



Noninvasive Molecular Detection of Colorectal Neoplasia Next Generation Approaches

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Disclosures

Relationship with Exact Sciences

- Mayo Clinic is equity investor
- Potential for future royalties to Mayo & Dr. Ahlquist for licensed IP
- Dr. Ahlquist serves as Senior Scientific Advisor

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Effective Test Detection

$$\text{Effective Detection} = S \times C \times A$$

S = sensitivity
C = compliance
A = access

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Screening Tools: Multiple Choices

	Accuracy	Cost	Invasive	Access
Stool blood	Low	Low	No	Unlim
Flex sig	Mod	Mod	Yes	Lim
Colon x-ray	Mod	Mod	Yes	Lim
Colonoscopy	High	High	Yes	Lim
CTC	High	High	Yes	Lim
Stool DNA	M-H	Mod	No	Unlim
Other	?	?	?	?
Ideal	High	Low	No	Unlim

CP1003356-27



Next Generation Molecular Tests Potential Advantages

- Noninvasive
- Avoidance of diet, medication, or cathartic preparation
- Off-site collection
- Wide accessibility
- Expanded value
 - Improved "effective" detection of CRN
 - Pan-GI cancer screening

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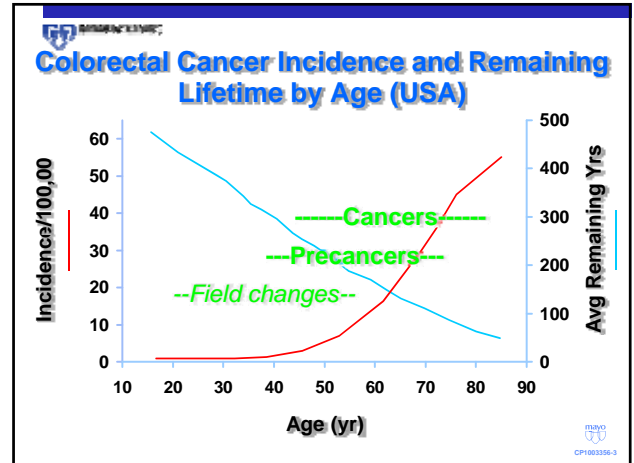


Improved Effective Detection of CR Neoplasia

Molecular Detection of CR Neoplasia

- Correct target lesions
- Broadly informative markers
- Marker stability
- High analytical sensitivity
- Full colorectal coverage
- Right medium
- Appropriate test frequency
- Effective test penetration

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Correct Target Lesions

- Curable stage cancer
- Advanced adenoma
- Serrated polyps*
 - Precursor for >20% of CRCs
 - Elderly, R>L colon
 - Sinister natural hx
 - BRAF mut, methylation
 - Often ignored as outcome

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

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Detection of precursor lesions essential for CRC prevention

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Broadly Informative Markers

- Well-established DNA alterations in CRC and precursor 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)

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Molecular Detection of CR Neoplasia

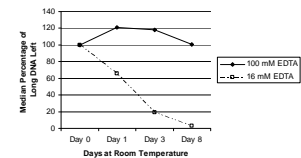
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Marker Stability in Stool

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

Zou et al. CEBP 2006; 15:1115



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Molecular Detection of CR Neoplasia

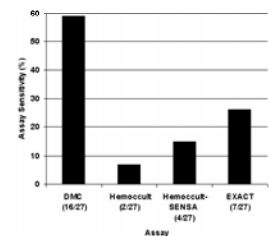
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High Analytical Sensitivity

- Stool DNA markers with adv adenomas
 - Median level M:WT ~0.5%
- Stool DNA assay detection limit:
 - 1st gen: >1%
 - Next gen: <0.1%
- Higher analytical sens yields higher clinical sens for adv adenomas

Zou et al. Gastroenterology 2009;136; 459



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Detection of CR Neoplasms by Next Generation Stool DNA Assay

Zou et al, DDW 2009

Subjects: 100 normals, 100 CR neoplasia

Marker panel: Alu, KRAS, APC, mBMP3

Detection at 90% specificity

Cancer Adv Ad	Sensitivity, % 86-91
≥1cm	63
≥2cm	78
≥3cm	90

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Full Anatomic Coverage of Colorectum

- Structural screening approaches biased toward left-sided CRC mortality reduction
 - Flex sig (Selby et al. NEJM 1992;326:653)
 - Colonoscopy (Baxter et al. Ann Intern Med 2009;150:1; Gupta et al. CGH 2005; 3:150)
- Ideal test as complement or alternative to structural screening would detect both R and L lesions

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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)*			
FIT	16	31	<0.0001
Ahlquist (n=4,482)**			
Hemoccult	9	21	0.06
HemoccultSensa	13	31	0.01
DNA (SDT2)	45	48	NS

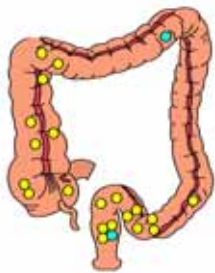
*Gastroenterology 2005;125:422 (colonoscopy as gold std)

**Ann Intern Med 2008;149:441 (colonoscopy as gold std, unbuffered stools)

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Colorectal Cancer Detection

Gastroenterology 2000;119:1219



Multi-target DNA test

Positive (●) 20

Negative (●) 2

Sensitivity 20/22=91%

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Stool vs Other Media?

- Markers likely shed into colorectal lumen (stool) sooner than into circulation
- DNA markers have been detected in plasma and in urine at rates 60-80% with CRC but 0-20% with adv adenomas
- RNA expression assays in circulating mononuclear cells may detect adv adenomas (Arber et al. DDW 2009)
- Other media (e.g. rectal mucin)?

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Altered APC in Plasma

	% (+)	% M:WT
Normals (10)	0	0
Large adenoma (11)	9	0.02
Cancer		
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

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Screen Test Frequency

- **Must accommodate natural hx**
- **Short-interval testing increases program sensitivity**
 - e.g. sensitivity 50% with 1st screen, increases to 75% with 2nd screen,
- **Long-interval testing may compromise program sensitivity**

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- **Effective test penetration**

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Effective Test Penetration

- **Effective Detection = S x C x A**
 - S = sensitivity
 - C = compliance
 - A = access
 - High compliance requires *user-friendly* test features
 - Wide access requires *high throughput* and *broad distribution*
- } **Test Penetration**

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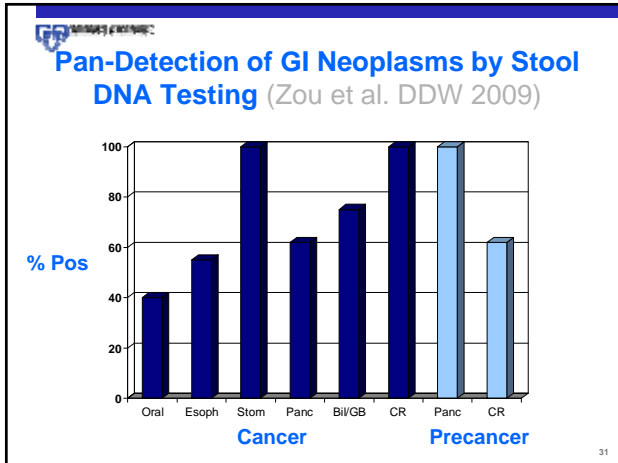
Pan-Detection of GI Neoplasia



Pan-detection of GI Neoplasia by Stool DNA Testing

- Shared exfoliative biology
- Aggregate rather than single site prevalence
- Potential for tumor site prediction using multi-marker panel
Taylor et al. DDW 2009
- Demonstrated feasibility

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- ### Summary
- Next generation molecular tests hold promise for expanded value
 - Improved CRN detection
 - Pan-GI cancer screening
 - Technical features can be engineered to deliver optimal performance
 - Validation and comparison studies needed

Other Future Approaches to Colorectal Cancer Screening

- Self-intubating colon scopes (e.g. Aeroscope)
- Swallowed video capsules

- ### Video Capsule Endoscopy
- Van Gossum et al. NEJM 2009; 361:3
- Multicenter European study
 - N = 328 referred patients
 - Colonoscopy on all
 - VCE completed in 93% within 10 hrs
 - Accuracy for advanced adenomas

Sensitivity	73%
Specificity	79%