The Biology of HPV Infection and Cervical Cancer

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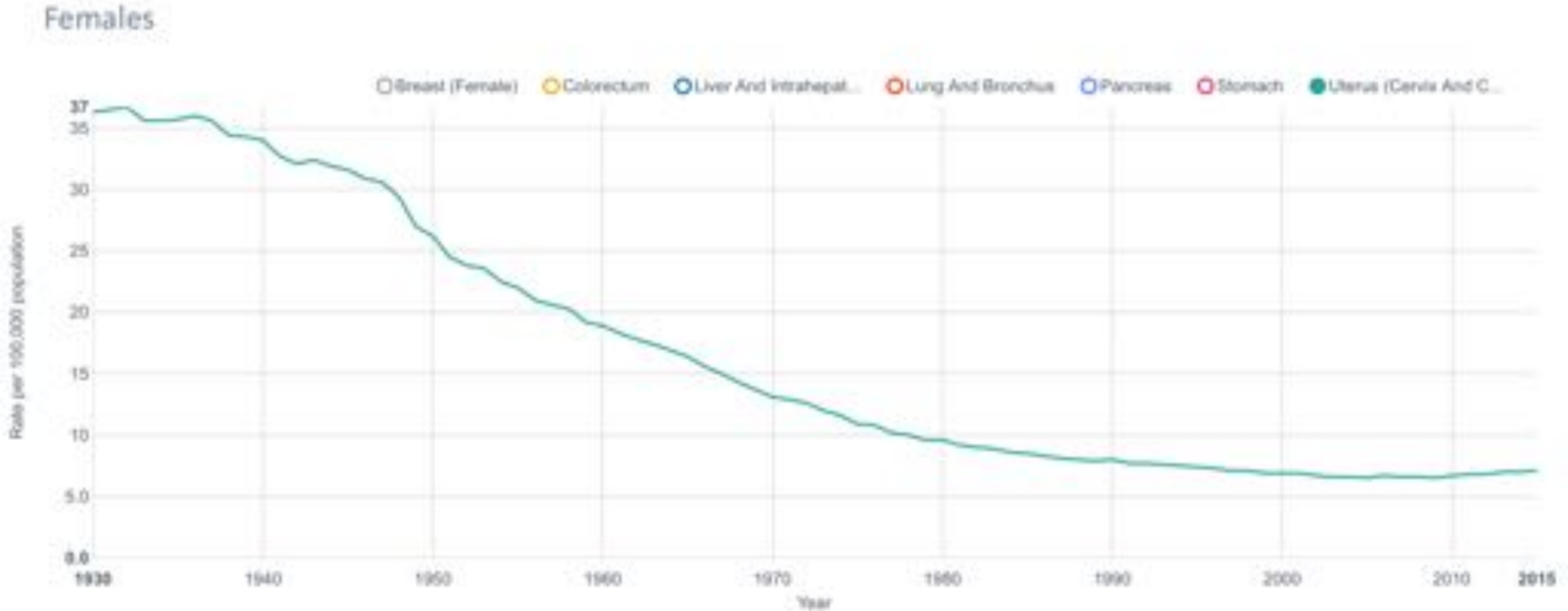
Learning Objectives

- Describe the key molecular events in HPV oncogenesis.
- Relate transient and persistent HPV infection to patient clinical history and cytomorphologic findings.
- Explain the integration of HPV testing and cytologic findings in cervical cancer screening follow up guidelines.
- Troubleshoot pitfalls in HPV testing.

The Pap test: A minimally invasive test for cancer and pre-cancer

- Originally developed by Dr. George Papanicolaou, immigrant to the US from Greece
- Early scientific work used vaginal smears to study the reproductive cycles of guinea pigs
- Developed a staining method that allowed identification of benign and malignant cells under the microscope
- Original papers were published in late 1910's-1920's
- Pap test widely adopted in the 1940's



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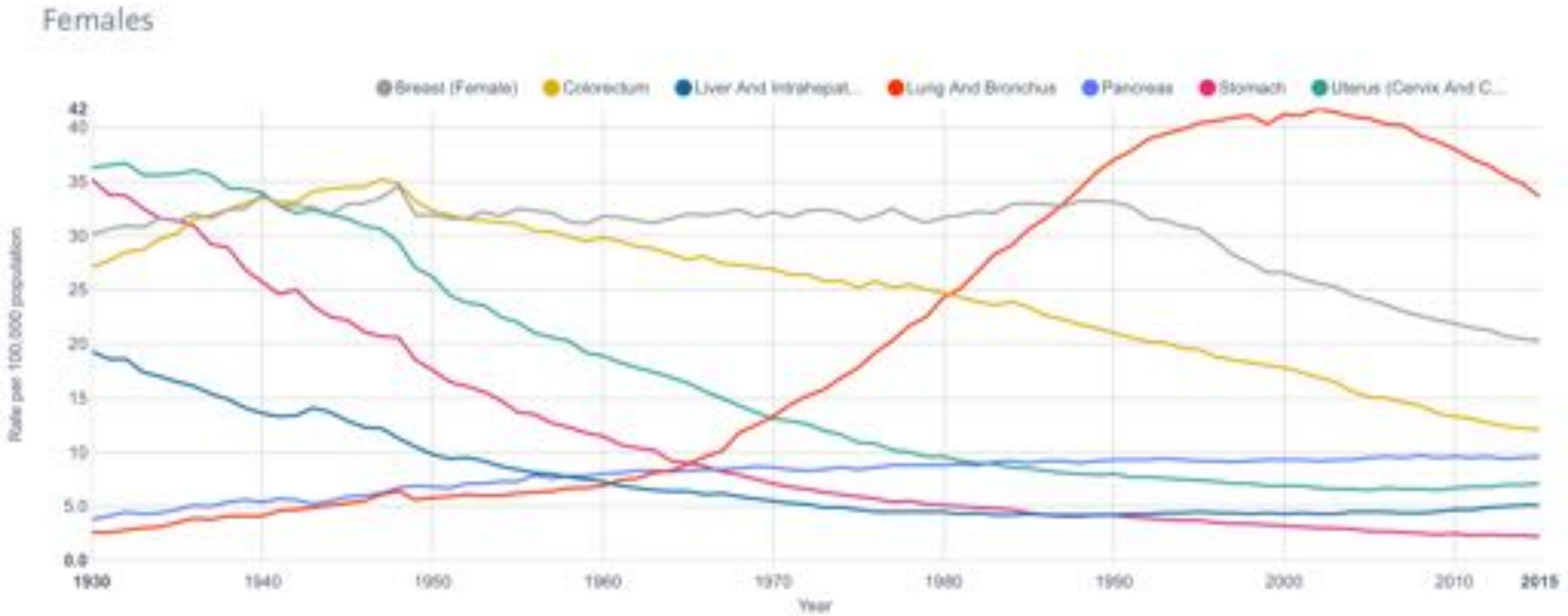

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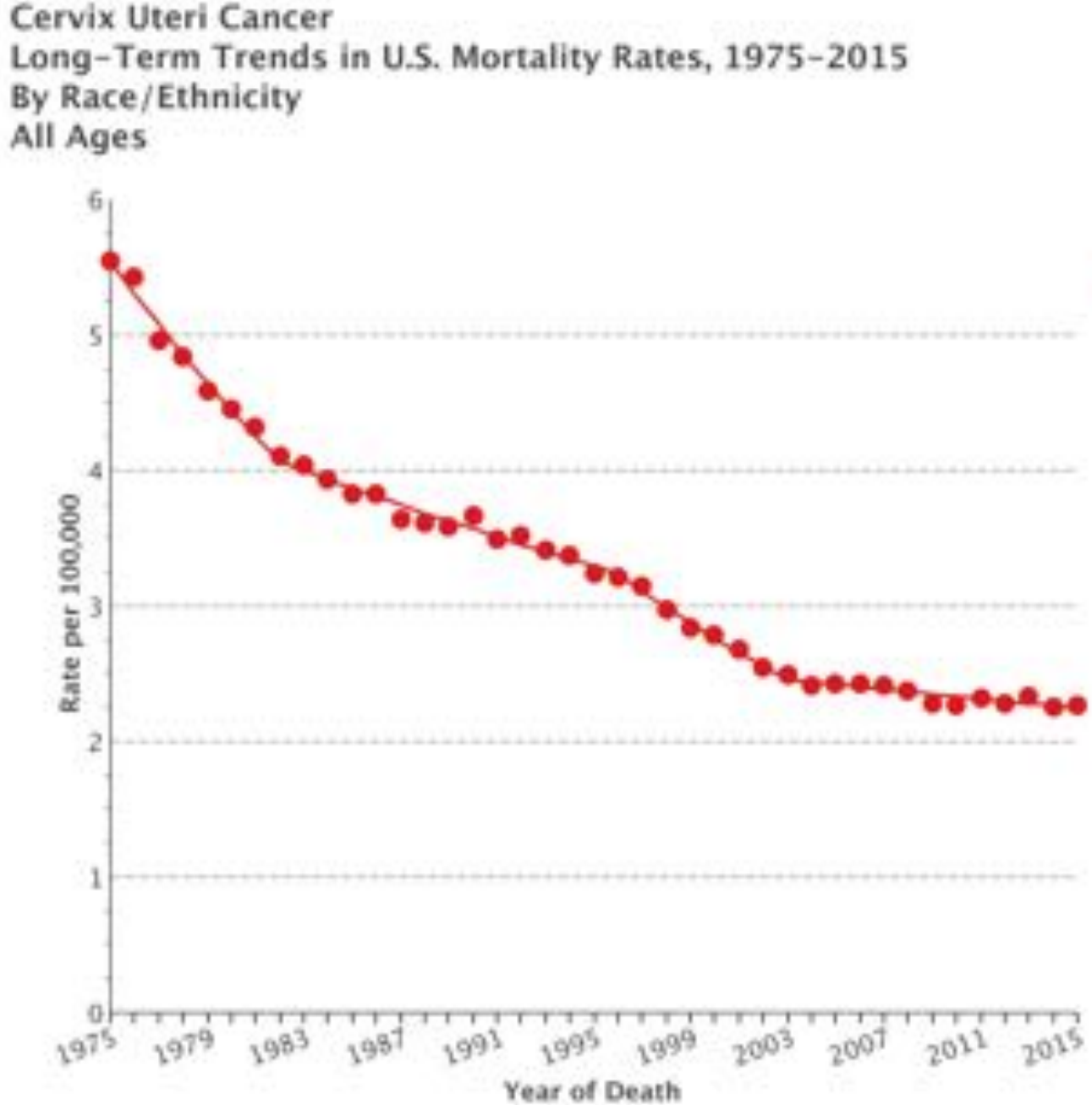
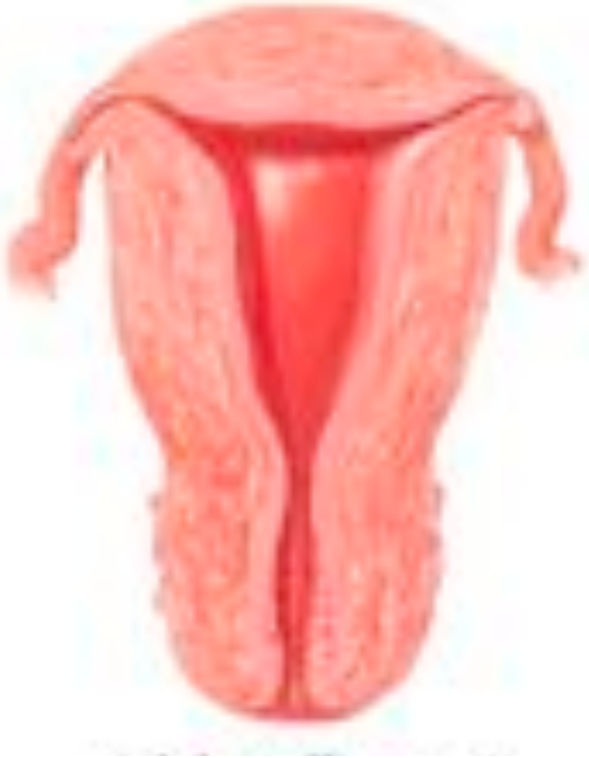
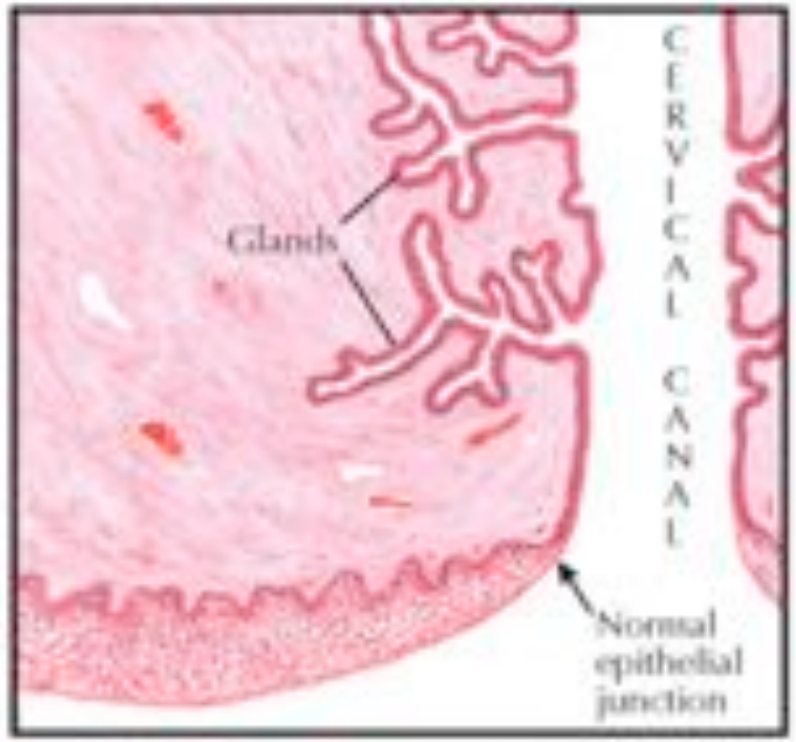


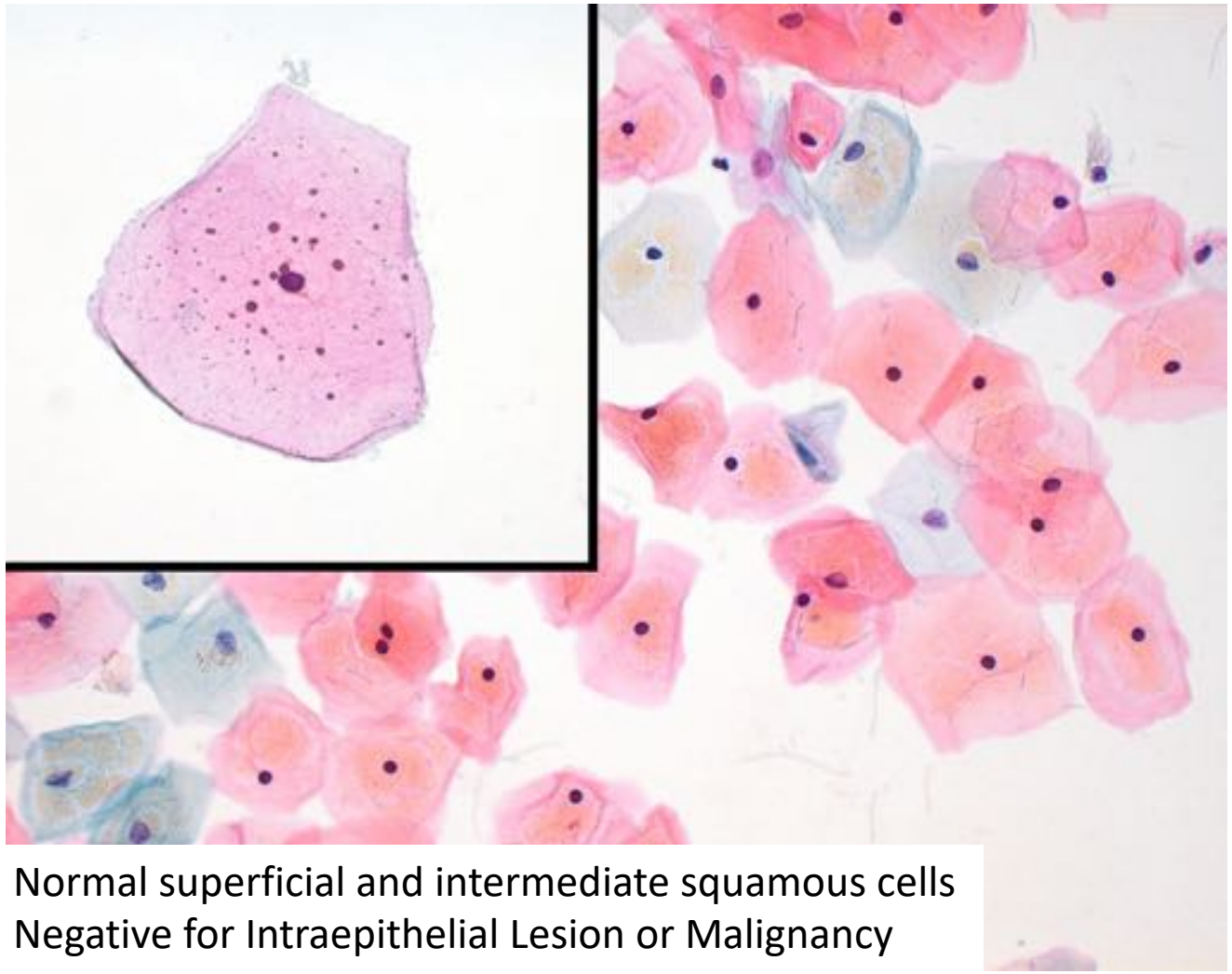
https://seer.cancer.gov/explorer/application.php



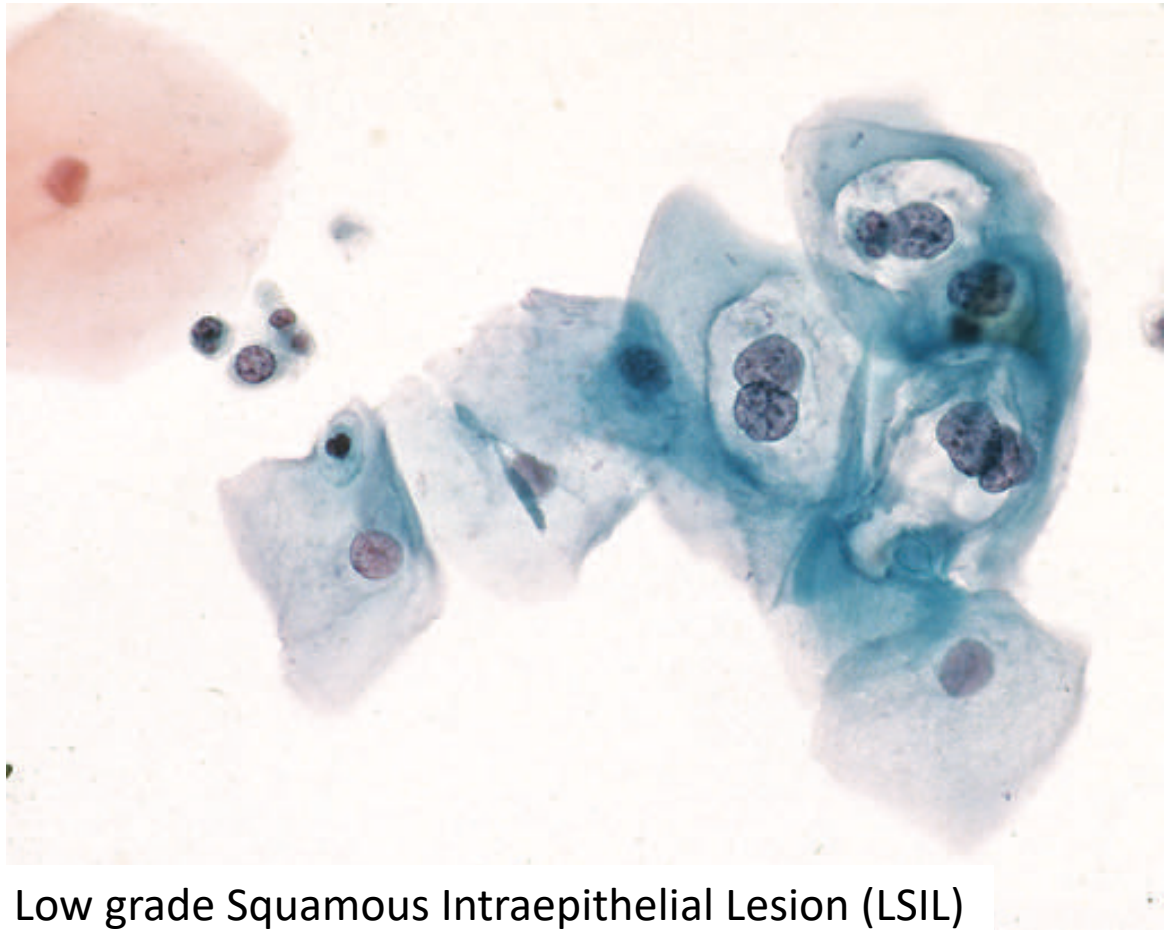


The Netter Collection of Medical Illustrations: The Reproductive System

The Pap test: Morphology



Normal superficial and intermediate squamous cells
Negative for Intraepithelial Lesion or Malignancy



Low grade Squamous Intraepithelial Lesion (LSIL)

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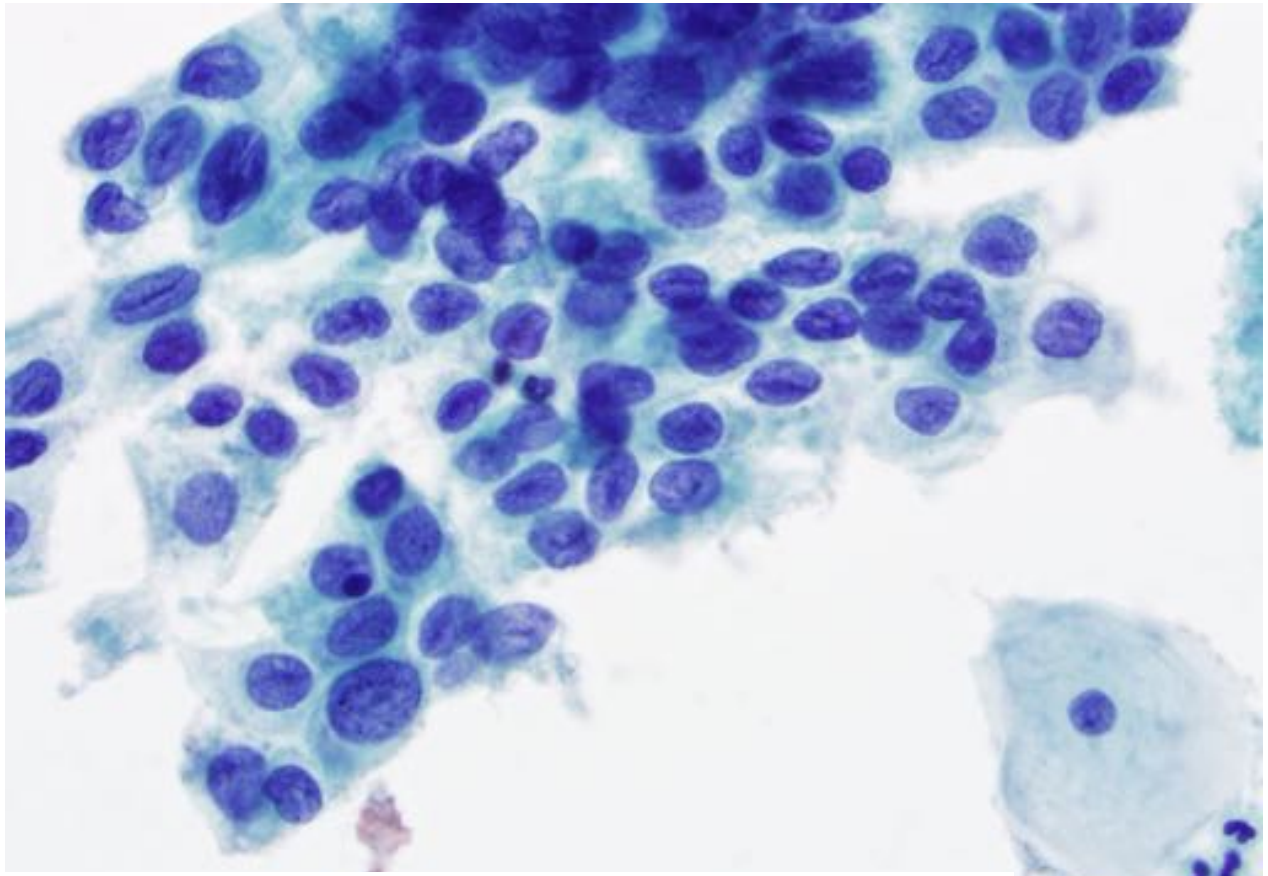
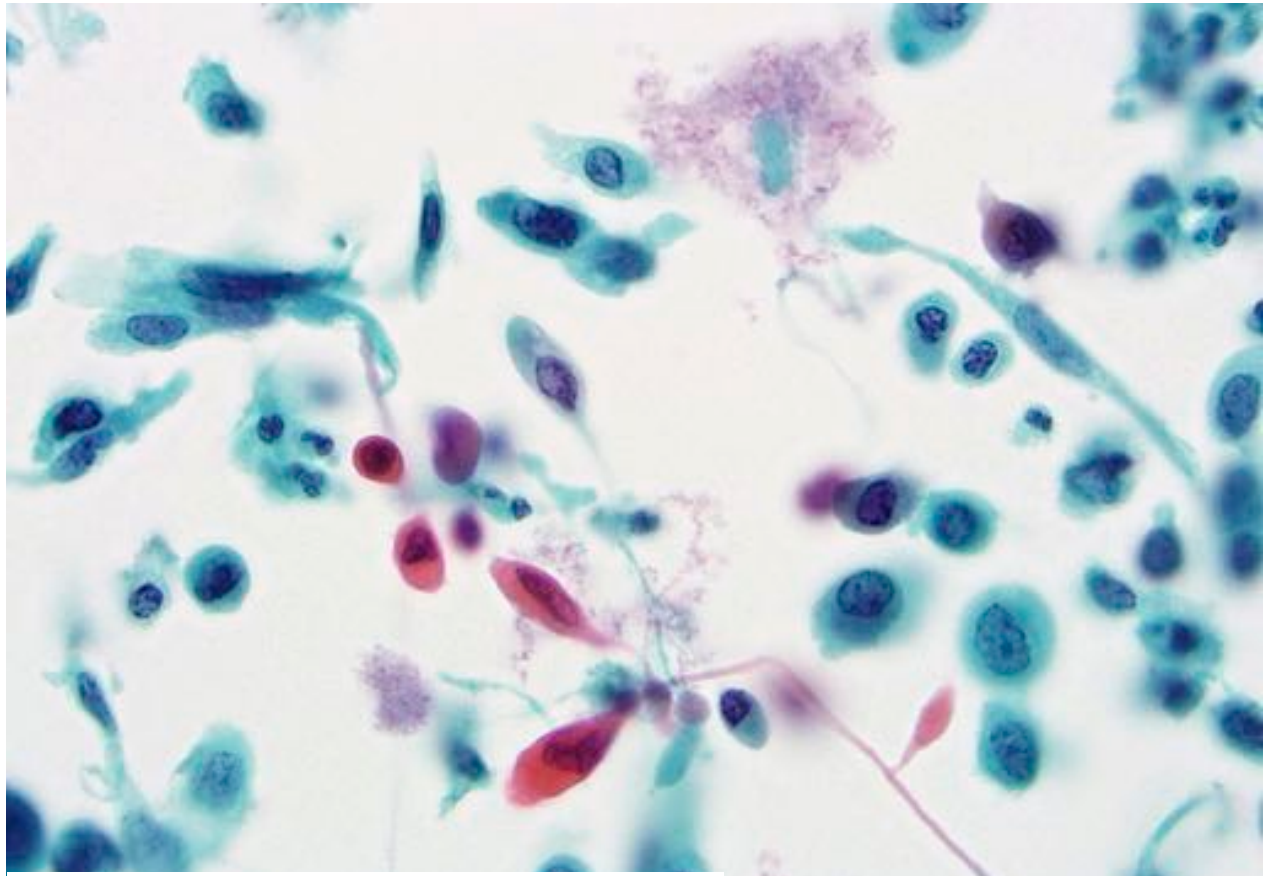


High grade Squamous Intraepithelial Lesion (HSIL)



Invasive Squamous Cell Carcinoma

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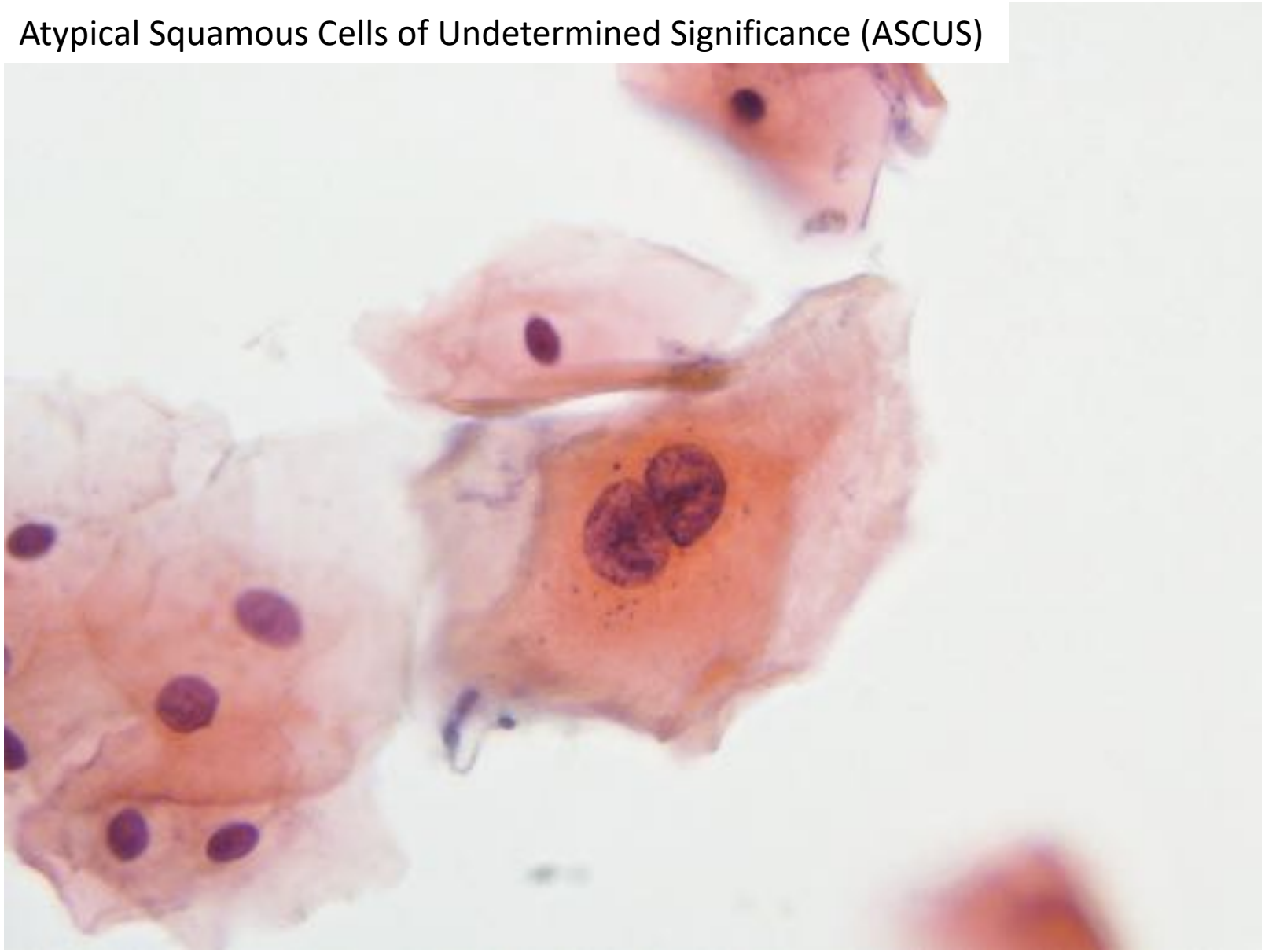
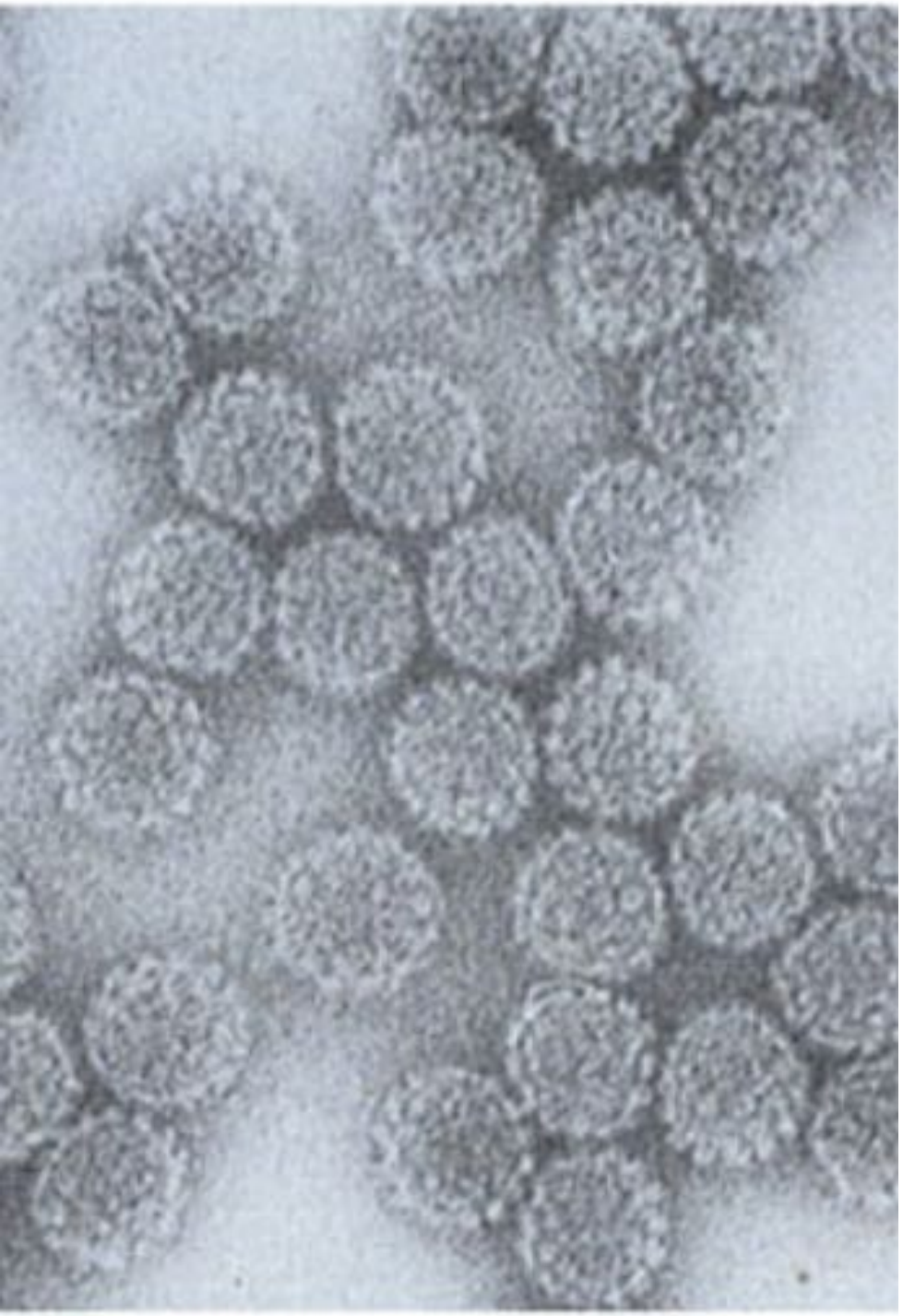
Atypical Squamous Cells of Undetermined Significance (ASCUS)



https://bethesda.soc.wisc.edu

The Pap test: A crucial component of cervical cancer prevention

- Primary prevention
  - HPV vaccination
  - Condoms (But what about areas not covered by condoms? How likely will patients be to use a condom for all contact, every time?)
  - Limiting sexual partners (But what about the partner's partners?)
- Secondary prevention
  - Pap test
  - Appropriate treatment and follow-up of dysplasia (precancerous lesions)



Human papillomavirus

- Non-enveloped, circular dsDNA virus
- Early genes E6 and E7 bind p53 and Rb
- Late gene L1 makes the major coat protein
- HPV types infect birds and mammals
- Infection is ubiquitous

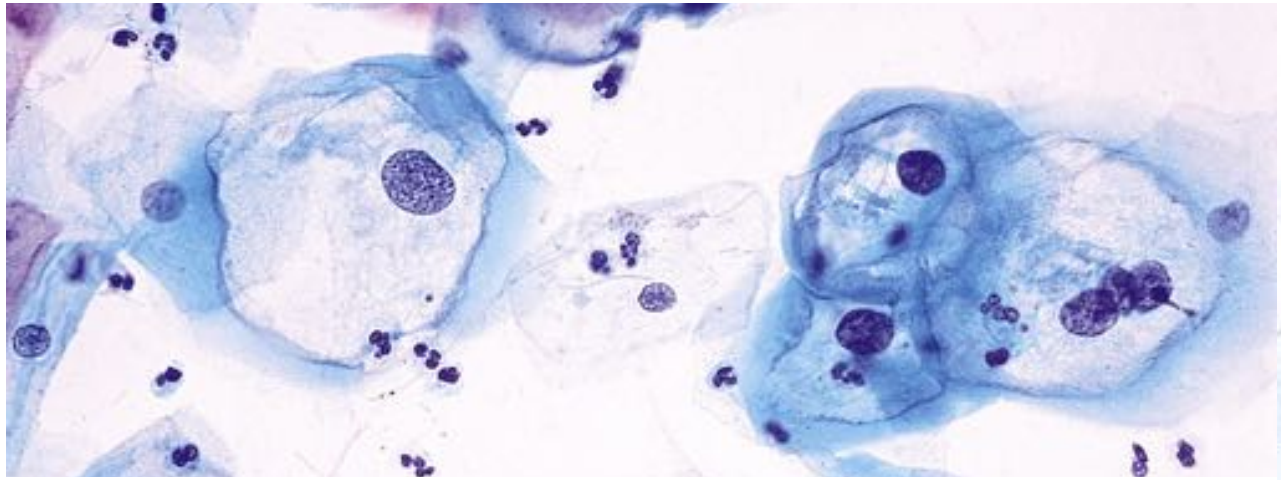
Doorbar J, et al. *Rev Med Virol*. 2015;25:2-23. doi:10.1002/rmv.1822

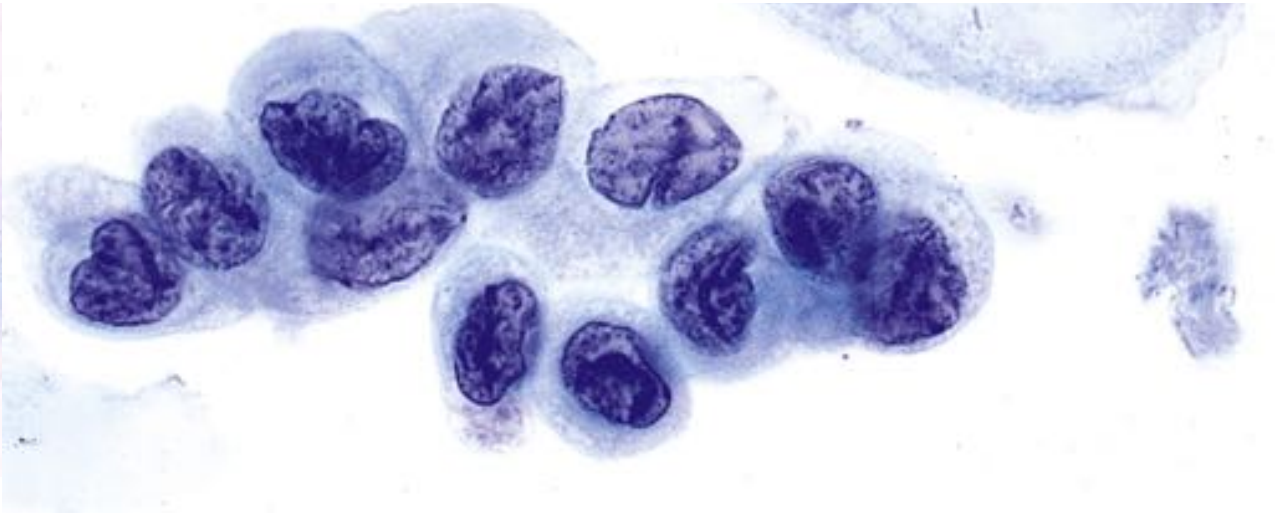
HPV-related disease

- Anogenital tract skin and mucosa – penile, vulvar, vaginal, cervical, and anal
- Oropharynx – tonsils and base of tongue
- Skin – most commonly low risk types, causing warts
- Papillomas of the respiratory tract and conjunctiva – usually low risk types

Low risk vs. high risk HPV infection

- Low risk HPV types can cause koilocytosis and condylomas, unlikely to cause cancer
- High risk HPV types can cause koilocytosis and condylomas, may progress to HSIL (high grade SIL) and cancer
- HPV testing almost uniformly refers to testing for **high risk HPV** types





HPV vaccination

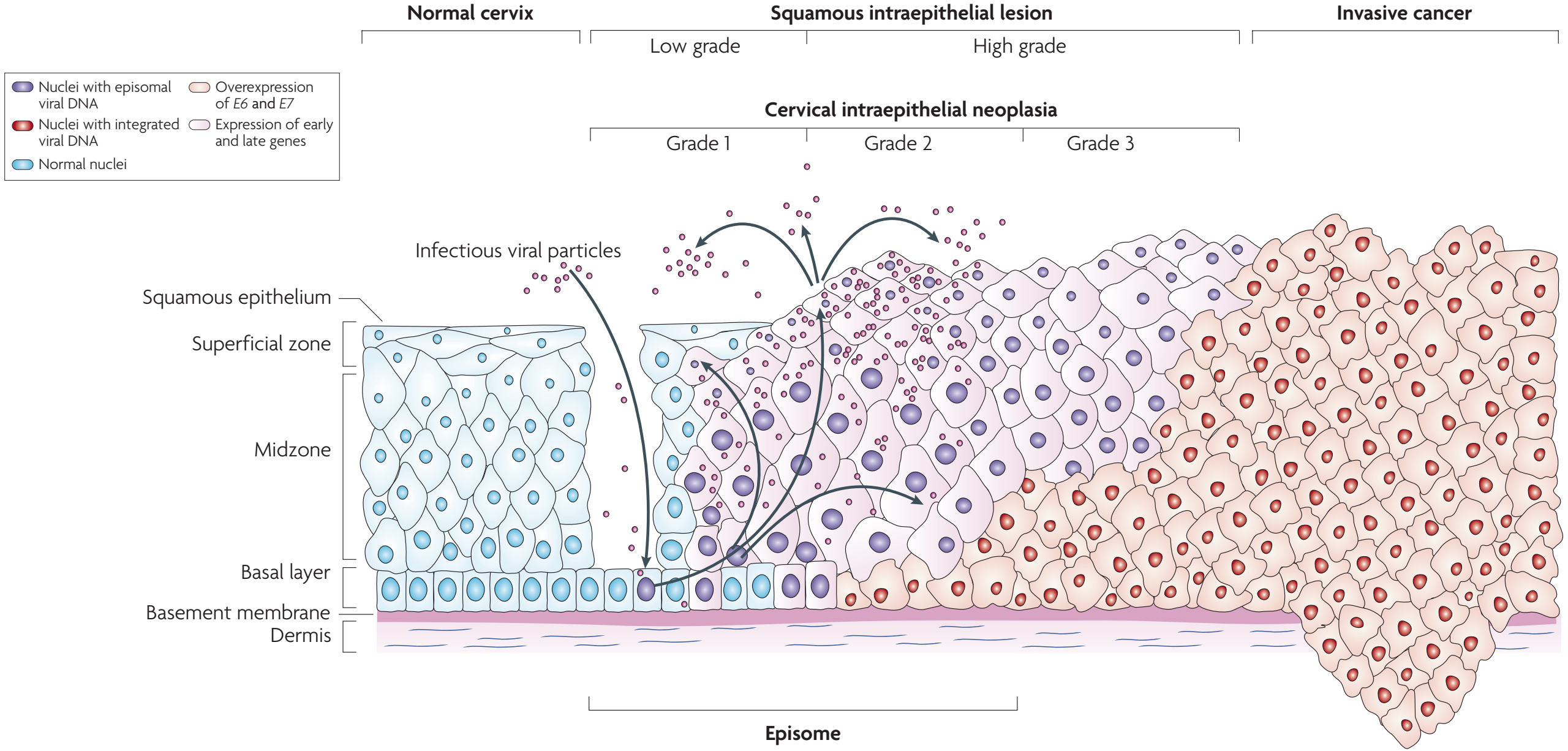
- Most effective at preventing infection when given prior to first exposure
- When given later, may still be effective in preventing infection by new HPV types

HPV vaccination

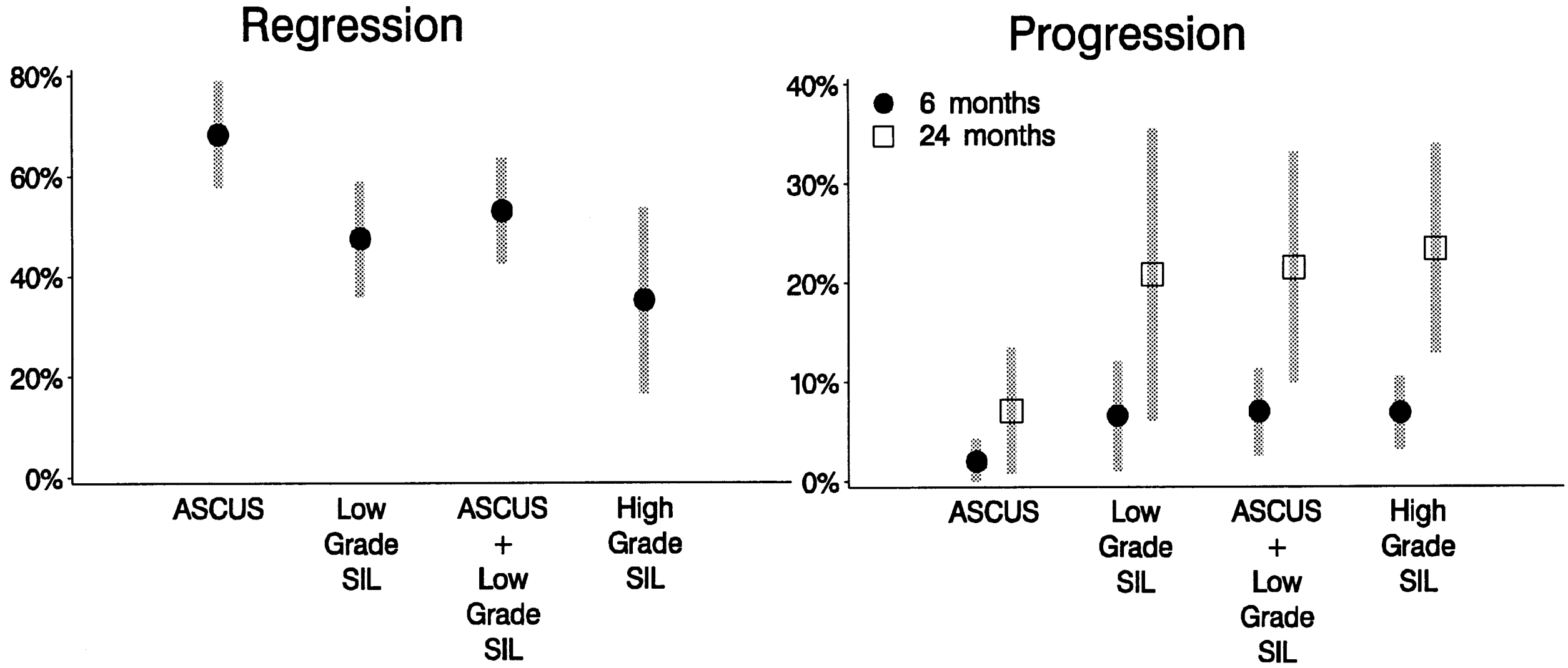
- Risks of HPV Vaccination: Allergic response to vaccine components, minor localized or febrile (fever) vaccine reactions
- Benefits of HPV Vaccination:
  - Boys: Reduced risk of genital warts, reduced risk of penile cancer, reduced risk of anal cancer, reduced risk of oropharyngeal cancer
  - Girls: Reduced risk of genital warts, reduced risk of cervical, vaginal, vulvar, and anal cancer, and reduced risk of oropharyngeal cancer
  - General public: Herd immunity
- https://www.cdc.gov/hpv/hcp/for-hcp-tipsheet-hpv.pdf
- https://wicancer.org/action-plans/hpv-vaccination-rates/

Gardasil 9 vaccine

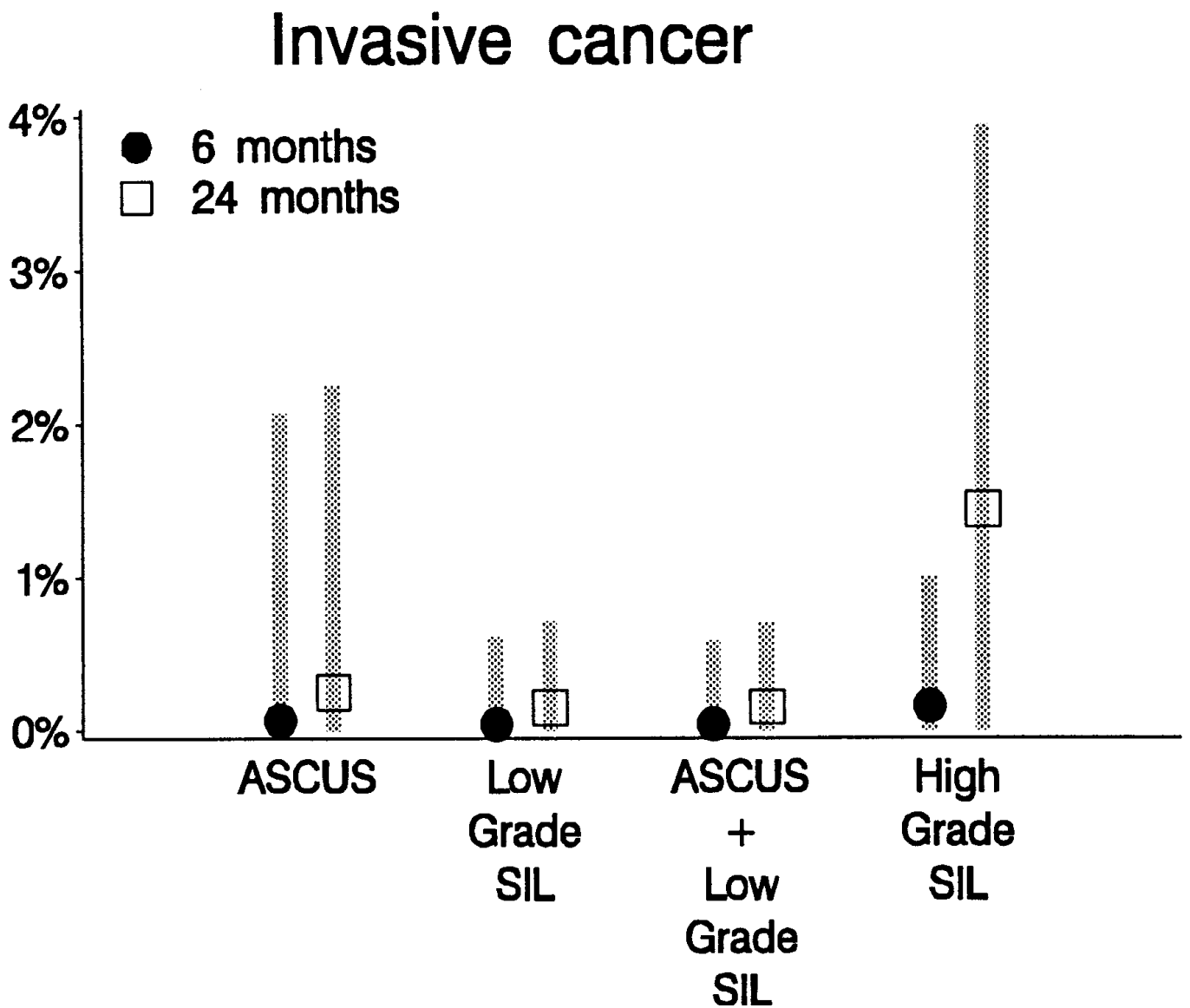
- L1 protein virus-like particles
- Protective agains:
  - 6, 11 – low risk, causing genital warts
  - **16, 18,** 31, 33, 45, 52 and 58 – high risk
- Recommended for boys and girls ages 11 or 12
- May begin as early as age 9, catch up recommended up to age 26 for women, 21 for men
- FDA approval recently extended to upper limit of age 45



Woodman CBJ, et al. *Nat Rev Cancer*. 2007. doi:10.1038/nrc2050



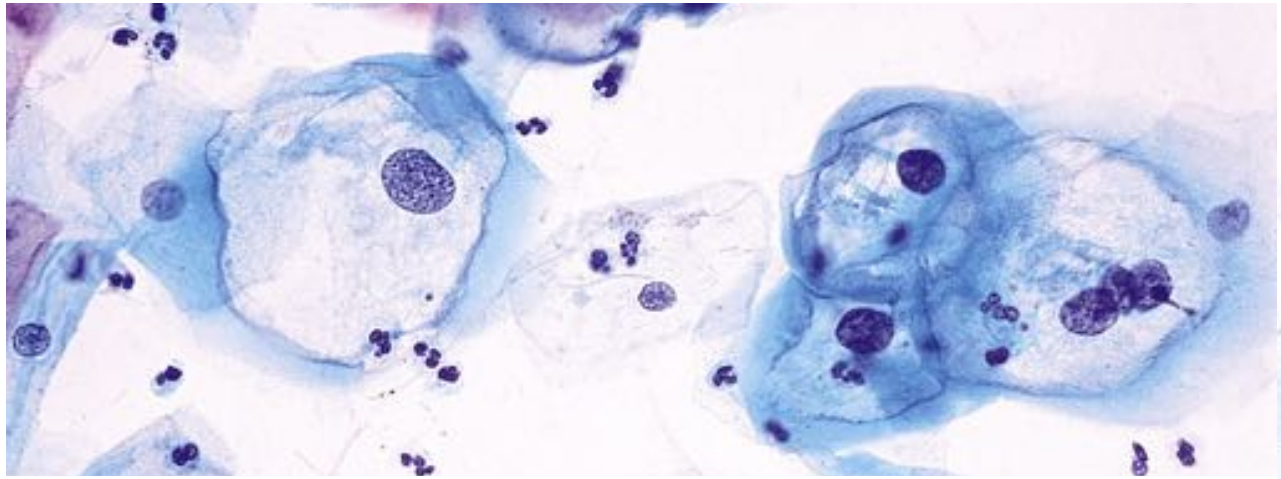
Melnikow J, et al. *Obstet Gynecol*. 1998.
https://www.ncbi.nlm.nih.gov/pubmed/9764690



Melnikow J, et al. *Obstet Gynecol*. 1998.
https://www.ncbi.nlm.nih.gov/pubmed/9764690

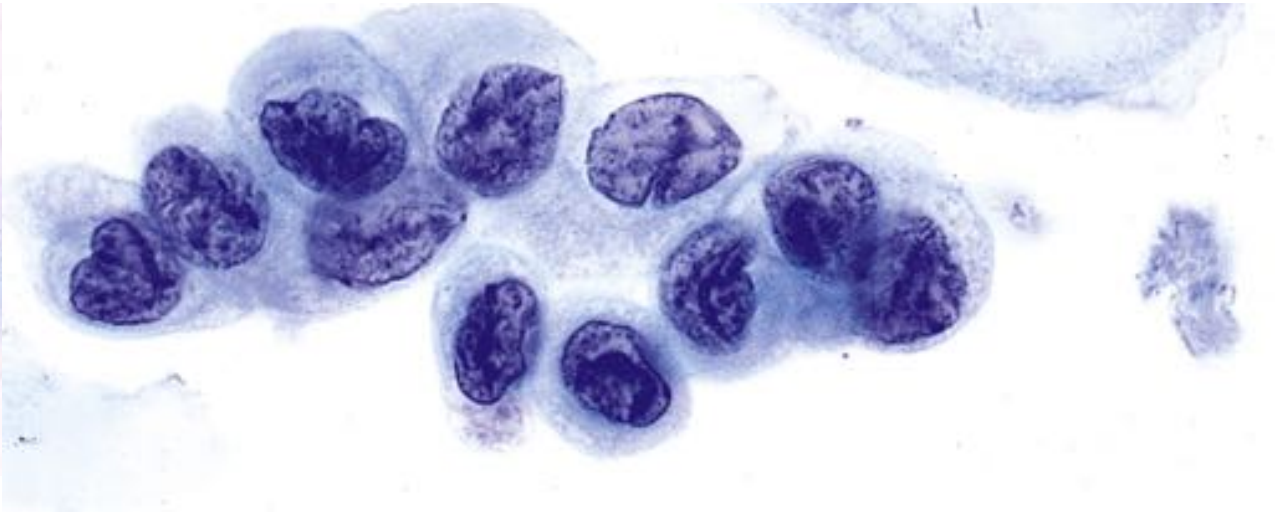
Transient HPV infection

- Usually LSIL/koilocytic changes
- Common in women in their 20s
- Regresses



Persistent HPV infection

- HSIL, koilocytes less likely
- More common in women in their 30s and up
- May lead to cancer

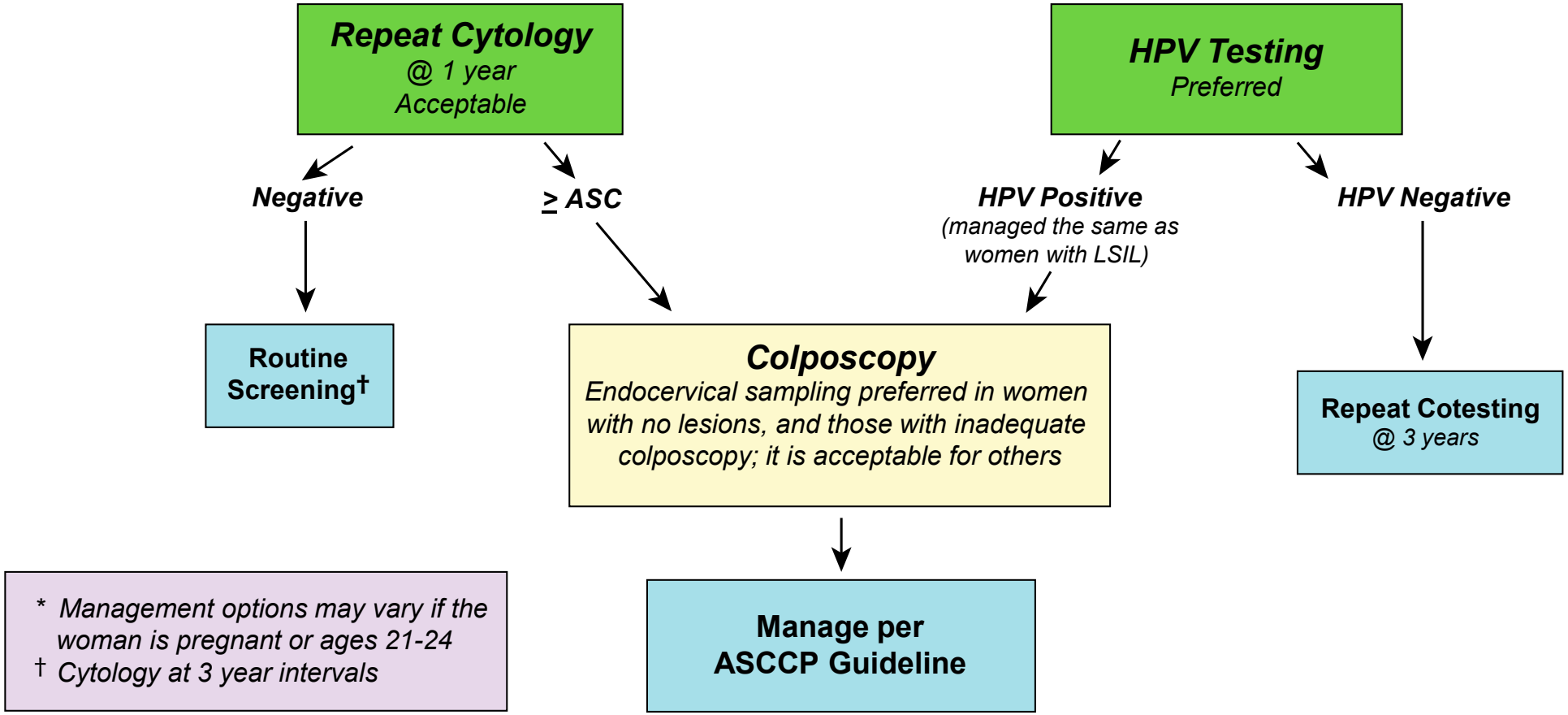


Routine Cervical Cancer Screening Guidelines

Age group	ASCCP 2012	USPSTF 2018
Under 21	No screening	No screening
Age 21-29	Cytology alone every 3 years	Cytology alone every 3 years
Age 30-65	Cytology alone every 3 years OR Cotesting with cytology and HPV testing every 5 years	Cytology alone every 3 years OR Cotesting with cytology and HPV testing every 5 years OR Primary screening with HPV testing along every 5 years
Over 65	Discontinue screening if adequately screening and not at high risk for cervical cancer	Discontinue screening if adequately screening and not at high risk for cervical cancer

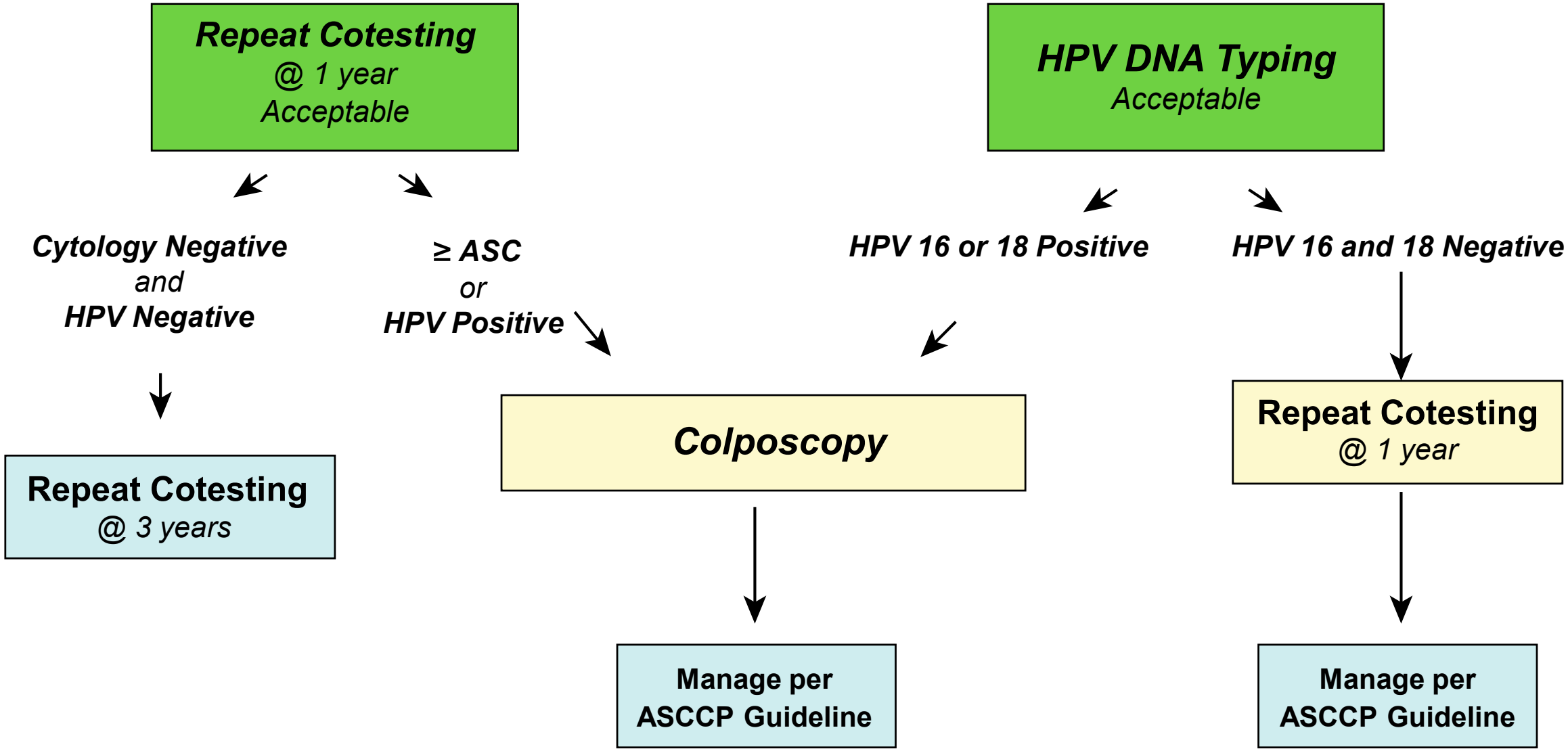
https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/cervical-cancer-screening2
http://www.asccp.org/Assets/fcd6fdab-0325-466b-a5cd-3c1c06cf0e66/635912171989730000/asccp-guidelines-pdf

*Management of Women with Atypical Squamous Cells of Undetermined Significance (ASC-US) on Cytology**



http://www.asccp.org/asccp-guidelines

Management of Women ≥ Age 30, who are Cytology Negative, but HPV Positive



http://www.asccp.org/asccp-guidelines

Test	Target Gene(s)	Target Biomolecule	Internal Control	Technology	HPV Types Detected	Fixative
Abbott RealTime High Risk HPV Assay	L1	DNA	Beta globin	PCR	**16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	ThinPrep
APTIMA	E6 and E7	mRNA	Spiked in	Transcription mediated amplification	**16, 18/45**, 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68	ThinPrep
Cervista	L1	DNA	HIST2H2BE	Isothermal DNA amplification, Invader FRET	**16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	ThinPrep
Cobas 4800 HPV Test	L1	DNA	Beta globin	PCR	**16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	ThinPrep
Hybrid Capture 2	L1	DNA	None	RNA probes and antibody detection of RNA:DNA hybrids	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	ThinPrep
BD Onclarity	E6 and E7	DNA	Beta globin	PCR	**16, 18**, 31, 45, 51, 52, and 59, (33, 56, 58, 66), (35, 39, 68)	SurePath

Adapted from Bibbo Comprehensive Cytopathology

Risks of screening

- False positives
  - Unnecessary biopsies and loop electrosurgical excision procedures
    - May lead to shortened cervix or cervical stenosis
    - Reduced fertility or incompetent cervix
- False negatives
  - Lost opportunity for early treatment
  - Lesions may present when already invasive or even metastatic, requiring more invasive treatment, impact of quality of life and survival
- Direct costs to patients
- Public health impacts
- Utilization of healthcare resources

Potential biological pitfalls of HPV testing

- 10% of invasive carcinomas may be HPV negative[1]
  - Presumed loss of HPV viral DNA in the tumor after acquisition of other mutations, such as DNA repair defects
  - L1 gene may be lost[2]

1. Flanagan MB. *Archives of Pathology & Laboratory Medicine*. 2018. doi:10.5858/arpa.2018-0001-RA
2. Burd EM. *Clinical Microbiology Reviews*. 2016. doi:10.1128/CMR.00013-15

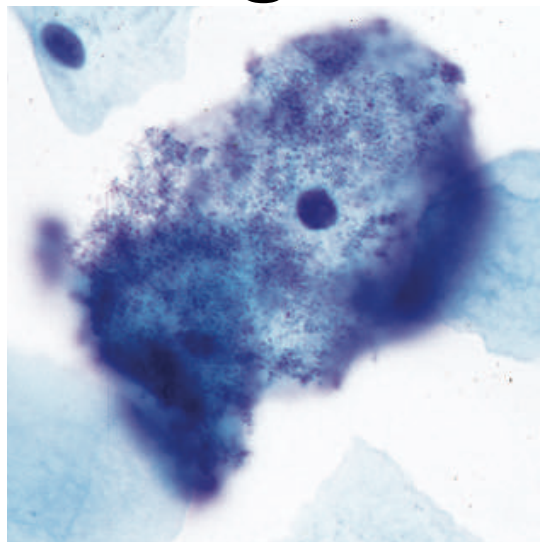
Table 2 Secondary genetic typing for 205 HSIL cases of found to be either totally negative for HPV or positive for other high-risk HPV genotypes other than HPV 16/18 on initial testing.

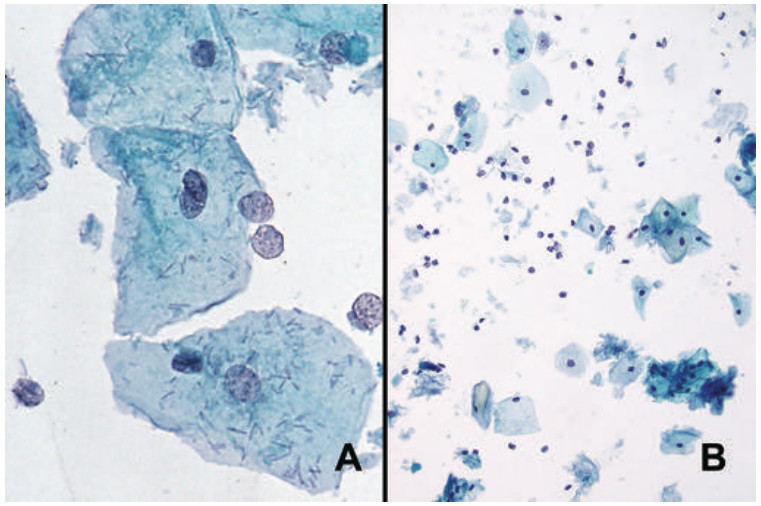
HPV type	Total				Single				Mixed				Risk
	N	Bx Conf	% HSIL cases	Bx Conf	N	Bx Conf	% HSIL cases	Bx Conf	N	Bx Conf	% HSIL cases	Bx Conf	
31	30	12	14.6	17.6	23	10	11.2	14.7	7	2	3.4	2.9	High
52	13	5	6.3	7.4	10	5	4.9	7.4	3	0	1.5	0.0	
58	13	6	6.3	8.8	11	4	5.4	5.9	2	2	1.0	2.9	
35	9	7	4.4	10.3	8	6	3.9	8.8	1	1	0.5	1.5	
45	7	3	3.4	4.4	1	1	0.5	1.5	6	2	2.9	2.9	
33	6	3	2.9	4.4	4	2	2.0	2.9	2	1	1.0	1.5	
59	6	2	2.9	2.9	4	2	2.0	2.9	2	0	1.0	0.0	
16	3	1	1.5	1.5	1	1	0.5	1.5	2	0	1.0	0.0	
56	1	0	0.5	0.0	1	0	0.5	0.0	0	0	0.0	0.0	
Negative	76	18	37.1	26.5									

McCarthy E, Ye C, Smith M, Kurtycz DFI. *Journal of the American Society of Cytopathology*. 2016. doi:10.1016/j.jasc.2016.08.001

Potential biological pitfalls of HPV testing

- Endogenous flora (coccobacilli including *Gardnerella sp.*, lactobacilli) and cytolysis may lead to interference
- Shifts in high risk HPV types with increasing HPV vaccination
  - Gardasil 9: 16, 18, 31, 33, 45, 52, 58
  - Most HPV tests: **16, 18, 31, 33**, 35, 39, **45**, 51, **52**, 56, **58**, 59, 66, 68
- Selective pressure on the L1 gene due to HPV vaccination





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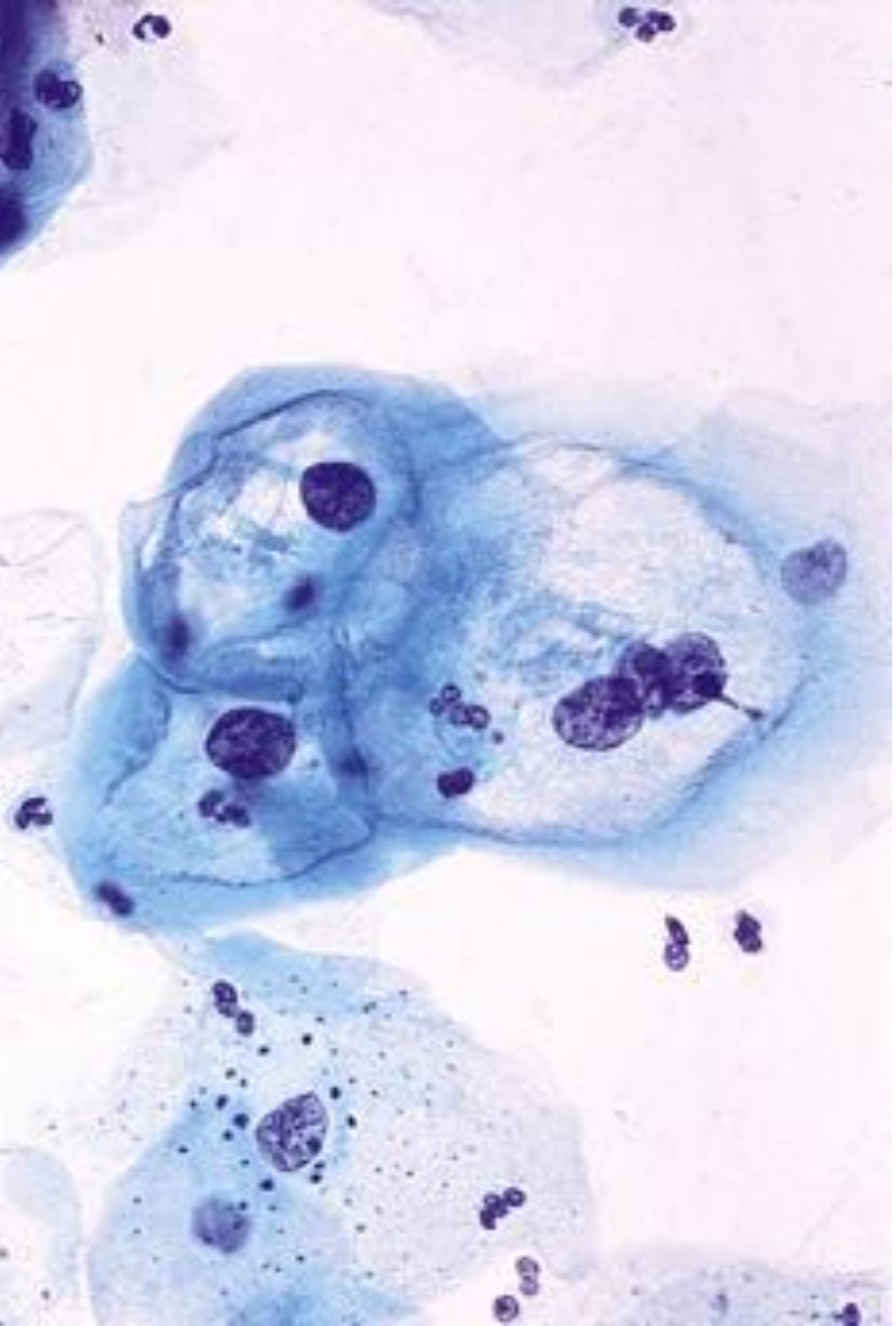
Technical pitfalls in HPV testing

- SurePath vs. ThinPrep
  - SurePath vials should contain the collection device, while ThinPrep should not
  - SurePath fixative contains a small amount of formaldehyde
  - Some laboratories have validated a boiling pre-processing step
    - Burd EM. *Clinical Microbiology Reviews*. 2016. doi:10.1128/CMR.00013-15
- Alternate collection methods/sources
  - Vaginal self-collection
  - Urine



On the horizon

- HPV primary screening
  - Australia and the Netherlands
  - Potential reflex to cytology
  - Concerns: PPV, NPV, colposcopy infrastructure
- HPV testing combined with other tests to improve specificity for precancerous lesions
  - DNA methylation
  - Gene expression
  - IHC staining
- CDC Grand Rounds: Preventing Cervical Cancer in the 21st century
  - https://www.cdc.gov/grand-rounds/pp/2019/20190125-cervical-cancer.html

Thank you!

Questions, comments, suggestions, or potential collaborations?

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