HPV-Negative Results in Women Developing Cervical Cancer: Implications for Cervical Screening Options

R. Marshall Austin MD,PhD
Magee-Womens Hospital of
University of Pittsburgh Medical Center (UPMC)
(raustin@magee.edu)

Progressive CIN3

Pap detection &
Clinical Ablation

Early Stage
(Asymptomatic)
Cervical Cancer

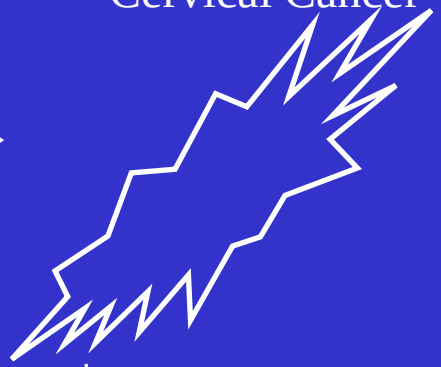
Outcome:

Incidence of
Cervical Cancer

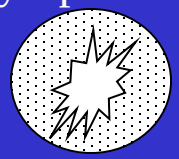
Early Stage
(Asymptomatic)
Cervical Cancer

Pap detection &
Clinical Ablation

Late Stage
(Symptomatic)
Cervical Cancer



+ Lymph Nodes

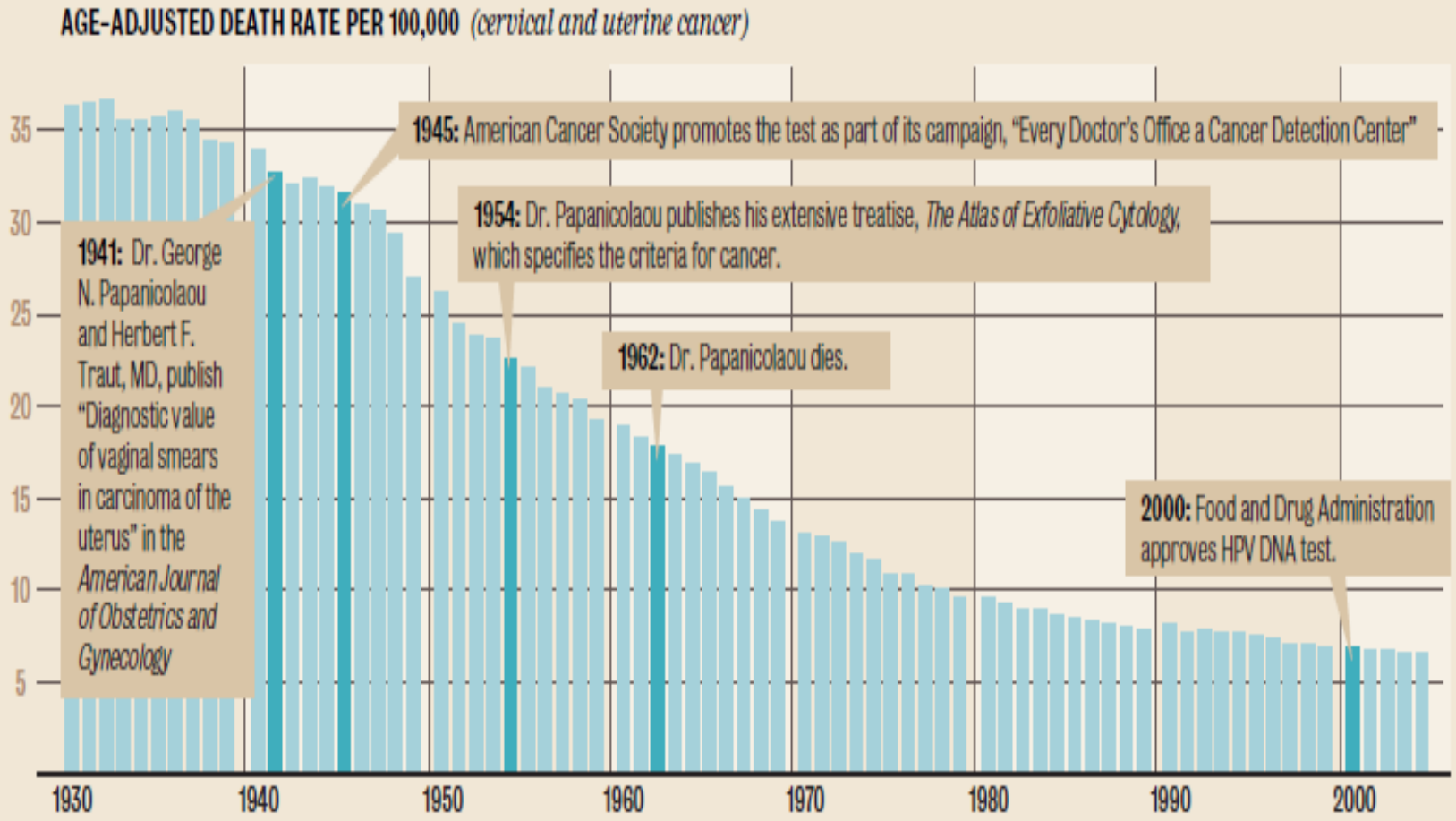


Outcome:

Morbidity and
Mortality due to
Cervical Cancer

MEASURING THE IMPACT

The Pap smear is credited with significantly decreasing the death rate from cervical cancer. Mortality rates for this disease alone are not available for all years, but the combined rates for this carcinoma along with uterine cancer went down by 82% from 1931 to 2004.



SOURCE: AMERICAN CANCER SOCIETY, CENTERS FOR DISEASE CONTROL AND PREVENTION

PUBLISHED IN THE SEPTEMBER 3, 2007 ISSUE OF AMERICAN MEDICAL NEWS. WWW.AMEDNEWS.COM

SCREENING FOR CERVICAL CANCER

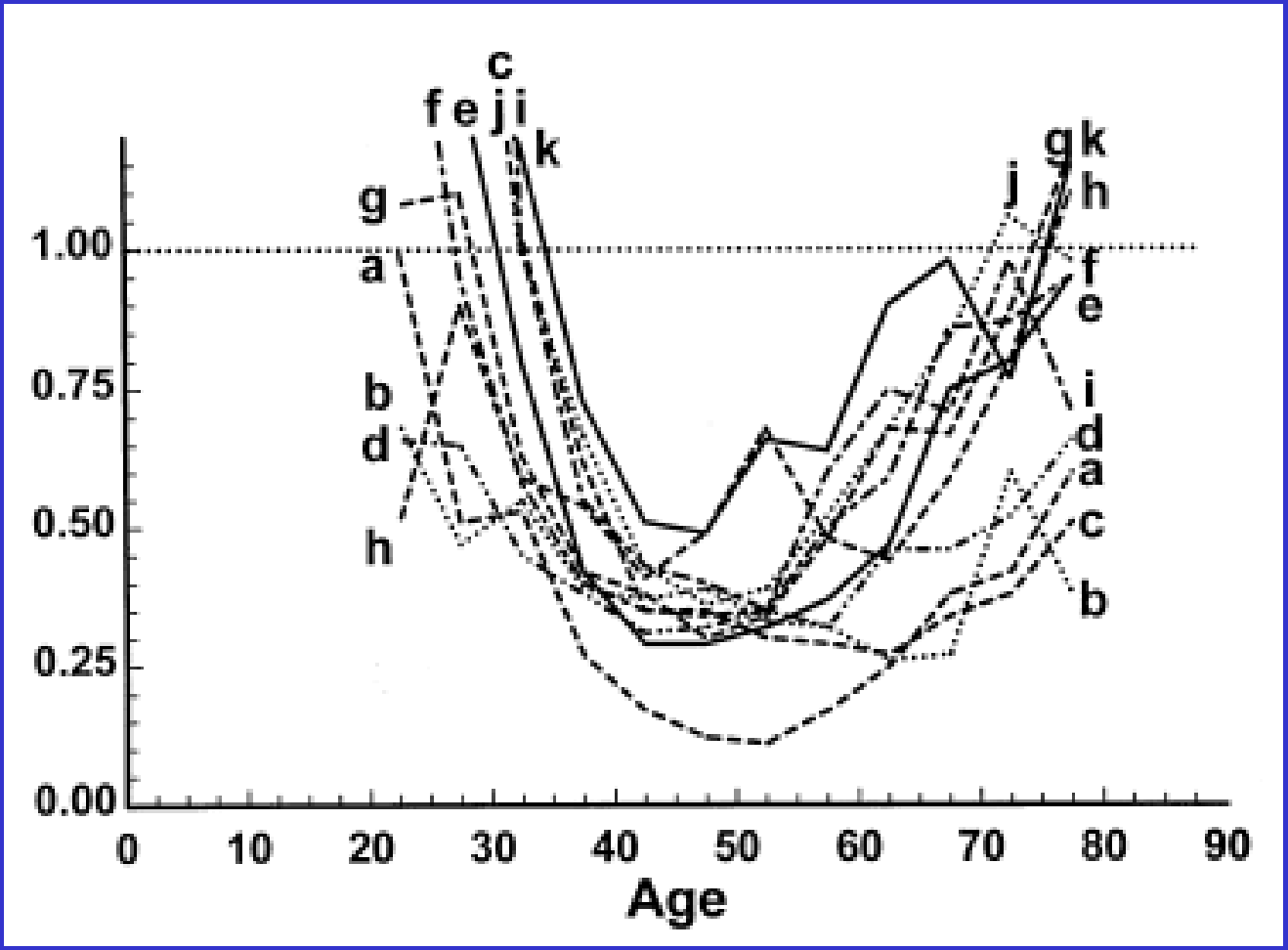
(SYSTEMATIC EVIDENCE REVIEW NUMBER 25)
US AGENCY FOR HEALTHCARE RESEARCH AND QUALITY

" Introduction of screening programs in populations naïve to screening reduces cervical cancer rates by 60% or more within three years of implementation."

"This reduction of morbidity and mortality is consistent and dramatic across populations."

Impact of Cervical Smear Screening on Cancer Rates Women of Varying Ages

Cancer Causes and Control 1997; 8: 755-763



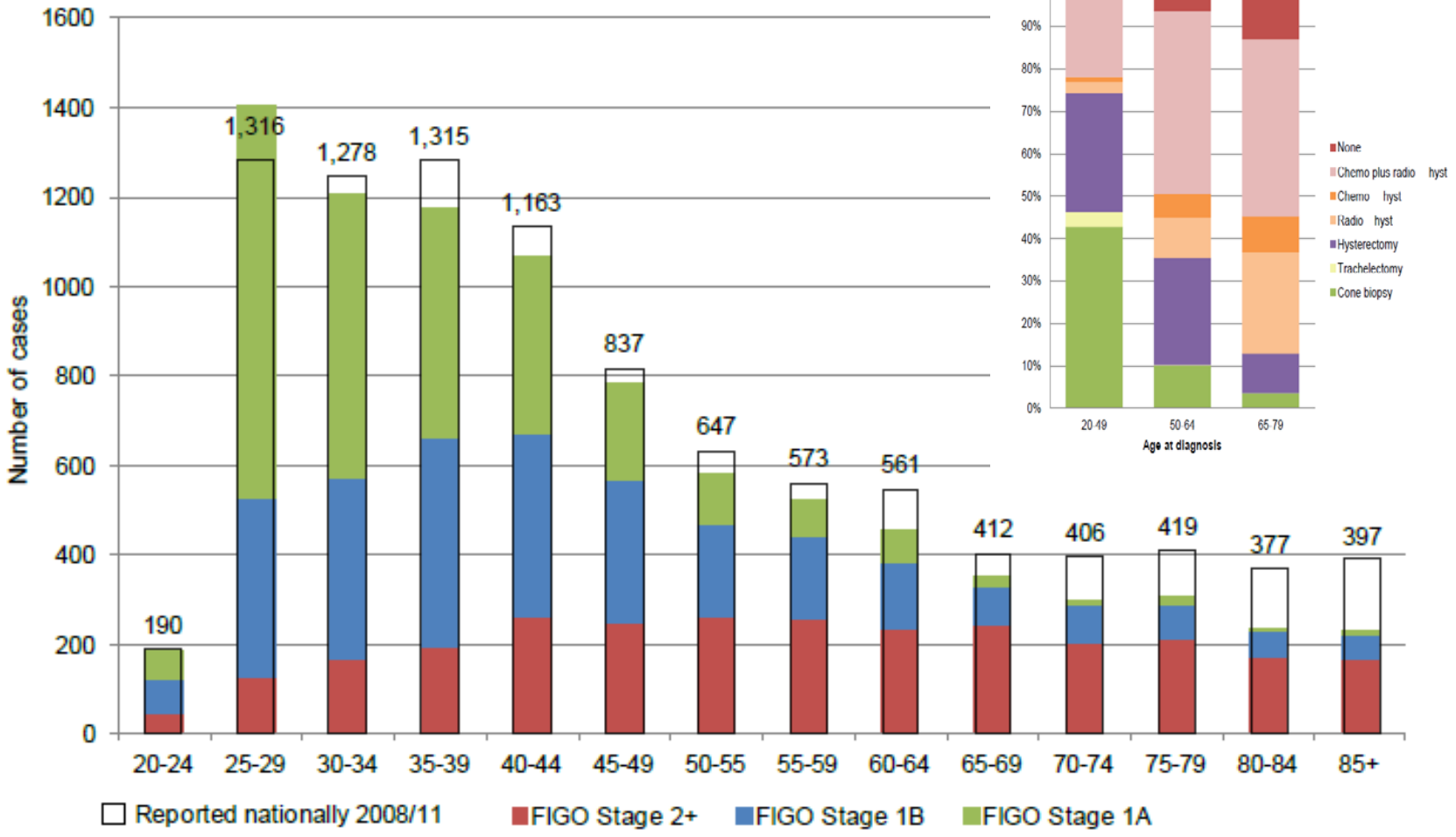
US Cervical Cancer Rates

(Uncorrected and Hysterectomy-Corrected Cervical Cancer Incidence Rates) Cancer 2014; 120: 2032-2038

Age Group	Uncorrected Rate	Corrected Rate
20-24	1.5/100,000	1.5/100,000
25-29	5.7/100,000	5.8/100,000
30-34	11.2/100,000	11.5/100,000
35-39	14.2/100,000	15.0/100,000
40-44	15.6/100,000	17.6/100,000
45-49	14.7/100,000	18.3/100,000
50-54	13.9/100,000	19.2/100,000
55-59	13.5/100,000	20.5/100,000
60-64	14.1/100,000	23.6/100,000
65-69	14.8/100,000	27.4/100,000

UK 2014 CxCa Audit Data

Figure 1 FIGO stage of cervical cancer cases: estimated percentage distribution, by age



Carcinogenic HPV said to be detectible in "virtually all" cervical cancers

Analysis of 932 specimens from women in 22 countries indicated prevalence of HPV DNA in cervical cancers worldwide = 99.7% [1]

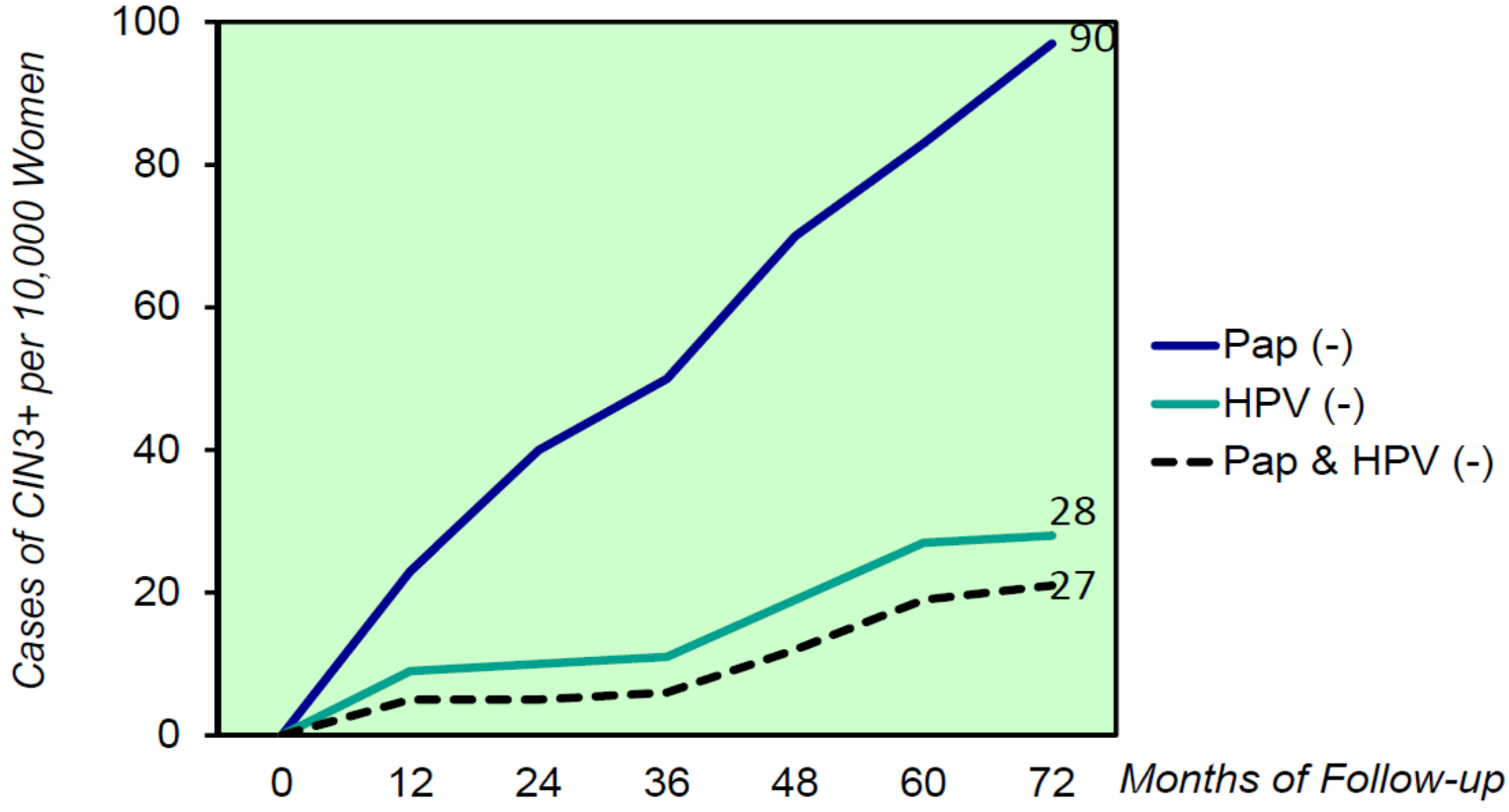
- International cervical cancer tissue samples which previously tested HPV-negative were analyzed for HPV DNA by 3 different polymerase chain reaction (PCR) -based assays (esp. E7 directed).[1]

"The extreme rarity of HPV-negative cancers reinforces the rationale for HPV testing in addition to, or even instead of cervical cytology in routine testing"

[1] Walboomers et al. J Pathol. 1999;189:12–19.

Risk of CIN3+ After Negative Screening Test

7 European follow-up studies; 24,295 women



Dillner *et al.* BMJ 2009;377

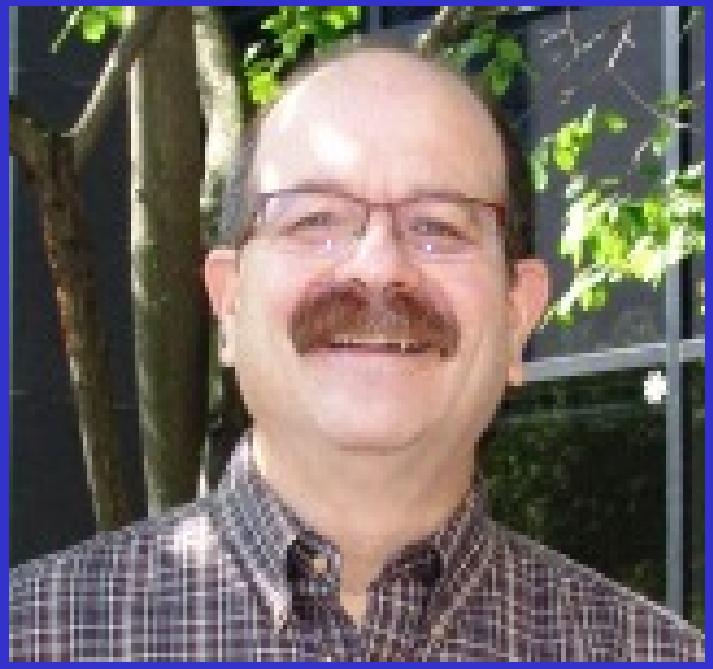
A New Approach for Cervical Cancer Prevention

- **HPV Vaccination** as the preferred new method for Primary Cervical Cancer Prevention.
- Move to primary **HPV screening at greatly extended screening intervals.**
- **Decrease costs by markedly decreasing clinical visits for screening and cytology and histology based testing (Secondary Prevention).**

Mark Schiffman MD, MPH

National Cancer Institute Senior Investigator
Division of Cancer Epidemiology & Genetics

"The high sensitivity of HPV DNA testing makes the test amenable to one or two screens in a lifetime."

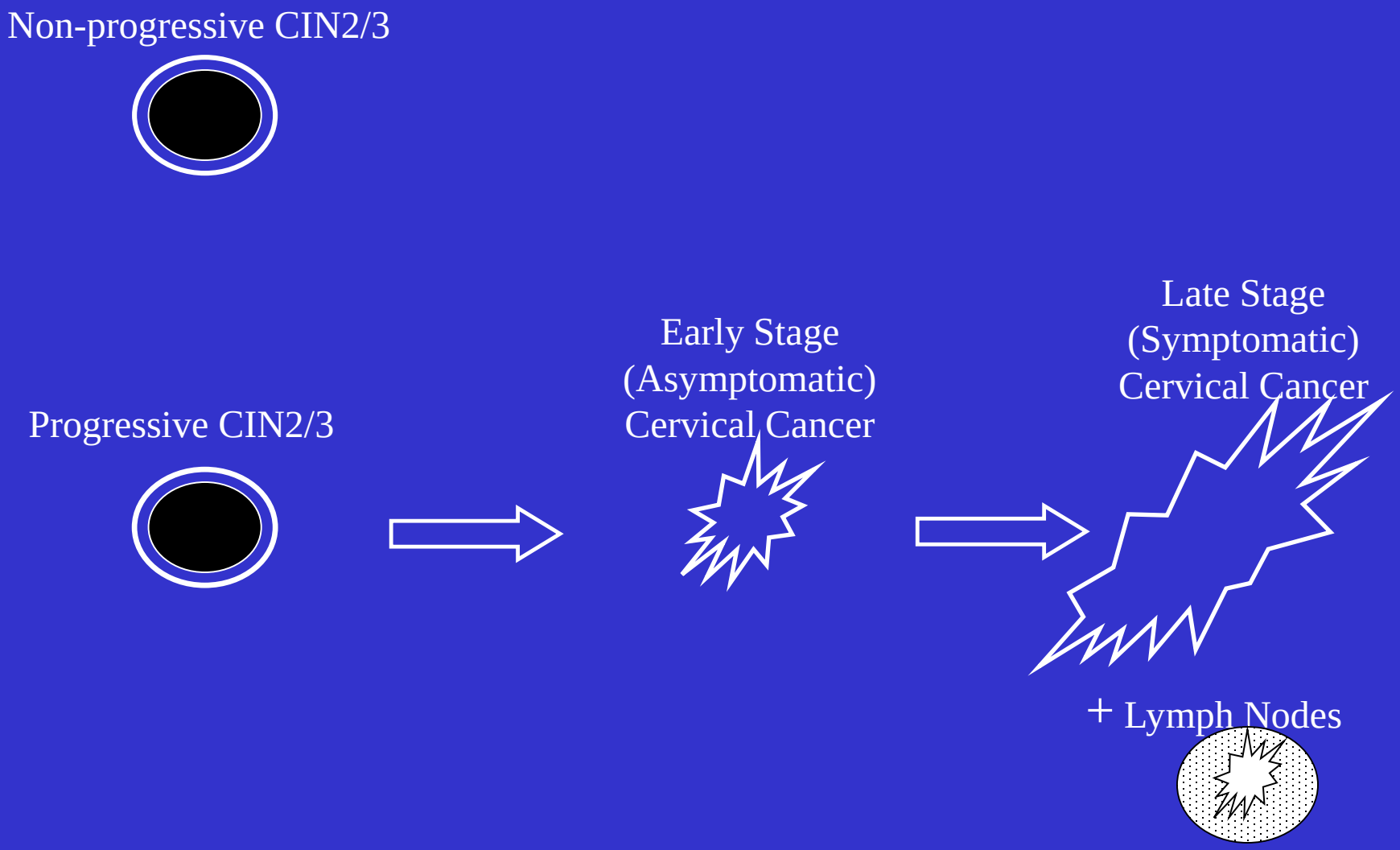


2015 NCI investigator site

http://dceg.cancer.gov/about/staff-directory/biographies/K-N/schiffman-mark

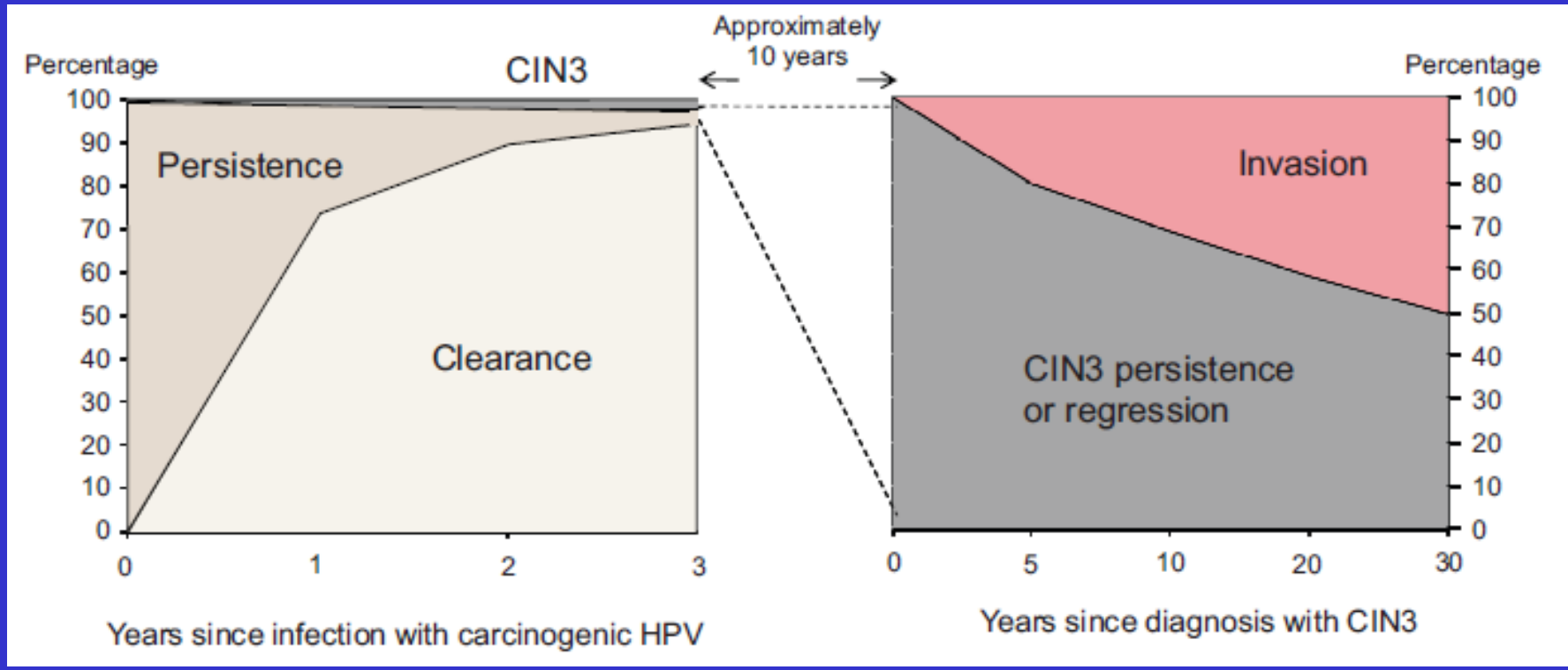
CIN2/3+ ≠ invasive Cervical Cancer

(CIN2/3+ used in trials as a “surrogate” for Cervical Cancer)



From Human Papillomavirus to Cervical Cancer

Obstet Gynecol 116: 177-185, 2010



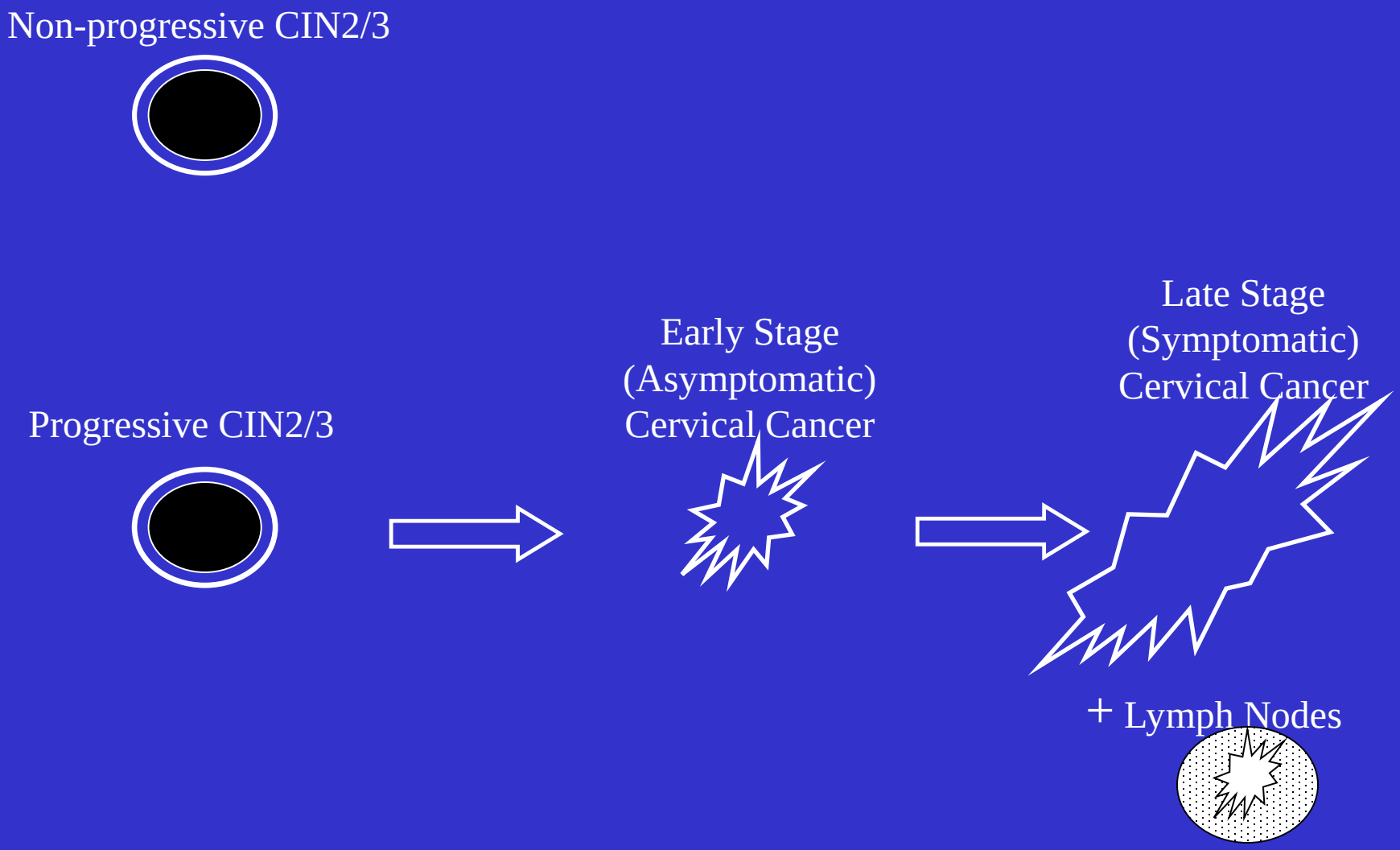
Natural History of Cervical Neoplasia and Risk of Invasive Cancer in Women with CIN3: A Retrospective Cohort Study

Lancet Oncol 2008; 9: 425-434 (New Zealand)

- In 143 women managed only by punch or wedge biopsy, the cumulative incidence of invasive cancer was **31% at 30 years**.
- In 593 women whose initial treatment was cone biopsy, the cumulative incidence of invasive cancer was **0.7% at 30 years**.

CIN2/3+ ≠ invasive Cervical Cancer

(CIN2/3+ used in trials as a “surrogate” for Cervical Cancer)



Assumptions

(J Med Screening 2013; 20: 99-103)

- **"Assuming HPV testing is 95% sensitive for cancers that would develop over the next 6 years…"**
- **Castanon A, Landry R, Sasieni P. How much could human papillomavirus testing reduce cervical cancer incidence and mortality?**

HC2 False Negative Rates with Cervical Cancer Diagnoses

Reference	Country	# Cervical cancers	Collection Vial	# Positive HC2	Negative HC2 (%)
JClinVirol 2006 35:264-9	China	475	Standard Transport Medium (STM)	427	48 (10.1%)
IntJGynCa 2009 19:924-8	Korea	198	STM	185	13 (6.6%)
IntJGynCa 2008 18:104-9	Brazil	168	STM	148	20 (11.9%)
BJOG 2015 122:119-127	Holland	136	STM	122	14 (10.2%)
ActaDematoven APA 2009; 18:94-103	Slovenia	95	STM	83	12 (12.6%)
	Total	1072		965	107 (10.0%)

HPV False Negative Rates Prior to Cervical Cancer Diagnoses

TIME PRIOR TO CxCA DX	HPV FALSE NEG RATE	# CxCA	REFERENCES
0	10%	1072	5 prior references
< 1 yr	19%	526	Cancer Cytopathology 2015; 123: 282-288
1-3 yrs	23%	26	Arch Path Lab Med 2015; 139: 184–188
< 5 yrs	31%	87	Lancet Oncology 2011; 12: 662-672
2.5- 8 yrs	42%	19	Lancet 2014; 383: 524- 532

Updated Kaiser Data (Eurogin 2016)

- From 2003 through 2013, 699 women underwent cotesting at Kaiser Permanente Northern California prior to the diagnosis of invasive cervical cancer.
- One hundred seventy (24%) of the 699 cervical cancer patients later diagnosed with had at least one HPV-negative cotest at some time interval prior to the diagnosis of cervical cancer.

Lancet 2014; 383: 524-532

- Ronco et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomized controlled trials.
- "the effect of HPV testing- as an alternative to regular screening- on incidence of invasive cancer has not been assessed adequately."

Lancet 2014; 383: 524-532

STUDY ARM	# CX CANCERS	≤ 2.5 YEARS	> 2.5 YEARS
EXPERIMENTAL (HPV)	44	25/233,709	19/419,500
CONTROLS (CYTOLOGY)	63	27/202,549	36/358,656
	107	52/436,258	55/778,156

Italy	(NTCC)	CONVENTIONAL SMEARS
Holland	(POBASCAM)	CONVENTIONAL SMEARS
Sweden	(SWEDESCREEN)	CONVENTIONAL SMEARS
England	(ARTISTIC)	LIQUID BASED CYTOLOGY

Increased Cervical Cancer Protection with Quality-Controlled UK LBC
Lancet 2014; 383: 524-532

LBC-UK ARTISTIC	FOLLOW-UP TIME	CxCaDx ≤ 2.5 YRS	CxCaDx > 2.5 YRS	NEGATIVE BASELINE
HPV	8 YRS	5	5	3
LBC	8 YRS	4	0	0

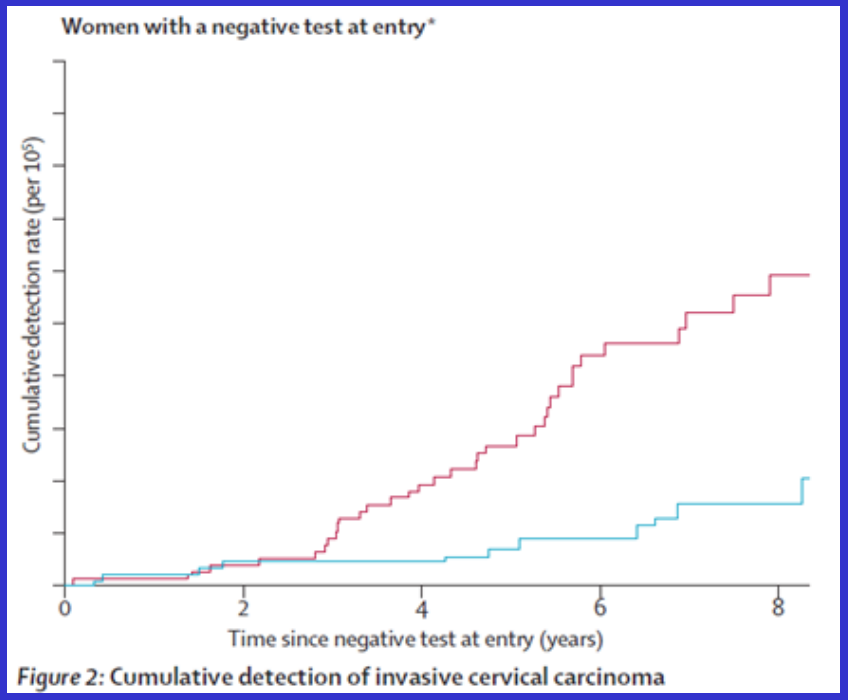
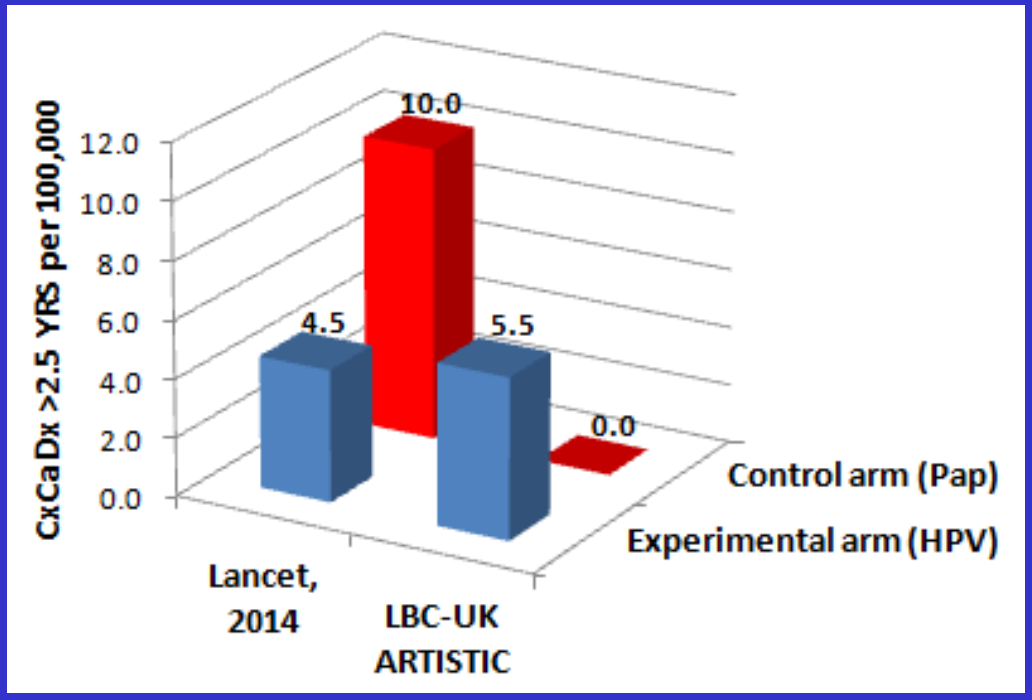


Figure 2: Cumulative detection of invasive cervical carcinoma

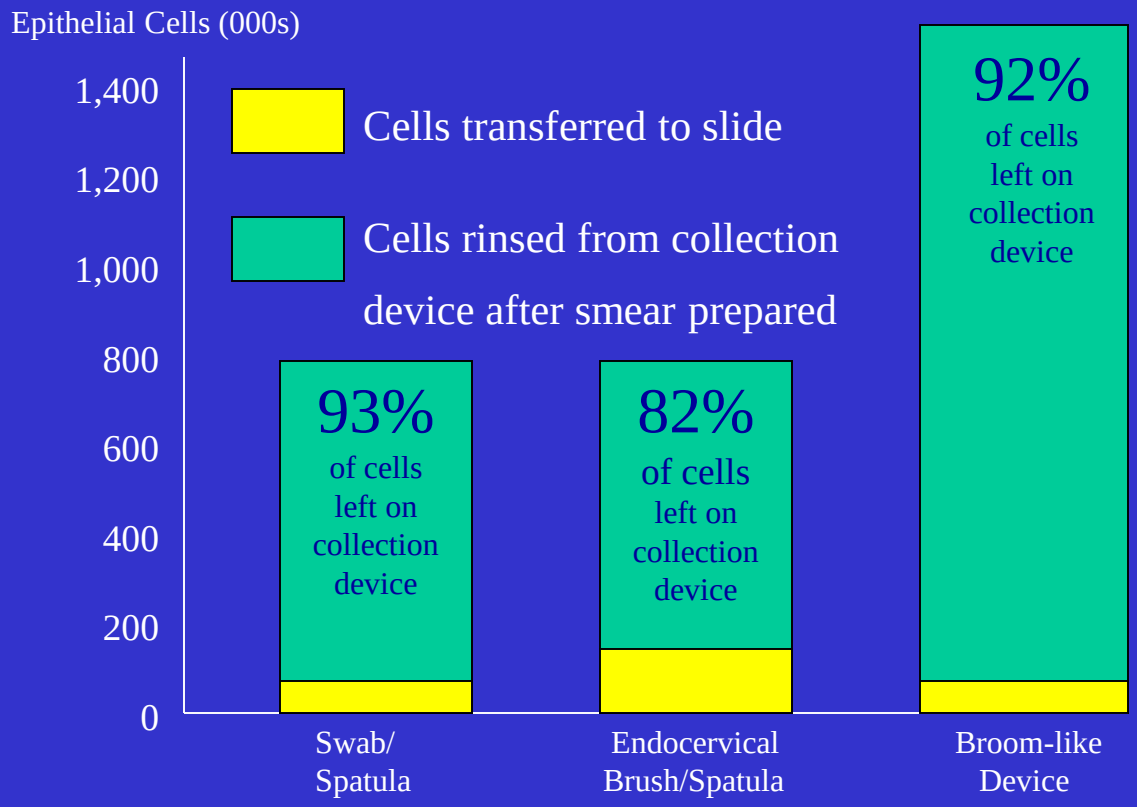


Lancet 2014; 383: 524-532

- 42% HC2 false negative rate in 8 of 19 cervical cancers diagnosed 2.5-8 years after onset of trials in the HPV trial arms.
- No increased protection from cervical squamous carcinoma in HPV trial arms.
- No cervical cancer protection in younger women under age 30 in HPV trial arms.
- Opposite results in UK ARTISTIC Trial using quality-optimized LBC screening.

Sampling Challenges

Percentage of Obtained Sample Transferred to Slide



More than 80% of the sample may be discarded with the collection device with the conventional Pap smear [1]

[1] Hutchinson ML, et al. *Am J Clin Pathol* 1994.

Conventional Pap Smears vs. Liquid-Based Cytology

- **Holland:** JAMA 2009; 302: 1757-1764
- **Italy:** BMJ 2007; 335: 28
- **UK:** Health Technology Assessment 2009; Vol. 13: No. 51

"It is difficult to escape the conclusion that LBC was more sensitive in ARTISTIC than earlier conventional cytology"

US Assessment of HPV Types in Cancers: Implications for Current and 9-Valent HPV Vaccines (JNCI (2015) 107(6): djv086)

- HPV testing on 2670 archival (1993-2005) tumor registry cervical cancer tissues
- New 9-Valent vaccine covers HPV genotypes detectible in 80.8% of cervical cancers.
- "The finding that nearly 10% of all cervical cancers tested negative and that the proportion of cervical tissue testing negative for HPV increased with age is consistent with other studies… we have no definitive explanation for these patterns"

HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases

Edyta C Pirog[1], Belen Lloveras[2], Anco Molijn[3], Sara Tous[4], Núria Guimerà[3], Maria Alejo[5], Omar Clavero[4], Joellen Klaustermeier[4,6], David Jenkins[3], Wim GV Quint[3], Francesc Xavier Bosch[4], Laia Alemany[4,6,7], Silvia de Sanjosé[4,6,7] and on behalf of the RIS HPV TT study group

Table 3 HPV positivity by histologic adenocarcinoma subtypes

Histologic adenocarcinoma subtypes	N	*N HPV+*	*%*	*Single HPV infection*	*%[a]*	*Multiple HPV infection*	*%[a]*	*HPV unknown*	*%[a]*
Classic	567	407	71.8	375	92.1	27	6.6	5	1.2
Not otherwise specified	36	5	13.9	4	80.0	1	20.0	0	0.0
Clear cell	30	6	20.0	5	83.3	1	16.7	0	0.0
Serous	24	6	25.0	6	100	0	0.0	0	0.0
Minimal deviation	12	1	8.3	1	100	0	0.0	0	0.0
Endometrioid	11	3	27.3	2	66.7	1	33.3	0	0.0
Mixed: serous and clear cell	2	0	0.0	–	–	–	–	–	–
Total	682	428	62.8	393	91.8	30	7.0	5	1.2

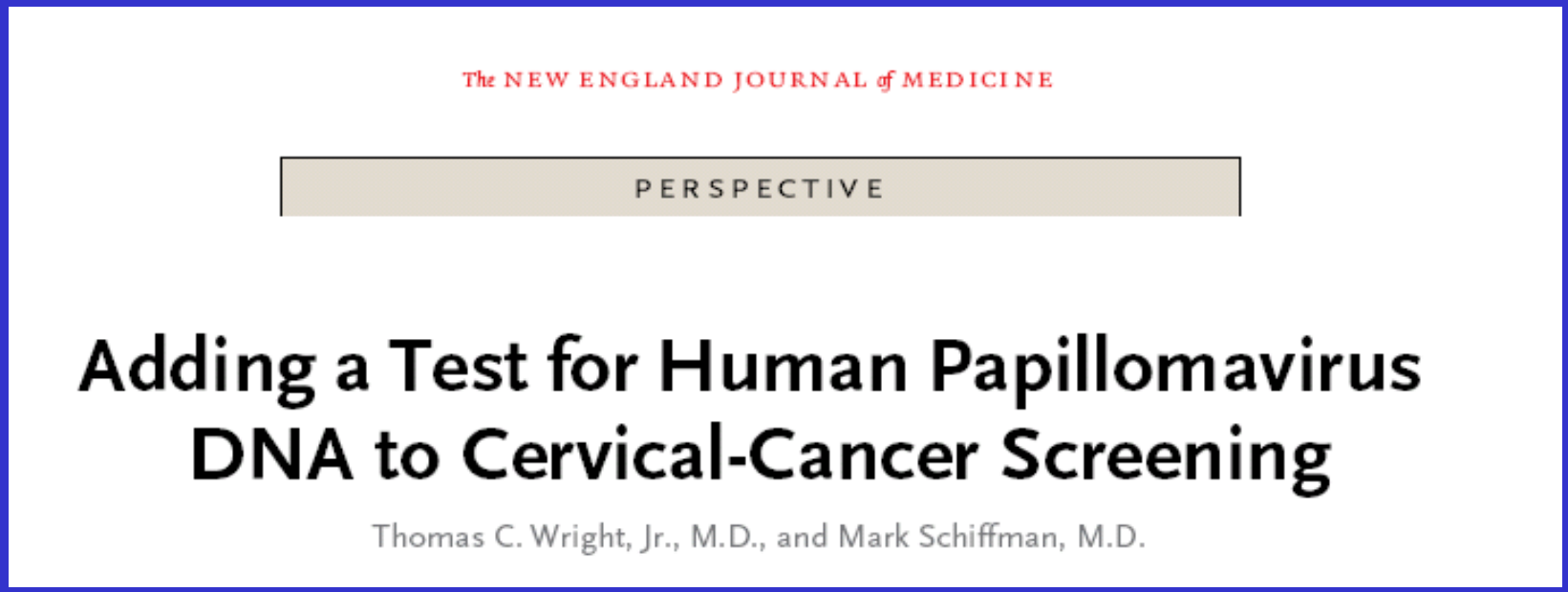
N+: HPV-positive adenocarcinomas; %: HPV positivity among HPV analyzed adenocarcinomas; %[a]: percent among HPV-positive cases; HPV unknown: DEIA +/$LiPA_{25}$ −.

Pirog et al Modern Pathology 2014 1–9

NEJM 348: 489-490, 2003 (Feb 6, 2003)

"Testing for HPV DNA will probably soon receive approval from the FDA

for use in conjunction with cytologic analysis in primary screening for cervical cancer in women 30 years of age and older"



The NEW ENGLAND JOURNAL of MEDICINE

PERSPECTIVE

Adding a Test for Human Papillomavirus DNA to Cervical-Cancer Screening

Thomas C. Wright, Jr., M.D., and Mark Schiffman, M.D.

US Cotesting and CxCa Diagnoses
(Cancer Cytopathology 2015; 123: 282-288)

Among the 526 cervical cancers

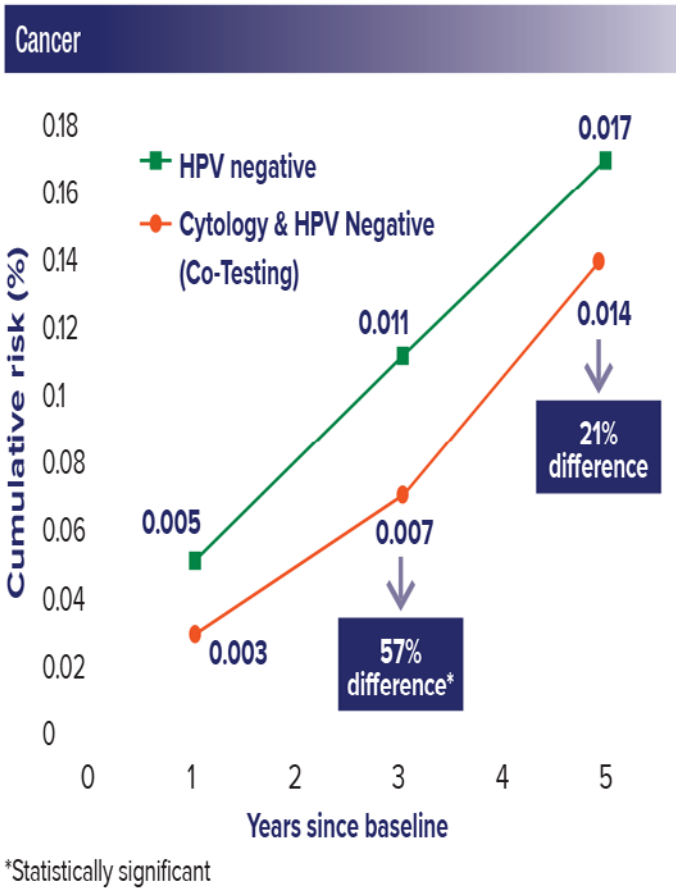
Test Result	n (%)
HPV negative	98 (18.6)
Pap negative	64 (12.2)
Cotest negative	29 (5.5)

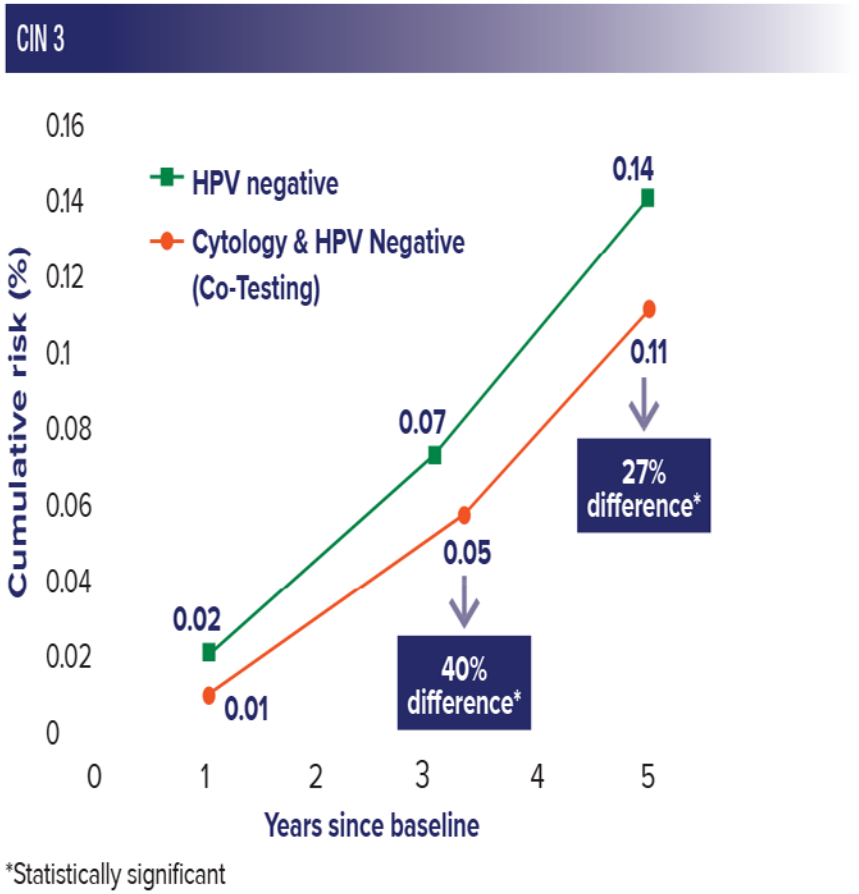
Among the 169 adenocarcinomas*

Test Result	n (%)
HPV negative	45 (26.6)
Pap negative	35 (20.7)
Cotest negative	14 (8.3)

* Adenocarcinoma verified as cervical in origin

Kaiser HC2 Cotesting Experience
(JNCI 2014; 106(8)





University of Pittsburgh Medical Center (UPMC)
Magee-Womens Hospital (MWH)
Department of Pathology
and School of Information Sciences
University of Pittsburgh

Cervical Screening and Risk Stratification using Bayesian Decision Science Technology

R. Marshall Austin, MD-PhD, Agnieszka Oniśko, PhD, Marek J. Druzdzel, PhD

Increased cervical cancer risk associated with extended screening intervals after negative human papillomavirus test results: Bayesian risk estimates using the Pittsburgh Cervical Cancer Screening Model (J Am Soc Cytopathol 2016; 5: 9-14)

- The analyzed database included cervical screening data collected over 10 years (2005-2014) at Magee Womens Hospital with 976,624 liquid-based cytology (LBC) results, 285,351 companion high-risk FDA-approved HPV test results from LBC vials, and 112,435 follow-up histopathologic results from surgical procedures with cervical tissue sampling..
- Histopathologic cervical cancer risk estimates for patients with prior double negative results with cervical LBC and from-the-vial HPV cotesting were computed using the PCCSM for women rescreened at intervals ranging from 1 to 9 years.
- Similar risks were computed for women with any negative HPV test result, not considering cytology results.

Cervical Cancer Risk after Double Negative Results or After Any Negative HPV Result (J Am Soc Cytopathol 2016; 5: 9-14)

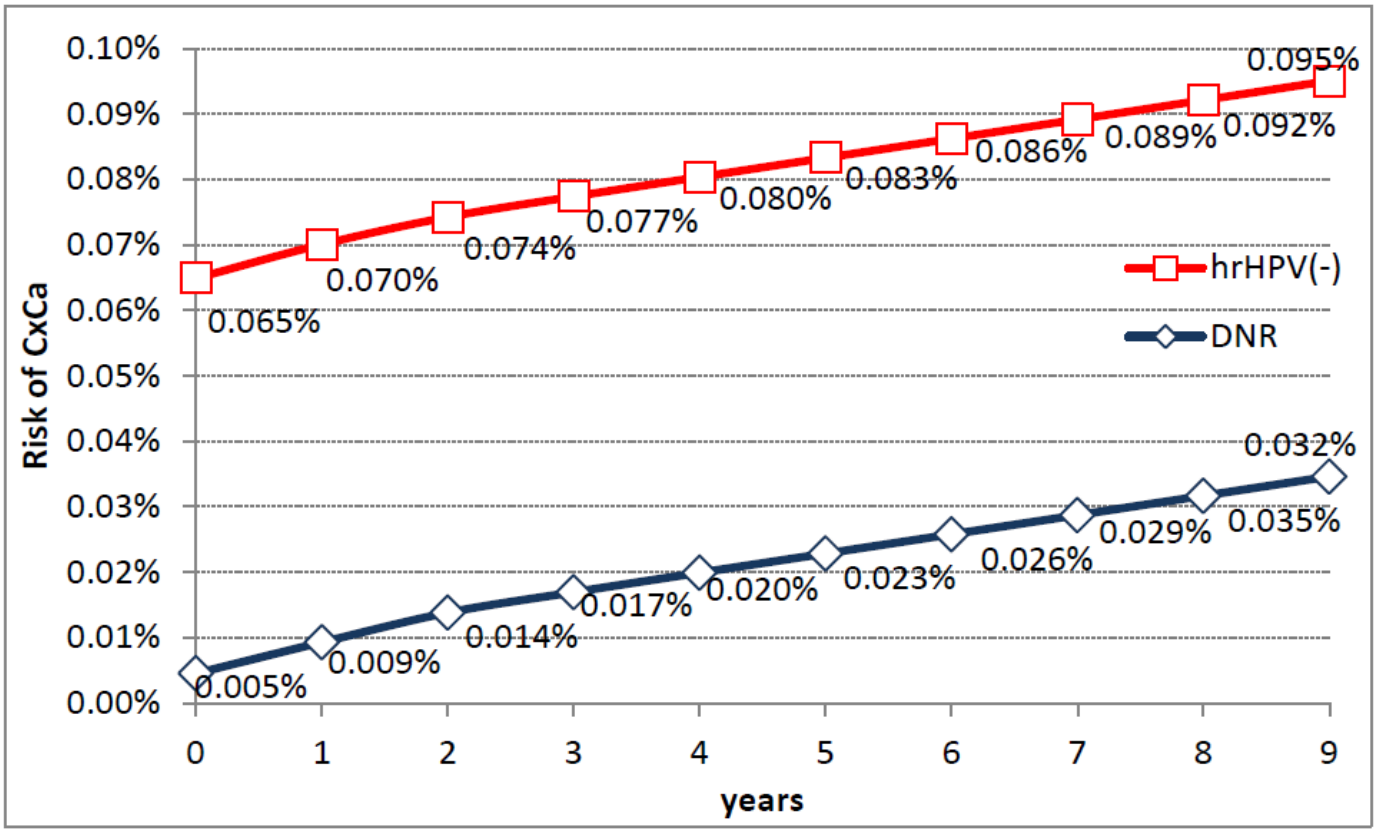
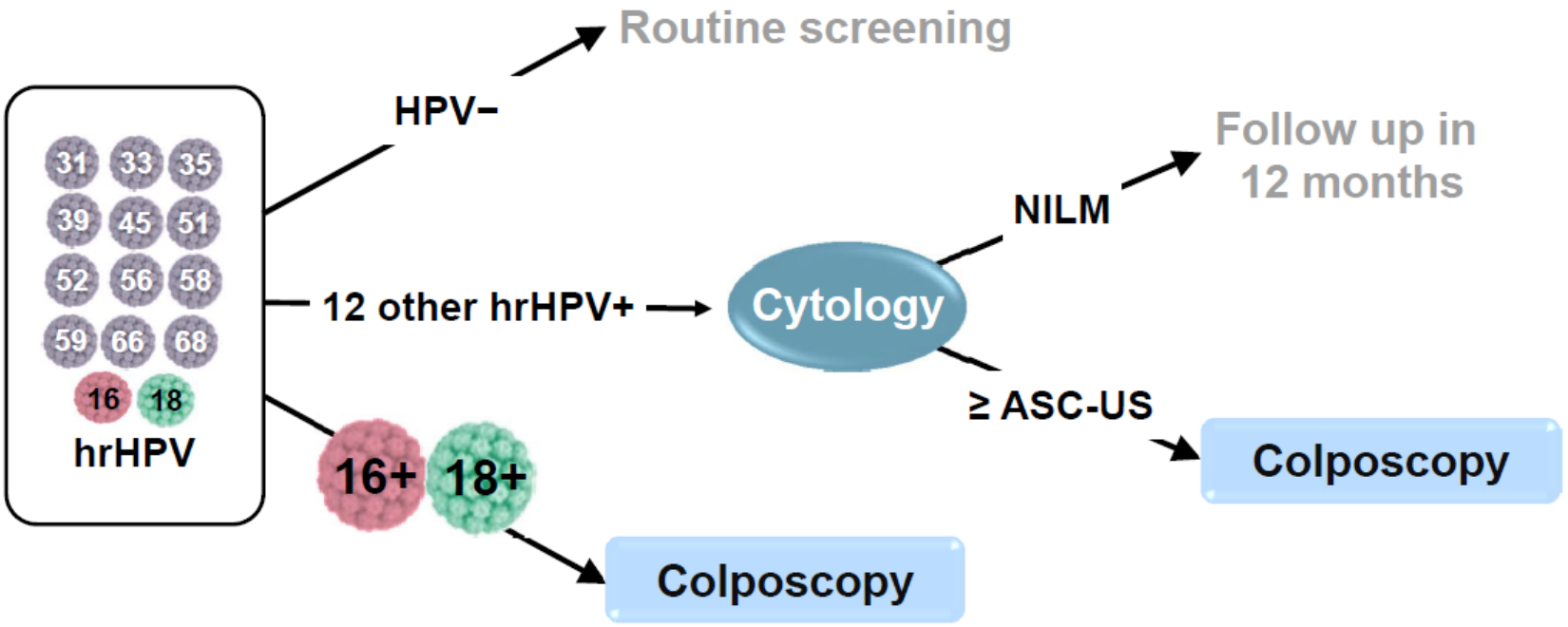


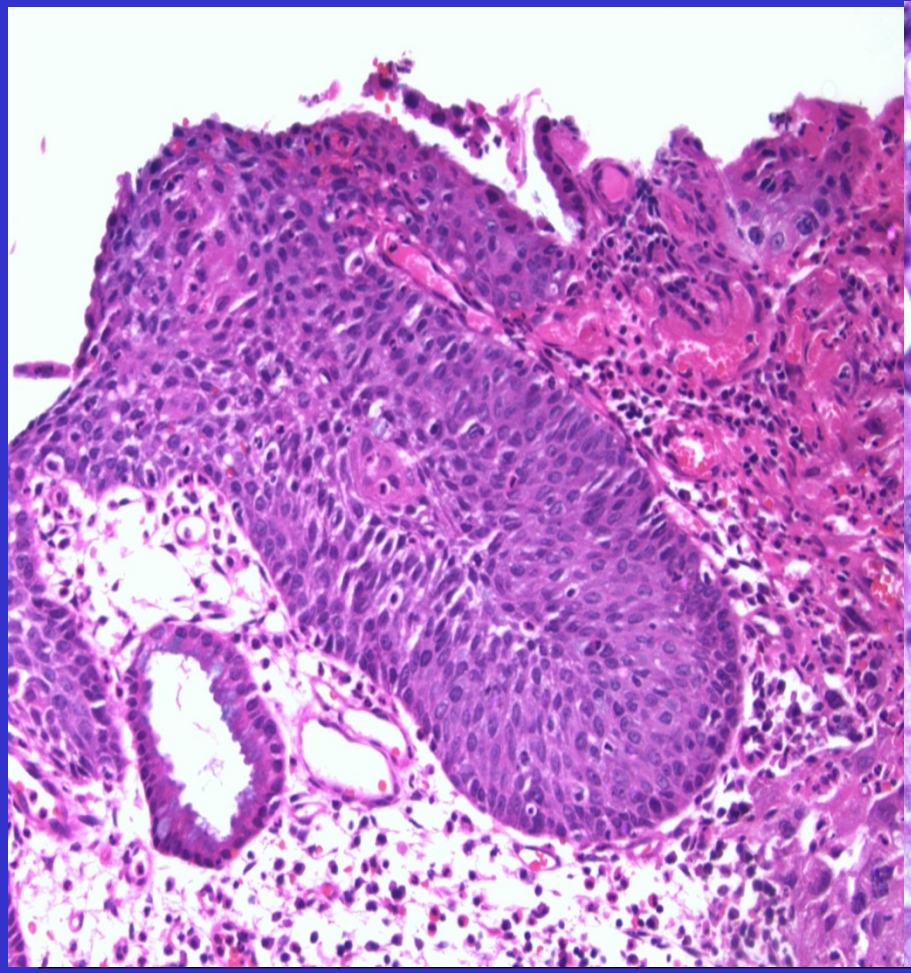
Figure 1: PCCSM risk projections for histopathologic diagnosis of invasive cervical carcinoma with extended rescreening intervals

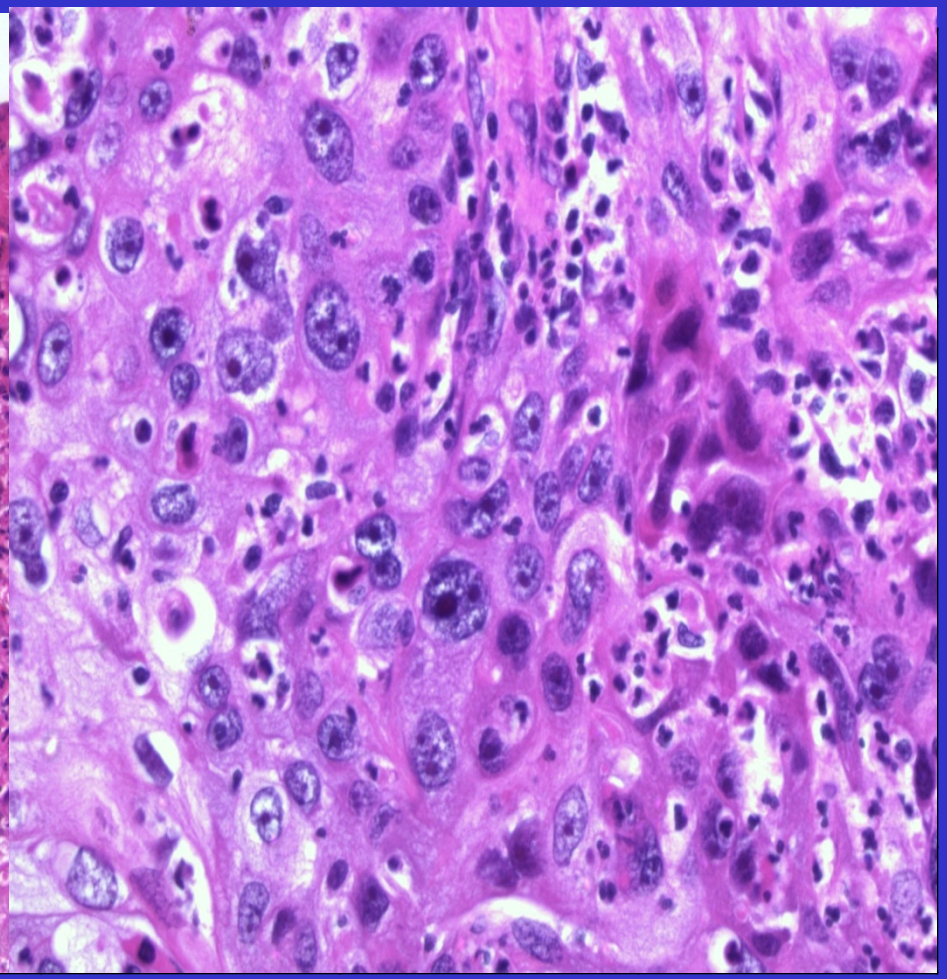
Primary HPV Screening

With HPV16/18 Genotyping and Reflex Cytology in women ≥ 25



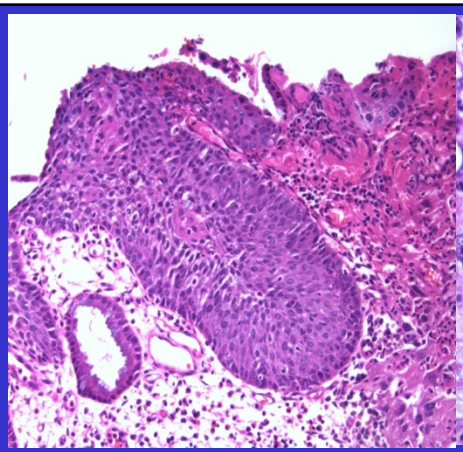
CIN3+ Trial Endpoint
(CIN3 or invasive cervical cancer)

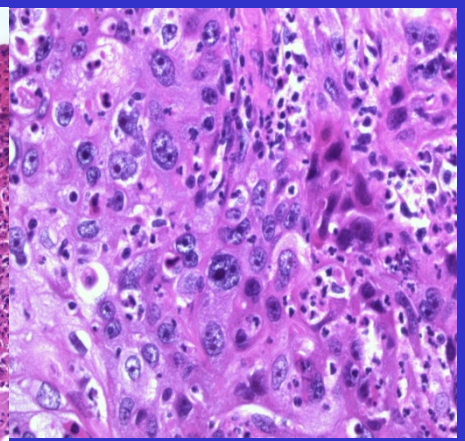




CIN3+ SENSITIVITY

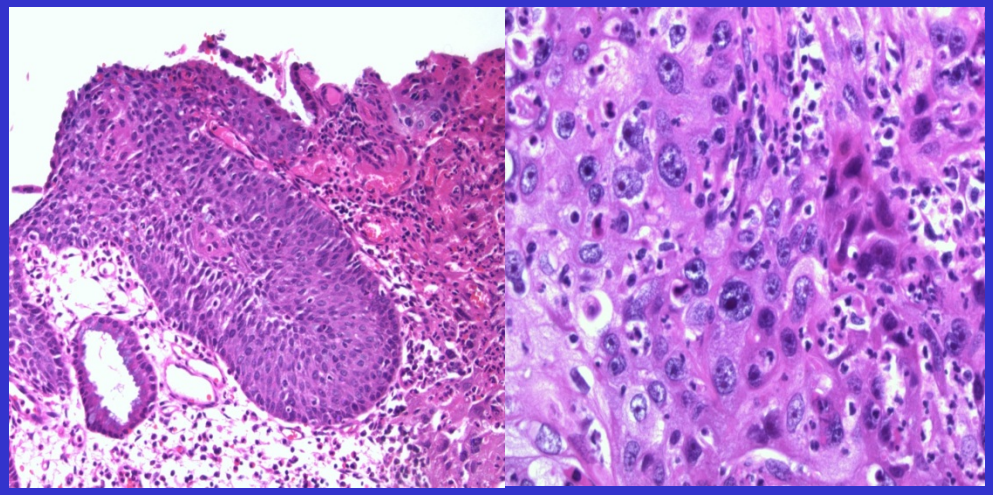
AGE GROUP	CERVICAL CANCER RATES/100,000 SEER DATA 2000-2009 Cancer 2014; 120: 2032-2038
25-29	5.8 annual CxCa/100,000
30-39	11.9-14.6 annualCxCa/100,000
40-49	18.1-18.6 annualCxCa/100,000
50+	19.8-27.4 annualCxCa/100,000





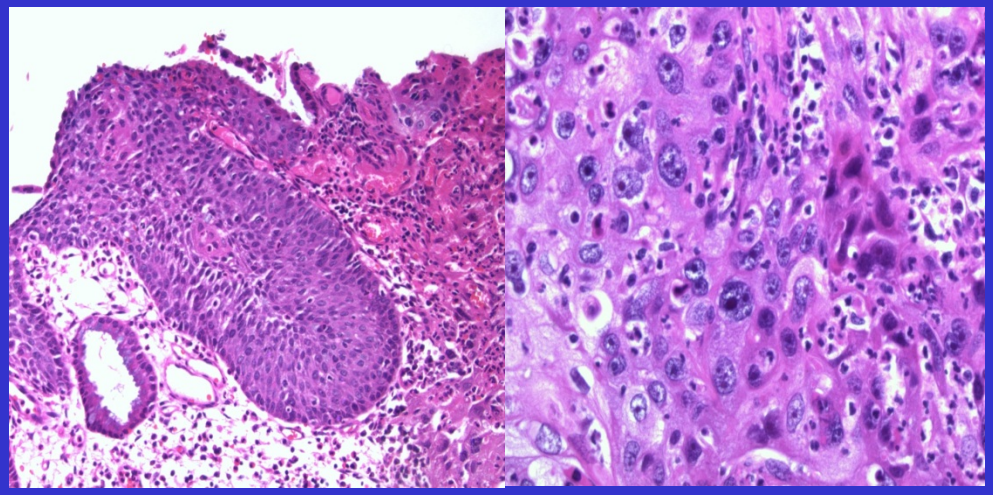
CIN3+ SENSITIVITY

AGE GROUP	CIN3+ SENSITIVITY (VERIFICATION BIAS-ADJUSTED)
≥25 (25+)	
≥30 (30+)	
≥40 (40+)	
≥50 (50+)	27.26%



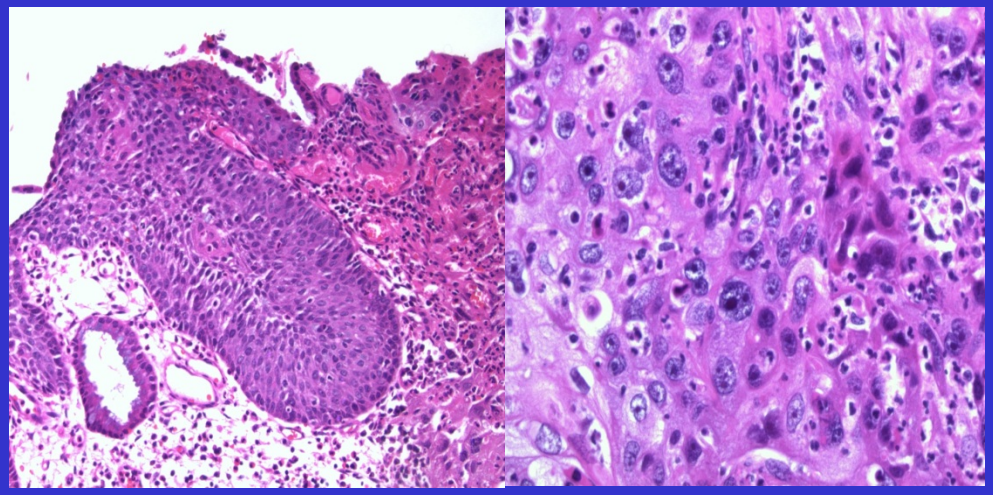
CIN3+ SENSITIVITY

AGE GROUP	CIN3+ SENSITIVITY (VERIFICATION BIAS-ADJUSTED)
≥25 (25+)	
≥30 (30+)	
≥40 (40+)	36.09%
≥50 (50+)	27.26%



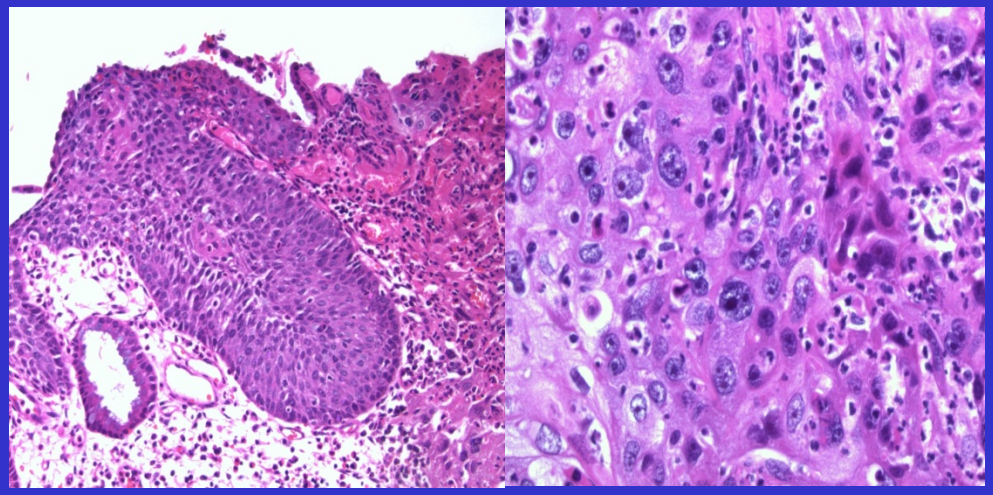
CIN3+ SENSITIVITY

AGE GROUP	CIN3+ SENSITIVITY (VERIFICATION BIAS-ADJUSTED)
≥25 (25+)	
≥30 (30+)	53.56%
≥40 (40+)	36.09%
≥50 (50+)	27.26%



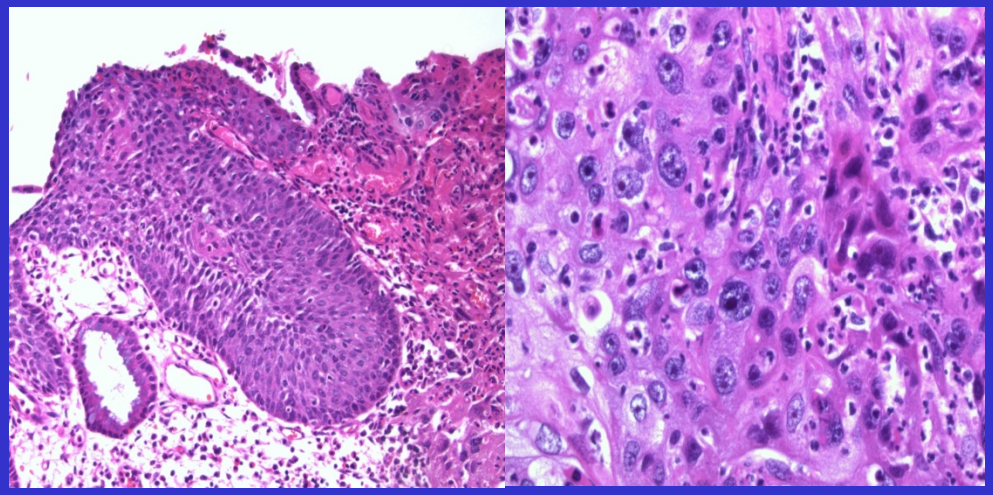
CIN3+ SENSITIVITY

AGE GROUP	CIN3+ SENSITIVITY (VERIFICATION BIAS-ADJUSTED)
≥25 (25+)	58.26%
≥30 (30+)	53.56%
≥40 (40+)	36.09%
≥50 (50+)	27.26%



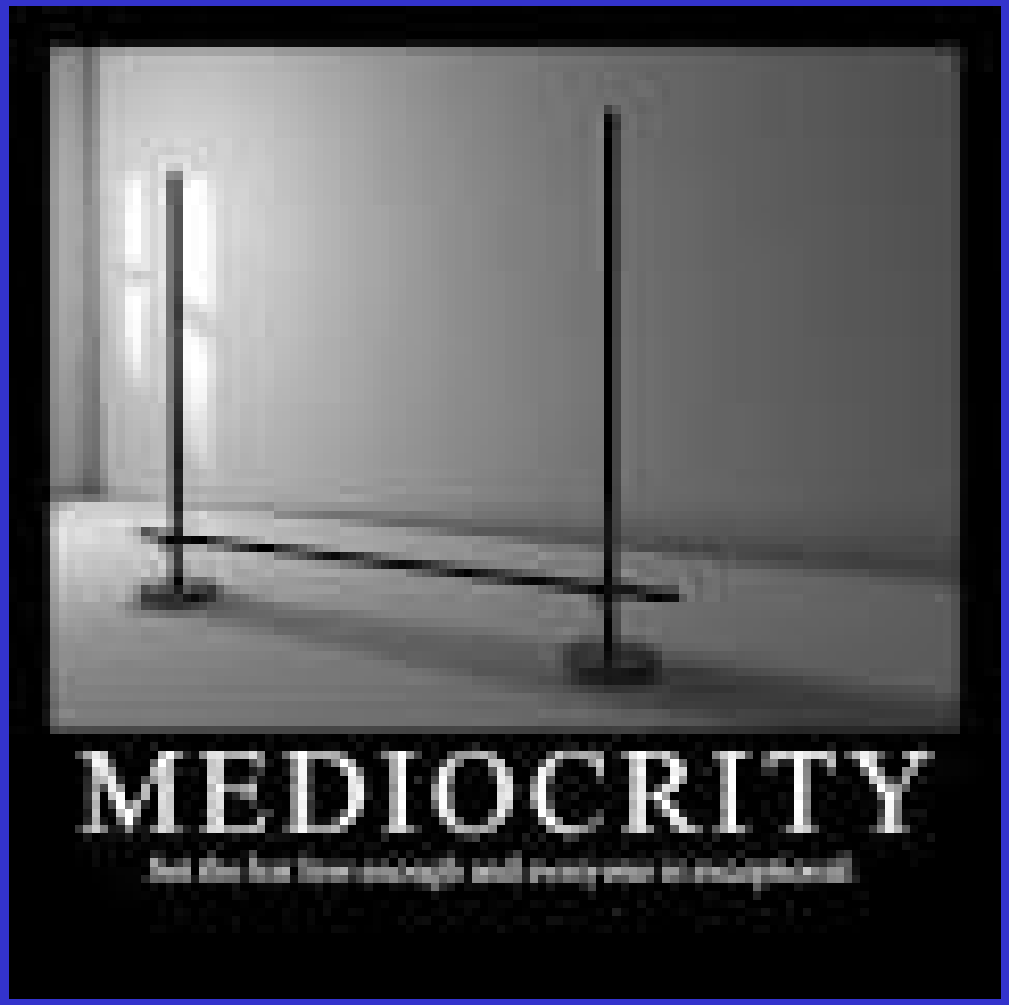
CIN3+ SENSITIVITY
FDA ATHENA TRIAL DATABASE

AGE GROUP	CIN3+ SENSITIVITY (VERIFICATION BIAS-ADJUSTED)
≥25 (25+)	58.26%
≥30 (30+)	53.56%
≥40 (40+)	36.09%
≥50 (50+)	27.26%



Why was an HPV testing algorithm with such low sensitivity for detection of CIN3+ (CIN3 or cervical cancer) approved by the FDA?

Suboptimal Cytology Laboratories (<50% Sensitivity) Selected for the ATHENA Trial



SENSITIVITY OF COBAS HPV PRIMARY SCREENING ALGORITHM FOR DETECTION OF CARCINOMA-IN-SITU (CIN3) OR INVASIVE CERVICAL CANCER (CIN3+) IN FDA ATHENA TRIAL DATABASE

AGE GROUP IN ATHENA TRIAL	COBAS CIN3+ VERIFICATION BIAS-ADJUSTED SENSITIVITY	CYTOLOGY CIN3+ VERIFICATION BIAS-ADJUSTED SENSITIVTY
≥ 25	58.26%	42.63%
≥ 30	53.56%	42.40%
≥ 40	36.09%	33.45%
≥ 50	27.26%	27.04%

2014 Meeting Materials of the Microbiology Devices Panel

FDA Executive Summary: March 12, 2014

Biopsy-confirmed CIN2+ Results Using LBC and Cobas HPV testing in routine clinical practice (Houston)

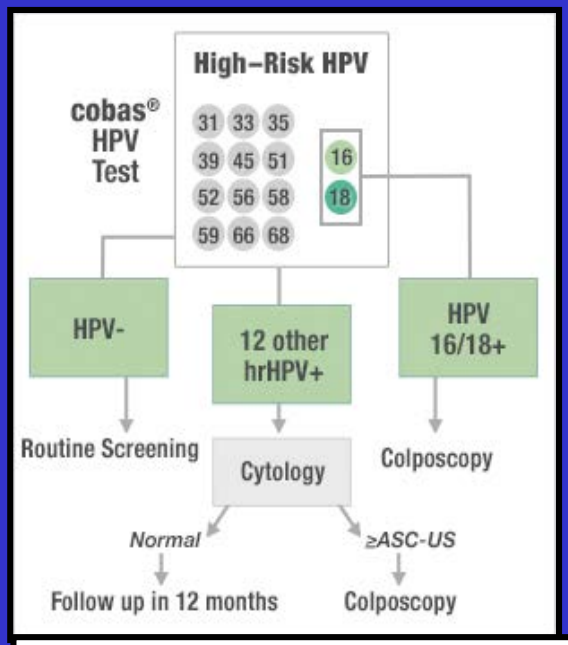
Test	Sensitivity, %
Pap alone	90.9%
Cobas HPV alone	91.2%
Co-testing	98.8%

(Cancer Cytopathology 2016; 124: 317-323)

Co-Testing Modelling Analysis
(J Womens Health 2016; 25: 1-6)

AGES 30-70 YEARS		
OUTCOMES	CO-TESTING	HPV PRIMARY
Cervix Cancers/10,000	57.61	79.02
Cervix Ca Deaths/10,000	23.06	43.47
Lifetime QALYs	23.0084	22.9861
Screening Costs (USD)	$1,319	$1,129
Treatment Costs (USD)	$1,007	$1,236
Total Costs (USD)	$2,326	$2,365

Cobas HPV Primary Screening vs PapHPV Co-testing



Detection of ≥CIN3 (CIN3+)
CIN3 or CxCancer (FDA)

- **58% (≥25 yrs)**
- **53% (≥30 yrs)**
- **36% (≥40 yrs)**
- **27% (≥50 yrs)**

WHY?

Increased Disease Detection

Informed Refusal with Full Knowledge of HPV False Negative Rates Prior to Cervical Cancer Diagnoses?

TIME PRIOR TO CxCA DX	HPV FALSE NEG RATE	# CxCA	REFERENCES
0	10%	1072	5 prior references
< 1 yr	19%	526	Cancer Cytopathology 2015; 123: 282-288
1-3 yrs	23%	26	Arch Path Lab Med 2015; 139: 184–188
< 5 yrs	31%	87	Lancet Oncology 2011; 12: 662-672
2.5- 8 yrs	42%	19	Lancet 2014; 383: 524- 532

Challenges in measuring effectiveness of new cervical cancer prevention policies

1) Few countries with effective school-based HPV vaccination programs for young girls.
2) Vaccinated cohort will not reach age of high cervical cancer incidence for decades.
3) HPV screening in most clinical trials has shown enhanced sensitivity but low specificity
4) Trials with a clinical endpoint of CIN2/3+ primarily reflect non-progressive intraepithelial lesions that are not a robust surrogate for measuring the impact of screening on invasive cervical cancer.
5) Long term observational studies on the impact of new screening methods on cervical cancer require decades and seek to measure an infrequent outcome.

Long Term Observational Studies in Countries Implementing HPV Screening will be of Great Interest

- At what rate will cervical cancers be diagnosed in patients screened by primary HPV testing?
- What will the cervical cancer stage distribution be in HPV-screened women diagnosed with cervical cancer?
- What HPV genotypes will be detected in HPV-screened women diagnosed with cervical cancers?