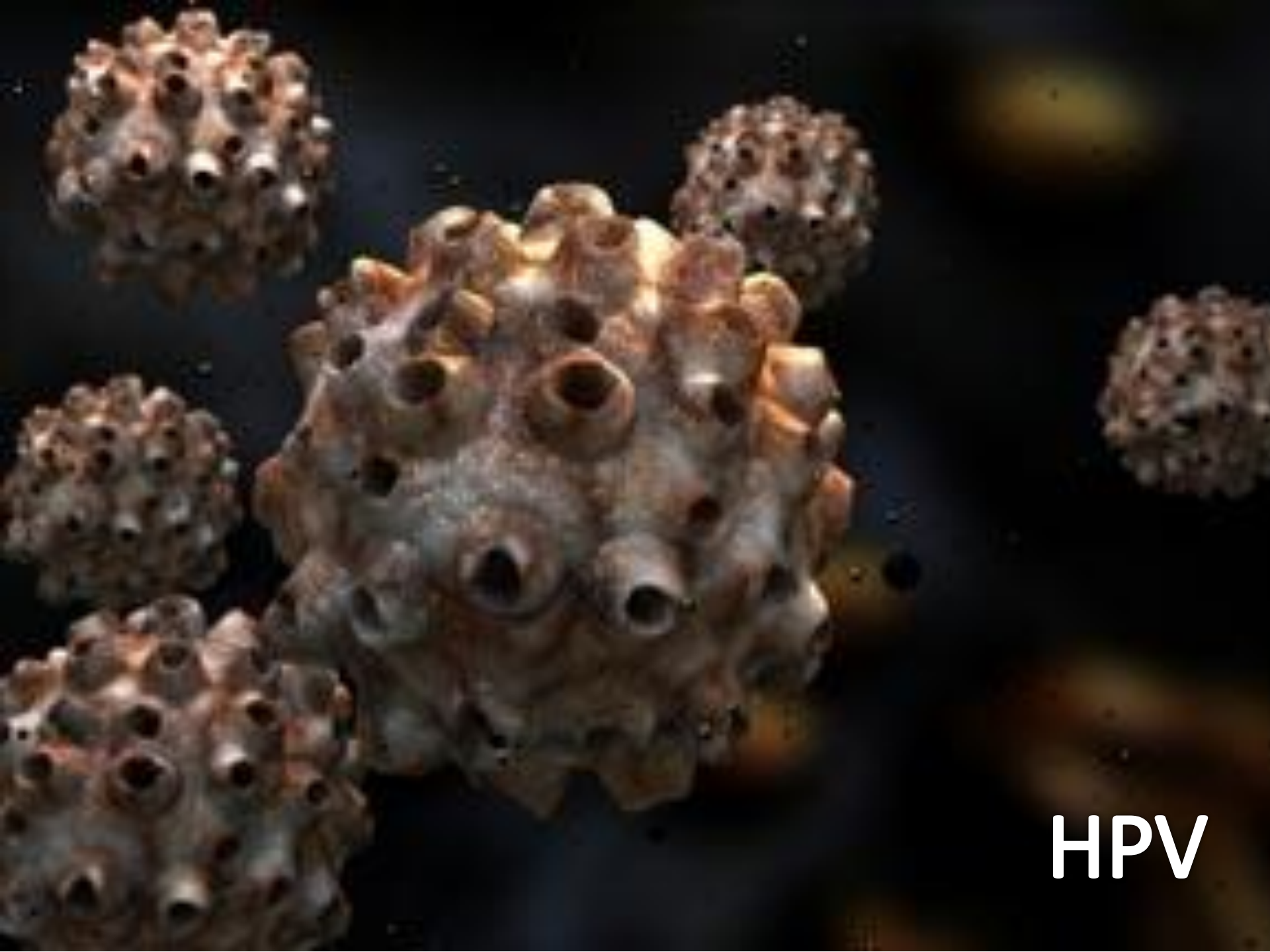

HPV


an evolving understanding of
an ancient and established virus

Disclosures-1

- Merck
  - Speaker Panel for Gardasil
- Hologic
  - Speaker Panel for Cervista and Thin Prep

Disclosures-2

Gynecologic Oncologist

Parent

Vaccine Enthusiast

Objectives

- HPV
- Cervical cancer
- The Cervix
- Epidemiology
- Screening tests
- Breast Cancer

Mortality over the years

- Pneumonia
- TB
- GI infections
- Cardiac disease
- Cerebrovascular dz
- Nephropathies
- Accidents
- Cancer
- Senility

- Cardiac disease
- Cancer
- Pulmonary causes
- Cerebrovascular dz
- Accidents

Concept of Cancer

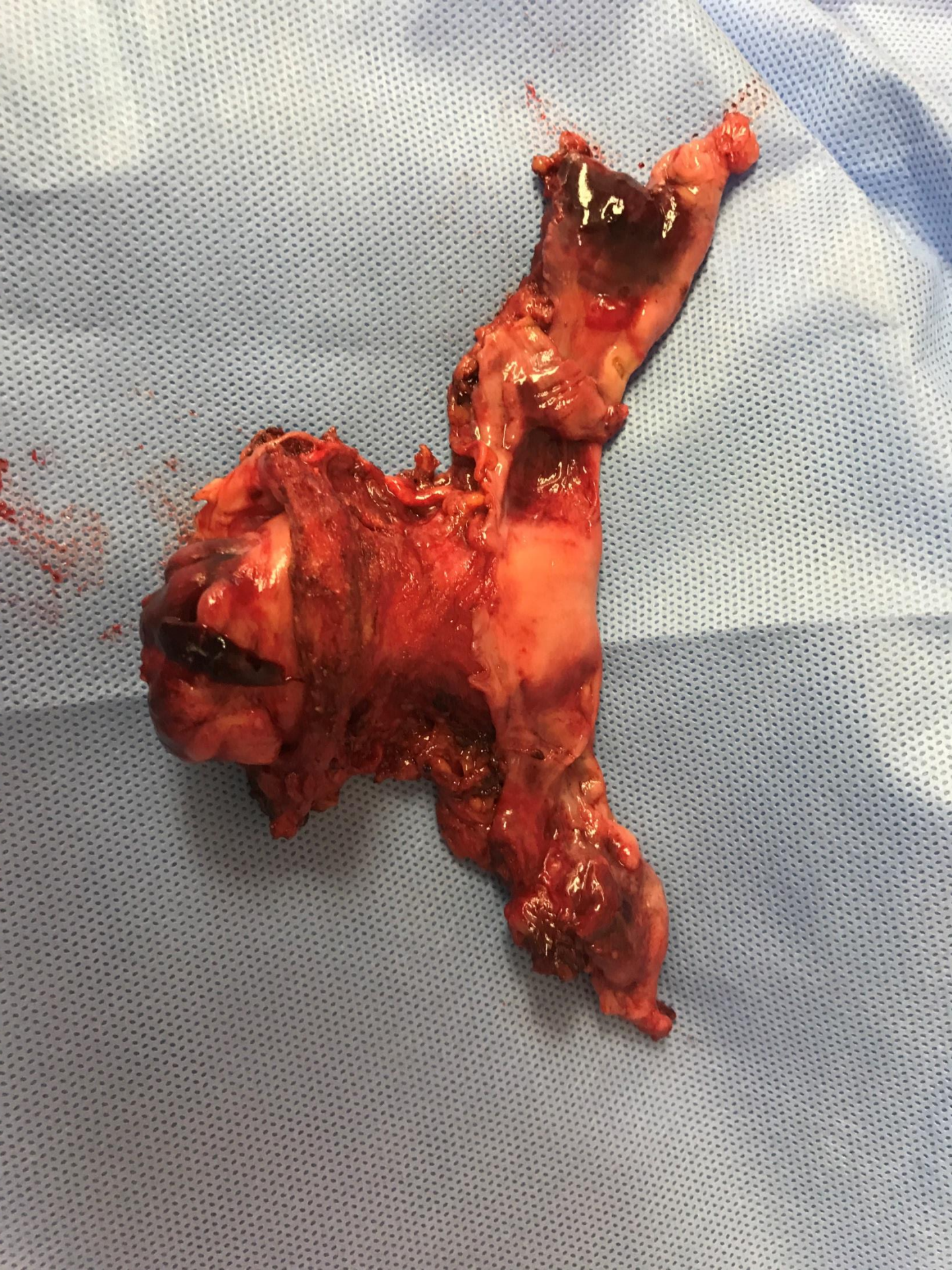
- Largely dependent upon knowledge of normal
- No concept of prodromal cancer
- Early diagnosis: desired, though treatment options limited
- Prevention: unclear

Screening Test

- Performed on asymptomatic people
- Common disease
- Plausible test
  - Cost, Access, Reliable
- Sufficient “lead time” to intervene
- Intervention that can prevent death or morbidity

SCREENING

- Public Health mechanism
- For the asymptomatic patient
- Cost to patient and society
- Balance benefit versus harm



Diagnostic Test

- A test performed on someone who is symptomatic and needs DIAGNOSIS
- Xray, blood, biopsy
- Pap smear can be diagnostic
  - Vaginal bleeding
- Mammogram can be diagnostic
  - Breast lump
- Colonoscopy can be diagnostic
  - Rectal bleeding

SCREENING TESTS

- xray
- pap smear
- mammogram
- colonoscopy
- PSA
- stool guaiac
- “get yourself tested” STI screening
- vision screening
- scoliosis screening
- TB screening
- mobile CT scans
- blood pressure

PAP SMEAR

- Age range
  - under 21
  - over 65
- Risk factors
  - HPV Negative
  - Sexual partners
  - iatrogenic immunosupression


SCREENING

Enter the Pap Smear

- Georgios Papanikolaou (1883-1962)
- 1920s describing the normal vagina
- 1928 presented his findings
- 1943 published his findings
- 1965 ACS recommended screening
- 1999 reflex HPV testing
- 2009 co-testing HPV and cytology
- 2014 HPV primary testing

Major Advances in Cervical Cancer Screening

1941
Pap Smear

1940s

1970s
Research by Harald zur Hausen linking HPV to cervical cancer[1]

1996
ThinPrep® Pap Test

1990s

1999
SurePath® Pap Test

1999
Hybrid Capture® 2 HPV Test

2003
ThinPrep® Imaging System

2000s

2006
Gardasil® HPV Vaccine

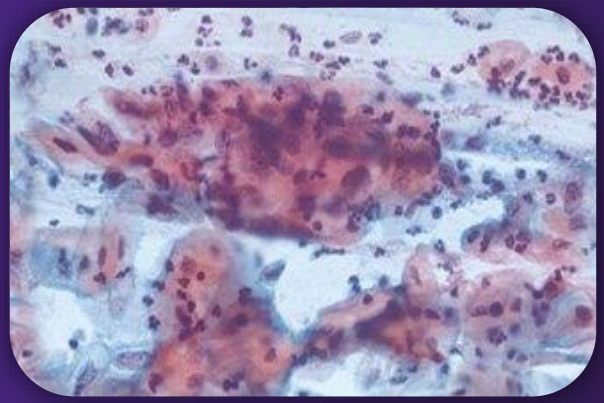
2006
ThinPrep® Receives Glandular Indication

2009
Cervista® HPV HR Test and Cervista® HPV 16/18 Genotyping Test

2009
Cervarix® HPV Vaccine

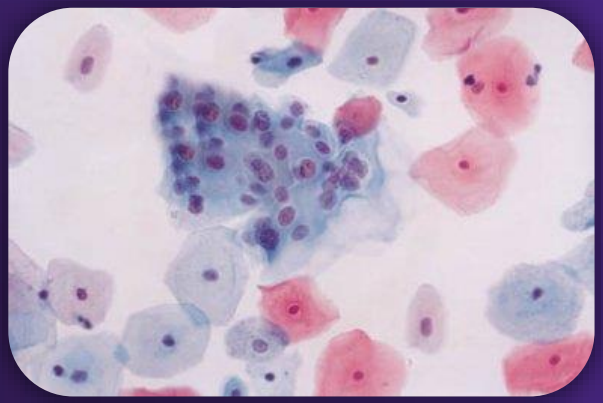
1. zur Hausen H. *Cancer Res*. 1976;36:794.

ThinPrep® Liquid-based Cytology: Mitigates Sampling Error and Improves Preservation



Conventional Smear

- Majority of cells discarded
- Nonrepresentative transfer of cells
- Clumping and overlapping of cells
- Obscuring material

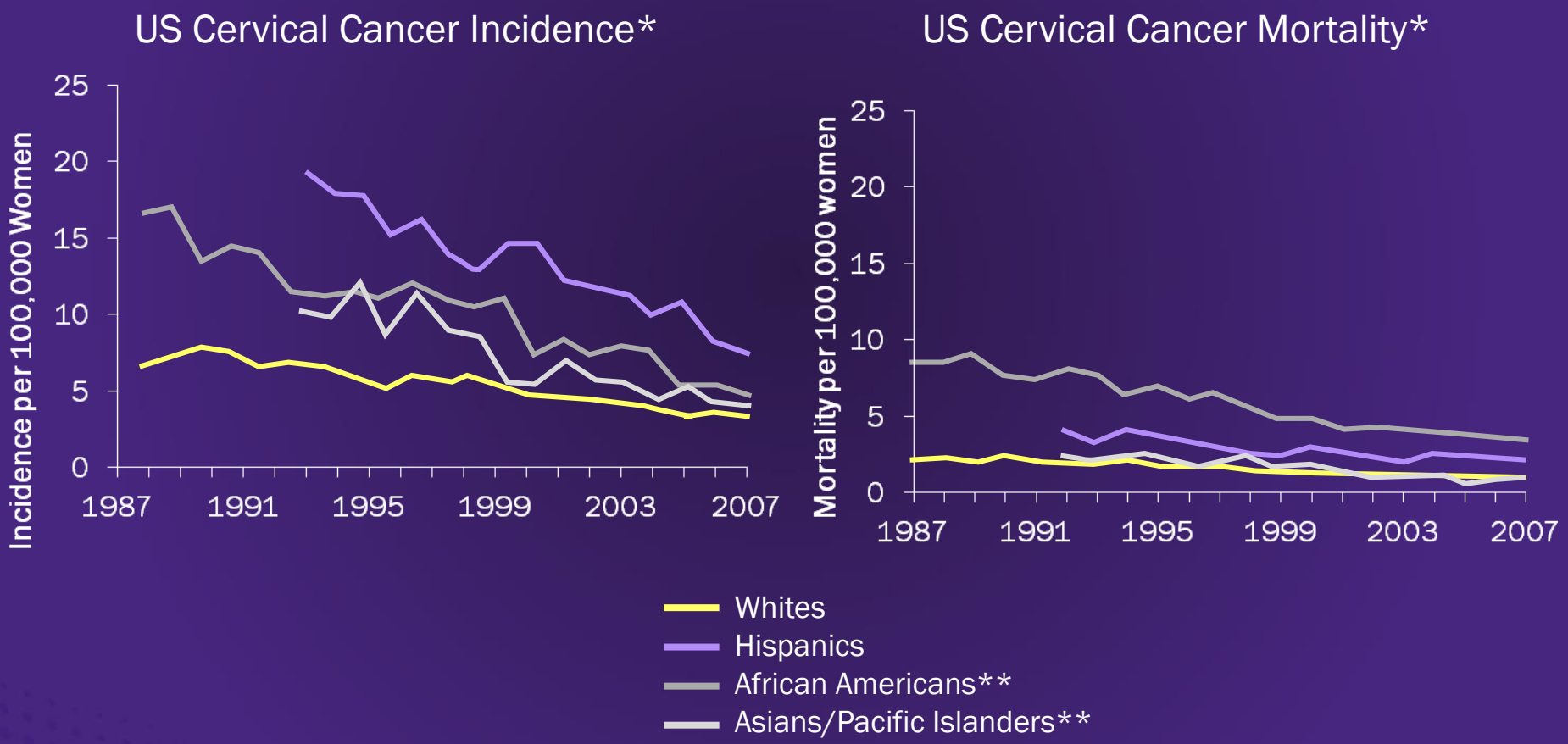


ThinPrep Pap Test

- Virtually all of sample is collected
- Randomized, representative transfer of cells
- Even distribution of cells
- Minimizes obscuring material

Source: Hutchinson ML, et al. *Am J Clin Pathol*. 1994;101:215-219.

Screening Has Dramatically Reduced Cervical Cancer Incidence



* Insufficient data available for time trend analysis for American Indians/Alaskan Natives.

** Incidence and mortality data not available before 1992.

http://www.cancer.gov/aboutnci/servingpeople/snapshots/cervical.pdf

Limitations of Cytology

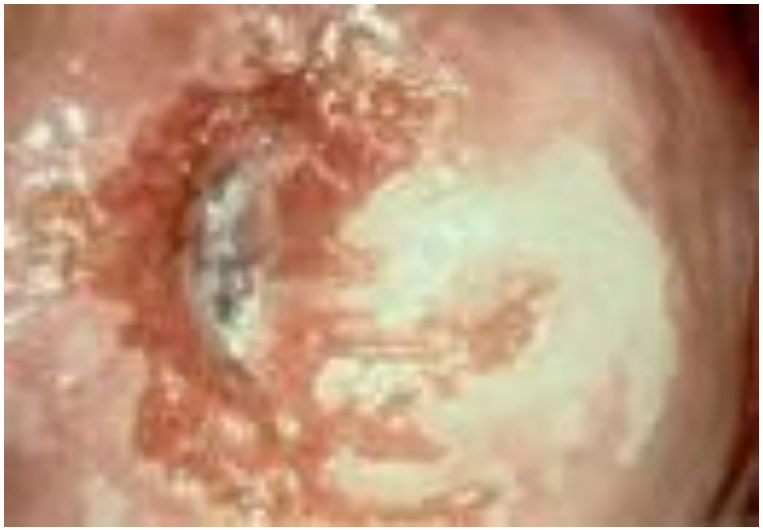
- Epidemiology
  - changing face of the disease
- Anatomy
  - screens ectocervix reliably but not endocervix
- Labor Intensive
  - limitations on cyto-technologist work force
- Cost

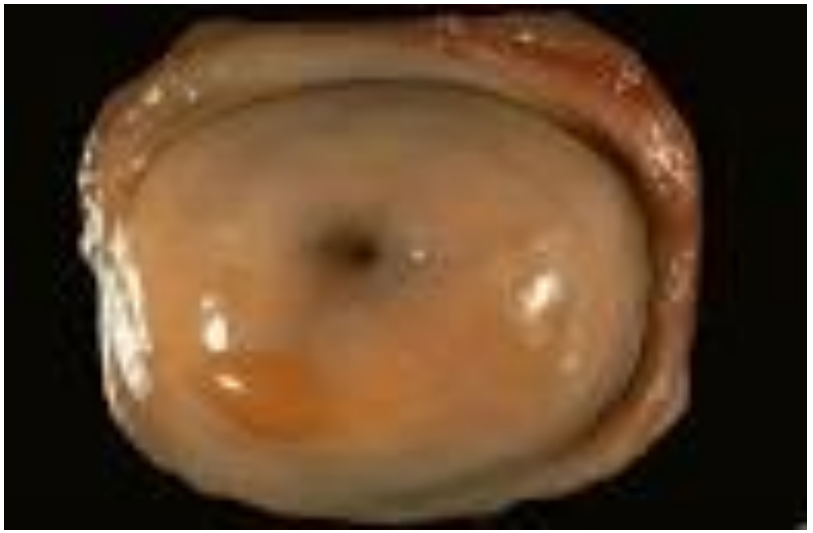
Advantages of HPV Screening

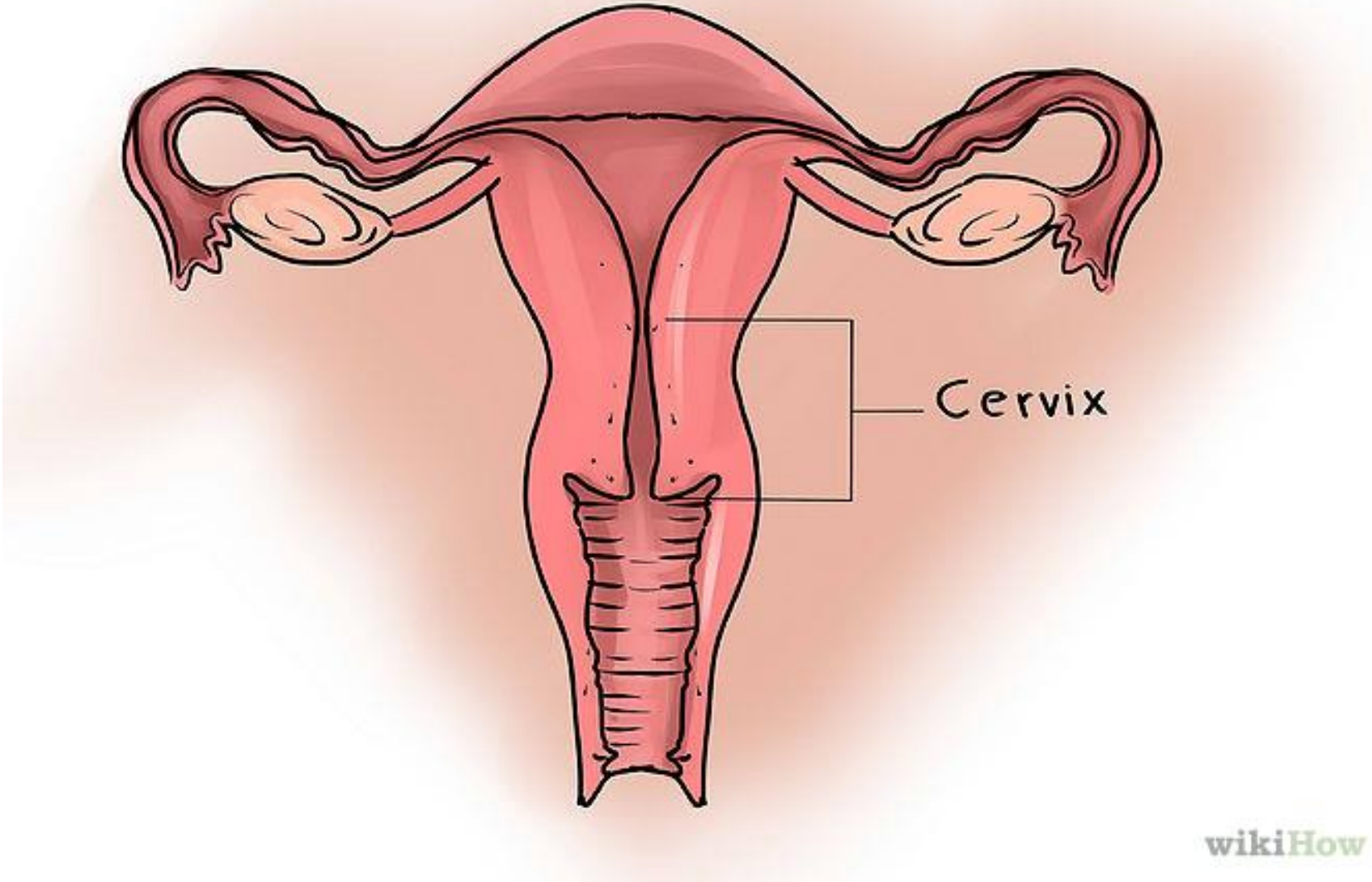
- HPV testing is more sensitive than cytology
- HPV testing has a high negative predictive value
- HPV testing is automatable and reproducible
- For women 30 and older, a negative Pap and HPV test would allow for a patient to extend to a 3-year screening interval[2]

1. Bulkmans NWJ, Berkhof J, Rozendaal L, et al. *Lancet* 2007; 370:1764-72.
2. *J Clin Oncol* May 2011 vol. 29 no. 15_suppl 1508

Changing Epidemiology






Cervix
wikiHow


HPV ONCOGENESIS

HPV in Cancer: History

- 1935 Francis Peyton Rous
  - caused skin cancer in rabbit
- 1972 Stefania Jablonska
  - identified HPV 5 in skin cancer
- 1976 Harald zur Hausen
  - hypothesized HPV cause of cervical cancer
- 1983 HPV 16
- 1984 HPV 18
- 2008 Nobel Prize in Medicine

Peyton Rous and Rous Sarcoma Virus (RSV)

1910

1966


HPV EPIDEMIOLOGY

Could YOU have HPV?

YES… if you have…

- a cervix
- a vagina
- a penis
- tonsils
- a throat
- an anus
- ever had sex

HPV is Ubiquitous

- 85% have come in contact with it
- The majority “clear it”
- More common in smokers
- More transmissible female to male
- Resides in epithelial layer
  - Basal layer required for replication
- Conserved in mammals
  - Across time and geography

“My patient is not at risk”

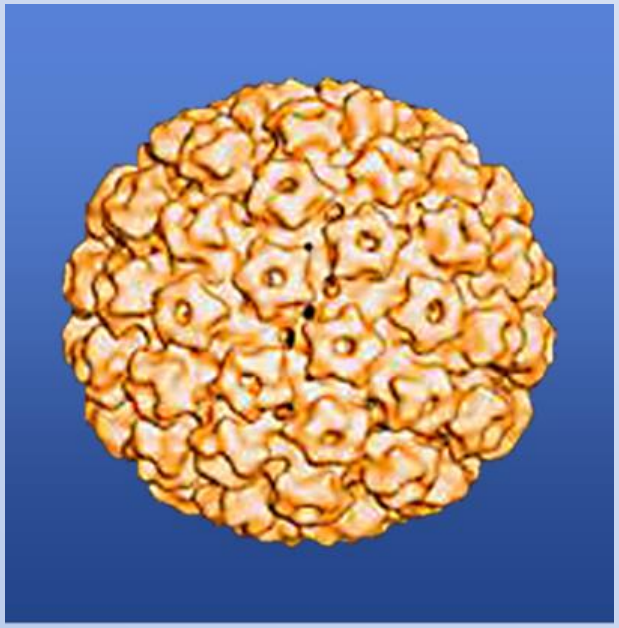
- Wife of Korean WHO Chief
- 4J school teachers
- Librarians, nurses
- The girl next door
- Anesthesiologists
- Social workers

WVCI WILLAMETTE VALLEY CANCER INSTITUTE AND RESEARCH CENTER

Human Papillomavirus (HPV) Is a Cause of Cervical Cancer

- Over 100 types identified[2]
- 30–40 anogenital[2,3]
  - 15–20 oncogenic[2,3] types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 58[4]
    - HPV 16 (54%) and HPV 18 (21%) account for the majority of cervical cancers worldwide[5]
  - Nononcogenic types include:

    6, 11, 40, 42, 43, 44, 54[4]
    - HPV 6 and 11 account for 90% of external genital warts[3]



Nonenveloped double-stranded DNA virus[1]

1. Howley PM. In: Fields BN, Knipe DM, Howley PM, eds. Philadelphia, Pa: Lippincott-Raven; 1996:2045–2076.
2. Schiffman M. *Arch Pathol Lab Med*. 2003;127:930–934.
3. Wiley DJ. *Clin Infect Dis.* 2002;35(suppl 2):S210–S224.
4. Muñoz N. *N Engl J Med*. 2003;348:518–527.
5. Smith J, et al. *Int J Cancer*. 2007;121:621-632.

HPV Facts: Most common STD in the U.S.

Approximately 20 million Americans are currently infected.[1]

- Estimated incidence of new cases 6 million per year[1]
- 80% sexually active adults in U.S. infected w/ at least one HPV type by age 50[1]
- Peak prevalence during adolescence and young adulthood
  - Among sexually active 15-24 year olds:
    - 74% new infections occur in this age group[2]
    - ~9.2 million currently infected[2]

1. Centers for Disease Control & Prevention, Rockville MD: CDC National Prevention Information Network; 2009
2. Weinstock H, et al. *Perspect Sex Reprod Health*. 2004;36:6-10.

"My patient is not at risk

- HPV is ubiquitous virus
- Risk assessment does not work
  - HIV
  - Hep B
  - HPV vaccination

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I'M A
VIRGIN
(But this is an old shirt)

US Cervical Cancer Statistics

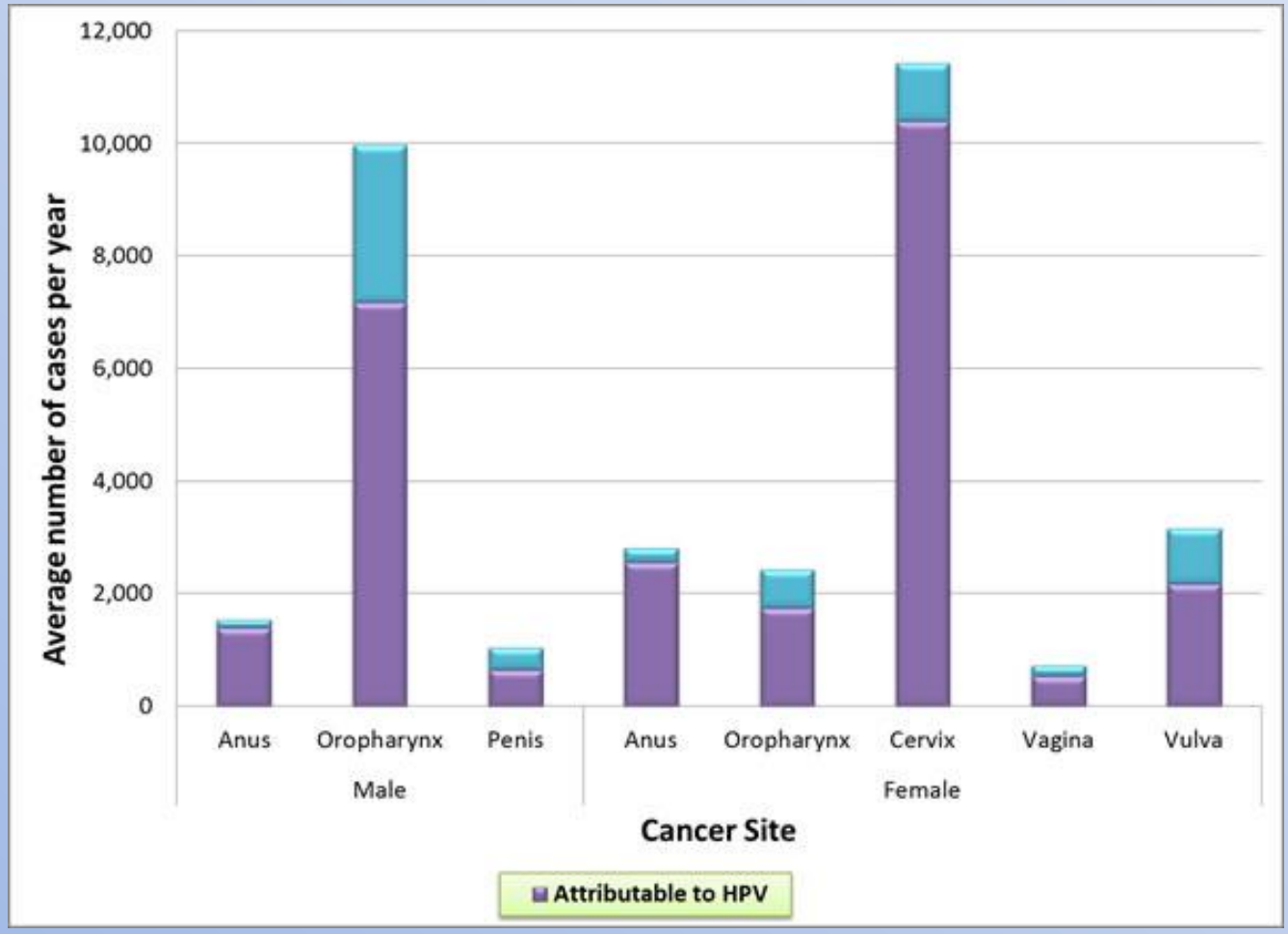
- Approximately 12,710 new cases/year[1]
- Approximately 4,290 deaths/year[1]

- Approximately 10 million cases of HPV infection without cytologic abnormalities[2]

- Approximately 1 million cases CIN 1[2]
- Approximately 300,000–700,000 cases of CIN2/3
- Direct cost of prevention and treatment of cervical cancer is $6 billion annually in the US

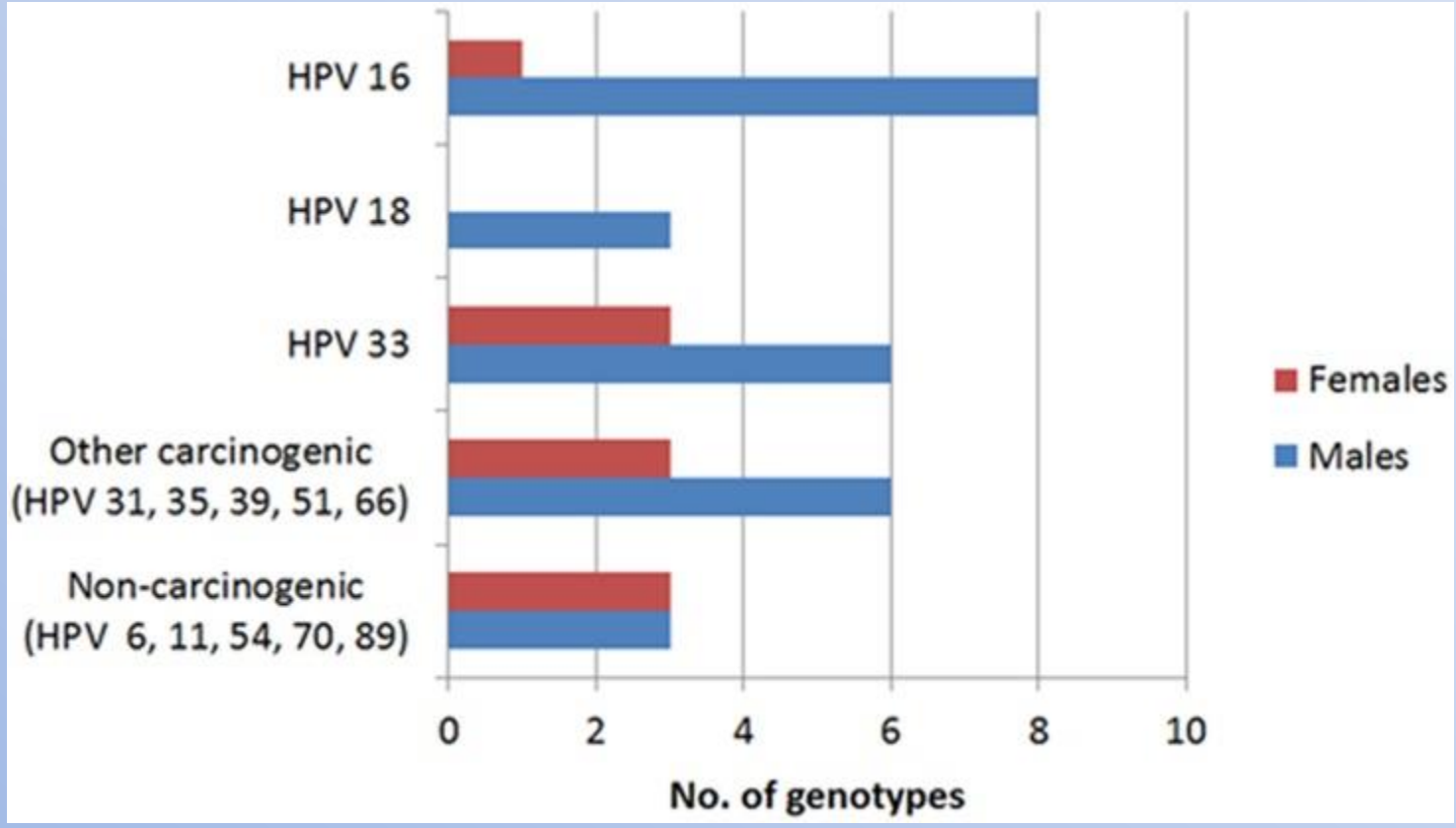
1. CA: *A Cancer J for Clinicians* 2011.
2. Parkin et al. *Int J Cancer*. 1993;54:594-606.

33,000 cancer cases annually



CDC Info

HPV in Laryngeal Cancers



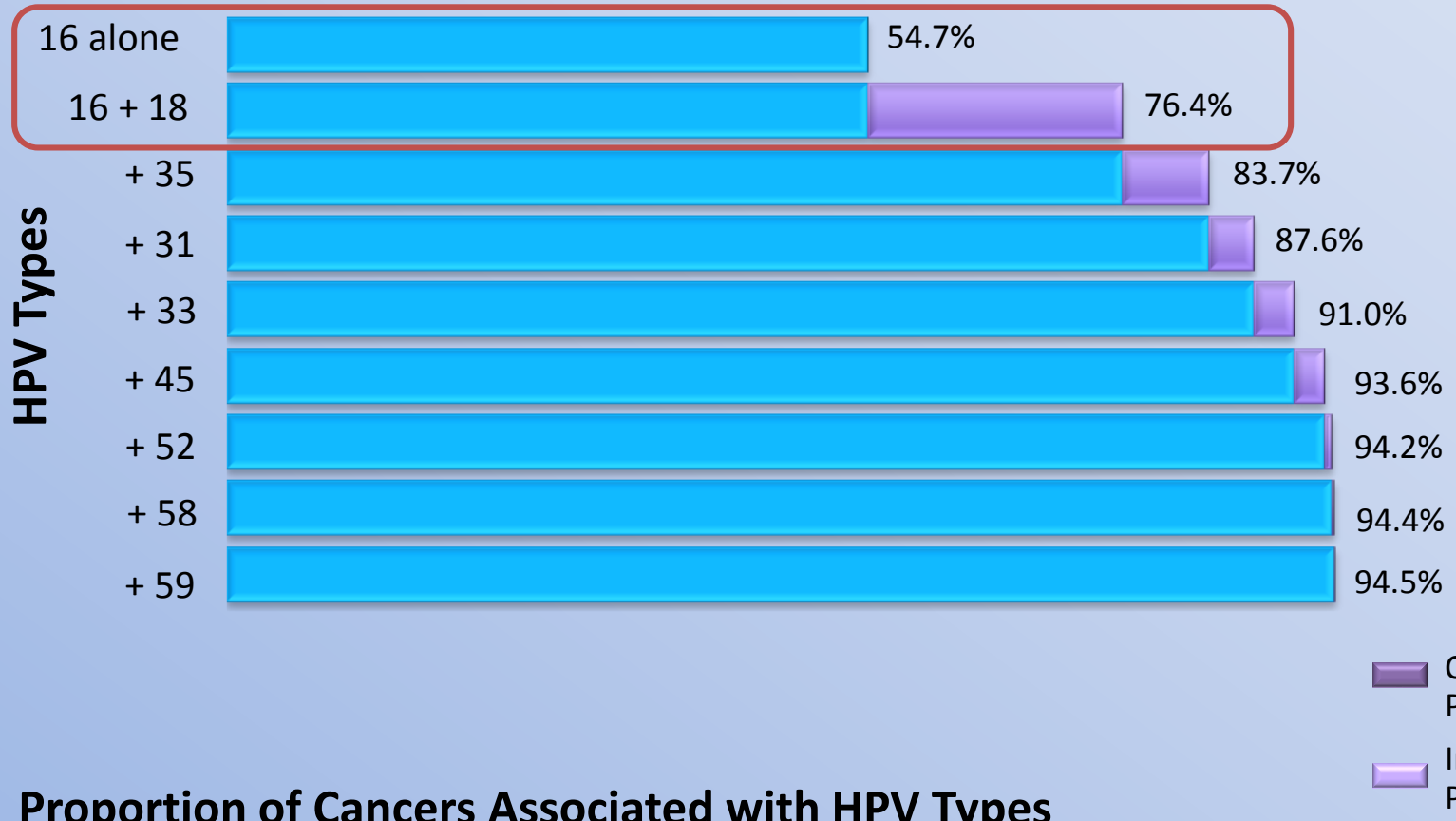
PLoS One 2014 Dec 29, 9(12)

Internationally

- 530,000 cases of cervical cancer
  - 230,000 deaths
- 30,000 cases of anal cancer
  - more female than male
- oropharyngeal cancer
  - new area of tabulation
- >600,000 cancer cases
  - >300,000 deaths

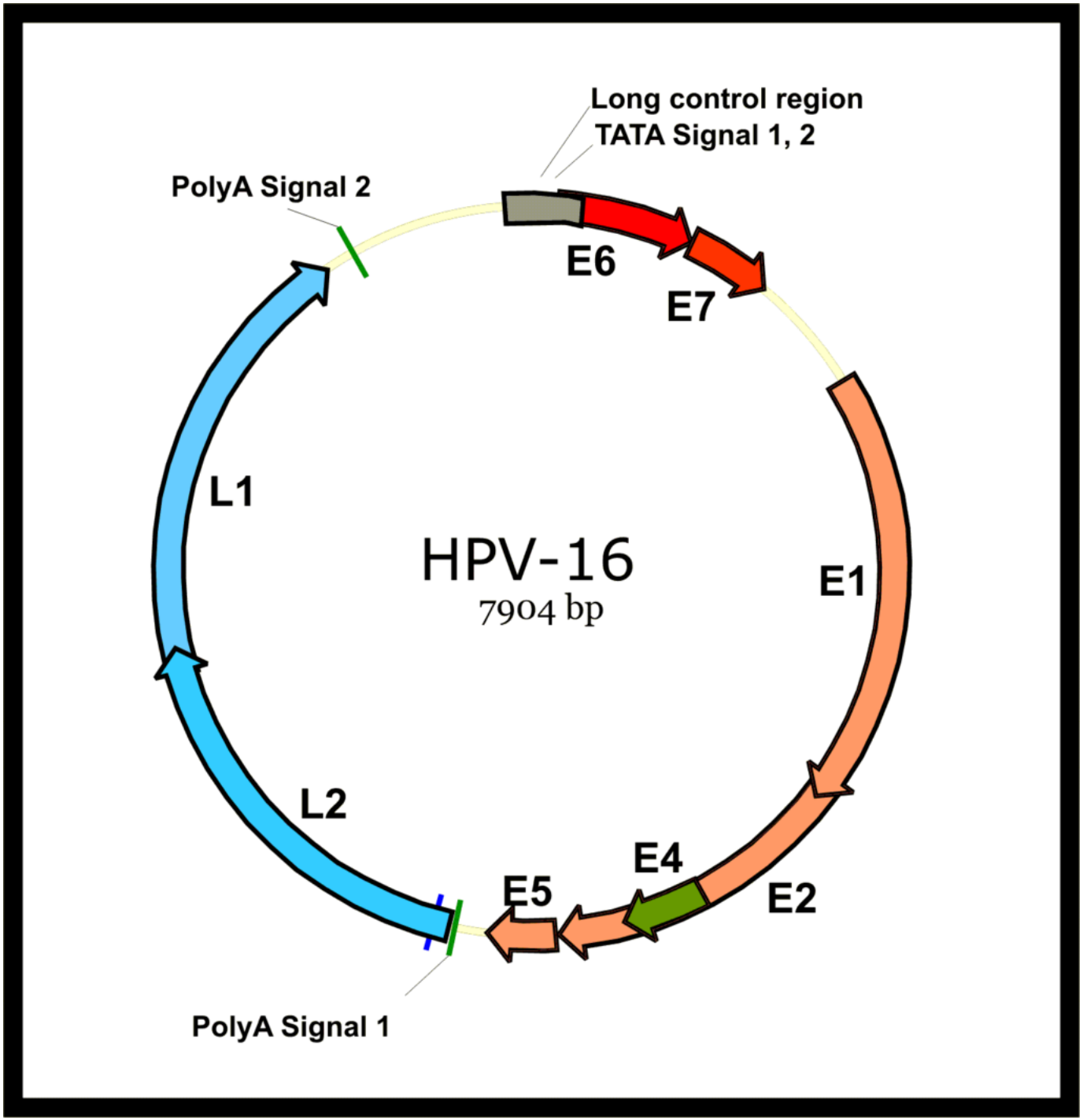
Why Are HPV 16/18 Important?

> 75% of Squamous Cancers in the United States Are Caused by HPV 16/18

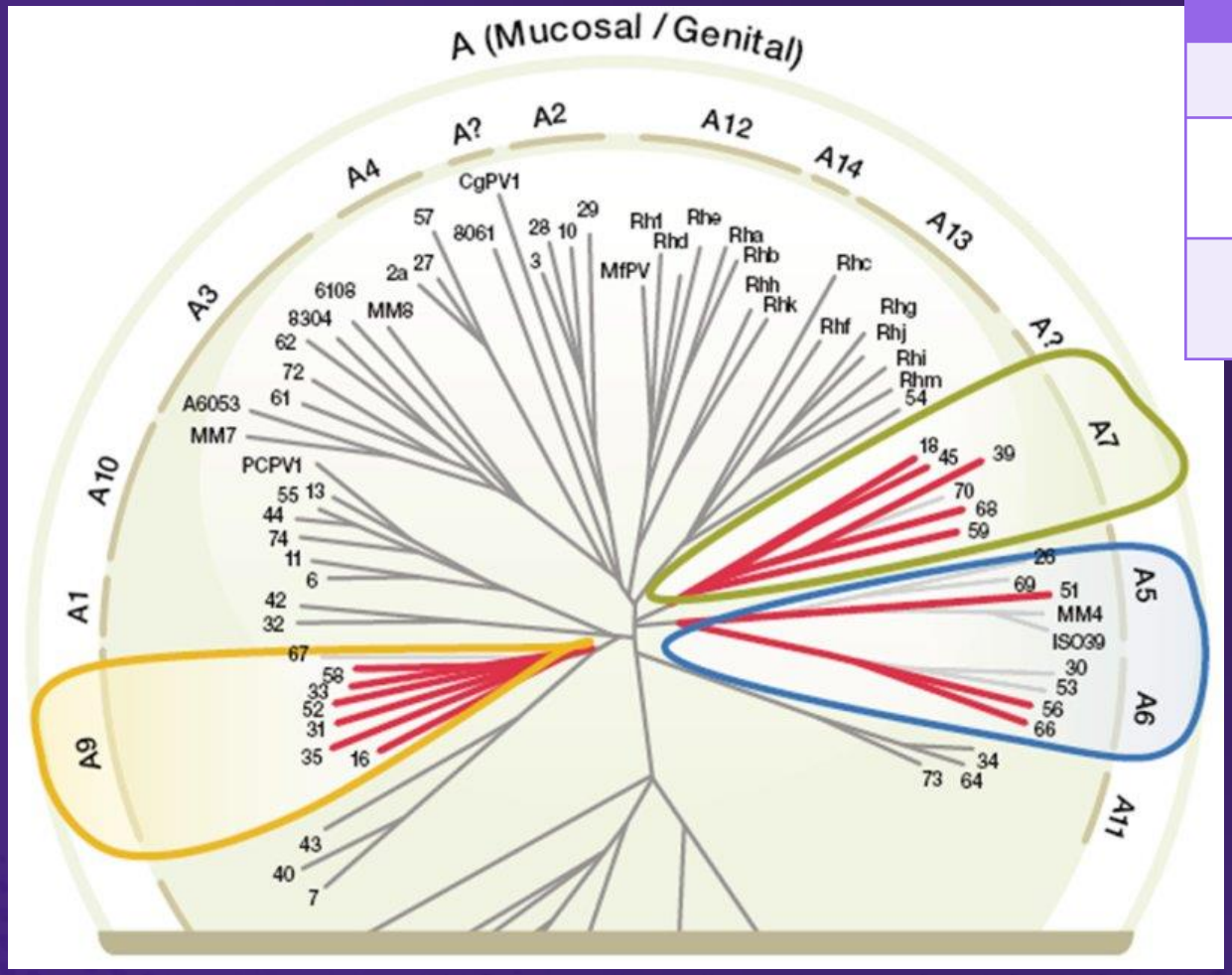


Proportion of Cancers Associated with HPV Types

Smith J, et al. *Int J Cancer*. 2007;121:621-632.


Long control region
TATA Signal 1, 2
PolyA Signal 2
E6
E7
E1
E2
E4
E5
L2
L1
HPV-16
7904 bp
PolyA Signal 1

Phylogenetic Tree Basis of Cervista® HPV HR Test Design

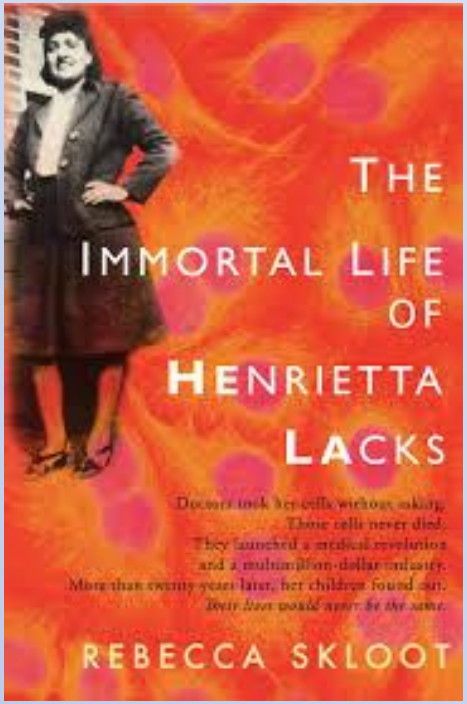


HPV Group	HPV Types
A5/A6	51, 56, 66
A7	18, 39, 45, 59, 68
A9	16, 31, 33, 35, 52, 58

Los Alamos National Laboratory. Human papillomaviruses: a compilation and analysis of nucleic acid and amino acid sequences. 1997. Available at: http://hpv-web.lanl.gov/.

How does HPV do it?

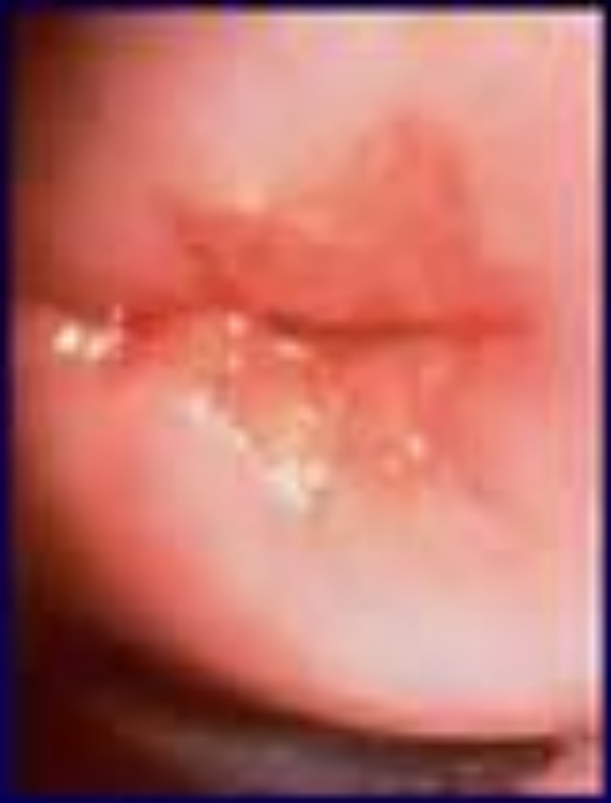
- interferes with the normal work of the cell
  - invades epithelium
  - evades “surveillance”
  - integrates into host genome
- E6
  - interrupts important work of p53
- E7
  - interrupts important work of RB
- Able to create immortal cell lines
  - HeLa cells: HPV 18



HPV enters the epithelium

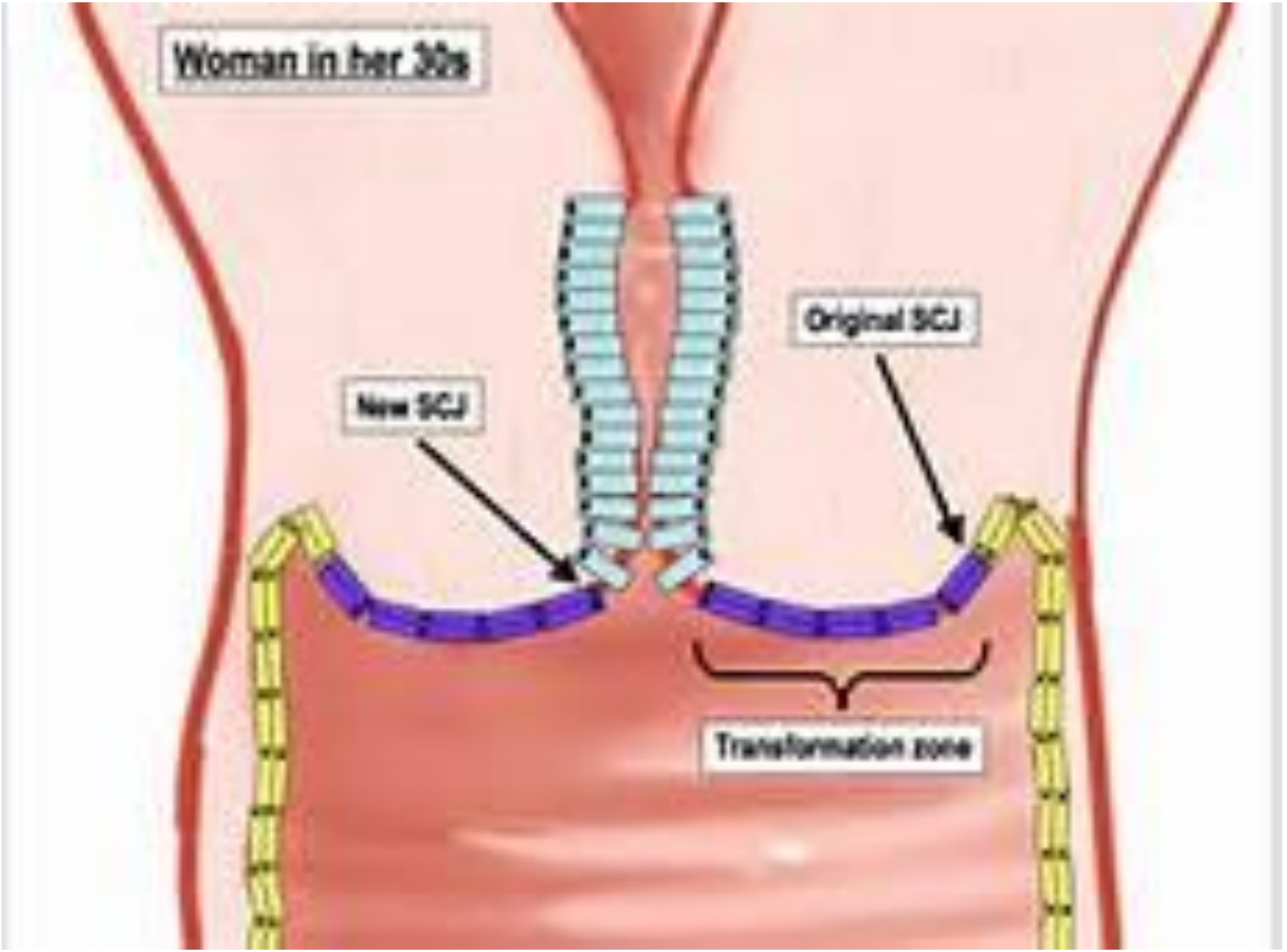
- trauma of intercourse
  - micro-abrasions
  - coexisting infections
- “dry” intercourse
- anal intercourse
- areas of metaplasia/ transition
- oropharyngeal trauma

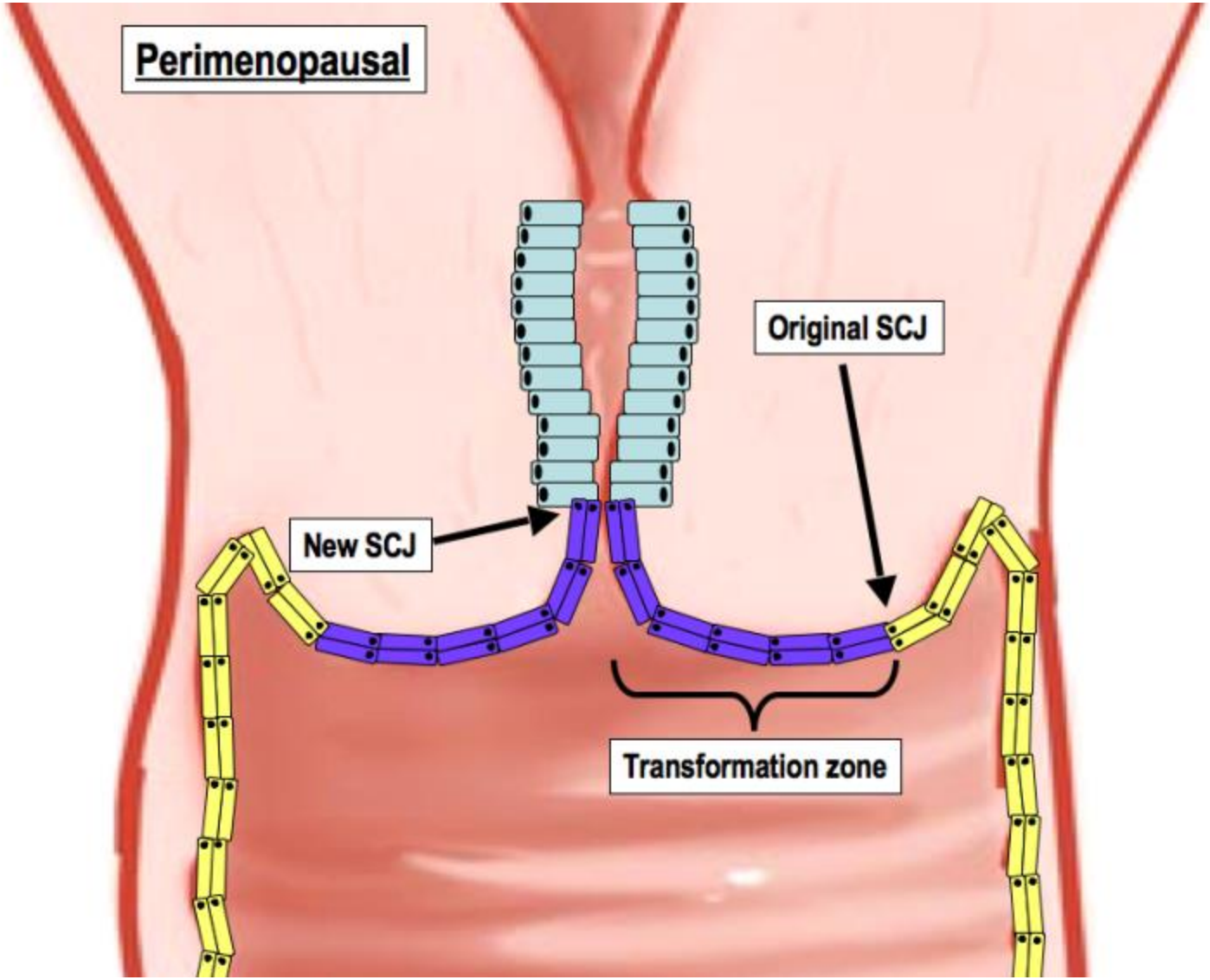
Normal Cervix with Ectopy

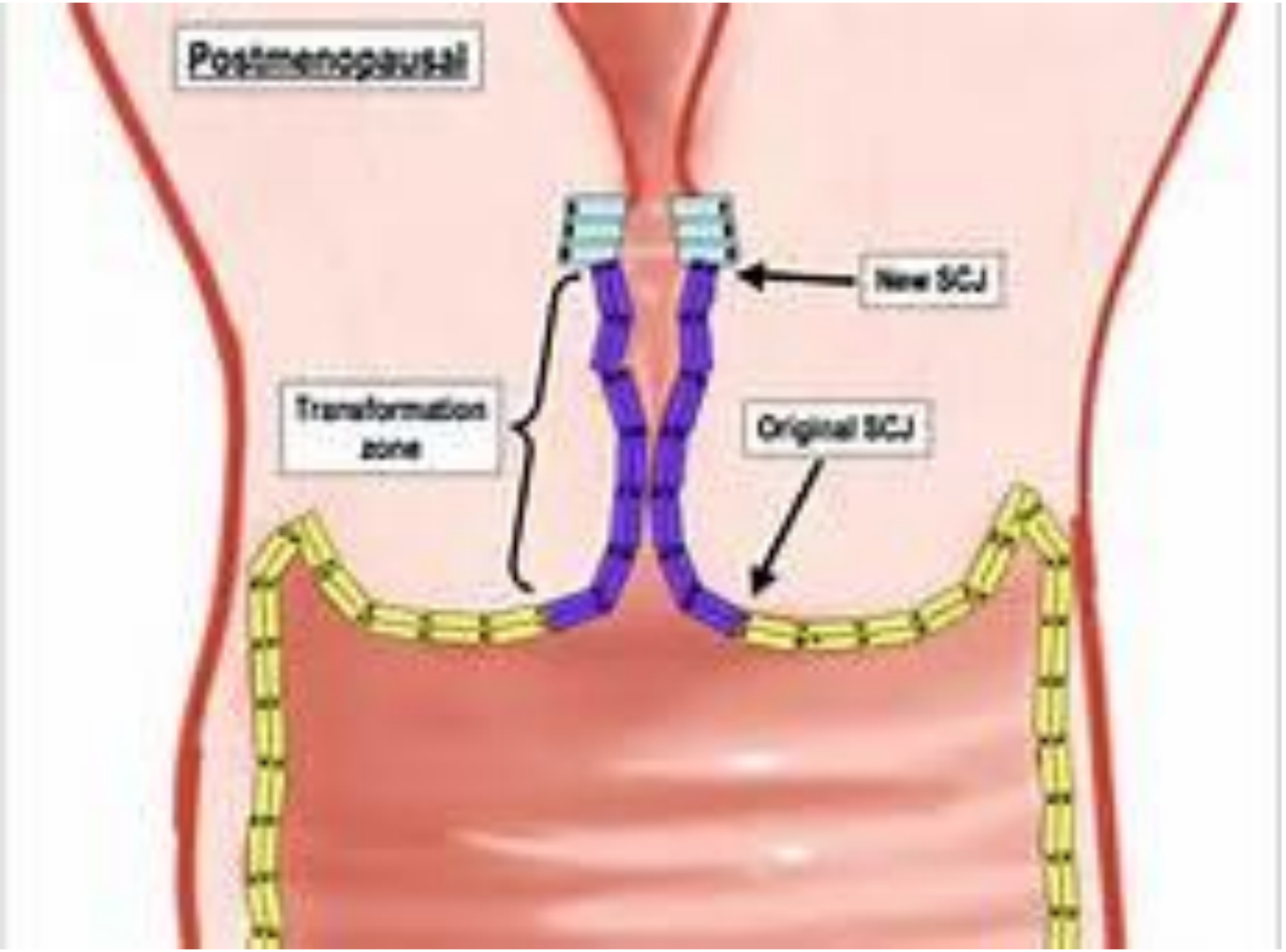


Source: Seattle STD/HIV Prevention Training Center at the University of Washington/
Diane E. Stevens

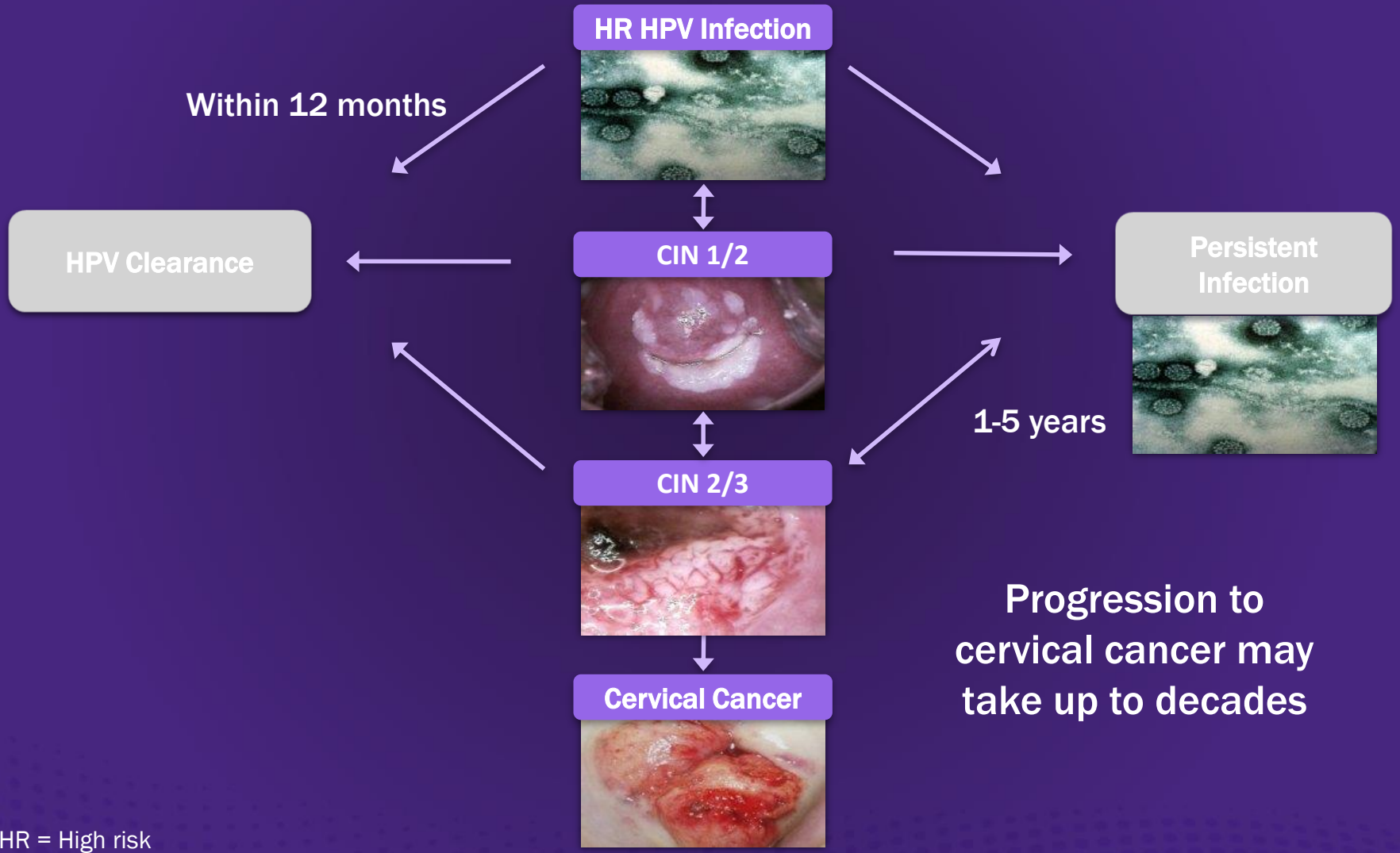
CDC


Woman in her 30s
New SCJ
Original SCJ
Transformation zone


Perimenopausal
Original SCJ
New SCJ
Transformation zone


Postmenopausal
New SCJ
Transformation zone
Original SCJ

Progression from HPV infection to Cervical Cancer

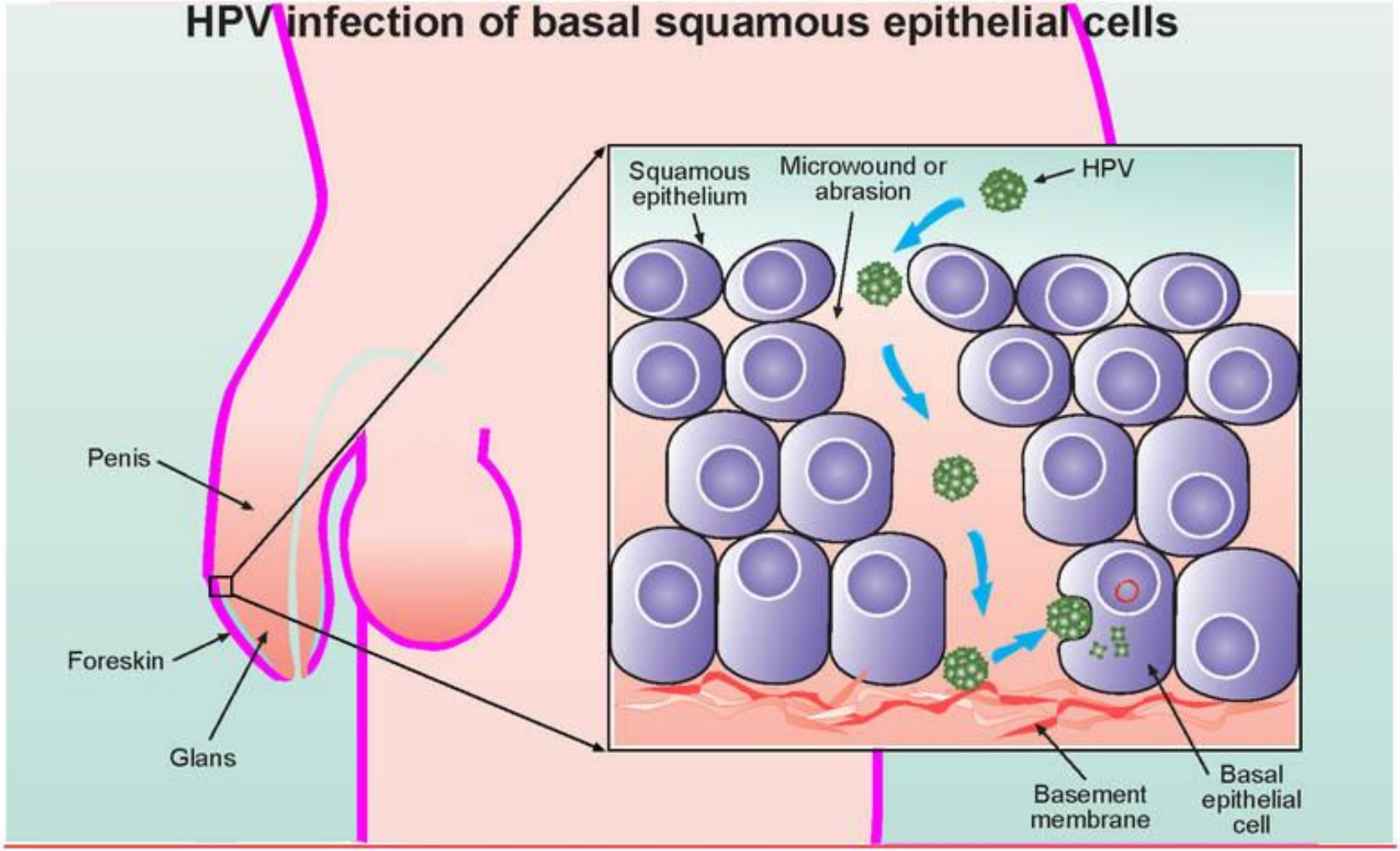


Progression to cervical cancer may take up to decades

HR = High risk
CIN = Cervical intraepithelial neoplasia

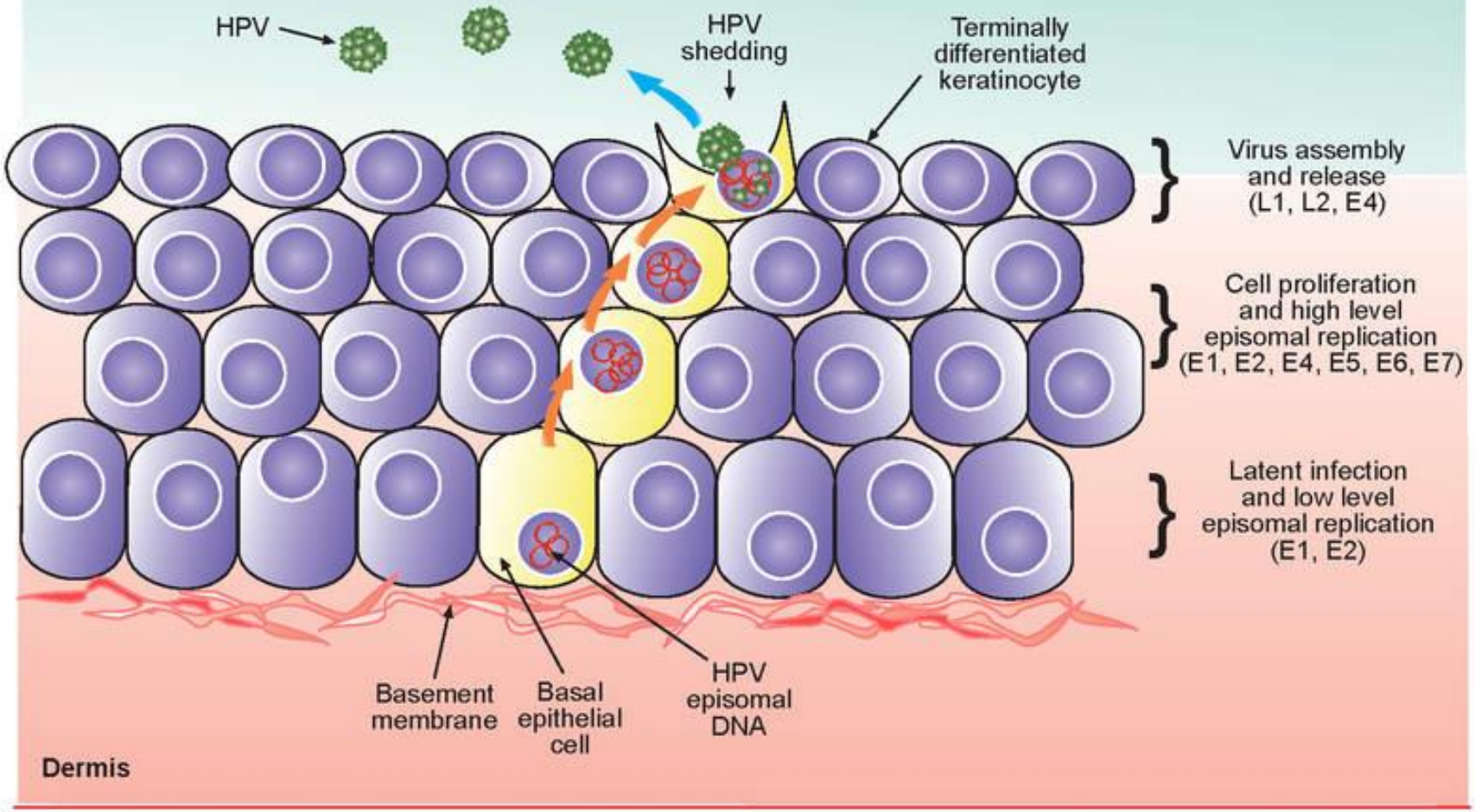
CDC. *Epidemiology and Prevention of Vaccine-Preventable Diseases*. 11th ed. 2009.

HPV infection of basal squamous epithelial cells



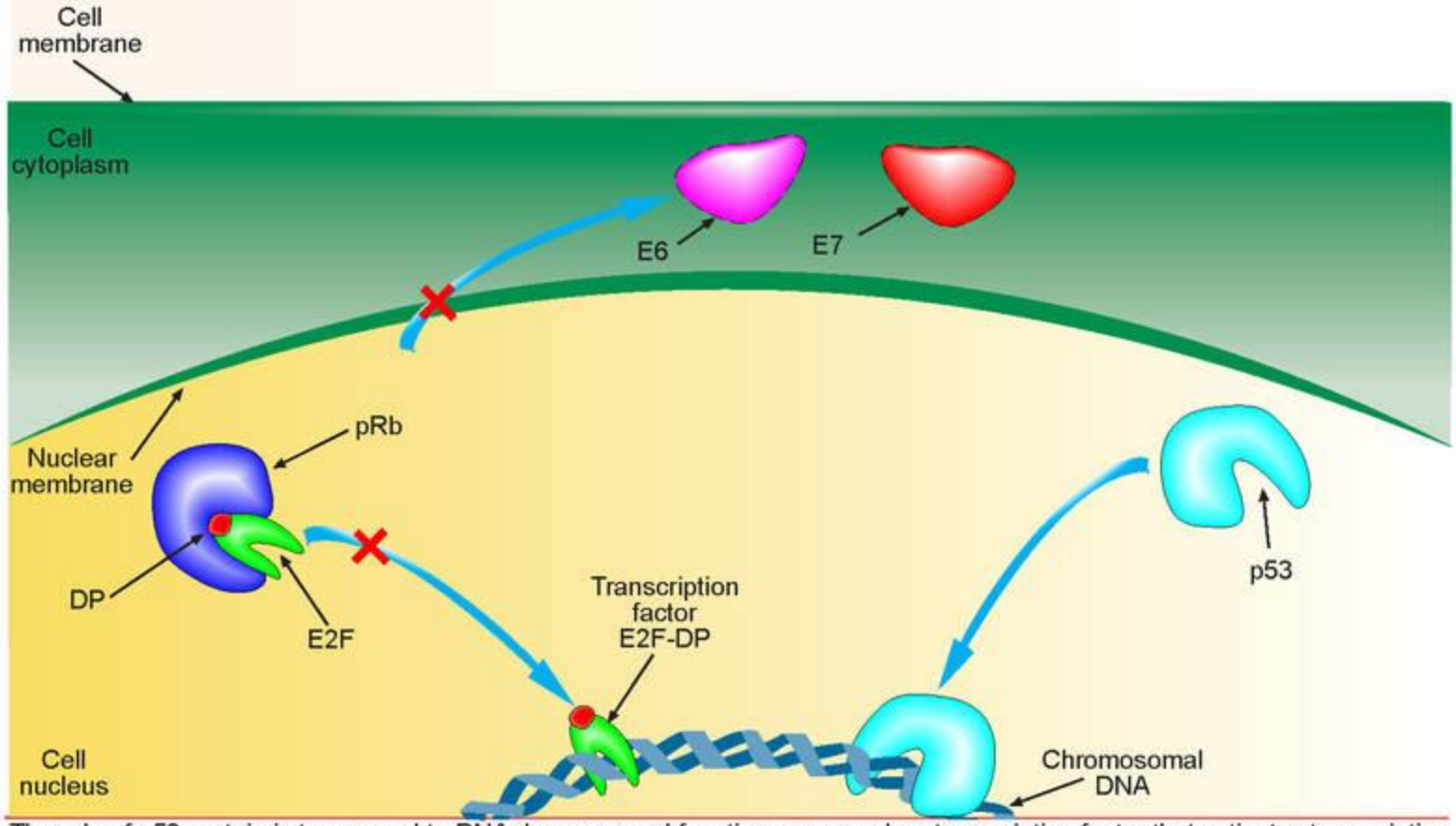
HPV targets the squamous epithelium found beneath the foreskin of the penis and also the cervix. Infection of basal epithelial cells is necessary for HPV replication and it is thought that virus particles gain access to these cells through microabrasion or microwounding that exposes the basement membrane. The L1 capsid protein on the surface of the HPV virion interacts with $\alpha 6\beta 4$ integrins that are upregulated on basal epithelial cells during wound repair. Interaction with $\alpha 6\beta 4$ integrin promotes internalisation of virus. Circumcision may reduce the risk of HPV infection via the removal of target cells present in the squamous epithelium beneath the foreskin. Antibodies to the capsid proteins L1 and L2 of HPV may be important in blocking attachment of virus to receptors on basal epithelial cells during microwound healing.

HPV replication in squamous epithelial cells



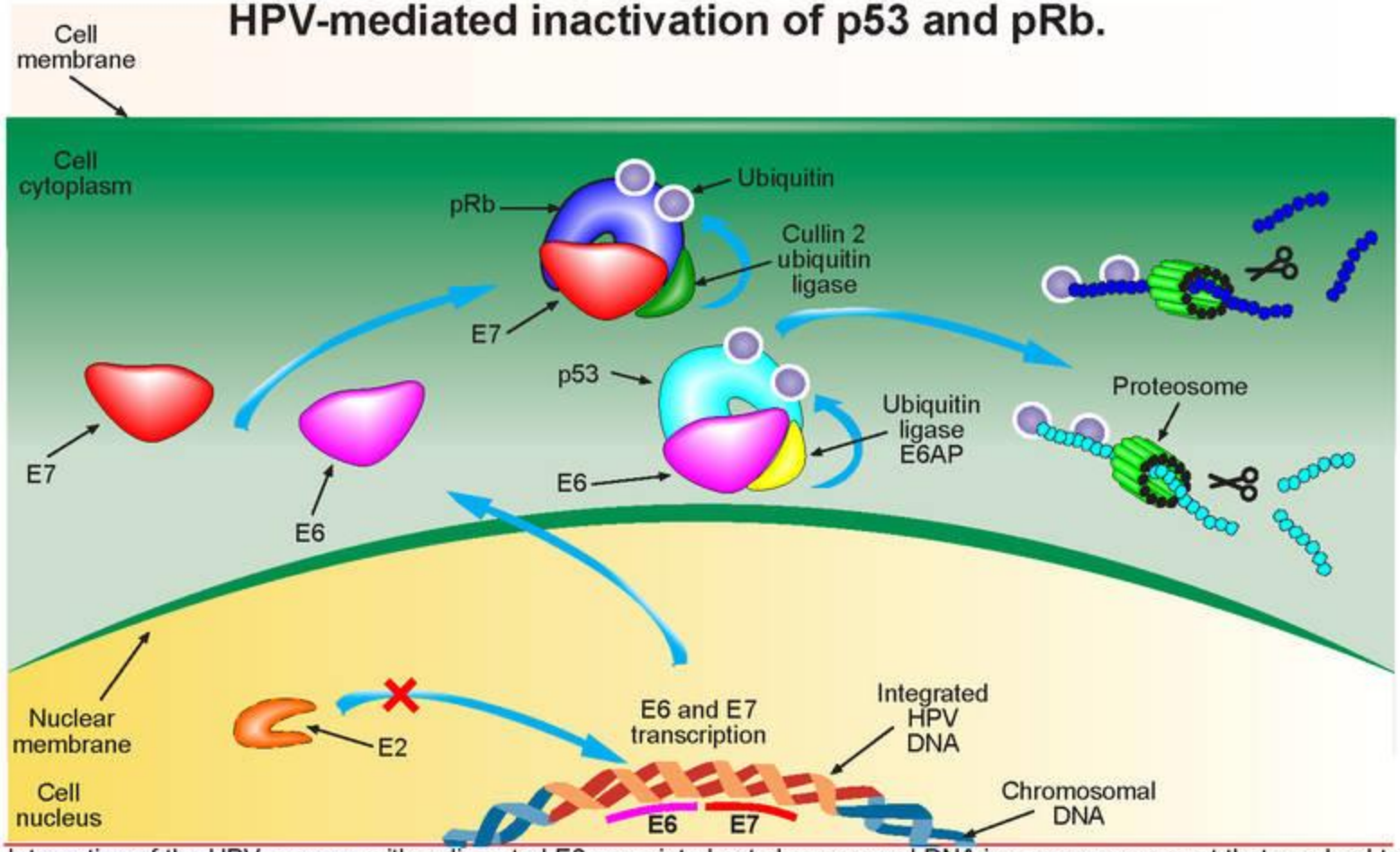
HPV takes advantage of the differentiation pathway of keratinocytes that are destined to die naturally (anoikis). Since HPV is not cytolytic and does not cause viraemia, there is no inflammation and subsequent activation of the immune system. Infection of basal epithelial cells establishes a latent infection with low level replication of the viral episome and minimal viral protein expression. Following differentiation of the keratinocyte, early HPV genes are expressed and the viral episome is further amplified to higher copy numbers. Viral late protein expression and virus assembly occurs during terminal differentiation of the keratinocyte and viruses are shed from the outermost layer of epithelial cells.

Normal function of p53 and pRb proteins.



The role of p53 protein is to respond to DNA damages and functions as a nuclear transcription factor that activates transcription of genes involved in arrest of the the cell cycle and induction of DNA repair systems or the induction of apoptosis. Retinoblastoma protein (pRb) functions to inactivate the transcription factor E2F-DP that is required to initiate transcription of genes involved in DNA replication. These two proteins are essential to prevent cells with damaged DNA from dividing and are known as tumour suppressor proteins. In the absence of HPV E6 and E7, p53 and pRb function normally and reduce the risk of malignant cell transformation.

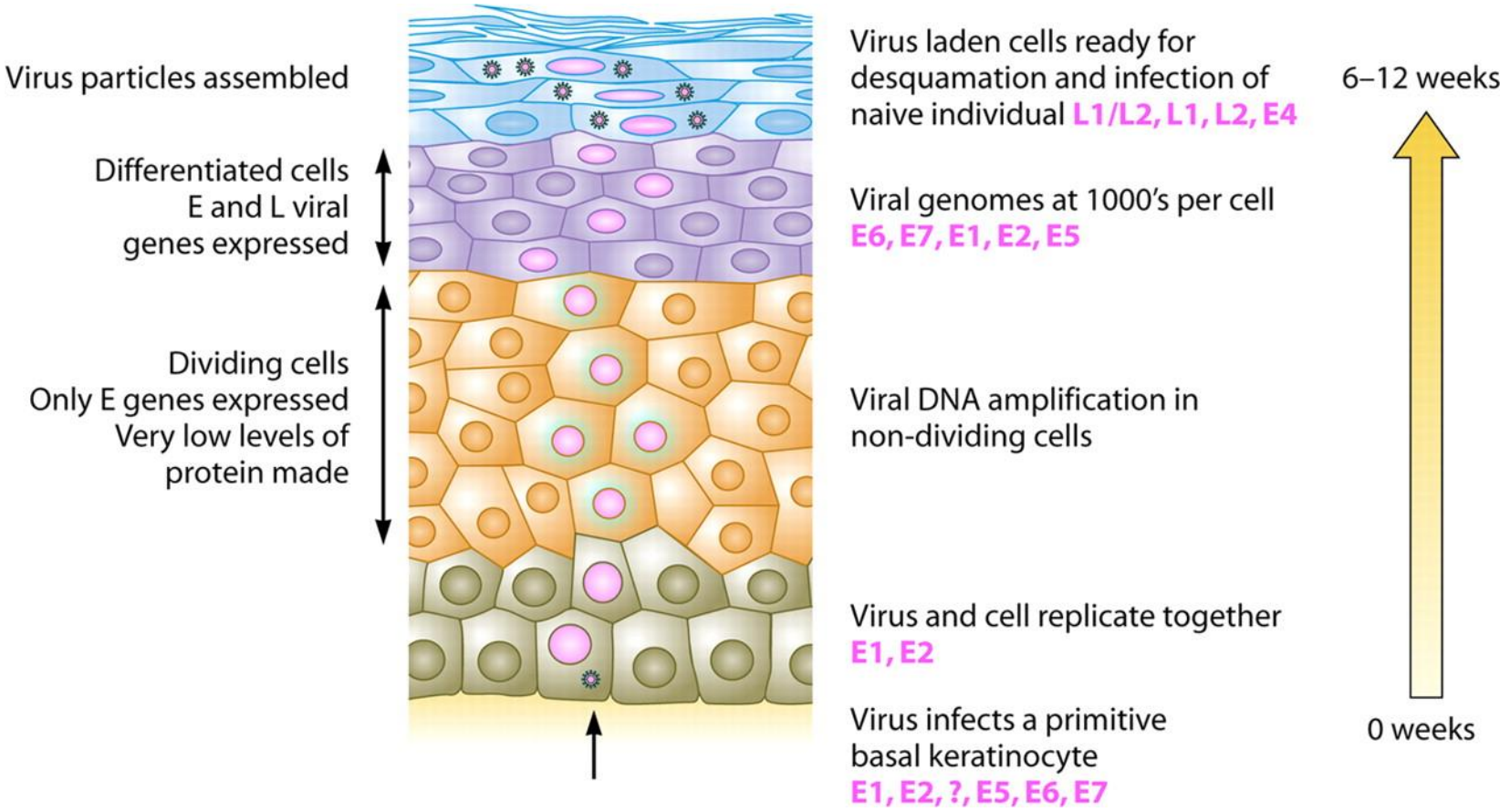
HPV-mediated inactivation of p53 and pRb.



Integration of the HPV genome with a disrupted E2 gene into host chromosomal DNA is a necessary event that can lead to the development of carcinoma. The E2 gene encodes a transcription factor that regulates the transcription of HPV E6 and E7 oncoproteins. In the absence of E2, increased synthesis of E6 and E7 protein occurs. E6 binds to p53 in the cytosol and also recruits the E6AP ubiquitin ligase that ubiquitinates p53 and targets it for proteosomal degradation. Similarly, HPV E7 binds to pRb in the cytosol and recruits the cullin 2 ubiquitin ligase that ubiquitinates pRb and promotes proteosomal degradation. Loss of cellular p53 and pRb tumour suppressor proteins allows a cell with DNA damages to divide and thereby increases the risk of cancer development.


TRAINED MY DOG TO IMPERSONATE 6
POLITICAL SEX SCANDALS AT THE SAME TIME.
~SARA CASM

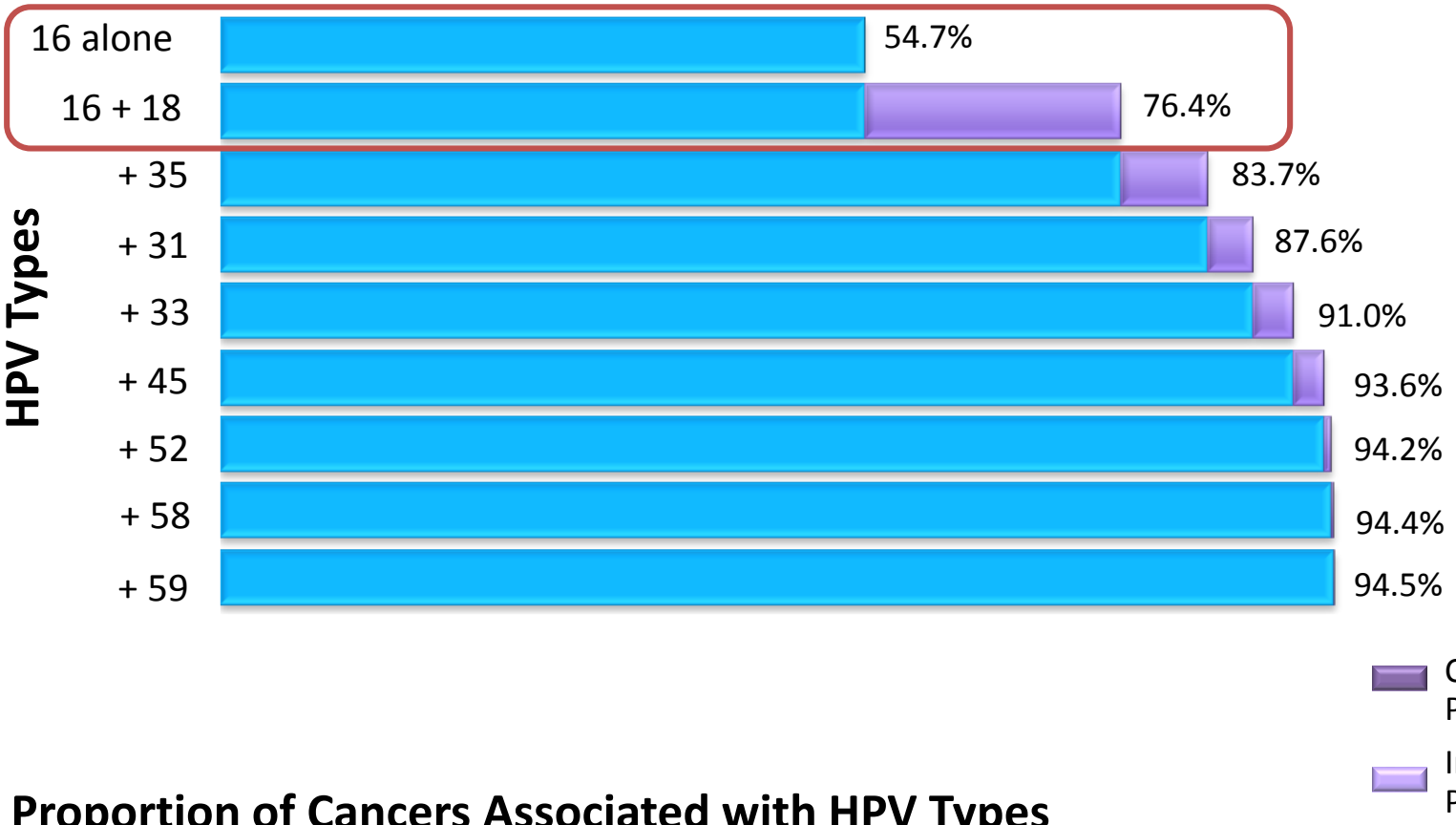
Papillomaviruses are absolutely species specific and tissue specific.



Stanley M A Clin. Microbiol. Rev. 2012;25:215-222

Why Are HPV 16/18 Important?

> 75% of Squamous Cancers in the United States Are Caused by HPV 16/18

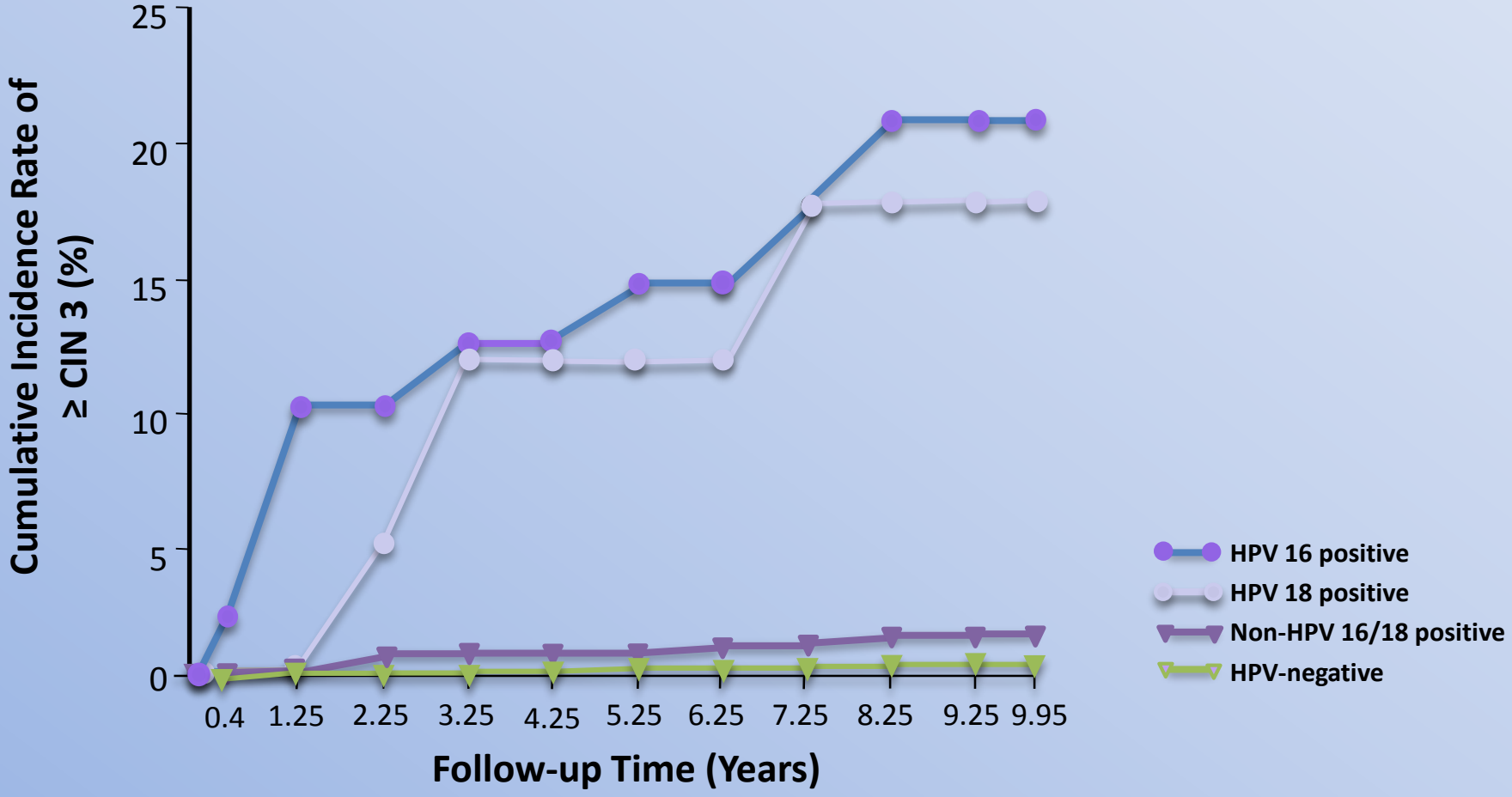


Proportion of Cancers Associated with HPV Types

Smith J, et al. *Int J Cancer*. 2007;121:621-632.

Risk Stratification with HPV Types 16 and 18 in Women ≥ 30 Years of Age with Negative Cytology

In women ≥ 30 years of age, 10-year cumulative incidence of ≥ CIN 3 was 20% and 18% for HPV 16 and 18, respectively



Khan MJ, et al. *J Natl Cancer Inst*. 2005;87(14):1072-1079.

Papanicolau to zur Hausen

Cervical Cancer Prevention: Get with the times…

“This dial phone has always worked for me…”

“My patients would never be able to understand a more modern test…”


FUTURE DIRECTIONS

Vaccines

- Gardasil 4 (Merck) FDA approved 2006
  - 6,11,16,18
- Cervarix (GSK) approved 2009
  - 16,18
- Gardasil 9 (Merck) FDA approved 2014
  - 6,11,16,18,31,33,45,52,58
- Vaccines are controversial

Vaccine Efficacy

- 99-100% immunogenicity
- 92-99% efficacy
- Decrease CIN3 17-33%
- Decrease colposcopy by 10%
- Decrease treatment by 25%
- Impeccable safety record

Vaccine Eligibility

- Gardasil 4 and Cervarix
  - Girls 9-26
  - Boys 9-26
  - Safety data exists for the “older woman”
- Gardasil 9
  - Girls 9-26

HPV adjunctive testing

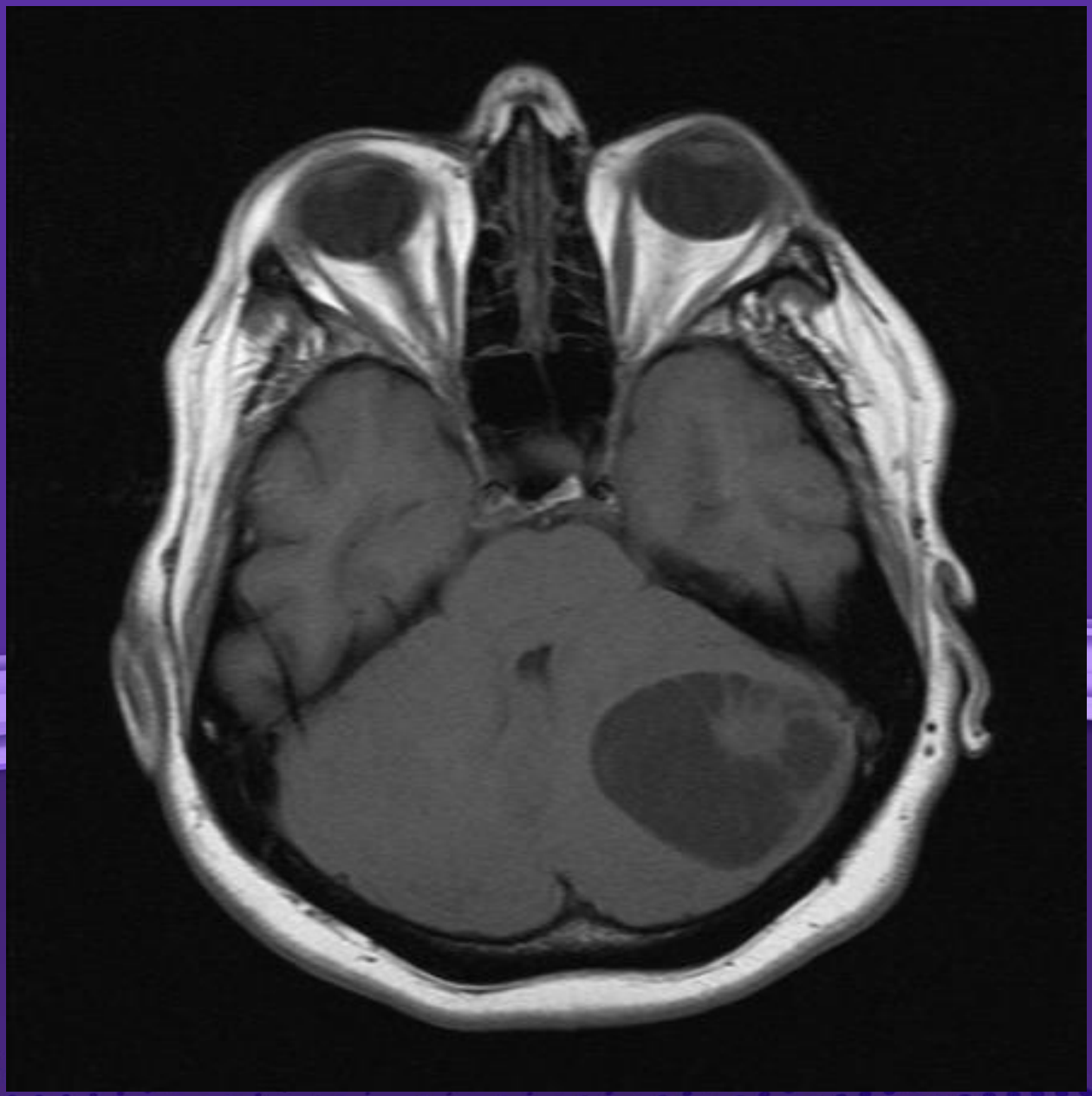
- Primary testing
  - controversial
- Cotesting
  - here to stay
- Enhanced HPV testing
  - Aptima test (Hologic) tests for mRNA
- Testing intervals
  - 3 lifetime screening tests?

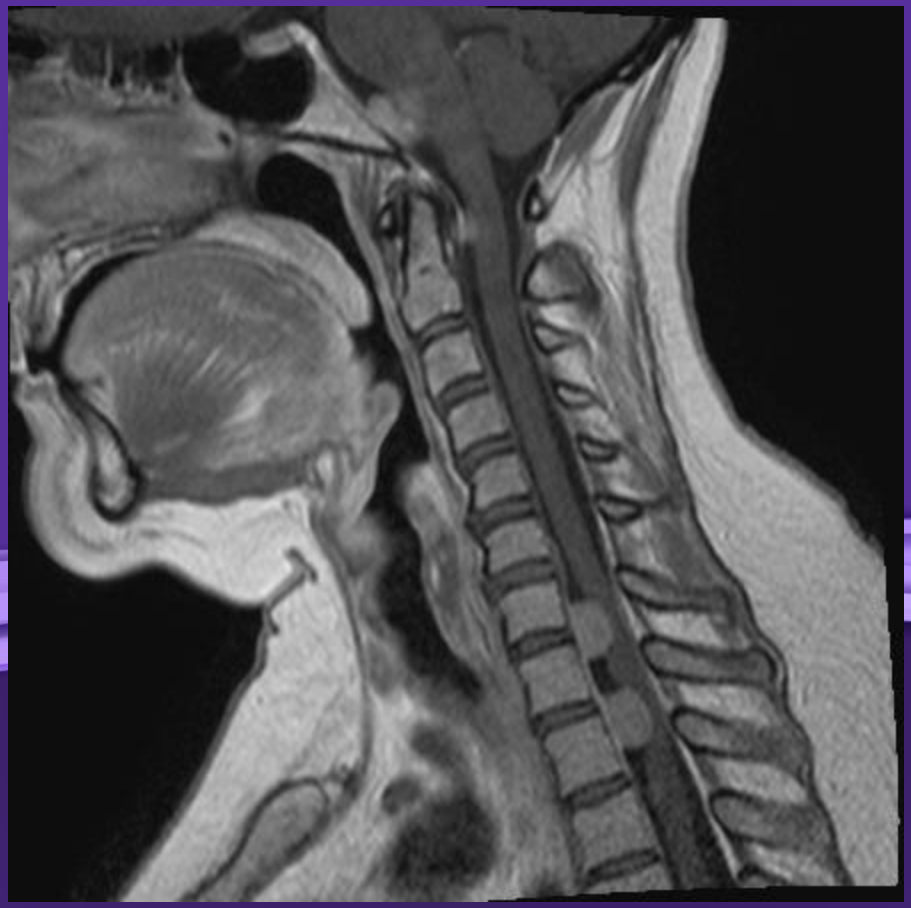
Oropharyngeal Cancers

- 20-40% are HPV positive
- HPV 16
- Better prognosis
- Decreased morbidity from scaled back treatment regimens

The Future

- Vaccines prevent cervical cancer
- Therapeutic “vaccines” eliminate any remaining HPV
- Cervical cancer goes the way of small pox
  - ? (and measles?)



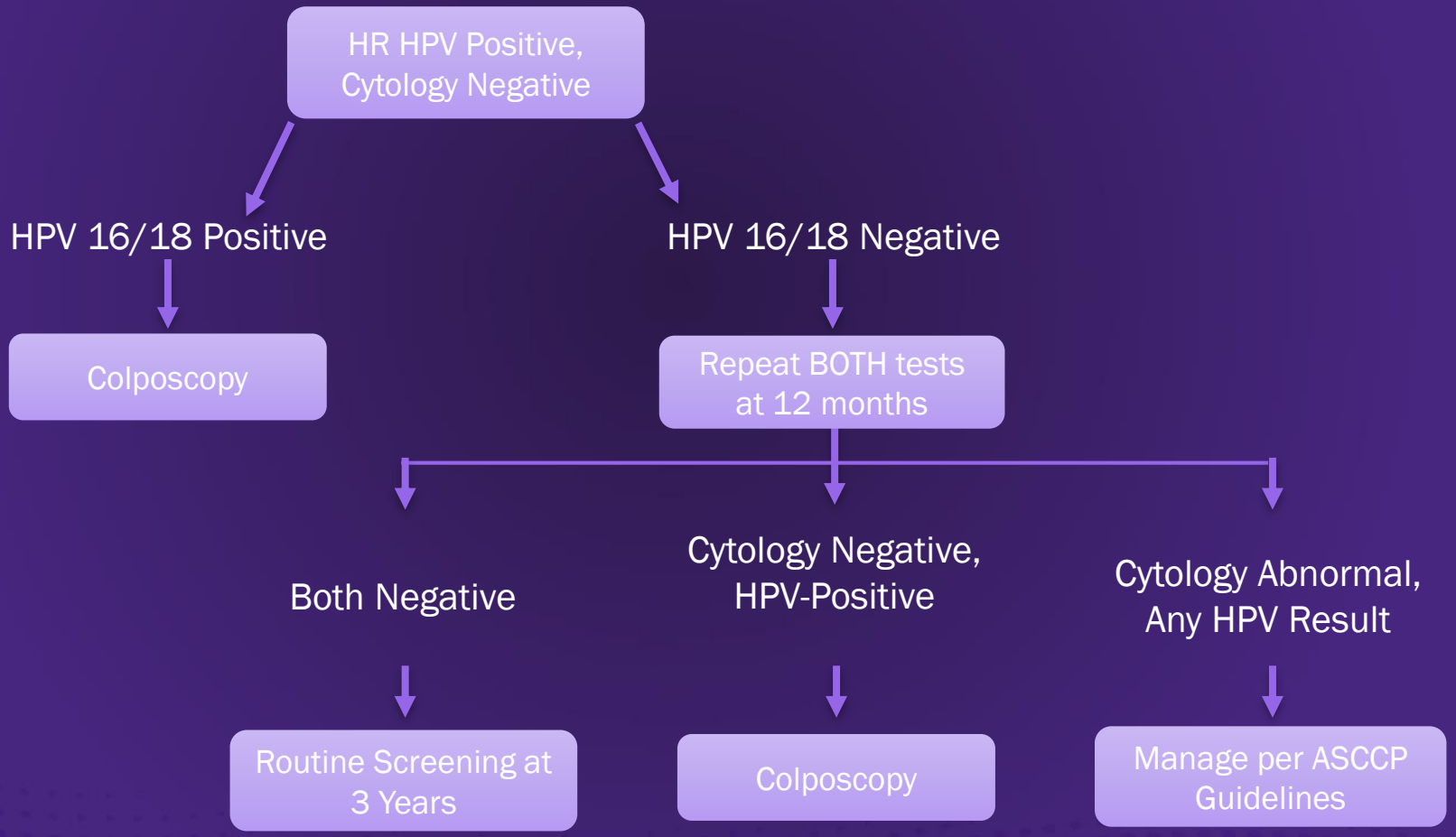


Adjunctive Testing (Pap + HR HPV)

ASCCP Algorithm for HPV Genotyping

For Resolution of Discordant Results in Reflex Testing Women ≥ 30 Years

Use of HPV Genotyping to Manage HPV HR[a]-Positive, Cytology-Negative Women 30 Years and Older



ASCCP = American Society for Colposcopy and Cervical Pathology

[a]Test that detects any of the 14 HR (oncogenic) types of HPV.

ASCCP. ASCCP Clinical Update. ASCCP: Hagerstown, MD; 2009.

ThinPrep® Imaging System with Dual Review™

1st Review

- Imager scans every cell and cell cluster on the slide, measuring DNA content

Image Processor

2nd Review

- Cytotechnologist reviews 22 fields containing "objects of interest"
  - Full slide screened if any cells judged abnormal

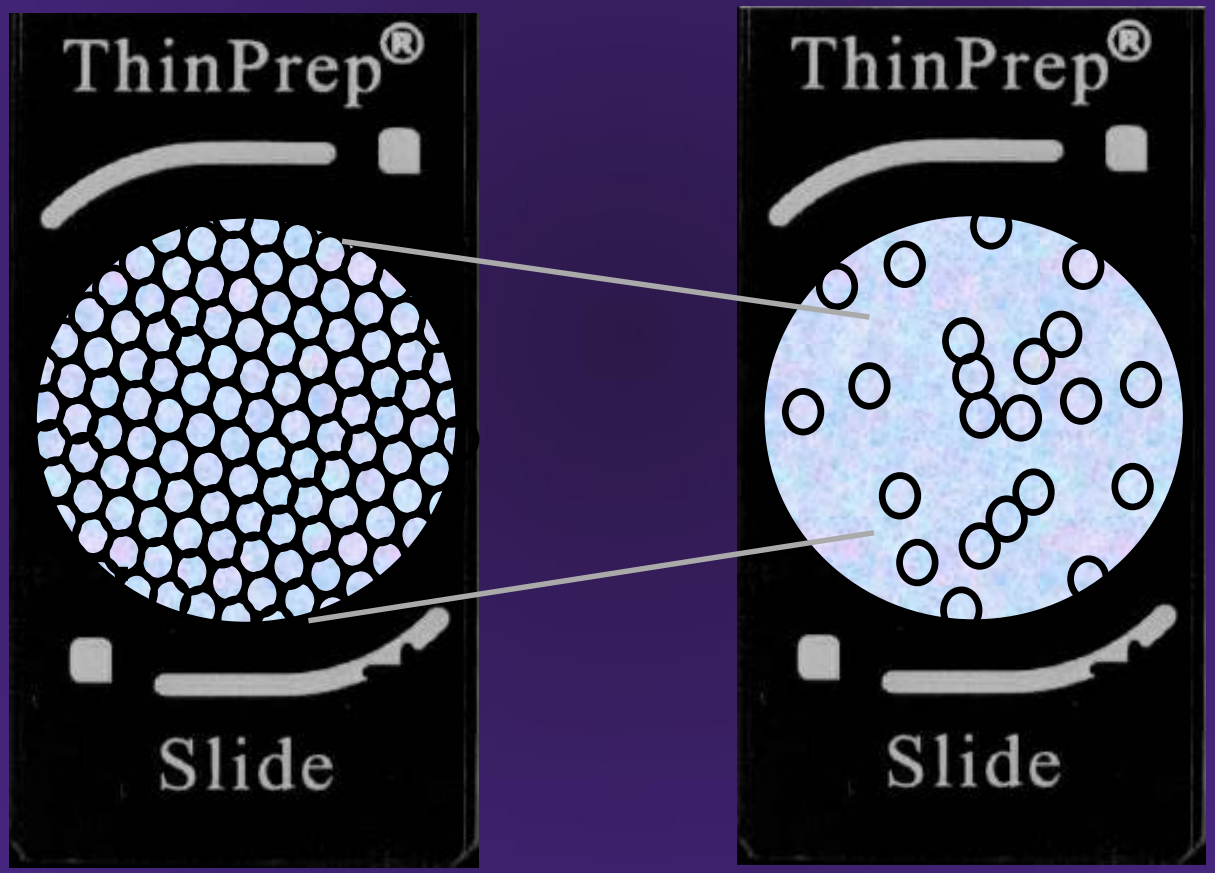
Review Scope

Clinical benefits over manually reviewed ThinPrep® Pap Test[1]:

- Increased sensitivity
- Increased specificity
- Reduced false-negative fraction

1. ThinPrep Imaging System Operation Summary and Clinical Information. Cytyc Corporation; 2003.

Imaging System Focuses Slide Review

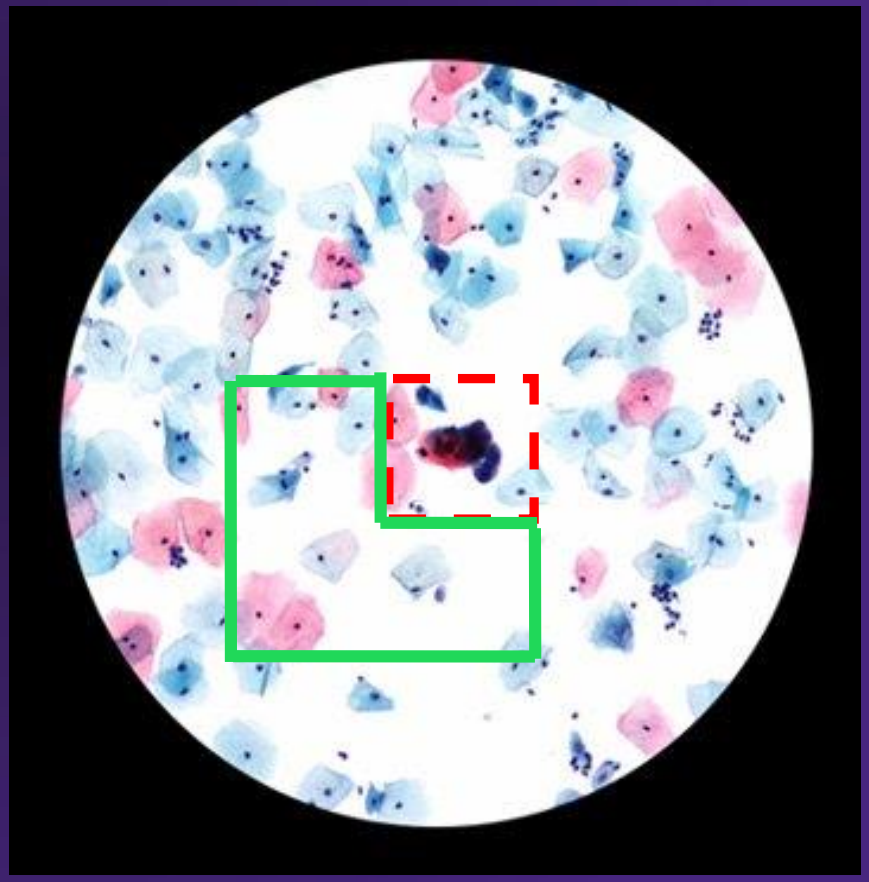


120 Fields of View → 22 Fields of View

1. ThinPrep Imaging System Operation Summary and Clinical Information. Cytyc Corporation; 2003.

ThinPrep® Imaging System Is Statistically More Sensitive Than Manual Screening

- TIS more sensitive than manual screening for:
  - ASC-US
  - Higher-grade lesions with equivalent specificity for ASC-US
  - LSIL
- Glandular malignancies not included in original studies, but data now support

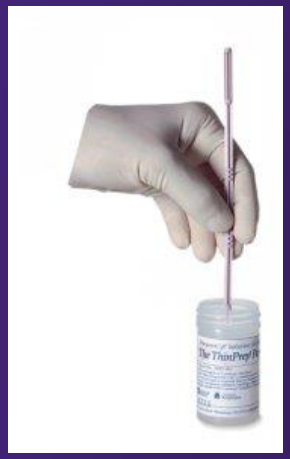


1. ThinPrep Imaging System Operation Summary and Clinical Information. Cytyc Corporation; 2003.

Cells Are Collected in Liquid for Laboratory Processing

Healthcare Provider Office

Sample collected



Laboratory

- Representative sample
- Even distribution of cells
- Minimal obscuring material

Dispersion/Collection/Transfer

BREAST CANCER

- Annual exams
  - Clinical exams and mammograms
- Epidemiologic concerns
  - parity, HRT, breastfeeding
- Treatment concerns
  - Tamoxifen, aromatase inhibitors
- Genetic concerns
  - BRCA mutation status

BREAST CANCER EPIDEMIOLOGY

- Being female (100:1)
- Age (stats determined through age 90)
- Exposure to estrogen
  - Obesity
  - Alcohol
  - Breastfeeding
  - Pregnancy history
  - HRT
- Prior radiation
  - lymphoma

“whether it would be permissible to make the ladies old more quickly by removing their ovaries…”

Schinzinger, 1889

SIXTY-EIGHTH ANNUAL MEETING

OF THE

BRITISH MEDICAL ASSOCIATION

Held at IPSWICH, July 31st, August 1st, 2nd, and 3rd, 1900.

PROCEEDINGS OF SECTIONS.

SECTION OF SURGERY.

HOWARD MARSH, F.R.C.S., President.

ON OÖPHORECTOMY IN CANCER OF THE BREAST.

By STANLEY BOYD, M.B., F.R.C.S.,
Surgeon, Charing Cross Hospital.

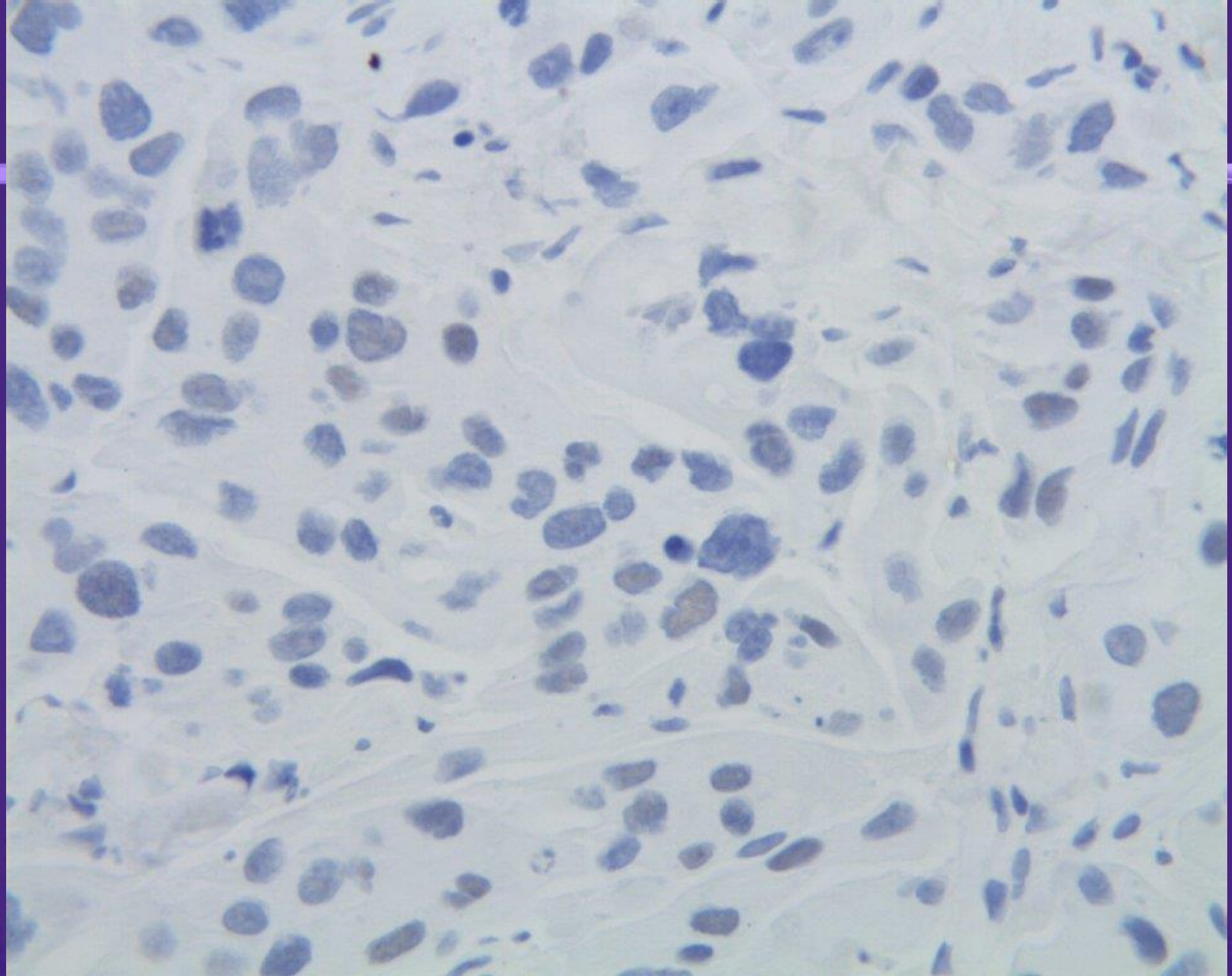
Distant History

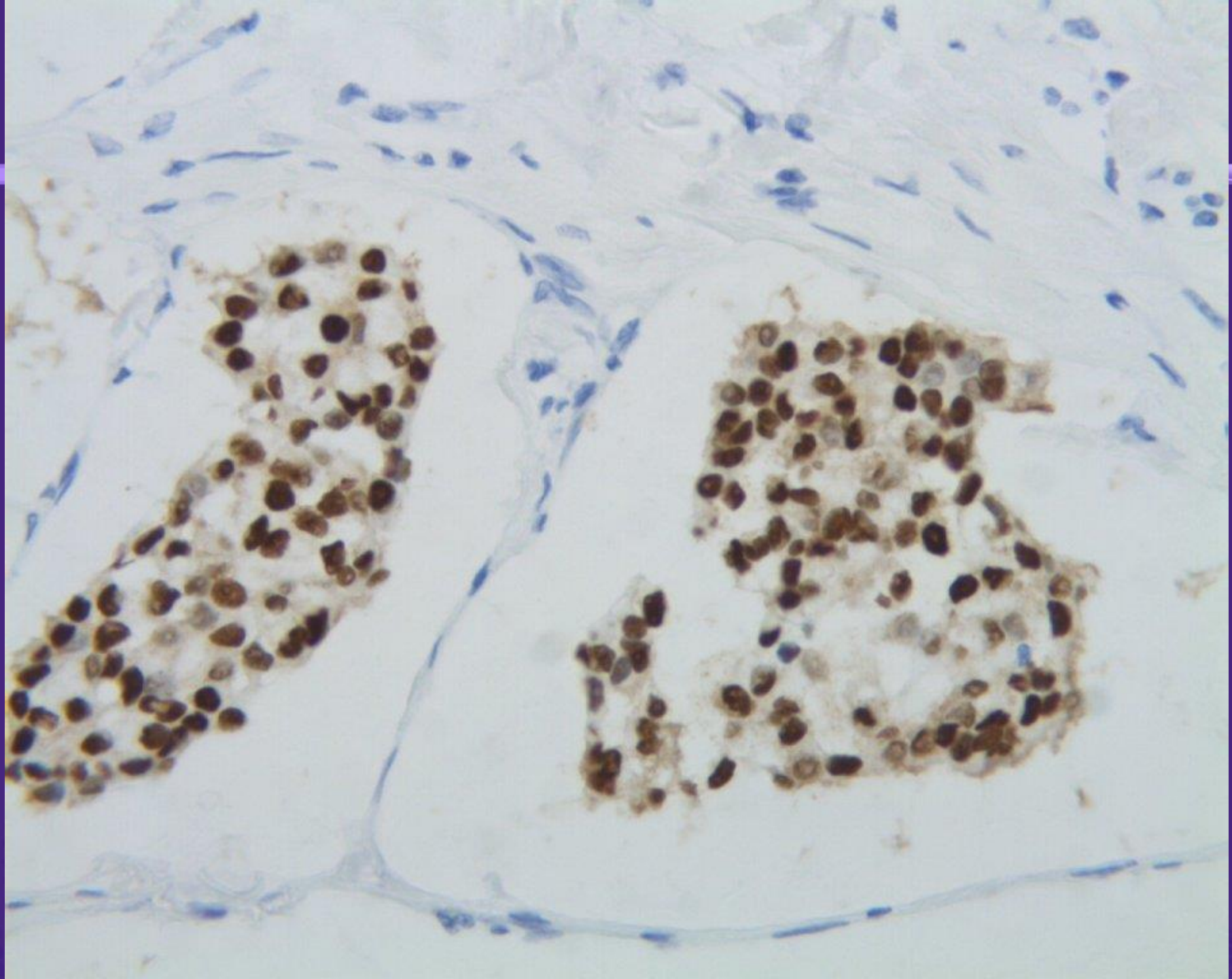
- Dr. George Beatson (Lancet, 1896)
  - Capitalized on experience of Scottish sheep farmers
  - Removed ovaries of a premenopausal patient with advanced breast cancer
- Dr. Stanley Boyd (Br Med Journal, 1900)
  - Reported on 46 patients
  - 37% response rate

V. Craig Jordan, “Why Tamoxifen” in Tamoxifen for the treatment and prevention of Breast Cancer, 1999

Endocrinology Unveiled

- 1923 “estrus stimulating principle”
  - “estrus” from the Latin “frenzy”
- 1962 synthesis of radioactive estradiol
  - Allowed identification of target tissues
- 1966 estrogen receptor isolated





Estrogen Receptor

- Separated tumors for treatment purposes
- Allowed stratification of tumors for prognostic purposes
- 1974: Estrogen Receptor discussed at NCI
  - ER + tumors had 60% response rate
  - ER- tumors had 10% response rate

Endogenous Hormonal Manipulation

- Obesity
- Pregnancy
- Breastfeeding
- Terminations
- Hormonal Contraception

Obesity

- Single most modifiable risk factor associated with diagnosis and prognosis
  - Complex association with medical comorbidities, therapy dosing
  - Multiple measures of obesity
- Across age ranges
  - Post and pre menopausal
- Associated with increased aromatase

Am J Epid; 2004;160:868-875 and *Cancer Epid Biomarkers Prev* 2005;14(7)1686-169