



Establishing a best panel of stool-based detection for non-invasive colorectal neoplasm screening

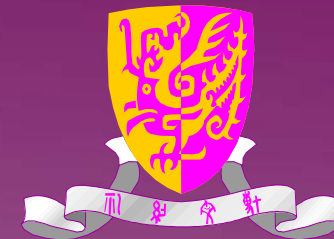
Jessie Qiaoyi Liang, PhD

Research Associate Professor
Department of Medicine and Therapeutics
The Chinese University of Hong Kong

香港中文大學醫學院

Faculty of Medicine

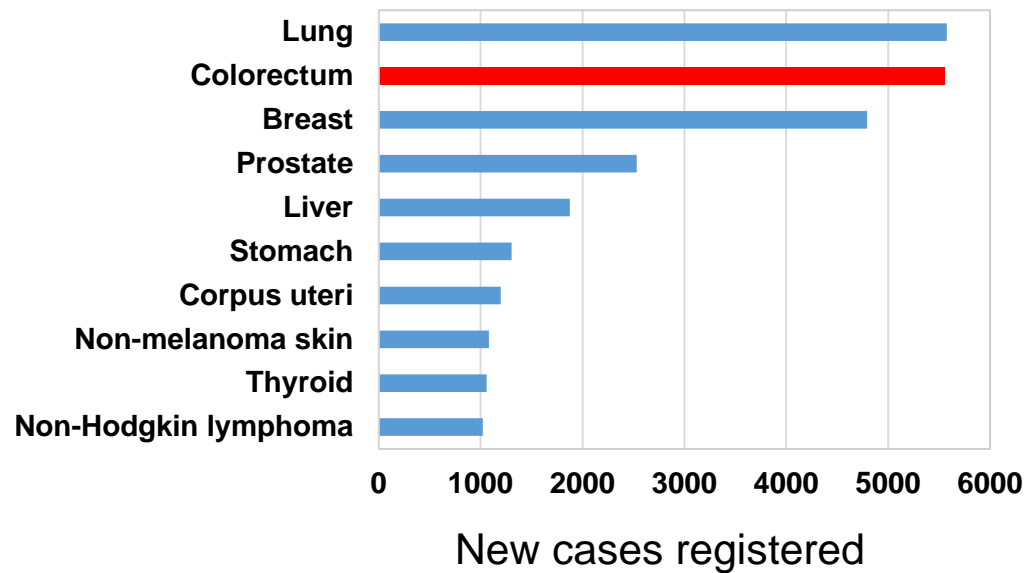
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Colorectal cancer (CRC) is a leading cancer globally

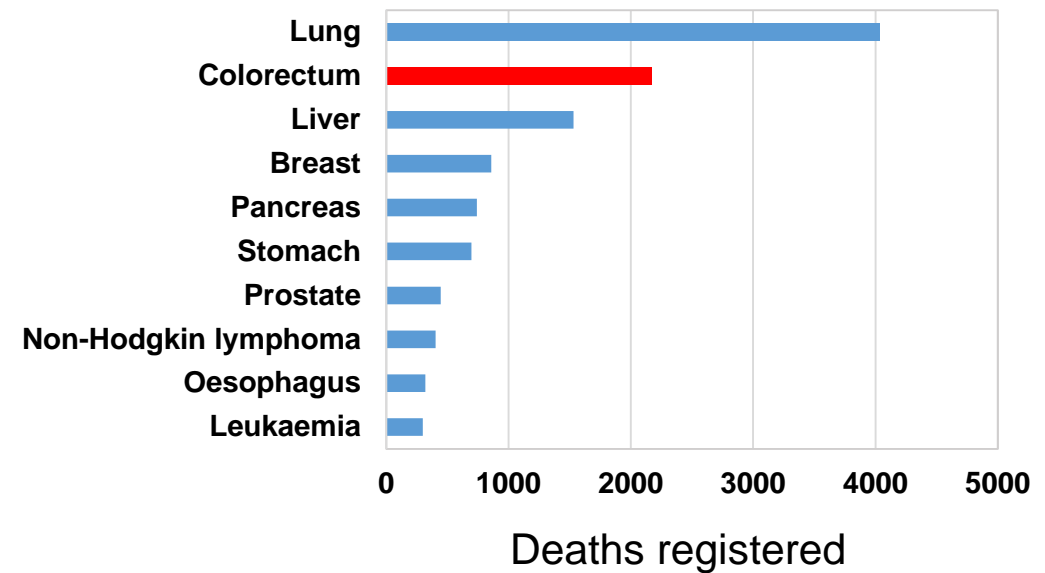
- **No.2 cancer and 2nd leading cause of cancer deaths in Hong Kong SAR**
- **2nd most common cause of cancer death in the USA**
- **3rd cancer in China and the incidence keeps rising**

Top 10 most common cancers 2019



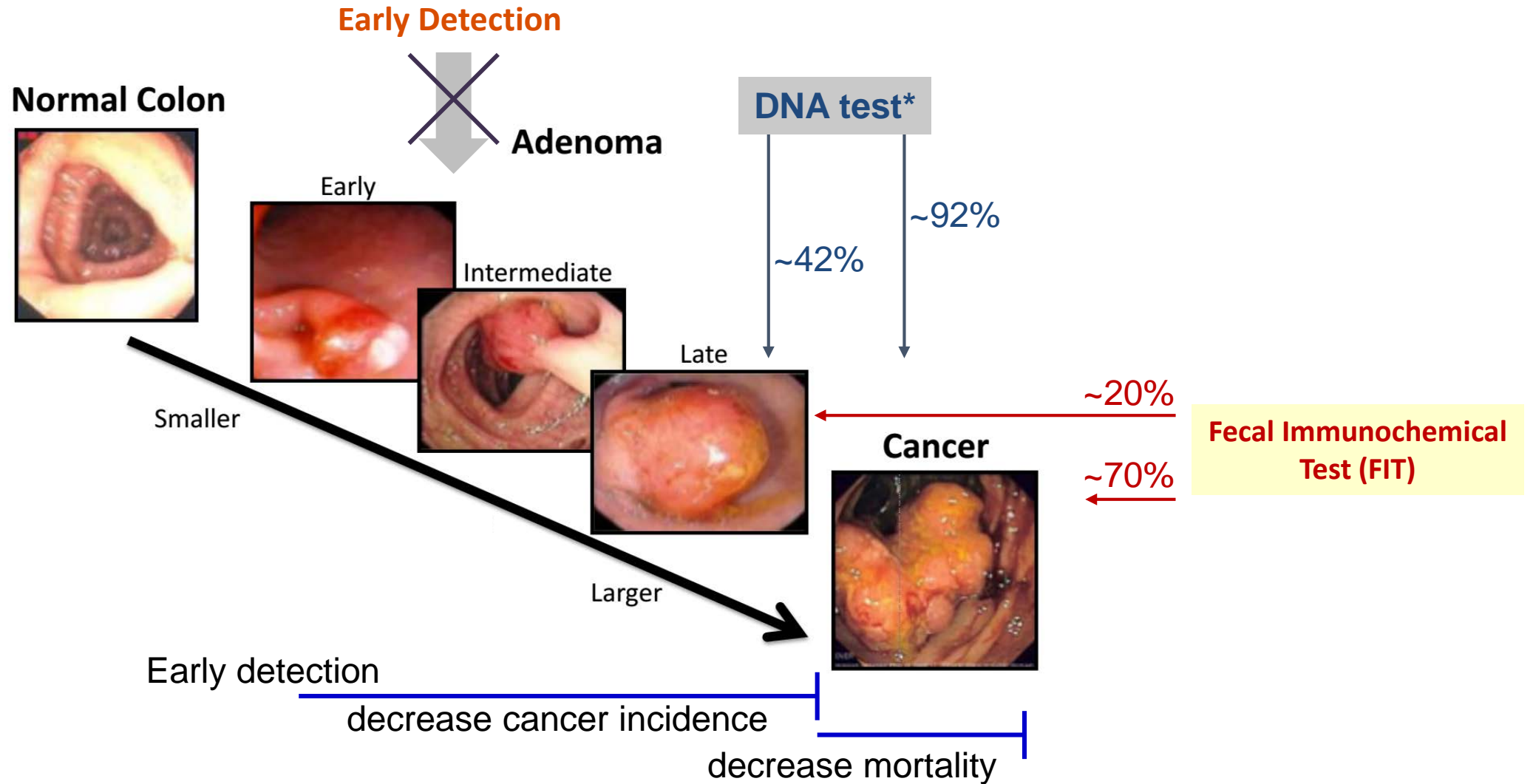
Data source: Hong Kong Cancer Registry, Hospital Authority

Top 10 causes of cancer deaths 2019



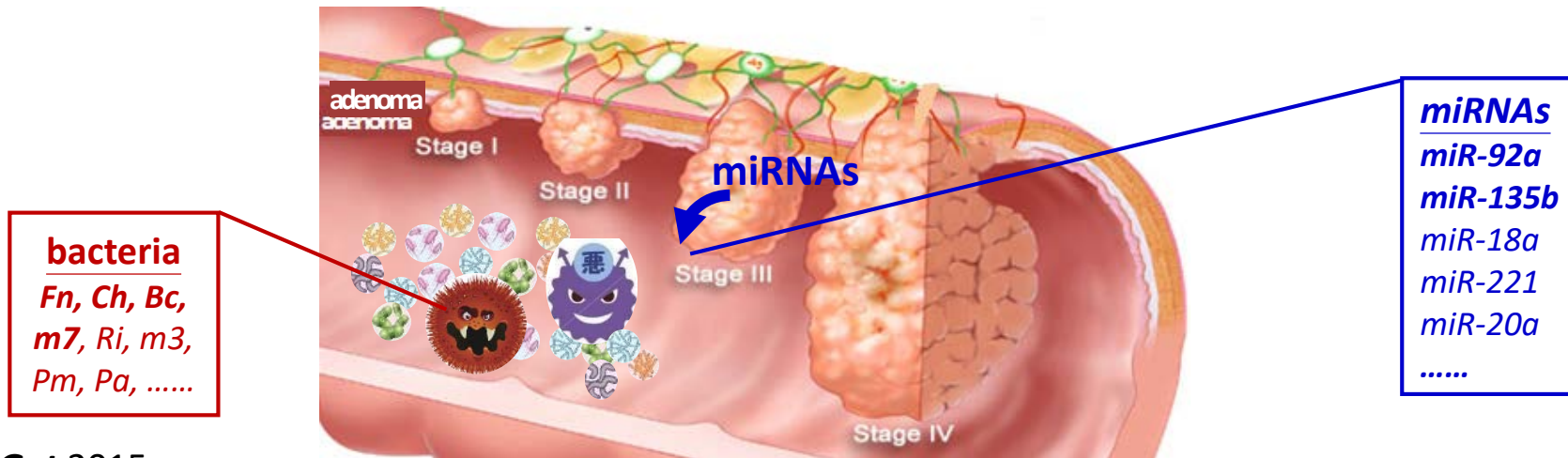
Siegel et al. CA Cancer J Clin. 2020
Sun et al. Cancer Biol Med. 2020

Colorectal neoplasm screening



Two types of fecal biomarkers

- Fecal bacterial markers - **environmental factors**
- Fecal miRNAs - **host cell biomarkers released into stools**

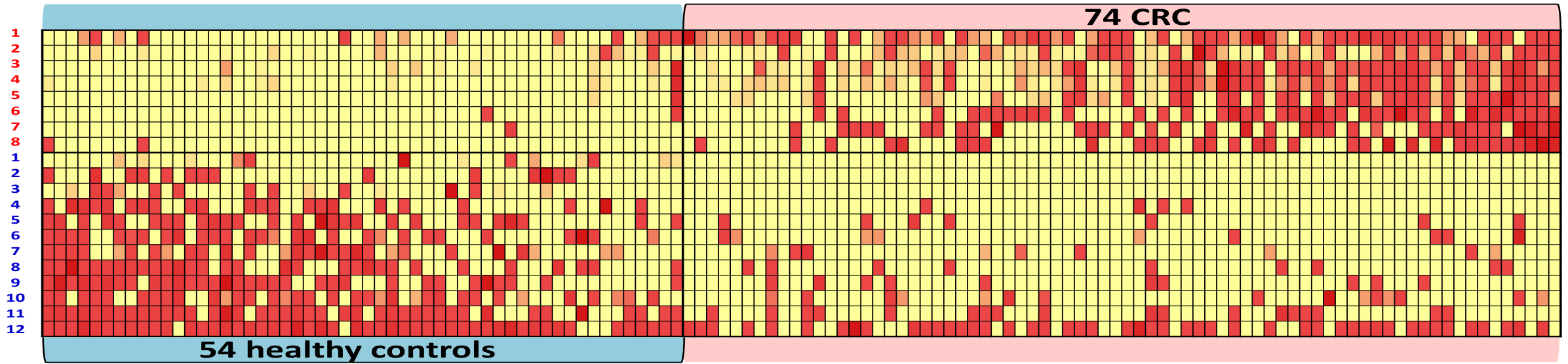


Gut 2015;
Clin Cancer Res 2017 ;
Gut 2020;
JGH 2021

- Proven good markers
- Stable, easy to detect

Gut 2012;
Clin Cancer Res 2014;
Plos One 2013;
Br J Cancer 2014;
Oncotarget 2016; etc

Our discovery: new bacterial markers for CRC



74 CRC vs 54 controls

8 enriched in CRC patients

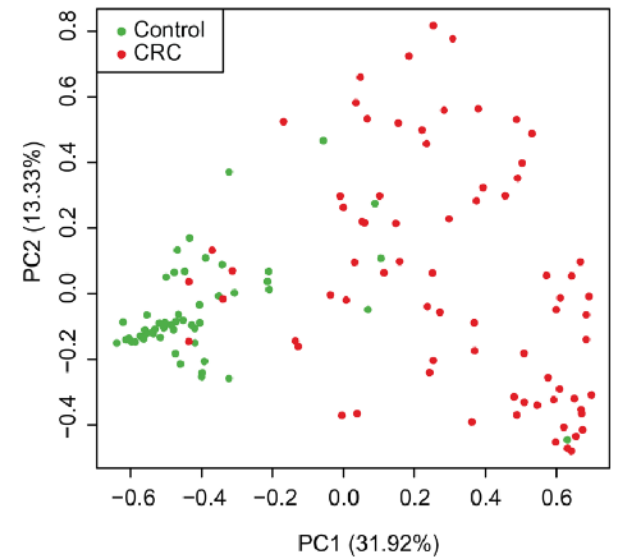
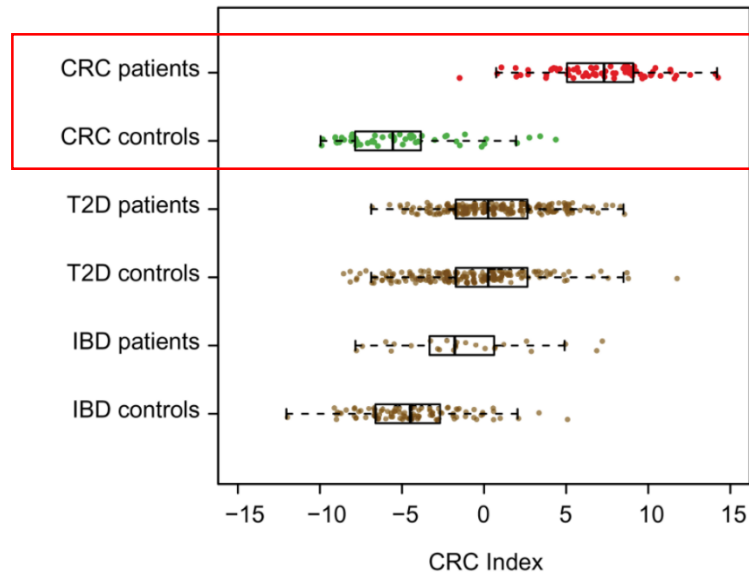
12 enriched in healthy subjects

$$I_j = \left[\frac{\sum_{i \in N} \log_{10}(A_{ij} + 10^{-20})}{|N|} - \frac{\sum_{i \in M} \log_{10}(A_{ij} + 10^{-20})}{|M|} \right]$$

(CRC_enriched) – (control_enriched)



CRC
specific



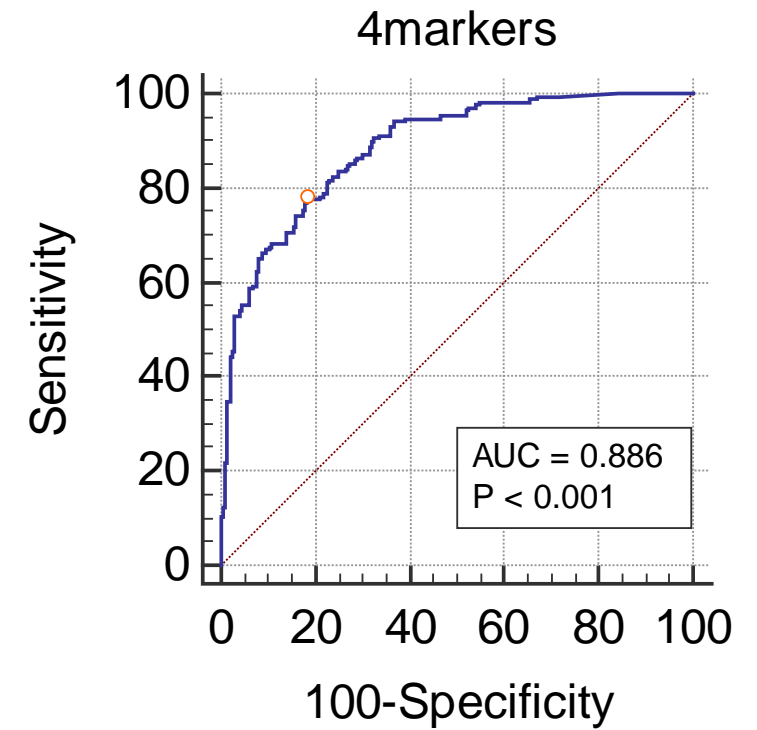
Fecal Bacteria for Non-Invasive Diagnosis of CRC

Fecal Bacteria Act as Novel Biomarkers for Non-Invasive Diagnosis of Colorectal Cancer.

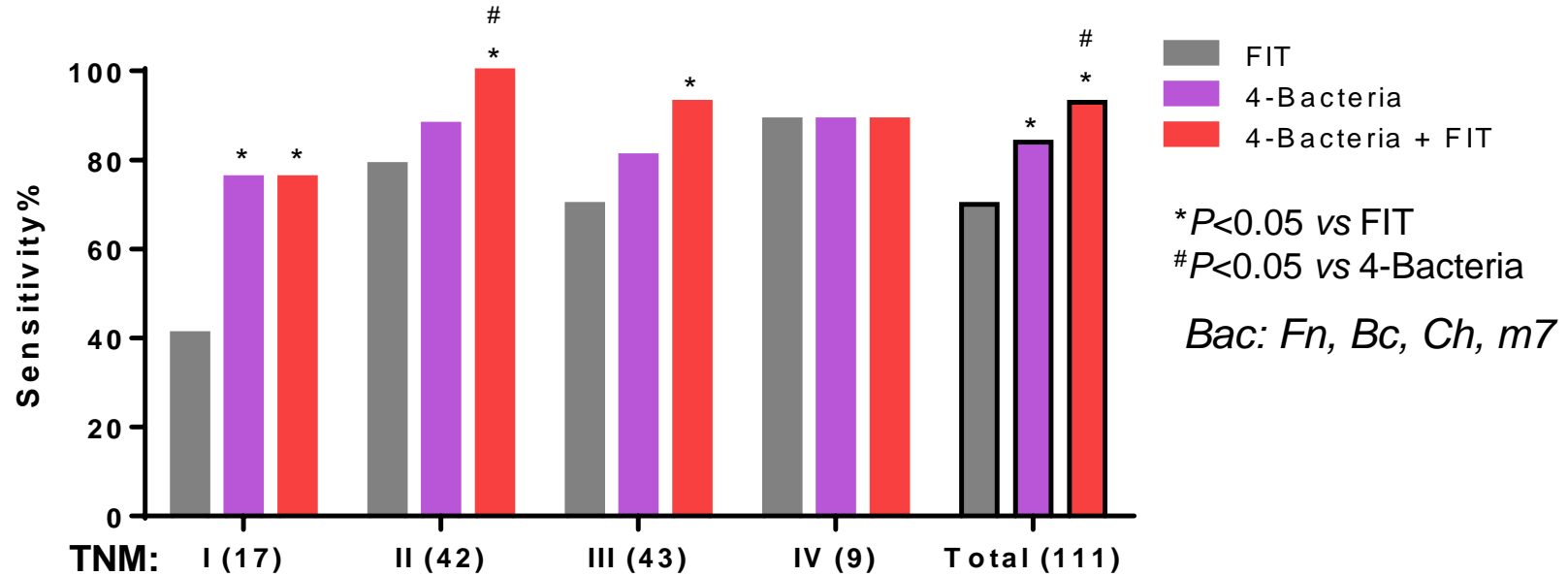
Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang JY, Brim H, Ashktorab H, Ng SC, Ng SS, Zheng S, Chan FK, Sung JJ, Yu J.

Clin Cancer Res. 2017 Apr 15;23:2061-70. doi: 10.1158/1078-0432.CCR-16-1599. Epub 2016 Oct 3.

Species	Label	Enrichment (metagenome)	Wilcoxon rank-sum test	
			P-value	q-value
<i>Fusobacterium nucleatum</i>	Fn	CRC	7.53E-08	2.61E-03
<i>Clostridium hathewayi</i>	Ch	CRC	5.71E-06	8.55E-03
in-house label 'm7'	m7	CRC	1.80E-07	3.24E-03
<i>Bacteroides clarus</i>	Bc	Control	2.64E-05	1.47E-02



Fecal Bacteria for Non-Invasive Diagnosis of CRC



For CRC	Bac+FIT	FIT
Sensitivity	92.8%	~70%
Specificity	81.5%	~95%



Article
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Citation
Tools



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Gut microbiota
Original article



A novel faecal *Lachnoclostridium* marker for the non-invasive diagnosis of colorectal adenoma and cancer

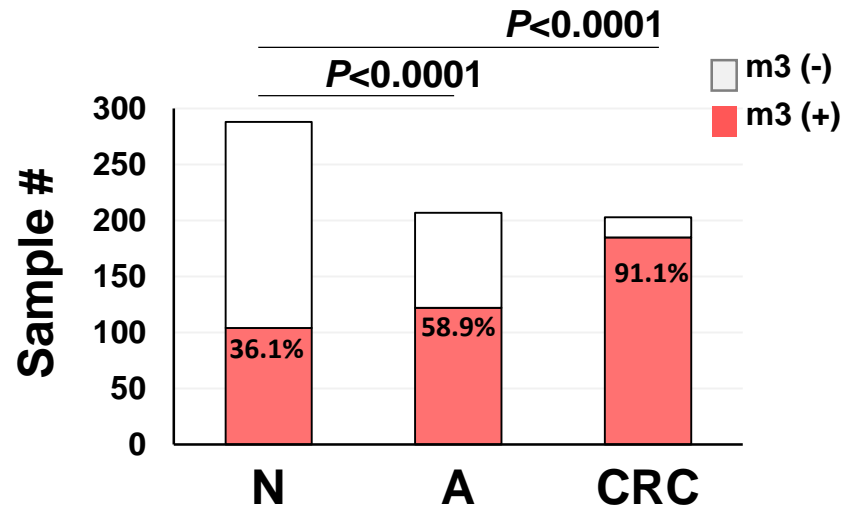
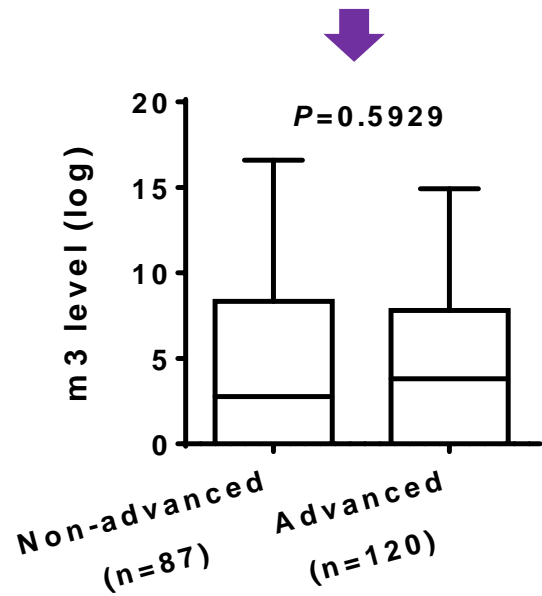
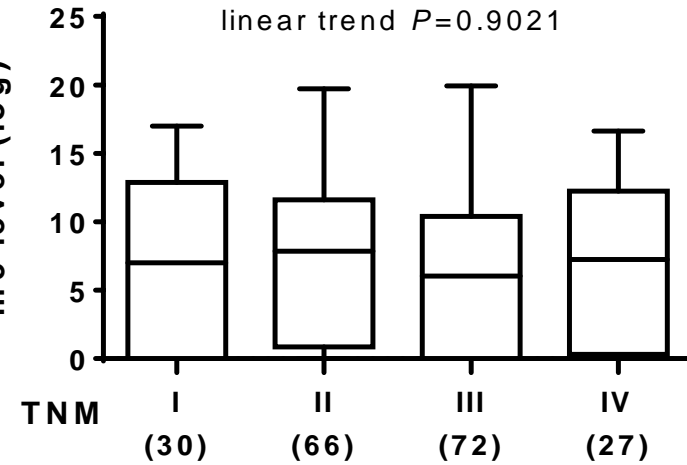
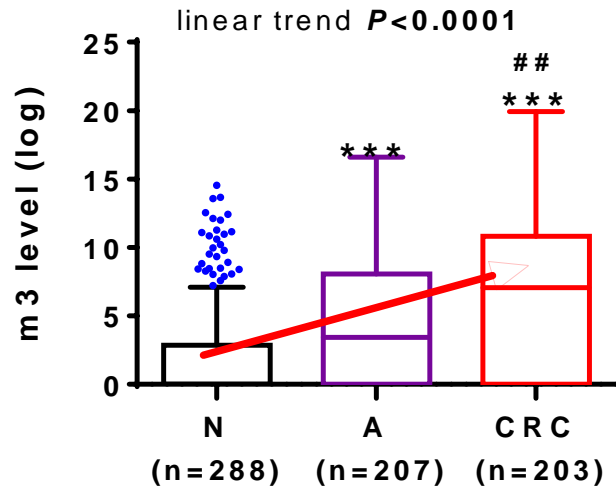
Jessie Qiaoyi Liang¹, Tong Li¹, Geicho Nakatsu¹, Ying-Xuan Chen², Tung On Yau¹, Eagle Chu¹, Sunny Wong¹, Chun Ho Szeto¹, Siew C Ng¹, Francis K L Chan¹, Jing-Yuan Fang², Joseph J Y Sung¹, Jun Yu¹

[Author affiliations](#)

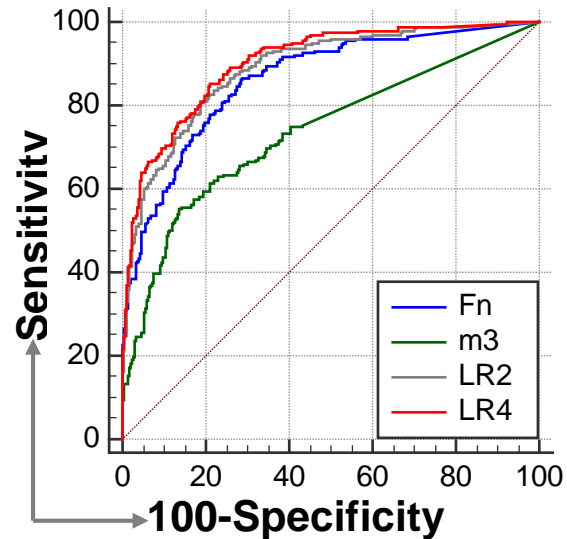
Abstract

Objective There is a need for early detection of colorectal cancer (CRC) at precancerous-stage adenoma. Here, we identified novel faecal bacterial markers for diagnosing adenoma.

Fecal level of 'm3' in CRC, adenoma and control subjects



A new panel for CRC detection



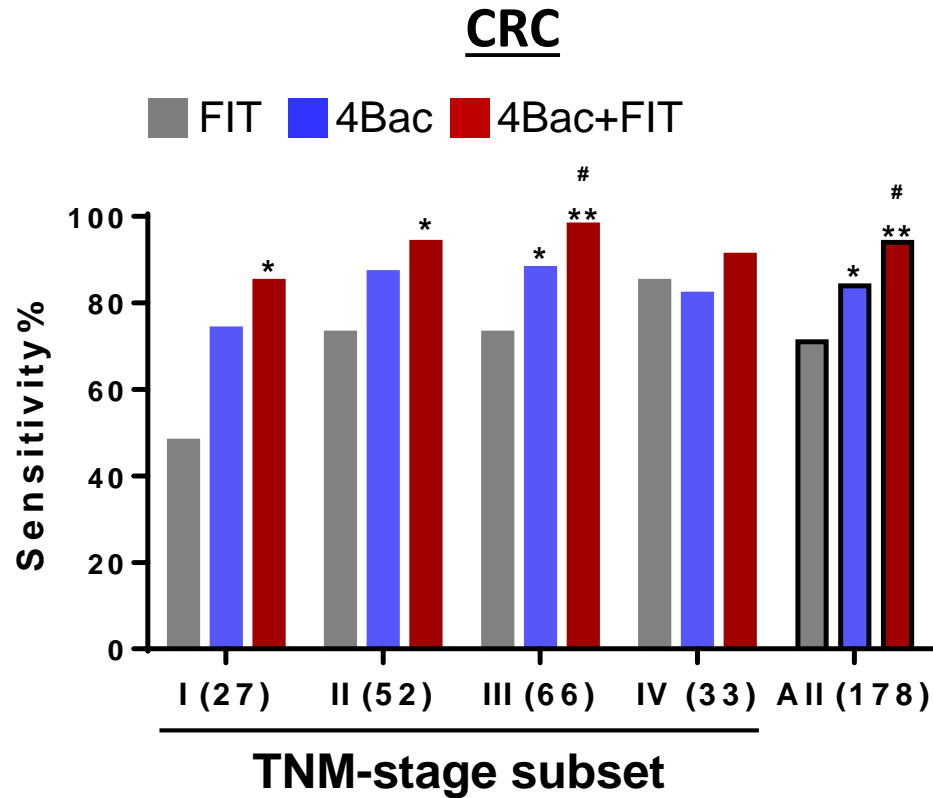
For CRC	AUC	SE	95% CI	Markers	P
Fn	0.863	0.0167	0.829 to 0.892	Fn	<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-left: 1px solid black; border-right: 1px solid black; height: 100px; margin-right: 5px;"></div> <div style="text-align: center; font-size: 8px;"> <p><0.0001</p> <p>0.006</p> <p>ns</p> <p>0.033</p> <p>ns</p> </div> </div>
m3	0.741	0.0227	0.699 to 0.779	m3	
LR2	0.891	0.0147	0.860 to 0.917	Fn,m3	
LR3	0.895	0.0144	0.865 to 0.921	Fn,m3,Bc	
LR4	0.907	0.0133	0.877 to 0.931	Fn,m3,Bc,Ch	
LR5	0.907	0.0133	0.877 to 0.931	Fn,m3,Bc,Ch,m7	

Panel I - 'Fn, Bc, Ch and m7' (AUC=0.892, 95% CI: 0.856 to 0.921) [*Clin Cancer Res* 2017]

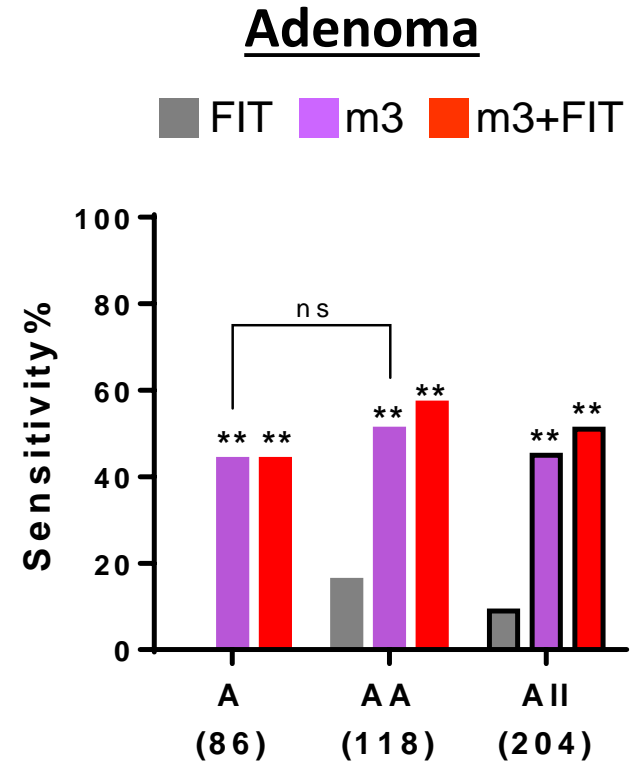


Panel II - 'Fn, Bc, Ch and m3' (AUC=0.907, 95% CI: 0.877 to 0.931) [*Gut* 2020]

Superior to FIT for CRC and adenoma



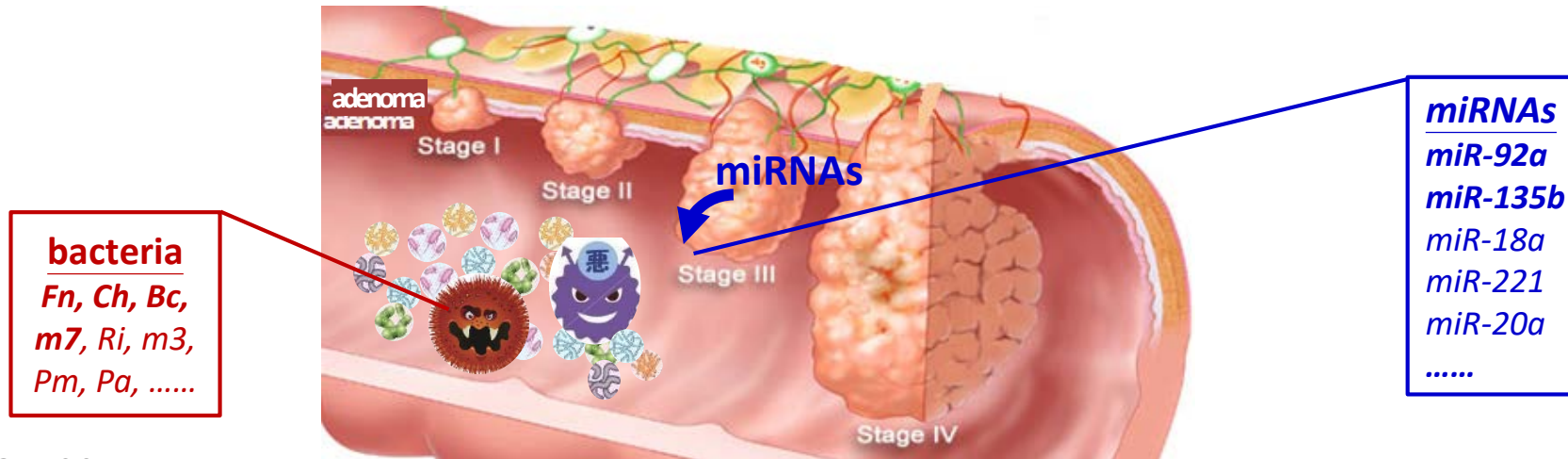
* $P < 0.05$ and ** $P < 0.001$ vs FIT
$P < 0.05$ and ## $P < 0.001$ vs 4Bac



** $P < 0.001$ vs FIT
A: non-advanced adenoma
AA: advanced adenoma

Two types of fecal biomarkers

- Fecal bacterial markers - environmental factors
- Fecal miRNAs - host cell biomarkers released into stools

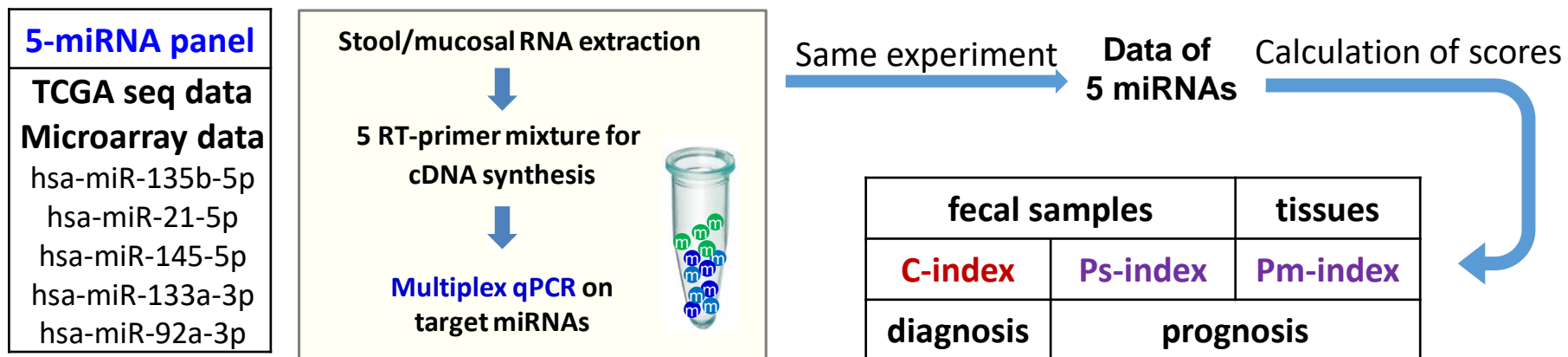


Gut 2015;
Clin Cancer Res 2017 ;
Gut 2020;
JGH 2021

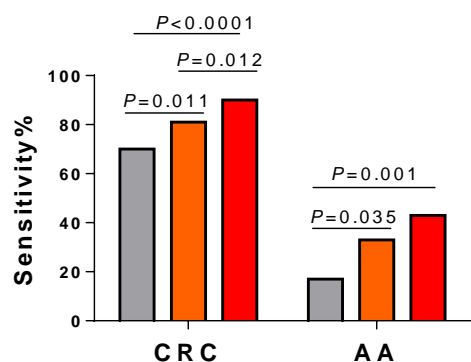
- Proven good markers
- Stable, easy to detect

Gut 2012;
Clin Cancer Res 2014;
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Oncotarget 2016; etc

A new miRNA panel for diagnosis and prognosis of CRC

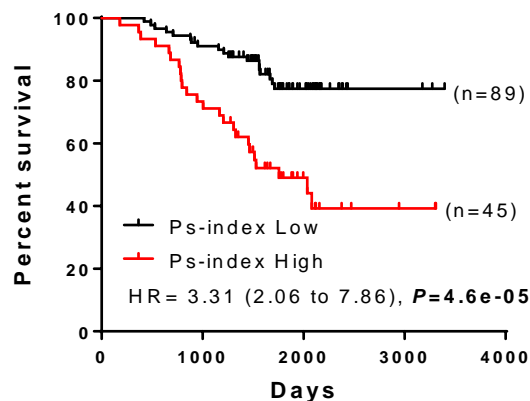


Diagnosis: C-index

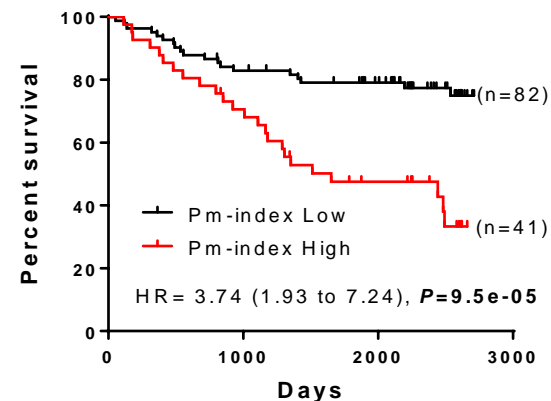


■ FIT ■ C-index ■ both
by Chi-square tests

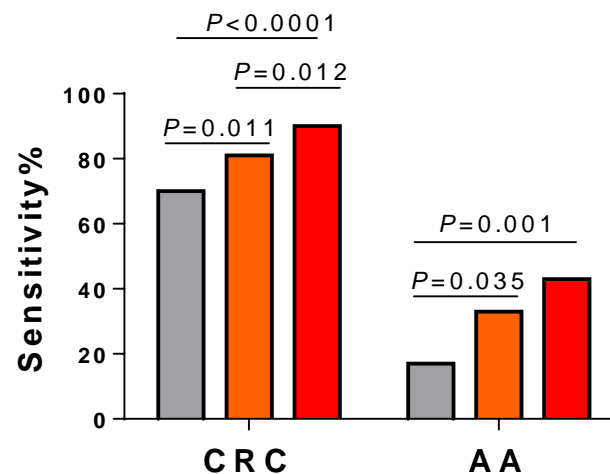
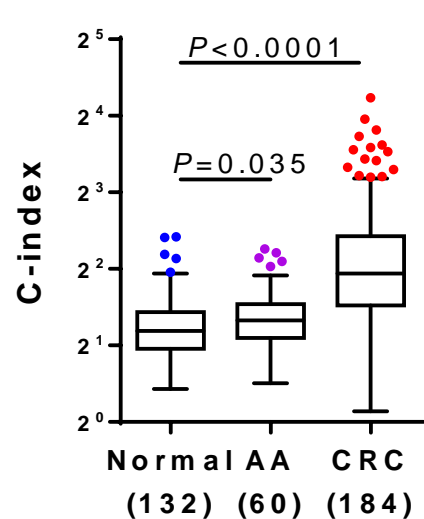
Prognosis: Ps-index



Prognosis: Pm-index



Comparison of C-index, FIT and their combination



■ FIT ■ C-index ■ both
by Chi-square tests

Cohort–Hong Kong (FIT)

CRC n=184

AA n=60

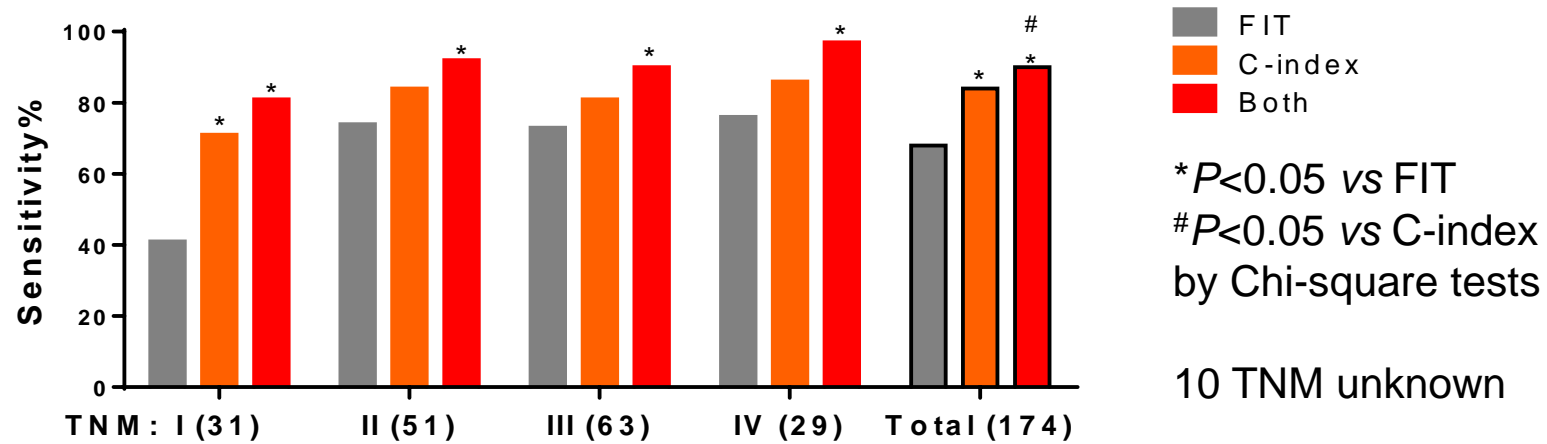
Normal n=132

Variables	CRC			AA		
	FIT	C-index	Both	FIT	C-index	Both
Sensitivity	70.1%	80.9%	90.2%	16.7%	33.3%	43.3%
Specificity	97.0%	80.3%	80.3%	97.0%	80.3%	80.3%

Combination with FIT increased the diagnostic performance of C-index for CRC and advanced adenoma.



C-index is more sensitive than FIT for CRC detection

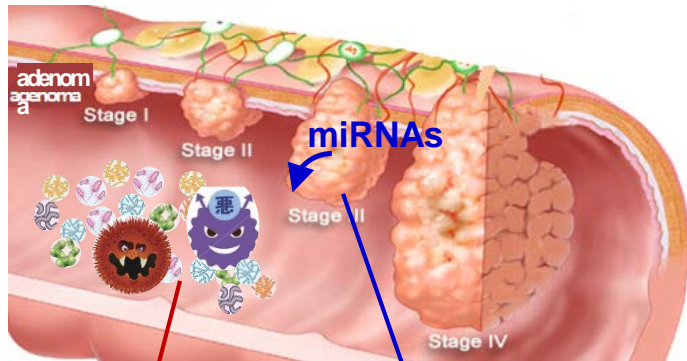


TNM stage	Sensitivity		
	FIT	C-index	Both
I	41.9%	71.0%	80.6%
II	74.5%	84.3%	92.2%
III	73.0%	81.0%	90.5%
IV	75.9%	86.2%	96.6%

Variable	FIT	C-index	Both
Sensitivity	68.4%	81.0%	90.2%
Specificity	97.0%	80.3%	80.3%
PPV	96.9%	85.1%	86.5%
NPV	69.2%	75.2%	85.5%



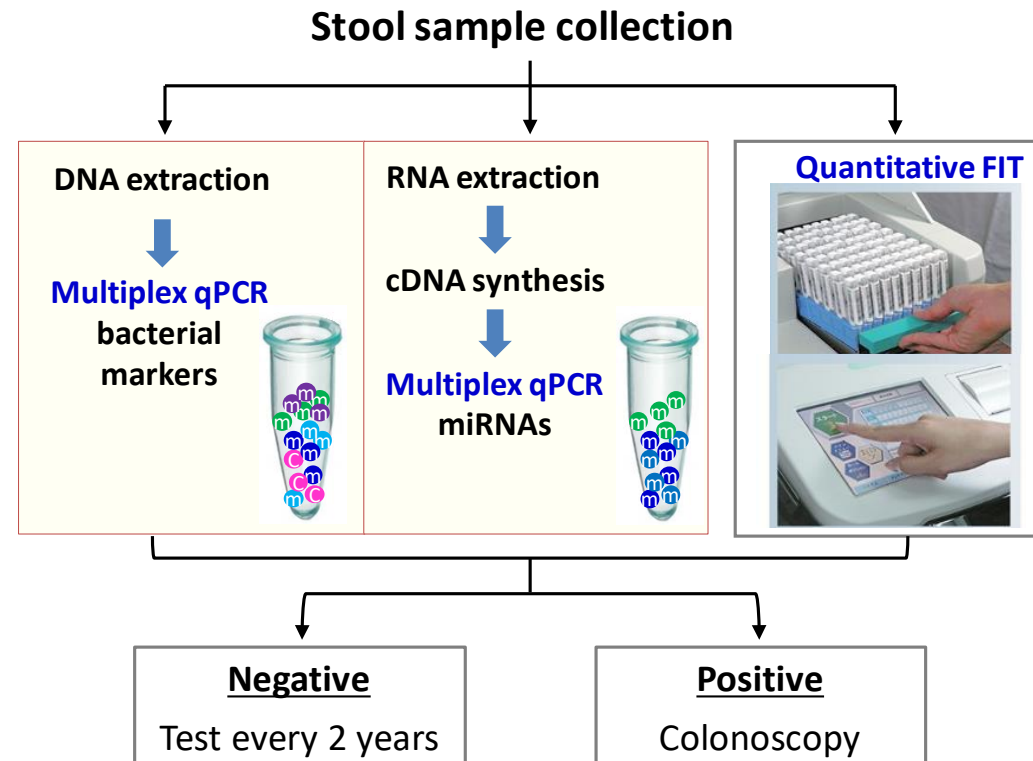
A new colorectal neoplasm screening method



Bacterial markers

miRNAs

New screening strategy



Statistical modeling

Statistical modeling of fecal biomarkers for CRC screening



The research team
co-developed Cologuard



Prof. John B. Kisiel
Mr. Douglas Mahoney

Research
team

Statistical modeling of our biomarkers

Statistical modeling methods:

- Logistic Regression-Lasso
- Multinomial Logistic Regression
- Random Forest classification
- others

cross-validation to avoid overfitting

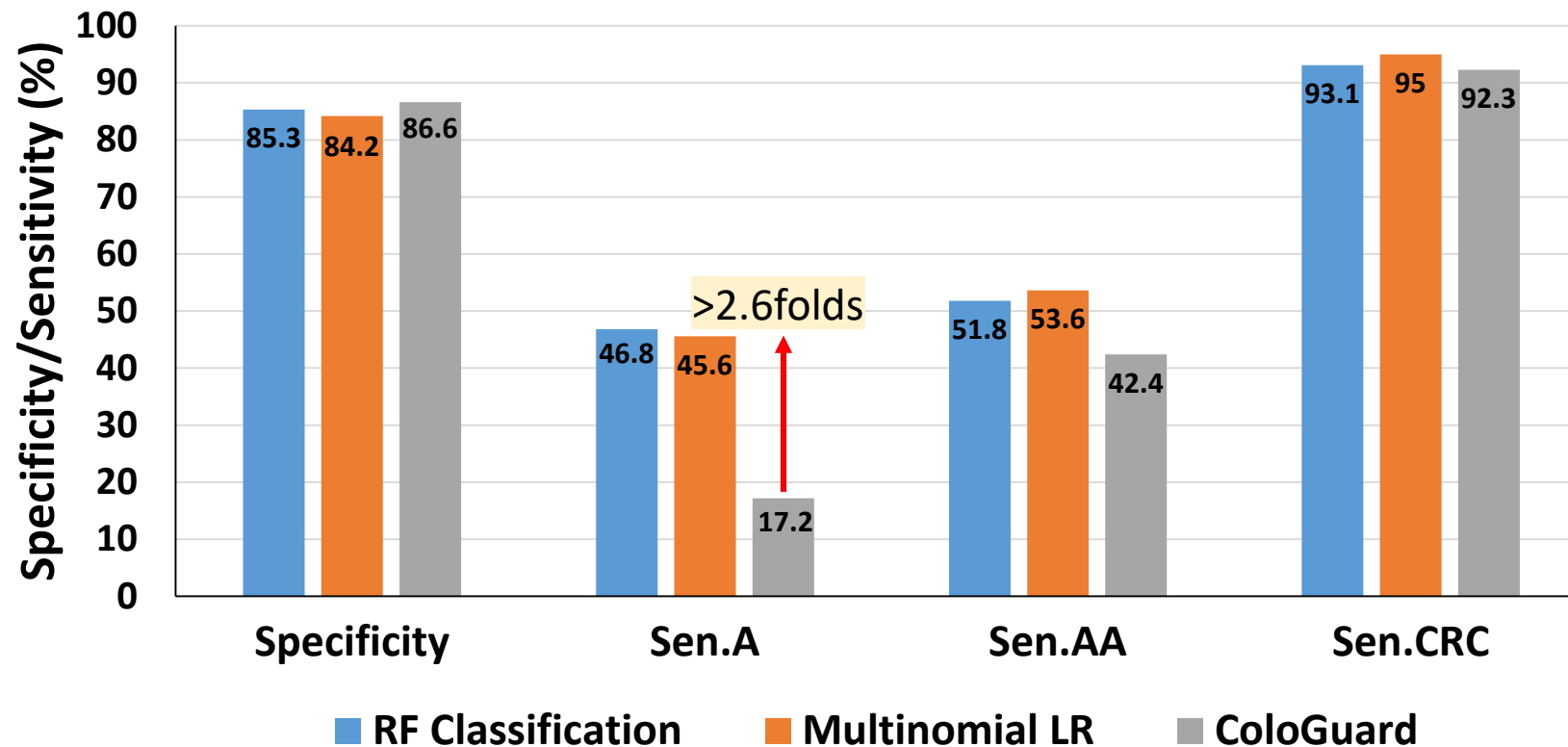
11 markers

Bacterial markers	Fecal miRNAs	Other
<i>Fn</i>	hsa-miR-135b-5p	FIT
<i>m3</i>	hsa-miR-21-5p	
<i>Ch</i>	hsa-miR-145-5p	
<i>Bc</i>	hsa-miR-133a-3p	
<i>m7</i>	hsa-miR-92a-3p	



Models combining bacterial markers, miRNAs and FIT

Models & performance	Specificity	Sen.A	Sen.AA	Sen.CRC
RF Classification (7)	85.3	46.8	51.8	93.1
Multinomial LR (9)	84.2	45.6	53.6	95.0



Research Outputs

Refereed Publication

- 1) **Liang JQ[#] (corresponding)**, Li T, Chen YX, Yau TO, Nakatsu G, Chu ESH, Wong S, Szeto CH, Ng SC, Chan FK, Fang JY, Sung JJ, Yu J[#]. A Novel Fecal Lachnoclostridium Marker for the Non-Invasive Diagnosis of Colorectal Adenoma and Cancer. *Gut* 2020 Jul;69(7):1248-1257. (IF2020: **23.059**)

Refereed conference papers

- 1) **Liang JQ**, Yau TO, Yau TCD, Szeto CH, Zhao FS, Chan FK, Sung JJ, Yu J. A novel microRNA panel for non-invasive diagnosis and prognosis of colorectal cancer. *Gastroenterology* 2019; 156(6), Suppl1, S-496. (IF2020: **22.682**)
- 2) **Liang JQ**, Wong SH, Szeto CH, Chu ESH, Lau H, Yu J, Sung JJ. Fecal microbial markers in colorectal cancer screening for symptomatic and asymptomatic subjects. *Gastroenterology* 2019; 156(6), Suppl1, S-600. (IF2020: **22.682**)
- 3) **Liang JQ**, Chan FK, Sung JJ, Yu J. Fecal Bacteria Act as Novel Biomarkers for Non-Invasive Diagnosis of Colorectal Neoplasm. *Digestion* 2019;99(1):104. (IF2020: **3.216**)
- 4) **Liang Q**, Chen YX, Wong S, Kwong TNY, Fang JY, Ng SC, Chan FK, Sung JJ, Yu J. A novel fecal bacterial marker for the non-invasive diagnosis of colorectal adenoma. *Gastroenterology* 2018; 154(6), Suppl1, S-110. (IF2020: **22.682**)

Patents

MicroRNA markers and quantification method for diagnosis and prognosis of colorectal neoplasm.

- 1) US patent. (US Provisional Application No. 62/816,724; Filing date: 11 Mar 2019)
- 2) PCT. (Application No. PCT/CN2020/078497; Filing date: 10 Mar 2020)
- 3) China patent. (Application No. TBA; Filing date: Apr 2020)
- 4) Hong Kong patent. (Application No. TBA; Filing date: Apr 2020)

Awards

- 1) Poster of Distinction, Digestive Disease Week 2019, San Diego, USA 05/2019
- 2) Scientific Accomplishment as an Early Career Investigator, American Gastroenterological Association, 05/2018

Acknowledgements

- **The Chinese University of Hong Kong**

Prof Jun Yu

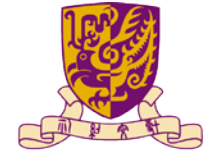
Prof Francis KL Chan

Ms Yao Zeng, Mr Payton Yau, Mr Jonathan Chiu

Prof Joseph JY Sung

Prof Siew Ng

Ms Effie Lau, Mr Yao Huang, Ms Dorothy Yau, others



香港中文大學
The Chinese University of Hong Kong



香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

- **Collaborators**

- **Prof. John B. Kisiel and Mr. Douglas Mahoney (Mayo Clinic, Rochester, USA)**
- Prof. Shu Zheng and Dr Yanqing Huang (Zhejiang University, Hangzhou, China)
- Profs. Jingyuan Fang and Yingxuan Chen (Shanghai Jiaotong University, Shanghai, China)
- Profs. Hassan Ashktorab and Hassan Brim (Howard University, Washington, USA)

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Thank You

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