HPV-Related Lesions of Gynecologic Tract: Current Challenges, Biomarkers, New Directions

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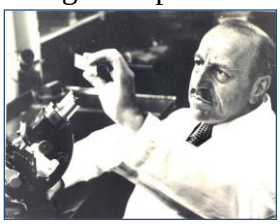
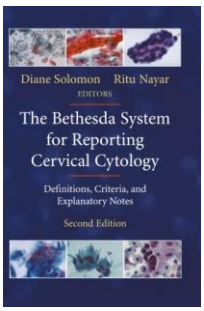
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Cervical cancer prevention

- 1928 - "… *first observation of cancer cells in a smear of the uterine cervix was one of the most thrilling experiences of my scientific career"*

Georgios Papanicolaou





- Pap test
- The Bethesda System for reporting Cervical Cytology

Cervical cancer prevention

- 1950-60s - Implementation of screening program for precancer
- 70-80% reduction in incidence of cervical cancer and mortality rates in high-resource countries
- The American Cancer Society's estimates
  - 0.7% of all new cancers in the U.S.
- Forth most common cancer affecting women worldwide
  - over 80% in developing countries
- Forth most common cause of cancer death in women worldwide

Cervical cancer pathogenesis

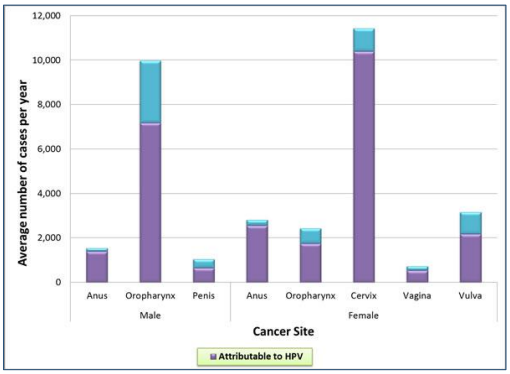
- 1976 – Human Papillomavirus plays an important role in the development of cervical cancer
- 2008 - The Nobel Prize in Physiology or Medicine

Harald zur Hausen

HPV-related cancers

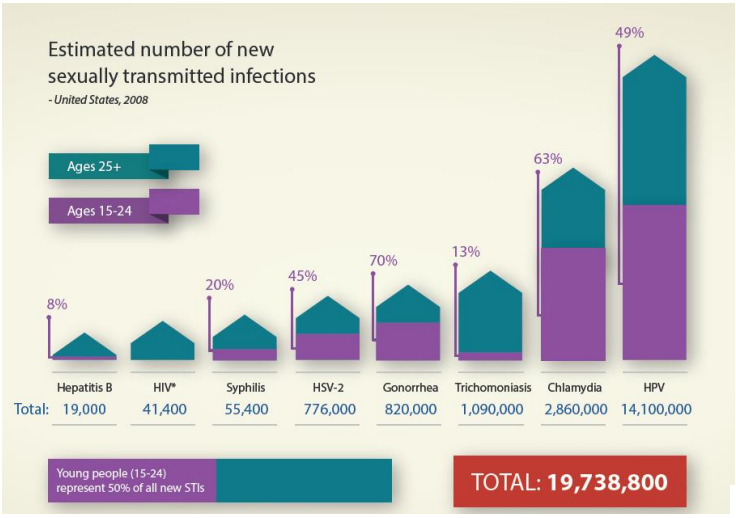
- 33,000 HPV-related cancers a year in the U.S.
- 90% of cervical cancers
- 90% of anal cancers
- 60% of vulvar cancers
- head and neck cancers oropharynx -70%

- majority caused by HPV 16 or 18



http://www.cdc.gov/cancer/hpv/statistics/cases.htm

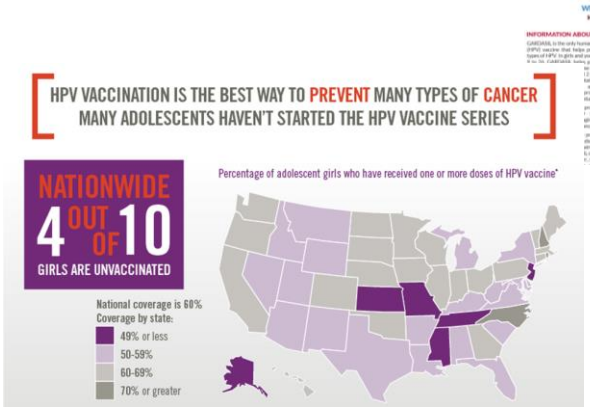
CDC FACT SHEET



http://www.cdc.gov/std/stats/STI-Estimates-Fact-Sheet-Feb-2013

HPV vaccine

- **Gardasil 4** (Merck) HPV 16,18, 6, 11
- **Gardasil 9** (Merck) HPV 16,18, 6, 11, 31, 33, 45, 52, 58
- **Cervarix** (GSK) HPV 16, 18



http://www.gardasil.com/

http://www.cdc.gov/hpv/infographics/vacc-coverage

HPV vaccine

Do we have to continue screening?

- Vaccine is prophylactic/not therapeutic
  - effective **pre-exposure** prophylaxis
  - no benefit in clearing existing infections
- Type-specific coverage (HPV16,18+, but not all)
  - unclear potential for cross-type immune response
- ~ X% of cervical cancers will continue to occur

Yes, we do…

Biomarkers of HPV-related lesions **Infection** vs. **Disease**

HPV infection

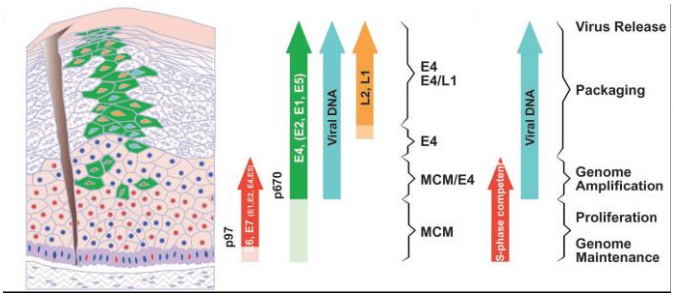
HR HPV detection
HPV genotyping
Cell cycle markers
(cyclin E, Ki-67, PCNA, MCM)
Markers of productive infection
(E4, L1, L2)

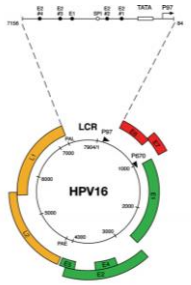
p16 ?

HPV integration
Methylation of HPV DNA
Copy number changes - 3q gain
Loss of markers of productive infection

Disease
True
Precancer

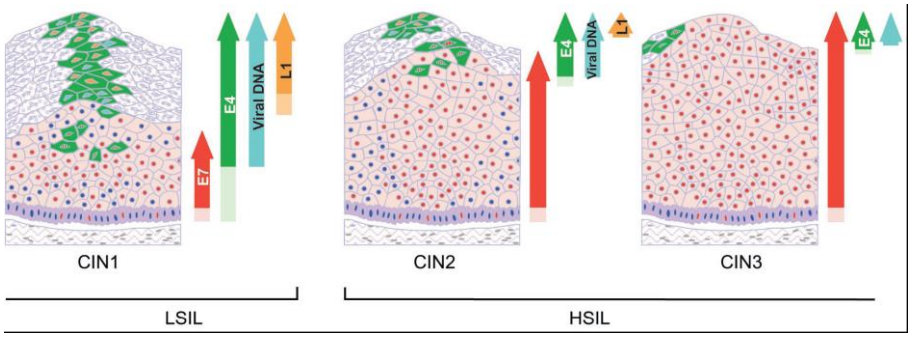
Organization of the HPV genome and the virus life cycle





J. Doorbar ,Clin Science 2006
Middleton, J Virol 2003

What is true disease?



Middleton et al, J Virol 2003

HPV integration

- Productive viral infection – episomal viral genomes
- Cervical carcinoma cell lines, HPV-related cancers – high frequency of integrated viral genomes
- Integration in high-grade SIL, i.e. precancer - **?**
- Driving mechanism of progression from productive infection to precancer - **?**

HPV integration

Frequency of integration is HPV type dependent

Table 2. Integrated HPV oncogene transcripts in cervical samples

	Normal	CIN 1	CIN 2	CIN 3	CxCa	Total	*P*
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n*	
HPV16	0/111 (0)	0/61(0)	5/83 (6)	27/141 (19)	33/60 (55)	456	0.0001
HPV18	0/22 (0)	0/6 (0)	0/13 (0)	0/8 (0)	33/36 (92)	85	0.0001
HPV31	0/22 (0)	0/16 (0)	0/29 (0)	3/29 (10)	2/14 (14)	110	0.0228
HPV33	0/23 (0)	0/20 (0)	0/35 (0)	0/28 (0)	7/19 (37)	125	0.0039
HPV45	0/8 (0)	0/5 (0)	0/12 (0)	6/10 (60)	20/24 (83)	59	0.0001
	0/186 (0)	0/108 (0)	5/172 (3)	36/216 (17)	95/153 (62)	835	0.0001

NOTE: *P*, the exact Cochran-Armitage Trend test.

Vinokurova et al, Cancer Res 2008

HPV integration

- Non-random HPV integration
  - integration hot spots/fragile sites with microhomology sequences
  - disruption of novel tumor suppressor genes
  - integration in the vicinity of mRNAs

Hu et al, Nature Genetics 47, 158–163 (2015)
Schmitz t al, PLoS One. 2012;7(6)

LAST project

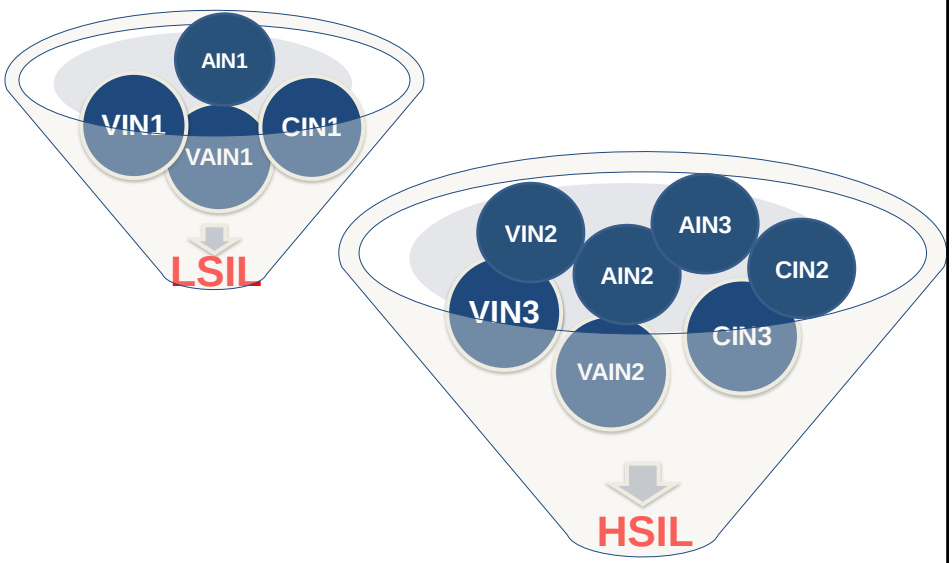
JOURNAL OF LOWER GENITAL TRACT DISEASE
Wolters Kluwer | Lippincott Williams & Wilkins
ARCHIVES of Pathology & Laboratory Medicine
International Journal of Gynecological Pathology

Original Article

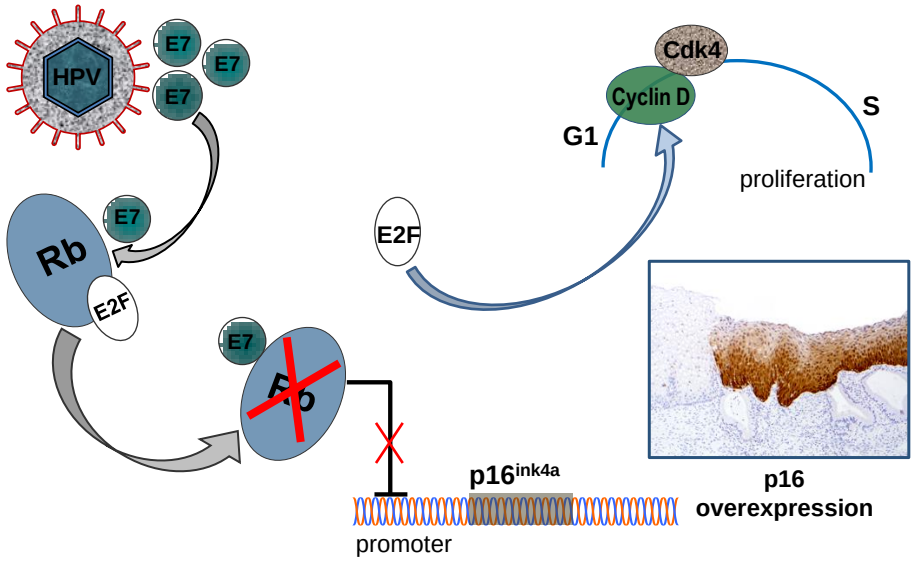
The Lower Anogenital Squamous Terminology Standardization Project for HPV-associated Lesions: Background and Consensus Recommendations From the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology

Teresa M. Darragh, M.D., Terence J. Colgan, M.D., J. Thomas Cox, M.D., Debra S. Heller, M.D., Michael R. Henry, M.D., Ronald D. Luff, M.D., Timothy McCalmont, M.D., Ritu Nayar, M.D., Joel M. Palefsky, M.D., Mark H. Stoler, M.D., Edward J. Wilkinson, M.D., Richard J. Zaino, M.D., David C. Wilbur, M.D., and For Members of the LAST Project Work Groups

LAST project
Nomenclature

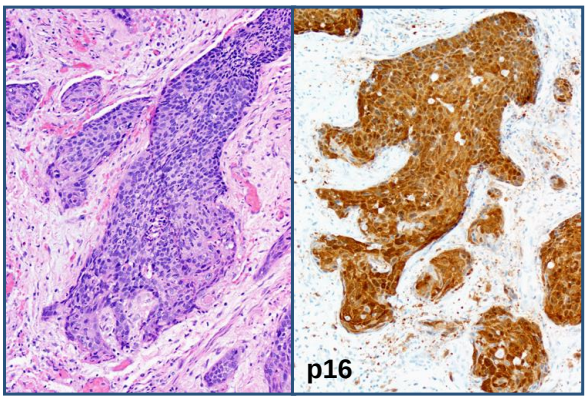


Mechanism of high-risk HPV-mediated p16 over-expression



P16 – marker of HPV-related squamous cell carcinoma

Cervix
Vulva
Anus
Oropharynx

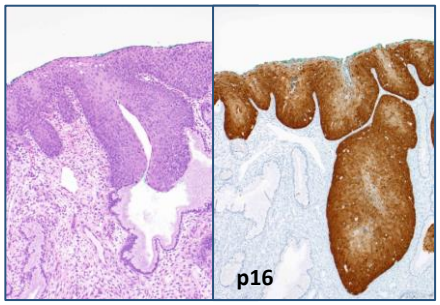


LAST project
Biomarkers in HPV-associated Lower Anogenital Squamous Lesions

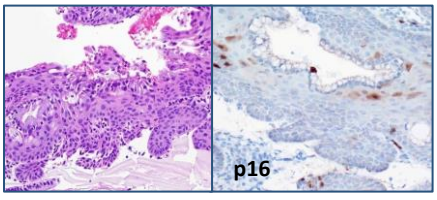
- **p16 IHC is recommended when the H&E morphologic differential diagnosis is between precancer (-IN 2 or -IN 3) and a mimic of precancer**
- If the pathologist is entertaining an H&E morphologic interpretation of -IN 2 (under the old terminology, which is a biologically equivocal lesion falling between the morphologic changes of HPV infection [low-grade lesion] and precancer), p16 IHC is recommended to help clarify the situation. Strong and diffuse block-positive p16 results support a categorization of precancer. Negative or non-block-positive staining strongly favors an interpretation of low-grade disease or a non-HPV-associated pathology.
- p16 is recommended for use as an adjudication tool for cases in which there is a professional disagreement in histologic specimen interpretation, with the caveat that the differential diagnosis includes a precancerous lesion (-IN 2 or -IN 3).
- WG4 recommends against the use of p16 IHC as a routine adjunct to histologic assessment of biopsy specimens with morphologic interpretations of negative, -IN 1, and -IN 3.

P16 – marker of HSIL

HSIL with endocervical gland involvement



Immature squamous metaplasia



LAST project
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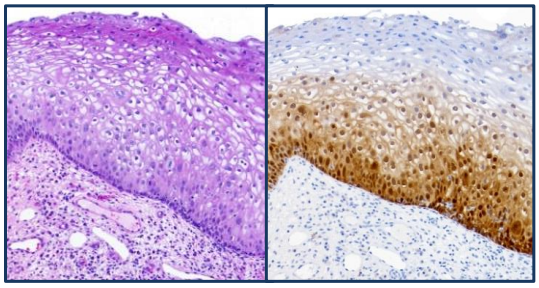
- 4. WG4 recommends **against** the use of p16 IHC as a routine adjunct to histologic assessment of biopsy specimens with morphologic interpretations of negative, -IN 1, and -IN 3

P16 in LSIL

- p16 is positive in 30-70% of LSILs
- Biologic potential of p16 positive LSIL is unknown
- Over 80% of LSILs contain HR HPV
- HPV 16 - most common type ~ 25%

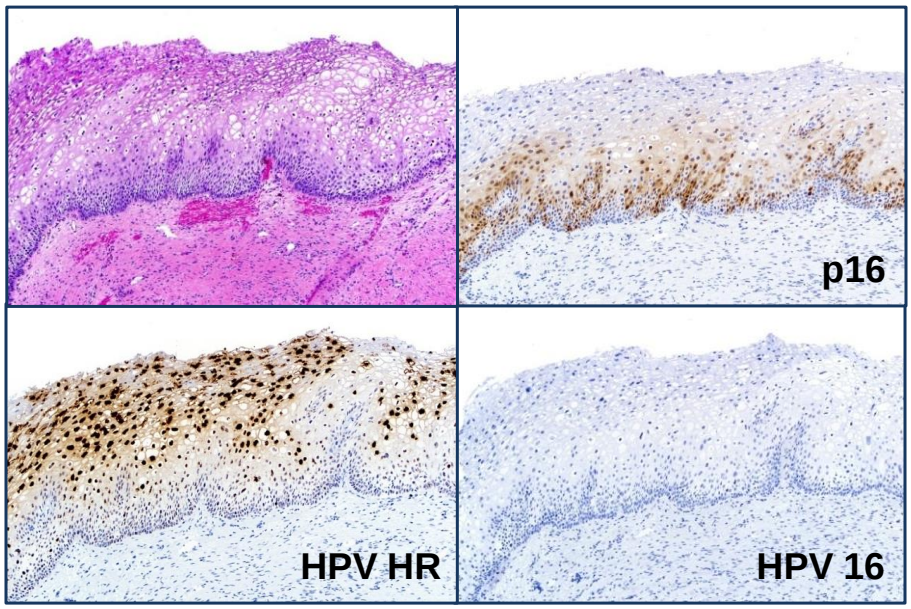


P16 in LSIL

- Study goal: to evaluate p16 expression in LSILs in correlation with HPV types
- 231 cervical specimens (biopsies, excisions) diagnosed as LSIL (CIN 1)
- p16 IHC - CINtec Histology kit (mtm laboratories)
- HPV typing - INNO-LiPA HPV Genotyping Extra (INNOGENETICS)
- HPV typing - in situ hybridization – RNA scope (ACD)

Yemelyanova et al, Mod Pathol 2013;26:299A

PCR- HPV 16, 54, 56, 66, 74



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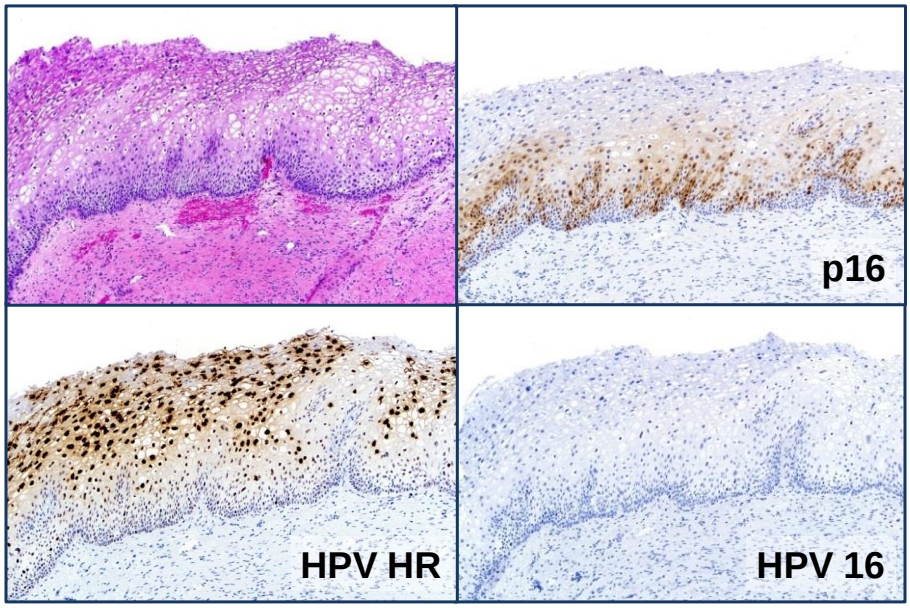
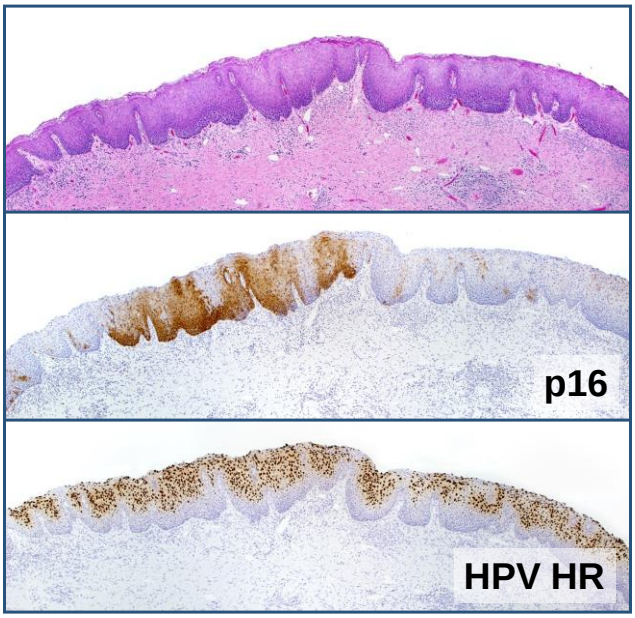
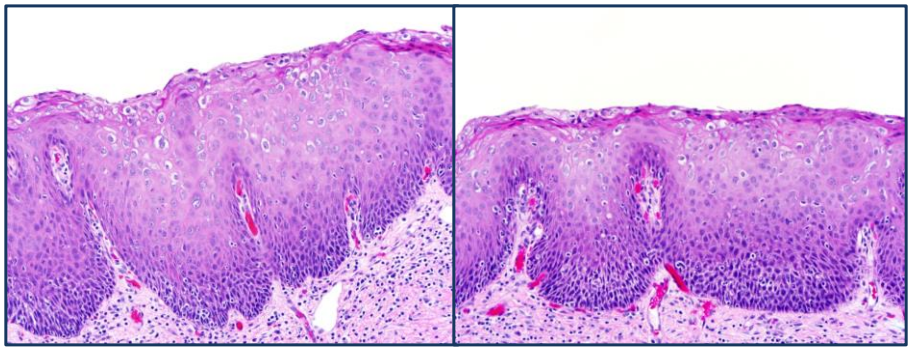
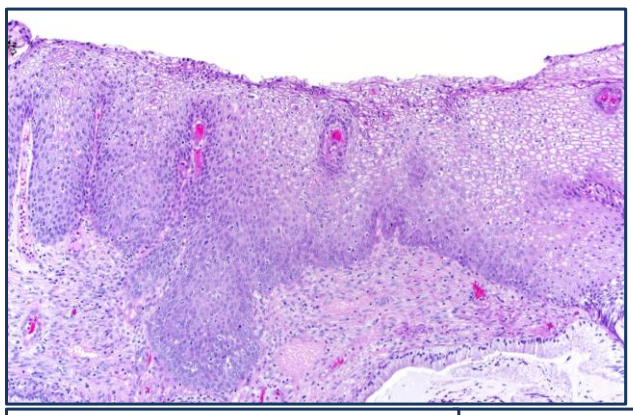




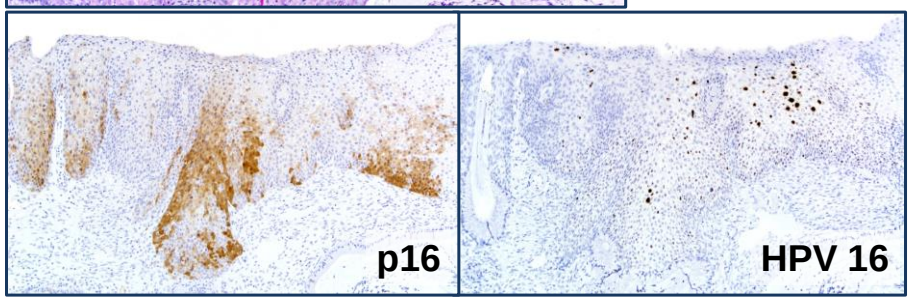
PCR- HPV 52

PCR- HPV 52





PCR- HPV 16



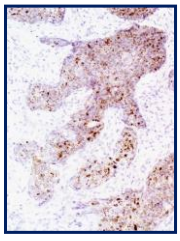
P16 in LSIL

- Positive p16 expression is observed in 59%
- Nearly all HPV16-related LSILs are p16 positive
- LSILs may display problematic patterns of p16 expression that are not readily interpretable as positive or negative
- Infections with multiple HPV types occur and can give rise to independent lesions with different biologic potential
- Detailed HPV-typing of the lesions is important while studying biologic potential of p16 positive LSILs

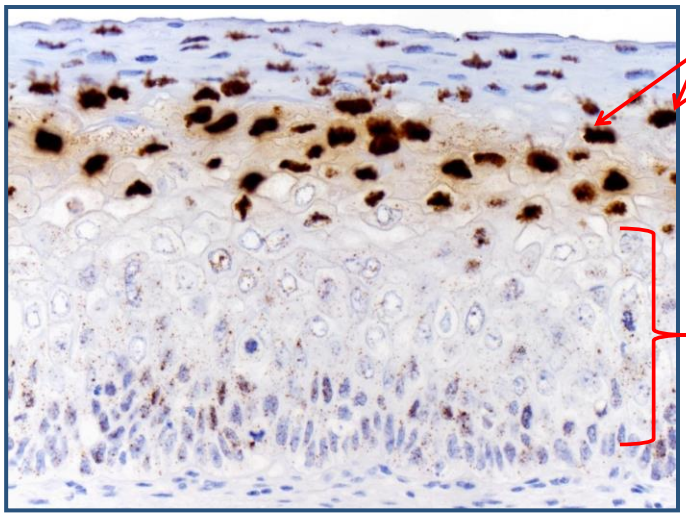
Yemelyanova, Mod Pathol 2013;26:299A

HPV detection

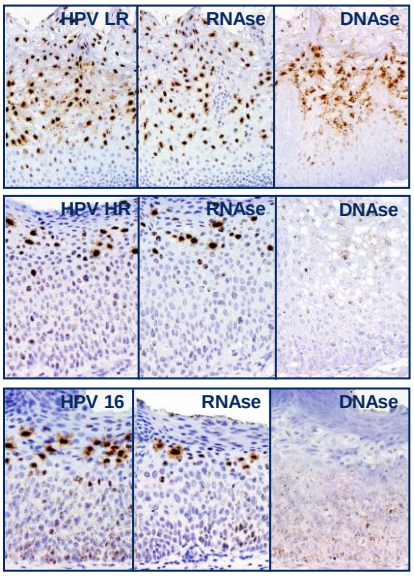
- Detection of HPV nucleic acid in solutions
  - DNA
  - mRNA
- In situ hybridization methods for FFPE
  - DNA-based
  - RNA-based (transcriptionally active virus)



In situ hybridization patterns – RNA scope
target – HPV E6/E7 transcripts



In situ hybridization patterns –RNA scope®
HPV E6/E7 transcripts



RNAscope® assay allows for detection of both HPV RNA and DNA in SILs

Yemelyanova et al, JMD 2013;15(6):936

HPV and cervicovaginal microbiome

- Five community-state types (CST)
  - I,II,III,V – dominated Lactobacillus spp.
  - IV – diverse anaerobic bacteria and low Lactobacillus spp.
- Maintenance of low pH by Lactobacillus spp is protective of STIs
- Lactobacillus-depleted microbiome – Bacterial vaginosis
  - increased transmission rates of STIs
- CST IV (Lactobacillus-depleted/ Atopobium-enriched)
  - slowest regression of HPV/ persistentce
- CST II (Lactobacillus gasseri –dominated) – rapid clearance of HPV
- CST IV - more commonly seen in women with HPV-related SIL

Brotman et al, JID 2014, 210: 1723
Mitra et al, nature.com/scientific reports

HPV and immunotherapy

- Adoptive T-cell therapy - Tumor-infiltrating T-cells (HPV E6/E7)

  – reported complete responses in metastatic cervical cancer
- Vaccine-based therapies

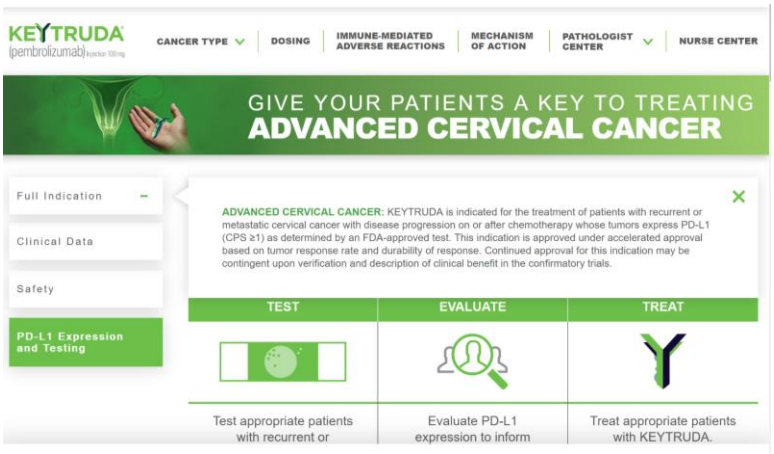
  - HPV E6 and E7 targets

  (vector-based, peptide-based, protein-based, NA-based vaccines)
- Immune-modulating agents - Immune checkpoints inhibitors

  - CTLA-4
  - PD-1/PD-L1 –Phase I/II trials

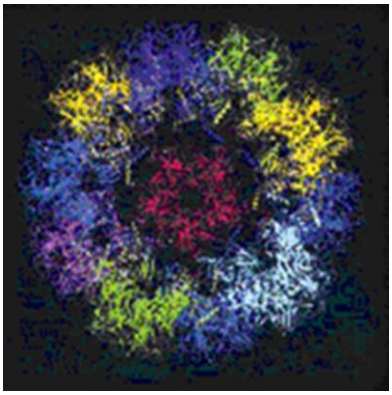
Stevanovic et al, JCO. May 2015
Eskander et al, Clin Therapeutics. 2015;37(1)

Immunotherapy in cervical cancer



Studying cervical cancer precursors Lessons learned

- Complex and dynamic system
  - Infections with multiple HPV types
  - Changes in immunologic milieu
  - Changes in hormonal environment
  - Host microbiome
- Constant evolution of infections/lesions
- Diagnostic procedures/treatment alter natural course



Thank you!

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