482)**			
<b>Hemoccult</b>	<b>9</b>	<b>21</b>	<b>0.06</b>
<b>HemoccultSensa</b>	<b>13</b>	<b>31</b>	<b>0.01</b>
<b>DNA (SDT2)</b>	<b>45</b>	<b>48</b>	<b>NS</b>

\*Gastroenterology 2005;125:422 (colonoscopy as gold std)  
\*\*Ann Intern Med 2008;149:441 (colonoscopy as gold std, unbuffered stools)

14

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 **Precursor Lesion Detection in CRC Screening**

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15

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## Stool vs Blood vs Urine?

- Biologically, precursor lesions should shed markers into colorectal lumen (*stool*) sooner than into circulation
- **DNA markers** have been detected in *plasma* and in *urine* at high rates 60-80% with CRC but 0-20% with adv adenomas
- Pilot data with **RNA expression** assays in circulating *mononuclear cells* suggest that adv adenomas can be detected

16

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## Altered APC in Plasma

	% (+)	% M:WT
Normals (10)	0	0
Large adenoma (11)	9	0.02
<b>Cancer</b>		
Dukes A (8)	63	0.04
Dukes B (8)	63	1.3
Dukes D (6)	100	8.1

Diehl et al, PNAS 2005;102:16368

17

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18

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19

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## Effective Test Penetration

- Effective Detection =  $S \times C \times A$ 
  - S = sensitivity
  - C = compliance
  - A = access } **Test Penetration**
- High compliance requires *user-friendly* test features
- Wide access requires *high throughput*

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## Molecular Detection of CRC Precursor Lesions

*Summary*

- Next generation tests show promise
- Technical features can be engineered to deliver optimal performance
- Phased validation and comparison studies needed

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