Lower Genital Tract Neoplasia – HPV and Beyond

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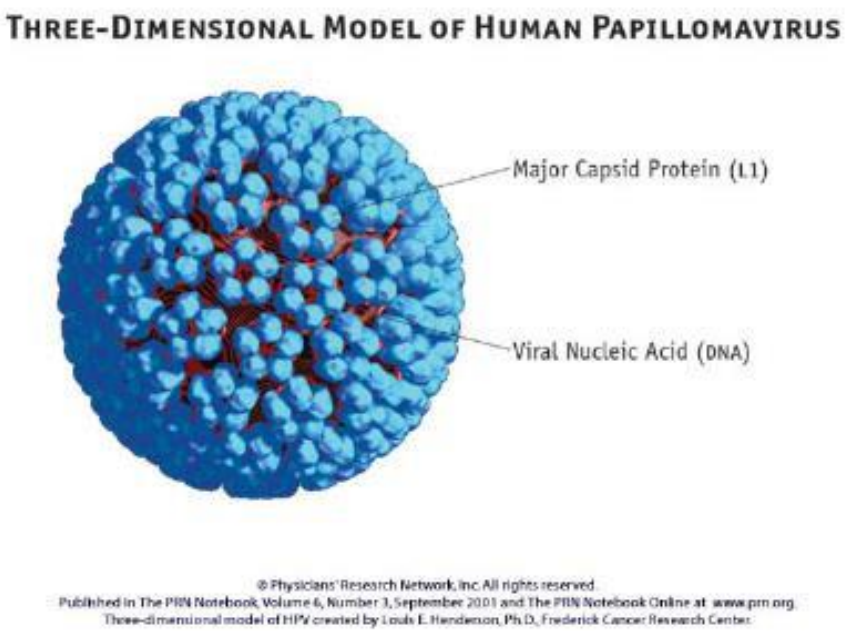
Outline

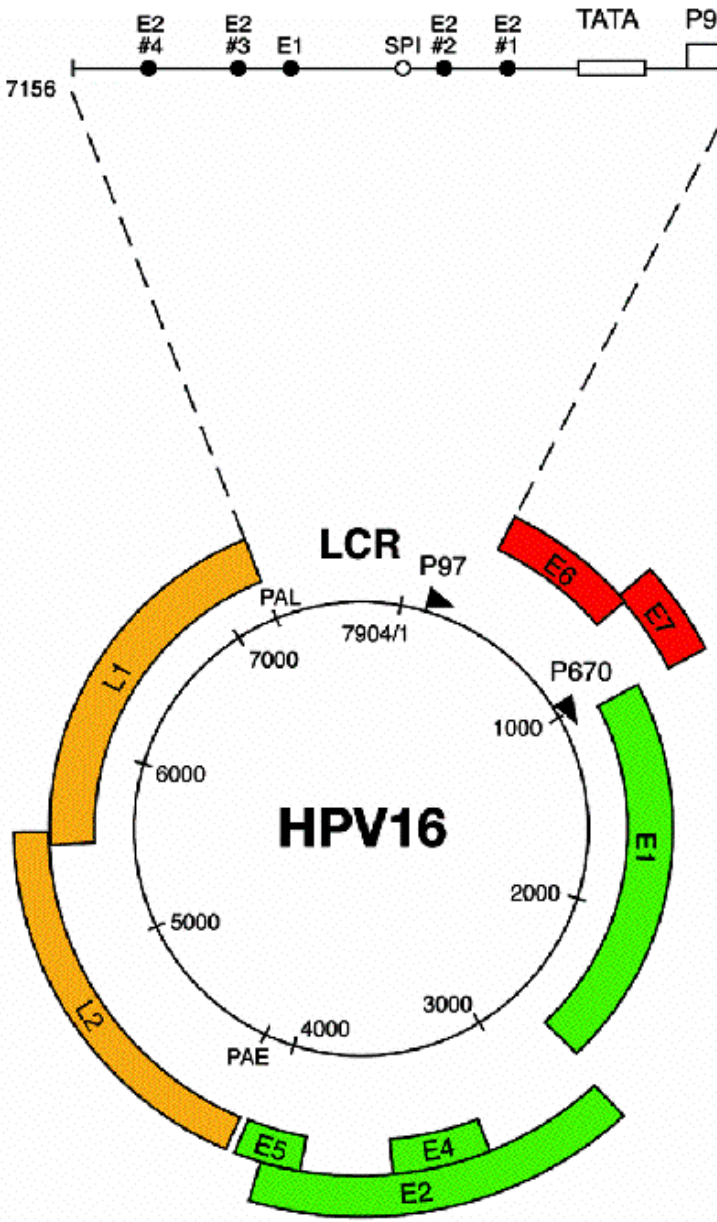
- HPV biology
  - Rationale for p16 as a biomarker for high-risk HPV infection
- Cervical lesions
  - WHO 2020 classification
  - Squamous lesions, HPV-associated and HPV-independent
  - Glandular lesions, HPV-associated and HPV-independent
- Vulval lesions
  - WHO 2020 classification
  - Squamous lesions, HPV-associated and HPV-independent

Human Papillomaviruses

- Non-enveloped, double-stranded DNA viruses
- 8 kb circular genome
- At least 200 genotypes
  - pave.niaid.nih.gov/



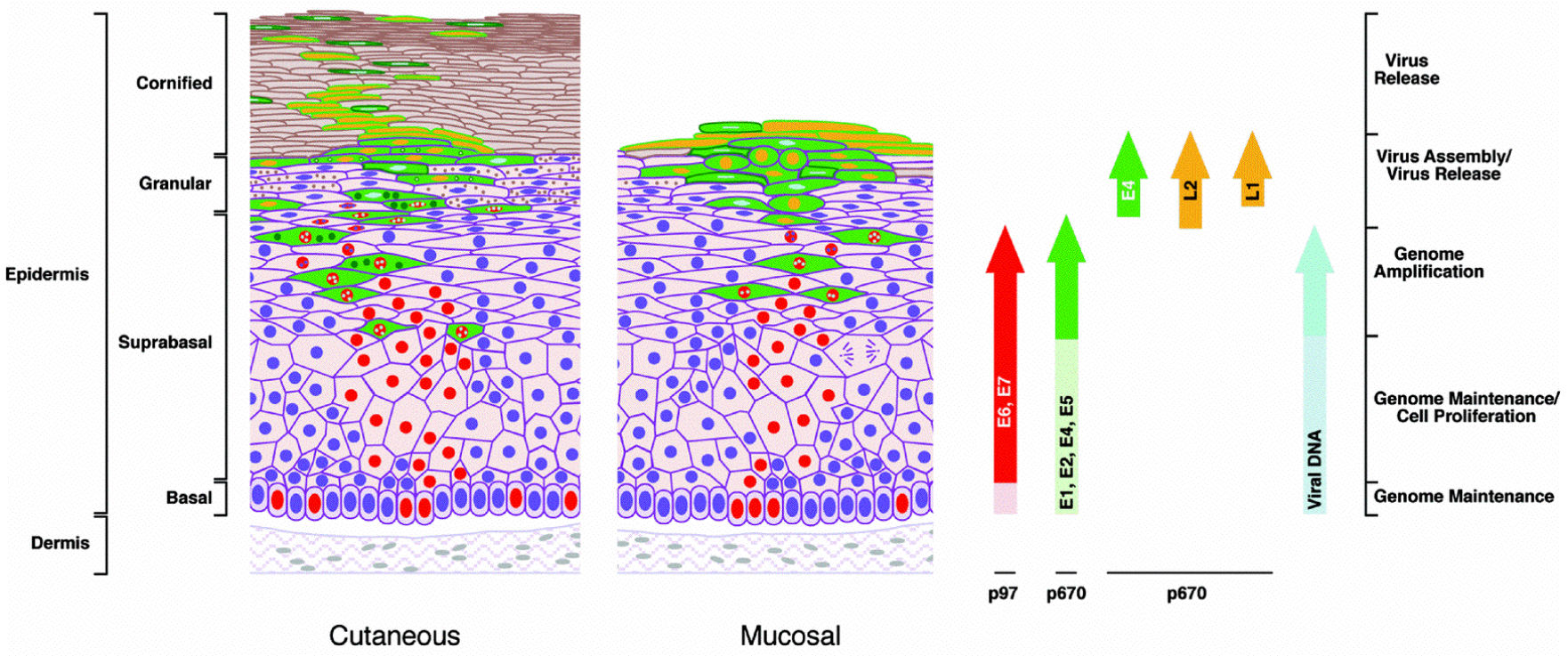
- Epitheliotropic
- High-risk vs low-risk HPV types
- Require squamous differentiation for replication



Molecular Organisation

Doorbar J *Clin Sci* 2006;110:525-41

The Papillomavirus Life Cycle



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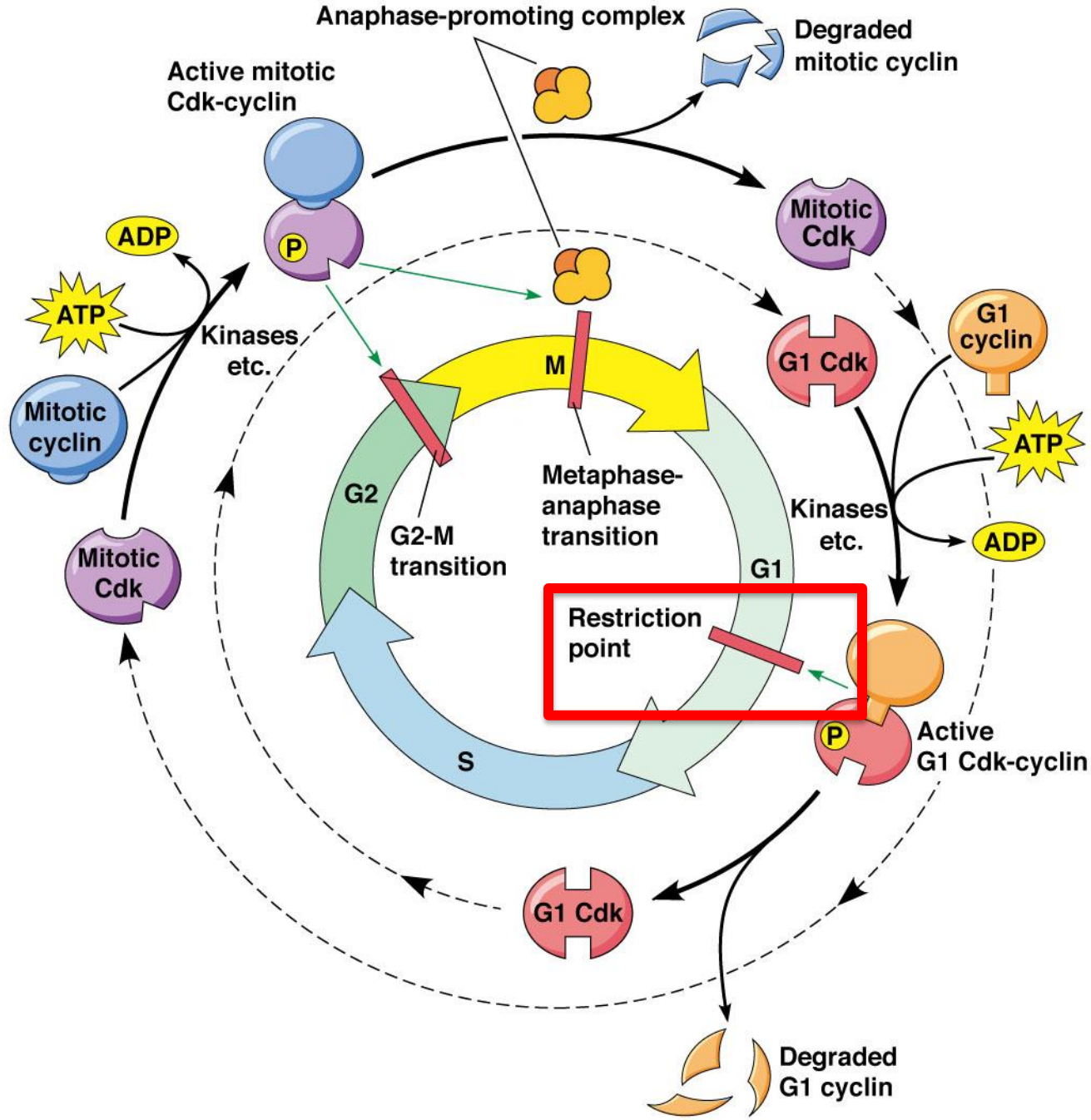
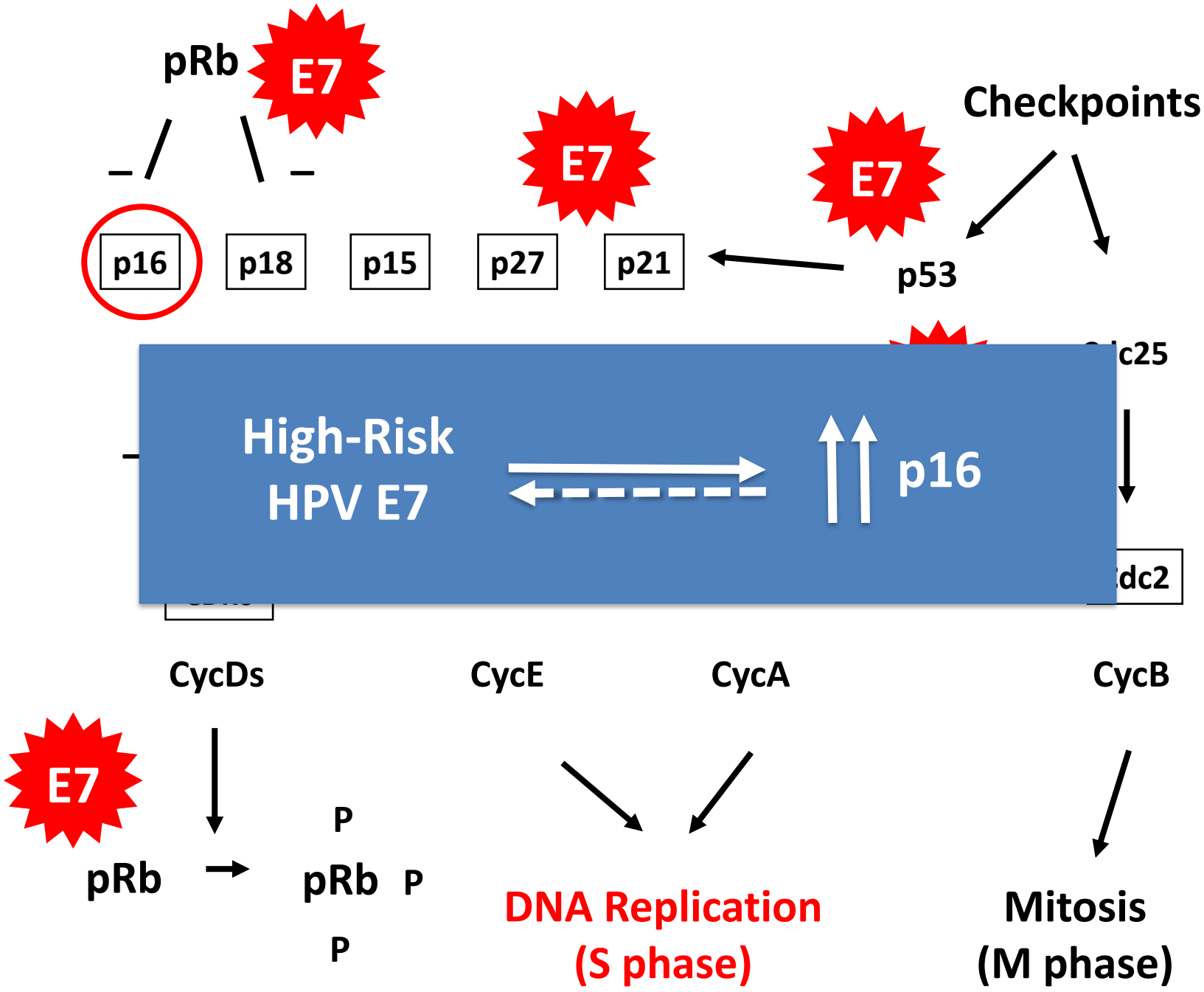
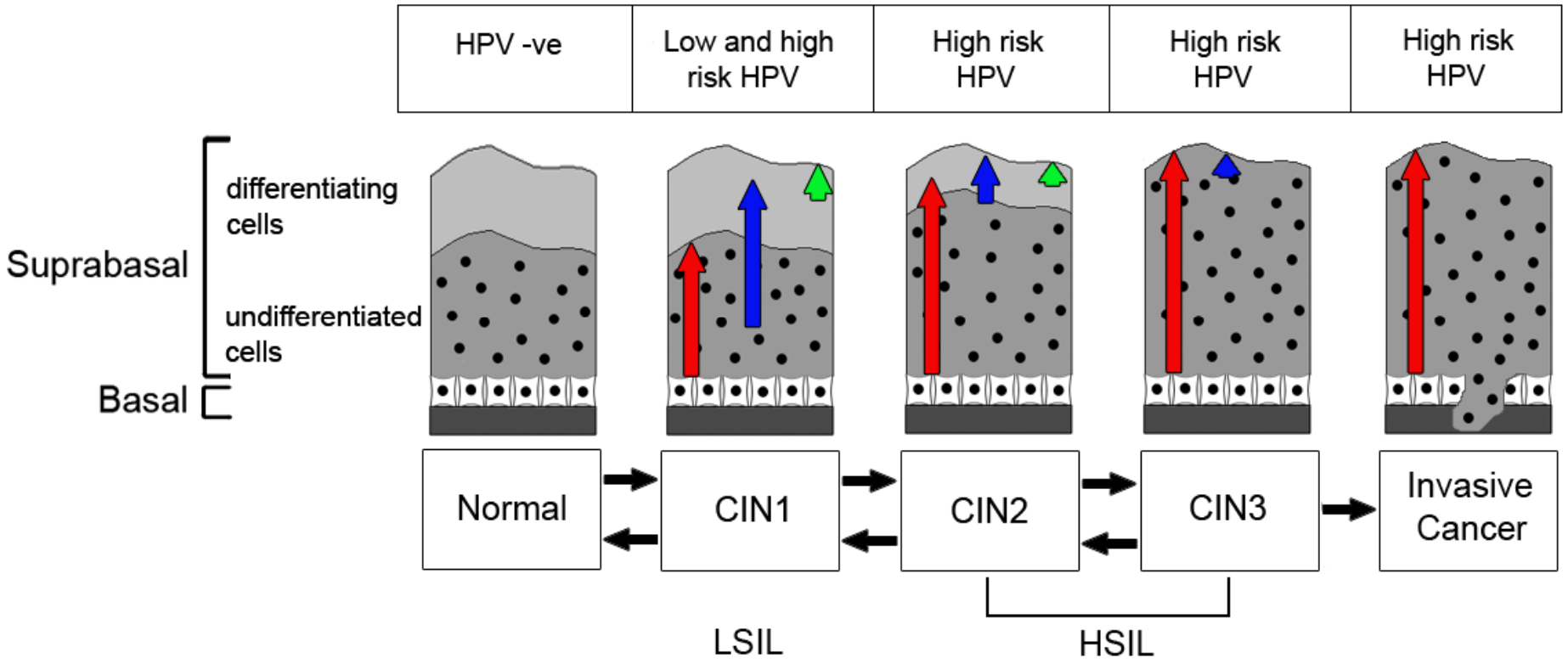
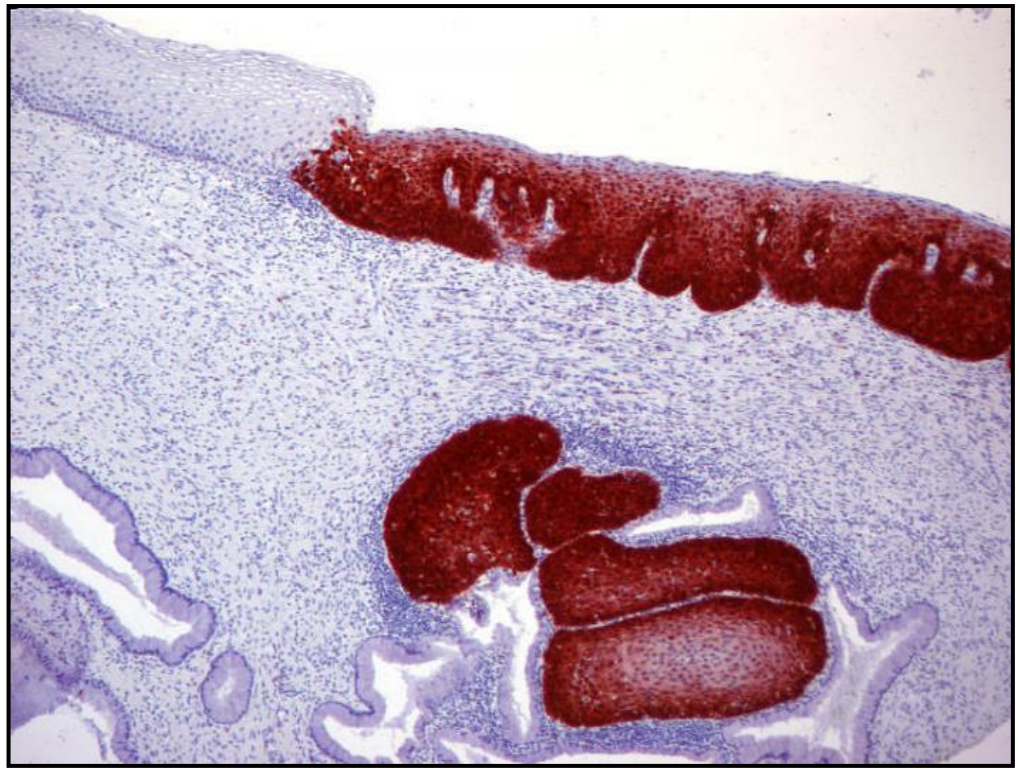


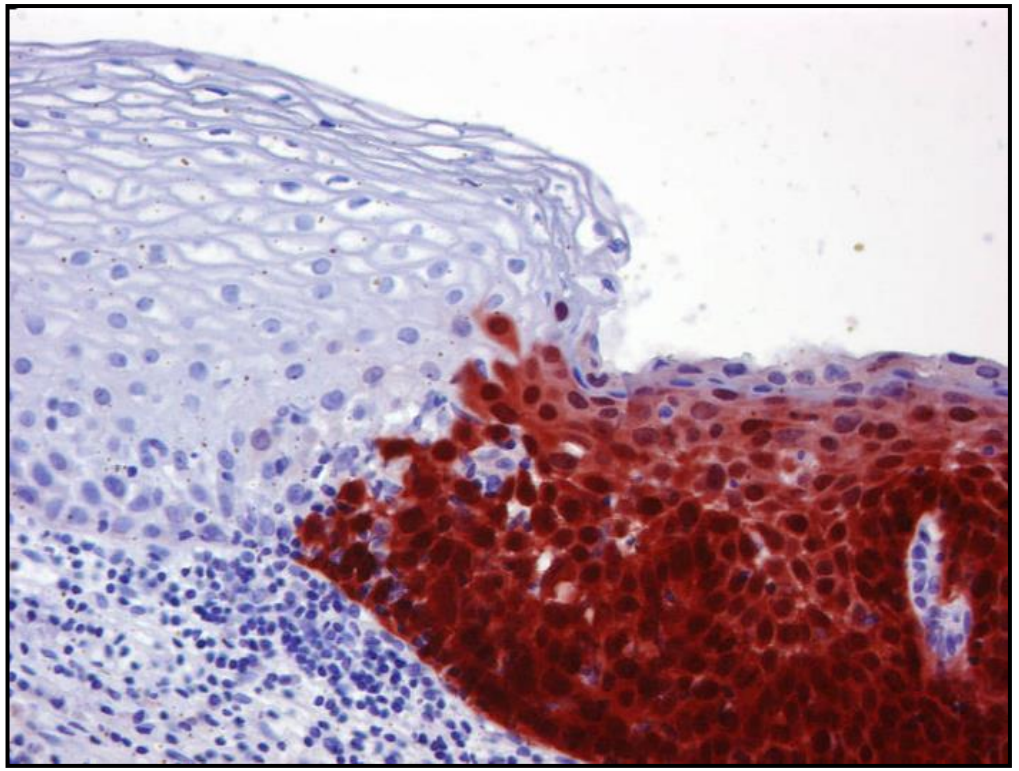

pRb
E7
E7
E7
Checkpoints
p16
p18
p15
p27
p21
p53
High-Risk
HPV E7
p16
c25
dc2
CycDs
CycE
CycA
CycB
E7
pRb
P
pRb
P
P
DNA Replication
(S phase)
Mitosis
(M phase)

HPV and Neoplastic Progression



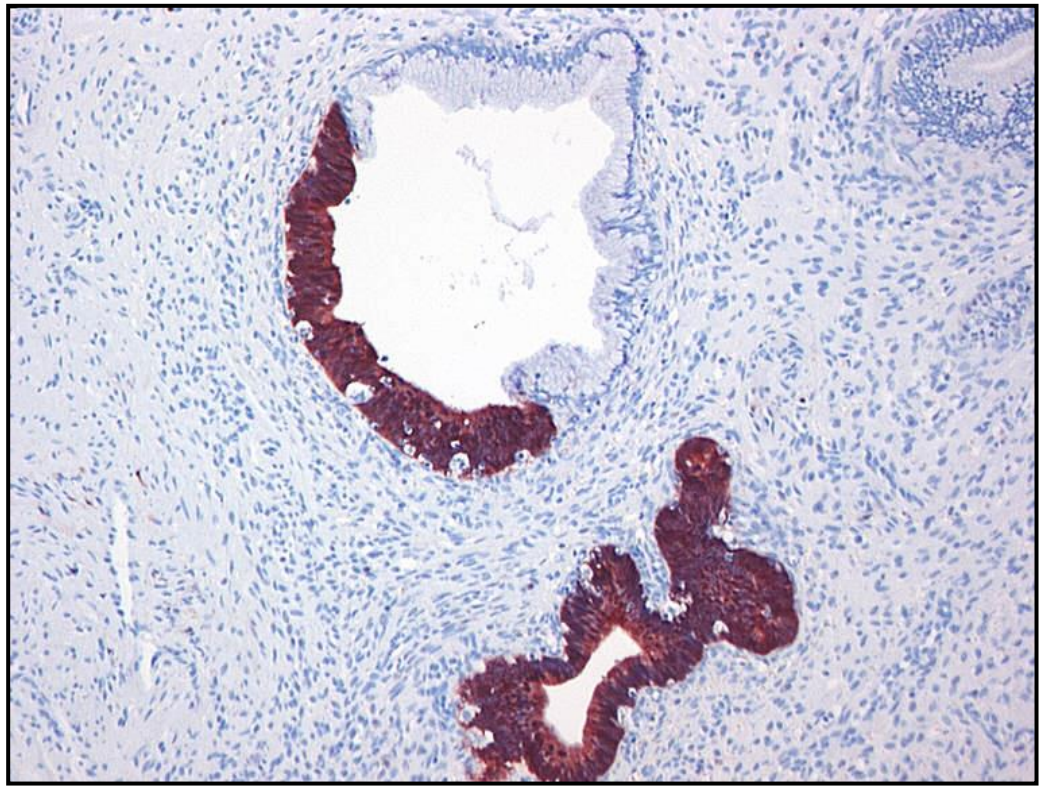
p16 in a Squamous Intraepithelial Lesion
Block-type Positivity

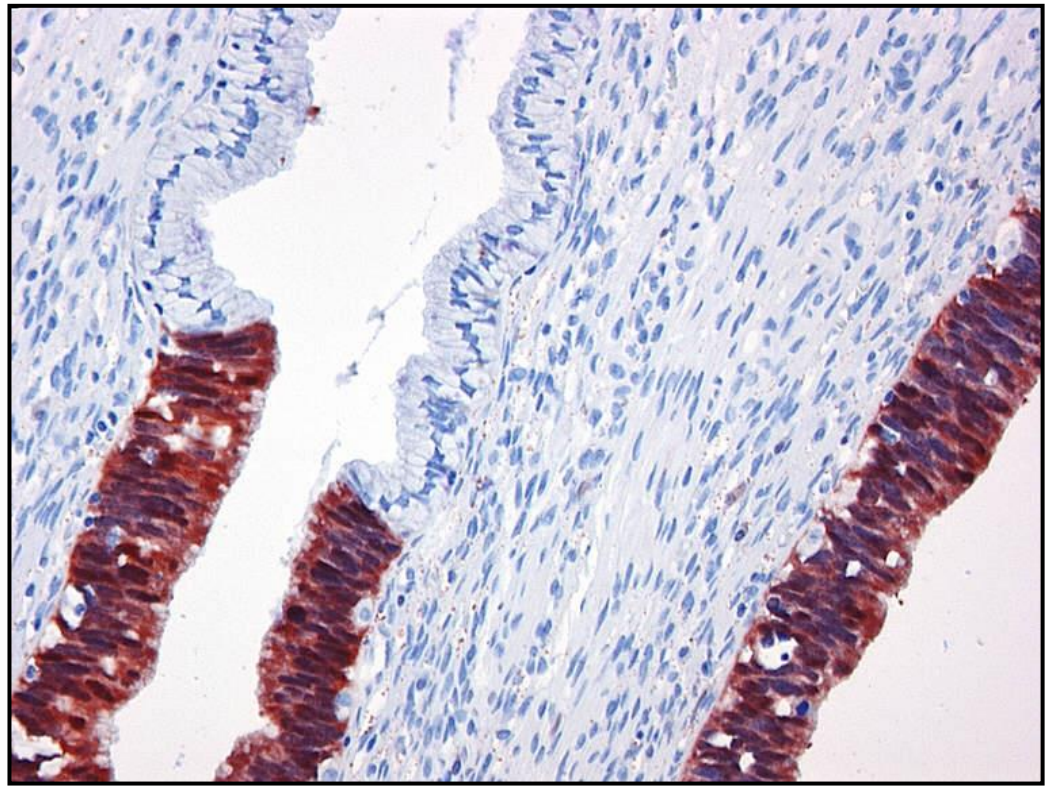




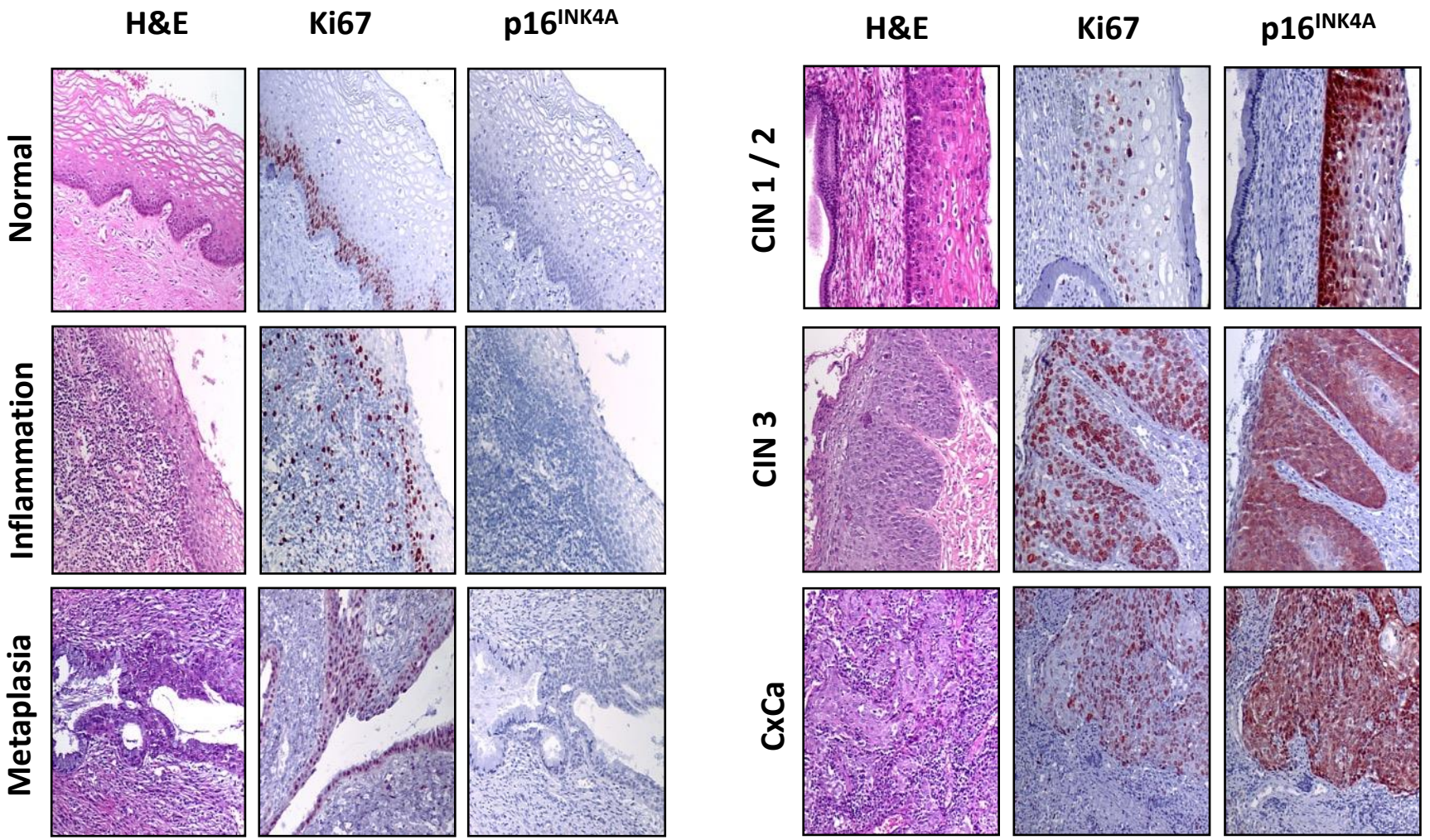
This is defined as continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upward involving at least one third of the epithelial thickness. The latter height restriction is somewhat arbitrary but adds specificity (Darragh et al Int J Gynecol Pathol 2013; 32: 76-11)

p16 in a Glandular Lesion
Diffuse positivity





p16 Immunohistochemistry



Klaes R et al Am J Surg Pathol 2002;11: 1389-99

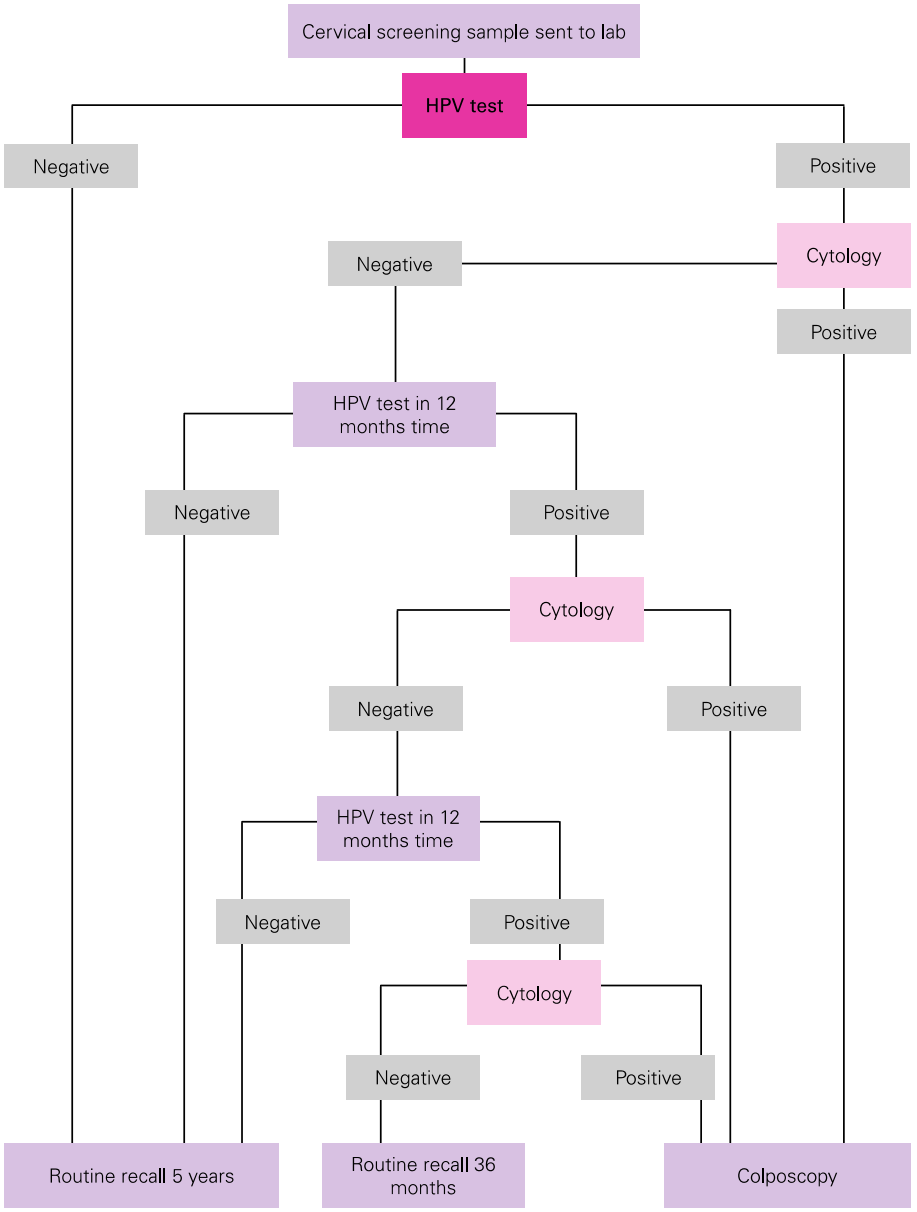
HPV-associated Neoplasia

- High vs low-risk HPV types
- Productive vs abortive/transforming infection
- Up-regulation of E6/E7 expression key
- p16 protein expression is a surrogate marker of high-risk HPV E7 expression

Issues with p16

- **p16 positive low-grade squamous intraepithelial lesions**
- p16 negative high-grade squamous intraepithelial lesions
- Tendency to up-grade – p16 does NOT grade squamous intraepithelial lesions
  - ‘Any identified p16-positive area must meet H&E morphologic criteria for a high-grade lesion to be reinterpreted as such’ (LAST)
- The effect of primary HPV testing
- False positive lesions (e.g. some gastric-type adenocarcinomas of cervix; occasional HPV-independent vulval squamous cell carcinomas)

Cervical screening protocol in Scotland – Primary HPV Testing

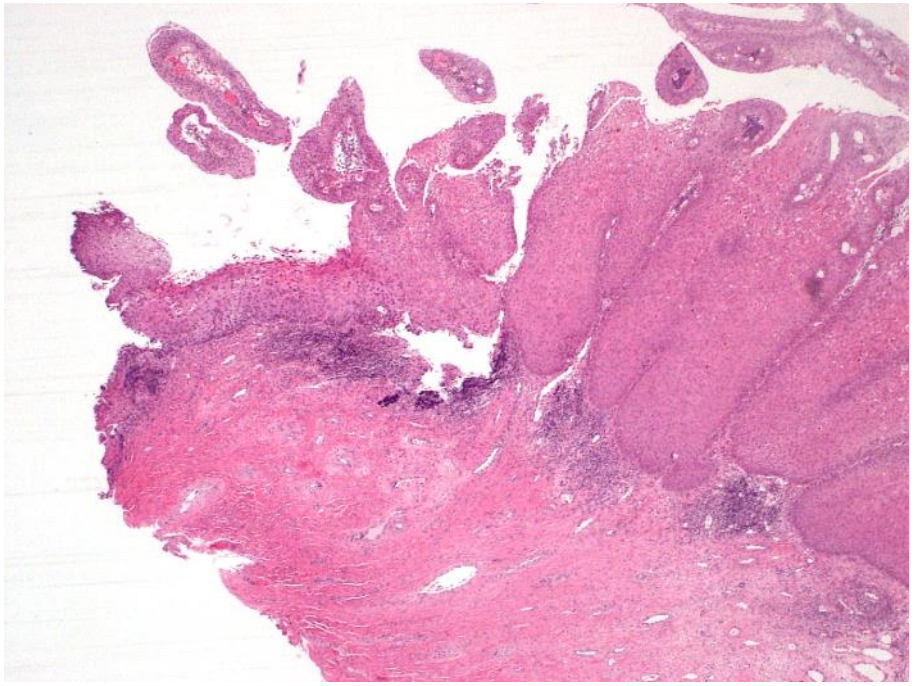


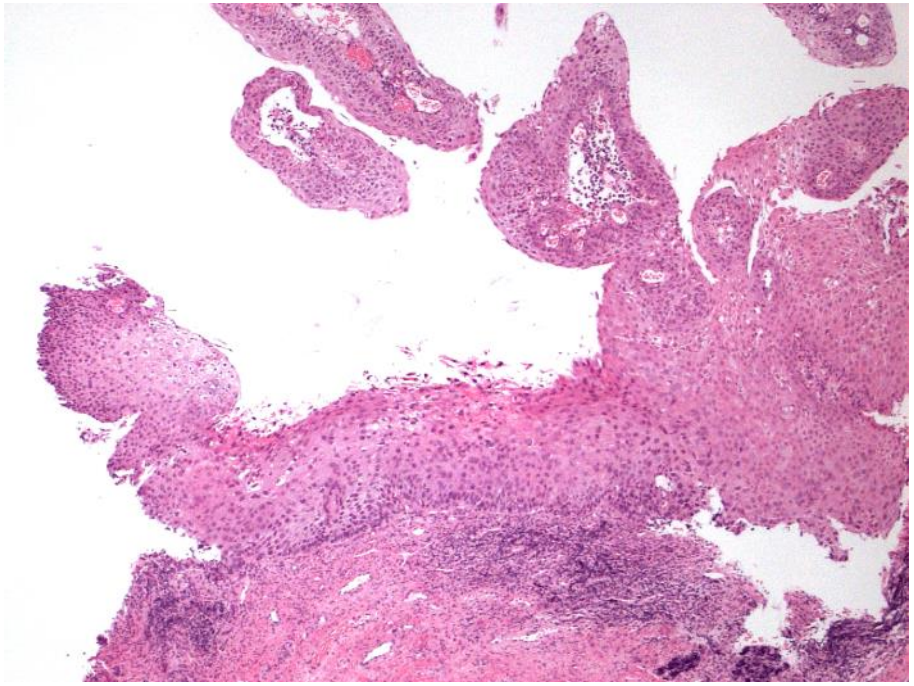
Consequence:
All biopsies and LETZs/LEEPs are from patients whose smears are high-risk HPV positive, cytology positive

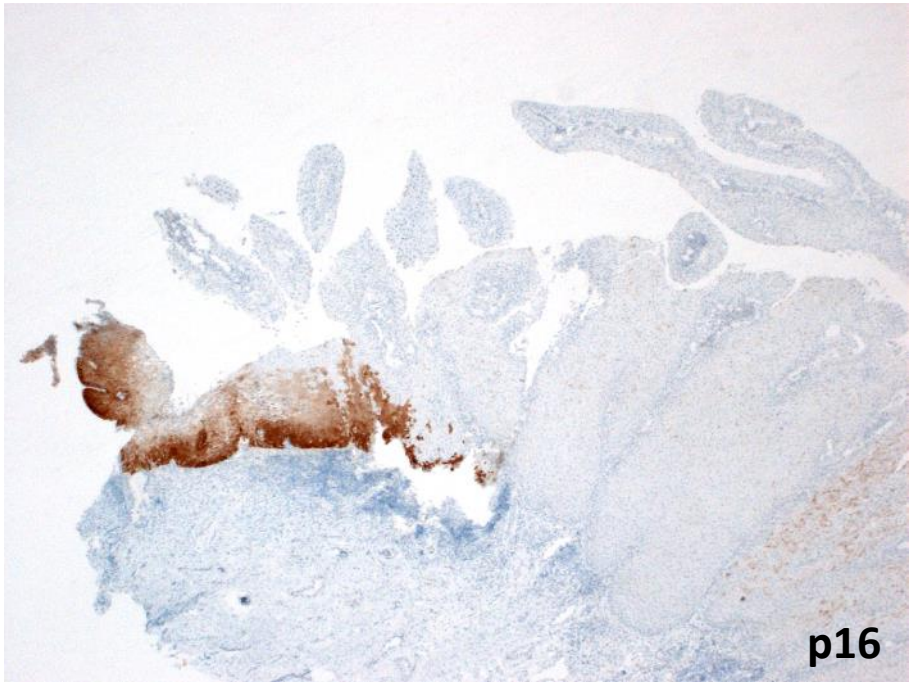
WHO Classification 2020, Cervical Squamous Lesions

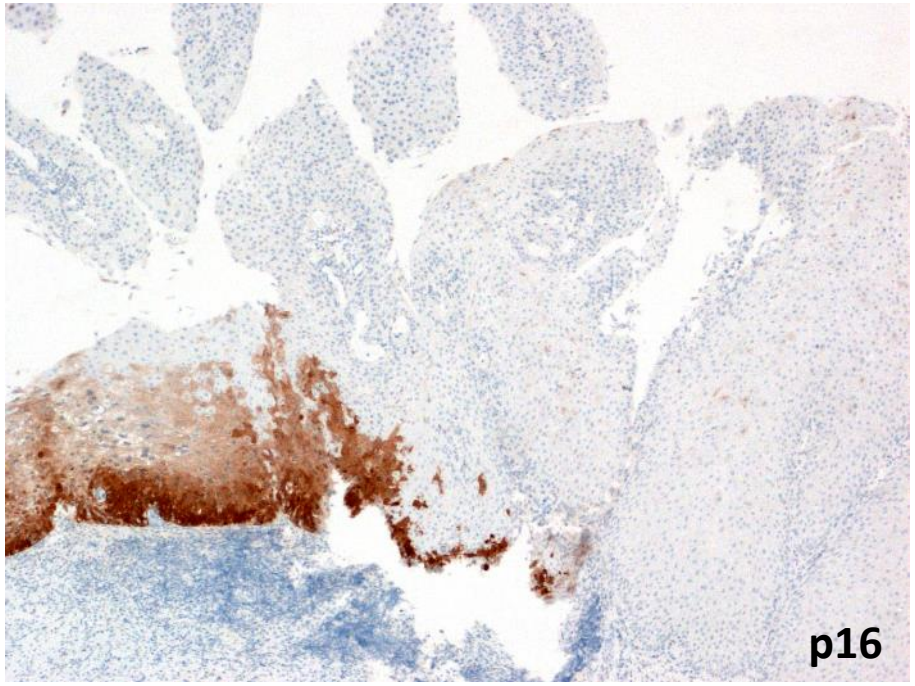
- Low grade squamous intraepithelial lesion (including CIN 1)
- High grade squamous intraepithelial lesion (CIN 2 and CIN 3)
- Squamous cell carcinoma, HPV-associated
- Squamous cell carcinoma, HPV-independent
- Squamous cell carcinoma, not otherwise specified (NOS)

- p16 immunohistochemistry is acceptable as a surrogate marker of HPV for HPV-associated tumours
- HPV DNA testing is recommended for HPV-independent tumours
- Up to 7% of squamous cell carcinomas are HPV-independent (Nicolas et al Mod Pathol 2019; 32: 1189-1196)
- The NOS category is acceptable only where p16/HPV testing are not available






p16


p16

WHO Classification 2020, Cervical Glandular Lesions

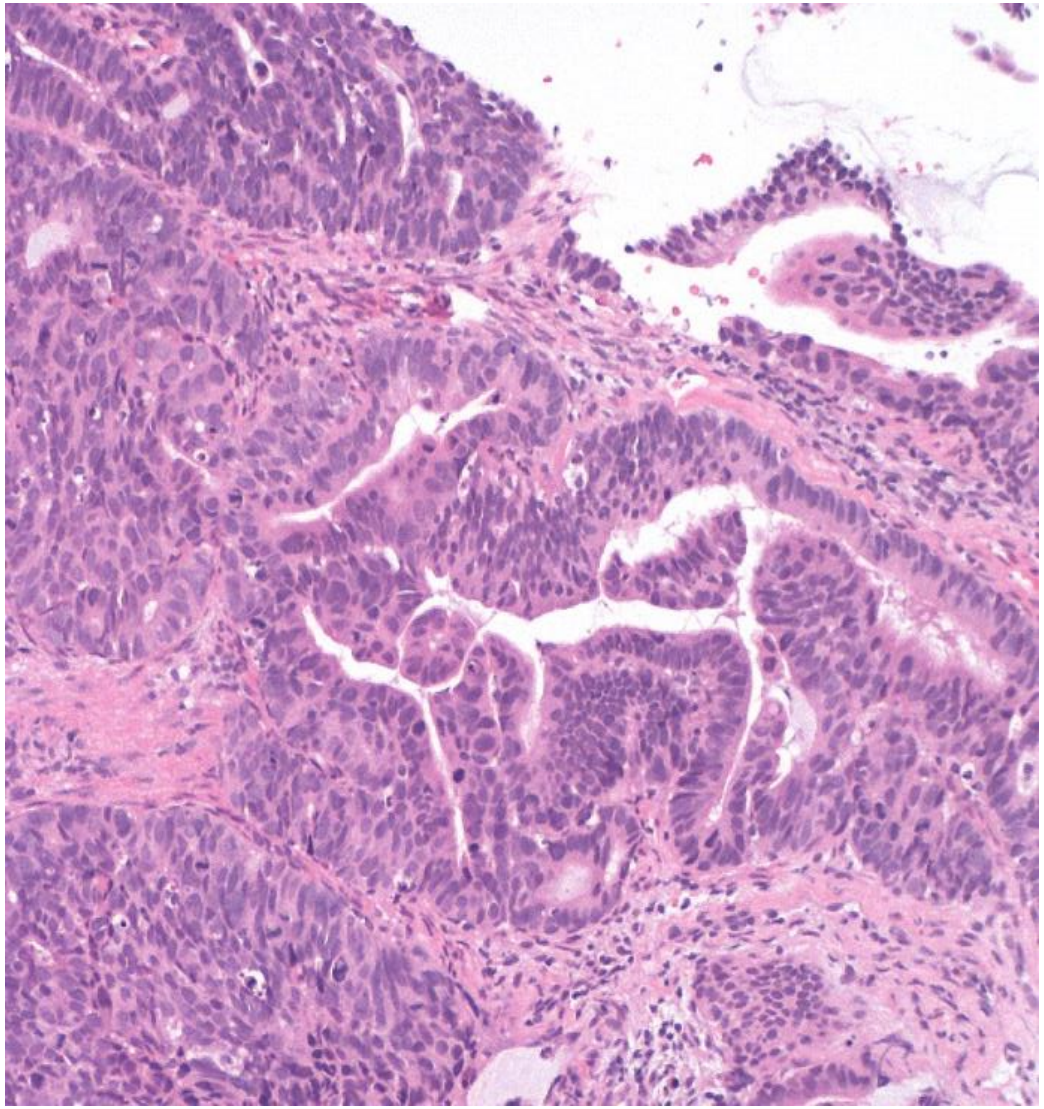
- Adenocarcinoma in situ, HPV-associated (High grade CGIN)
- Adenocarcinoma, HPV-associated (p16 positivity not essential for diagnosis)
  - Usual type
  - Mucinous type

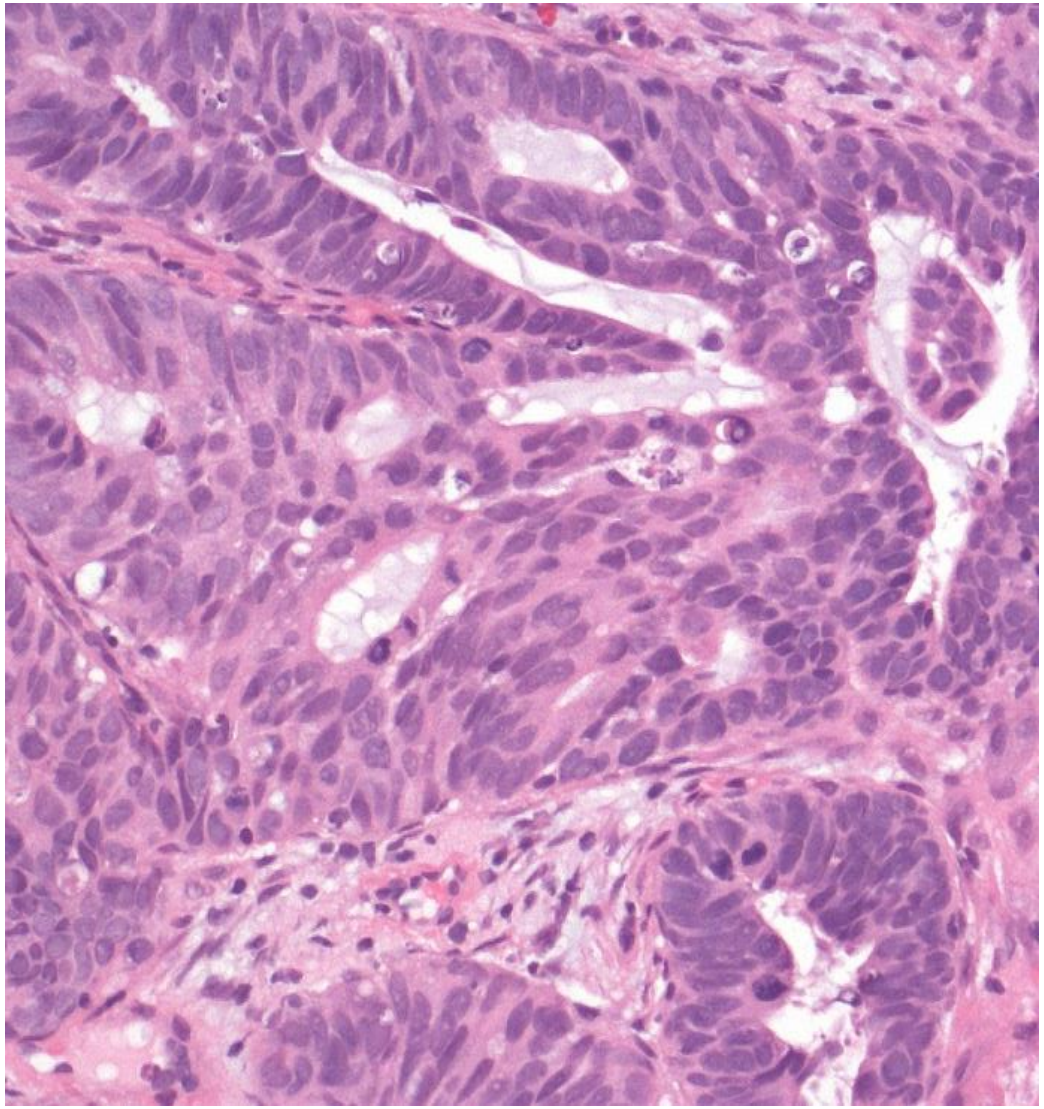
- Adenocarcinoma in situ, HPV independent (including LEGD)
- Adenocarcinoma, HPV-independent, gastric type
- Adenocarcinoma, HPV-independent, clear cell type
- Adenocarcinoma, HPV-independent, mesonephric type
- Other adenocarcinomas (includes very rare endometrioid carcinomas associated with endometriosis)

HPV and Glandular Neoplasia

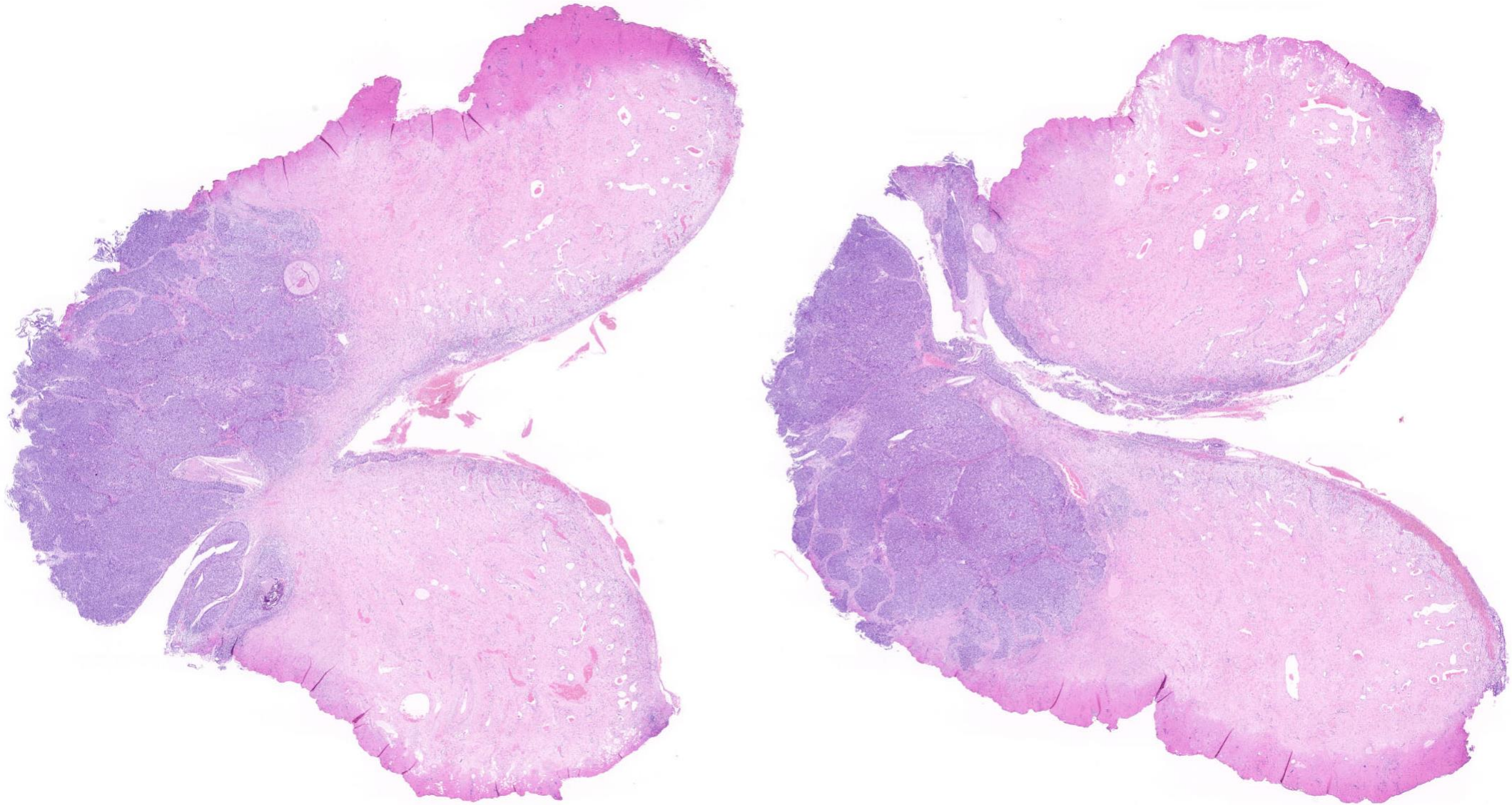
- Endocervical epithelium does not support HPV replication
- Productive infection does not occur
- HPV-related lesions of the endocervical epithelium are neoplastic
- They most likely arise from reserve cell or junctional cell infection, possibly with early HPV integration

Adenocarcinoma, HPV-associated

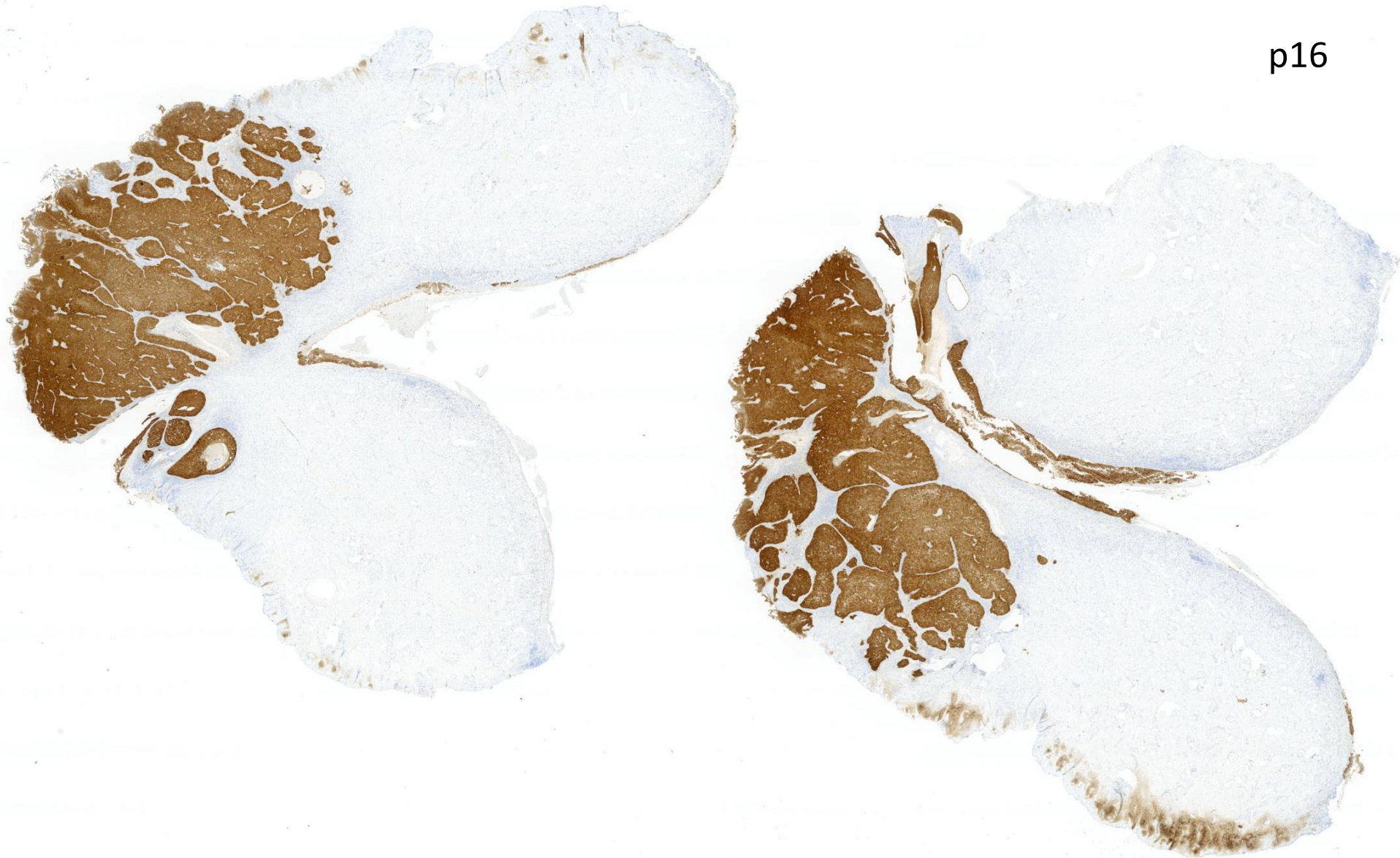




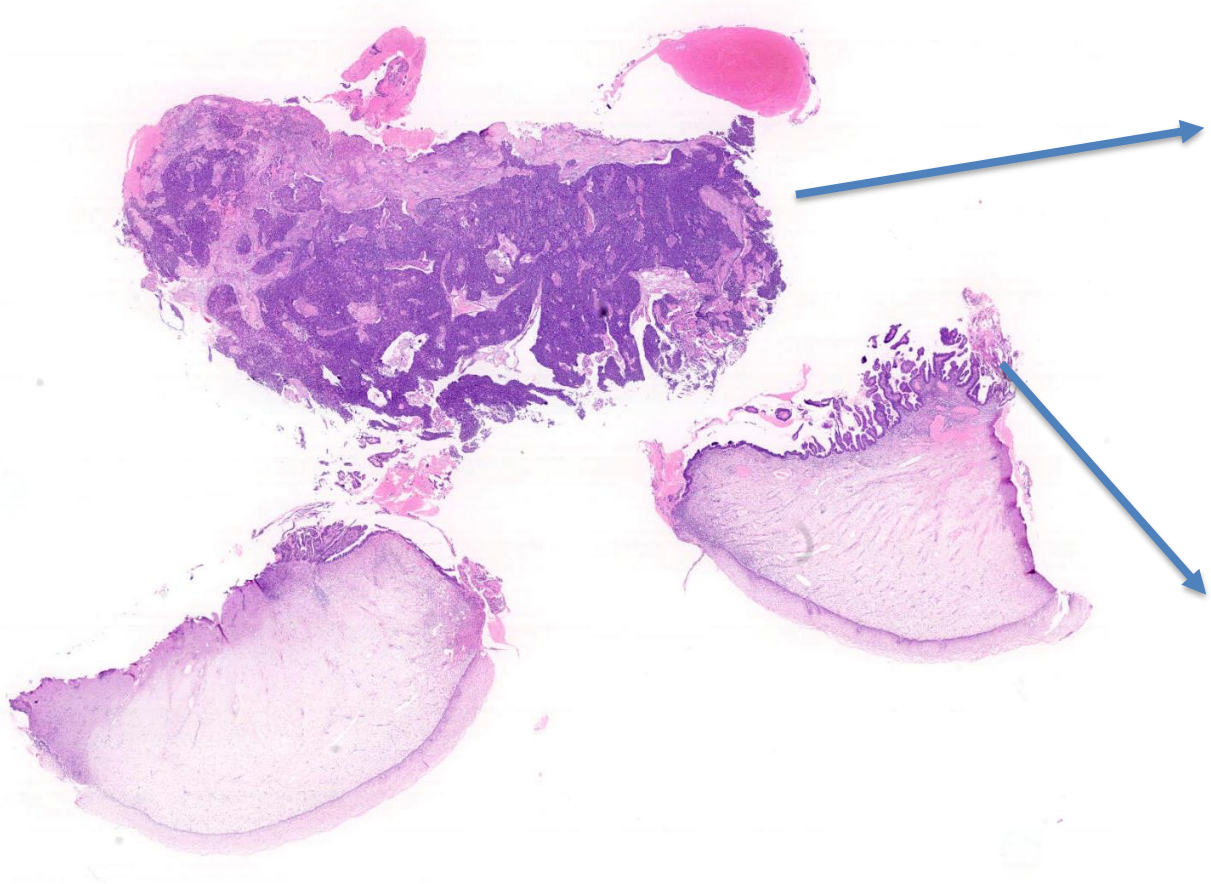
Adenocarcinoma, HPV-associated



Adenocarcinoma, HPV-associated



Mixed Adenocarcinoma and High-grade Neuroendocrine Carcinoma, HPV-associated



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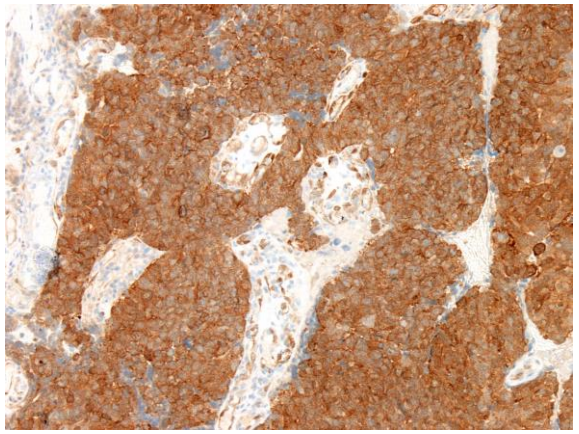
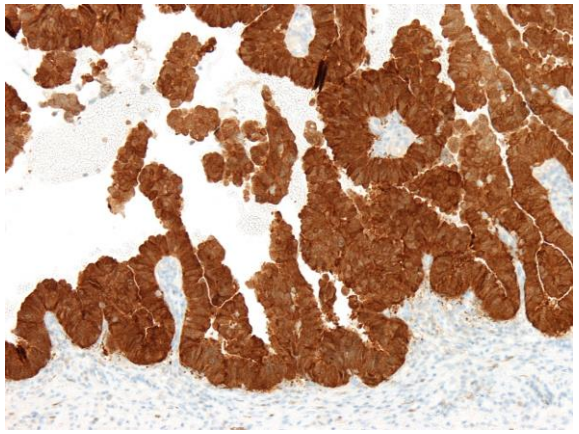
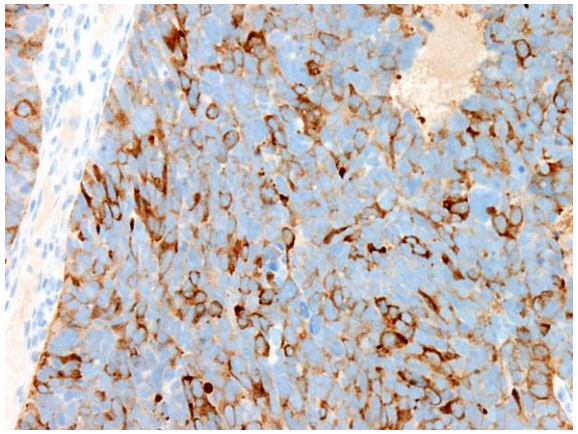
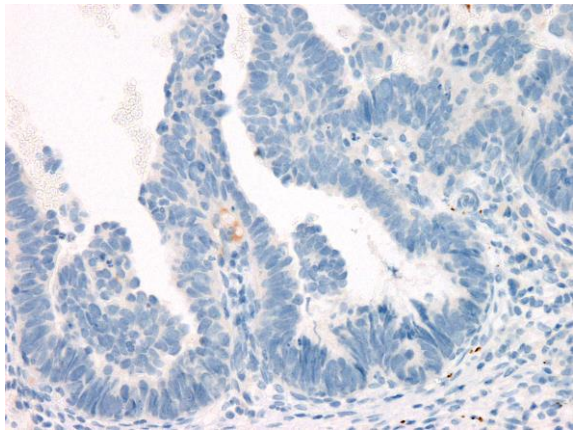




Synaptophysin





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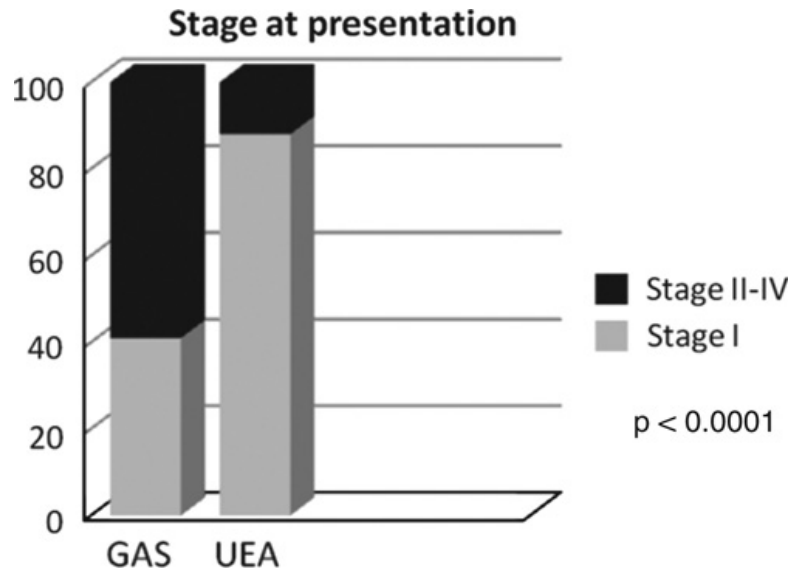
Gastric-type Cervical Adenocarcinoma

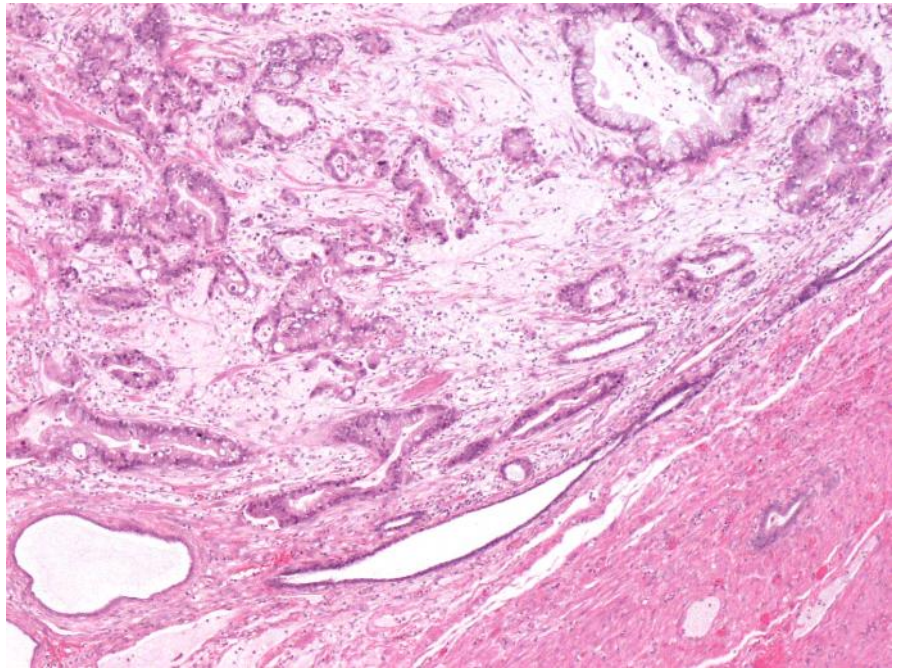
Outcomes	GAS (n = 38), n (%)	UEA (n = 139), n (%)	P*
NED	20 (52.6)	126 (91)	< 0.001
AWD	3 (7.9)	1 (1)	
DOD	15 (39.5)	12 (8)	
Outcomes	**Non-MDA (n = 26), n (%)**	**MDA (n = 12), n (%)**	**P***
NED	13 (50)	6 (50)	0.66
AWD	3 (12)	0 (0)	
DOD	10 (38)	6 (50)	

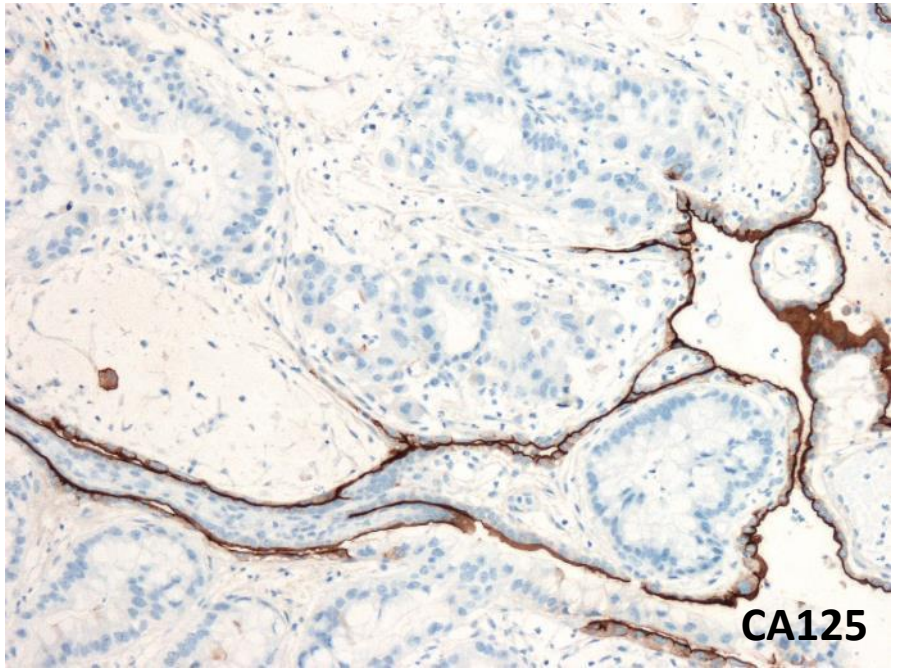
*2-sided exact.

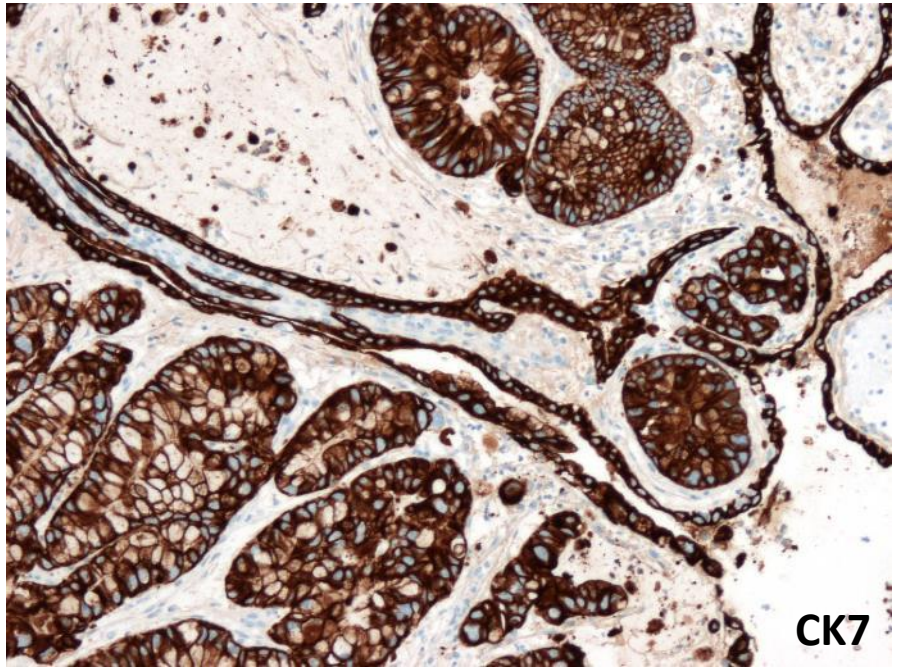
PMH	n (%)
None	33 (82.5)
Peutz-Jeghers syndrome	1 (2.5)
Li-Fraumeni syndrome with breast cancer	1 (2.5)
Breast cancer	2 (5.0)
Colon cancer	1 (2.5)
Wilms + pheochromocytoma + renal cell carcinoma	1 (2.5)
Mucinous BAC lung (KRAS mutation)	1 (2.5)

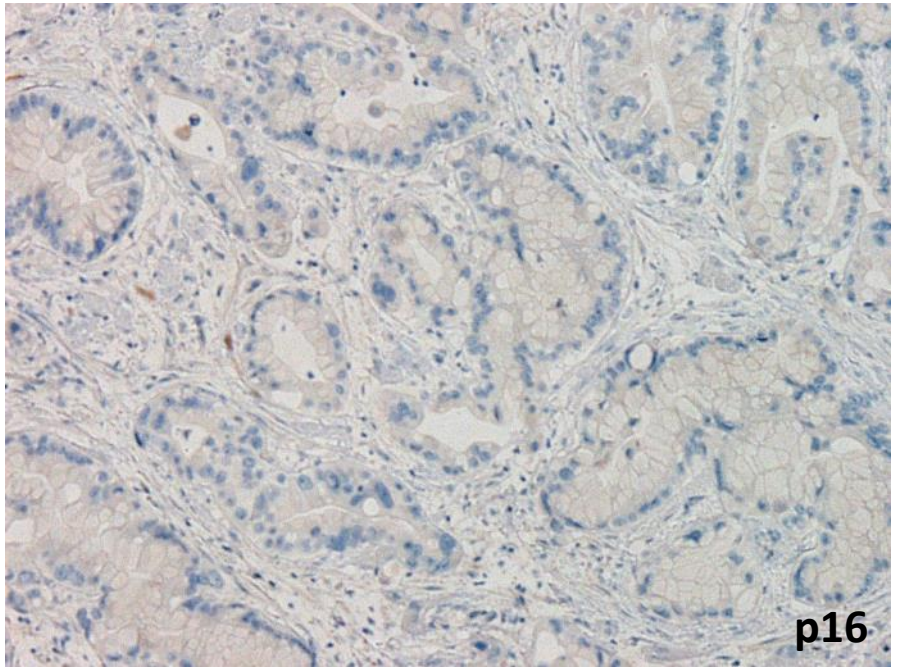


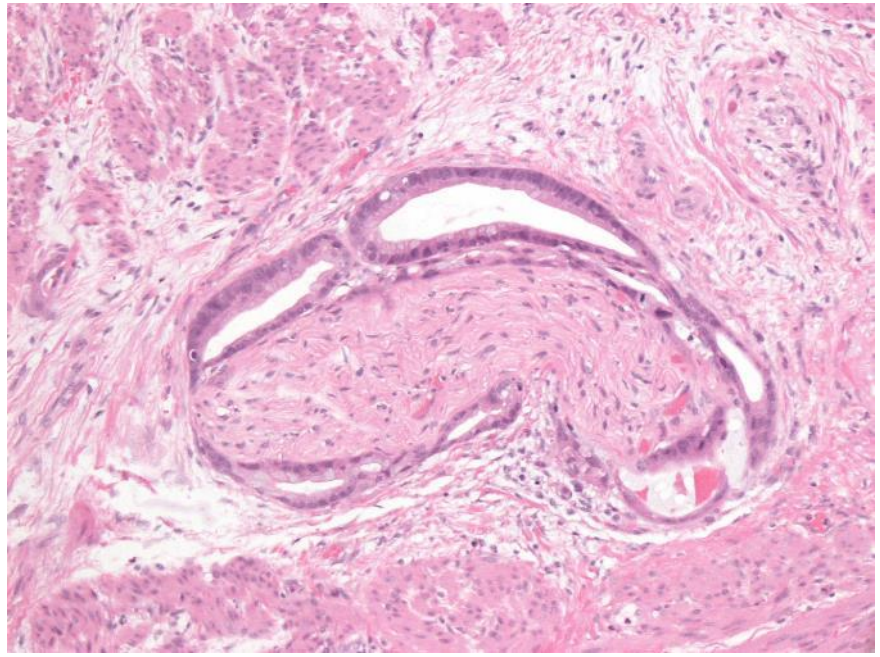
Karamurzin et al, Am J Surg Pathol 2015; 39: 1449-1457

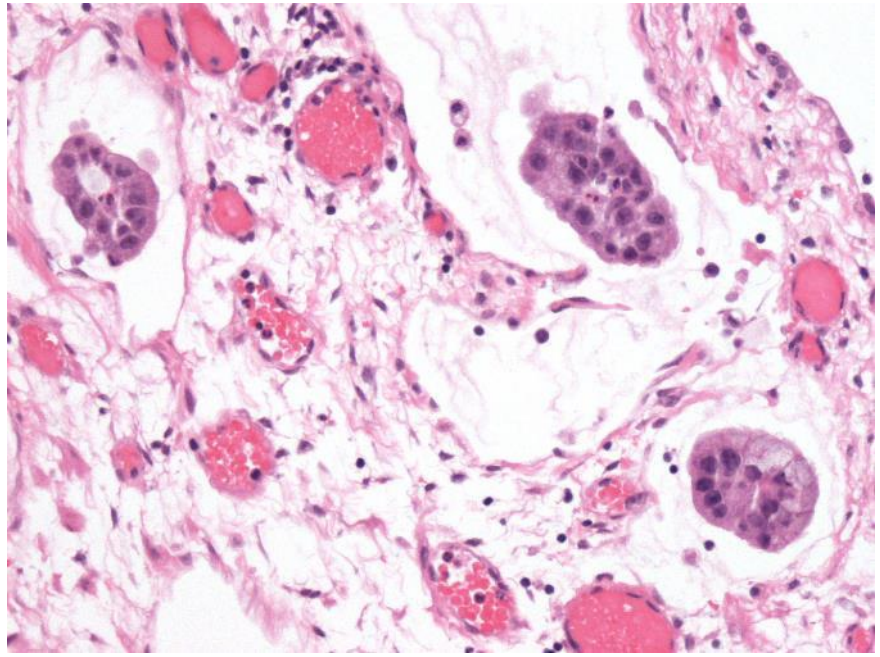


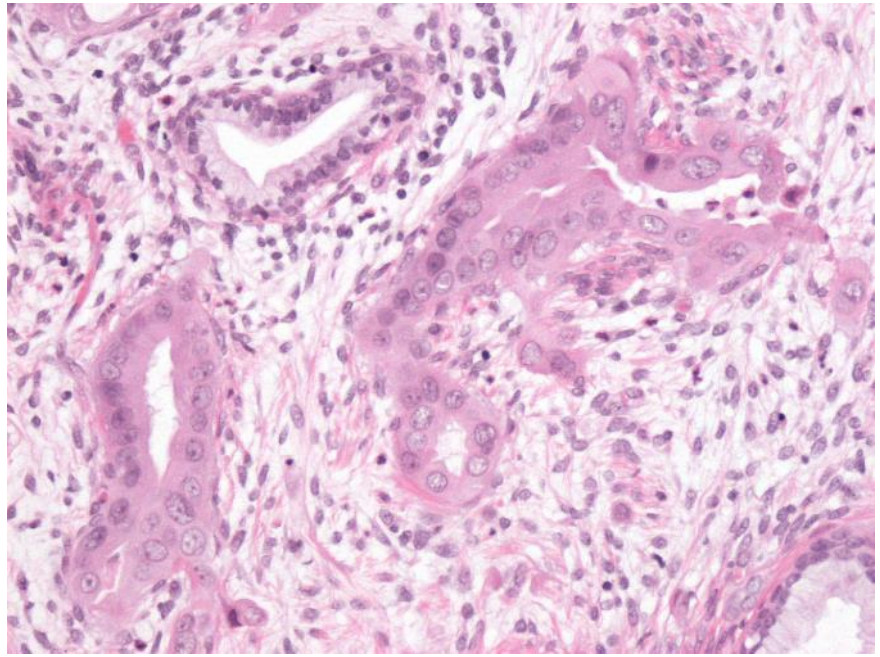

CA125

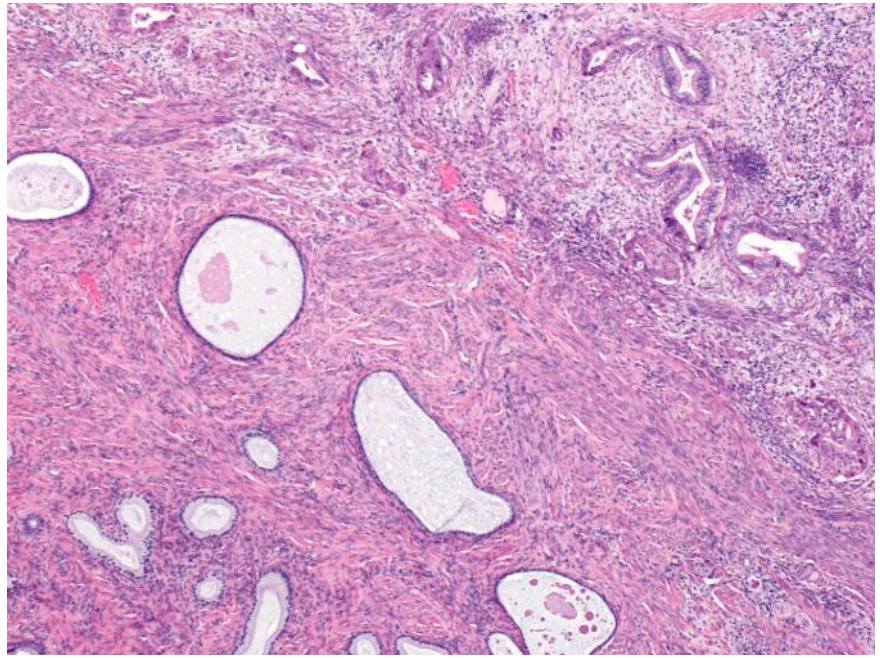

CK7


p16

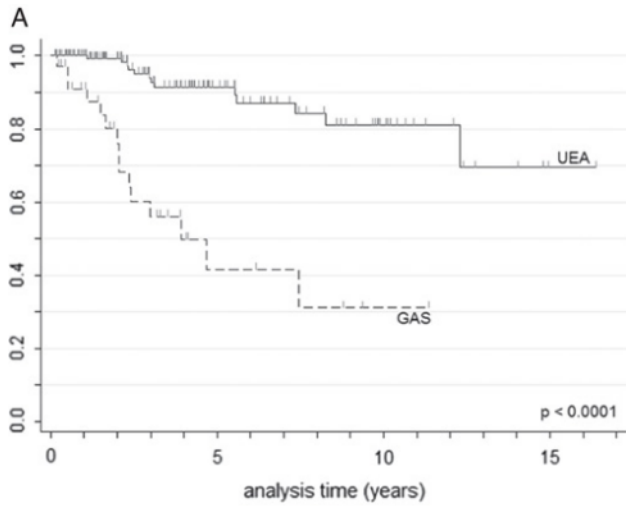


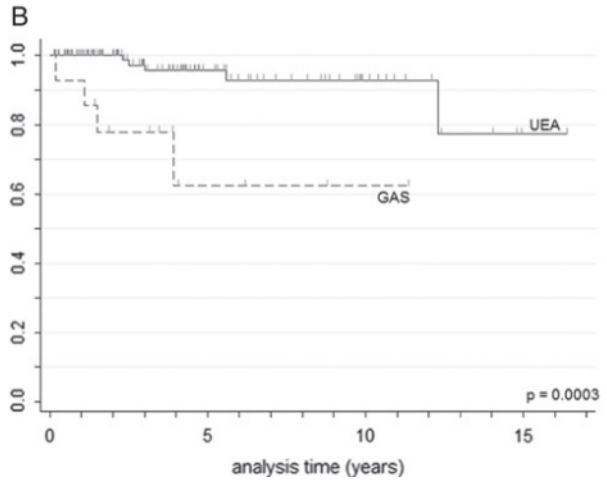




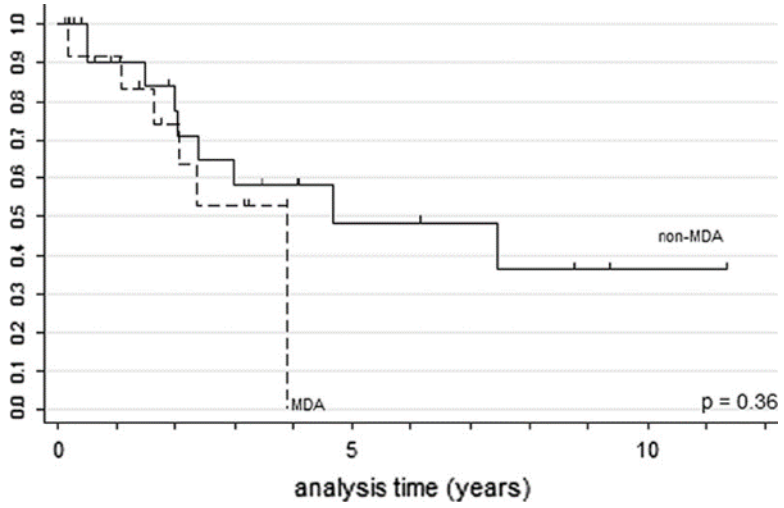


Gastric-type Cervical Adenocarcinoma





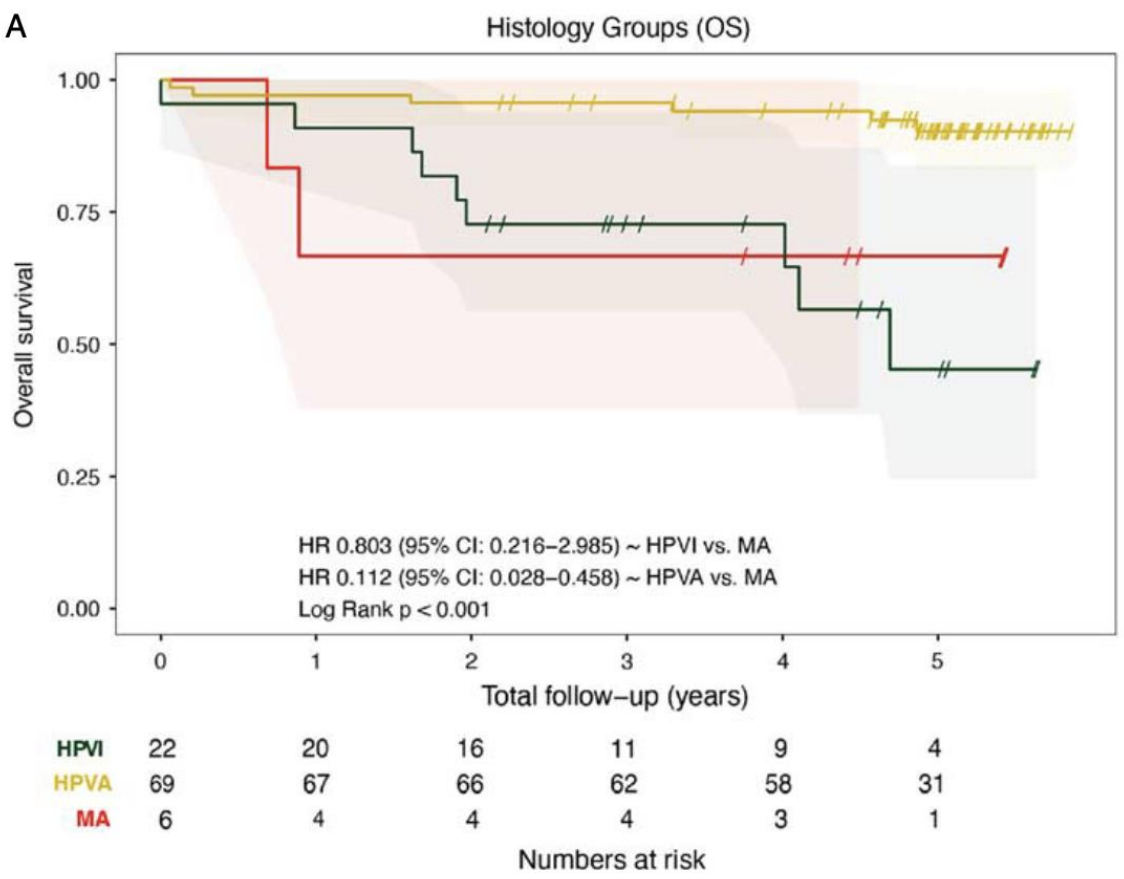
A – all stages; B – stage I

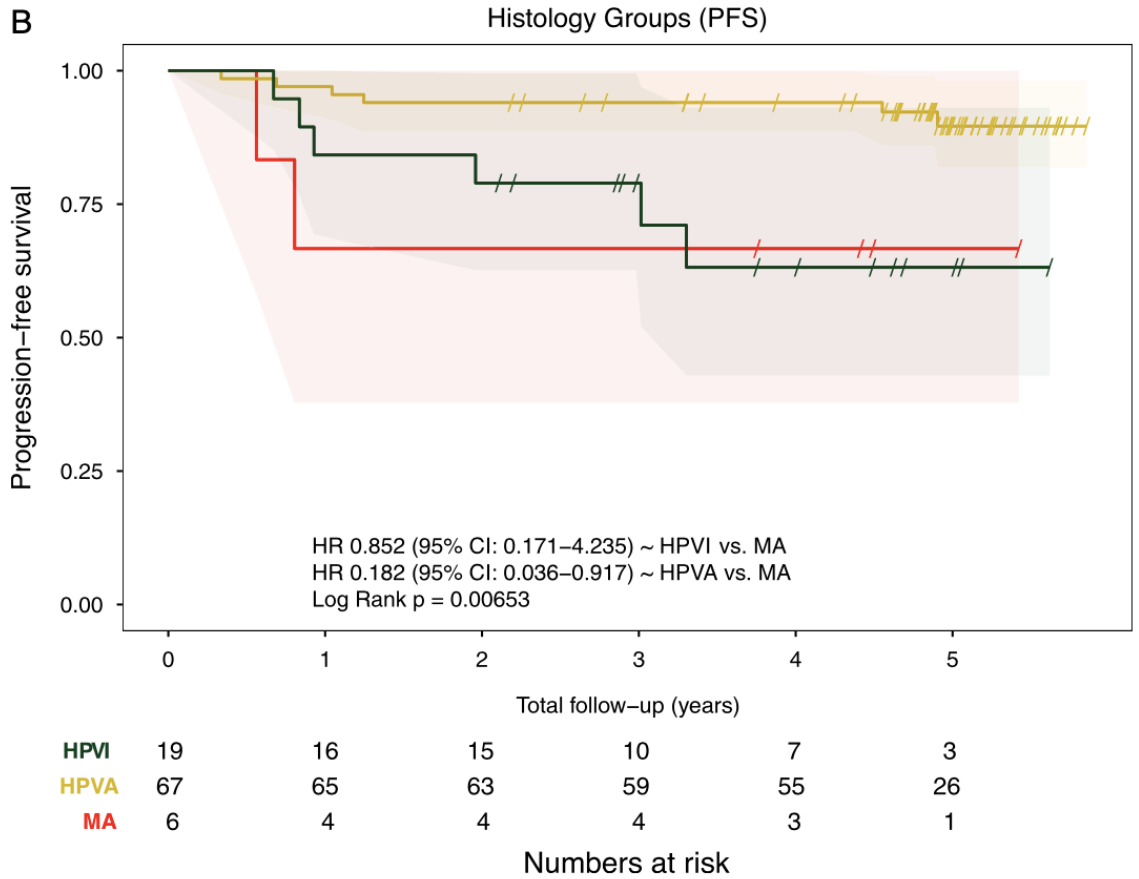


	GAS	UEA
Precursor lesions	LEGH	Adenocarcinoma in situ
Location	Upper endocervical canal	Transformation zone
HPV associated	No	Yes
p16 IHC	Negative or focal	Diffusely positive
Presentation	Often at high stage	Uncommonly high stage

Karamurzin et al, Am J Surg Pathol 2015; 39: 1449-1457

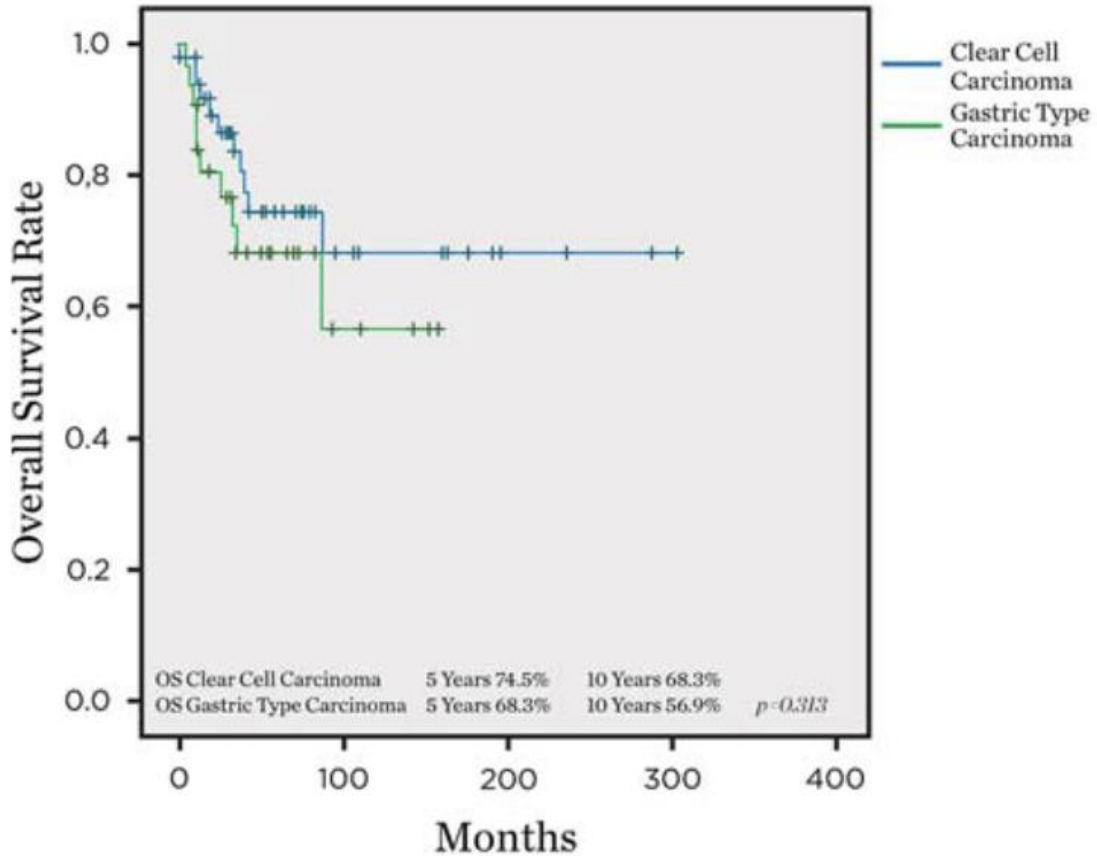
Cervical Mesonephric Adenocarcinoma

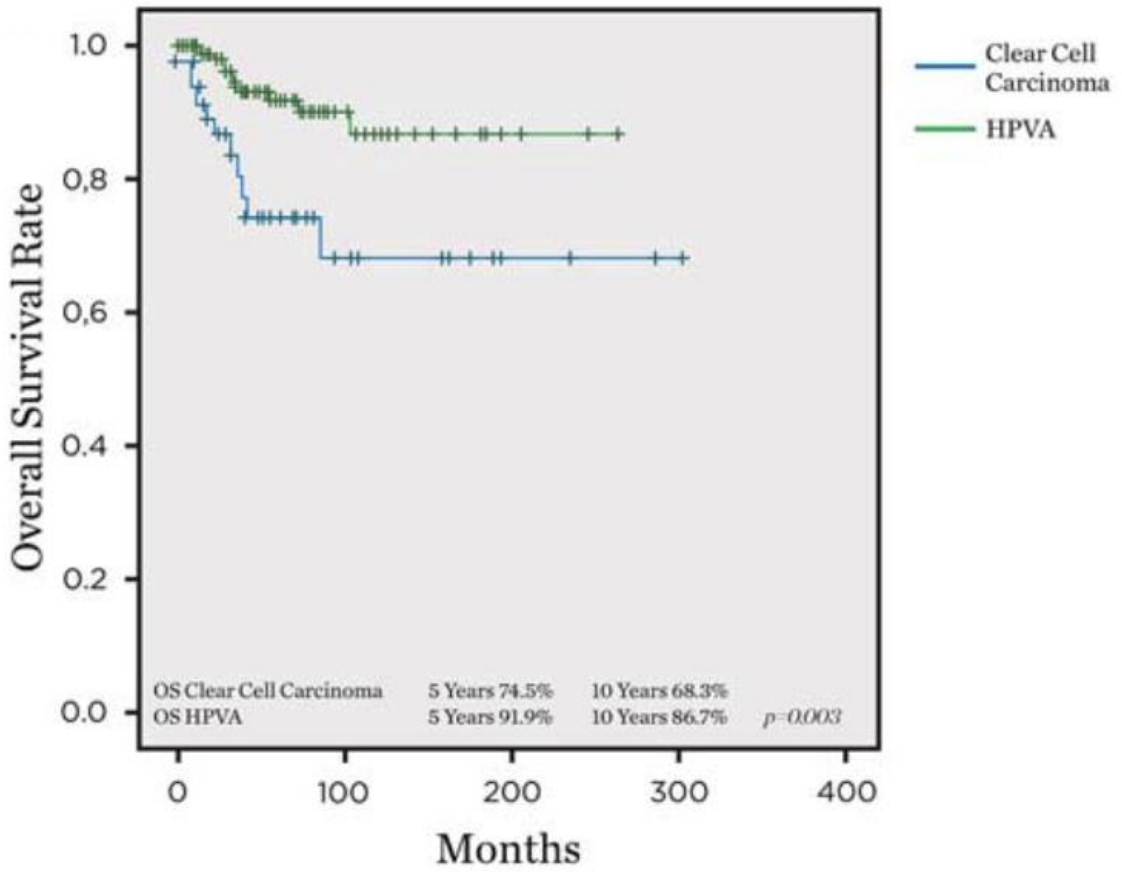




Pors et al, Am J Surg Pathol 2021; 45: 498-506

Clear Cell Carcinoma of Cervix





Stolnicu et al, Am J Surg Pathol 2022; 46: 765-773

WHO Classification 2020, Vulval Squamous Lesions

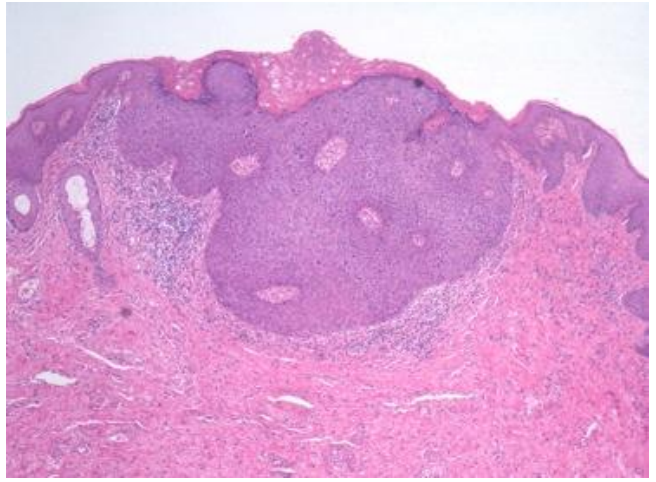
- Low-grade SIL (usually condyloma)
- High-grade SIL (VIN 2/3)
- Differentiated vulvar intraepithelial neoplasia (dVIN)
  - Differentiated exophytic vulvar intraepithelial lesion (DEVIL)
  - Vulvar acanthosis with altered differentiation (VAAD)
- Squamous cell carcinoma, HPV-associated
- Squamous cell carcinoma, HPV-independent
- Squamous cell carcinoma, NOS

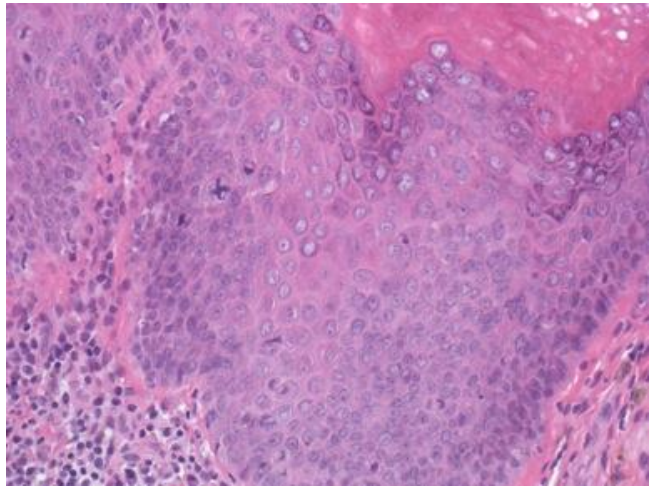
Green = associated with low-risk HPV
Red = associated with high-risk HPV
Blue = not associated with high-risk HPV
Black = unknown

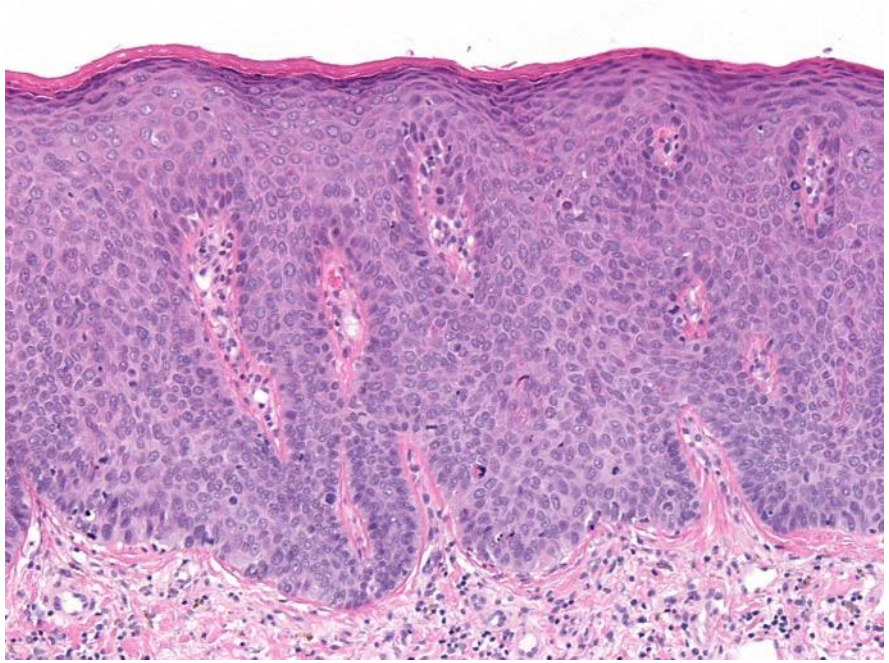
Two Pathways to Vulval Neoplasia

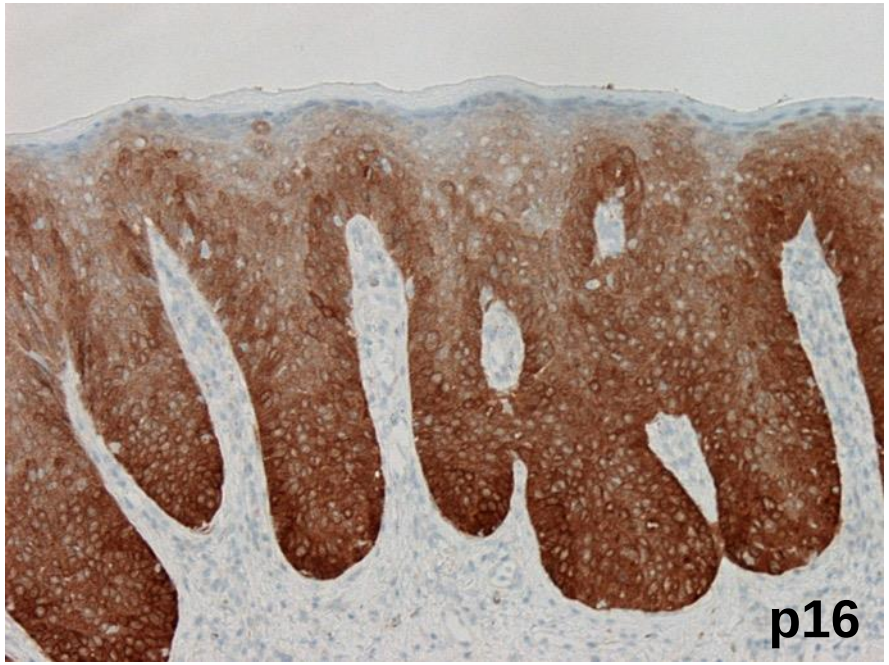
HPV-related

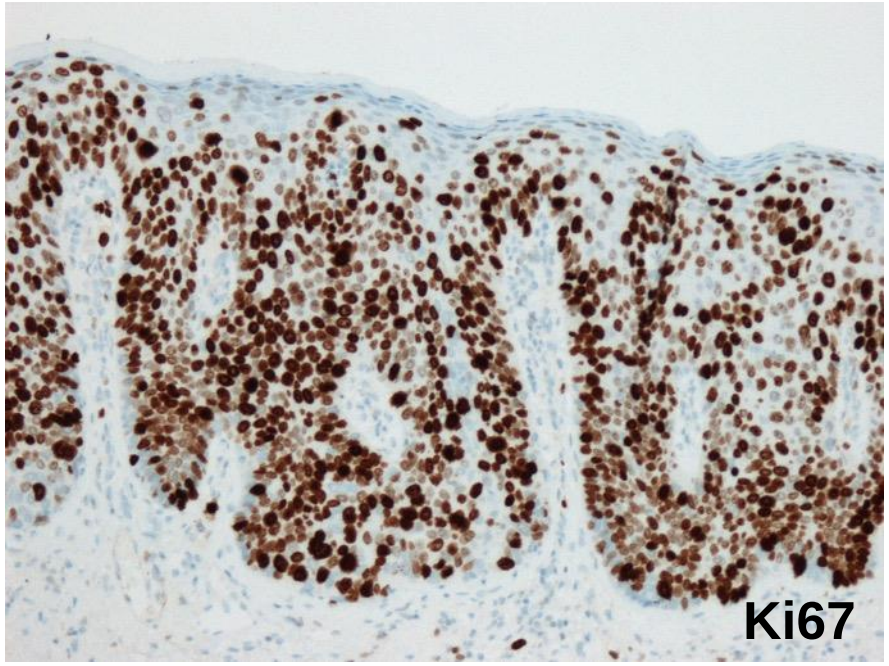
- Young women
- Warty/basaloid (undifferentiated) vulvar intraepithelial neoplasia (VIN)
- Warty/basaloid carcinoma
- Associated with other intraepithelial lesions
- Same HPV types as CIN
- Predominance of HPV 16
- Mechanisms probably similar
- p16 is a surrogate marker







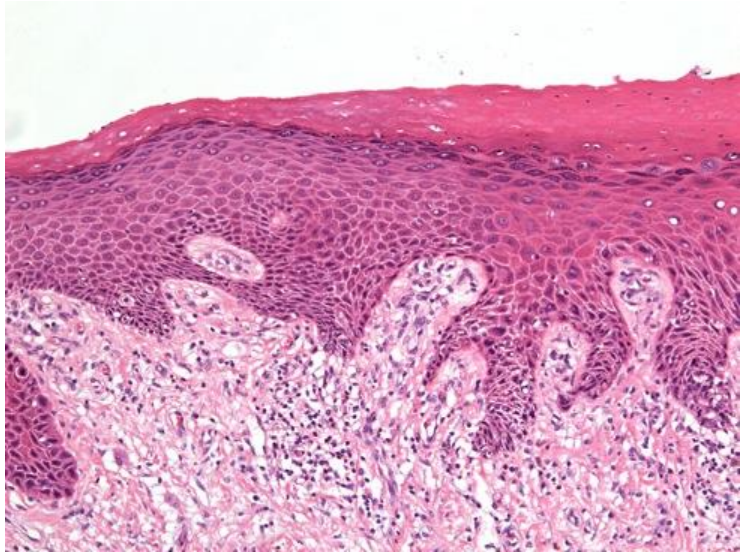




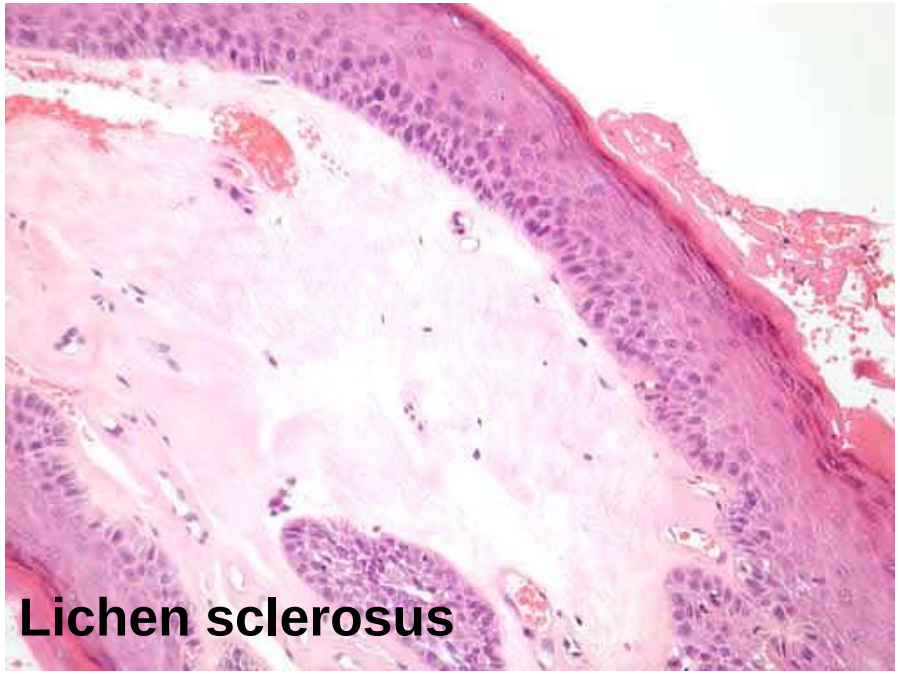
HSIL
(Usual-type VIN)

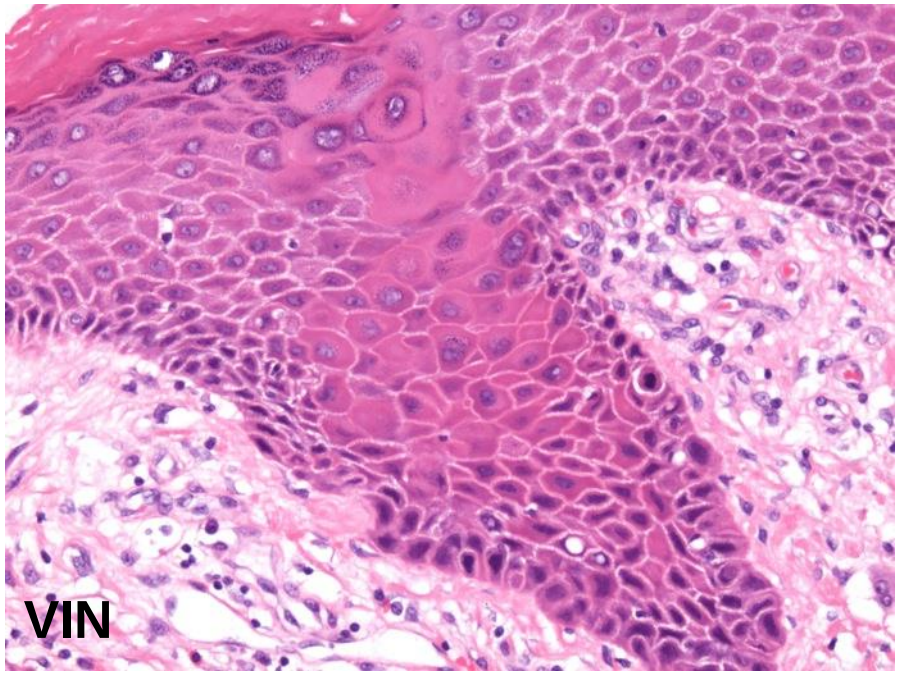
Two Pathways to Vulval Neoplasia

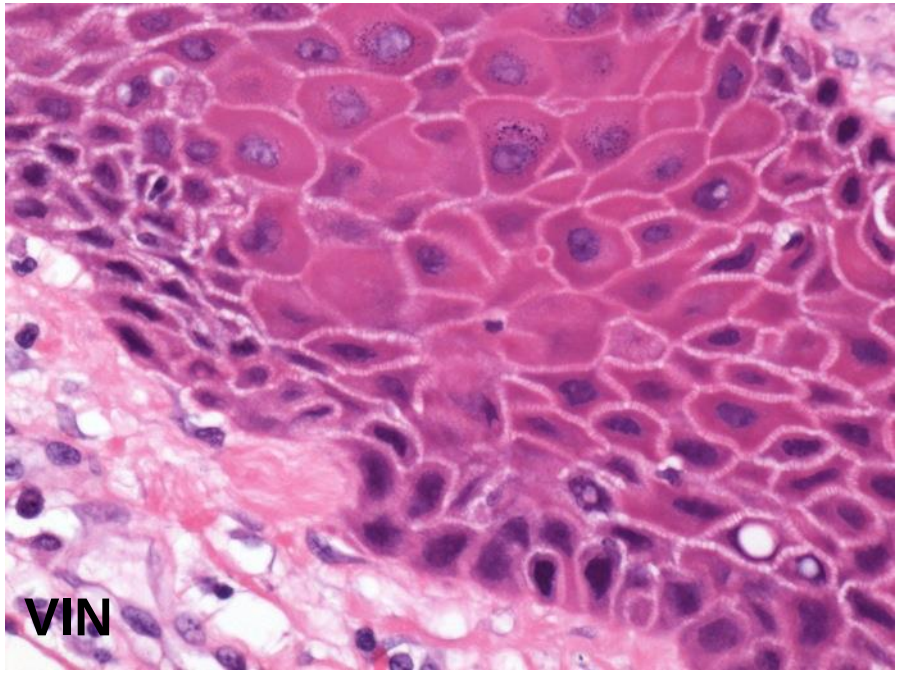
HPV-independent

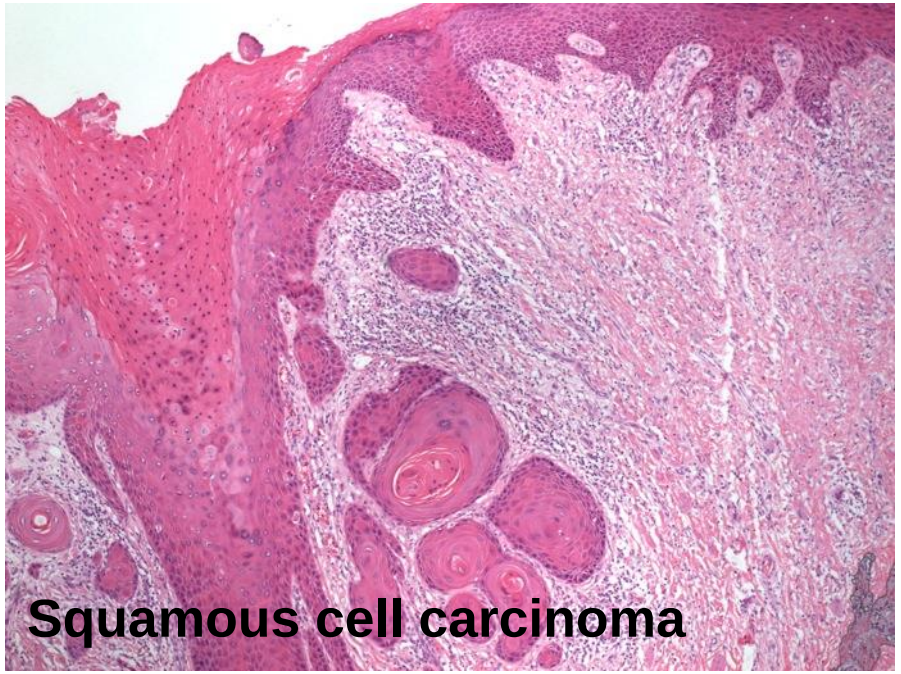


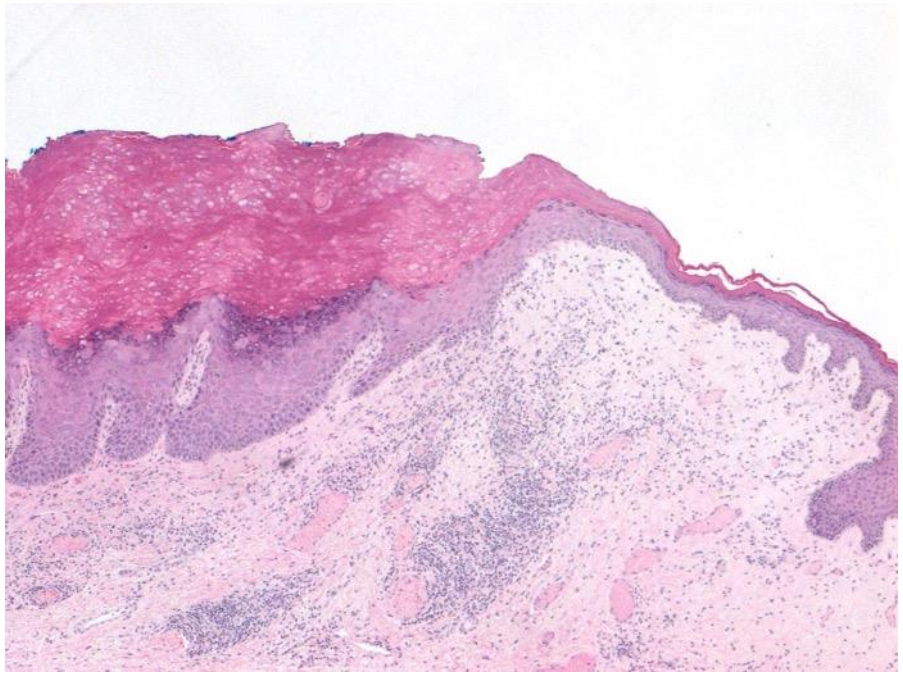
- Older women
- Associated with lichen sclerosus
- HPV-independent precursor lesions
  - Differentiated (simplex type) VIN
  - Vulval acanthosis with altered differentiation (VAAD)
  - Differentiated exophytic vulval intraepithelial lesion (DEVIL)
- Often well differentiated squamous cell carcinoma but clinically aggressive
- p16 typically negative

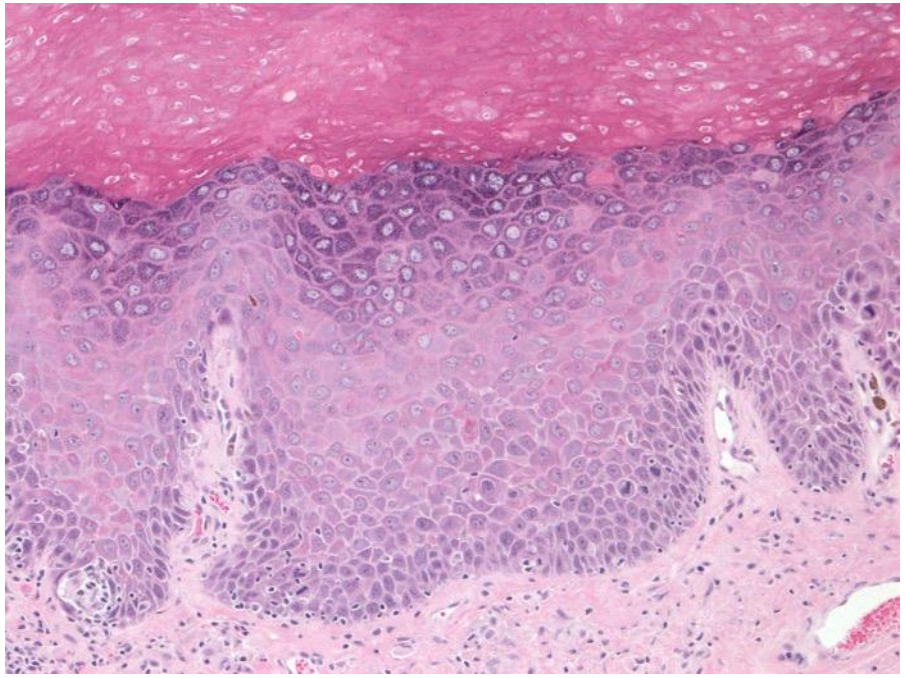

Lichen sclerosus

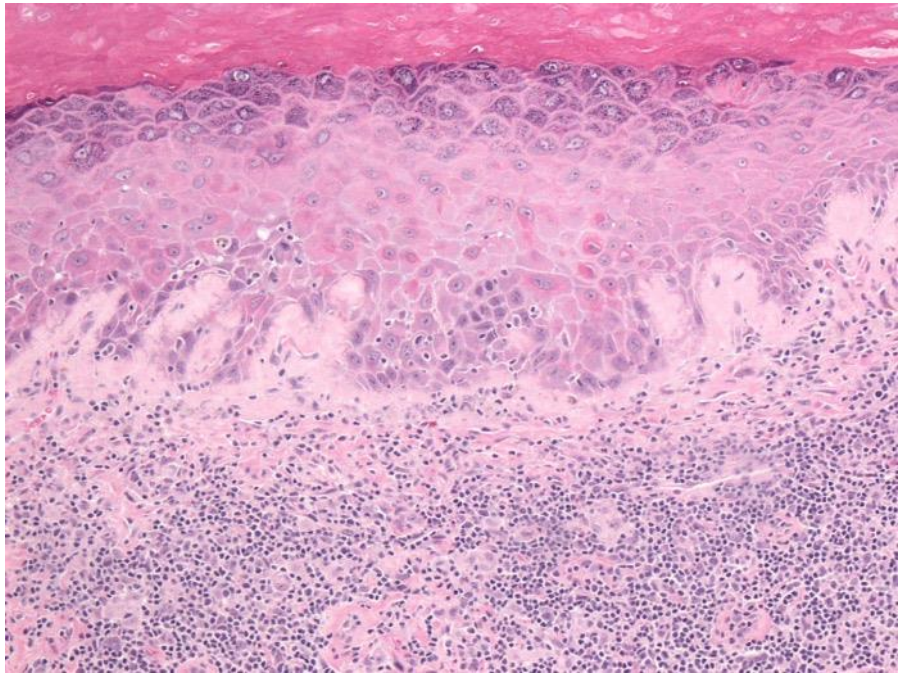

VIN

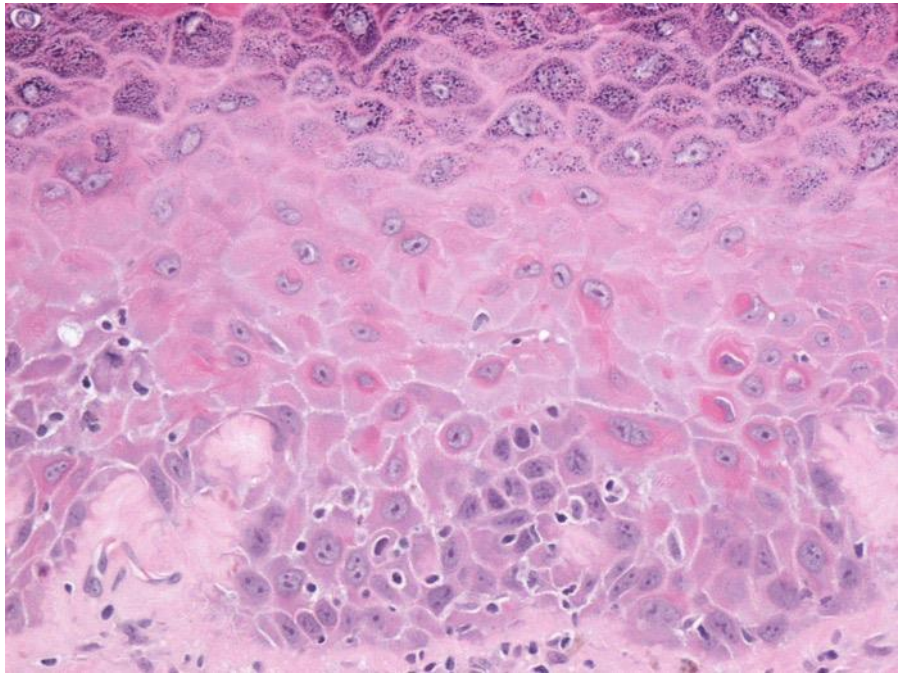

VIN

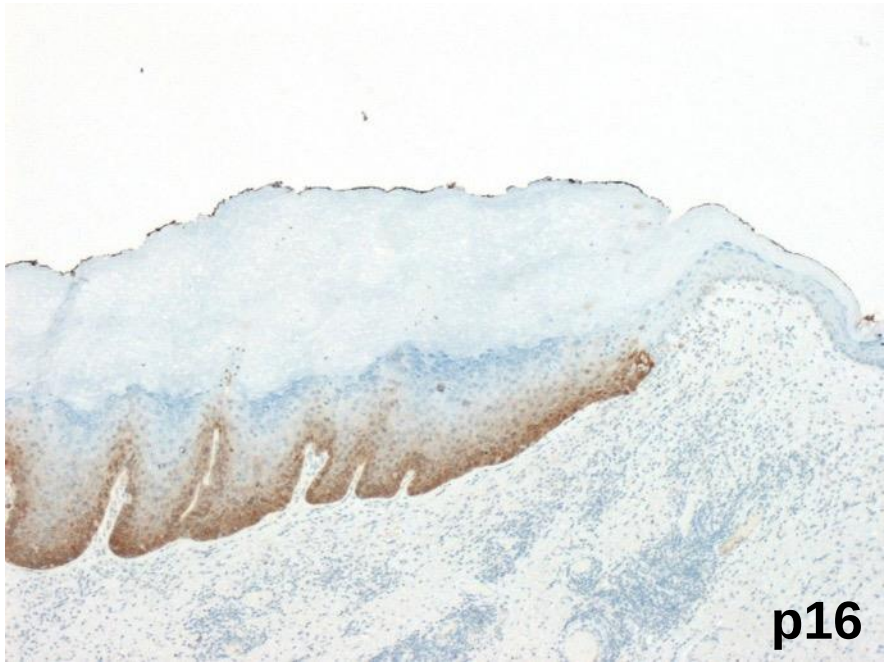

Squamous cell carcinoma

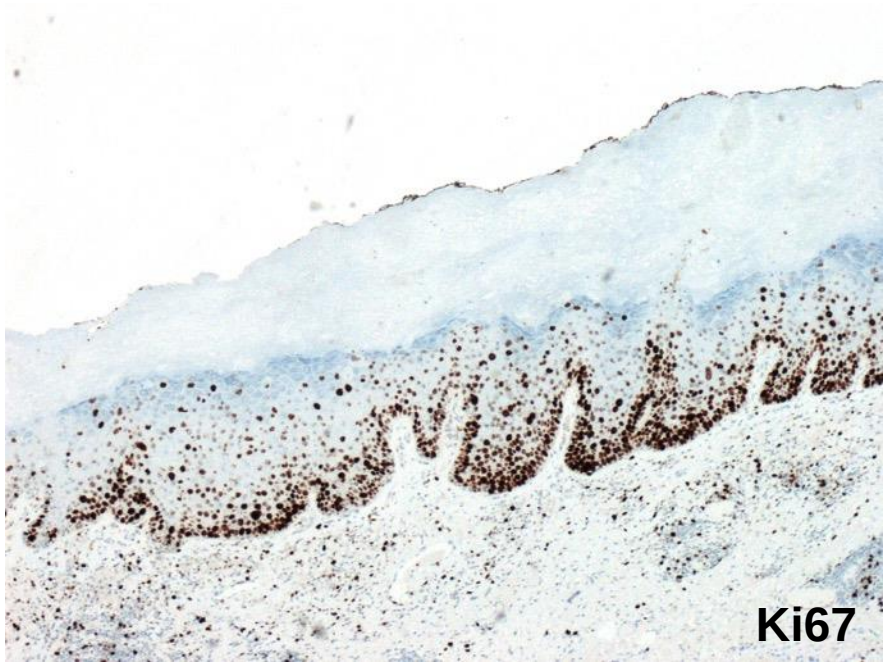


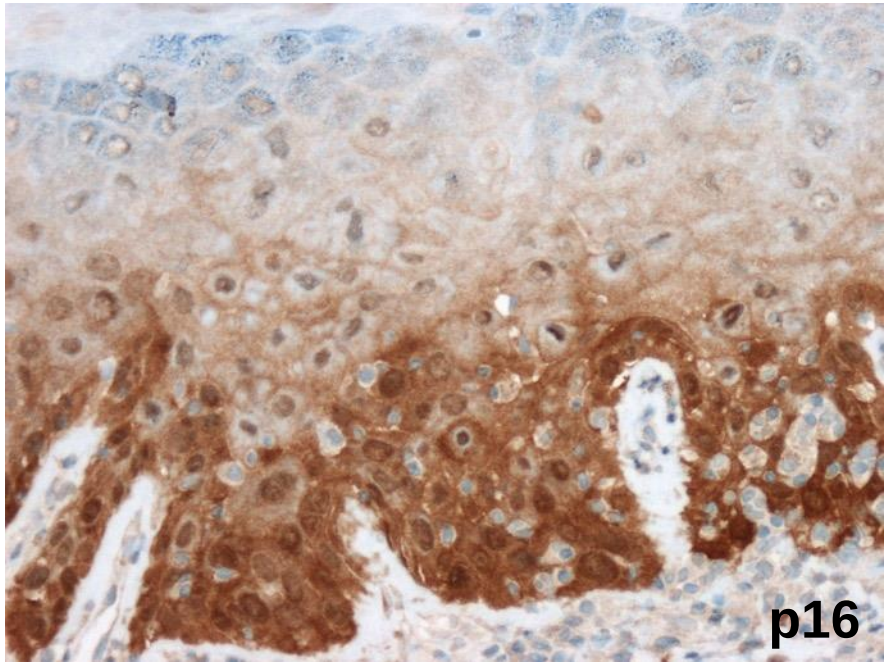


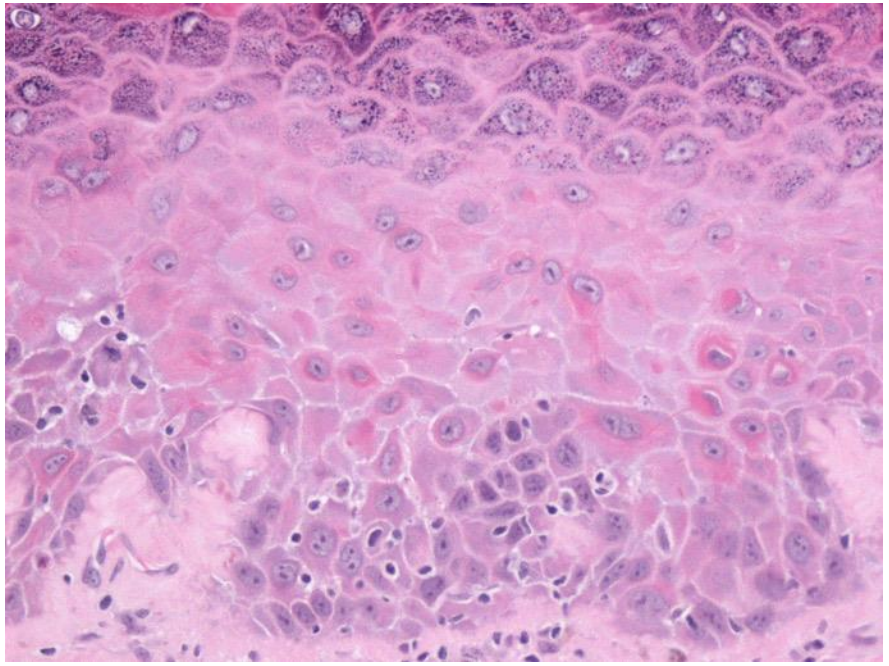


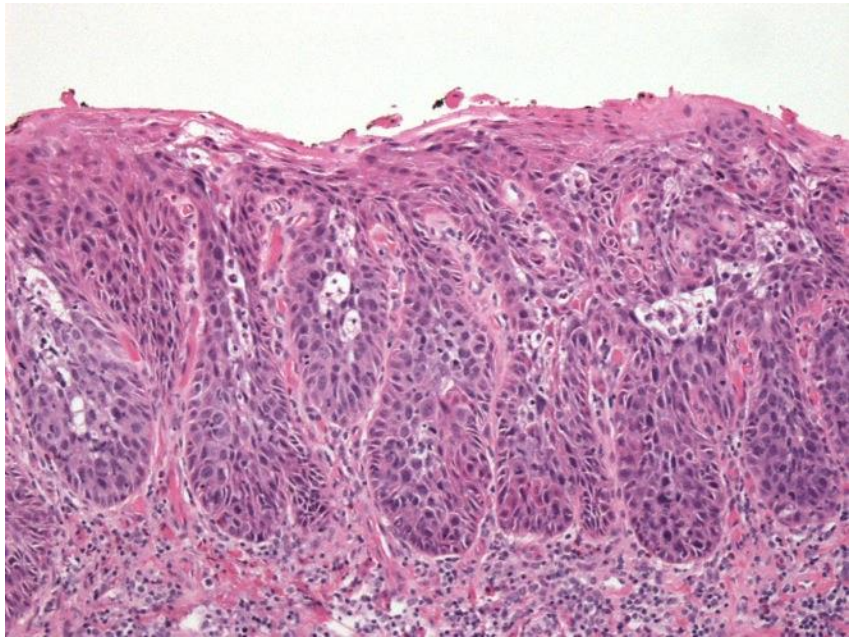


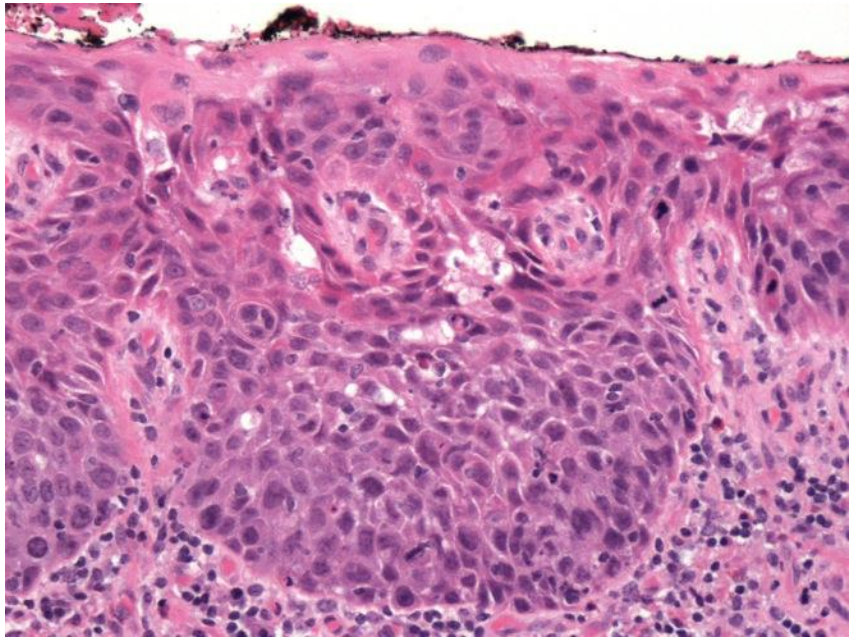

p16

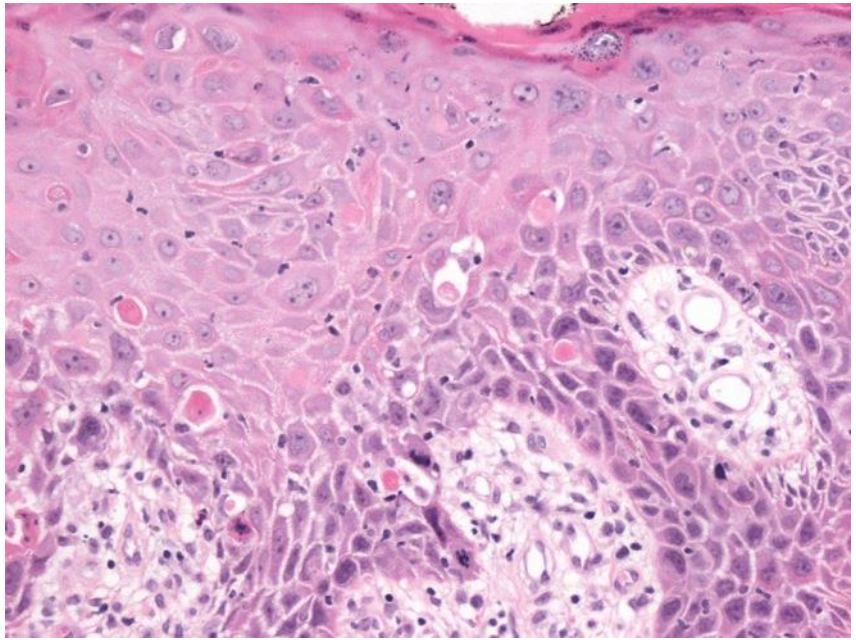

Ki67

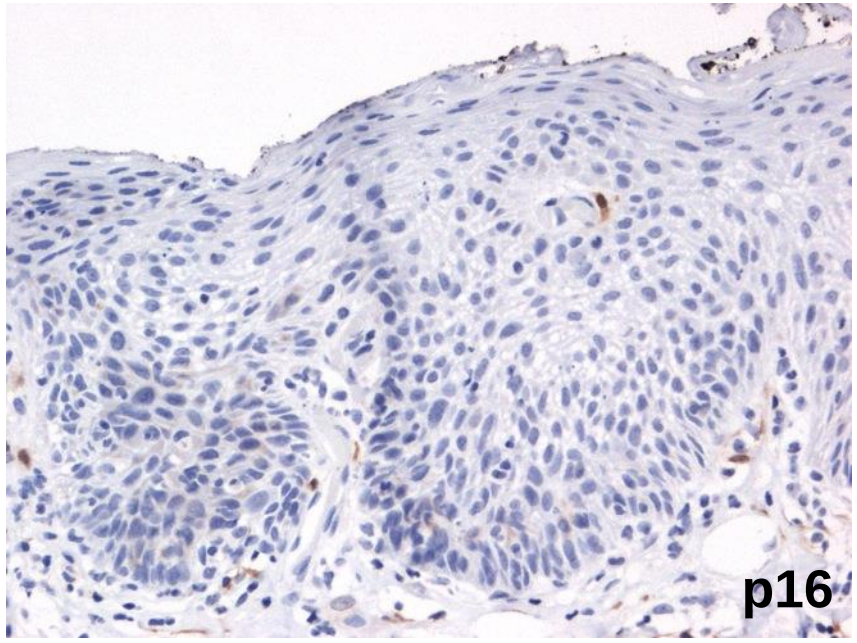

p16










p16

HPV and Morphology

- 69.5% basaloid SCC HPV positive (n=326)
- 11.5% keratinising SCC HPV positive (n=1234)

- 90.3% usual type VIN HPV positive (n=535)
- 48.9% differentiated type VIN HPV positive (n=48)

de Sanjose et al *Eur J Cancer* 2013; **49:** 3450-61

WHO Classification 2020, Vulval Squamous Lesions

- Low-grade SIL (usually condyloma)
- High-grade SIL (VIN 2/3)
- Differentiated vulvar intraepithelial neoplasia (dVIN)
  - Differentiated exophytic vulvar intraepithelial lesion (DE-VIL)
  - Vulvar acanthosis with altered differentiation (VAAD)
- Squamous cell carcinoma, HPV-associated
- Squamous cell carcinoma, HPV-independent
- Squamous cell carcinoma, NOS

Green = associated with low-risk HPV
Red = associated with high-risk HPV
Blue = not associated with high-risk HPV
Black = unknown

dVIN and HPV-independent SCC

Modern Pathology (2020) 33:1595–1605
https://doi.org/10.1038/s41379-020-0524-1

USCAP

ARTICLE

Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with *TP53* mutation status

Basile Tessier-Cloutier[1,2] · Kim E. Kortekaas[3] · Emily Thompson[2] · Jennifer Pors[1,2] · Julia Chen[2] · Julie Ho[2] · Leah M. Prentice[4] · Melissa K. McConechy[4] · Christine Chow[5] · Lily Proctor[6] · Jessica N. McAlpine[6] · David G. Huntsman[2,5,7] · C. Blake Gilks[1,2,5] · Tjalling Bosse[8] · Lynn N. Hoang[1,2,5]

Histopathology 2020, 77, 92–99. DOI: 10.1111/his.14109

Histopathology

Performance of the pattern-based interpretation of p53 immunohistochemistry as a surrogate for *TP53* mutations in vulvar squamous cell carcinoma

Kim E Kortekaas,[1] Nienke Solleveld-Westerink,[2] Basile Tessier-Cloutier,[3] Tessa A Rutten,[2] Mariëtte I E Poelgeest,[1] C Blake Gilks,[3] Lien N Hoang[3,*] & Tjalling Bosse[2,*]
[1]*Department of Obstetrics & Gynecology,* [2]*Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands, and* [3]*Department of Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, BC, Canada*

Modern Pathology (2021) 34:508–518
https://doi.org/10.1038/s41379-020-00651-3

USCAP

ARTICLE

Molecular characterization of invasive and in situ squamous neoplasia of the vulva and implications for morphologic diagnosis and outcome

Basile Tessier-Cloutier[1,2] · Jennifer Pors[1] · Emily Thompson[2] · Julie Ho[1] · Leah Prentice[3] · Melissa McConechy[3] · Rosalia Aguirre-Hernandez[3] · Ruth Miller[3] · Samuel Leung[4] · Lily Proctor[5] · Jessica N. McAlpine[5] · David G. Huntsman[2,4] · C. Blake Gilks[1,4] · Lynn N. Hoang[1,4]

Histopathology 2021, 79, 975–988. DOI: 10.1111/his.14451

Histopathology

DEVIL, VAAD and vLSC constitute a spectrum of HPV-independent, p53-independent intra-epithelial neoplasia of the vulva

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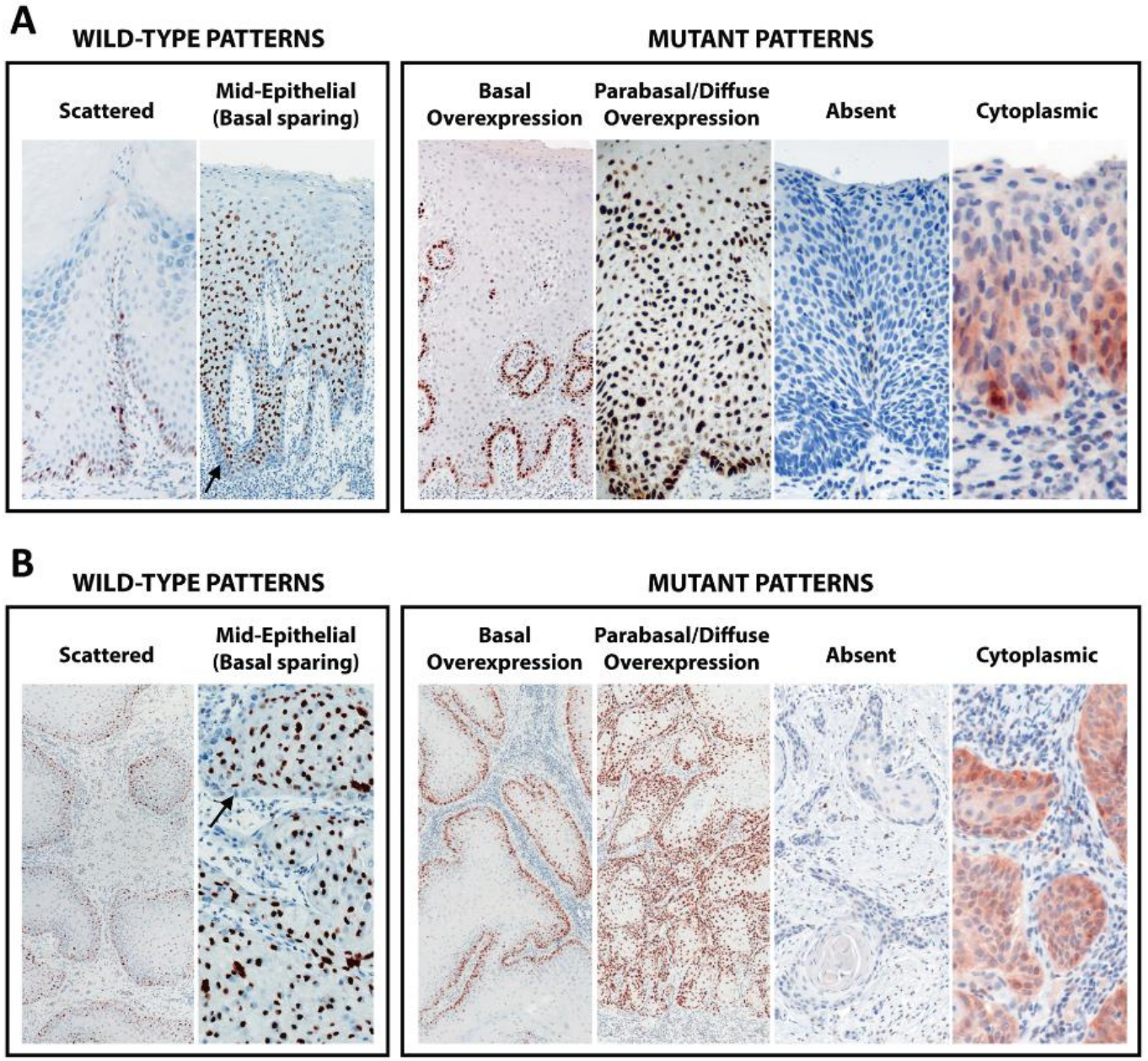
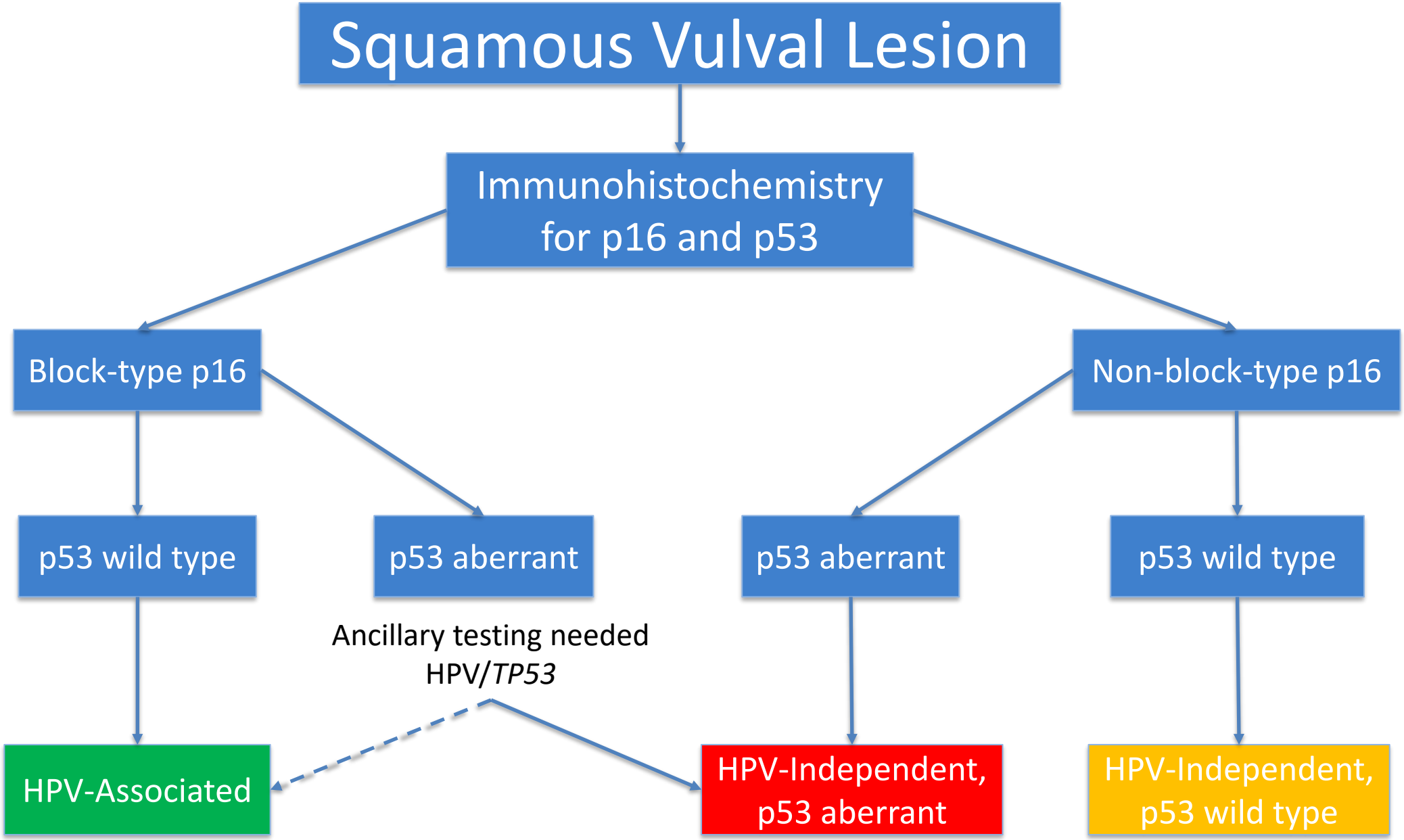


p53 Expression Patterns

From Tessier-Cloutier et al, Mod Pathol 2020; 33: 1595-1605


Squamous Vulval Lesion
Immunohistochemistry for p16 and p53
Block-type p16
Non-block-type p16
p53 wild type
p53 aberrant
p53 aberrant
p53 wild type
Ancillary testing needed
HPV/*TP53*
HPV-Associated
HPV-Independent, p53 aberrant
HPV-Independent, p53 wild type

Remaining Issues

- Potential therapeutic relevance
  - Imiquimod
  - Other agents
  - Surgical management
- Refinement of clinicopathological studies and clinical trials using HPV-based classification
- Further molecular investigation of *TP53*-mutant and wild-type HPV-independent squamous lesions, including VAAD and DE-VIL

Summary

- HPV infection dominates lower genital tract pathology
- Squamous lesions are associated with both low- and high-risk HPV infection, glandular lesions with high-risk HPV infection
- p16, Ki67 and p53 immunohistochemistry are useful for the diagnosis and stratification of HPV-associated and HPV-independent lesions of the lower genital tract
- HPV-independent lesions are increasingly recognised and are included in the WHO 2020 classification
- HPV testing and vaccination are changing disease distribution

e.g. Falcaro et al, Lancet 2021; 398: P2084-P2092