

# Anal Dysplasia

Michelle Inkster MD PhD

Digestive Disease and Surgical Institute  
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# Disclosures

- None
- We thank our colleagues in:
  - Infectious disease
  - Cytology
  - Surgical Pathology
  - Internal Medicine/LGBT
  - Pathology
- For their support and encouragement along the way

# Goals

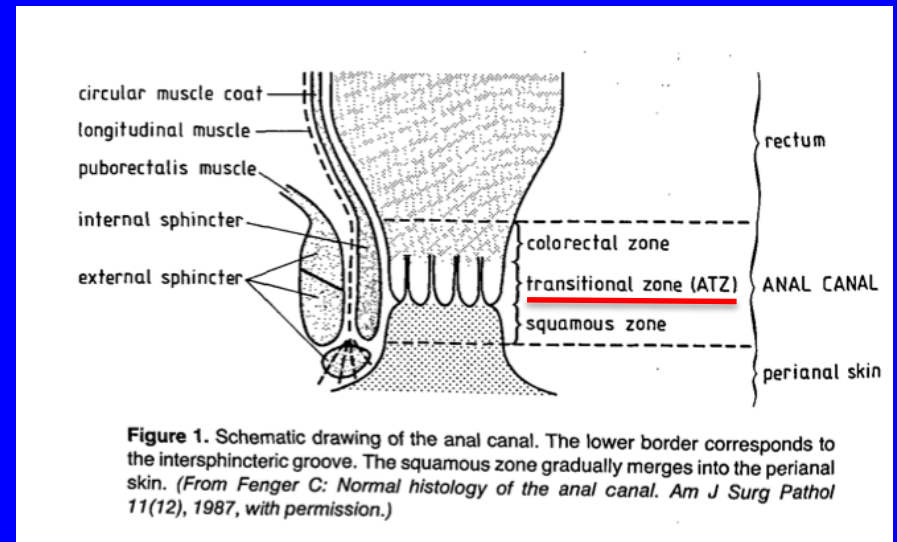
- Know the importance of the anal transitional zone
- Know the parallels between the diagnosis of cervical cancer and of anal cancer
- Be able to discuss the role of vaccination for prevention of disease
- Learn about potential new developments in treatment

# What is dysplasia

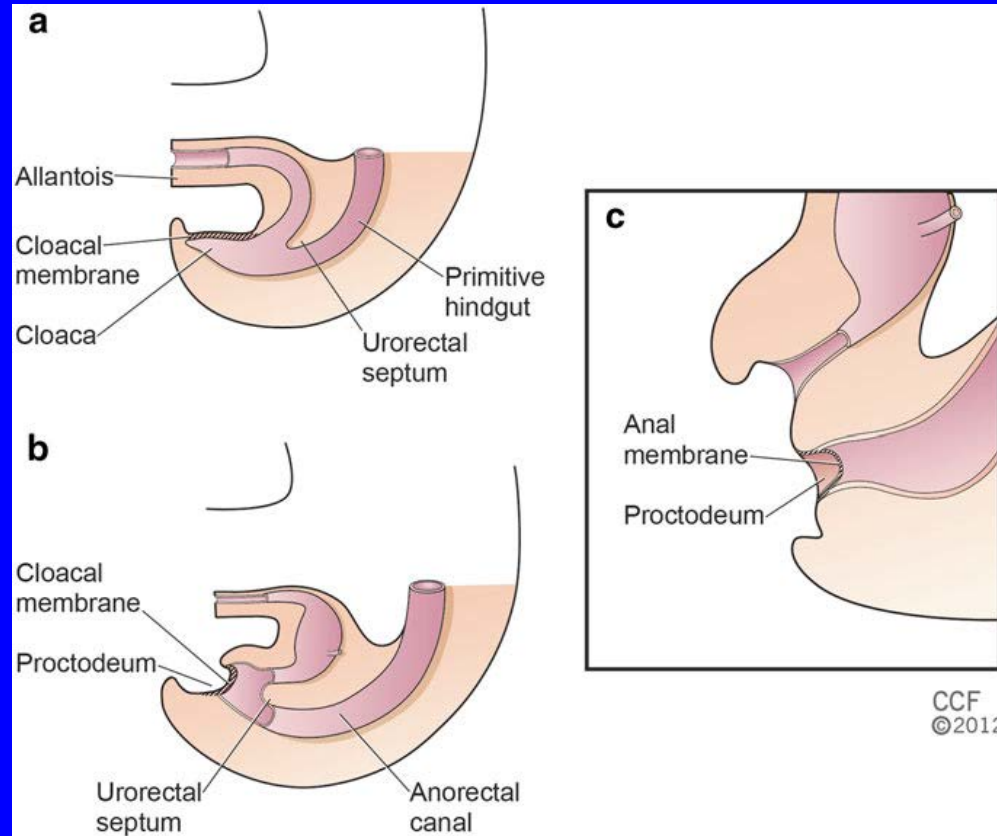
- **Dysplasia** – cells look abnormal under the microscope but are not cancer
- **Hyperplasia** – an increase in the number of cells in an organ or tissue
- Both hyperplasia and dysplasia may become cancer
- **Metaplasia** – reversible transformation of one undifferentiated cell type to another undifferentiated cell type – may be part of the normal maturation process –as if the original cells are not robust enough to withstand their environment so transform to another type

# Anal transitional zone (ATZ)

- 1987 – Fenger
- “the zone interspersed between uninterrupted colorectal type mucosa above and uninterrupted squamous epithelium below”



# Cloacal region in embryos at successive stages of development



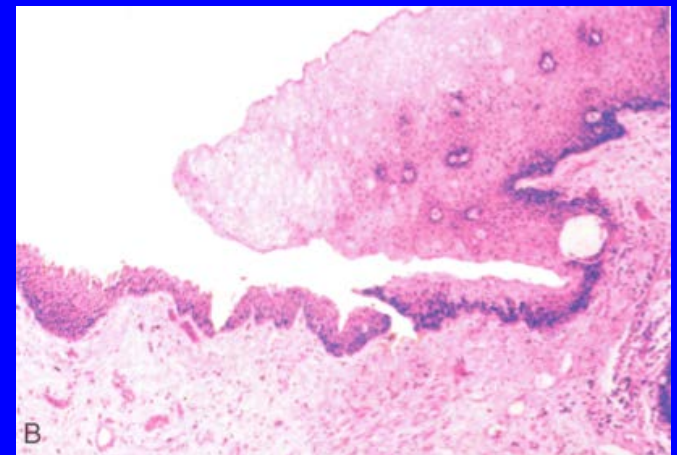
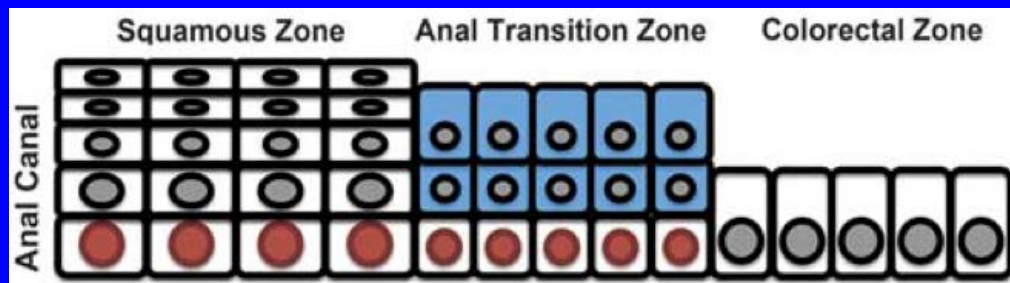
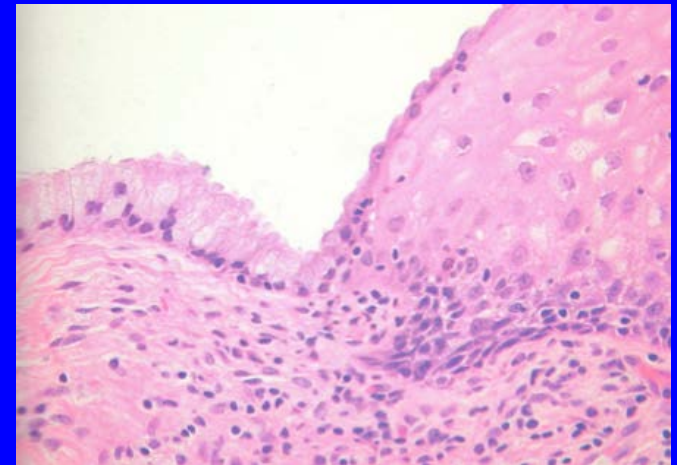
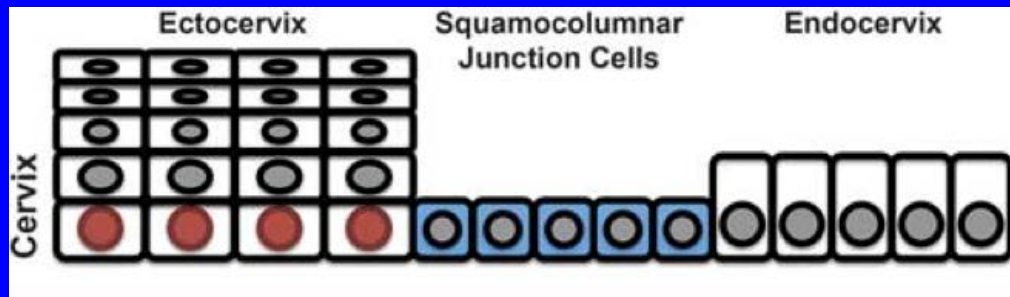


## Cervical transformation zone

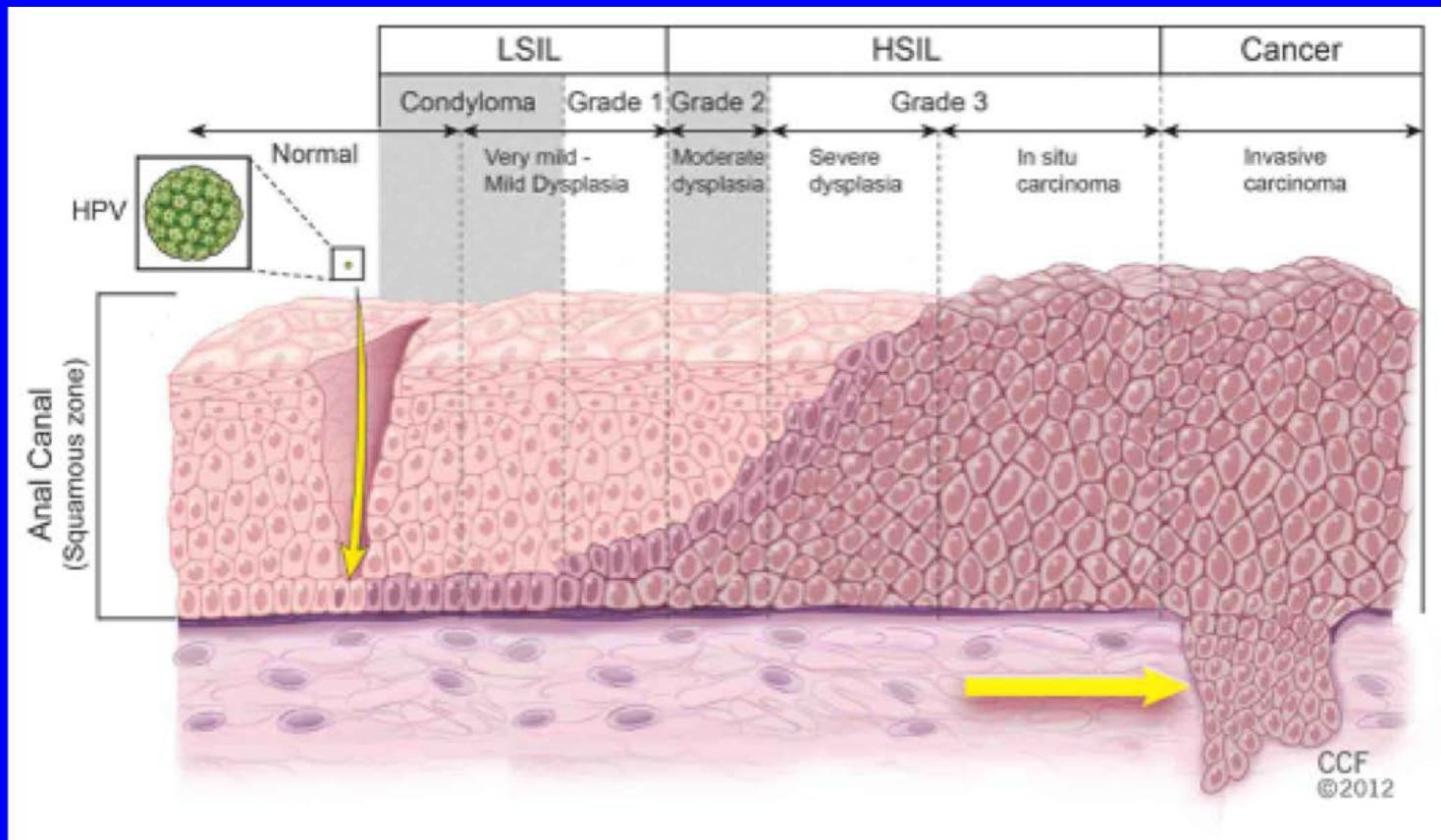
- Has a single layer of squamocolumnar cells
- Has direct contact with the basement membrane
- ?more susceptible to HPV infection

## Anal transitional zone

- Has more than one layer of cells
- Not in direct contact with the basement membrane
- The dominant cell type is metaplastic rather than squamocolumnar



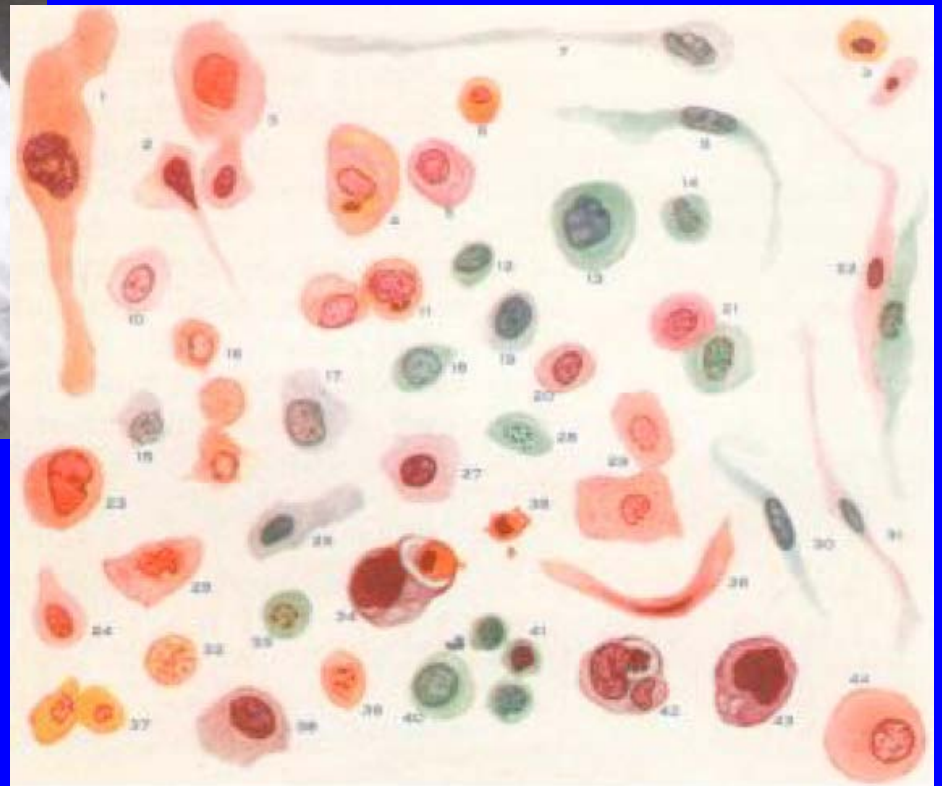




# George Papanicolaou



1920

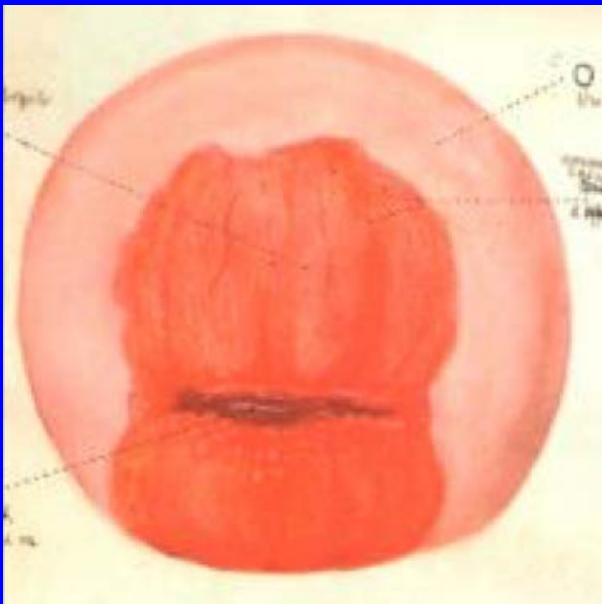


# Cytology

- ASCUS – atypical squamous cells of undetermined significance
  - Some cells were mildly abnormal
- LSIL – low grade squamous intraepithelial lesion
  - rarely becomes cancerous
- HSIL - high grade squamous intraepithelial lesion
  - Moderate or severe dysplasia – can transform into cancer over time

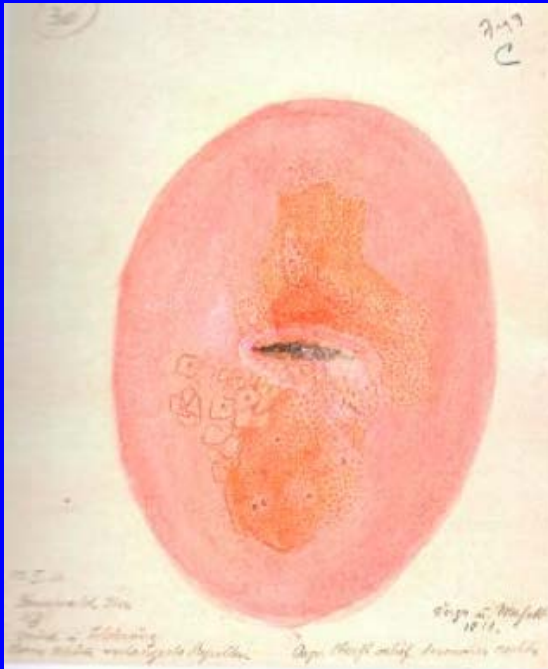
# Colposcopy

- Hinselmann 1925
- Drew the cervix with the transformation zone



# Colposcopy with acetic acid

- Also Hinselmann 1938





# Role of acetic acid

- Acetic acid interacts with both normal and neoplastic cells
- Causes them to swell and change color
- Cells with an increase in the nuclear:cytoplasmic ratio are transiently white
- The surrounding normal squamous epithelium remains pink in the cervix
- In the rectum the cells also stain

# Warts are similar



Perianal warts seen  
on routine colonoscopy

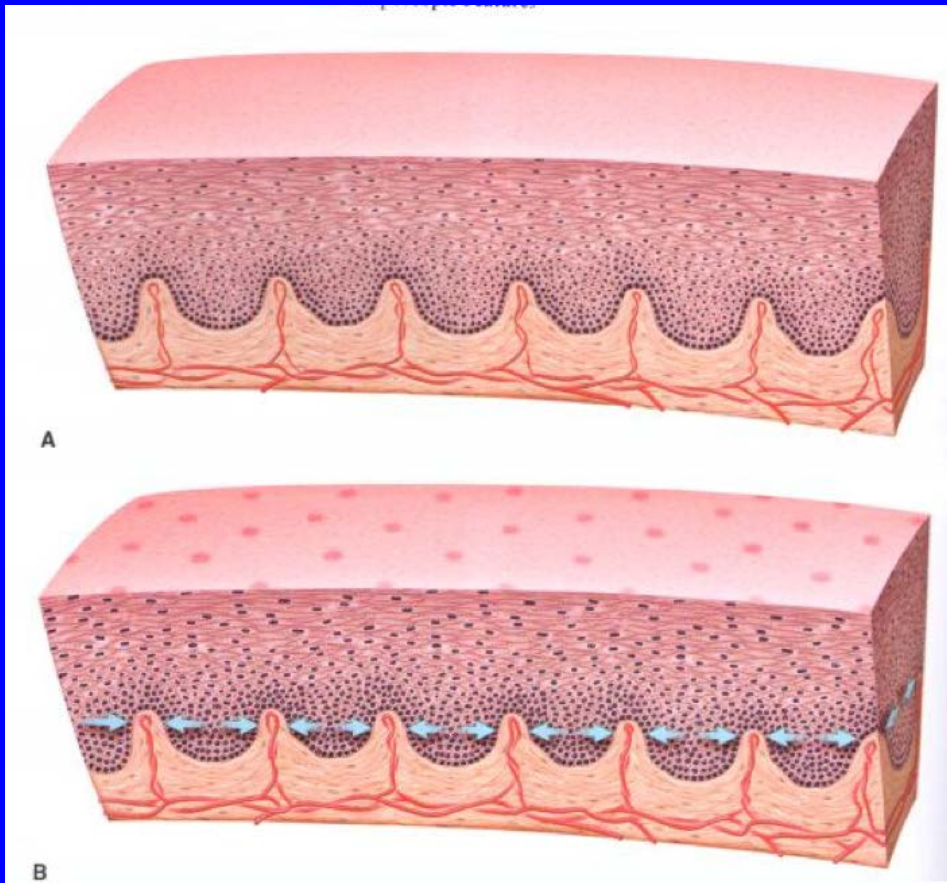


Vaginal condyloma

# Punctation

Loop capillaries are normal fine caliber  
With even distribution

Neoplastic cells exert pressure in the capillaries.  
Venous occlusion causes the vessels to dilate





# Mosaicism



Rows of hairpin capillaries  
coursing perpendicular to  
the epithelial surface



punctation

mosaicism

# What causes anal dysplasia?

- Human papillomavirus (HPV) is considered the cause of anal dysplasia
- The virus is thought to be more than 350 million years old
- Treatment for warts was known in the time of Hippocrates
- There are more than 200 types

# Human Papillomaviruses

- Diverse group
- Different epithelial tropisms
- Different life-cycle strategies
- Many are low risk
  - Difficult to manage in immunosuppressed people
  - Papillomatosis and sometimes cancer
- High risk
  - Almost all instances of cervical cancer
  - Large proportion of anogenital cancer
  - Growing number of head and neck cancers



## Harald zur Hausen

Extracted HPV DNA from  
plantar warts

Did not react to genital  
warts or other skin  
warts

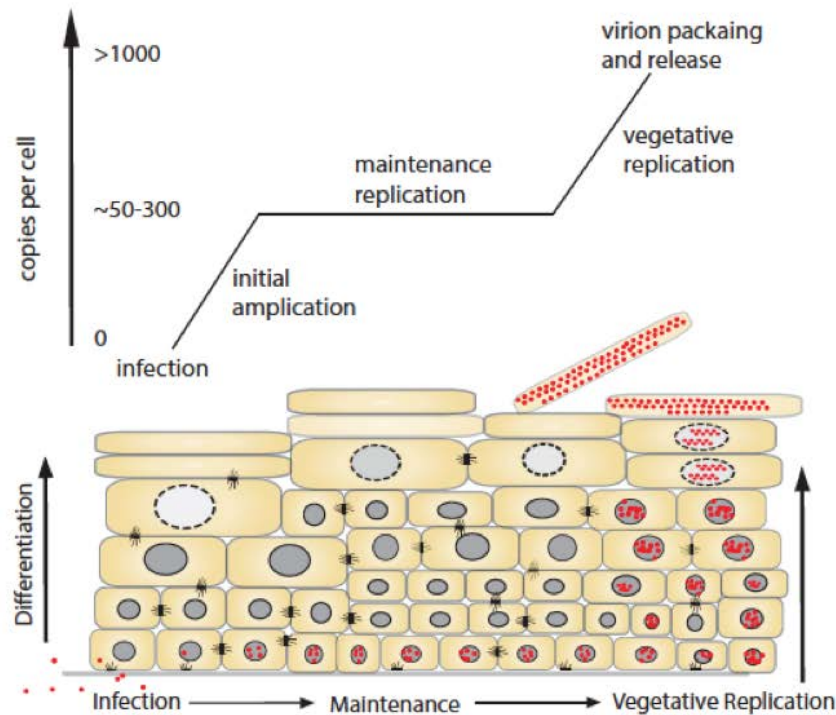
Concluded papilloma  
virus not a single virus  
but many

# Anogenital intraepithelial neoplasia associated with HPV

- AIN – anal intraepithelial neoplasia
- CIN - cervical intraepithelial neoplasia
- KIN – keratinocyte intraepithelial neoplasia
- PIN – penile intraepithelial neoplasia
- VIN – vulvar intraepithelial neoplasia

# HPV and HIV

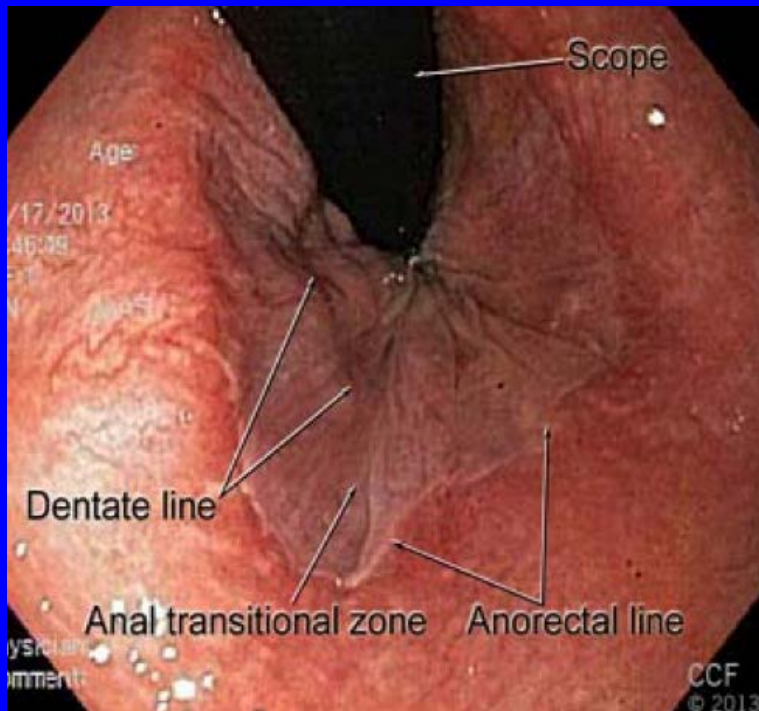
- HIV-1 *tat* protein transactivates the HPV LCR
- Leads to invasion of basement membranes
- Invasion may be mediated by integrins
  - *tat* binds integrins
- Person infected with genital HPV is at greater risk of contracting HIV - ?biological mechanism



**Figure 1.** Phases of human papillomavirus (HPV) DNA replication. Following infection, an initial amplification event boosts HPV DNA copy number to 50–300 per cell in the basal, or proliferative, epithelial compartment. Copy number is maintained until the vegetative phase, which occurs during a productive HPV infection. The vegetative (productive) phase is characterized by a second, differentiation-dependent HPV DNA amplification that produces DNA for packaging in virions and sloughing within terminally differentiated cells from the epithelial surface.



# Narrow band imaging



White light

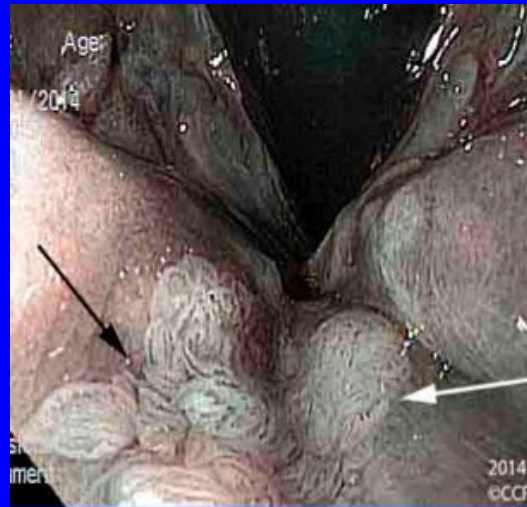


Narrow band

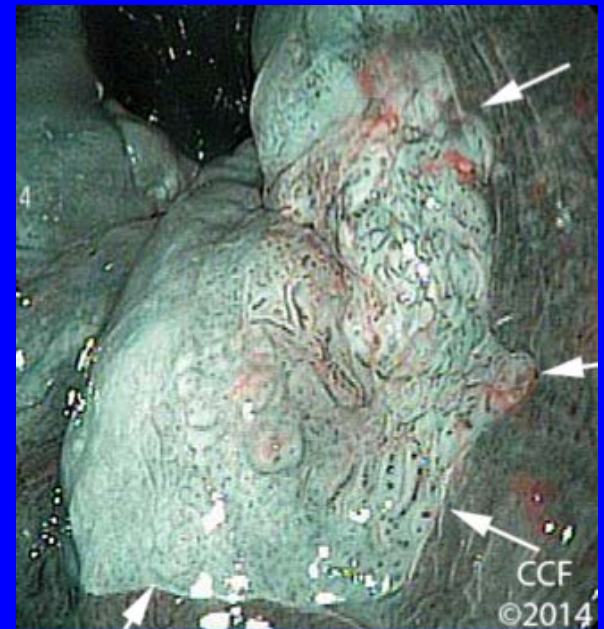




White light



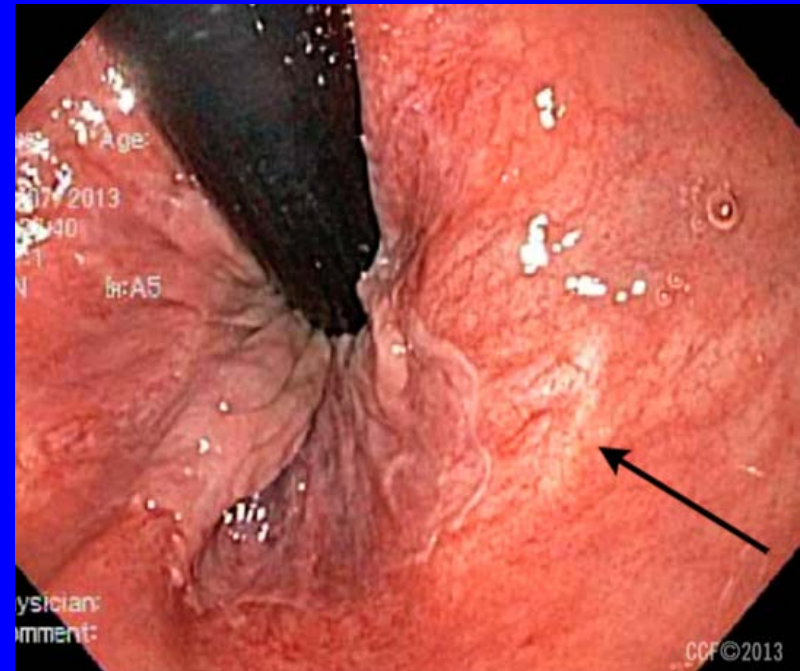
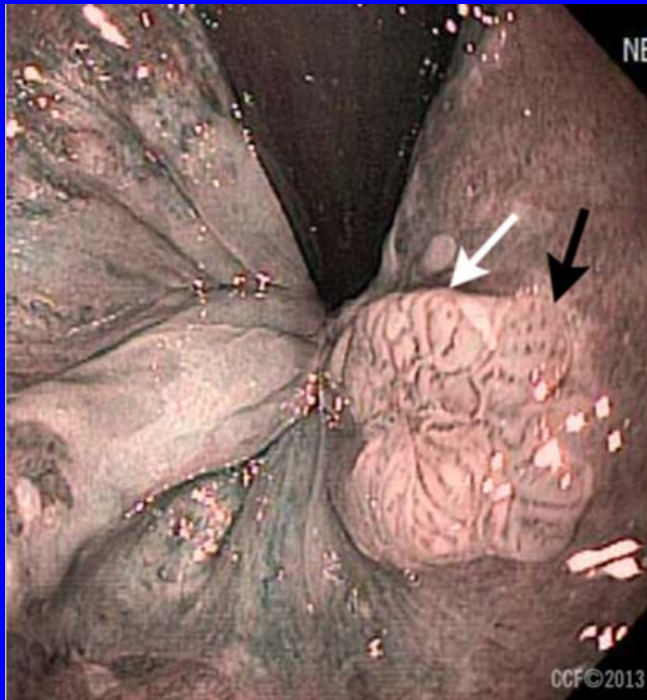
Narrow band



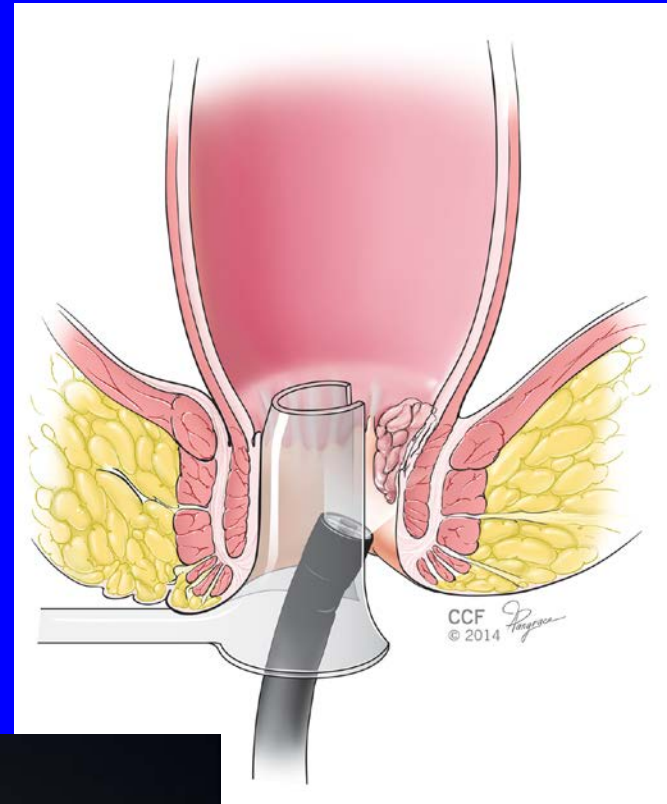
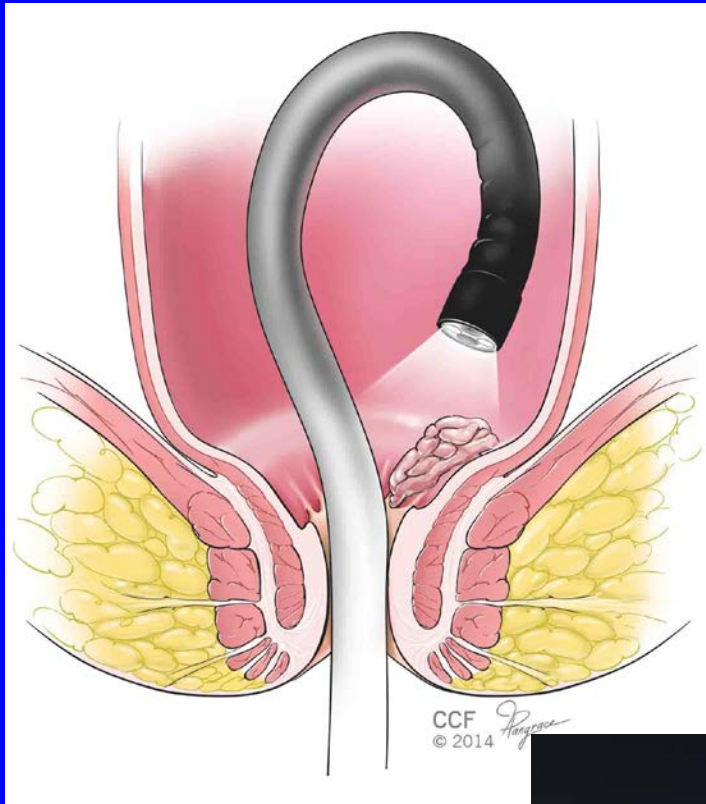
Narrow band  
with acetic acid



# Narrow band imaging with acetic acid (NBIA)



# Anospec<sup>®</sup> lighted anoscope





**Table 2. Chromoendoscopy (NBIA) in search of anal dysplasia in 260 individuals with abnormal anal cytology**

		Histopathology				
		SIL/SCC (%)	LSIL (%)	HSIL (%)	SCC	SIL not found
Cytology	N					
ASCUS	141	63/141 (44.7)	46/141 (32.6)	24/141 (17.0)	0 (0)	74/141 (52.5)
LSIL	100	73/100 (73)	54/100 (54)	19/100 (19)	1/100 (1)	25/100 (25)
HSIL	19	13/19 (68.4)	7/19 (36.8)	6/19 (31.6)	0 (0)	5/19 (26.3)

ASCUS=atypical cells of undetermined significance; LSIL= low grade squamous intraepithelial lesion; HSIL – high grade squamous intraepithelial lesion; SCC = squamous cell carcinoma. These results are for first time chromoendoscopy.

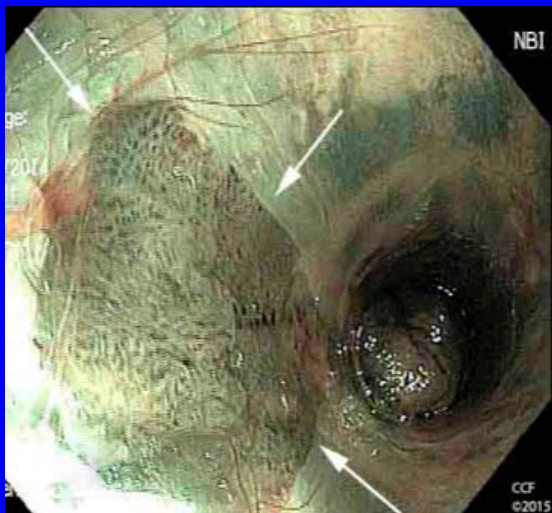
# Further testing

- Anal cytology is a poor predictor of anal dysplasia in HIV positive patients
- The correlation between cytology and histology is poor
- In our own studies 44.7% of patients with ASCUS have intraepithelial dysplasia or cancer
- This is typical of other studies both for the cervix and the anus
- Sturgis (CCF) introduced a quality control measure for cytology – the transitional zone must be present on all slides for a diagnosis to be made

# Anal cancer

- Estimated 90,000 cases worldwide
- Estimated 8,000 cases in the USA
- Risk factors
  - Low CD4 count
  - Persistent high risk HPV infection
  - Infection with multiple HPV types
  - Anoreceptive intercourse
  - History of cervical cancer
  - Tobacco use
  - Solid organ transplant
- In the HAART era
- ~40/100,000 for HIV +
- ~78/100,000 for HIV+/ MSM

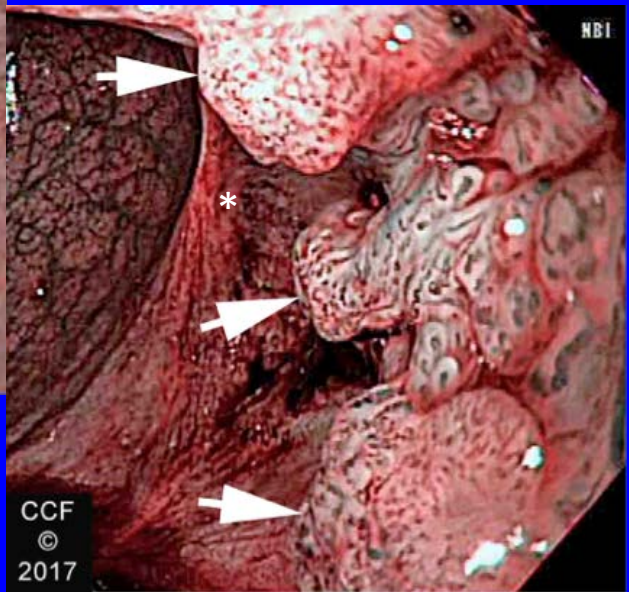
# Anal cancer



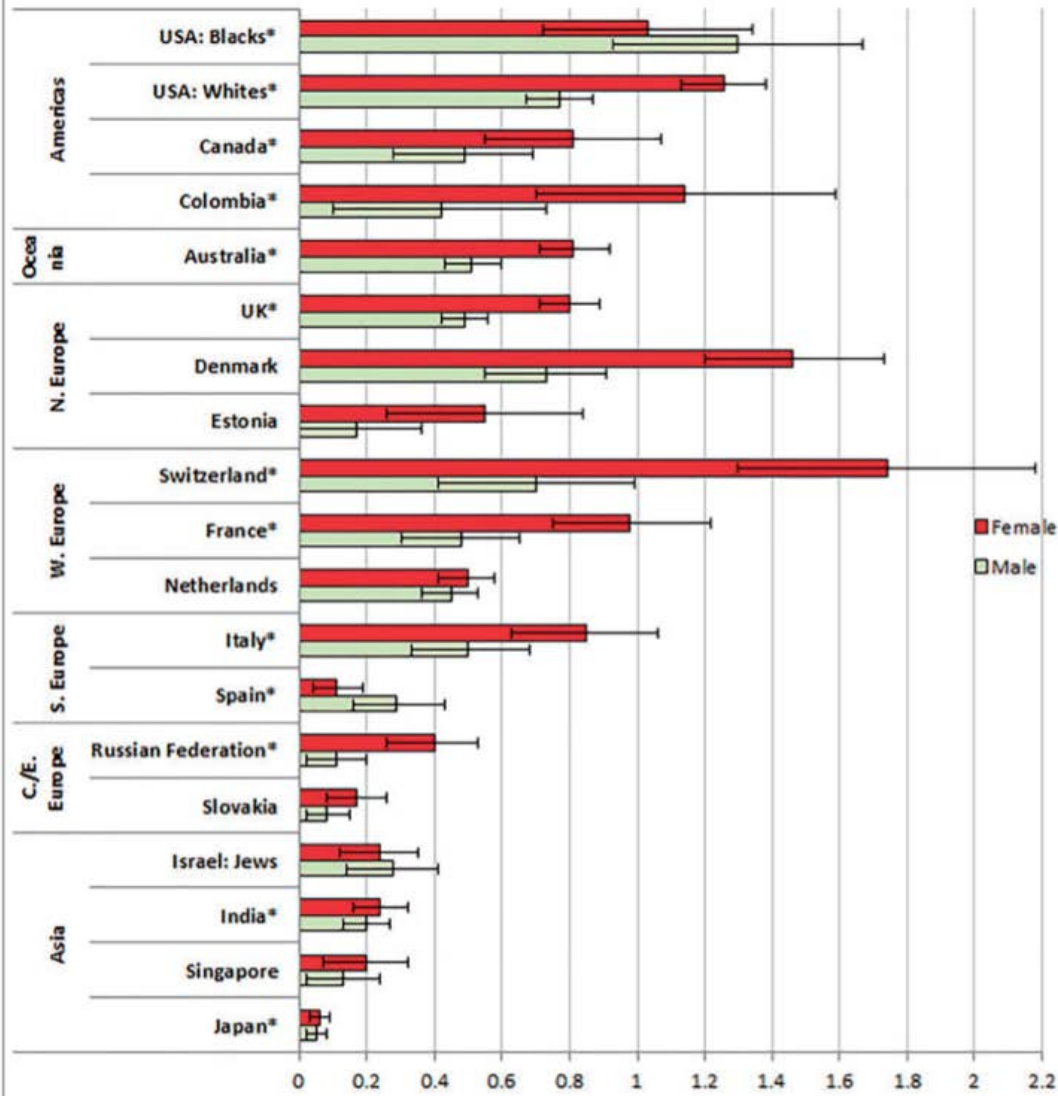
Seen through a lighted anoscope



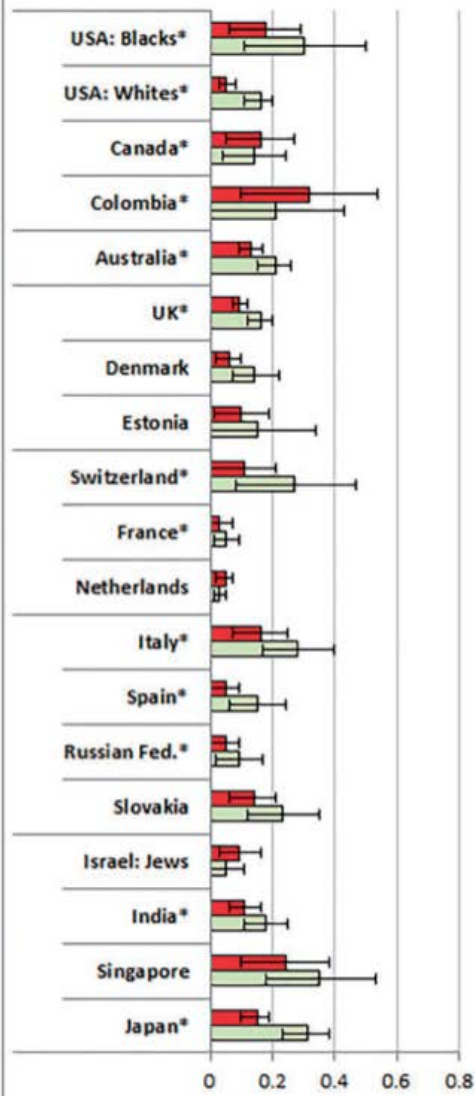
Retroflexed view  
\* Site of prior hemorrhoidectomy



### Squamous cell carcinoma



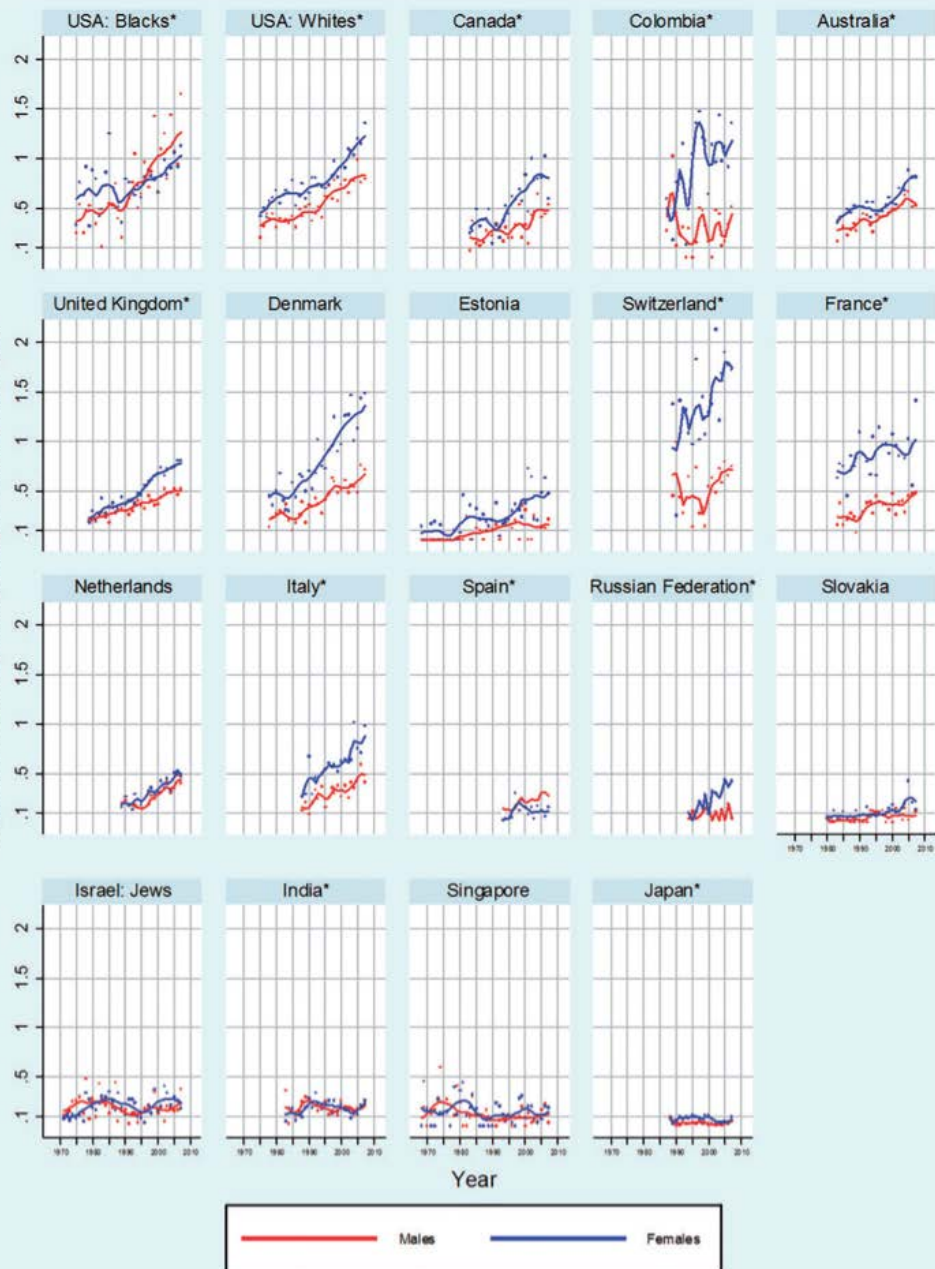
### Adenocarcinoma





# Age associated incidence of anal squamous cancer by sex

Age-standardized (World) rate per 100 000, all ages

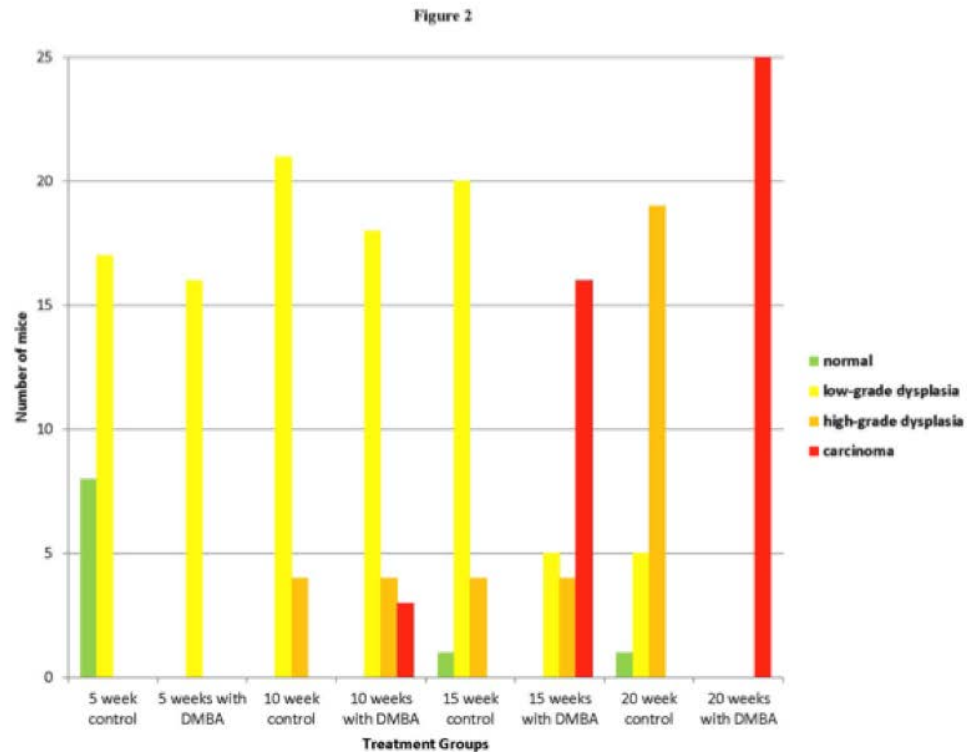


\* Regional data

# Transgenic mouse model for anal dysplasia

- 2010 – Lambert
  - HPV 16 E6 and E7 mice bred
  - E6 and E7 expressed in the anal transitional zone
  - DMBA – dimethylbenz[a]anthracene applied topically
  - 50% of DMBA-treated HPV 16 E6 E7 mice showed overt signs of tumors
  - Confirmed by histology
  - Biomarkers similar to HPV + precursors of human anal cancer

# DMBA induced anal cancer in transgenic mice



# Other modes of treatment

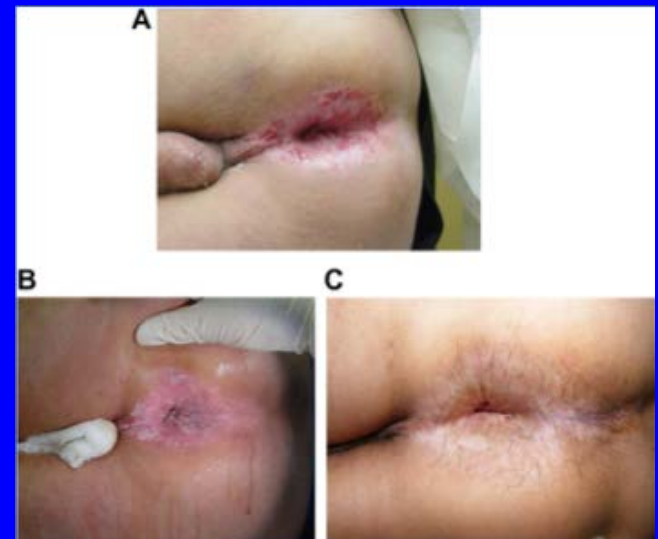
- Photodynamic therapy
- Antiviral particles
- Radiofrequency ablation
- Cryotherapy
- Cidofovir
- Rapamycin
- Imiquimod
- 5-fluorouracil



# Medical therapy

There are no FDA approved products for anal dysplasia – all are off-label

- Aldara, Imiquimod, Condylox, Podofilox, 5FU
  - Side-effects
  - Pain, burning and irritation
  - at best uncomfortable
  - at worst treatment limiting



# PDT

- The first phototherapy experiment was in the late 19<sup>th</sup> century - Finsen used a heat filtered lamp to treat *lupus vulgaris*
- 1913 – Meyer-Betz had general skin sensitivity after exposure to sunlight - he had injected himself with hematoporphyrin
- 1978 - Thomas Dougherty
- Treated 113 cutaneous or subcutaneous tumors and observed total or partial resolution in 111.

# Condyloma accuminata



Scissors, Cautery, Scalpel



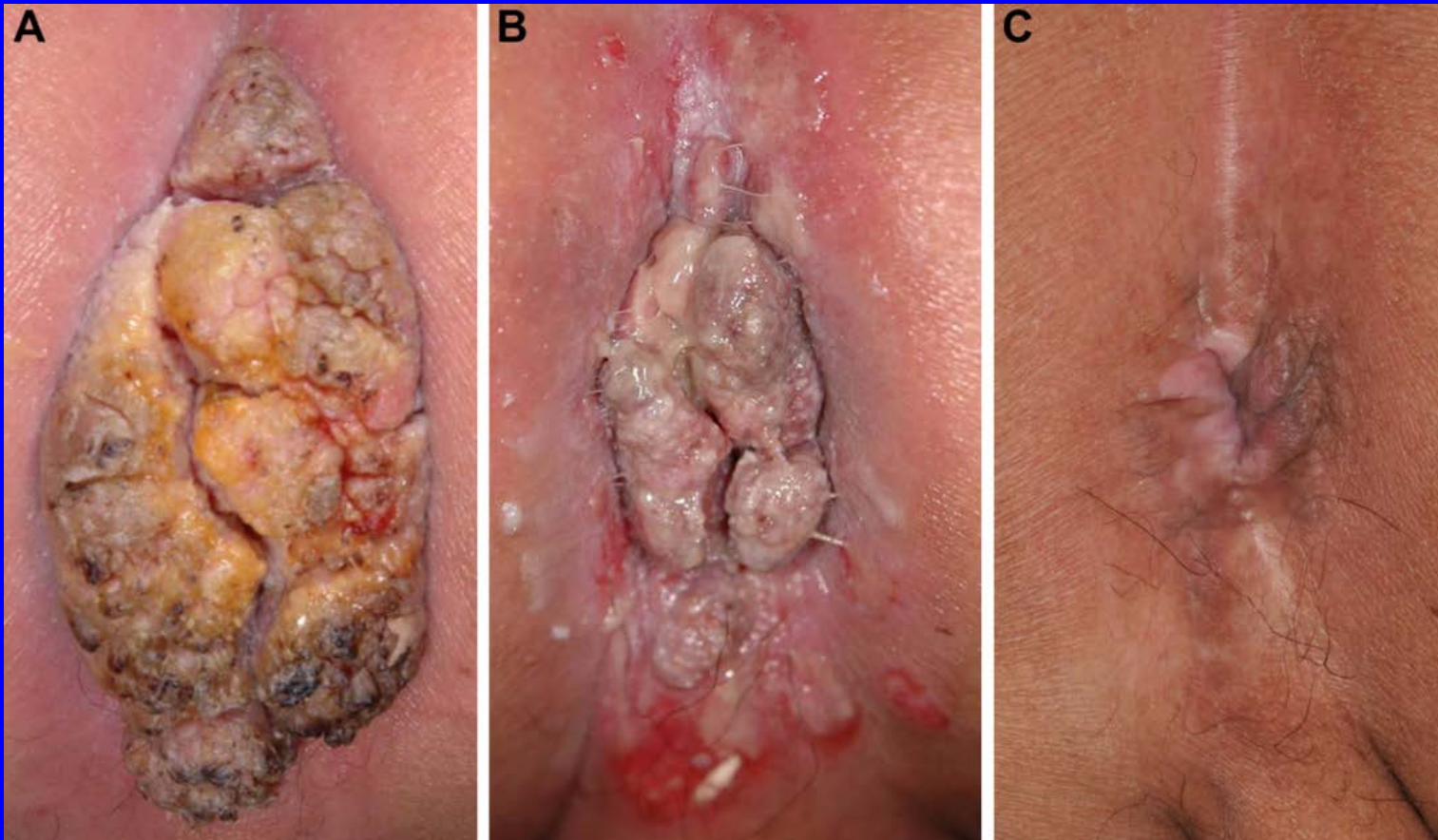


# Condyloma acuminata

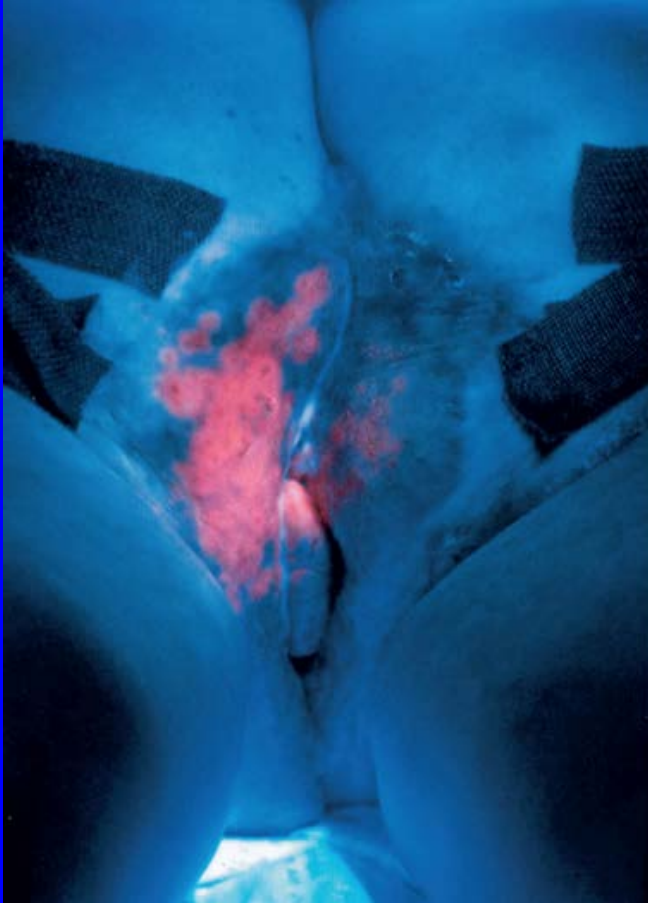




# Buschke-Lowenstein tumor treated with pdt



# Treatment of AIN III



# PDT side-effects

- Burning
- Swelling
- Infection
- Redness
- Activation of HSV
- Skin peeling
- Stay out of the sunlight  
for 24-48 hours

- Radiofrequency ablation – Goldstone 2017



- 21 HIV negative patients with HSIL
- Evaluated every 3 months x 12 months
- Minimal side-effects
- Increased BMI – increased recurrence

- Infrared coagulator – Goldstone

- 2004 – 65% developed new lesions within median of 217 days
- HIV more lesions develop



- **Cryotherapy**

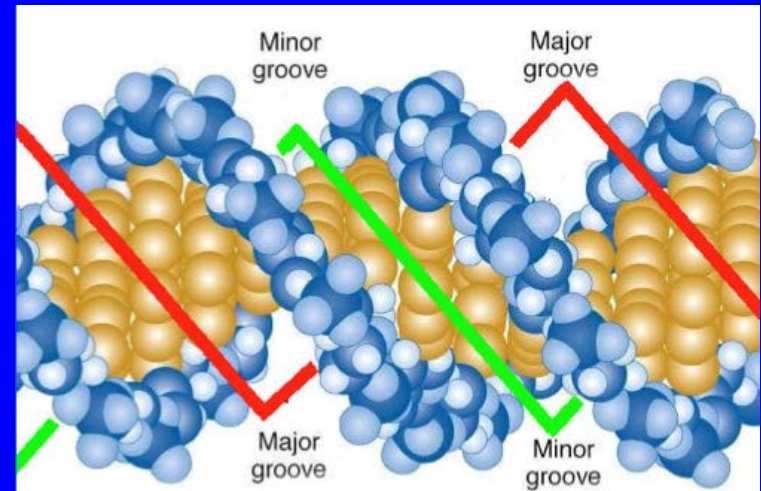
- Instruments range from hand held to large units
- Freeze with liquid nitrogen in the office
- Carbon dioxide laser treatment – needs the operating room with an extractor
- Needs oral or local anesthesia
- Excellent healing





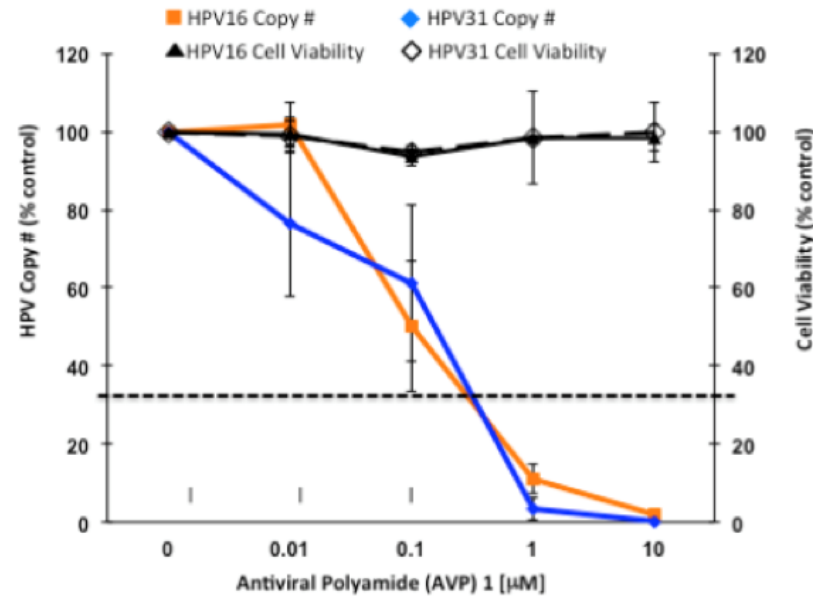
# Antiviral peptides

- Distamycin A and other naturally-occurring minor groove DNA binding agents
- Toxicity
- Lack of specificity
- Synthetic analogues that bind to AT rich regions within high risk HPVs induce rapid loss of HPV episomes without toxicity





- Antiviral peptides trigger noncytotoxic loss of HPV
- No cytotoxicity
- Cell replication not required – arrest of cells in S or M phase makes no difference
- Trigger viral DNA elimination
- Do not interact with host DNA or integrated viral DNA
- May be a promising approach to eliminate HPV



**Figure 2.** Antiviral polyamide 1 potently induces rapid loss of HPV episomes in the absence of cytotoxicity. The 48 h. dose response curves show loss of episomes for two different viruses (HPV16 and HPV31) while at the same time exhibiting no cell toxicity. Note that AVP1 drives episomes to nearly undetectable levels. The black, dashed line represents theoretical viral DNA levels following treatment with a putative “perfect” viral replication inhibitor after two cell cycles in 48 h.

# Future directions for detection

DNA methylation of HPV has been validated as an accurate method for the detection of CIN2 and CIN3 – cervical cancer

Testing was developed because of the need to minimize unnecessary colposcopy

Methylation of human genes may be detected up to 7 years before cancer is discovered

Cervical cancers have higher levels of methylation than CIN2 – Can this be used as a diagnostic tool?

- Hypermethylation has been known to occur in cancer cells for many years
- Many genes modified by hypermethylation have tumor suppressor function
- Mutations are irreversible
- HPV-associated cancers are associated with the p16 tumor suppressor gene

- HPV has greater sensitivity than cytology
- HPV has poorer specificity than cytology
- HPV16 is the most common type
- High risk progressive lesions from other types would not be identified if only HPV16 were checked
- Human genes and viral genes can undergo hypermethylation
- DNA methylation may therefore be useful for triaging because of greater specificity

# p16 tumor suppressor gene

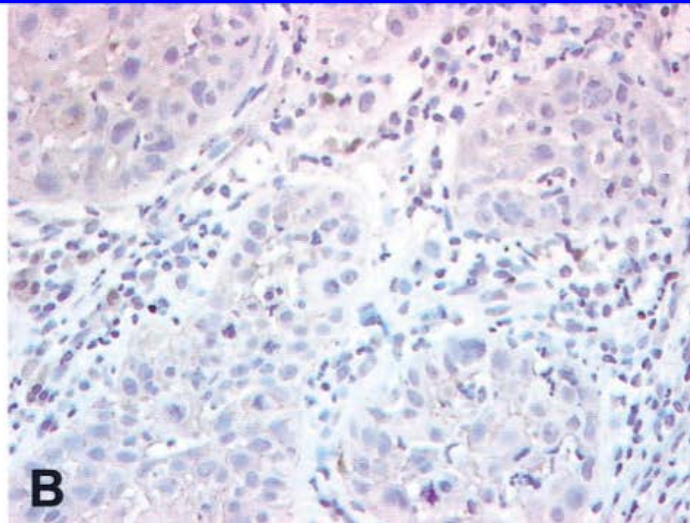
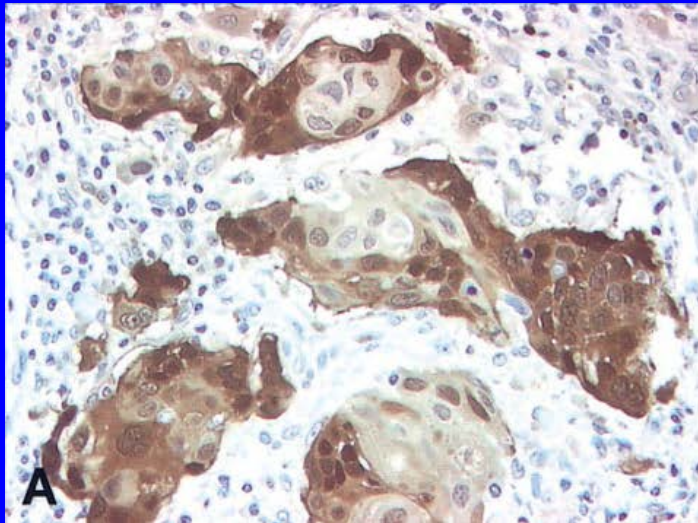
- p16 is the most commonly altered gene in human malignancies
- hypermethylation of the p16 gene causes
  - transcriptional down-regulation
  - transcriptional silencing
- one of the major mechanisms of p16 gene inactivation in various types of cancer, including cervical carcinoma



# p16 staining

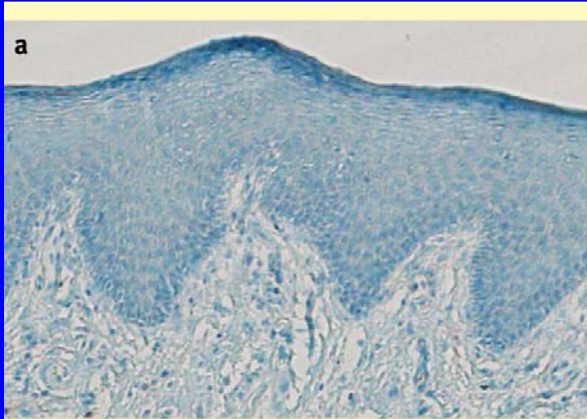
HPV positive tonsillar CA

HPV negative tonsillar CA

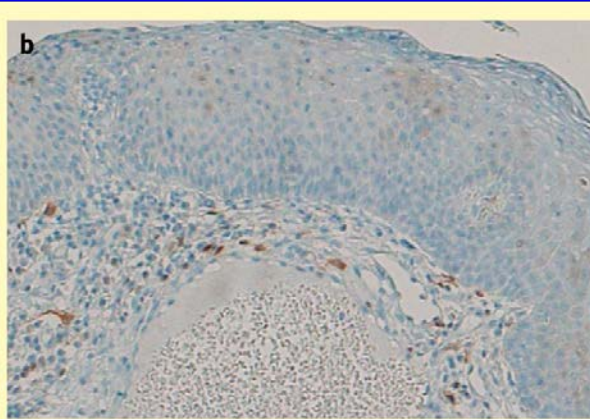


# P16 staining anal dysplasia

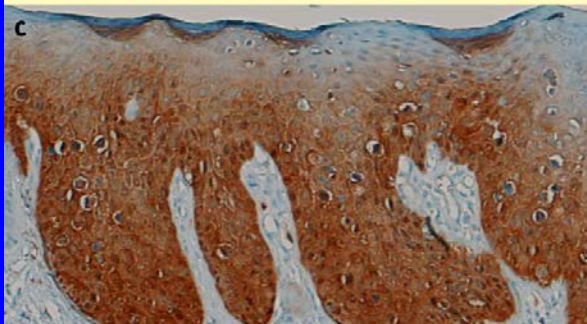
Normal



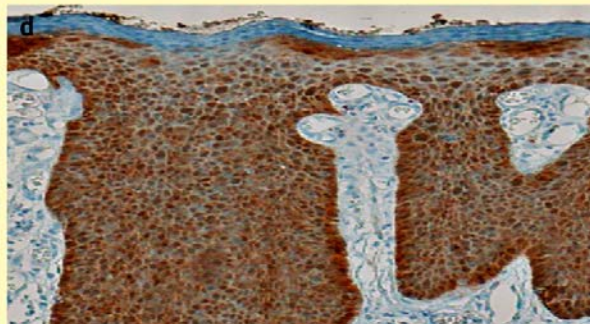
AIN I



AIN II



AIN III



# Improving detection

- Lorincz et al 2016
  - Used methylation of HPV and a tumor suppressor gene to determine whether high levels of methylation would be associated with anal dysplasia.
- Used host gene *EPB41L3* (tumor suppressor)
  - This is highly methylated in cancer of lung, Cervix, ovary and breast



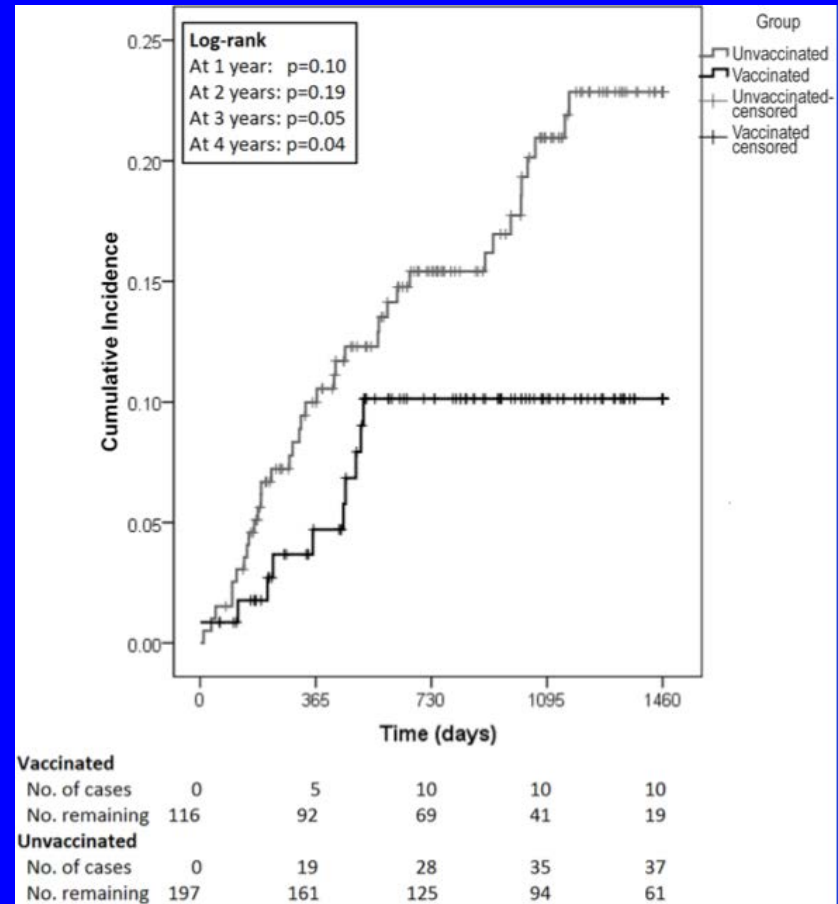
# HPV methylation and anal cancer

- DNA methylation testing of HPV and human genes validated for CIN2 and CIN3
- Levels persist and increase over time with persistent HPV16 infection
- All of the anal cancers studied were positive for HPV DNA methylation similar to cervical cancer
- ?HPV DNA methylation can be used to indicate which lesions will progress from AIN to cancer

# VACCINATION

## Time to anal condyloma development

- MSM  $\geq$  26 years
- h/o anal condyloma

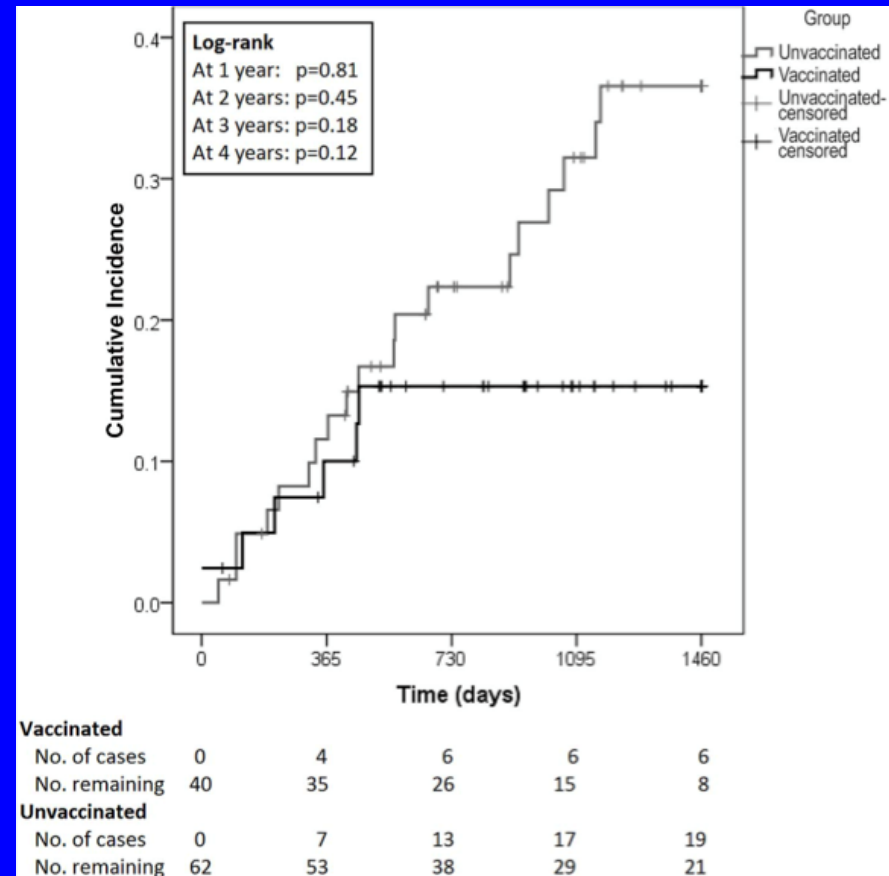




# VACCINATION

## Time to recurrent anal condyloma

These two studies suggest that older MSM patients may be protected



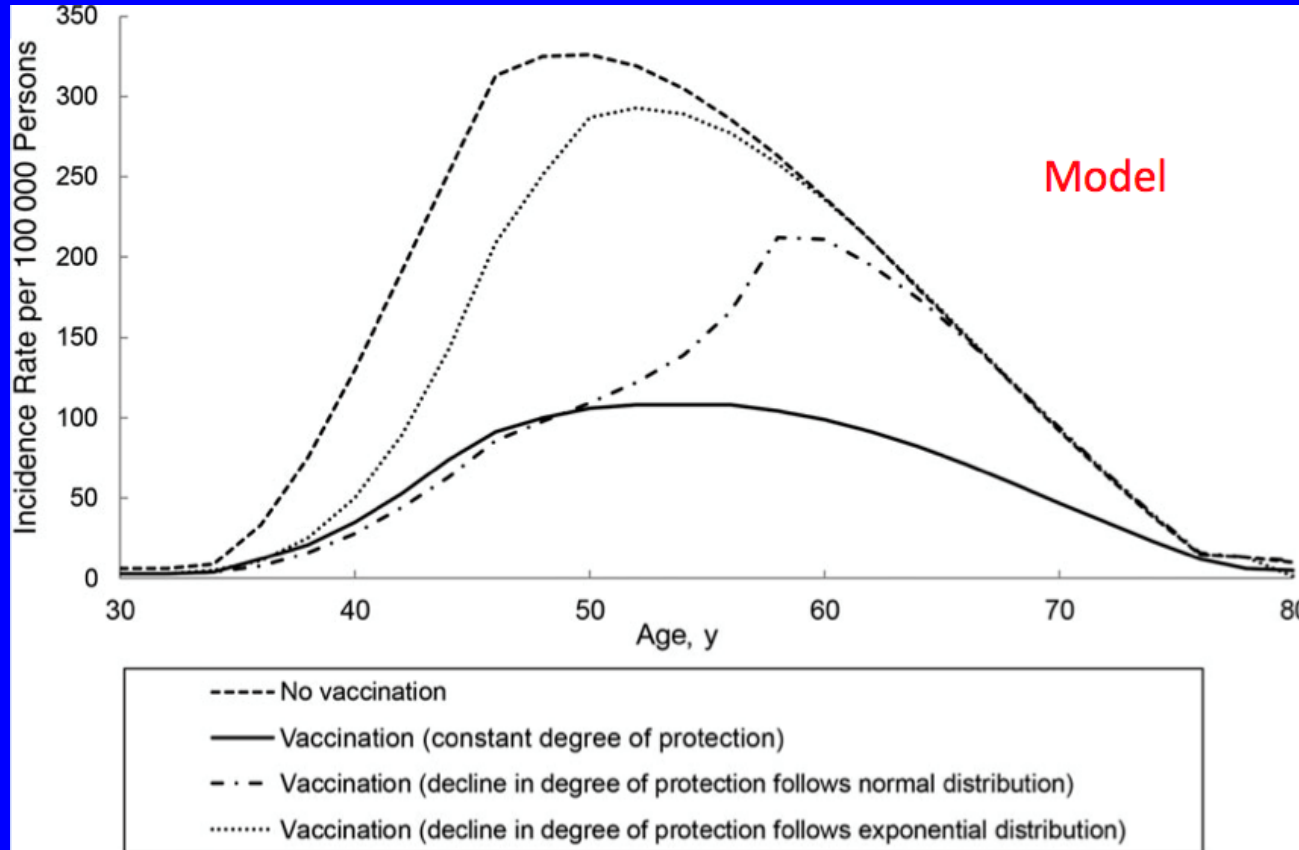
# Wales

- Welsh Government April 3, 2017
- New HPV vaccination program for:
  - MSM up to the age of 45
  - transgender men and women
  - sex workers
  - HIV positive men and women
- This was introduced after the Joint Commission on Vaccination and Immunization for the UK published a policy statement (2015)

# Canada

- Recommendations for HPV Vaccination for MSM
- Numerous public health authorities/organizations, such as Canada's National Advisory Committee on Immunization (NACI) (8) and the US Advisory Committee on Immunization Practices (2), have issued recommendations for HPV vaccination of males.
- **NACI recommendations:**
  - Routine HPV vaccination of males between the ages of 9 and 26 years.
  - HPV vaccine may be used in males over 26 years of age who have not been previously vaccinated.
  - MSM older than 26 years of age are still viable candidates for the HPV vaccine for the following reasons:
    - Individuals may be re-infected with HPV types to which they have been previously exposed

# Long term outcome of adding HPV vaccine to the AIN treatment regimen in HIV positive men who have sex with men



Vaccination decreased the lifetime risk of anal cancer by 63% in this model