Out of Africa: developing automated assays for early detection and monitoring of disease burden in breast, cervical and prostate cancer

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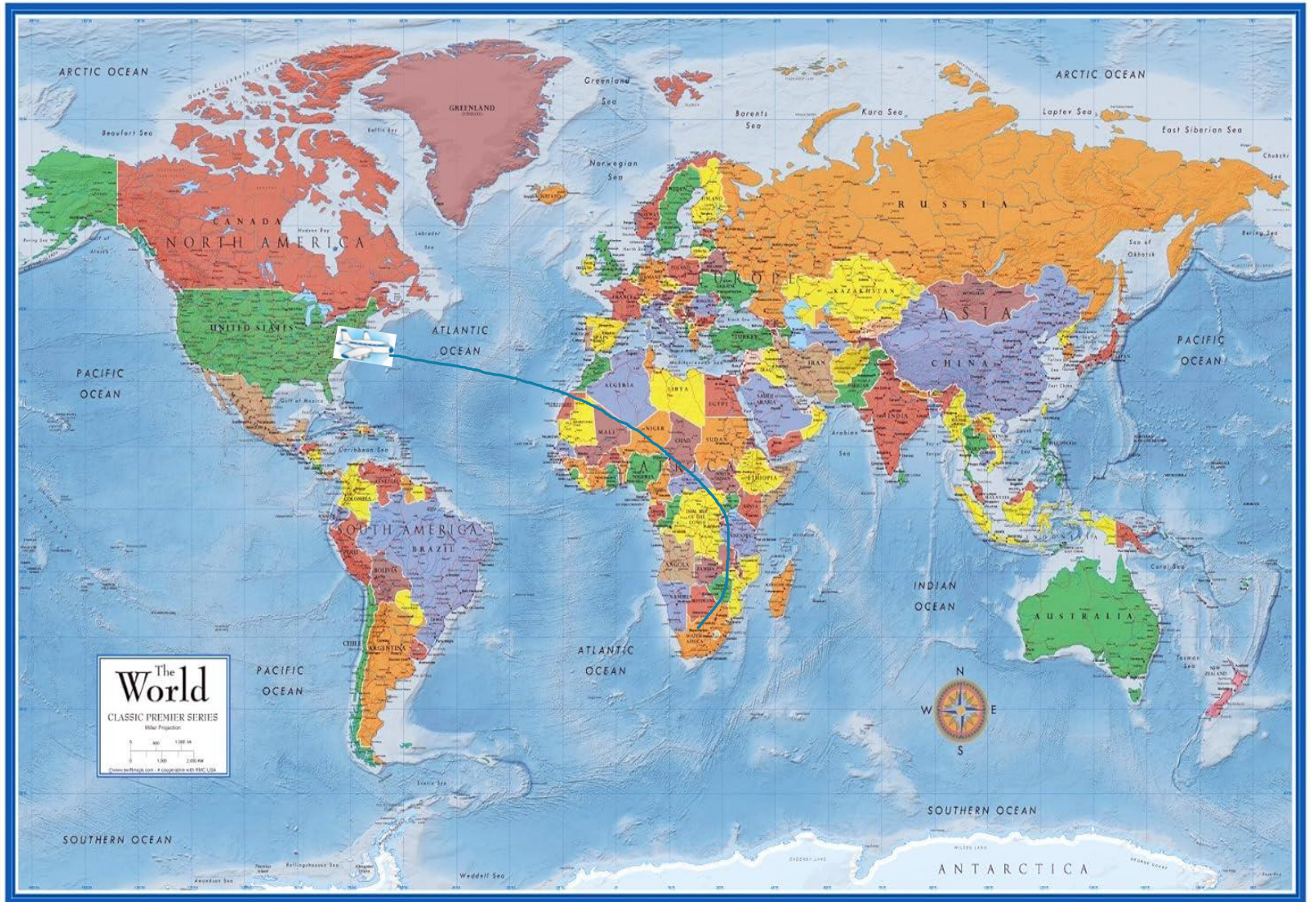
Disclosures

- **Competing interests:**
- Part of this research was funded by Cepheid through a research agreement with JH/Dr. Sukumar.
- **NON FDA Approved use of drugs or products referenced in this presentation:** The Cepheid GeneXpert® cartridge for the Breast Cancer Monitoring Assay (BCMA) is for Research Use Only, and is not FDA approved, and has not been reviewed by any regulatory body

Saraswati Sukumar, Ph. D

South Africa-our intro to CHBAH

Susan Harvey, M.D.
Breast Radiology, JH



Dr. Maureen Joffe, PhD,
WITS Foundation

Superwoman

South Africa-CHBAH

- Gauteng Province -~25 million – most populous and poor province
- 10,000 patients/day at CHBAH
- Nearly 2-300 breast cases/clinic day each week

Soweto –a periurban population in transition

What is the excitement about?

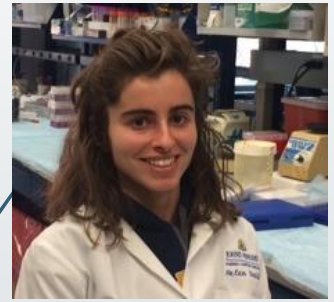
Findings from 1990-2016

1. Hypermethylated genes are commonly observed in breast cancer
2. Hypermethylated genes are potent markers of the presence of disease

Cancer Res 1999, Lancet Oncology 2000, Clin Cancer Res. 2006, 2008, Cancer Res, 2011, 2014, JCO 2017, CRC 2022, Clin CR 2023, Clin Epi 2024

Our team…

Bradley Downs, Ph.D



Claudia Mercado Rodriguez, MS

Mary Jo Fackler, Ph.D

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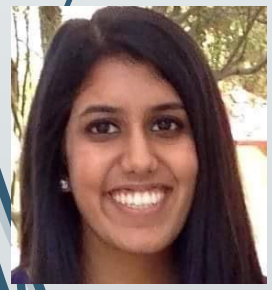
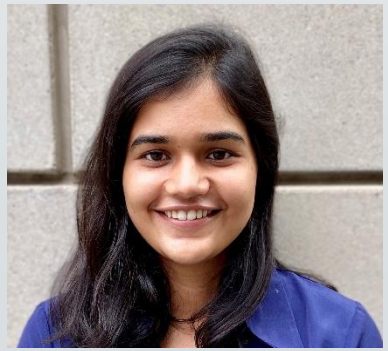
Romy Klein-Kranenbarg, MD



Rupali Sood, BS
(MD, 2019)

Dani Meir-Levi
(MD, 2023)

Madison Pleas, BS



Anu Soni, MBBS

Sara Sukumar

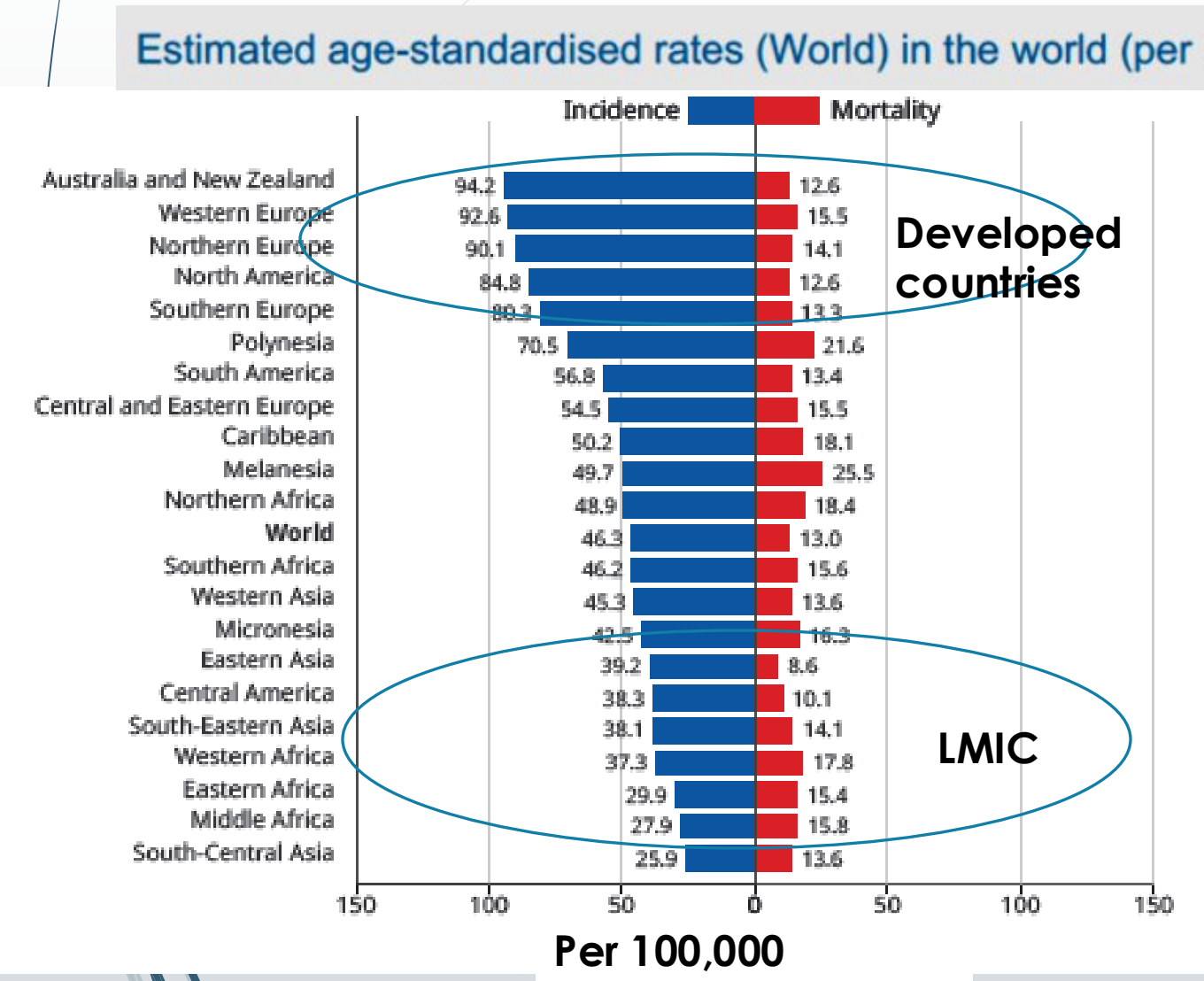
Why go global?

The Magnitude of the Problem

- Global Burden of Breast Cancer is Increasing
  - **Breast cancer is the second most common cancer in the world.**
  - **Affects women everywhere**

Globocan 2022 estimates there were nearly 2.3 million new breast cancer cases detected worldwide in 2022, *with 665,000 deaths due to metastatic breast cancer* (https://doi.org/10.3322/caac.21834).

Problem: BC outcome is different in low and middle income countries



Incidence (blue bars) of breast cancer is far higher in the developed countries than in LMIC.

In contrast, mortality (red bars) is very similar for both LMIC and developed countries

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018). CA Cancer J Clin. 68(6):394–424.

Reasons For Disparity

- **Disparity in timely detection and care is responsible for most of the cancer deaths in underdeveloped countries.**

  Ref: Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. Soerjomataram I et al, Lancet. 2012;380:1840–1850

- **Lack of screening, minimal hospital facilities, delayed diagnosis-few pathologists per population**

  Ref: Oncologic Care and Pathology Resources in Africa: Survey and Recommendations Ann M. Nelson, Danny A. Milner, Timothy R. Rebbeck, and Yawale Iliyas, J Clin Oncol 34:20-26. 2015

Fact: Mammography is impractical due to costs and difficulty in implementation

The Problem

Low- and middle-income countries (LMICs) suffer 50% of the total death burden due to breast cancer. Why?

In most developing countries, population mammographic screening is not accessible and women present at clinic with palpable breast lesions.

Delays in referral to tertiary hospitals occur; there are insufficient number of pathologists to provide timely diagnosis.

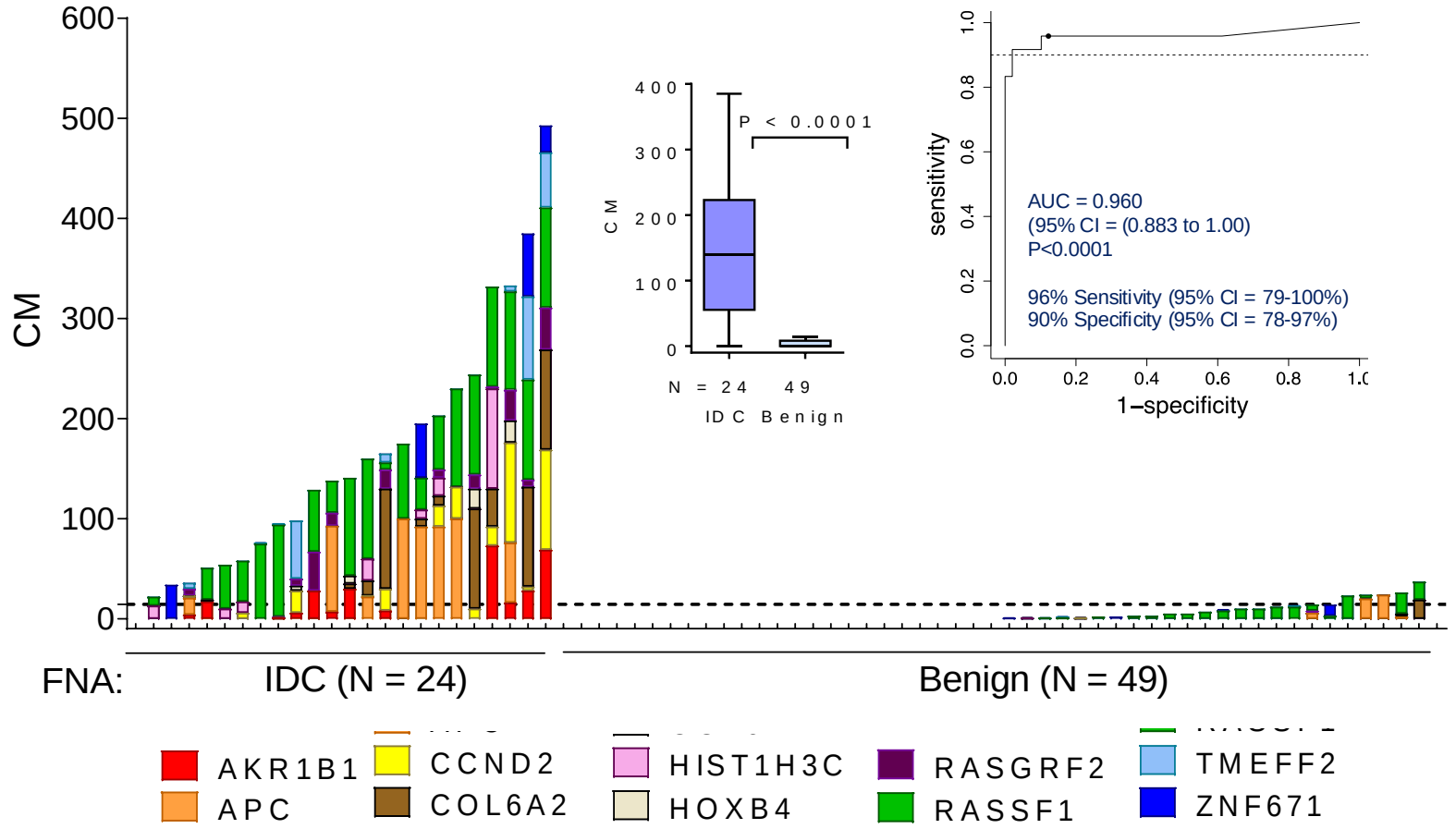
There is an urgent need for innovative solutions to prioritize high-risk lesions for pathology review since most lesions are benign and can be managed in primary care settings.

(Globocan 2020).

Our vision

- Test cells from the suspicious lesion with a fast, automated cancer detection assay to provide accurate identification of the lesion as malignant or benign
- **End Goal: The test should enable rapid prioritization of patients with suspected cancer for further pathological evaluation and care at a regional care center**

Pilot Study- Archival Breast Fine Needle Aspirates Of Suspicious Breast Lesions



Downs et al, Clin Cancer Res 2019, 25, 6357-

Fernando Schmitt, (Porto Univ.), and Gary Tse (Chinese Univ HK)

Step 1 Conclusions- Discovery, Training And Testing

- We identified a 10-gene panel that distinguishes between benign vs. malignant breast disease with high sensitivity and specificity with AUC (area under the curve) of 0.95 in samples from China, Africa and America
- The same panel of methylated genes performs equally well in three different ethnic populations and all different IHC subtypes of tumors
- The assay displays high performance characteristics in a pilot study of archival FNA samples

Cepheid GeneXpert® System
DNA Detection-Cartridge* Workflow

FNA from palpable breast lesions on slides

Add scraping of FNA to Lysis Reagent vial, add PK Vortex to mix

Precipitate DNA with Ethanol

Convert DNA template with sodium bisulfite

Insert cartridge (2 hr)

Transfer template DNA to 1 GX Methylation cartridges

Insert cartridges (1.5hr)

*For Research Use Only. Not for use in diagnostic procedures. Assay made available to investigators under a sponsored research agreement. Not reviewed by any regulatory body.

The goal of our current research

Develop an inexpensive, single-cartridge automated assay

Funded by NIH RO1-Academic- Industrial partnership, 2022-27
Teams: Hopkins, Cepheid, and WITS Foundation, Johannesburg

Cepheid GeneXpert® System
DNA Detection-Cartridge* Workflow

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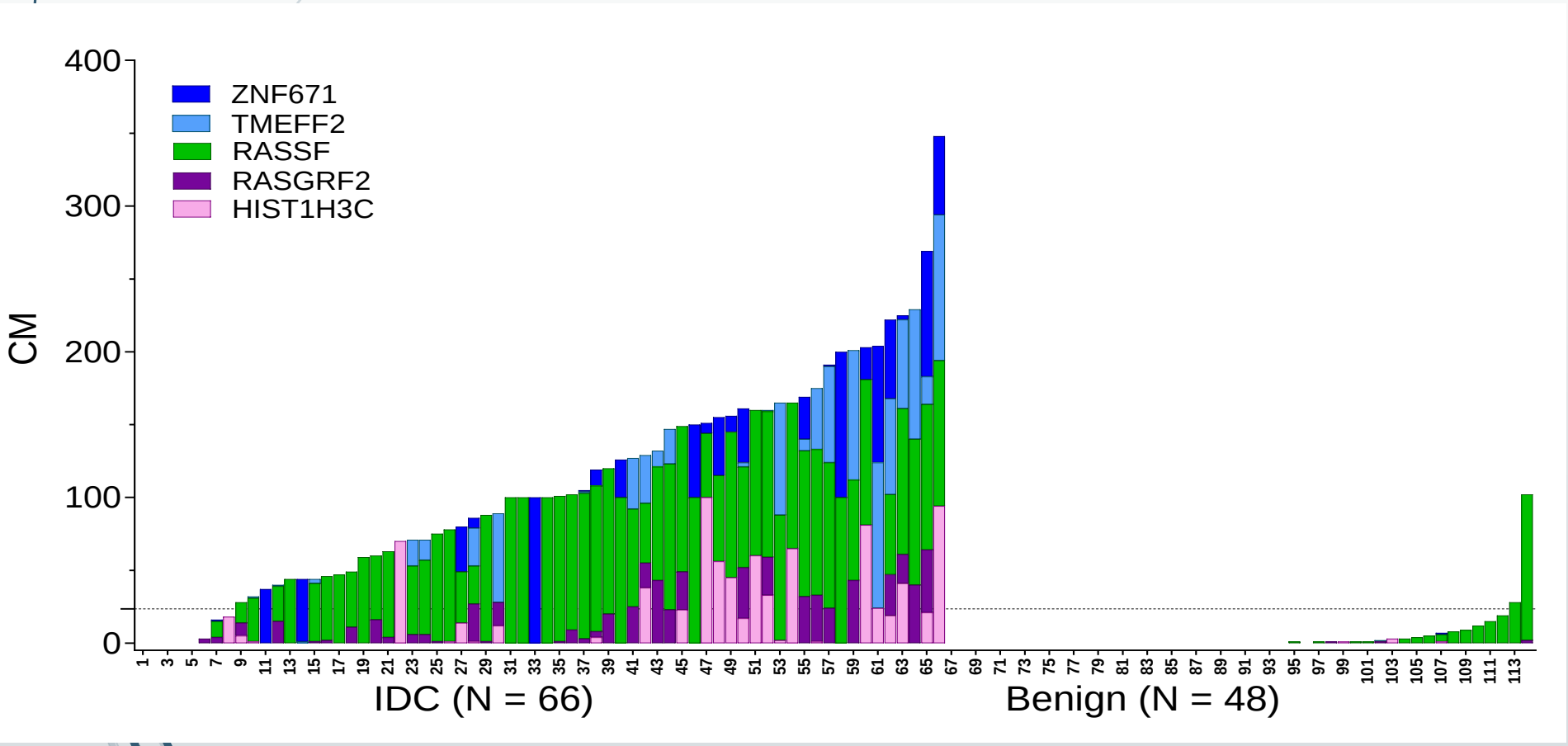
Analytical validation of the new panel of 5 genes in SA study FNA samples

Total N= 114 patient FNA analyzed in Breast Cancer Detection cartridge



Sensitivity: 91%
Specificity: 94%
Accuracy: AUC: 0.931
Threshold for normal :15.5 CM units

Fackler, Pleas, Joffee, Chen, Michelow, …Sukumar, unpublished

Testing the Clinical Utility

- **A prospective case/control clinical study** has been initiated at Chris Hani Baragwanath Hospital (BARA) (PI: Dr. Nivashini Murugan)
- Technical validation: N= 130
- **Validation Study: N=560**, FNA of 560 women with palpable lesions. Distribution: 60% malignant, 40 % benign

Rapid accrual

Total to date- 517 enrolled in the study within 24 months

Advantages of the GeneXpert* cartridge system

- The simplified assay, data analysis and interpretation are automated, leaving little margin for operator-errors.
- This newly developed methylation cartridge* could be easily adapted for detection of other cancers.
- A positive assay will lead to expedited pathology, and rapid treatment.

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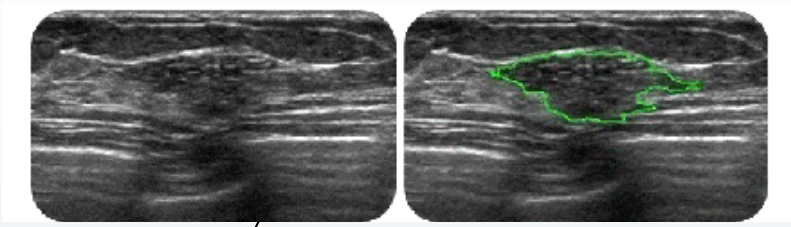
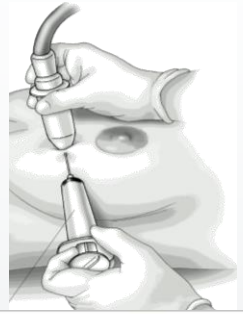
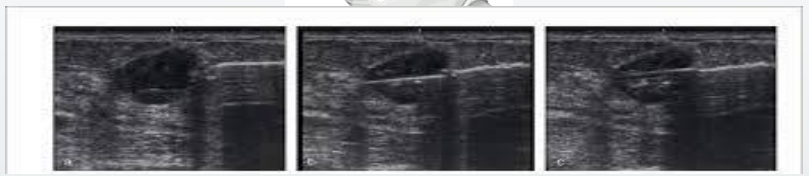
Our Vision : Fast Detection of Breast Cancer in Screening Centers in Developing Countries

Step 1: Breast Screening- Ultrasound+AI



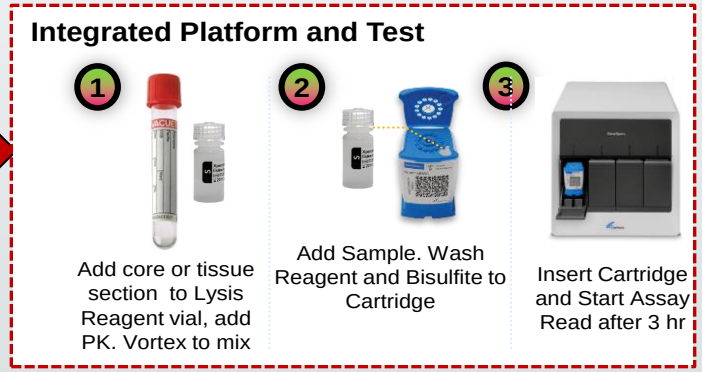


Step 2: Ultrasound- guided FNA of suspicious lesion



Step 3: Molecular BC Assay

FNA



Step 4: Pathology Lab

Step 5: Conventional therapy (surgery, chemo/tam)

"+" Expedited review by pathologist

"+" Expedited treatment

Assay-based Prioritization

Molecular Subtyping, 1.5 h

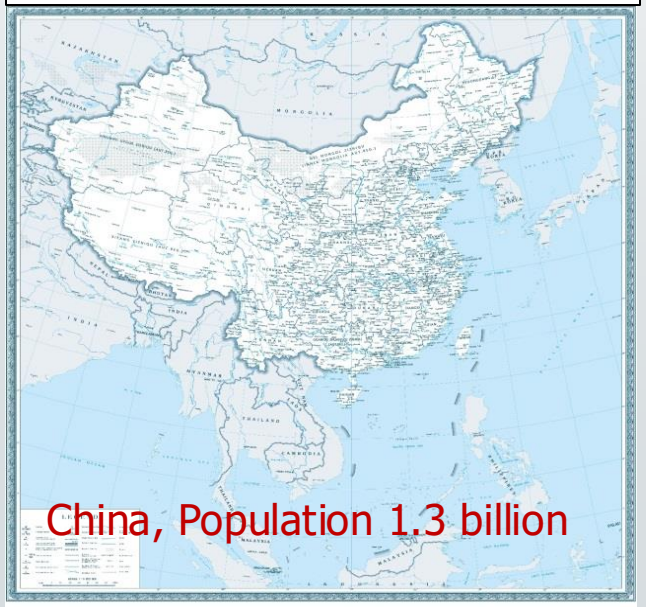
- **ER**
- **PR**
- **HER2**
- **Ki67**

*For Research Use Only. Not for use in diagnostic procedures. Cartridges made available to investigators under a sponsored research agreement. Not reviewed by any regulatory body.

Why will this approach succeed?

- CEPHEID has >25,000 instruments in centers throughout the world including Africa and India for TB and other infectious disease testing
- Mini battery operated units are available

S. Africa, Population: 50.9 million



IMPACT

- Millions of women will be screened by ultrasound
- **Automated diagnosis and subtyping will be performed within hours of arrival at the clinic.**
- Treatment would start rapidly. For example, women with ER+ breast cancer can be administered oral tamoxifen pills. This act alone will extend life for more than 5 years in nearly 70% of the women with breast cancer.
- Screening and timely diagnosis is key to reducing mortality due to breast cancer

Cancer-specific DNA methylation provides powerful CANCER detection markers

New Cases and Deaths for Common Cancers in 2020

CANCER SITE	NO. OF NEW CASES (% all SITES)	NO. OF NEW DEATHS (% all SITES)
Lung	2,206,771 (11.4)	1,796,144 (18.0)
Prostate	1,414,259 (7.3)	375,304 (3.8)
Colon	1,148,515 (6.0)	576,858 (5.8)
Rectum	732,210 (3.8)	339,022 (3.4)
Cervix uteri	604,127 (3.1)	341,831 (3.4)

CA CANCER J CLIN 2021;71:209–249

Cervical Cancer

Fackler MJ, Pleas M, Li Y, Soni A, Xing D, Cope L, Ali S, Van Le Q, Van Nguyen C, Pham HT, Duong LM, Vanden Berg E, Wadee R, Michelow P, Chen WC, Joffe M, Fjeldbo CS, Lyng H, Sukumar S. **Discovery and technical validation of high-performance methylated DNA markers for the detection of cervical lesions at risk of malignant progression in low- and middle-income countries.** Clin Epigenetics. 2024 Apr 20;16(1):56. doi: 10.1186/s13148-024-01669-z. PMID: 38643219; PMCID: PMC11032610.

What is the need for a molecular test for cervical cancer?

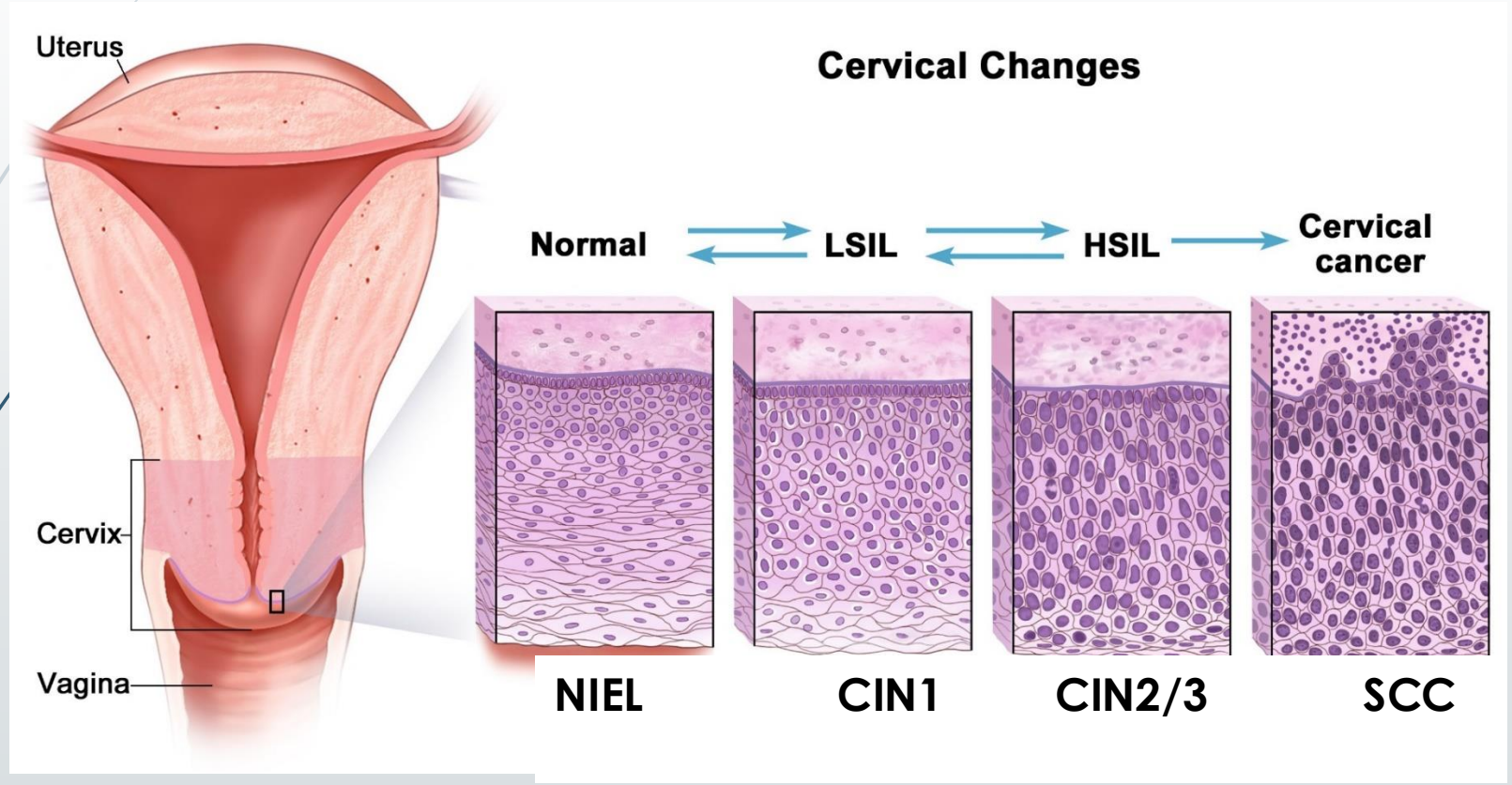
The WHO recommends HPV testing for cervical cancer screening.

However, most HPV-positive women do not develop cervical cancer in their lifetime. Yet, they all undergo colposcopy and oftentimes unnecessary biopsies

Concern is for the small percentage who have chronic HPV infection, who progress to invasive cervical cancer.

We need an objective test for detecting cervical cancer and for distinguishing between women with high risk cervical lesions who need to be treated, and those with low risk lesions who do not.

Cervical Cancer- Stages of progression

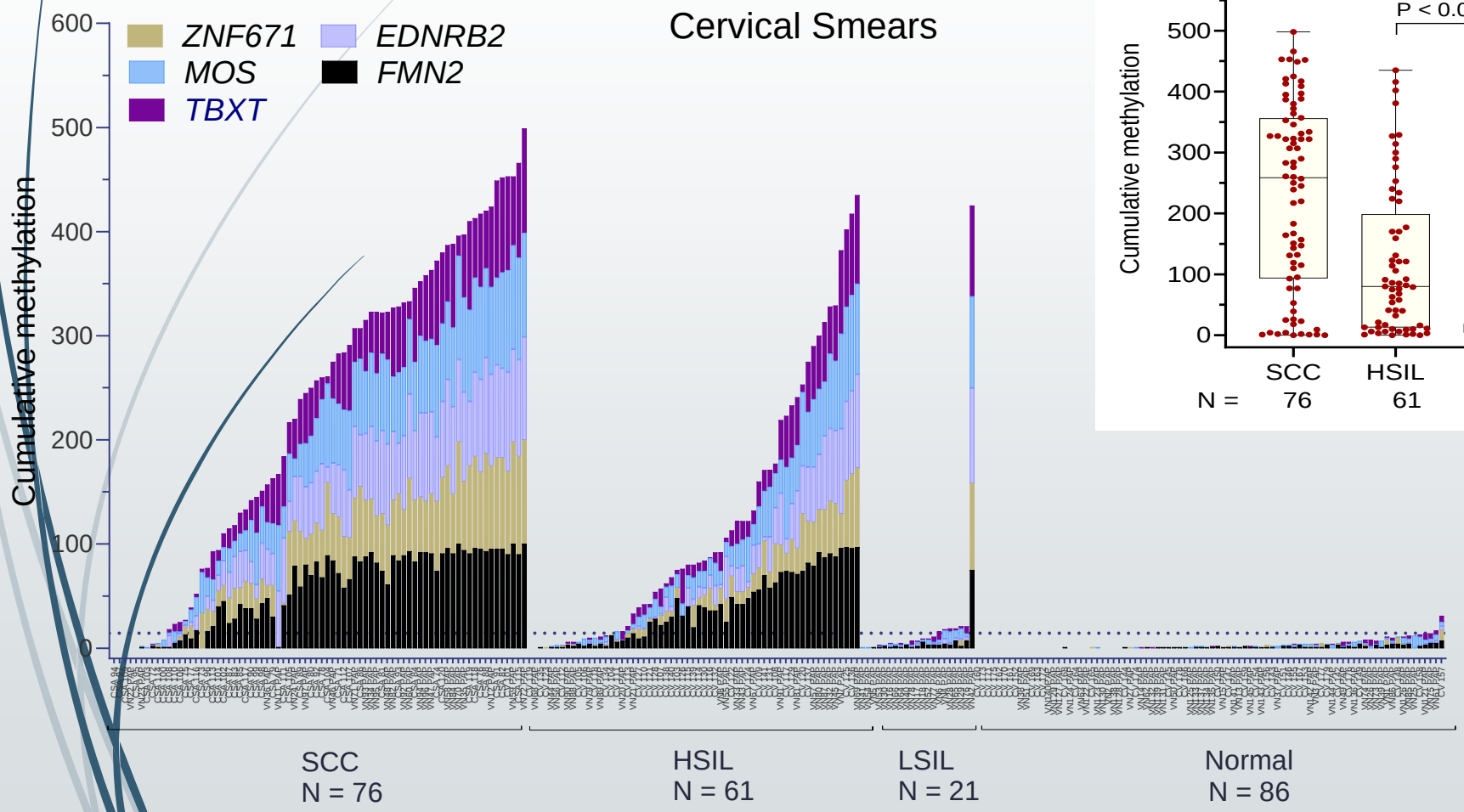


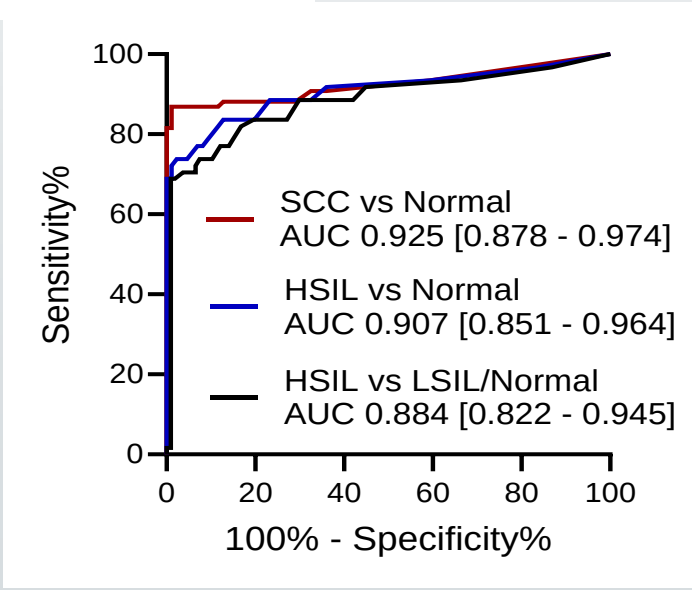
Cytology

Histopathology

Analysis of DNA methylation marker panels in PAP smears

Detection of cervical cancer and high-grade lesions in cervical smears

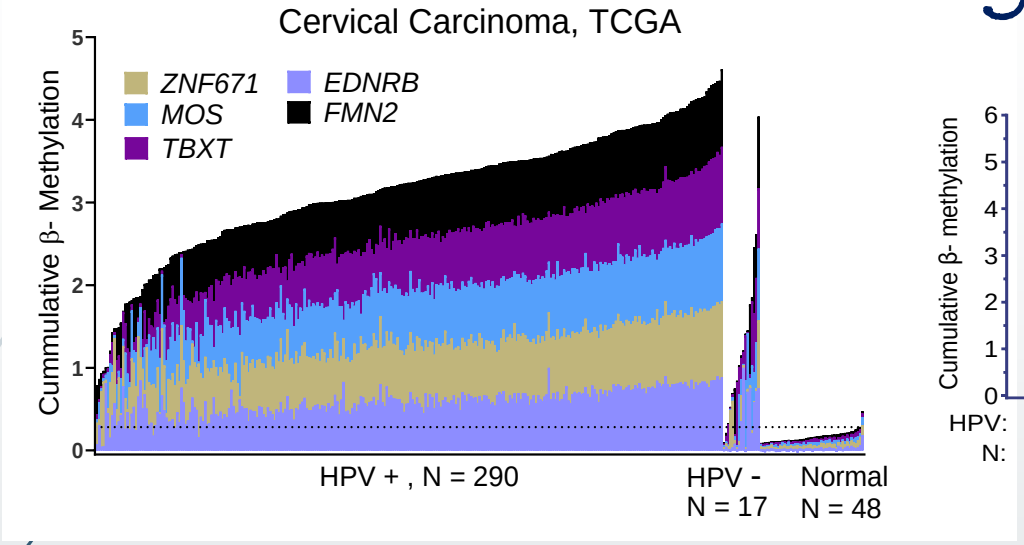


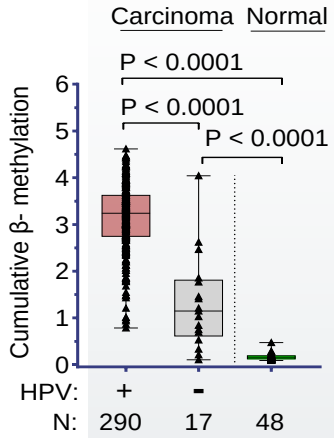


Fackler MJ, Pleas M et al, Clin Epigenetics. 2024 Apr 20;16(1):56.

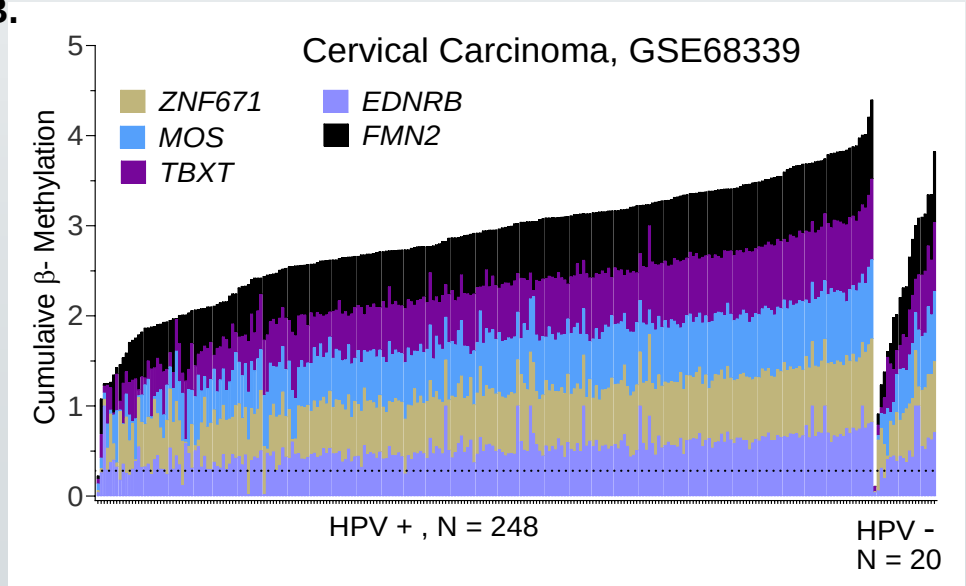
The 5-methylation marker panel detects both HPV+ and HPV-negative carcinomas

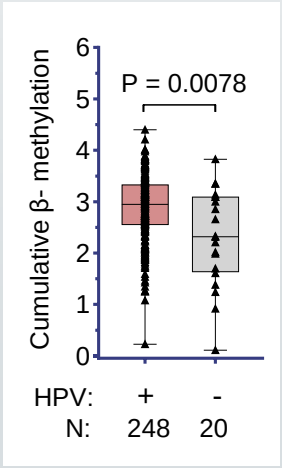
A.





B.

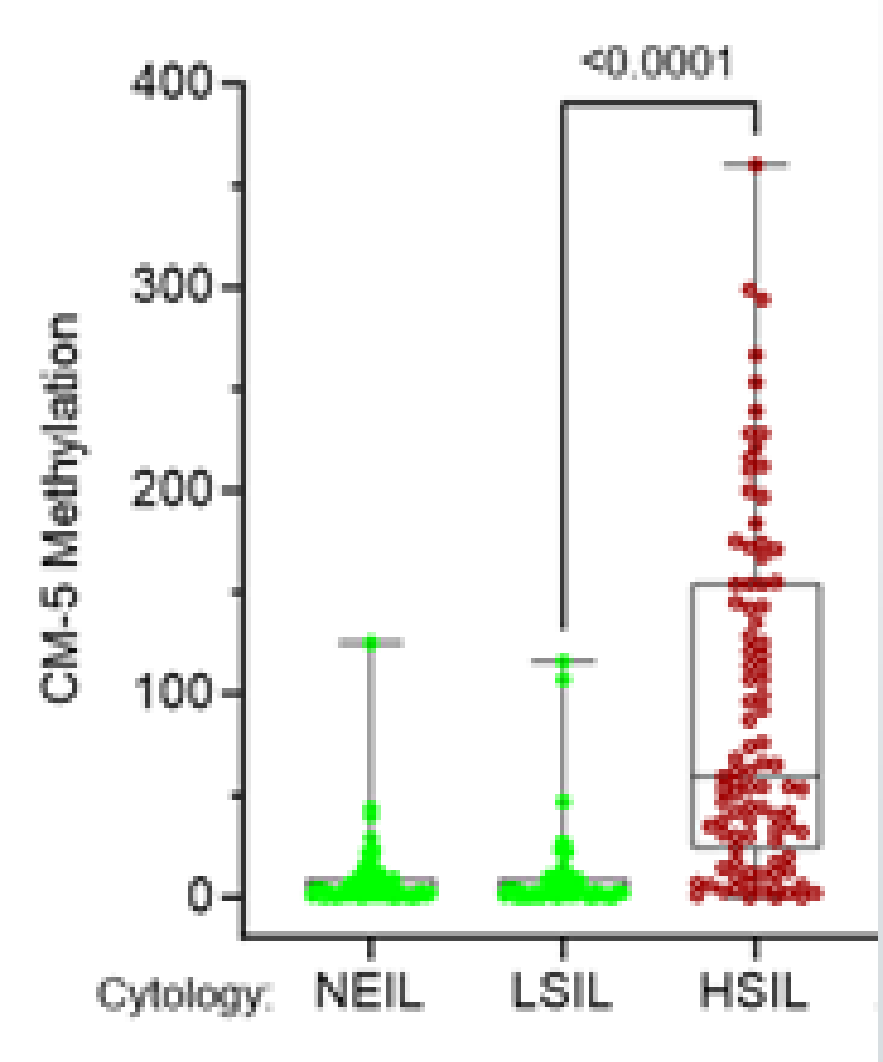




Fackler MJ, Pleas M et al, Clin Epigenetics. 2024 Apr 20;16(1):56.

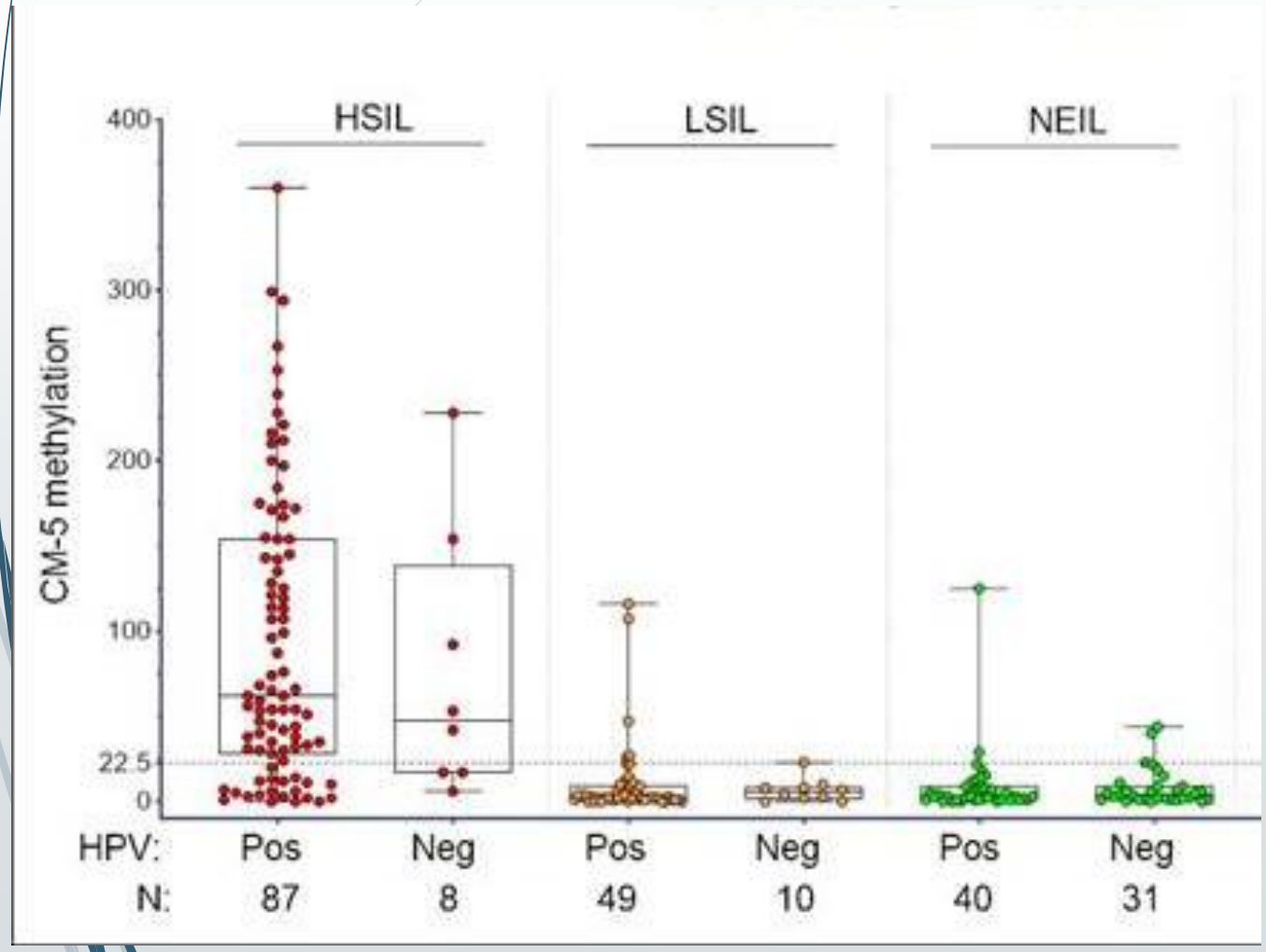
A pilot study on 250 samples of liquid-based cell (LBC) samples from a screening clinic in South Africa

Methylation of 5-gene panel in cervical brush LBCs- pilot study in SA, N=250



At 22.5 CM as cutoff for normal, HSIL is detected at 75.9% sensitivity at 90% specificity, an AUC of 0.886 compared to LSIL/NIEL or LSIL alone. N=250

Correlation of HPV status, cytological diagnosis and methylation of 5-gene panel



HPV+ identifies 91% of high-grade lesions

Methylation is far more specific than HPV positivity in identifying concerning disease

Will the two tests, together, improve both sensitivity and specificity of detection of SCC and HSIL?

The method has the potential to prioritize colposcopy to those who need it.

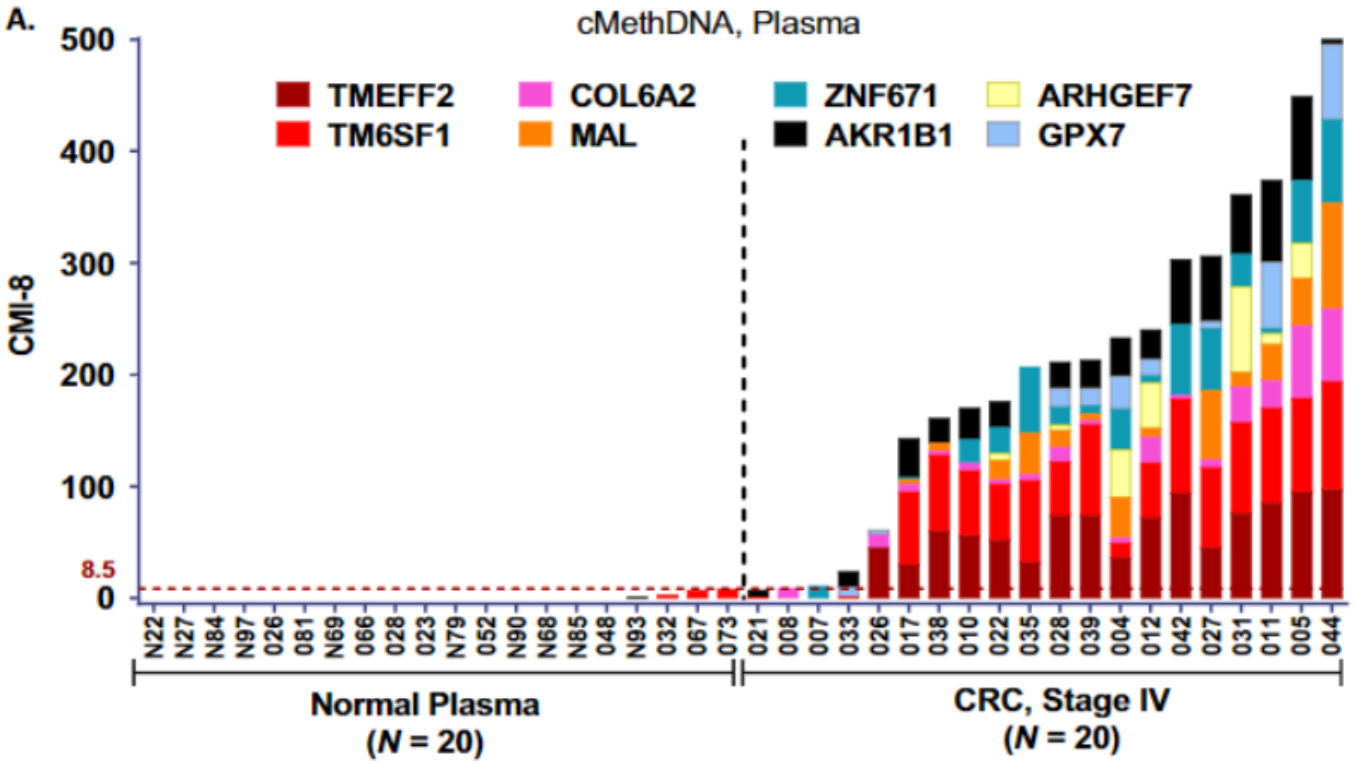
Conclusions

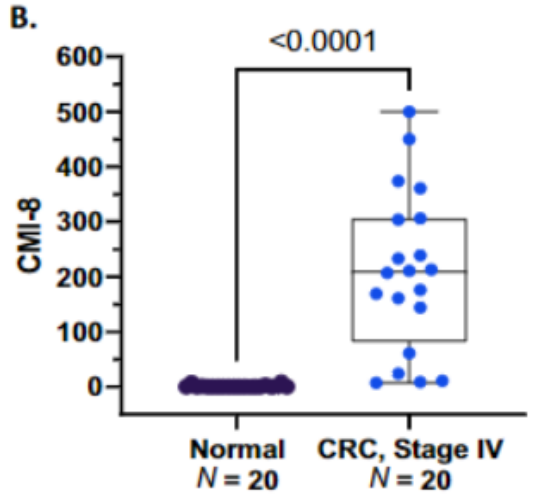
1. Methylated markers have been combined with HPV testing and cytology to improve sensitivity and specificity for the triaging process. Among the commercially available tests using both, QIAsure shows the highest sensitivity (77%) of detection of CIN3 at a specificity of 78.3%.
2. Our test achieved a sensitivity of 76% and specificity of 90% for detecting CIN3.

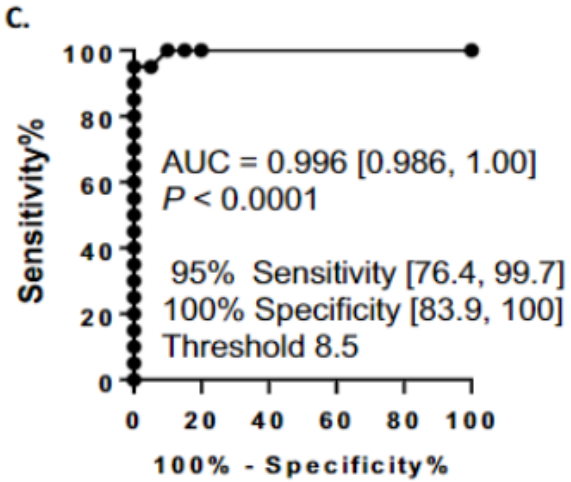
What next? Develop an affordable, simple-to-use PCR-based methylation detection test.

We need a commercial partner

Stories for another day-A blood test for CRC







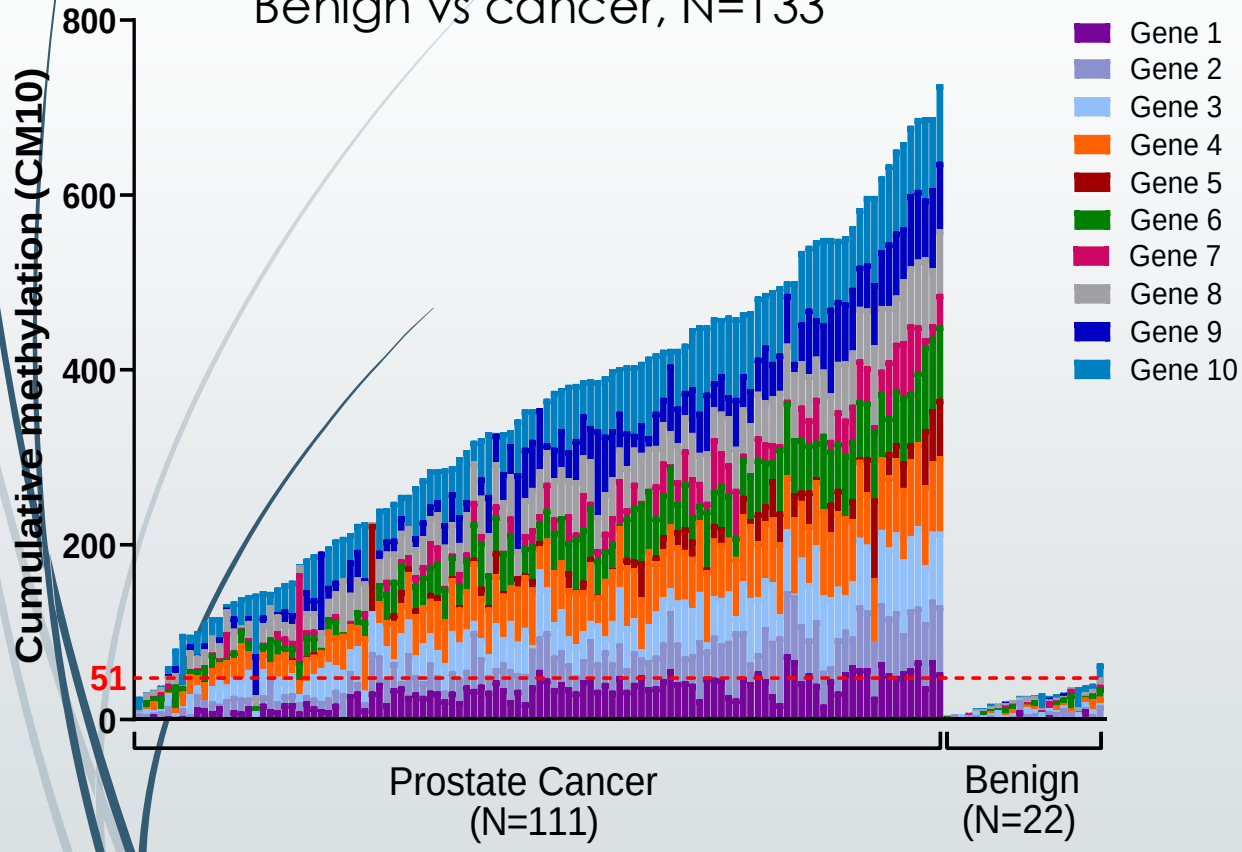
Klein-Kranenbarg et al, Clin Epigenetics, 2021, 13: 218

Validation studies initiated at AOU at Ile-Ife with Dr O. Alatise MD and MSKCC with Peter Kingham, MD

Funded by Academic-Industrial Partnership NIH-RO-1 2023-2028

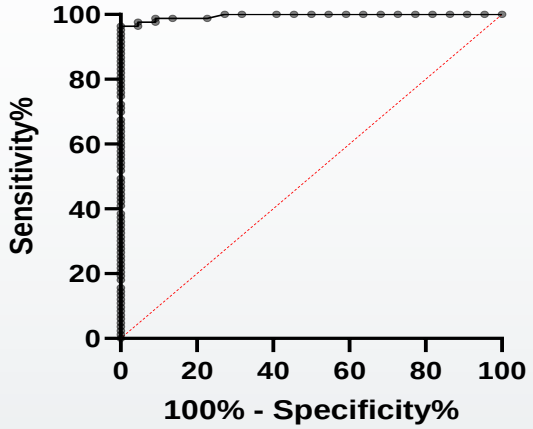
A promising 10-gene panel for prostate cancer

Benign vs cancer, N=133



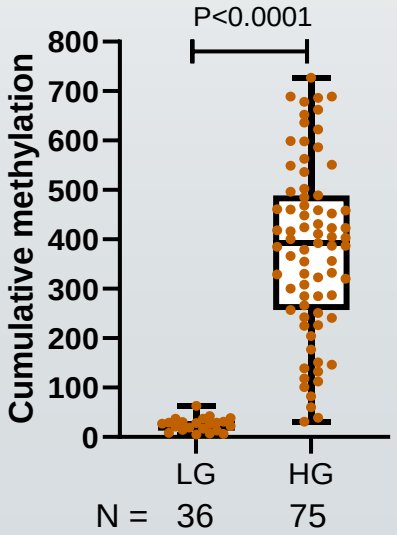
FFPE tissues from South Africa

Benign vs cancer



AUC=0.995 (95%CI: 0.987-1.00}		
Threshold	Sensitivity %	Specificity %
>51.0	97.59 [91-98]	95.45 [78-99]

Low grade vs High grade PCa



2 methylated genes plus age

For patients >55 years old		
Area under the ROC curve		
Area	0.7923	
Std. Error	0.04804	
95% confidence interval	0.6981 to 0.8865	
P value	<0.0001	
Data		
Controls (LG)	36	
Patients (HG)	57	

Gang Yu, Mary Jo Fackler Eunice Van den Berg, Adam Botha, Maureen Joffee, Carl Chen, Sukumar. unpublished

Conclusions

Clinical validation is ongoing-

- In South Africa for detecting breast cancer using FNA to prioritize patients with cancer (60% cancer, 40% benign) for expedited care
- In Nigeria for detecting CRC among patients with chronic GI bleeding and other symptoms who are undergoing colonoscopy (70% benign, 30% cancer)

Other cancers in the pipeline-

- To detect high-risk lesions of cervical cancer (HSILs) that need colposcopy and pathology, and just follow women with low-risk, often disappearing HPV+ lesions (LSIL)
- Prostate cancer- a test in urine or blood to detect prostate cancer in LMICs with no PCa screening
- A blood test to distinguish low-grade vs high-grade PCa,
- monitor the response of metastatic PCa to therapy,
- and detect disease recurrence

Our Study Team

Sukumar Laboratory, SKCCC at JH

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Cervical Cancer SPORE, DRP

GI Malignancies SPORE, DRP

NIH NATIONAL CANCER INSTITUTE

Cepheid A better way.

And to our survivors and activists…