MAYO CLINIC

LEVERAGING METHYLATED DNA MARKERS IN THE DETECTION OF OVARIAN AND ENDOMETRIAL CANCERS

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DISCLOSURE OF RELEVANT FINANCIAL RELATIONSHIP(S) WITH INELIGIBLE COMPANIES

- Dr. Bakkum-Gamez is listed as an inventor on intellectual property jointly owned by Mayo Clinic and Exact Sciences.

REFERENCES TO OFF-LABEL USAGE(S) OF PHARMACEUTICALS OR INSTRUMENTS

- Data presented is research-only.
- No references to off-label usage of pharmaceuticals or instruments.

OBJECTIVES

- Overall: Focus on bench-to-bedside cancer detection biomarker development in endometrial cancer (EC) and ovarian cancer (OC)
- Current clinical state in EC diagnostics
- Sampling of EC biomarker work
- Mayo Clinic methylated DNA marker pipeline in EC and OC
- Ongoing work and future directions

THE NUMBERS – POSTMENOPAUSAL BLEEDING AND ABNORMAL UTERINE BLEEDING

- Postmenopausal bleeding (PMB) chief complaint 5% office GYN evaluations
  - PMB: affects 4-11% women in their lifetime
    - 10% with PMB have EC
  - 50M postmenopausal women in the US

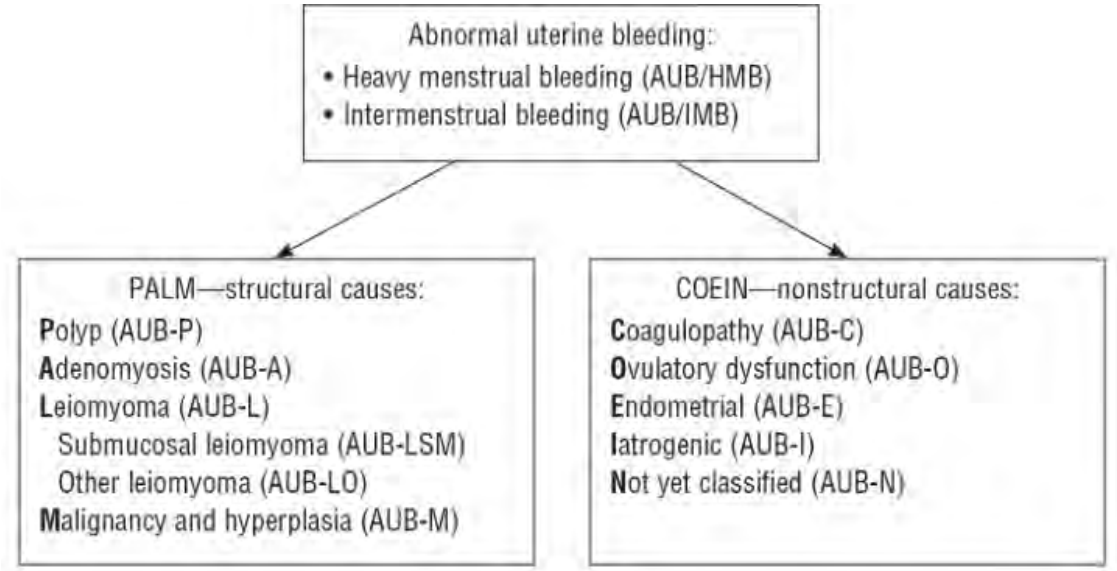
- Abnormal uterine bleeding (AUB) chief complaint for 70% of *peri-/postmenopausal* GYN outpatient evaluations
  - AUB: affects 1.4M women/year in US age 18-50
    - Perimenopause: is bleeding change normal or abnormal?
    - 5% with perimenopausal AUB have EC

Cozza, et al. Eur. Rev. Med. Pharmacol Sci. 2017.
Moodley and Roberts, J Obstet Gynaecol. 2004.

WORK UP FOR AUB OR PMB

- Any of the following:
  - Transvaginal ultrasound
  - Office hysteroscopy
  - Sonohysterogram

  - Endometrial sampling – criterion standard*
    - Office-based biopsy
    - D&C



***ACOG Guidelines → All women ≥45 yo, endometrial sampling should be performed as first-line test**
Plus: Endometrial sampling should also be performed in women <45 yo with obesity or other EC risk factors

  - Hysterectomy may be needed for diagnostic purposes if benign etiology cannot be ruled out

ACOG Cmte Opinion, Number 557, Reaffirmed 2020.
Munro, et al. Int J Gynecol Obstet. 2011

CHALLENGES WITH CURRENT DIAGNOSTIC APPROACH

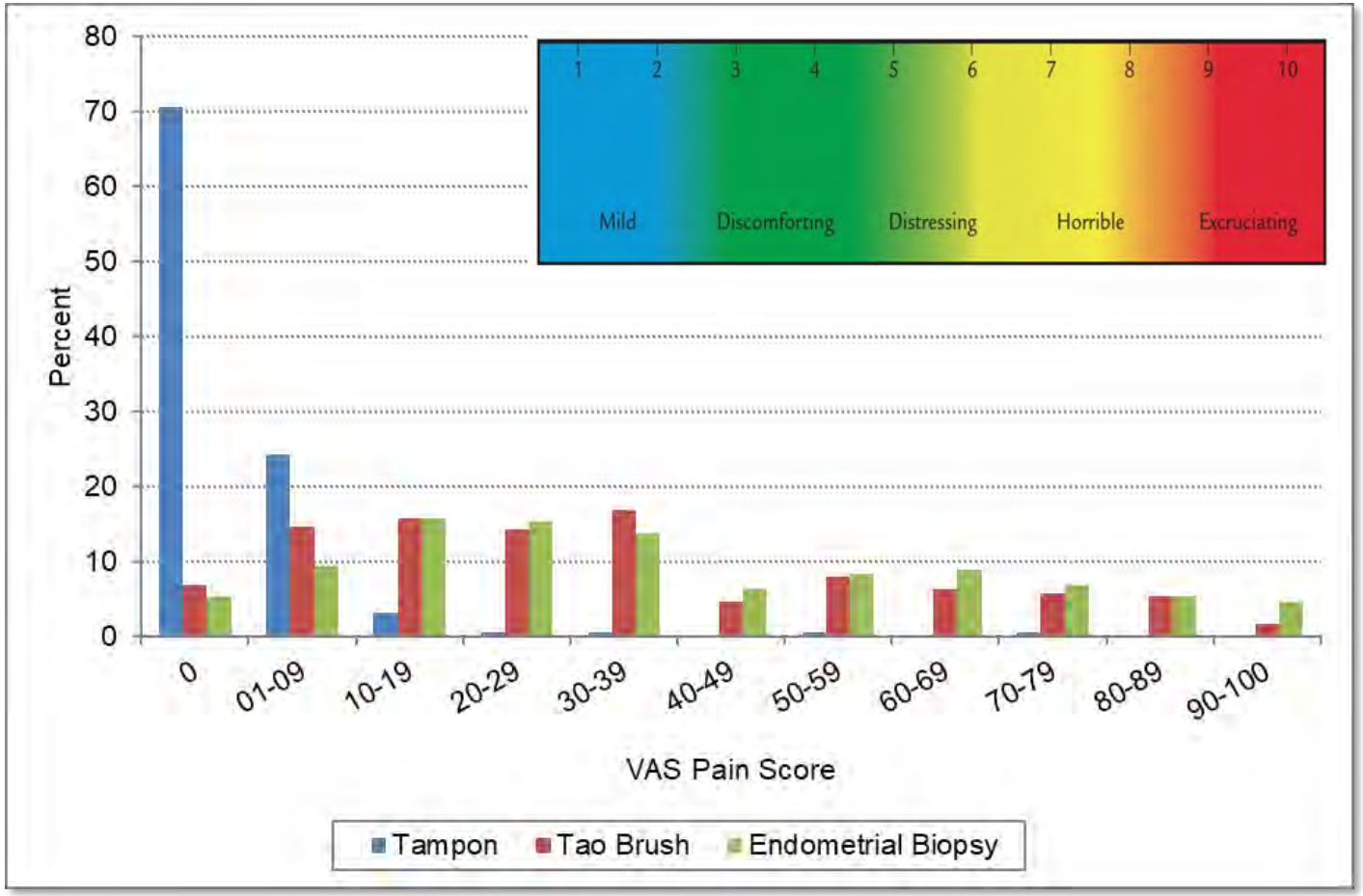
- Patient needs to see a provider to perform every test
  - Access
  - Lay population awareness of symptoms
  - Diversity in patient perception of AUB

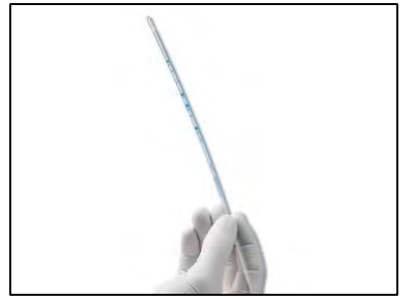
- Prior benign-pathology therapies can preclude successful endometrial sampling
  - For example: Endometrial ablation (40% fail endometrial sampling)
    - Endometrium found to be 100% present in post-ablation hysterectomies
    - Major surgery to dx small % EC

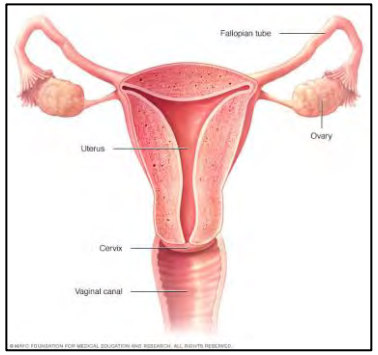
Gadzinski, et al. Obstet Gynecol. 2014.
Ridgeway, et al. J Clinc Trans Sci. 2021.

PATIENTS EXPERIENCE PAIN WITH OFFICE BIOPSY

Visual Analog Scale (VAS) pain scores among **190 patients** with VAS scoring available for all three sampling methods (EMB, Tao brush, tampon)







Bagaria, et al. Gyn Onc. 2021.

LEVERAGING CARCINOGENESIS IN BIOMARKER DEVELOPMENT

- What molecular changes happen between benign and cancer?
  - DNA mutations, epigenetic changes, CNVs
  - Microorganism genomes

Altered transcription and translation

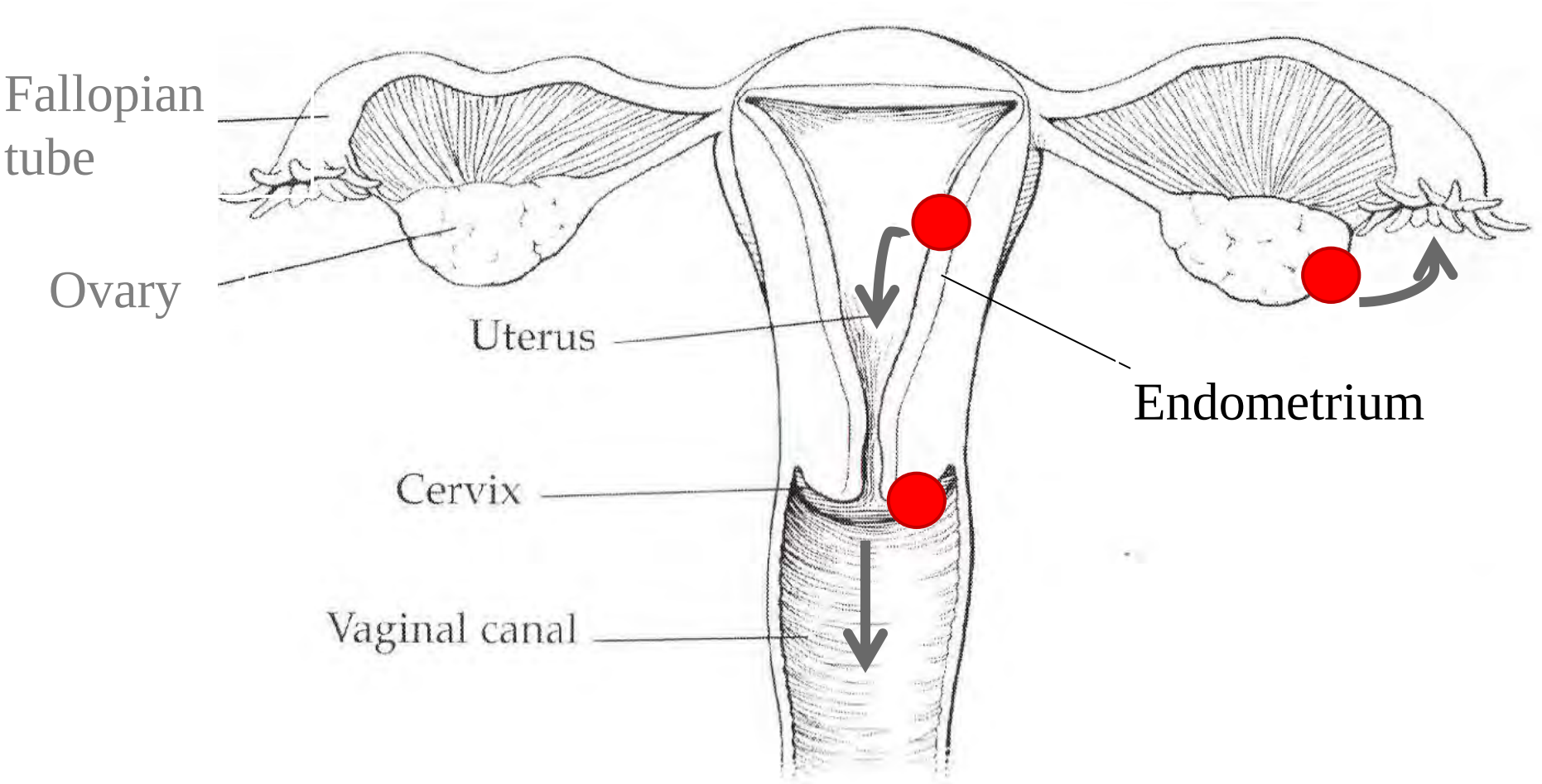
- Can we use these changes as signals to detect precancerous and early stage EC?
- Can we detect those changes in minimally-invasively collected biospecimens?
- Can we leverage self-collected biospecimens?

COLOGUARD™ MULTI-TARGET STOOL DNA TEST: A STORY OF SELF-SAMPLING SUCCESS

- Simple device for collection & mailing
- Preservative buffer
- Targets multiple markers
  - Methylated *BMP3* & *NDRG4*
  - Mutant *KRAS*
  - *β-actin* (human DNA)
  - Hemoglobin (FIT)
- Sensitive multiplex DNA assay (QuARTS)
- Screening in average risk population*; not a diagnostic test*
- **FDA-Approved: August 2014**

Slide courtesy of John Kisiel, MD.
Mayo Clinic Gastroenterology.

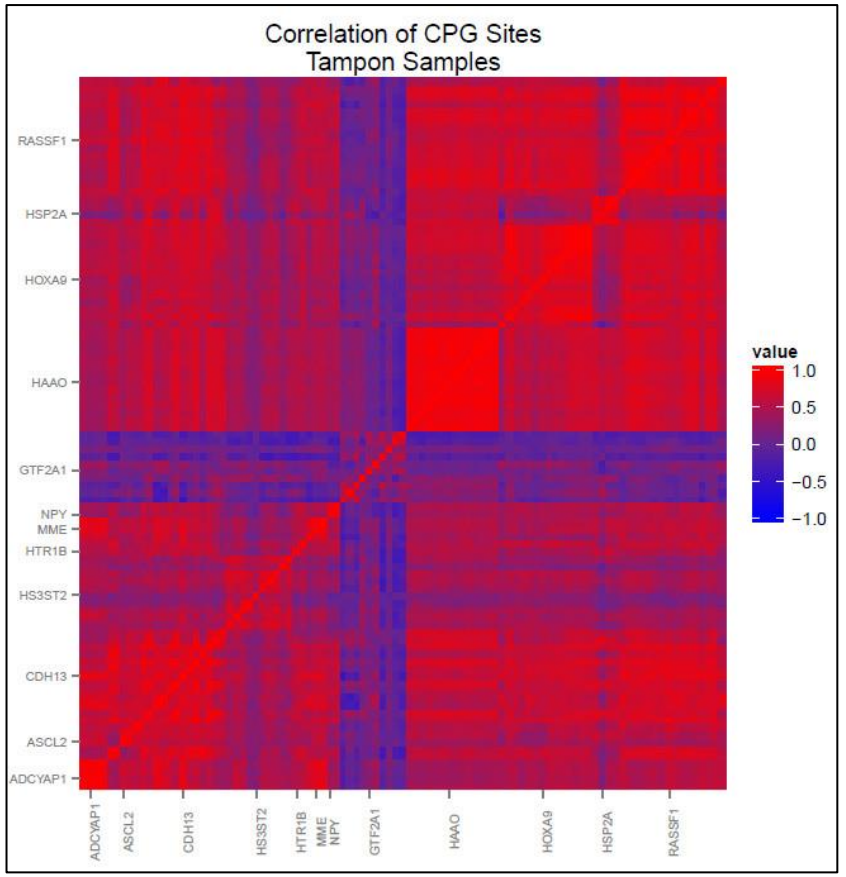
A NOVEL LIQUID BIOPSY: VAGINAL FLUID



Fiegl, et al. Ca Epi Bio Prev. 2004.
Finan, et al. Gyn Onc (SGO Abstracts). 2012.
Bakkum-Gamez, et al. Gyn Onc. 2015.

DNA METHYLATION IN VAGINAL TAMPONS: MAYO PROOF-OF-PRINCIPAL

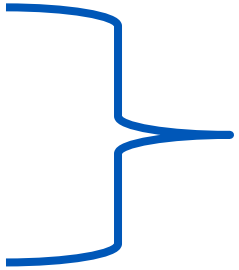
- 38 EC; 28 benign controls ≥45 yrs of age
- Clinically indicated hysterectomy
- 12 genes, 97 CpGs
- Pyrosequencing
  - Tampon DNA
  - Tao brush endometrial cytology DNA
- Consistent methylation across CpGs
- Higher mean methylation in EC v. benign in 9 genes (*ADCYAP1, ASCL2, CDH13, HS3ST2, HTR1B, MME, HAAO, HOXA9*, and *RASSF1*)
- Similar results from tampon and direct endometrial brushing



Bakkum-Gamez, et al. Gyn Onc. 2015.

DISCOVERING NOVEL MOLECULAR MARKERS OF GYNECOLOGIC CANCER

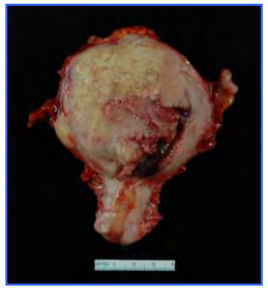
- Endometrial cancers of 5 predominant histologies
  - Grade 1/2 endometrioid
  - Grade 3 endometrioid
  - Clear cell
  - Carcinosarcoma
  - Serous



Type II; aggressive histologies

- *Ovarian cancers (4 predominant histologies), cervical cancers (2 histologies)
- Benign endometrial menstrual phases, cervical, vaginal, tubal tissue, endometrial hyperplasias, cervical dysplasia, blood buffy coat
- **Methylome sequencing on tissue-derived DNA**

ENDOMETRIAL CANCER – 3-STEP FOCUSED PIPELINE



I. Discovery & Technical Validation

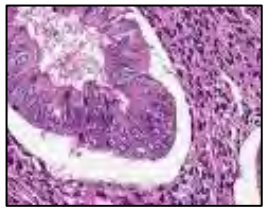
Specimen Source: Fresh frozen EC, BE, and BCV tissues

Cohort:

Endometrial Cancer (n=69)				
16 Grade 1/2 Endometrioid	16 Grade 3 Endometrioid	11 Serous	11 Clear Cell	15 Carcinosarcoma

Benign (n=80)		
44 BE	18 Buffy Coat	18 BCV

Methods: Whole methylome sequencing (RRBS) (Discovery) & quantitative methylation-specific PCR (qMSP) (Technical validation)



II. Biological Validation

Specimen Source: Independent archived FFPE EC, BE, AEH, and endometrial hyperplasia without atypia tissues

Cohort:

Endometrial Cancer (n=141)				
34 Grade 1/2 Endometrioid	31 Grade 3 Endometrioid	27 Serous	19 Clear Cell	30 Carcinosarcoma

Benign (n=112)
112 BE

Endometrial hyperplasia (n=59)
24 Without Atypia
35 AEH

Method: qMSP

III. Tampon Pilot

Specimen Source: Prospective tampon-collected vaginal fluid samples

Cohort:

Endometrial Cancer (n=100)					
49 Grade 1/2 Endometrioid	9 Grade 3 Endometrioid	24 Serous	4 Clear Cell	9 Carcinosarcoma	5 Mixed

Benign (n=92)
92 BE

Endometrial hyperplasia (n=36)
25 Without Atypia
11 AEH

Method: qMSP

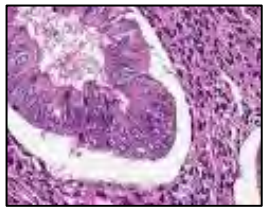
Bakkum-Gamez, et al. *Gyn Onc.* July 2023.

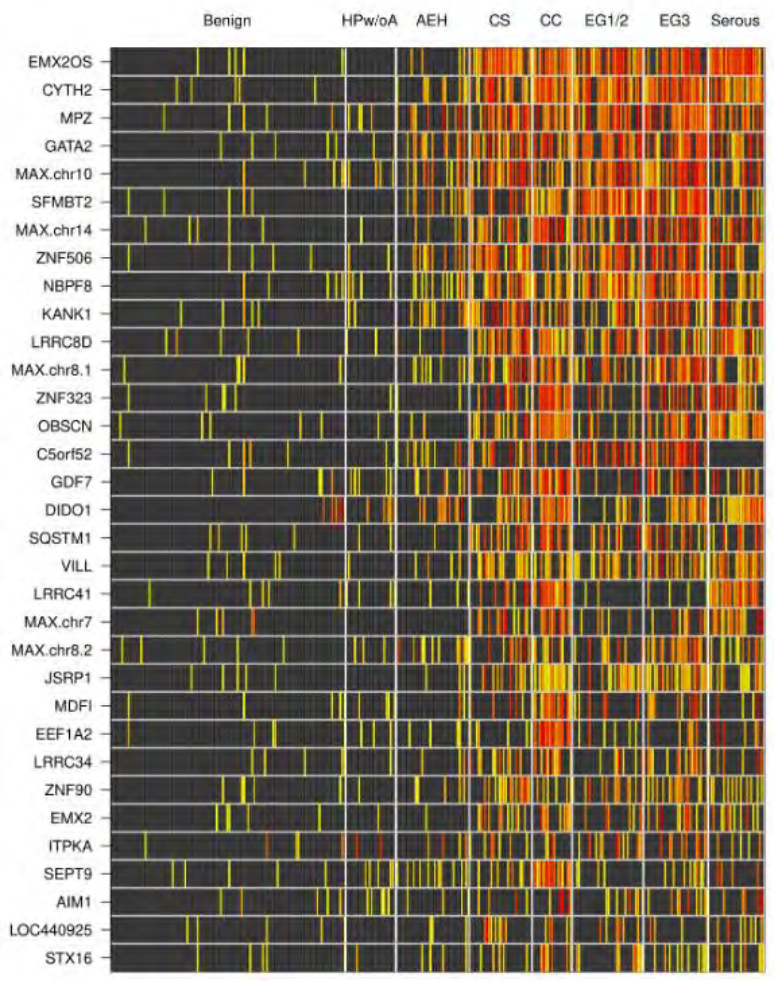
INDEPENDENT TISSUE VALIDATION AMONG HISTOLOGIES

All EC	AUC	FC	Clear Cell EC	AUC	Serous EC	AUC	Carcinosarcom	AUC	Endometrioid E	AUC
EMX2OS-RS	0.95	29.31	CYTH2-FS	1.00	EMX2OS-RS	1.00	EMX2OS-RS	0.96	NBPF8-FS	0.97
NBPF8-FS	0.92	14.57	OBSCN-FS	0.99	LRRC41(8188)-FS	0.94	ZNF506-FS	0.94	MAX.chr8.1451	0.94
CYTH2-FS	0.89	16.18	EMX2OS-RS	0.97	DIDO1-FS	0.94	MAX.chr10.226	0.94	EMX2OS-RS	0.93
MAX.chr10.226244	0.89	77.62	SEPT9-FS	0.97	OBSCN-FS	0.91	NBPF8-FS	0.93	SFMBT2.894(1	0.92
MAX.chr8.145103	0.88	22.51	MAX.chr14.1030	0.97	CYTH2-FS	0.90	ZNF90-RS	0.90	END.JSRP1-FS	0.91
JSRP1-FS	0.87	3.73	EEF1A2-RS	0.95	MAX.chr8.145103829	0.88	VILL-RS	0.88	OBSCN-FS	0.89
ZNF90-RS	0.87	6.43	MDFI-FS	0.95	LRRC8D-FS	0.87	KANK1-FS	0.87	MAX.chr10.226	0.88
SFMBT2.894(1000	0.86	22.62	SFMBT2.894(10	0.93	VILL-RS	0.87	JSRP1-FS	0.87	MAX.chr8.1451	0.88
OBSCN-FS	0.86	14.72	GDF7-FS	0.93			LRRC8D-FS	0.86	CYTH2-FS	0.87
MPZ-FS	0.85	112.07	MAX.chr8.14510	0.93			MAX.chr8.1451	0.86	ZNF90-RS	0.87
LRRC8D-FS	0.85	17.70	JSRP1-FS	0.92			OBSCN-FS	0.85	MPZ-FS	0.87
DIDO1-FS	0.85	214.16	DIDO1-FS	0.92						
			MPZ-FS	0.91						
			LRRC34-RS	0.91						
			SQSTM1-FS	0.90						
			ZNF90-RS	0.90						
			ZNF323-FS	0.90						
			LRRC8D-FS	0.90						
			VILL-RS	0.90						
			LRRC41(8188)-F	0.90						
			MAX.chr10.2262	0.88						
			NBPF8-FS	0.86						

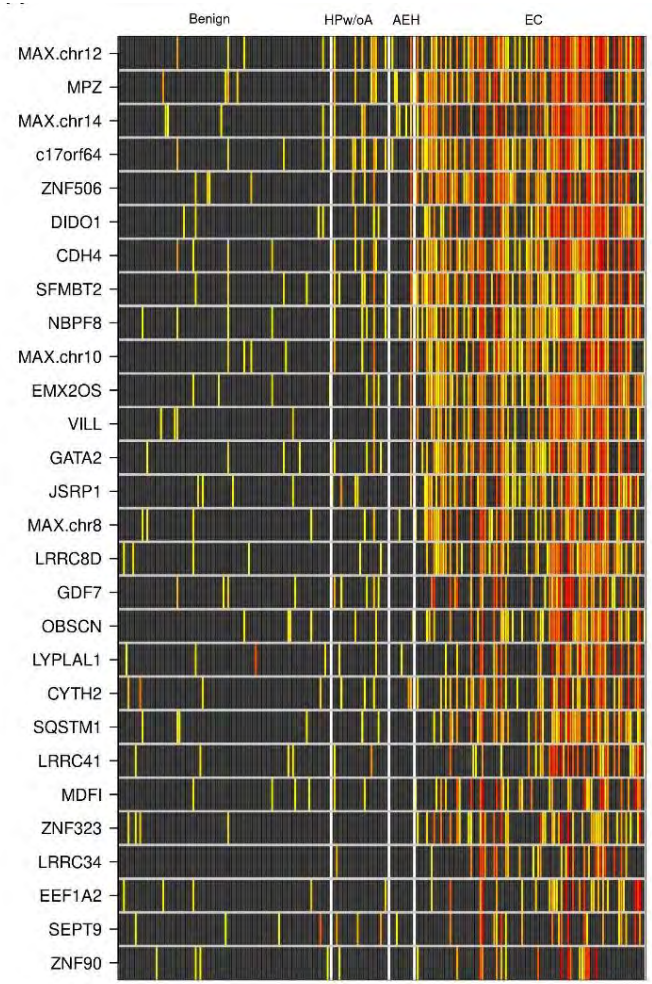
Unpublished data

TRANSLATION FROM DISCOVERY TO CLINICAL PILOT: TESTING APPLICATION





Biological tissue-based validation
33 MDMs



Tampon clinical pilot; independent subjects
29 MDMs

Bakkum-Gamez, et al. *Gyn Onc.* July 2023.

CLINICAL TAMPON PILOT – BUFFER ENHANCEMENT

- 93 women ≥45 yo with benign AUB/PMB
- 100 EC
- Early buffer (PBS only pre 2/14/17) v. current buffer (PBS/EDTA)
- PBS/EDTA buffer: 52 benign; 57 EC
- 29 MDM-panel
- **Greater discrimination in PBS/EDTA buffer**

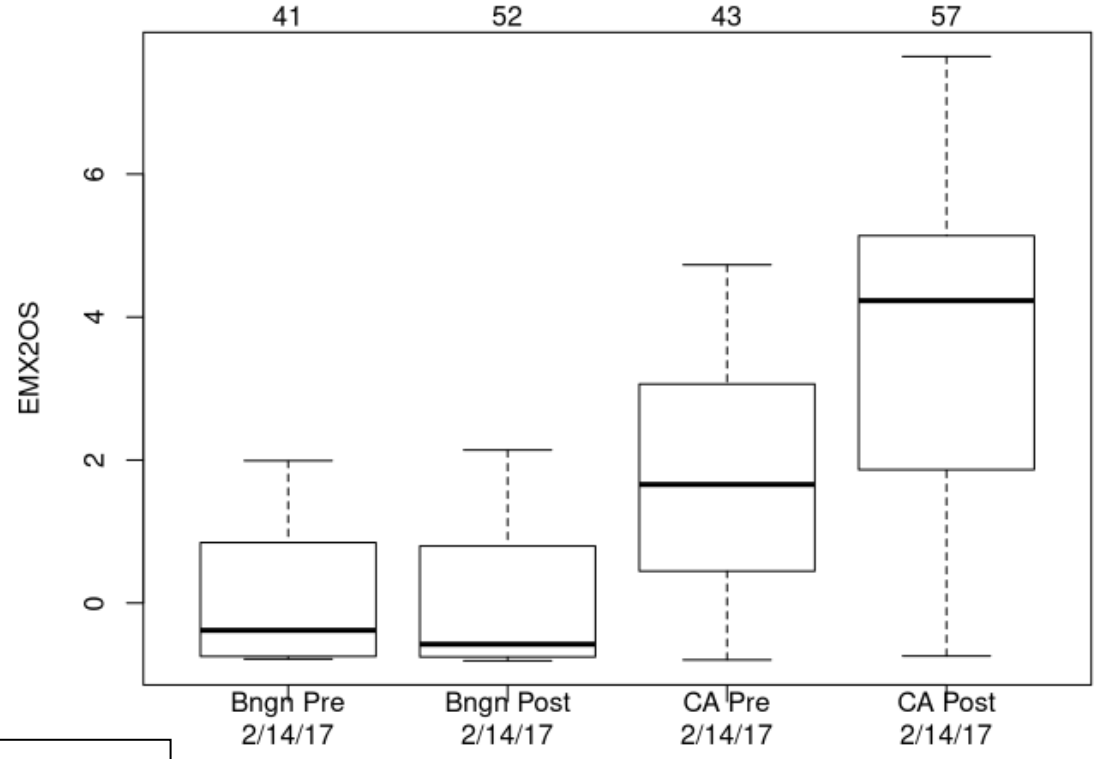


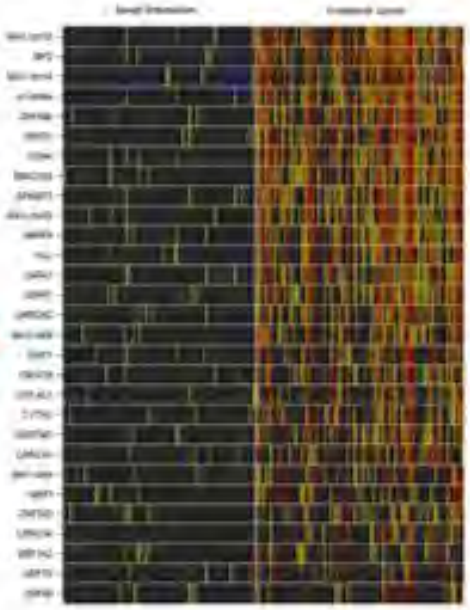
TABLE: Distribution of endometrial cancer (EC) histologies and cross-validated sensitivity by methylated DNA marker panel at 95% specificity in PBS/EDTA tampon buffer (N=57 ECs).

EC histology	Endometrioid	Serous	Carcinosarcoma	Clear cell	Mixed
N	20	23	9	3	2
Sensitivity at 95% specificity % (95% CI)	85% (62-97%)	78% (56-93%)	89% (52-99%)	67% (9-99%)	50% (1-99%)

Bakkum-Gamez, et al. *Gyn Onc.* July 2023..

Endometrial Cancer

- Novel biomarker **discovery**: RRBS, frozen tissue (EC and pan-GYN tract) DNA
- Stringent methylated DNA marker (MDM) filtering criteria (ROC, fold-change, control background
- Blinded, biological **validation**: msPCR, FFPE tissue (EC and pan-GYN tract) DNA
- Testing in vaginal fluid collected via tampon from women with and without EC**: msPCR for 29 top performing MDMs



PBS/EDTA tampon buffer

Specificity 96% (87-99%)
Sensitivity 82% (70-91%)
AUC 0.91

*Same approach performed in ovarian cancer, translated to plasma
**Majority of women enrolled from the Midwest catchment

IMPACT: ***PARADIGM-SHIFTING***
Licensed IP through Mayo Clinic Ventures; phase II trial (NCT05051722)

Bakkum-Gamez

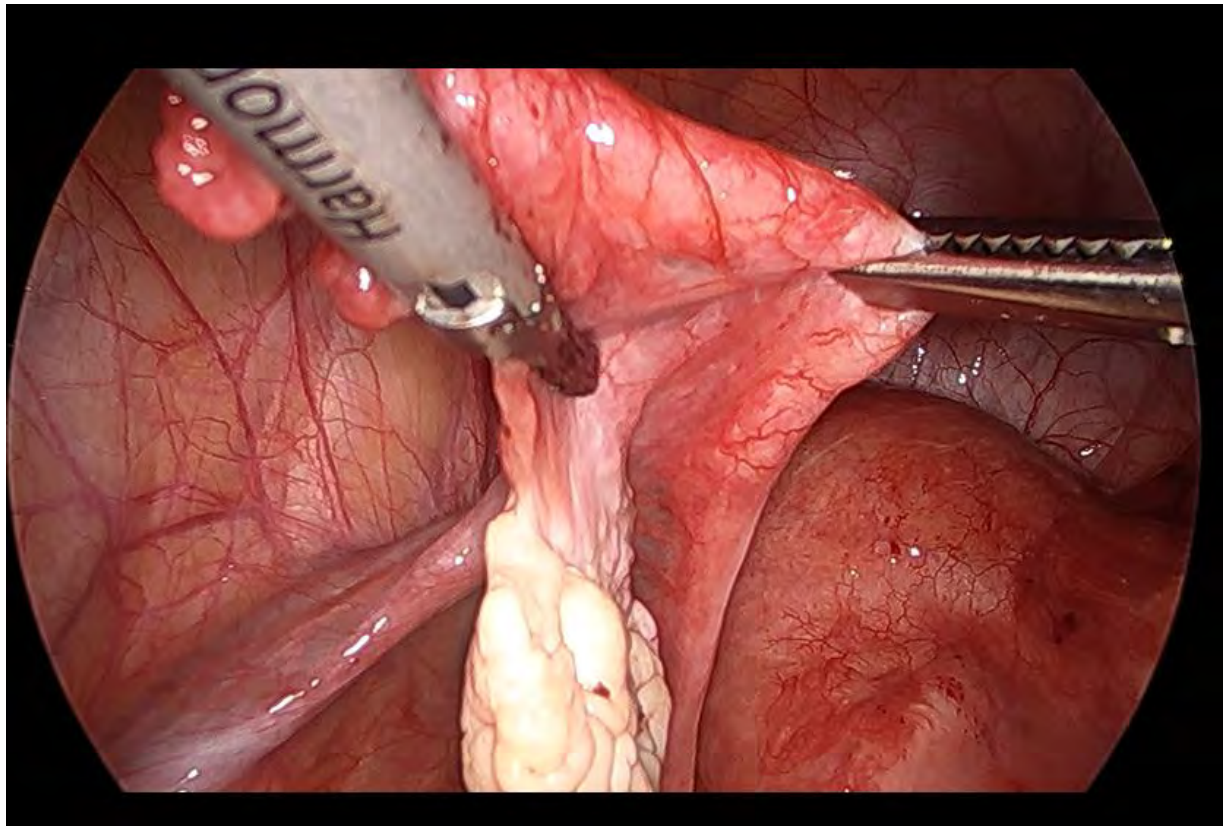
Sherman

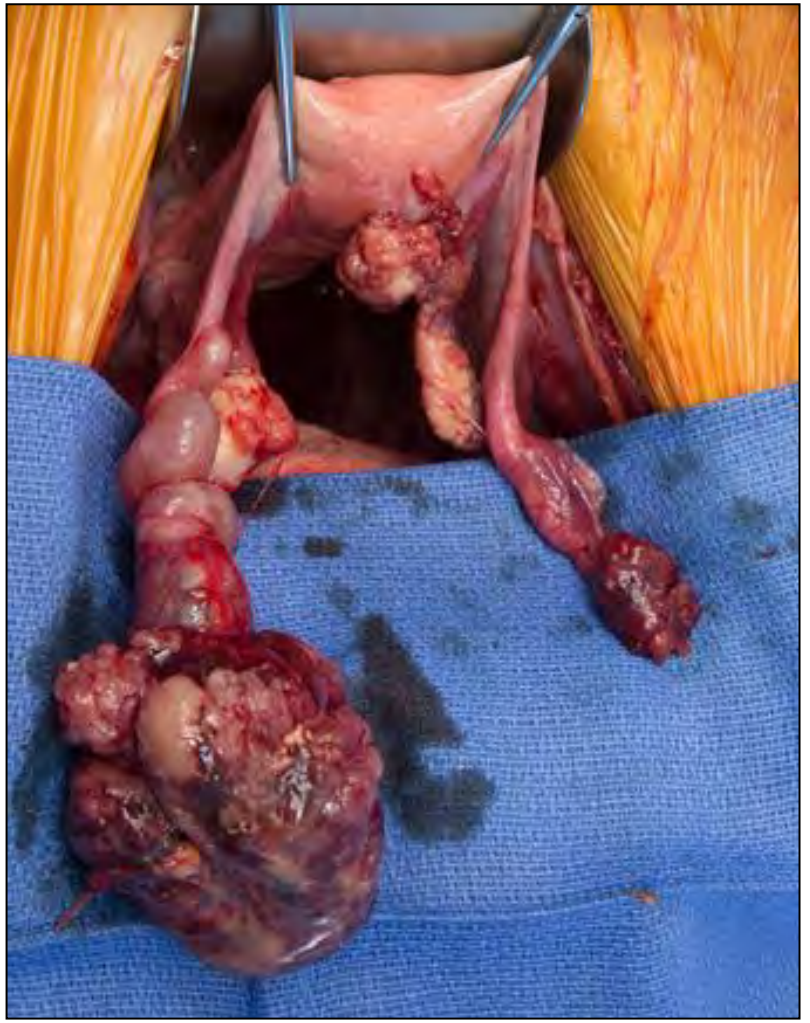
Shridhar

T2016-001-03
P50 CA136393
P30 CA015083
(CCITLA)

Gynecol Oncol, 2022
Gynecol Oncol 2023; 174:11-20. PMID: 37141817

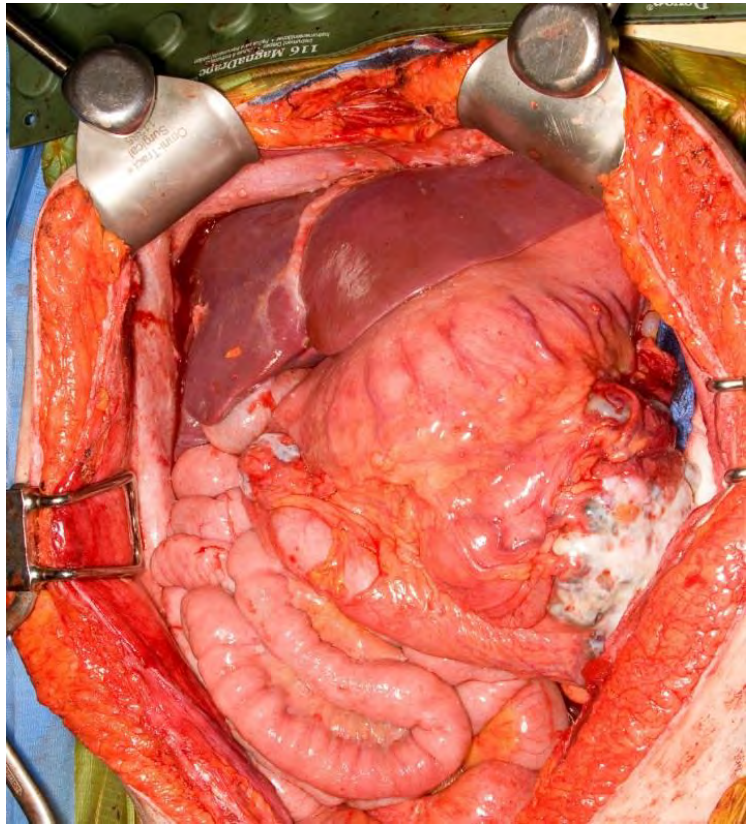
METHYLATED DNA MARKERS (MDMS) IN THE DETECTION OF OVARIAN CANCER





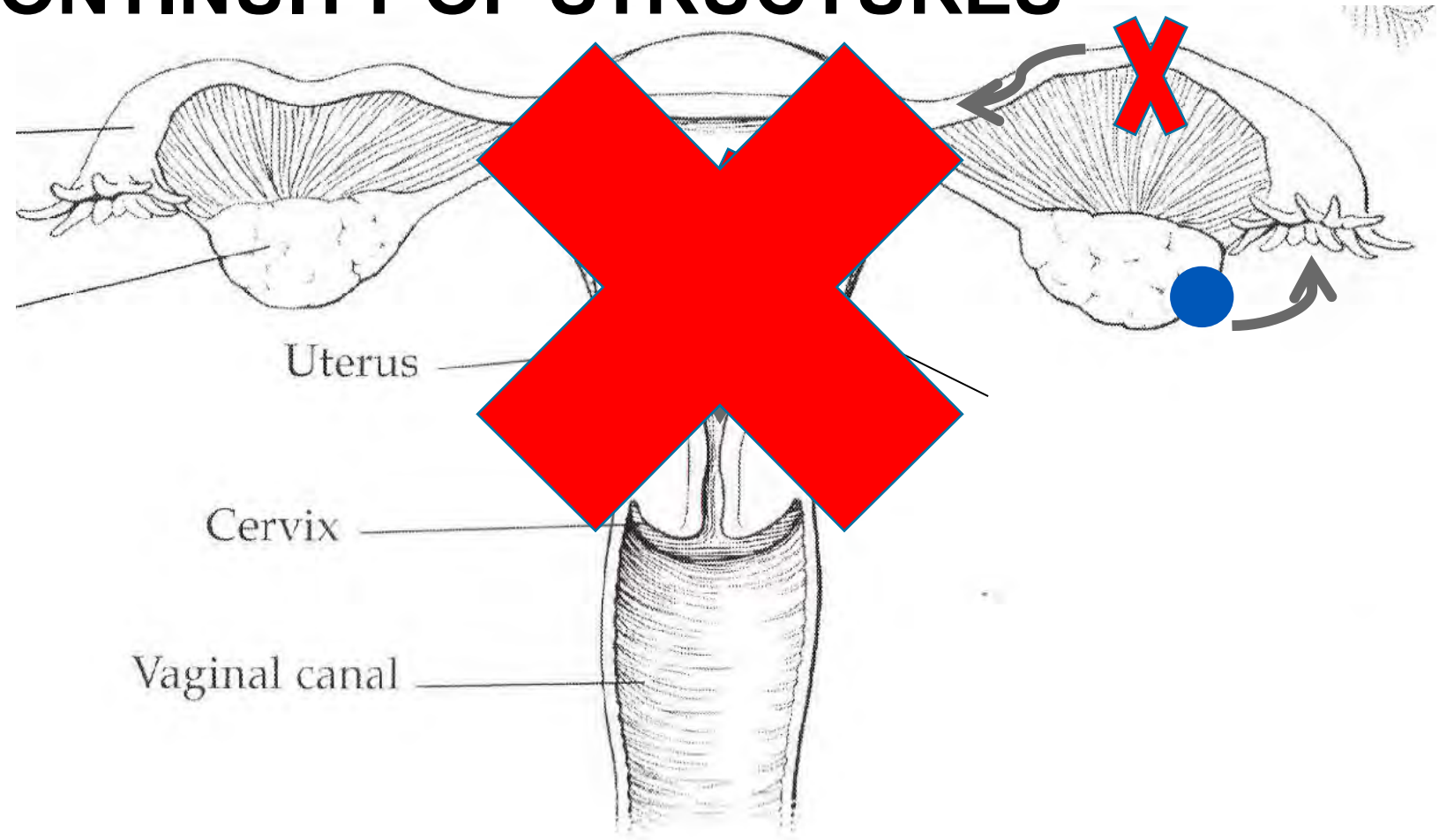
DESIGNING A SUCCESSFUL EARLY DETECTION TEST: REACHING FOR NIRVANA IN OVARIAN CANCER

1. High sensitivity to detect small volume, earliest stage disease
2. Encompass broad histologies
3. Generalizability to average-risk and elevated risk women
4. **Effective even in settings of altered gynecologic anatomy**
5. Widely accessible
6. Minimally invasive
7. Operator independent (automated)
8. Reasonable cost
9. For effective screening, needs to decrease mortality from EOC



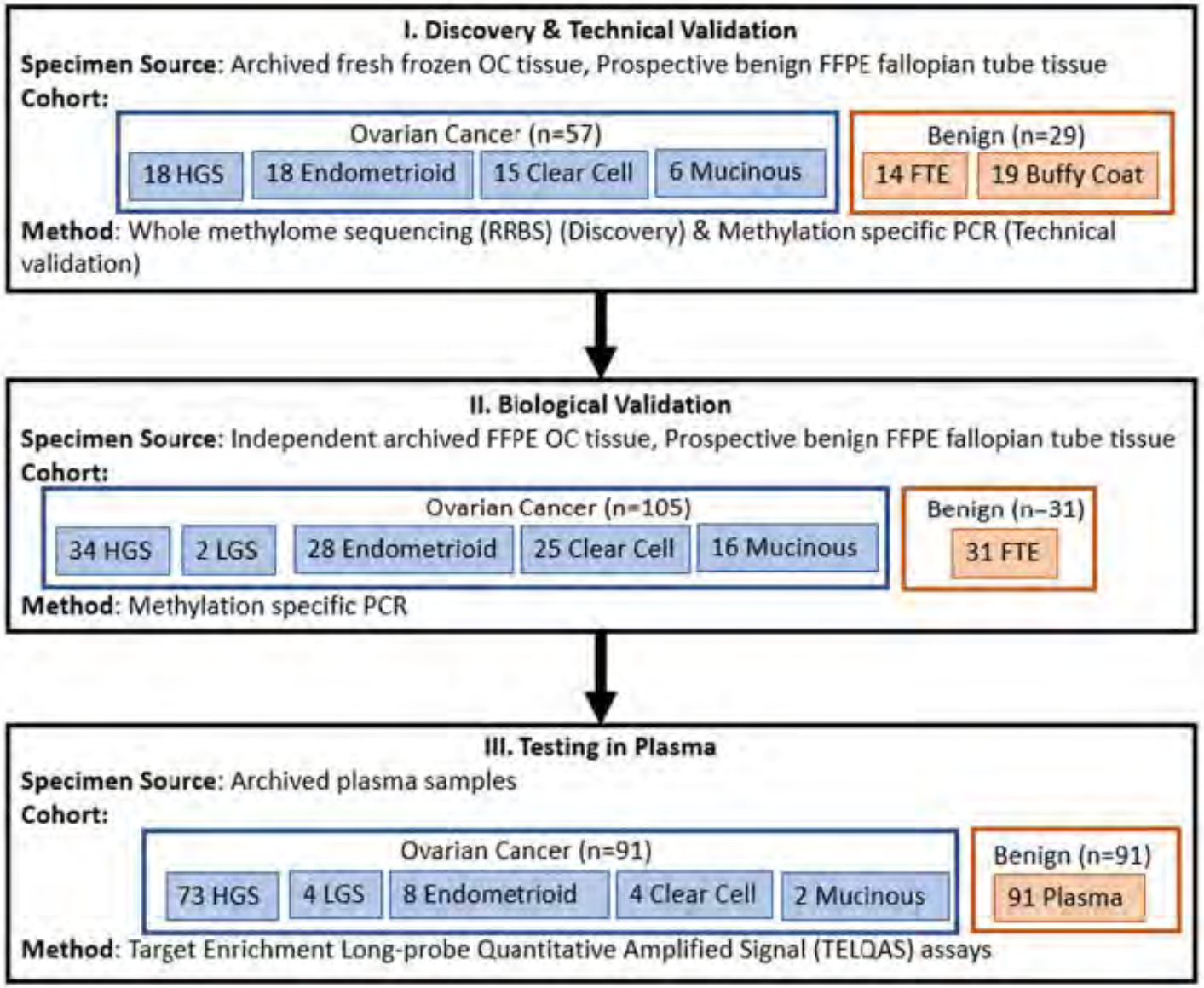
(uspreventiveservicestashttps://www.uspreventiveservicestaskforce.org/uspstf/kforce.org)

ANATOMIC LIMITATIONS—*IDEAL* CONTINUITY OF STRUCTURES



Caveat: Potential for continuity to be disrupted with clinically indicated procedures.

OVARIAN CANCER – THE 3-STEP FOCUSED PIPELINE

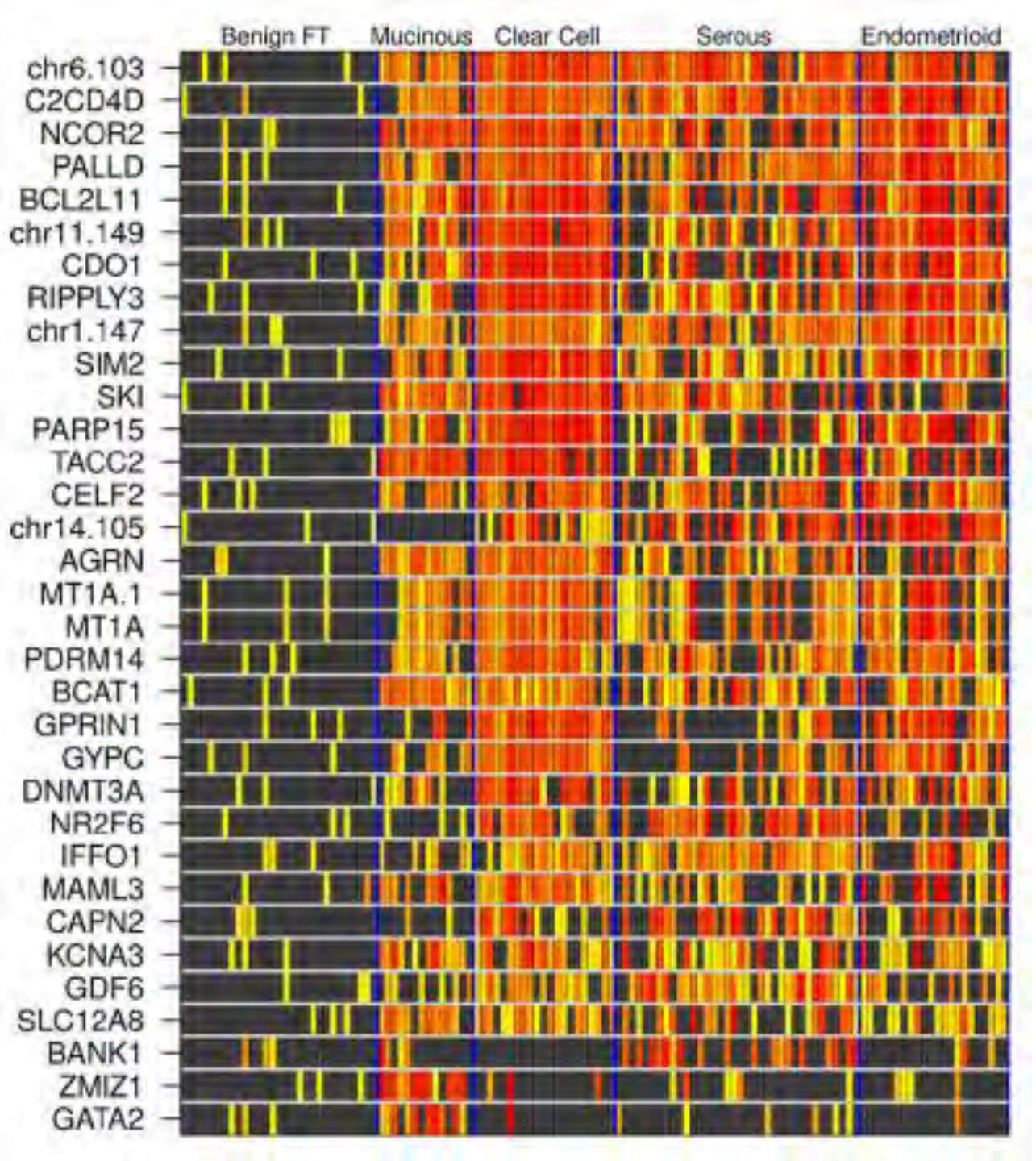


→ Discovery: 526 genes differentially methylated in OC
→ 54 selected for technical validation
→ 44 candidate genes AUC>0.90

→ 33 best performing genes in technical validation

→ 11 candidate genes/methylated DNA markers (MDMs)

Marinelli, et al. *Gyn Onc.* June 2022.



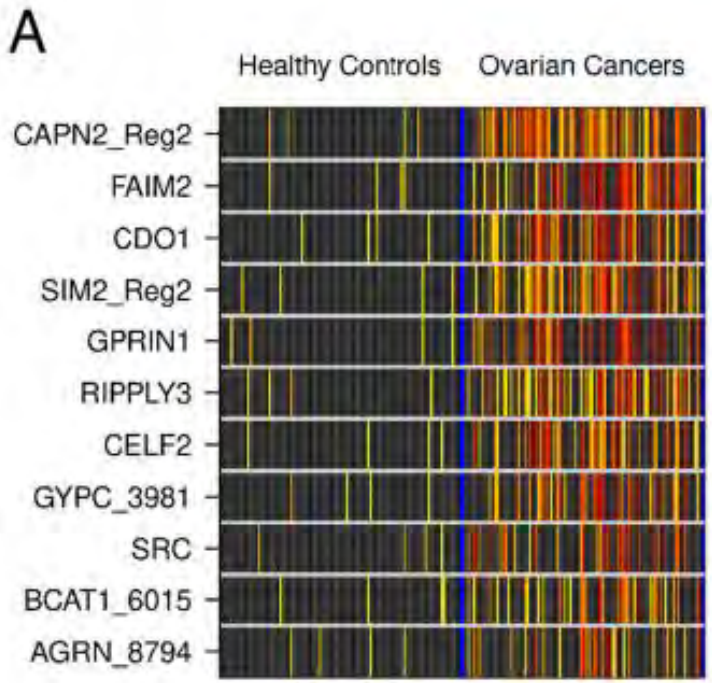
BIOLOGICAL TISSUE VALIDATION – 33 MDMS

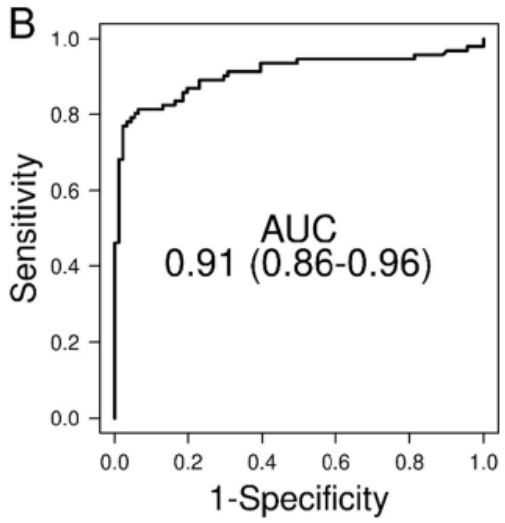
Characteristic	Ovarian Cancer (n=105)	Benign Fallopian Tube Controls (n=31)
Age, years (median [IQR])	60 [54-68]	53 [45-62]
BMI, kg/m2 (median [IQR])	28 [23.2-32.9]	26.7 [24.3-33.2]
Pregnancies (median [IQR])	2 [0-3]	3 [2-4]
Live births (median [IQR])	2 [0-3]	2 [1-3]
Race		
White	95 (90%)	27 (87%)
Non-White	5 (5%)	3 (10%)
Unknown	5 (5%)	19 (3%)
Tobacco Use		
Current	11 (10%)	2 (6%)
Previous	28 (27%)	7 (23%)
Never	62 (59%)	22 (71%)
Unknown	4 (4%)	0 (0%)
Menopausal Status		
Premenopausal	13 (12%)	19 (61%)
Perimenopausal	6 (6%)	0 (0%)
Postmenopausal	77 (73%)	12 (39%)
Unknown	9 (9%)	0 (0%)
Histology		
High grade serous	34 (32%)	-
FIGO grade 1/2 endometrioid	21 (20%)	-
FIGO grade 3 endometrioid	7 (7%)	
Clear cell	25 (24%)	-
Well differentiated mucinous	16 (15%)	-
Low grade serous	2 (2%)	
Stage		
I	50 (48%)	-
II	7 (7%)	-
III	39 (37%)	-
IV	9 (9%)	-

Marinelli, et al. *Gyn Onc.* June 2022.

OVARIAN CANCER PLASMA CLINICAL PILOT: 11 MDMS

Characteristic	Ovarian cancer ($n = 91$)	Healthy controls ($n = 91$)
Age, years (median [IQR])	61 [57–68]	61 [58–66]
BMI, kg/m^2 (median [IQR])	27.25 [24.66–30.5]	27.64 [22.95–31.45]
Pregnancies (median [IQR])	2 [1–3]	2 [1–4]
Live births (median [IQR])	2 [1–3]	2 [1–3]
Race		
White	75 (83%)	89 (98%)
Non-White	13 (14%)	1 (1%)
Unknown	3 (3%)	1 (1%)
Tobacco Use		
Current	9 (10%)	13 (14%)
Previous	23 (25%)	26 (29%)
Never	59 (65%)	52 (57%)
Menopausal Status		
Premenopausal	7 (8%)	9 (10%)
Perimenopausal	5 (5%)	5 (5%)
Postmenopausal	74 (81%)	77 (85%)
Unknown	5 (5%)	0 (0%)
Histology		
High grade serous	73 (80%)	–
FIGO grade 1 or 2 endometrioid	8 (9%)	–
Clear cell	4 (4%)	–
Low grade serous	4 (4%)	
Mucinous	2 (2%)	–
Stage		
I	10 (11%)	–
II	5 (5%)	–
III	64 (70%)	–
IV	12 (13%)	–
CA-125 U/mL (Median [IQR])	358.4 [119.2–1044.8]	8.6 [5.8–12.5]





OC histology	Serous	Clear cell	Endometrioid	Mucinous	Mixed
N	76	4	8	2	1
Sensitivity at 95% specificity % (95% CI)	83% (73 - 90%)	75% (19 - 99%)	50% (16 - 84%)	50% (13 - 99%)	100% (3 - 100%)

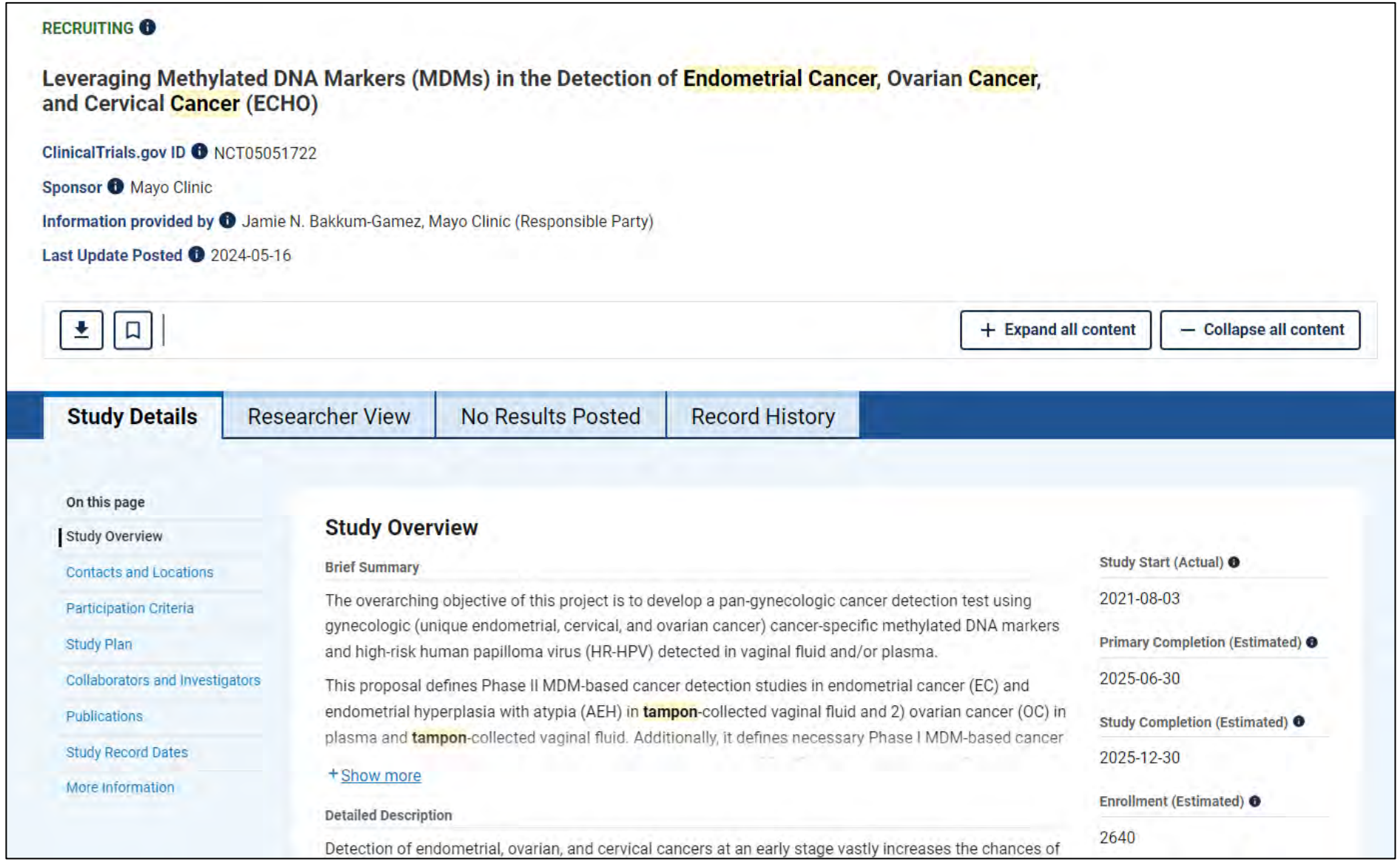
Marinelli, et al. *Gyn Onc.* June 2022.

SOME IMPORTANT CONSIDERATIONS

- Experiments in biospecimen compartment→ Supernatant, pellet, or homogenate?
- Experiments in stability in at-home collections
  - How many days does the DNA remain stable?
  - Impact of variation in shipping temperatures → Ambient environmental temperature in summer, winter extremes?
  - “People are human” → variability inherent in self-collection

- Marker selection refinement → MDM down-selection
- Independent validation of MDM panel
- Potential complementary molecular markers
- Voice of the customers → patients’ and providers’ preferences
- **Optimizing diversity of at-risk patients → Generalizability of the test**

NCT05051722: MAYO CLINIC'S ECHO STUDY



RECRUITING

Leveraging Methylated DNA Markers (MDMs) in the Detection of Endometrial Cancer, Ovarian Cancer, and Cervical Cancer (ECHO)

ClinicalTrials.gov ID NCT05051722

Sponsor Mayo Clinic

Information provided by Jamie N. Bakkum-Gamez, Mayo Clinic (Responsible Party)

Last Update Posted 2024-05-16

+ Expand all content — Collapse all content

Study Details | Researcher View | No Results Posted | Record History

On this page

- Study Overview
- Contacts and Locations
- Participation Criteria
- Study Plan
- Collaborators and Investigators
- Publications
- Study Record Dates
- More Information

Study Overview

Brief Summary

The overarching objective of this project is to develop a pan-gynecologic cancer detection test using gynecologic (unique endometrial, cervical, and ovarian cancer) cancer-specific methylated DNA markers and high-risk human papilloma virus (HR-HPV) detected in vaginal fluid and/or plasma.

This proposal defines Phase II MDM-based cancer detection studies in endometrial cancer (EC) and endometrial hyperplasia with atypia (AEH) in **tampon**-collected vaginal fluid and 2) ovarian cancer (OC) in plasma and **tampon**-collected vaginal fluid. Additionally, it defines necessary Phase I MDM-based cancer

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Detailed Description

Detection of endometrial, ovarian, and cervical cancers at an early stage vastly increases the chances of

Study Start (Actual) 2021-08-03

Primary Completion (Estimated) 2025-06-30

Study Completion (Estimated) 2025-12-30

Enrollment (Estimated) 2640

Clinicaltrials.gov

CELEBRATING AN INCREDIBLE TEAM!

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Exact Sciences

Shared Resources/ Cores
BAP
PRC
GAC
Biostatistics

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External Collaborating Institutions

Generous study participants!

THANK YOU!

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