Spectrum of Vulvar Squamous Neoplasia

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Objectives

- Understand the etiologic subgroups of vulvar squamous cell carcinoma
- Recognize precursor lesions and distinguish them from benign mimics
- Identify and be able to interpret relevant ancillary tools

Vulvar Squamous Cell Carcinoma

WHO Classification of Female Genital Tumours 5th Edition

HPV-Associated	HPV-Independent

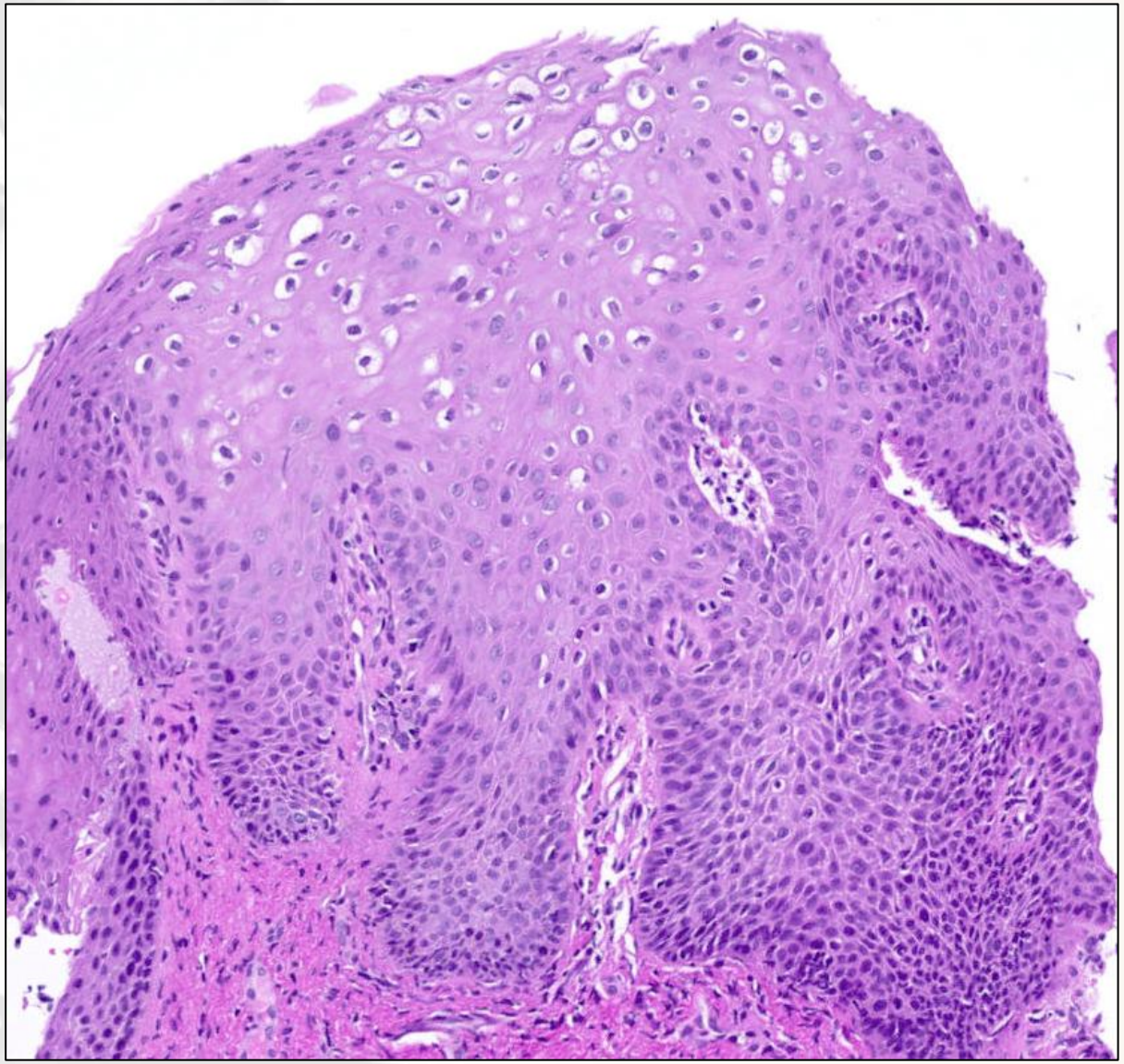
HPV-Associated	HPV-Independent *TP53* Mutant	HPV-Independent *TP53* Wild-Type

Kortekaas KE, et al. Vulvar cancer subclassification by HPV and p53 status results in three clinically distinct subtypes. Gynecol Oncol. 2020 Dec;159(3):649-656.

Standard Precursor Lesions

Low-Grade Squamous Intraepithelial Lesion (LSIL/VIN1)

- Most are condylomatous
  - “Condyloma acuminatum”
  - Exophytic verrucous/warty architecture
  - Hyperkeratosis and hypergranulosis
  - Broad, rounded papillae
  - Koilocytes +/-
- Usually low risk HPV subtypes (6, 11 most common)
- Flat LSIL rare at this site
  - In contrast to cervical LSIL

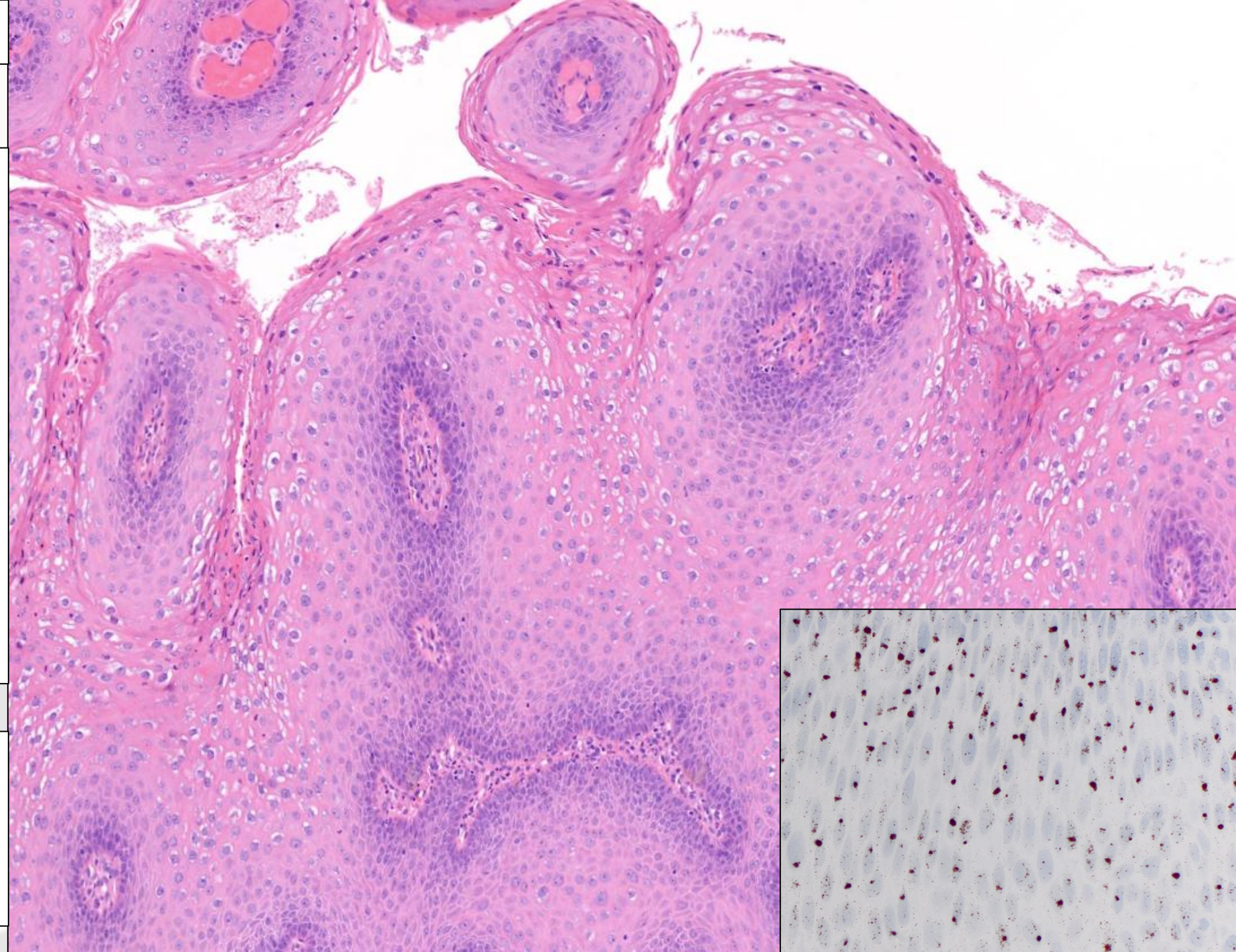


Condylomatous LSIL

- Koilocytes most prominent between papillae
- Degree of koilocytic change variable
  - May be absent in some despite the presence of low-risk HPV
- Mitotic activity confined to lower 1/3
- Lacks high-grade atypia
- Immature (high N:C) ratio cells confined to lower 1/3

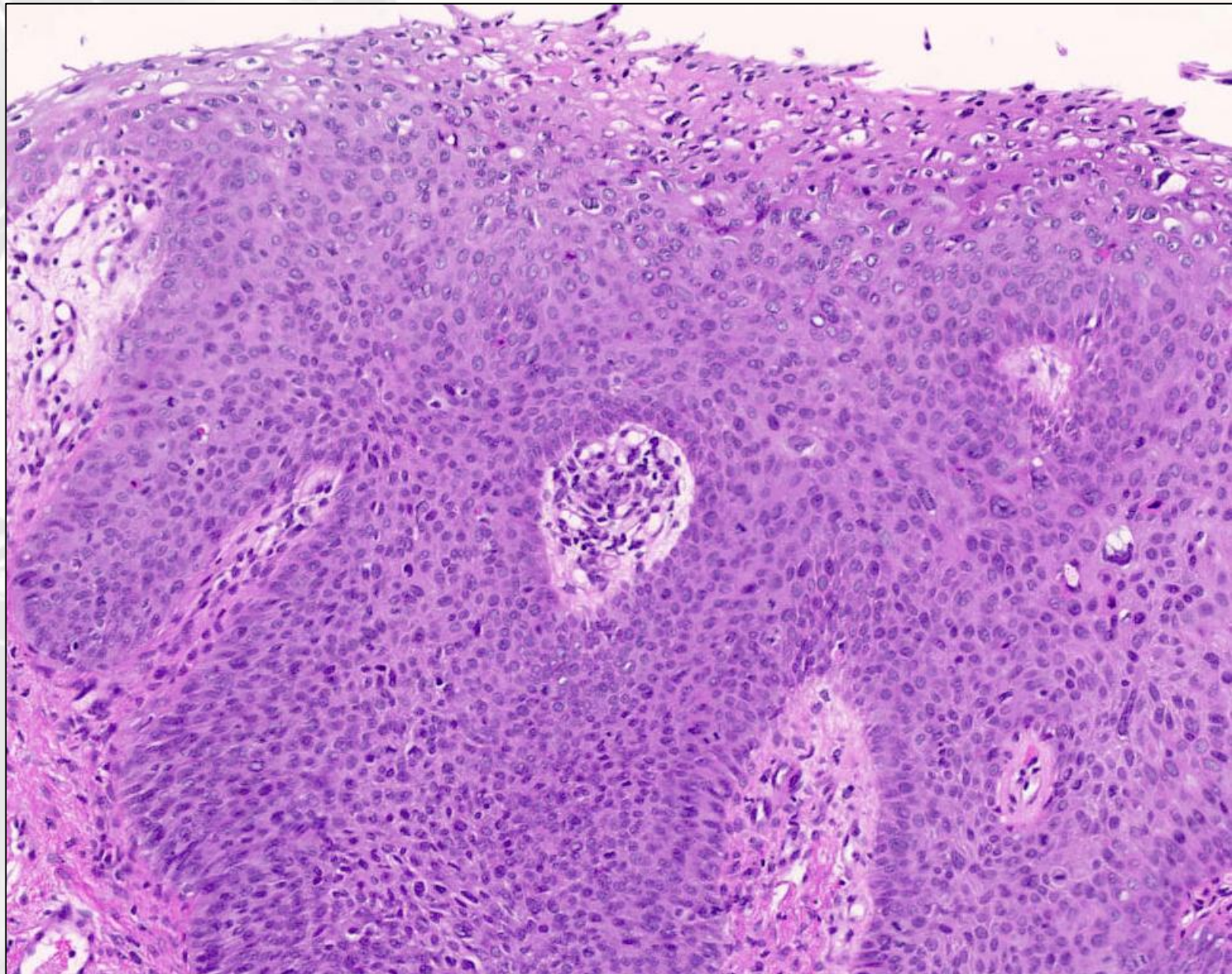
Ancillary studies

- P16 patchy/negative
- In-situ hybridization HPV 6/11



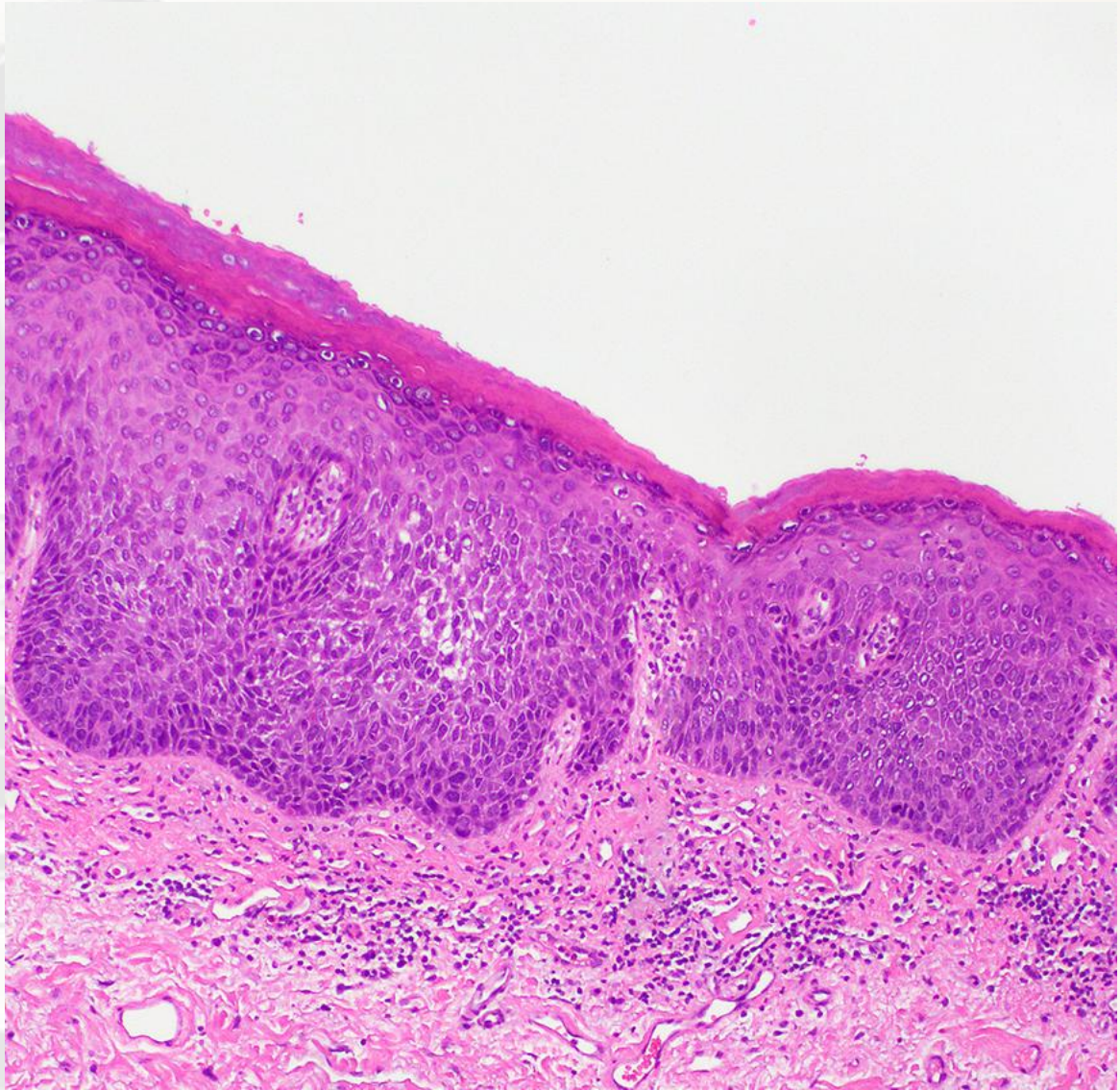
Risk of Progression

- Most vulvar LSIL does not progress to HSIL/SCC
- Rare progression to HSIL (pictured)
  - Typically associated with high-risk HPV subtypes
  - May be co-infected with both low and high-risk HPV
  - Diffuse p16 expression
- Rare reports of invasive squamous cell carcinoma associated with low-risk HPV



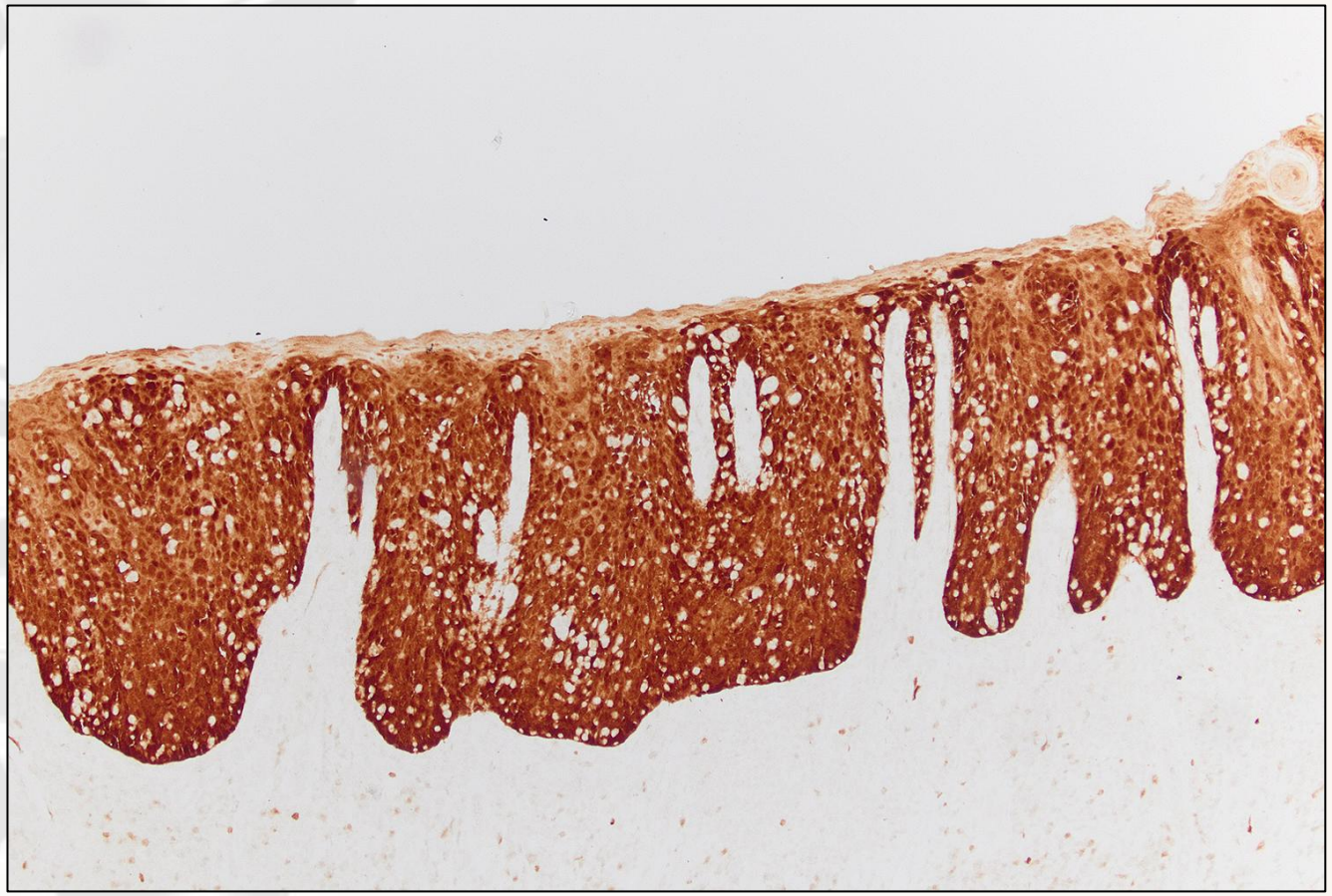
High-Grade Squamous Intraepithelial Lesion (VIN2/3)

- Much more frequent than LSIL in vulva
- HPV type 16 in >70%
- May be flat or exophytic (condylomatous)
- Risk of progression to invasive squamous cell carcinoma
- Immature, high N:C ratio cells extending to middle (VIN2) or upper (VIN3) 1/3 of epithelial thickness
- Mitoses in middle or upper 1/3



p16 in HSIL

- “Block” positivity
  - Diffuse nuclear and cytoplasmic staining
- Stratum granulosum and stratum corneum may be spared
- Surrogate for HPV infection only in sites where HPV-associated carcinomas are common
  - Consider combining with p53



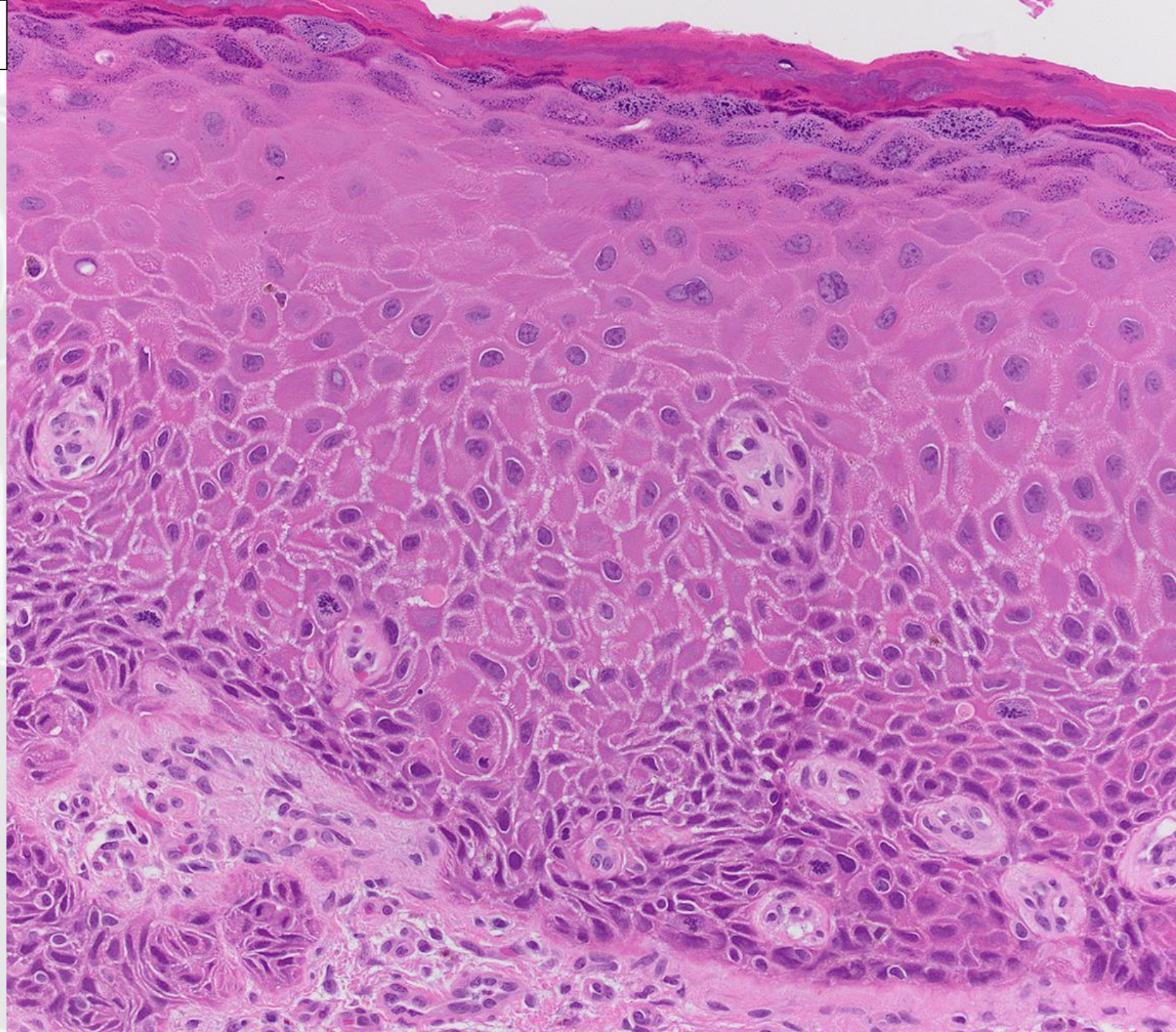
HPV-Independent, p53 Mutant Precursors

- Differentiated vulvar intraepithelial neoplasia (dVIN)
- Unrelated to HPV infection
- Peak incidence age 60-70
  - May occur earlier
- Associated with vulvar dermatoses
  - Lichen sclerosus
  - Lichen planus
- Aggressive clinical course
  - High rate of progression to invasive SCC (up to 90% in some studies)

Differentiated VIN

Essential Features

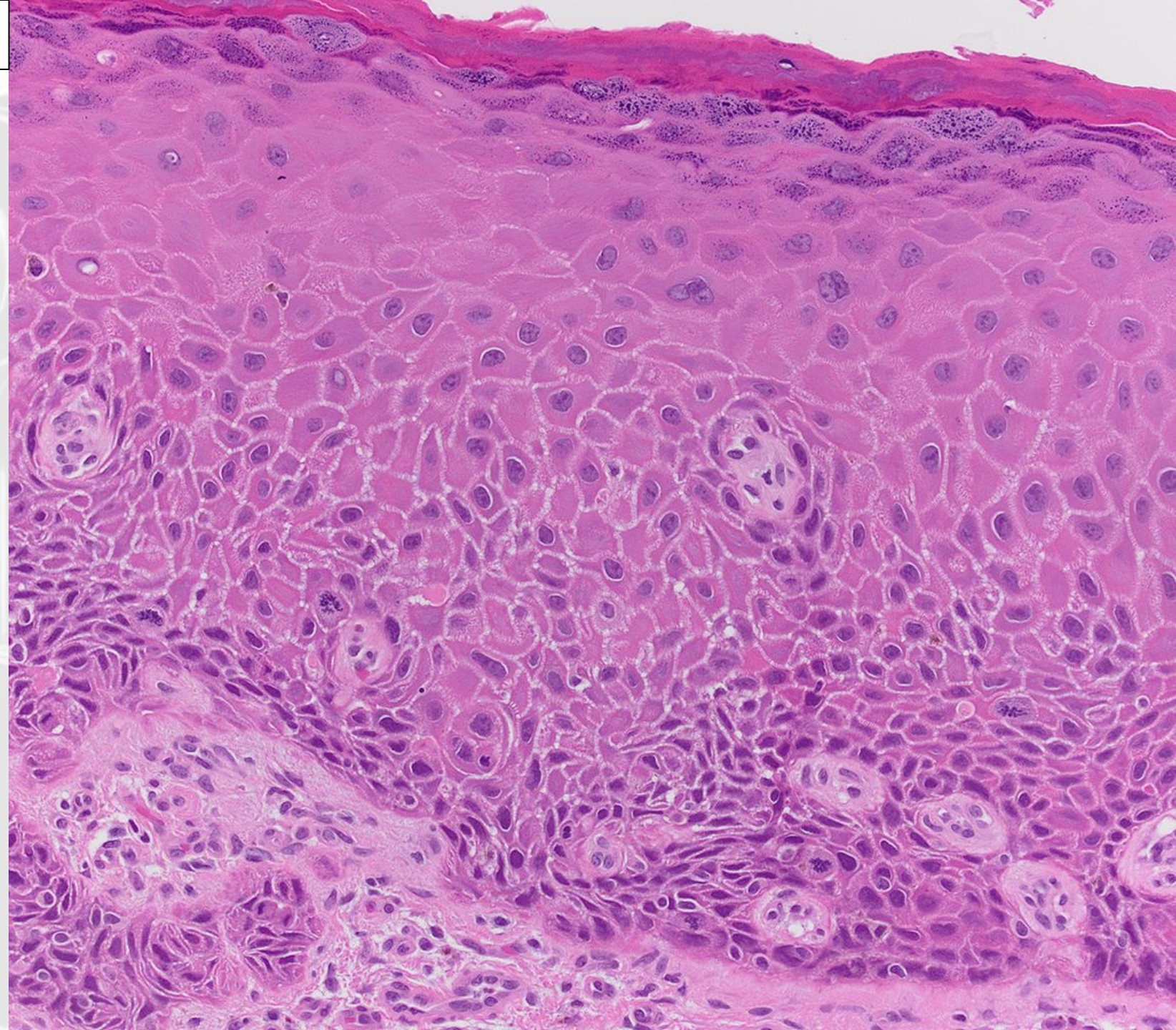
- Basal atypia
  - Cytologic atypia confined to lower 2-3 cell layers
  - Hyperchromasia, nuclear enlargement, prominent nucleoli
- Aberrant maturation
  - Premature keratinization (hypereosinophilic keratinocytes below the stratum granulosum)
  - Keratin pearls
- Mutant pattern of p53**



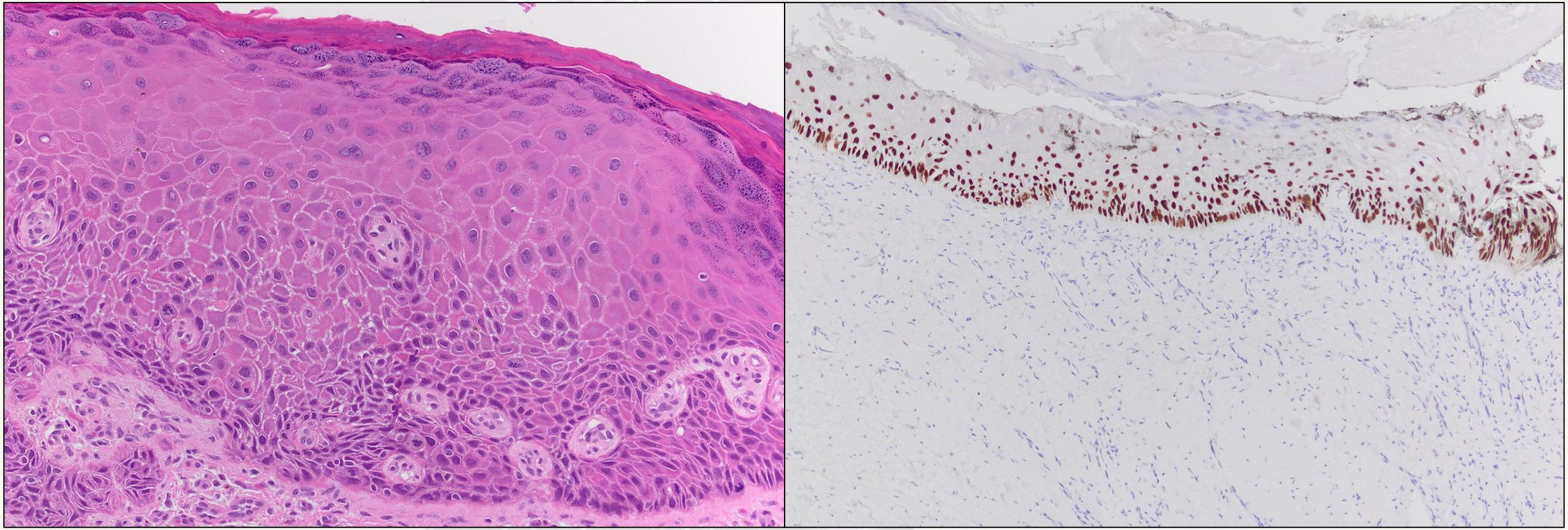
Differentiated VIN

"Soft" Features

- Acanthosis and parakeratosis
- Spongiosis with prominent intercellular bridges
- Elongation and anastomosis of rete ridges
- Inflammation is often not a feature
  - Complicated by superimposed dermatoses

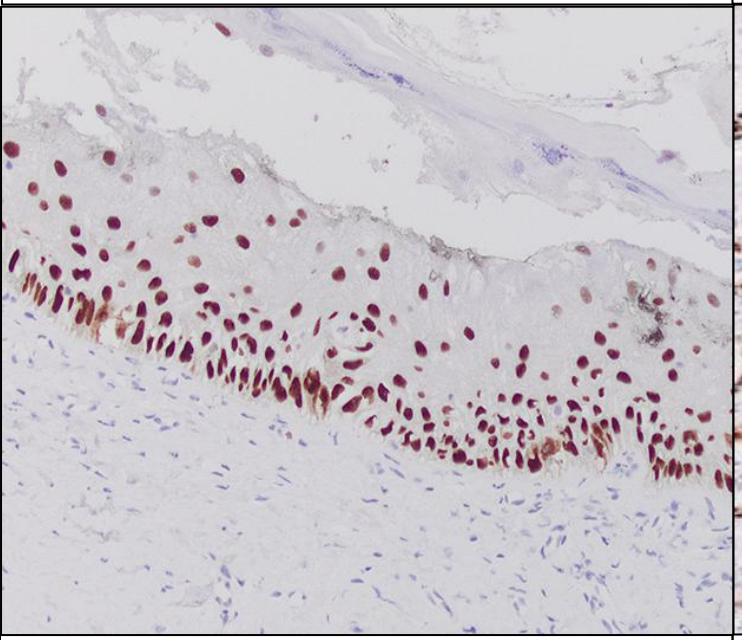
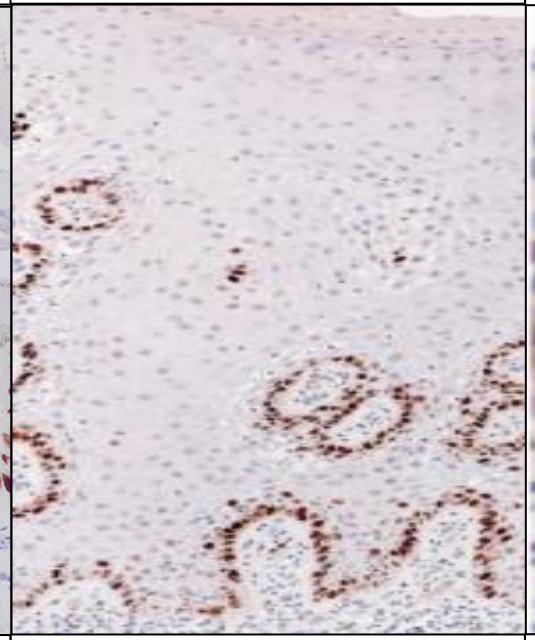
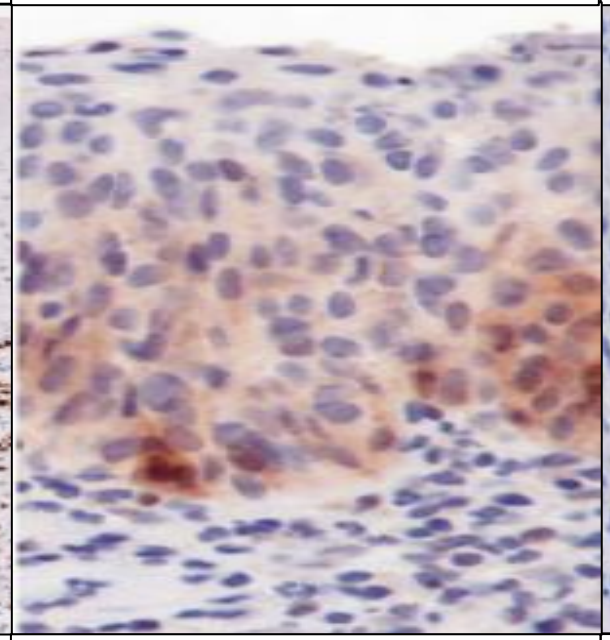
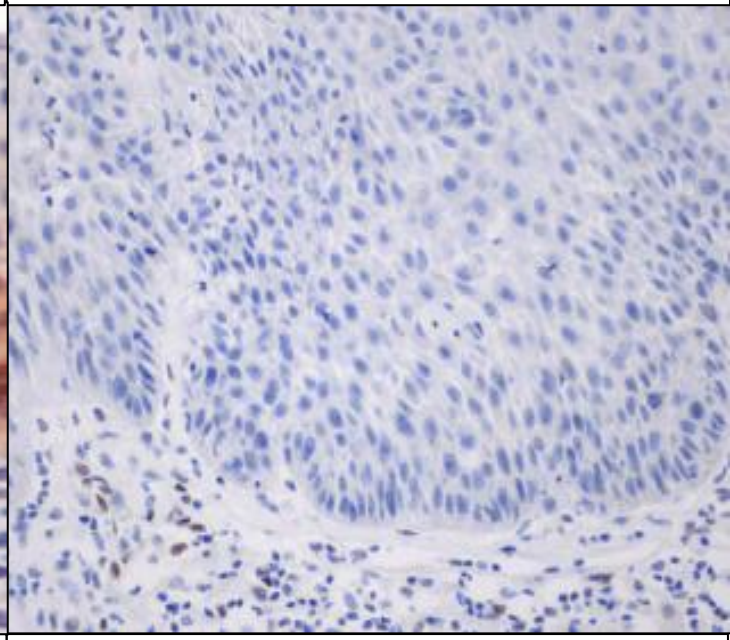


dVIN with Aberrant p53 pattern



- Multiple possible patterns of abnormal p53

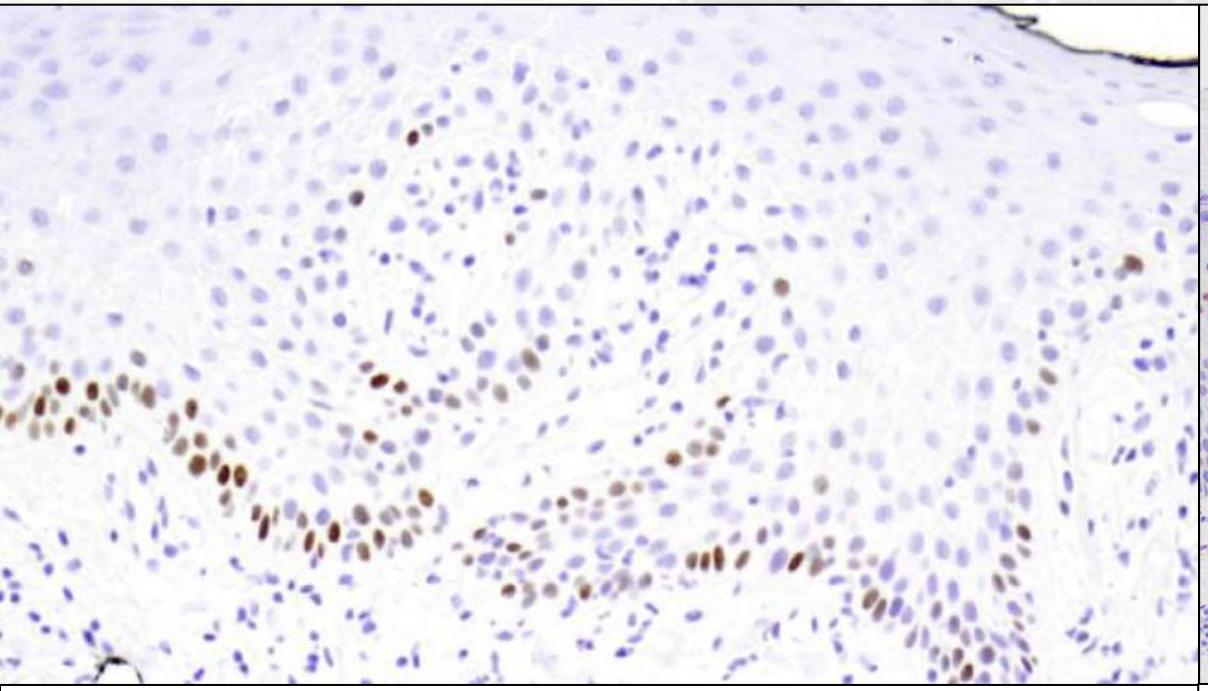
Abnormal p53 Staining Patterns

Basal + Parabasal Overexpression	Basal Overexpression	Cytoplasmic	Null
			
- Diffuse strong expression in basal and first several parabasal layers - ~75% of dVIN	- Diffuse strong linear basal layer expression - 5-10% of dVIN	- Cytoplasmic expression only - Rare (<5% of dVIN)	- Absence of nuclear staining with positive internal control - 10-15% of dVIN

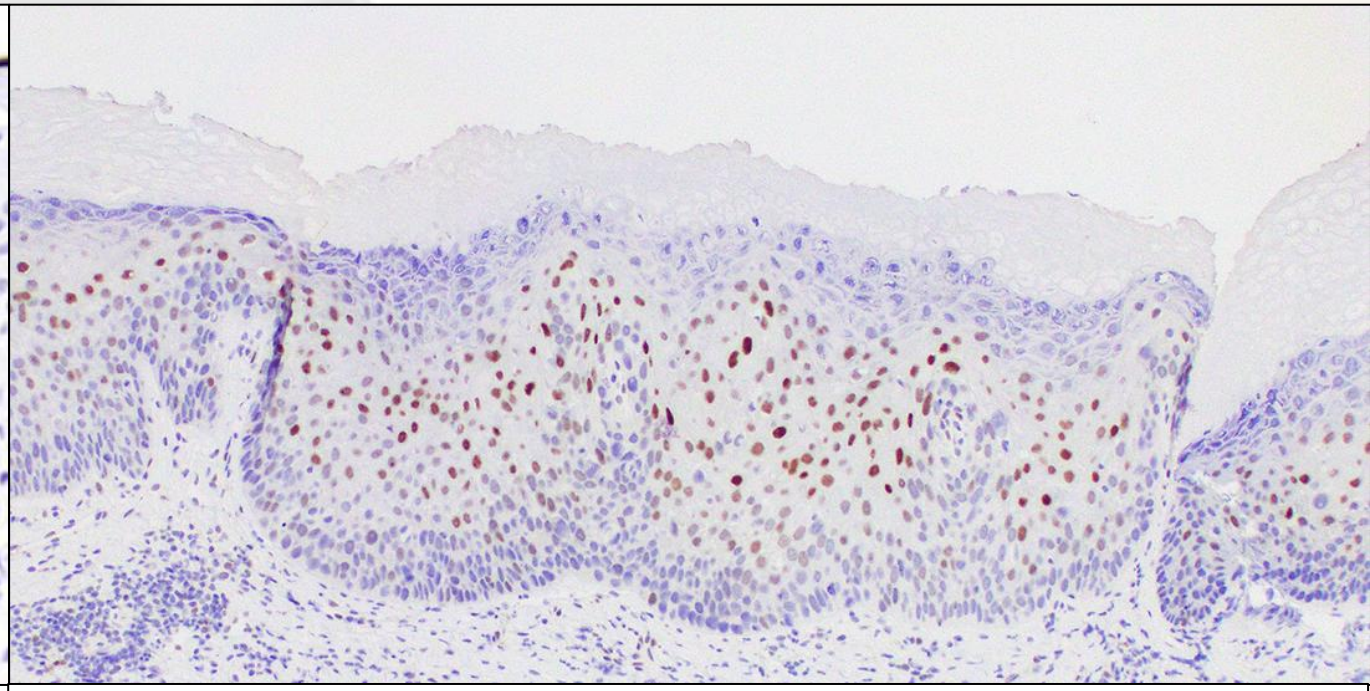
Liu YA, et al. Comparison of p53 immunohistochemical staining in differentiated vulvar intraepithelial neoplasia (dVIN) with that in inflammatory dermatoses and benign squamous lesions in the vulva. Histopathology. 2021 Feb;78(3):424-433.

Tessier-Cloutier B, et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. Mod Pathol. 2020 Aug;33(8):1595-1605.

Wild-type (normal) p53 patterns



- Patchy, incomplete staining of basal layer
- Positive cells have variable staining intensity



- Suprabasal accentuation
- Common in HPV-associated (“usual”) VIN

Jeffreys M, et al. Accentuated p53 staining in usual type vulvar dysplasia-A potential diagnostic pitfall. Pathol Res Pract. 2018 Jan;214(1):76-79.

HPV-Independent, p53 Wild-Type Precursors

- Varied nomenclature historically
  - Differentiated exophytic vulvar intraepithelial lesion (DEVIL)
  - Vulvar acanthosis with altered differentiation (VAAD)
  - Verruciform lichen simplex chronicus (vLSC)

- Current difference of opinion
  - Parra-Herran et al.
    - Verruciform acanthotic vulvar intraepithelial neoplasia (vaVIN)
    - Unifying terminology for all HPVI, p53 WT precursor lesions
    - Reflects neoplastic nature
  - International Society of the Study of Vulvovaginal Diseases (ISSVD)
    - Vulvar aberrant maturation (VAM)

HPV-Independent, p53 Wild-Type Precursors

- Clonal neoplastic processes
  - Mutations in *PIK3CA*, *TERT* promoter, *HRAS*, *ARID2*
- Progression to squamous cell carcinoma in 27-46%
- Treatment
  - Conservative excision (margins may be minimal)
  - Close clinical follow-up in select cases

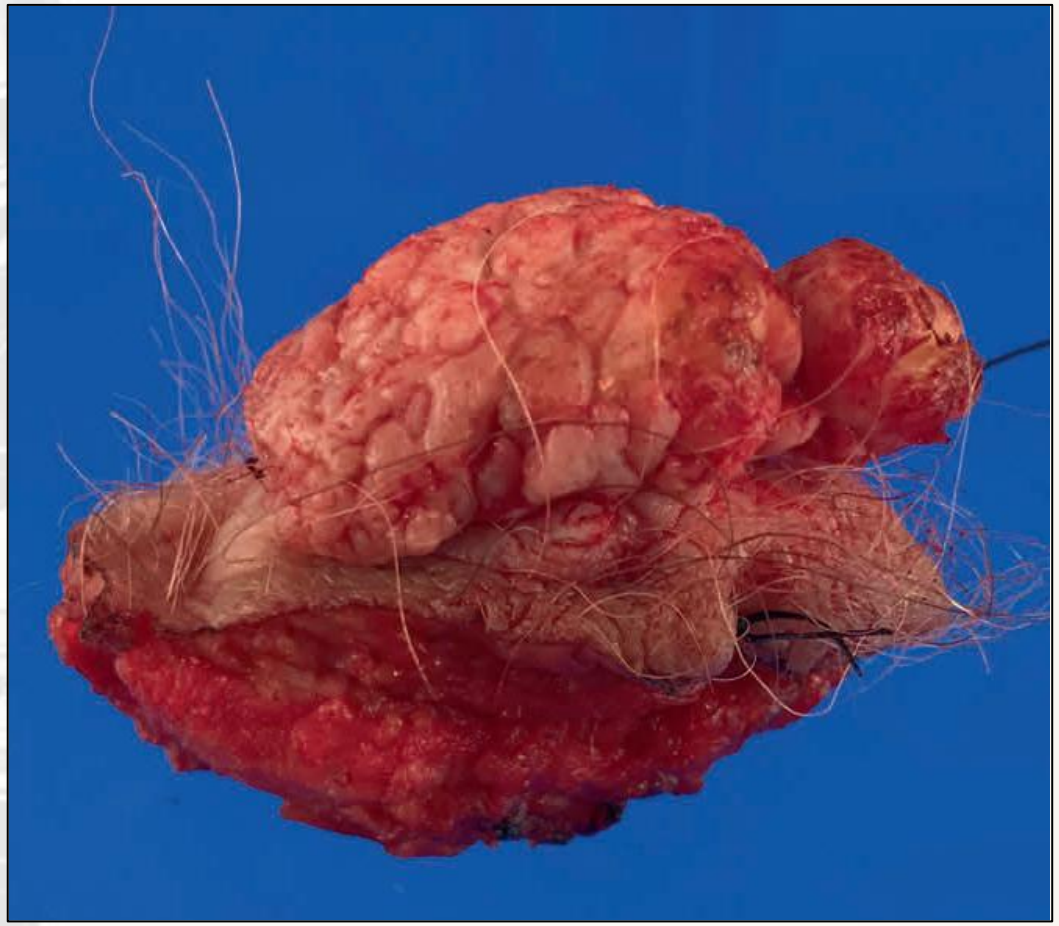
Roy SF, Wong J, Le Page C, Tran-Thanh D, Barkati M, Pina A, Trinh VQ, Rahimi K. DEVIL, VAAD and vLSC constitute a spectrum of HPV-independent, p53-independent intra-epithelial neoplasia of the vulva. Histopathology. 2021 Dec;79(6):975-988.

Clinical Features: vaVIN

- Age 50-90
- Discrete solitary exophytic lesion
  - Raised white or erythematous mass
  - “Cauliflower” or plaque-like appearance

Should not be a diffuse process

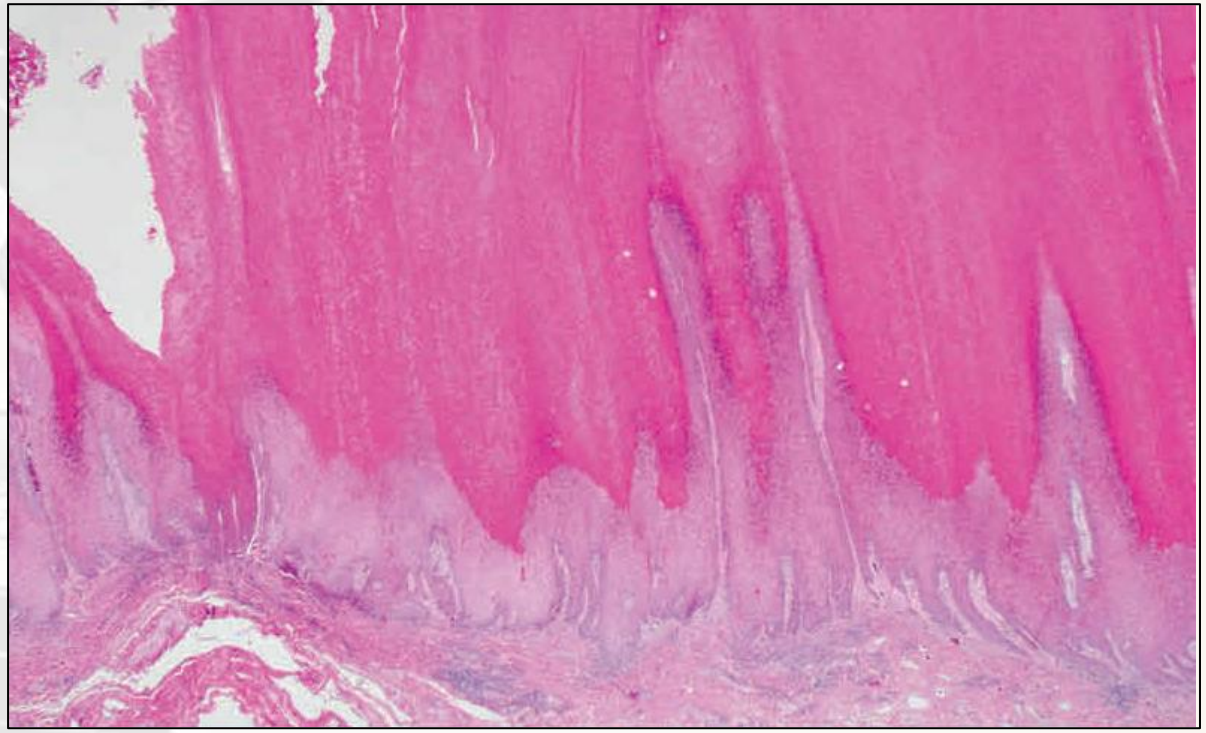
- Suggests inflammatory etiology



Parra-Herran C, Nucci MR, Singh N, Rakislova N, Howitt BE, Hoang L, Gilks CB, Bosse T, Watkins JC. HPV-independent, p53-wild-type vulvar intraepithelial neoplasia: a review of nomenclature and the journey to characterize verruciform and acanthotic precursor lesions of the vulva. Mod Pathol. 2022 Oct;35(10):1317-1326.

Pathologic Features: vaVIN

- Acanthotic and/or verruciform architecture
- Altered squamous differentiation
  - Hyperkeratosis
  - Parakeratosis
  - Either:
    - Hypogranulosis and cytoplasmic pallor (formerly VAAD)
    - Hypergranulosis (formerly vLSC)
- Absent cytologic atypia**



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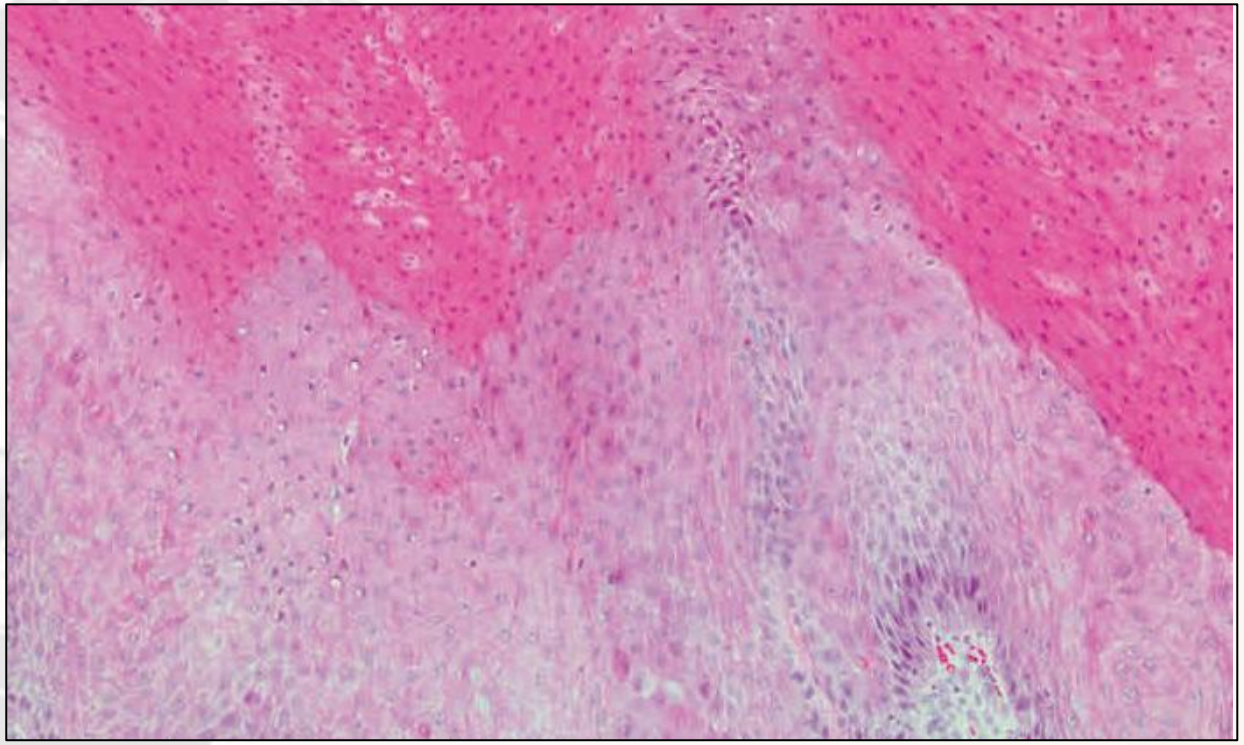

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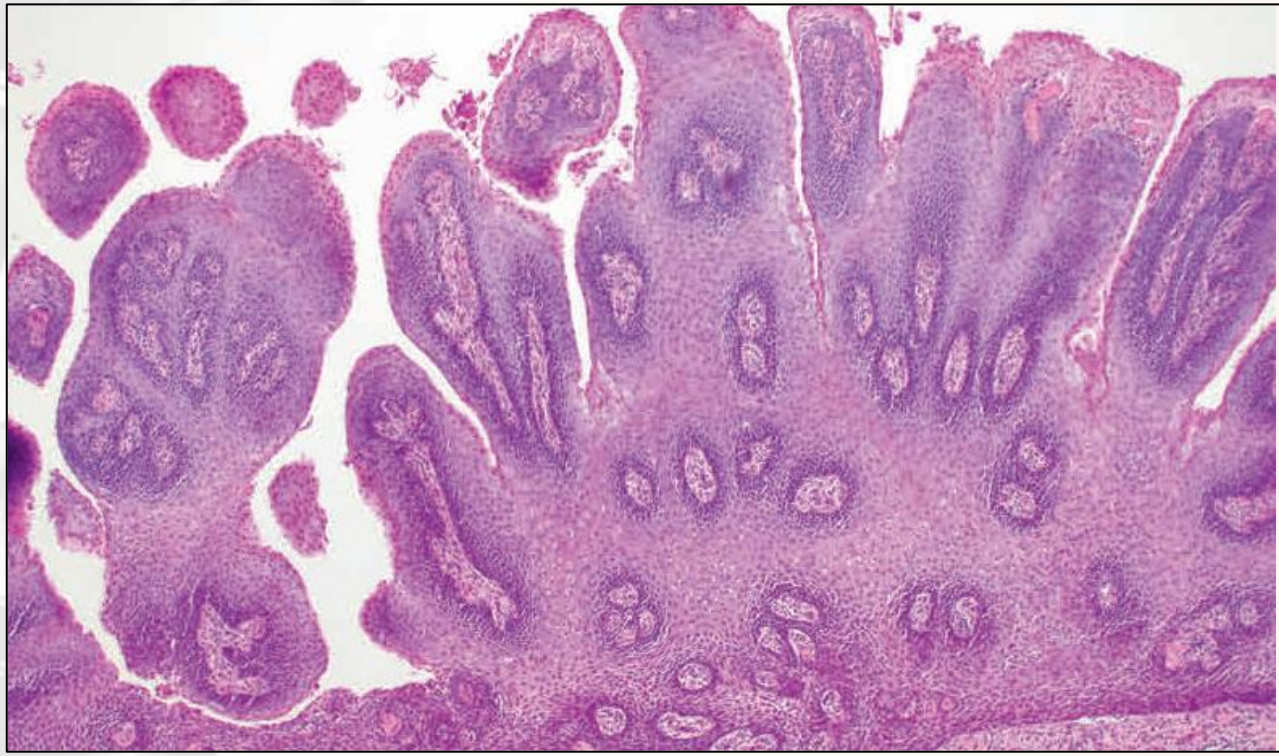

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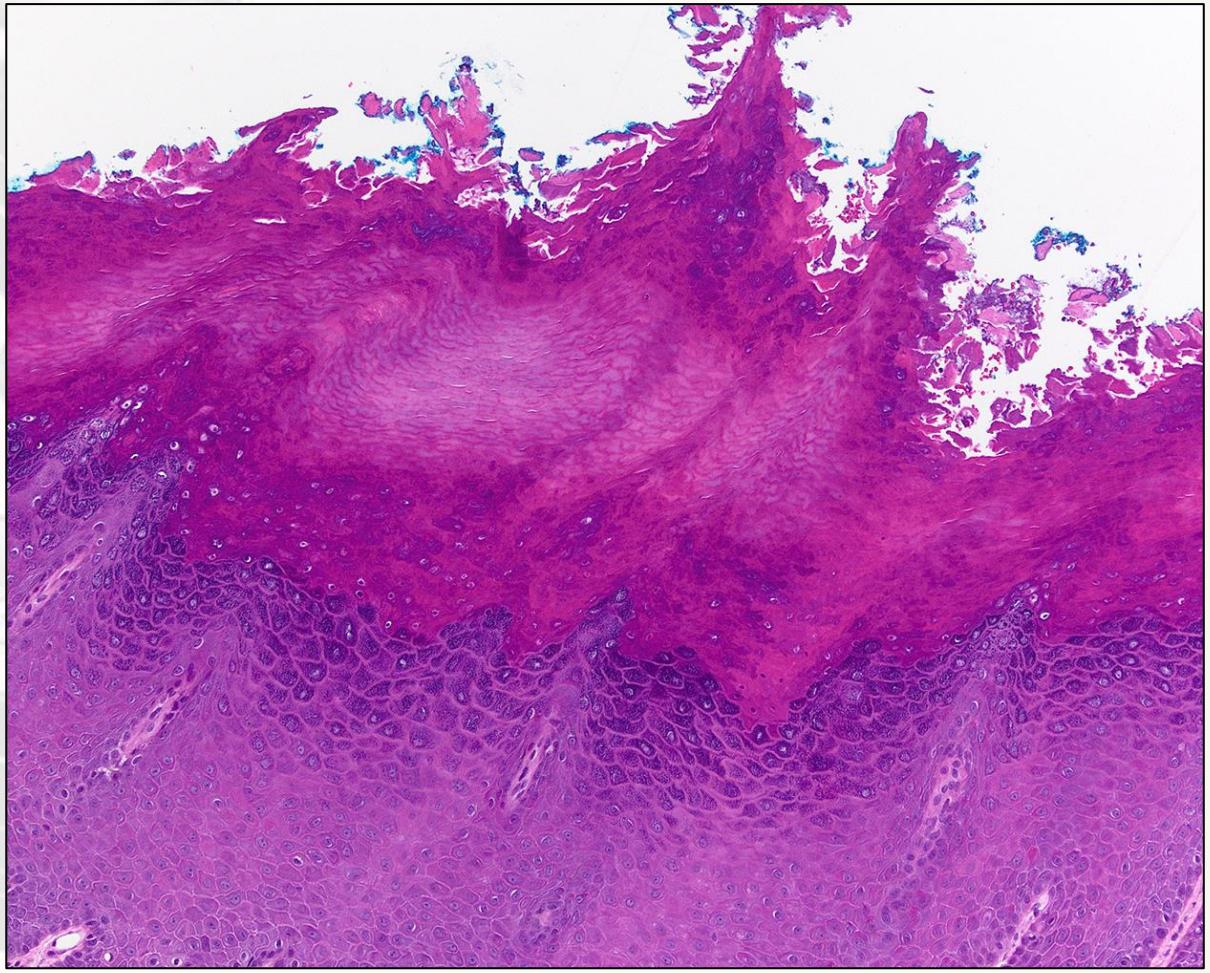

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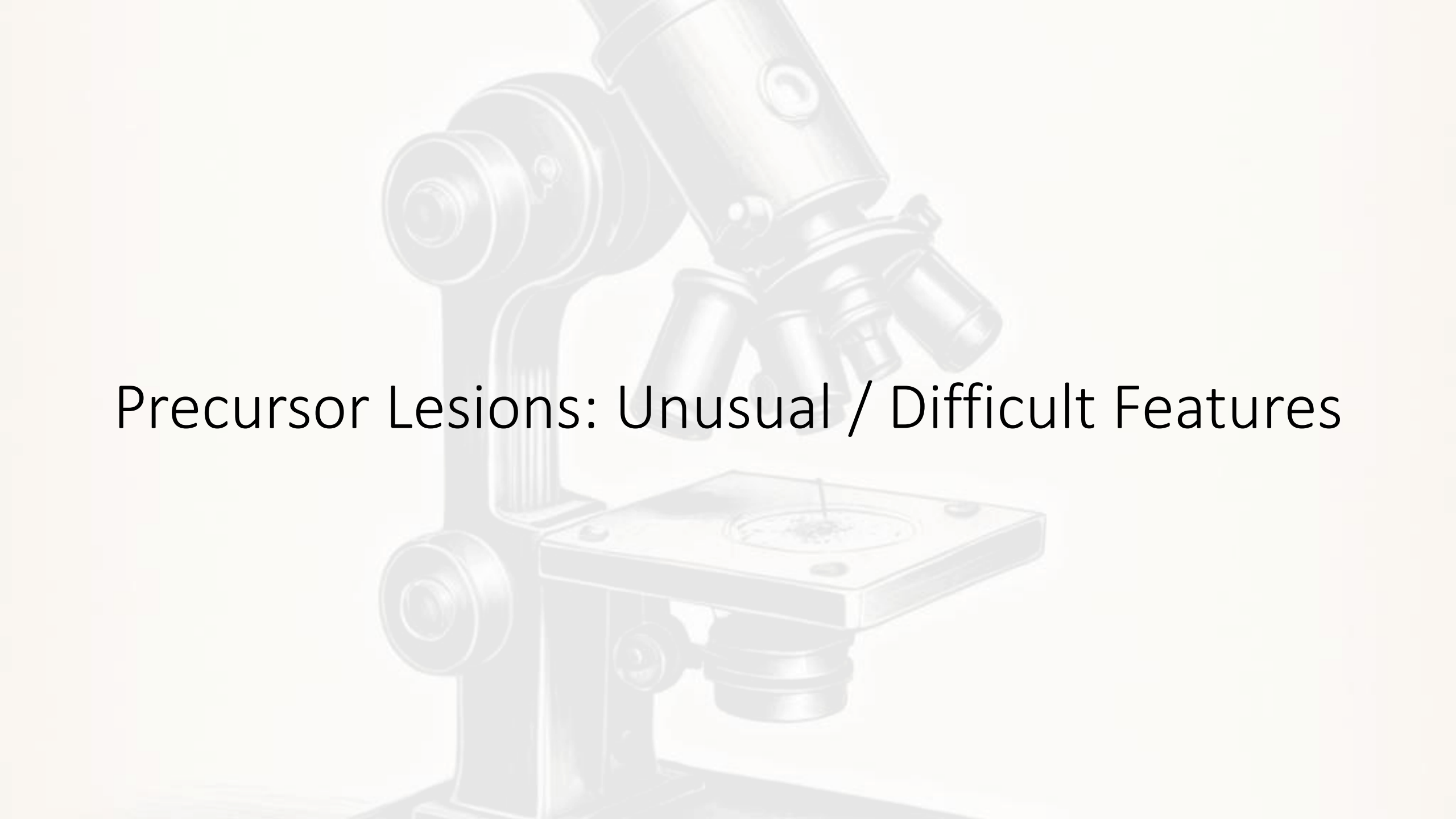
Summary: Essential Features of vaVIN

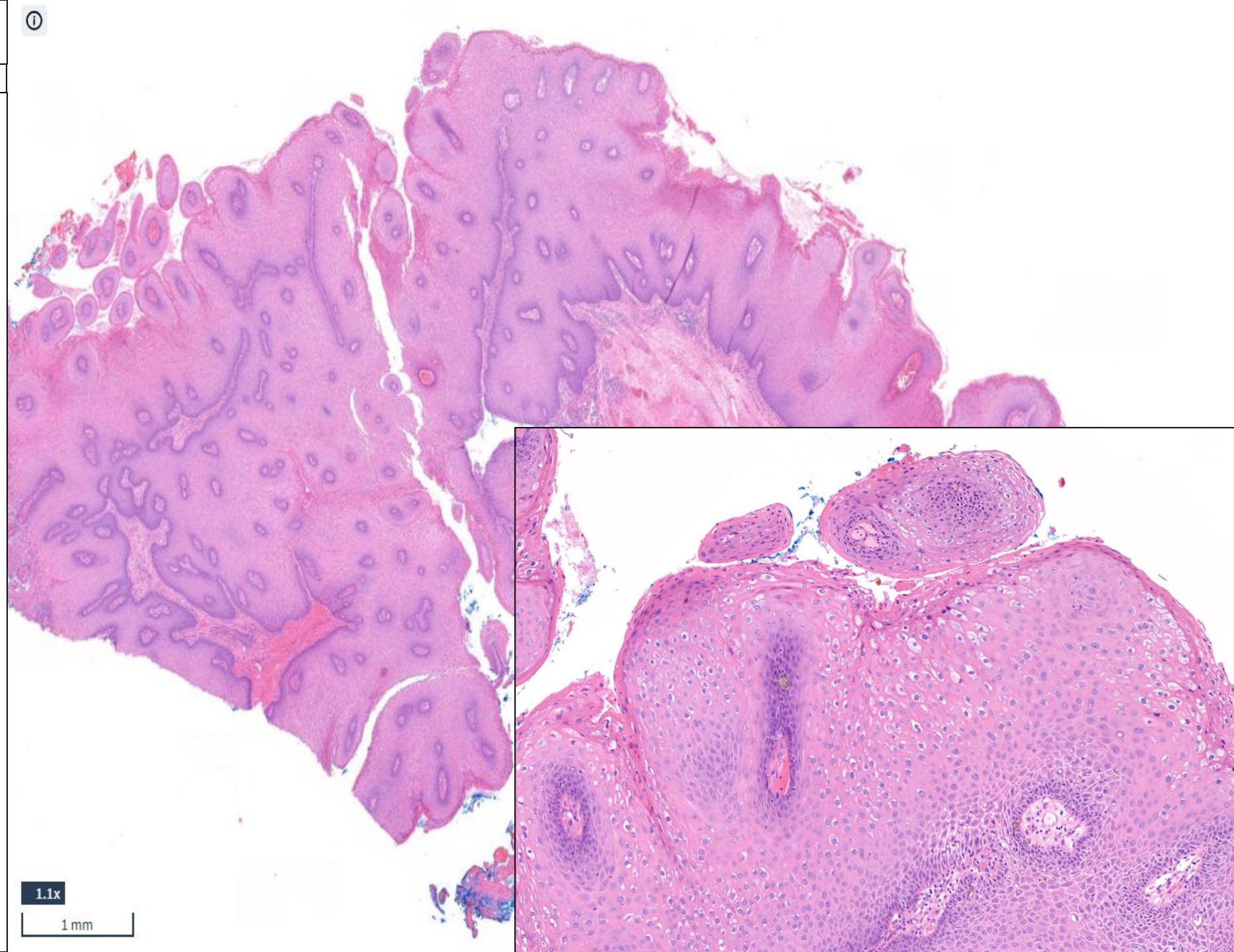
- Discrete lesion
- Very thick with abnormal maturation
- No cytologic atypia / koilocytic change
- Immunohistochemistry
  - Wild-type p53
  - p16 patchy or negative



Precursor Lesions: Unusual / Difficult Features

“Giant Condyloma”

- “Buschke-Löwenstein tumor”
- May be several cm in size
- Papillomatosis
- Hyperkeratosis
- Hypergranulosis
- Variable koilocytic change
- Tangential sectioning may generate endophytic appearance
  - No true invasion
- P16 patchy/negative
- p53 wild-type
- DDx: vaVIN
- HPV 6/11 (can confirm with in-situ hybridization)
- Rarely develop focal high-grade dysplasia with block-positive p16

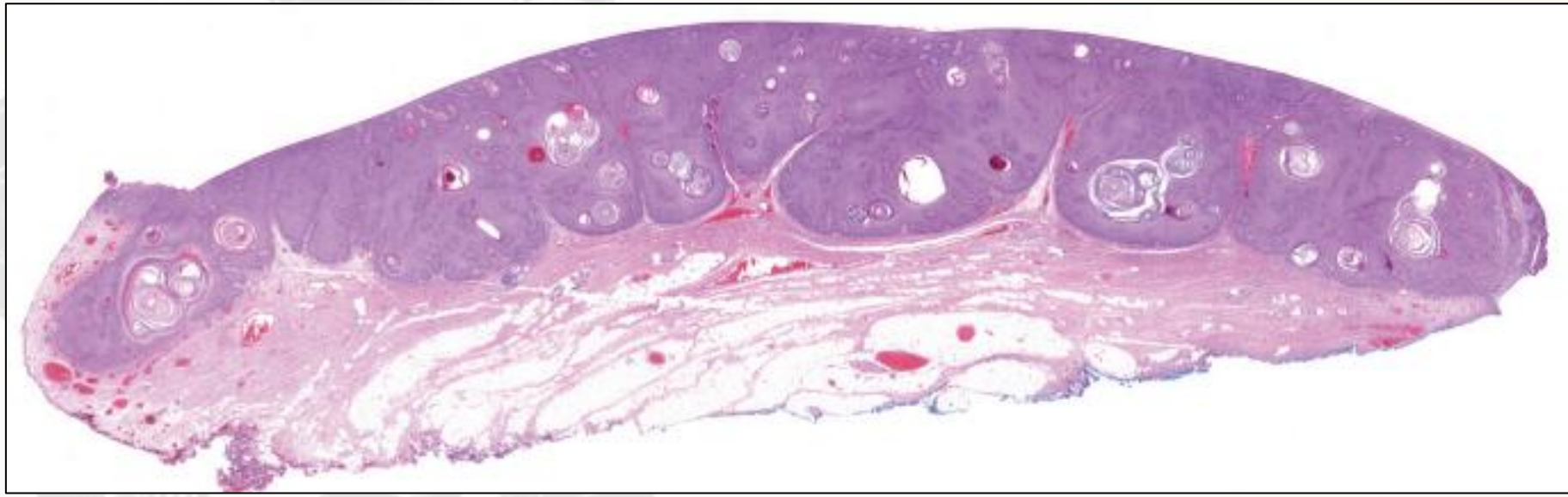


HPV and Genital Seborrheic Keratosis

- Unlike typical cutaneous SK, HPV has been identified in genital SK
- May co-occur with typical condyloma acuminatum
- Typically lack koilocytic change
- Variety of HPV types identified
  - HPV 6, 16, 42, 44, 53
  - Includes both low- and high-risk subtypes

Practical tips

- If lack of cytologic atypia
  - Diagnose SK and note occasional association with HPV at genital sites
- If significant atypia
  - Diagnose HSIL
- p16 helpful in identifying high-grade atypia



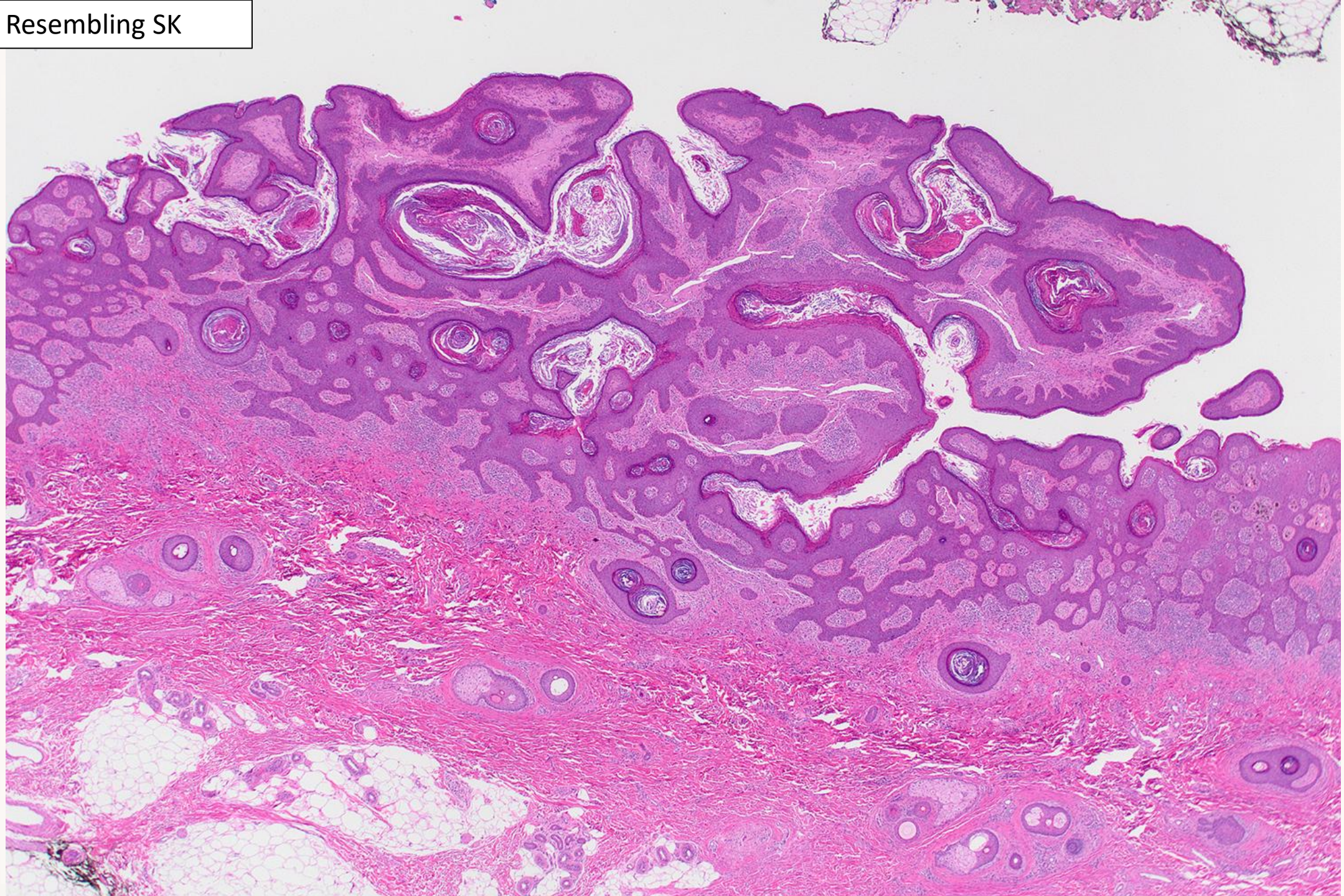
*Similar “Seborrheic keratosis-like” lesions in the vulva/cervix have been associated with HPV 42

Talia KL, McCluggage WG. Seborrheic Keratosis-like Lesions of the Cervix and Vagina: Report of a New Entity Possibly Related to Low-risk Human Papillomavirus Infection. Am J Surg Pathol. 2017 Apr;41(4):517-524.

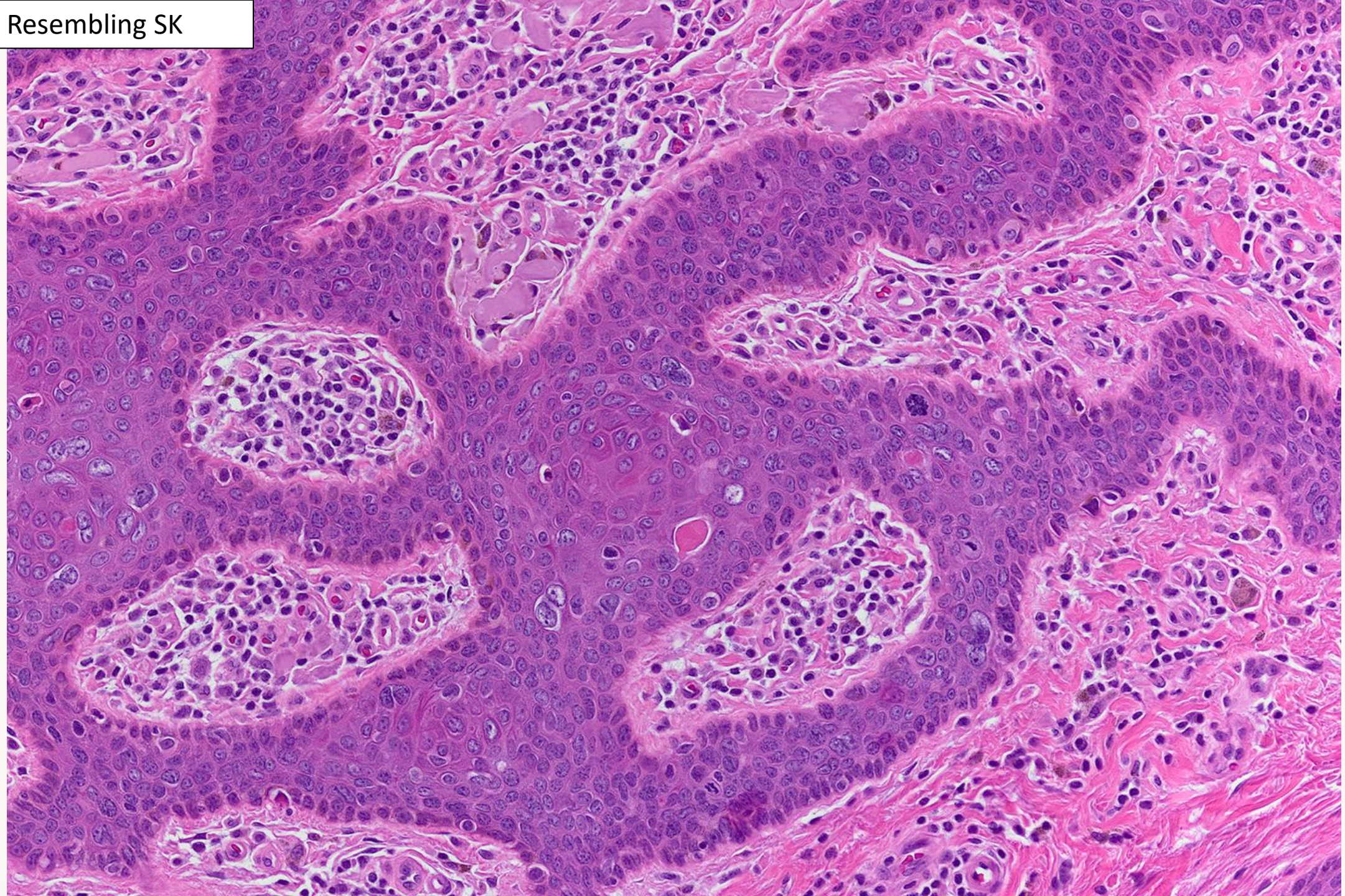
Talia KL, Hawkes D, Zhang G, Jamison J, Shanks J, Yang B, Soslow R, McCluggage WG. HPV42: A Common Low-Risk HPV Type Associated With Distinctive Cervicovaginal and Cutaneous Neoplasia. Am J Surg Pathol. 2025 Oct 1;49(10):992-1003.

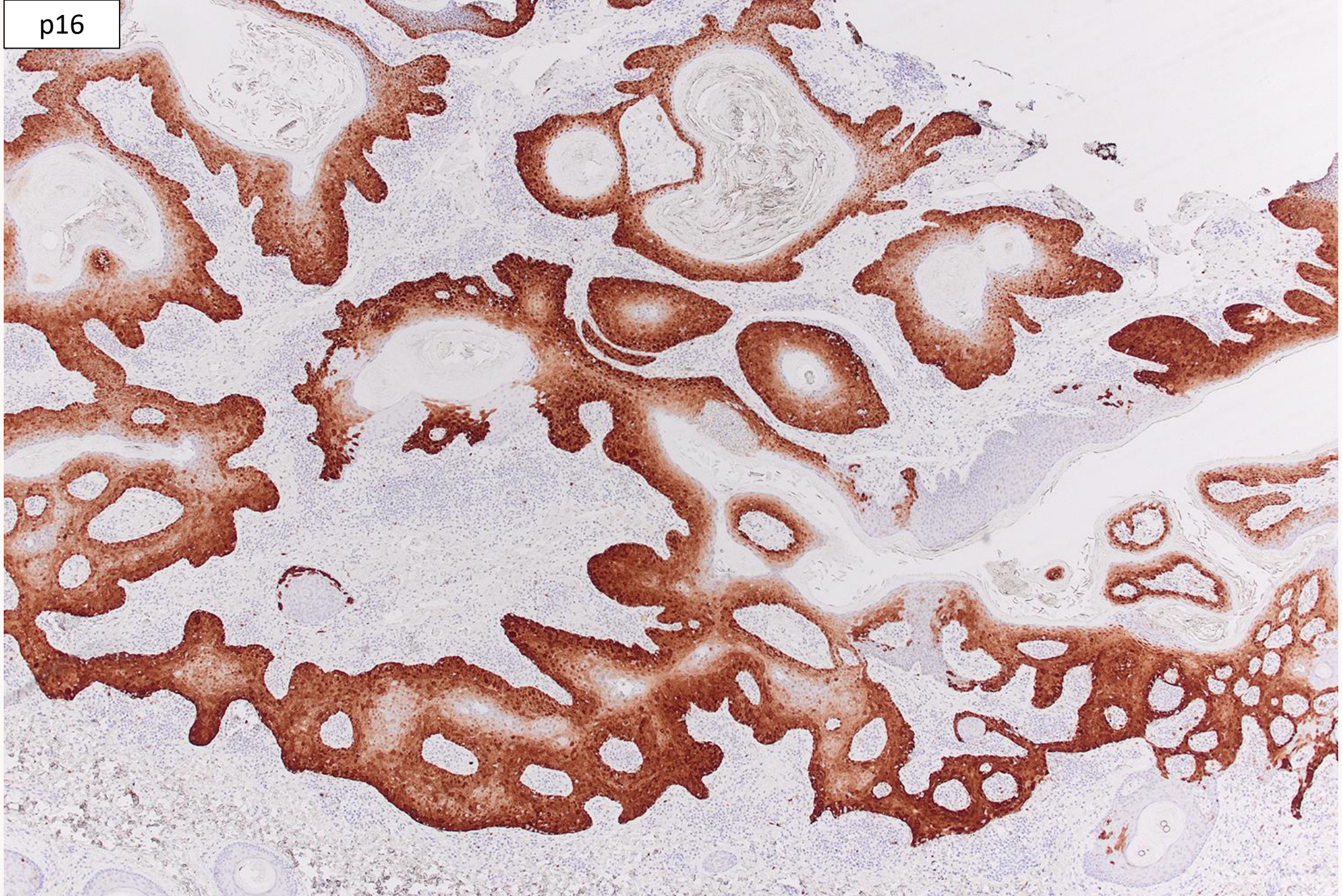
Dasgupta S, van Eersel R, Morrel B, van den Munckhof HAM, de Geus VA, van der Hoeven NMA, van de Sandt MM, Piso-Jozwiak M, Quint WGV, van der Avoort IAM, Koljenović S, Ewing-Graham PC, van Kemenade FJ. Relationship of human papillomavirus with seborrheic keratosis of the female genital tract - a case-series and literature review. Histol Histopathol. 2021 Dec;36(12):1209-1218.

HSIL Resembling SK



HSIL Resembling SK

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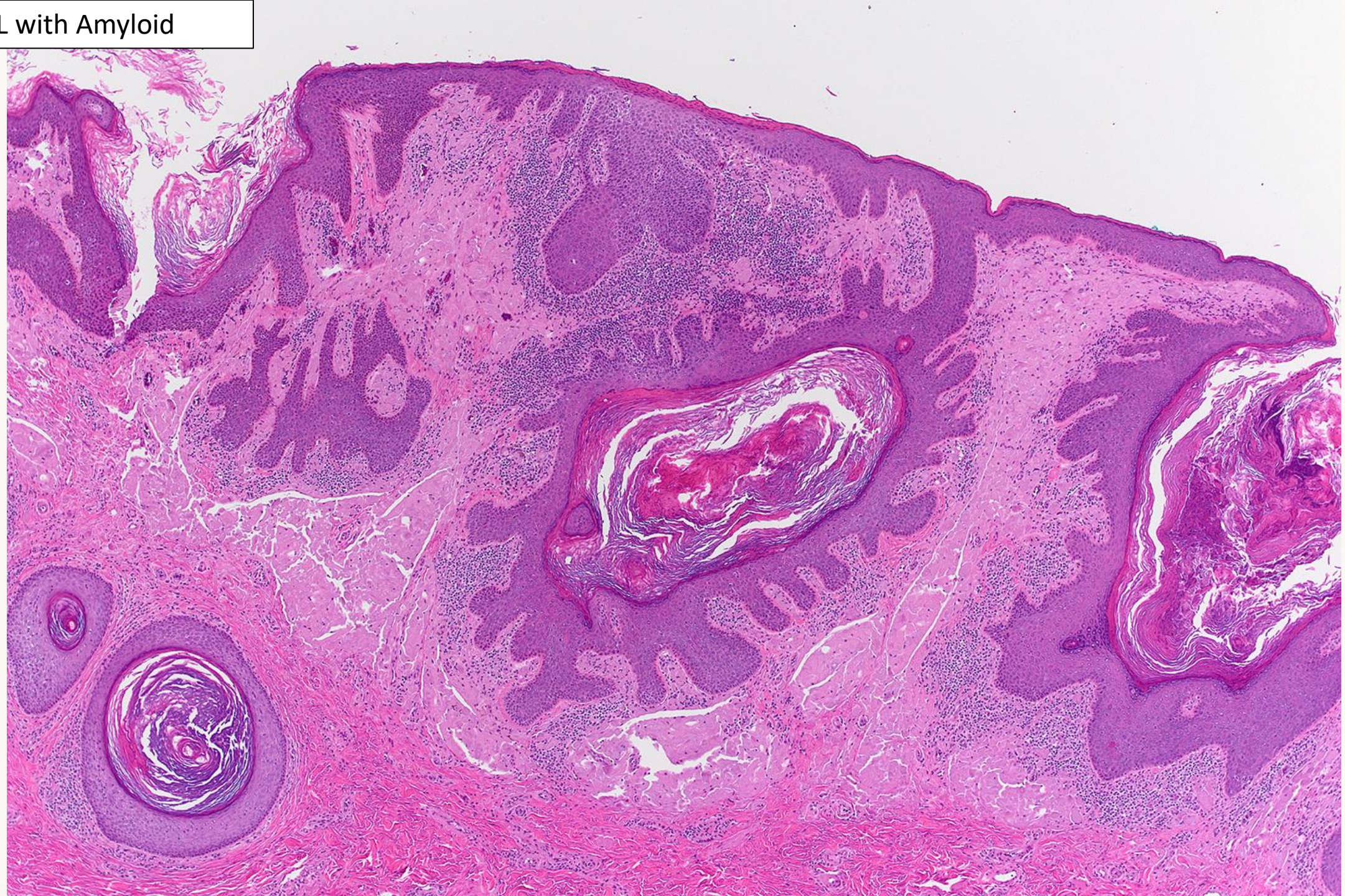
HSIL with Amyloid



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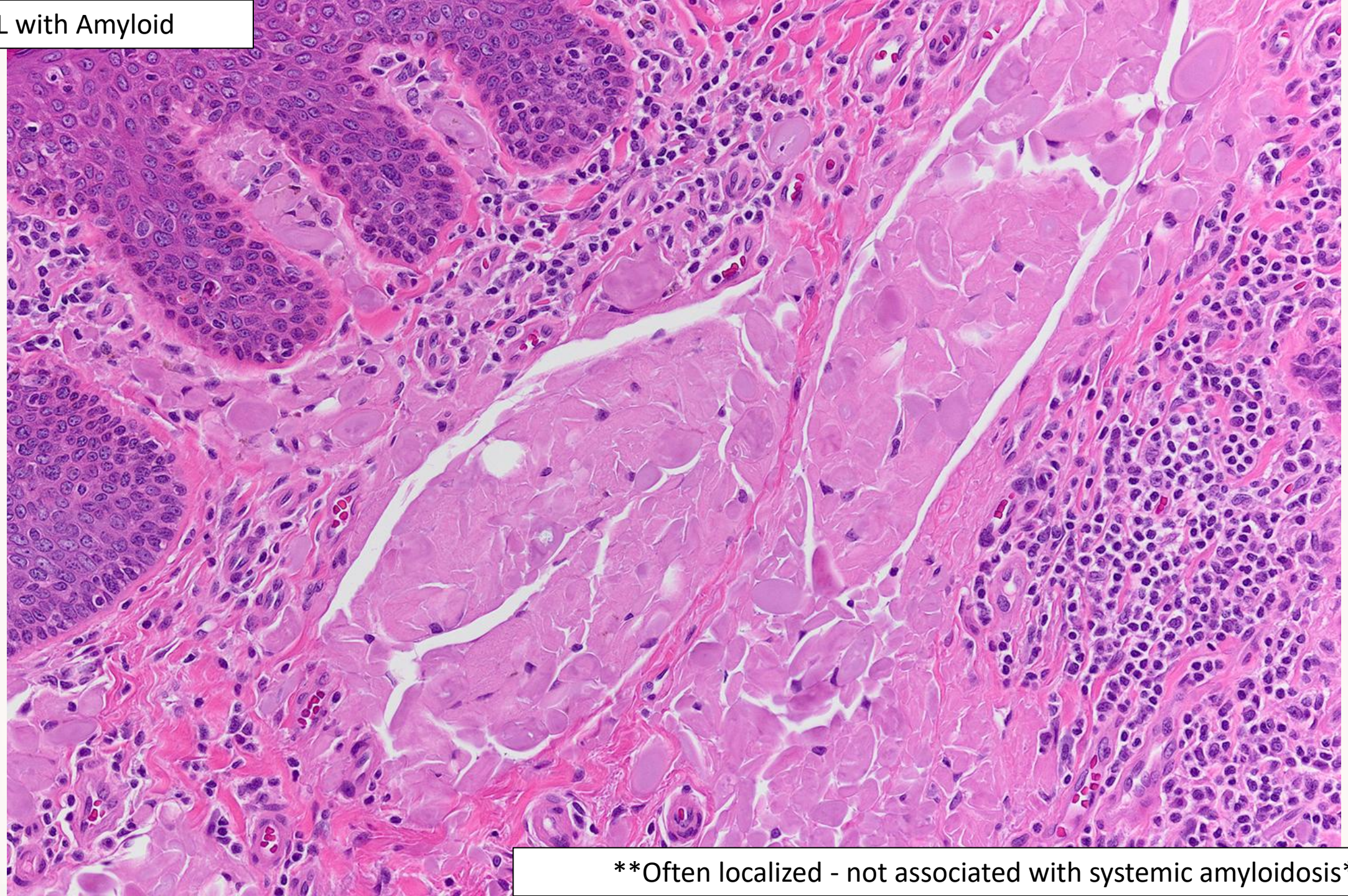
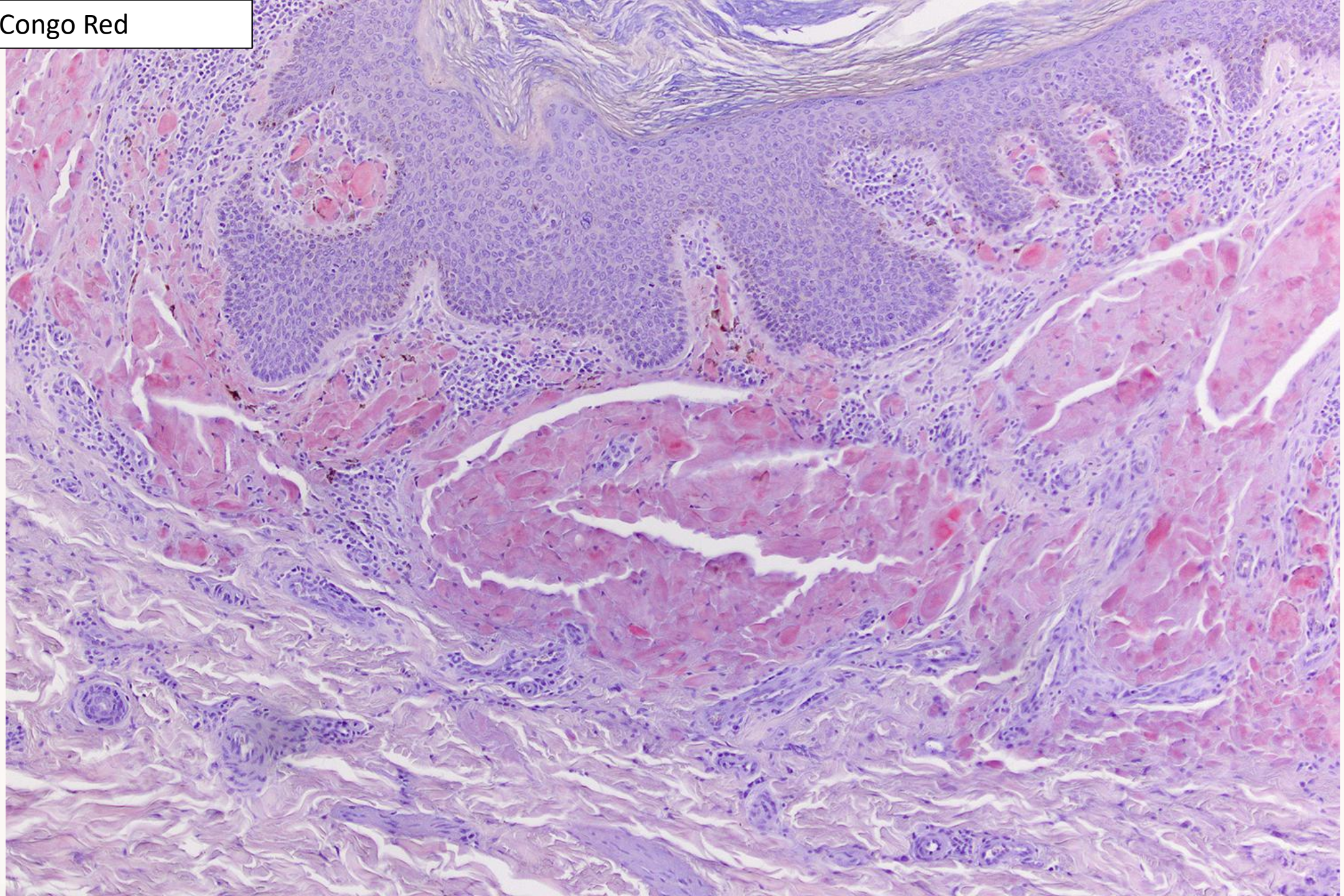


Often localized - not associated with systemic amyloidosis

Congo Red



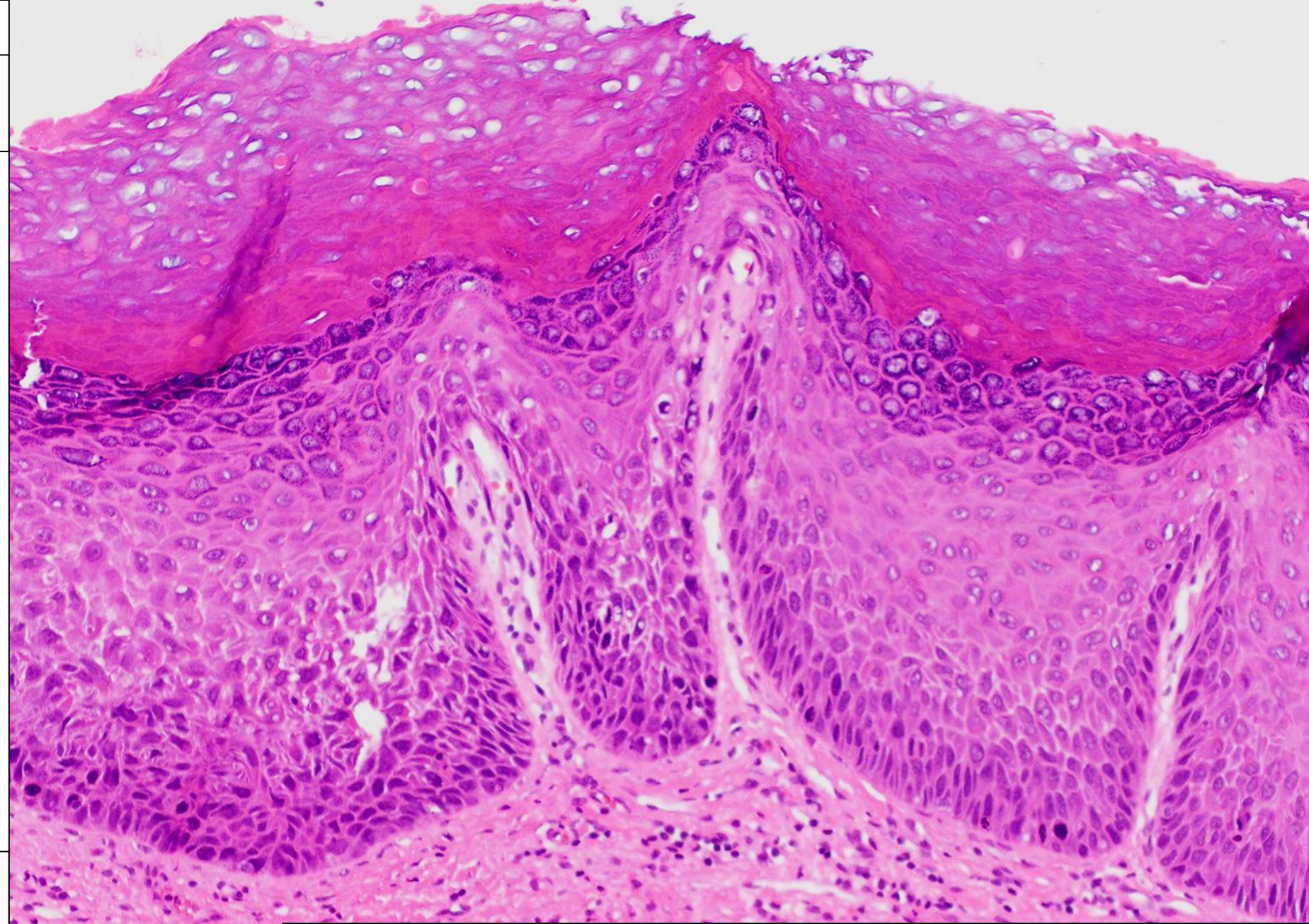
Deceptive Patterns of HSIL

HSIL with Superimposed Inflammatory Changes

- Hyperkeratosis, hypergranulosis, and maturation may mask atypia in superficial layers

Distinguishing from dVIN:

- Hyperchromasia in at least the first 3-4 parabasal layers
- Full-thickness atypia (up to granular cell layer)
- Only rare aberrant basal keratinization compared to dVIN



Watkins J, et al. Classic Vulvar Intraepithelial Neoplasia With Superimposed Lichen Simplex Chronicus: A Unique Variant Mimicking Differentiated Vulvar Intraepithelial Neoplasia. Int J Gynecol Pathol. 2019 Mar;38(2):175-182.

Deceptive Patterns of HSIL

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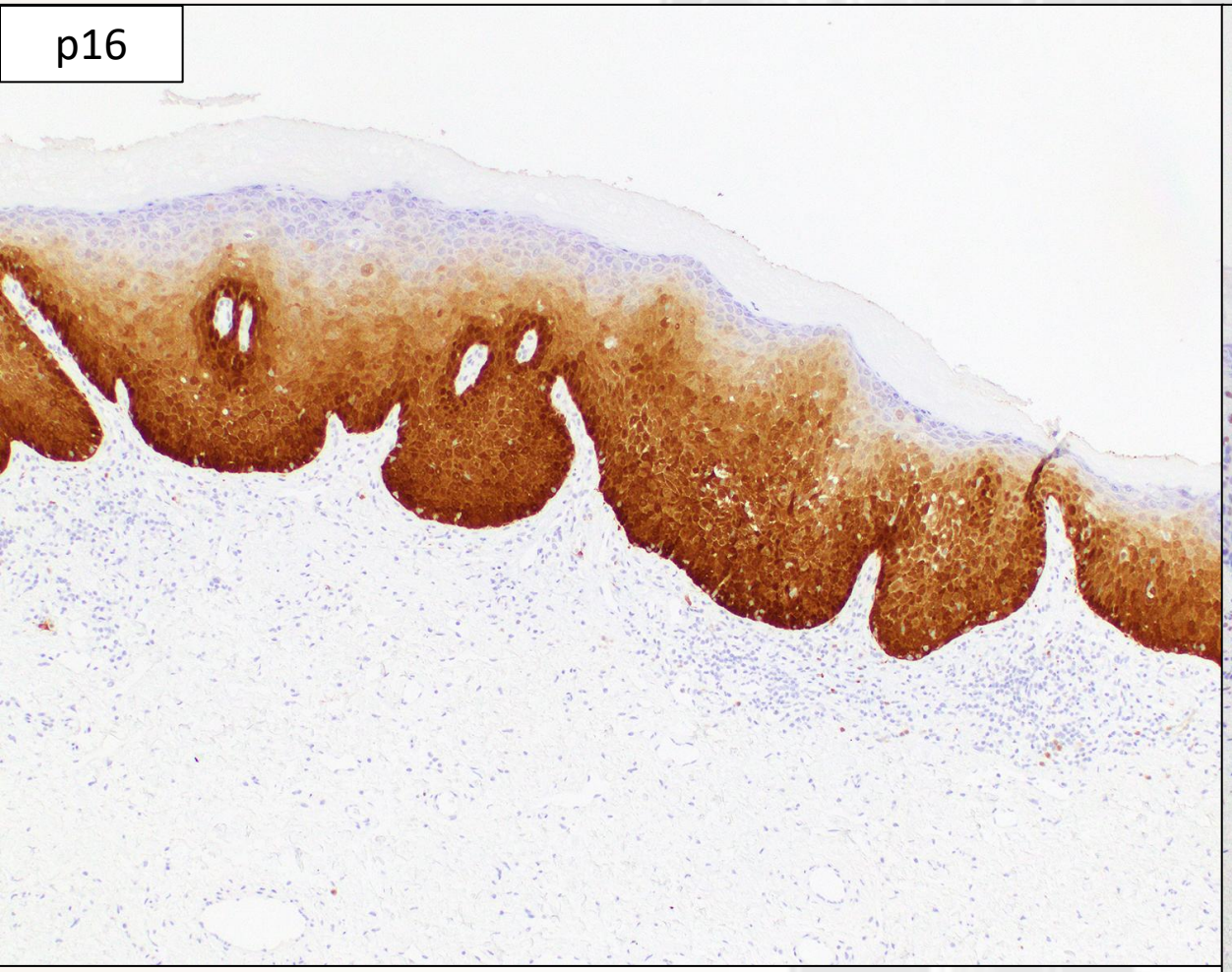
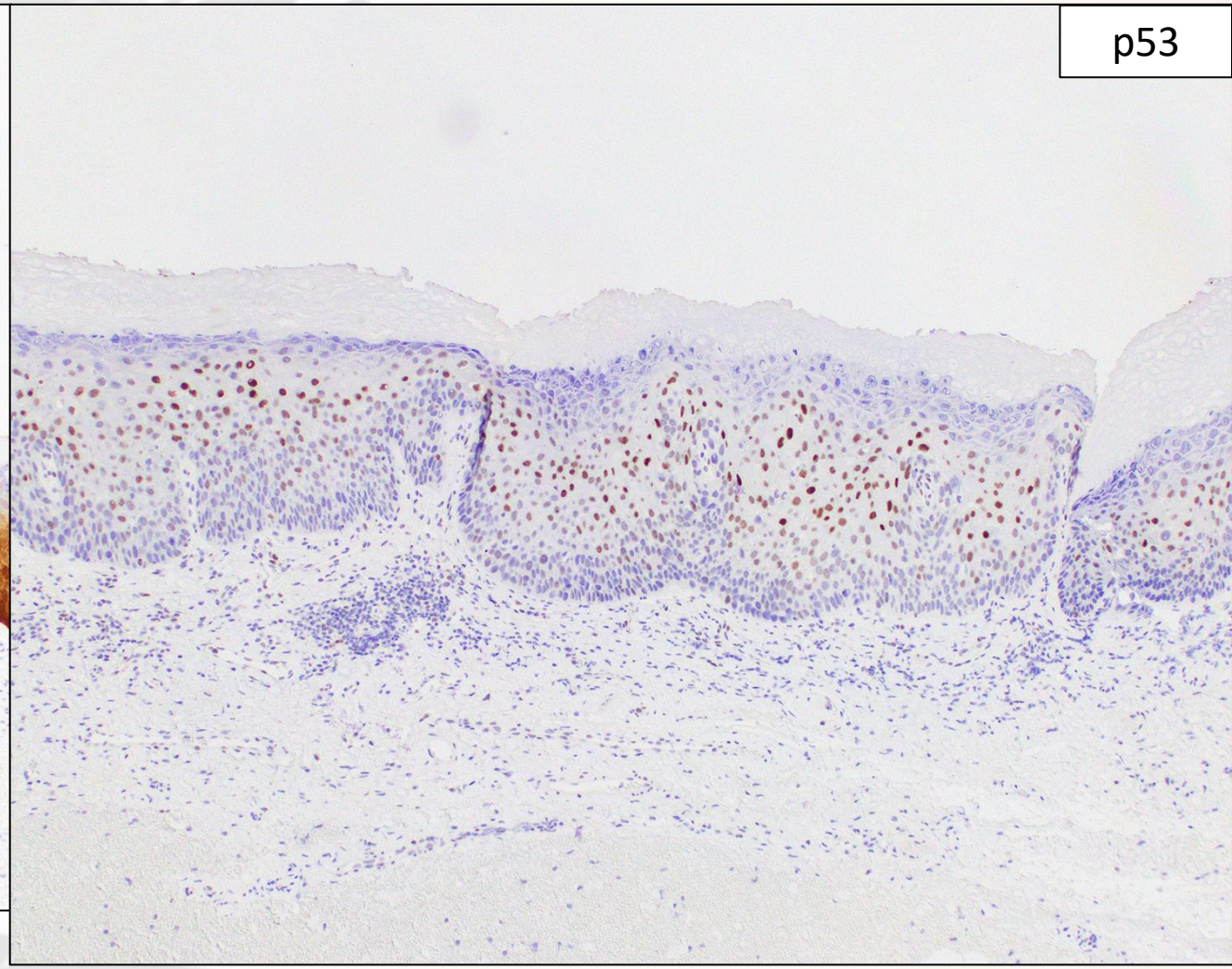


p53



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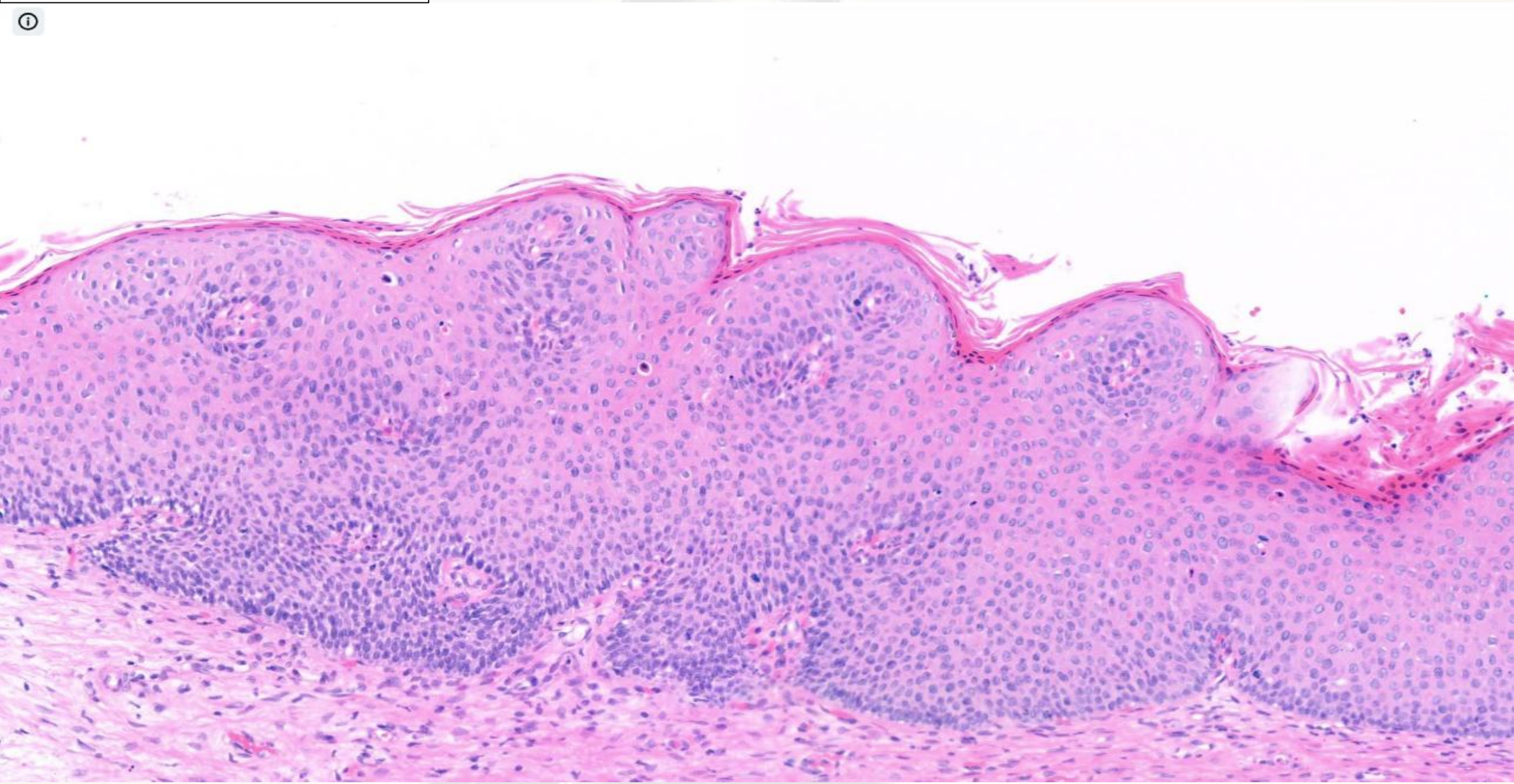

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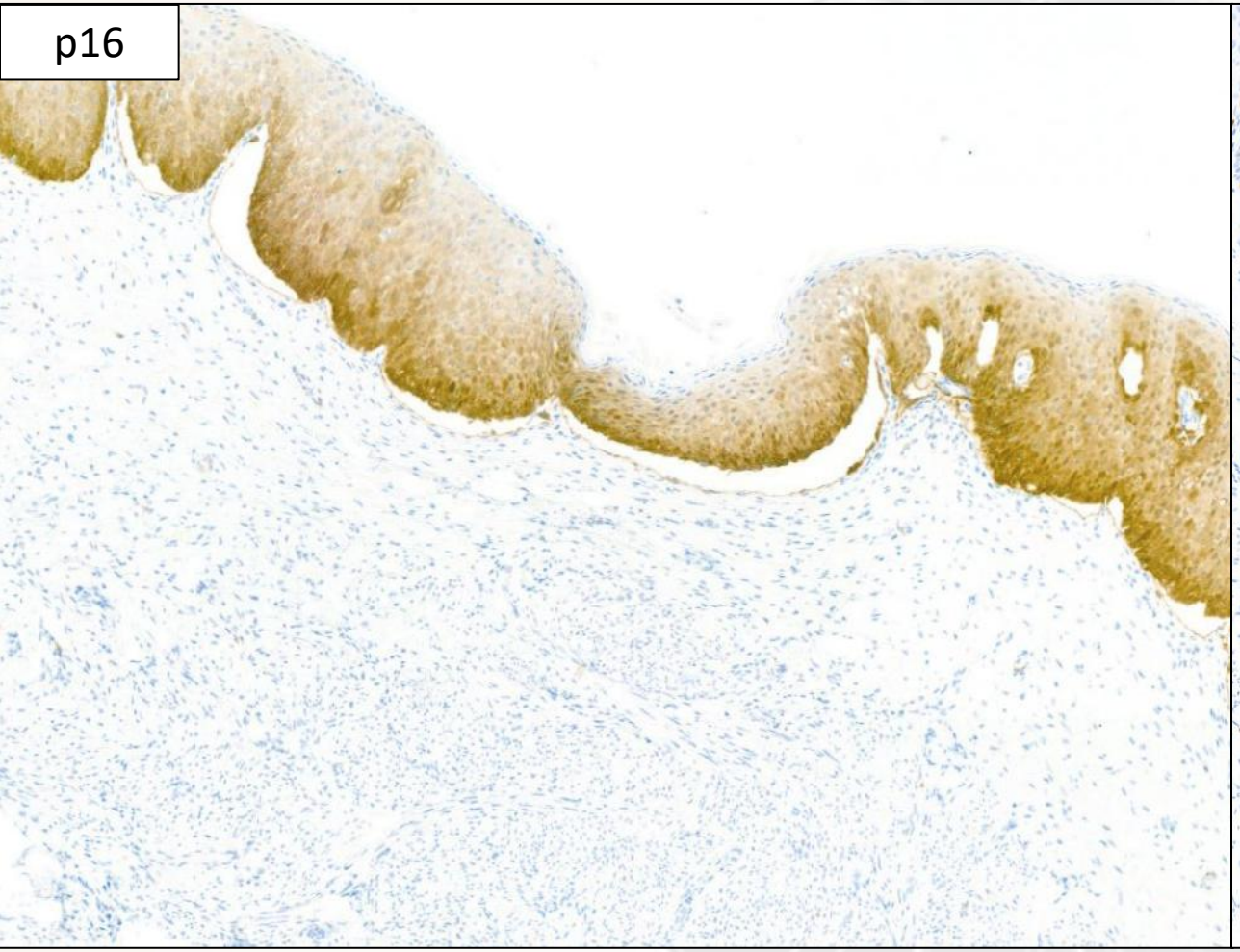

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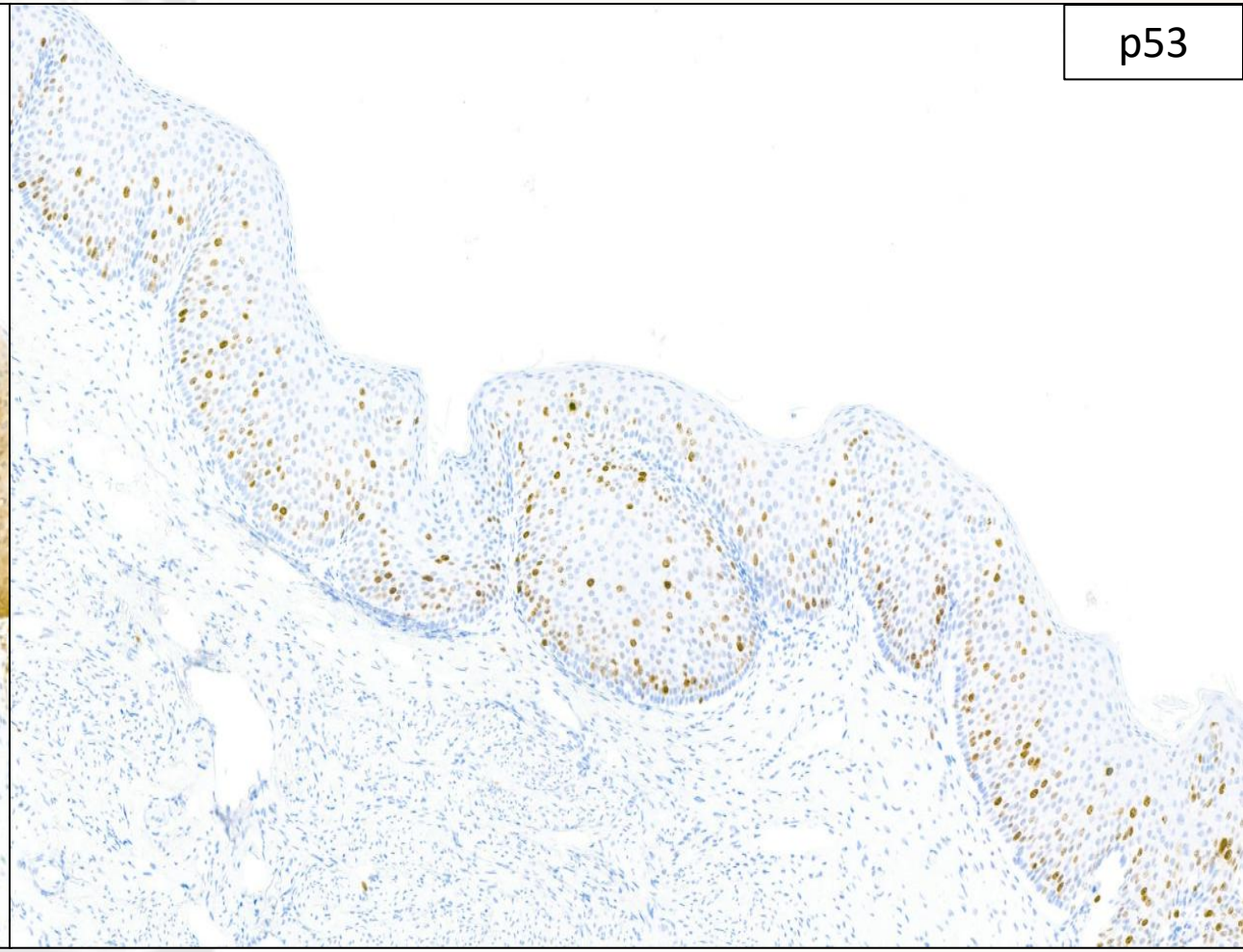

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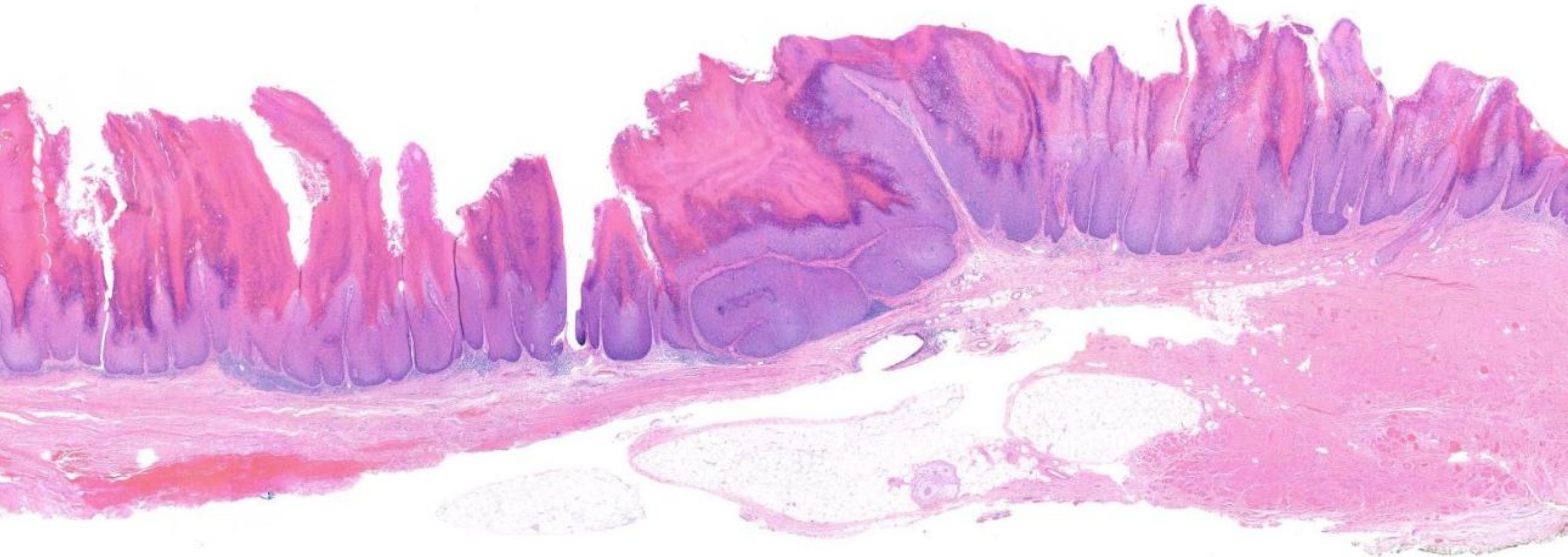
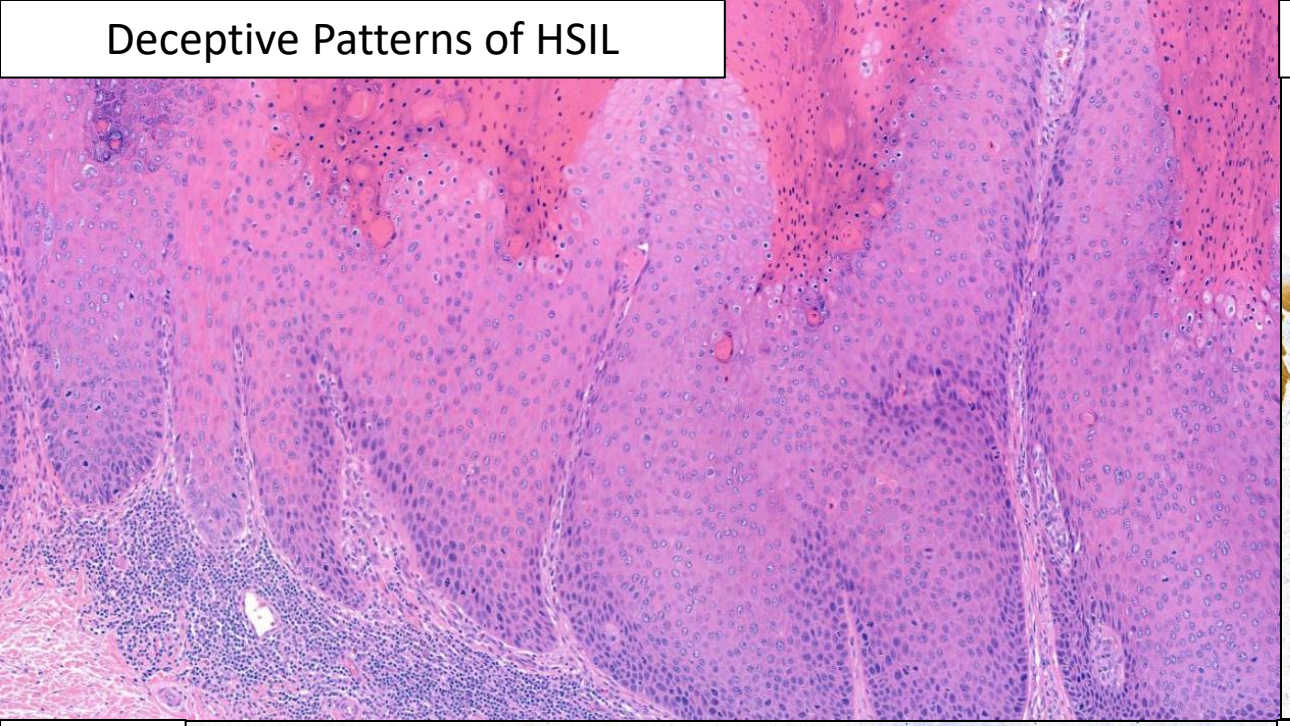
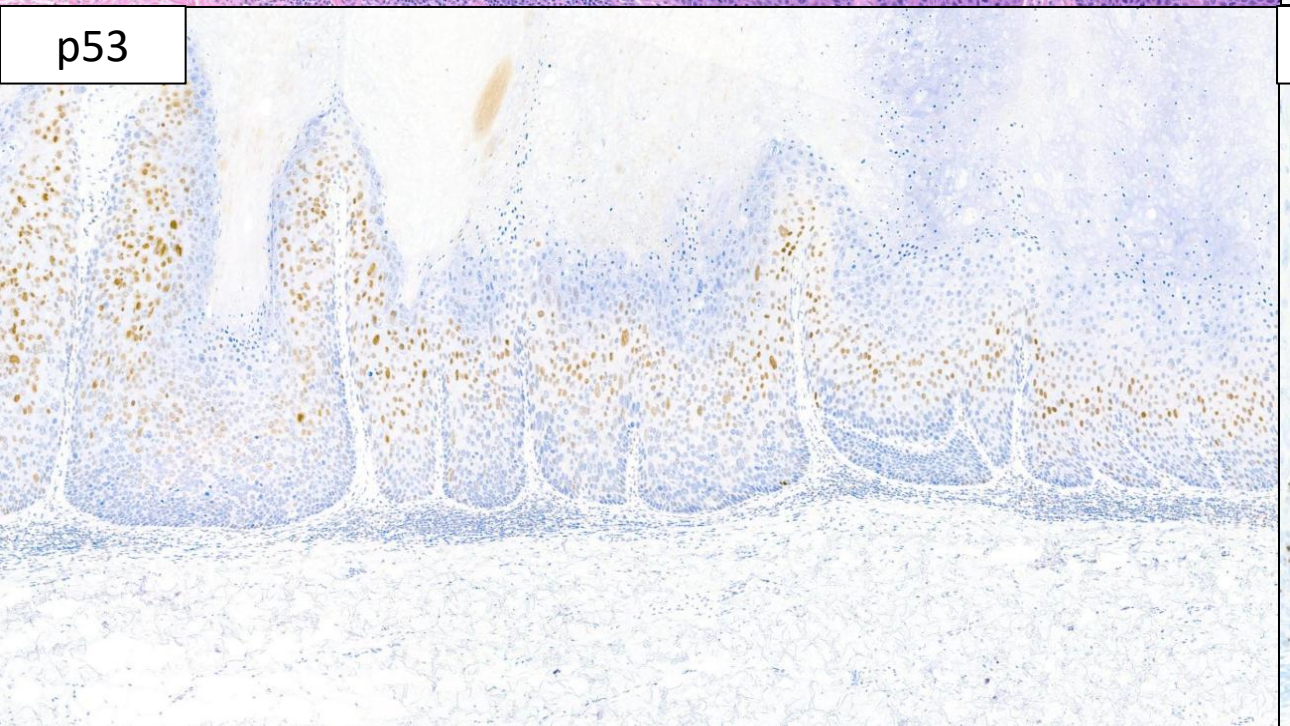


Deceptive Patterns of HSIL

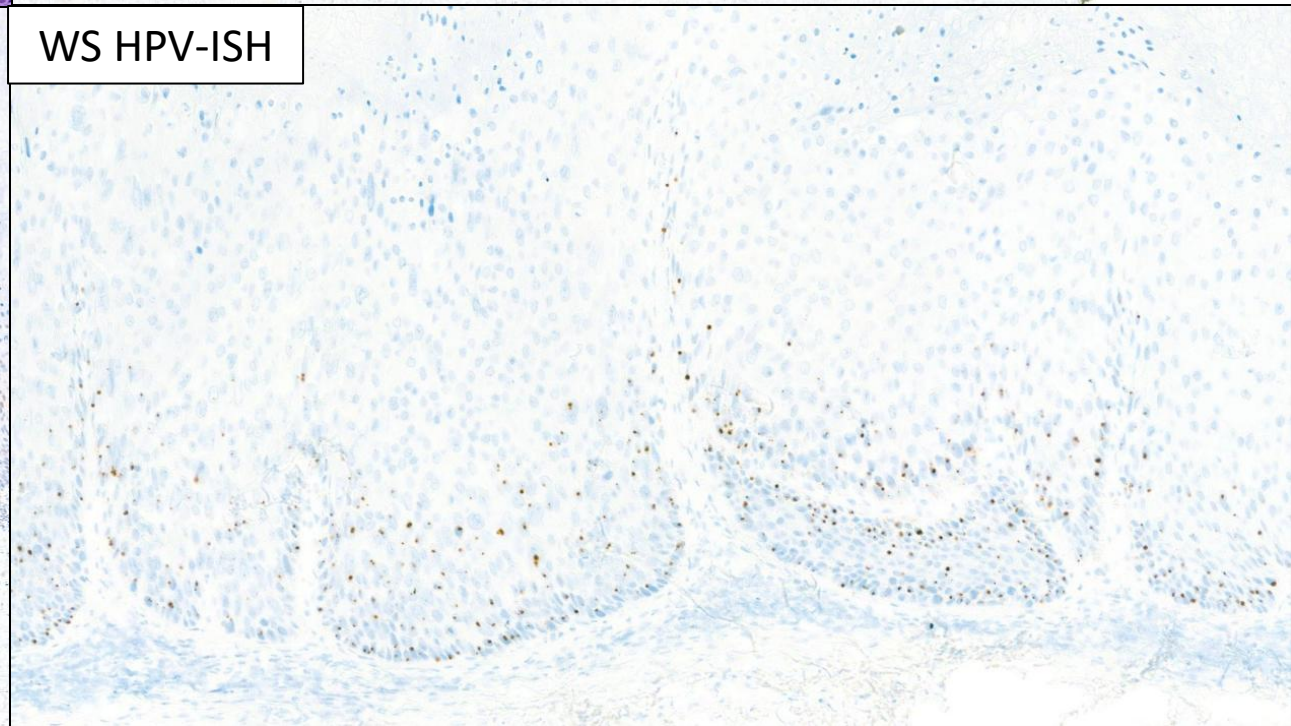


p16

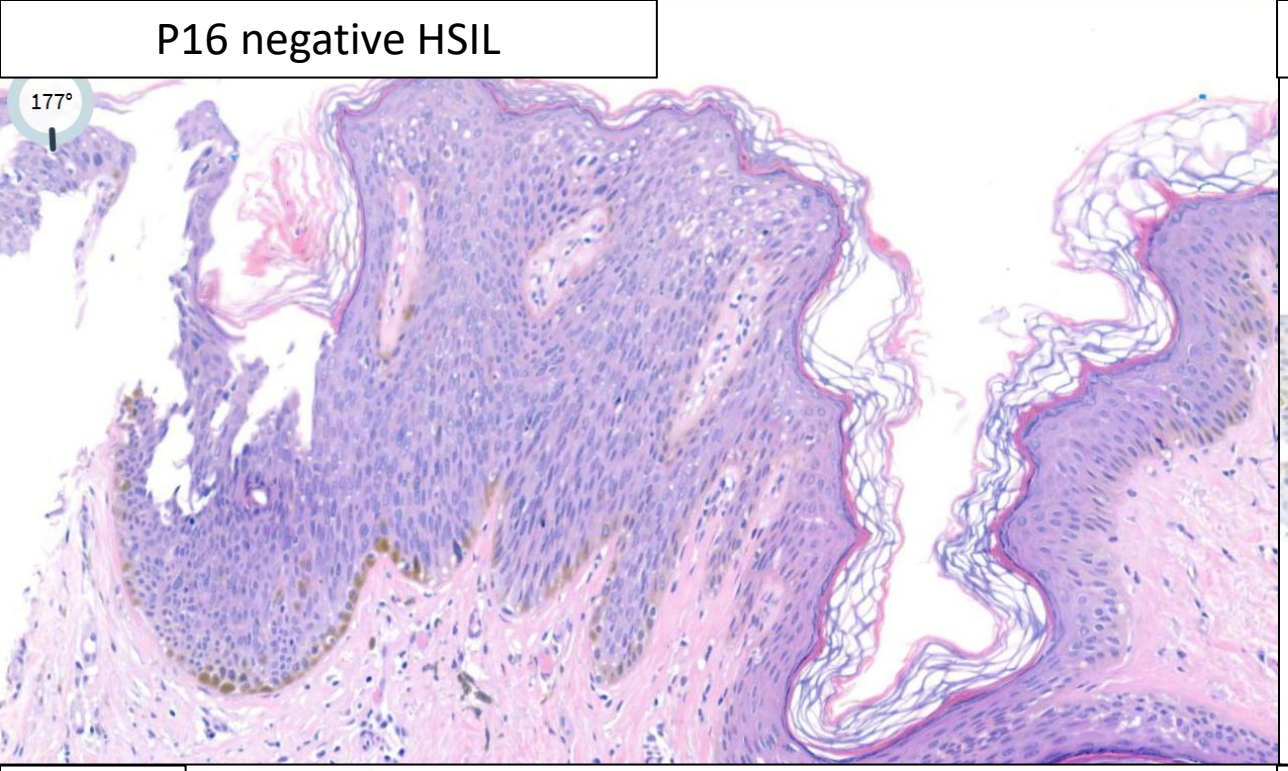
p53



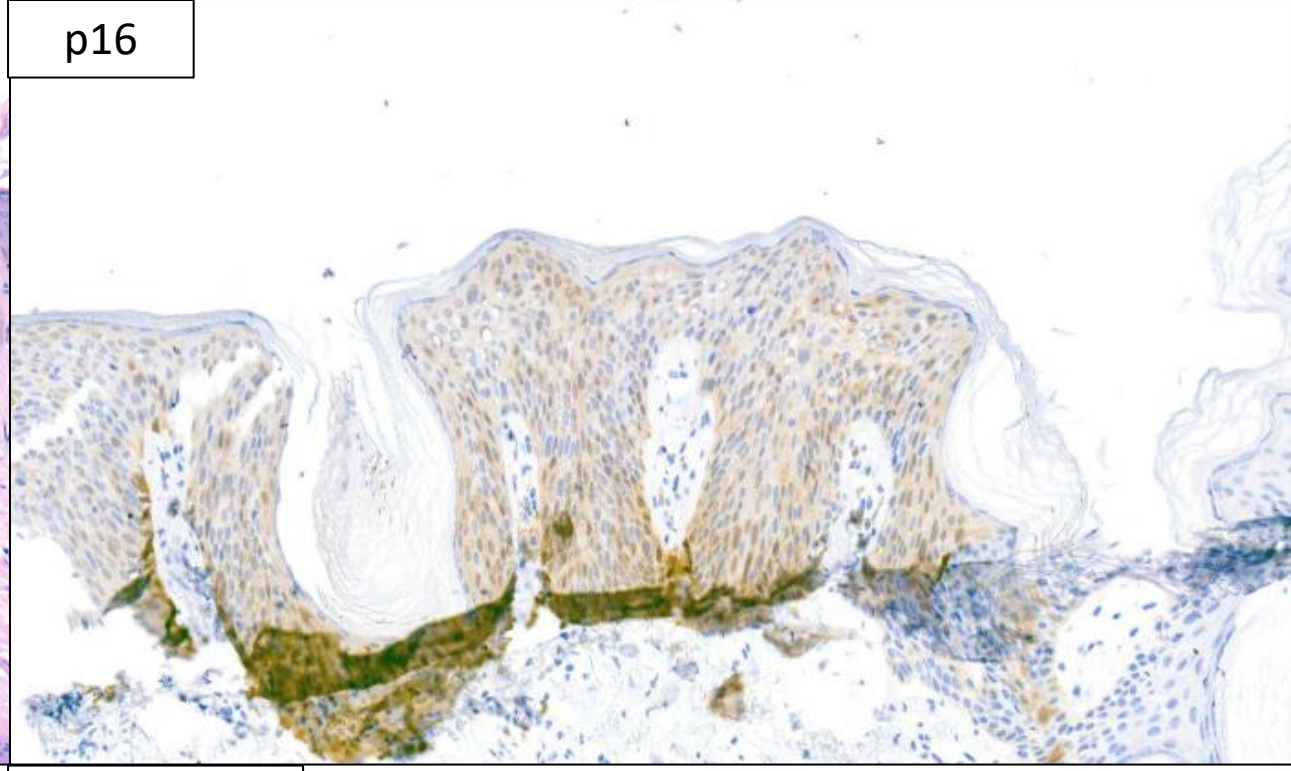
WS HPV-ISH



P16 negative HSIL



p16



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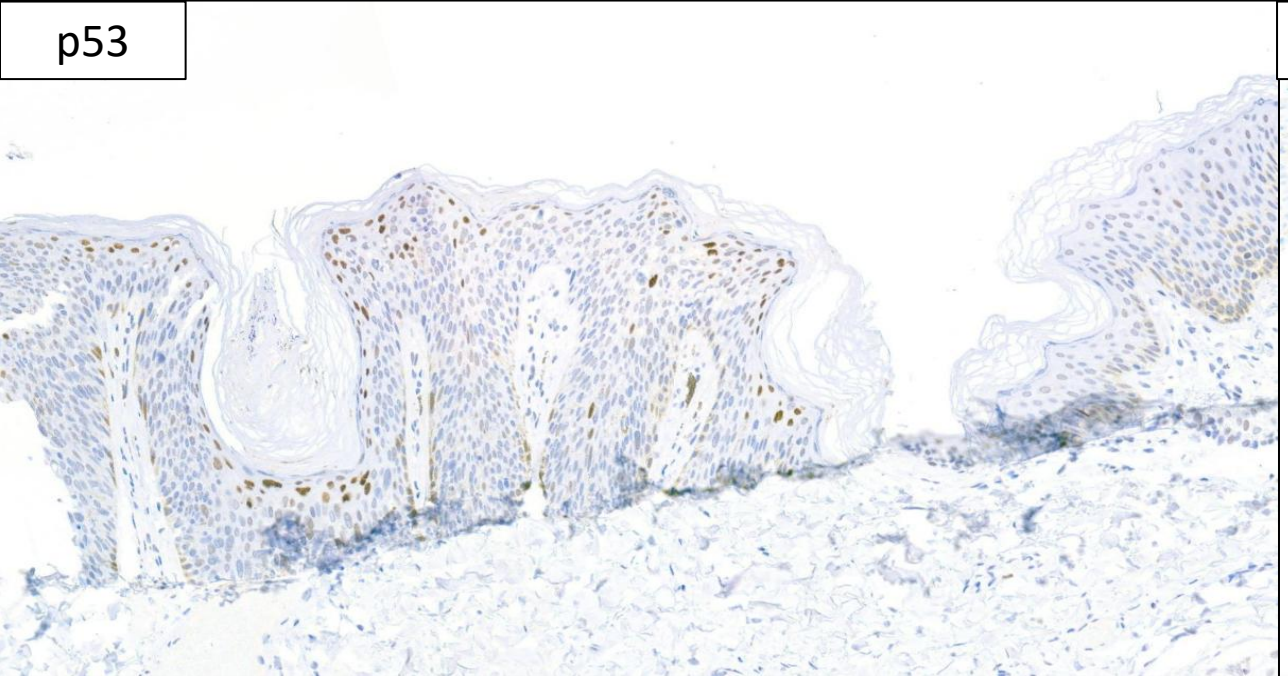
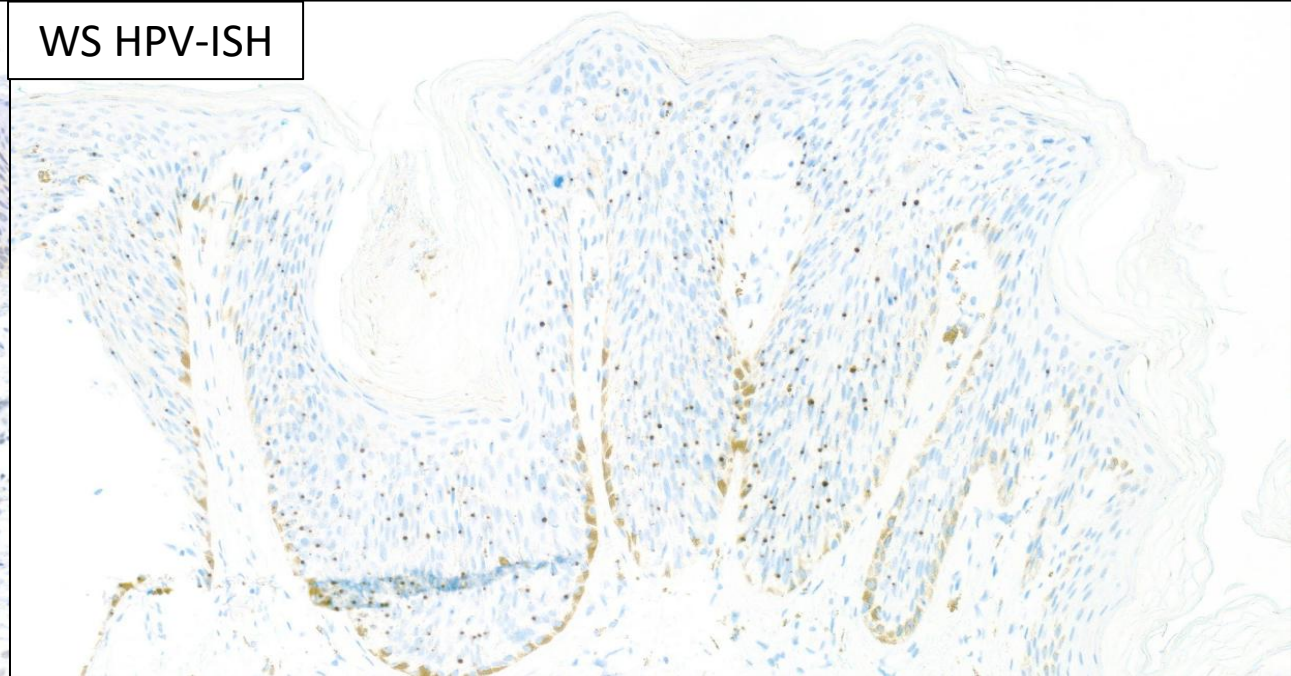
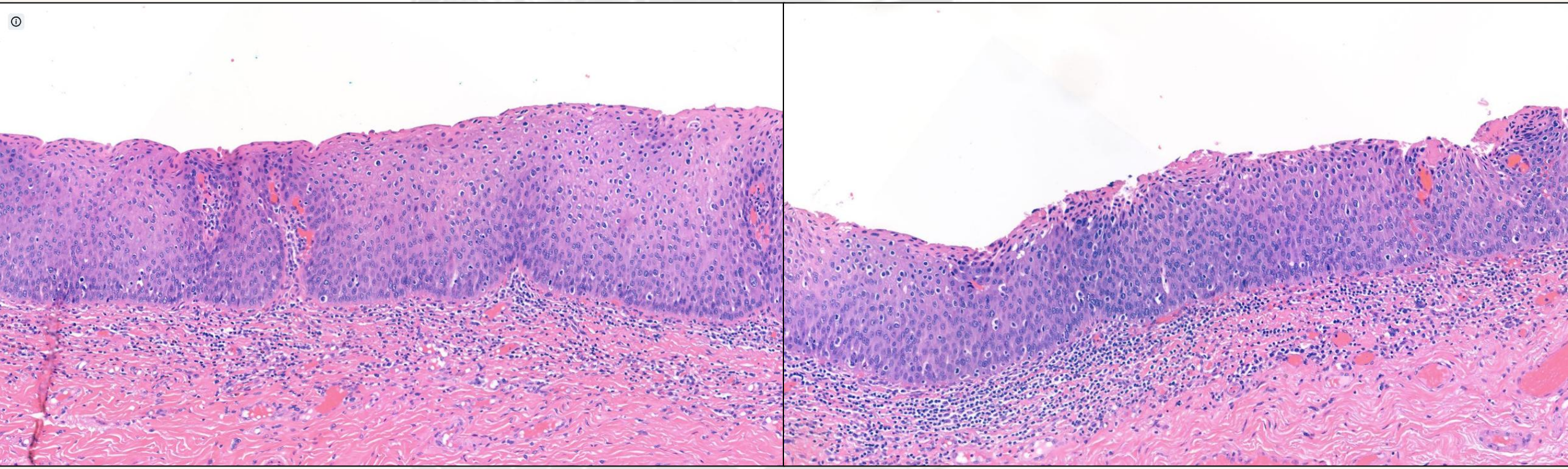


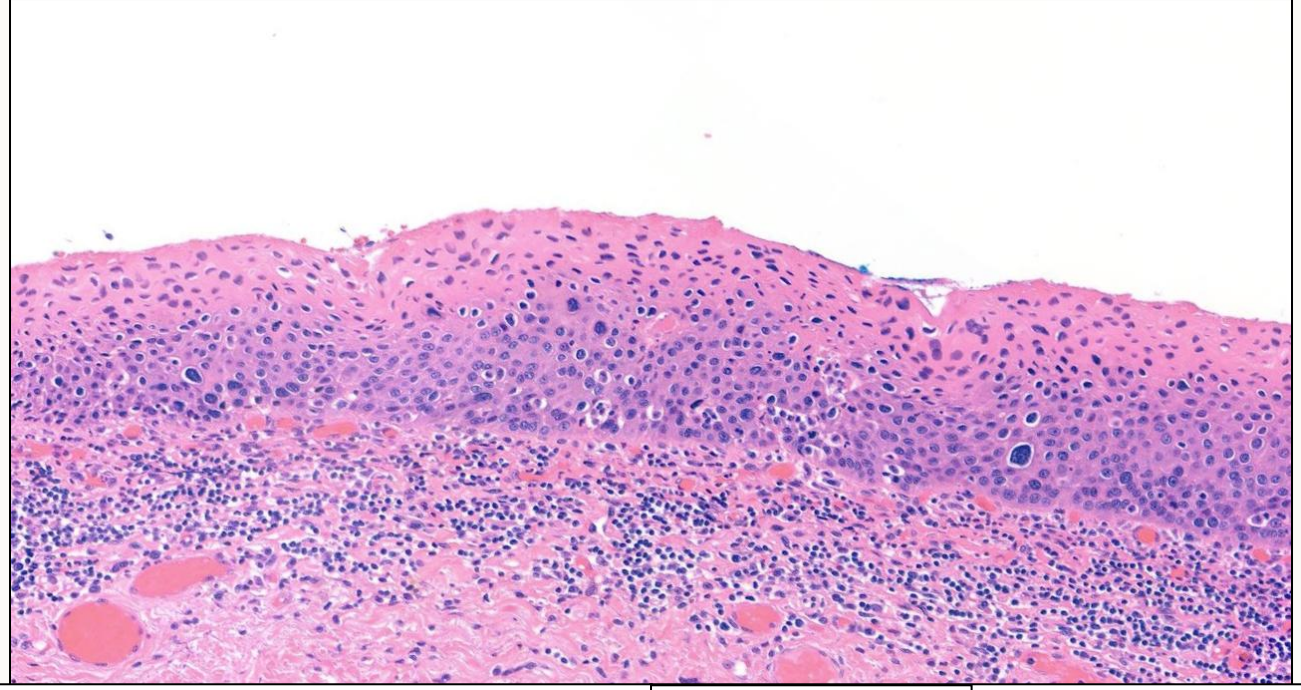
WS HPV-ISH



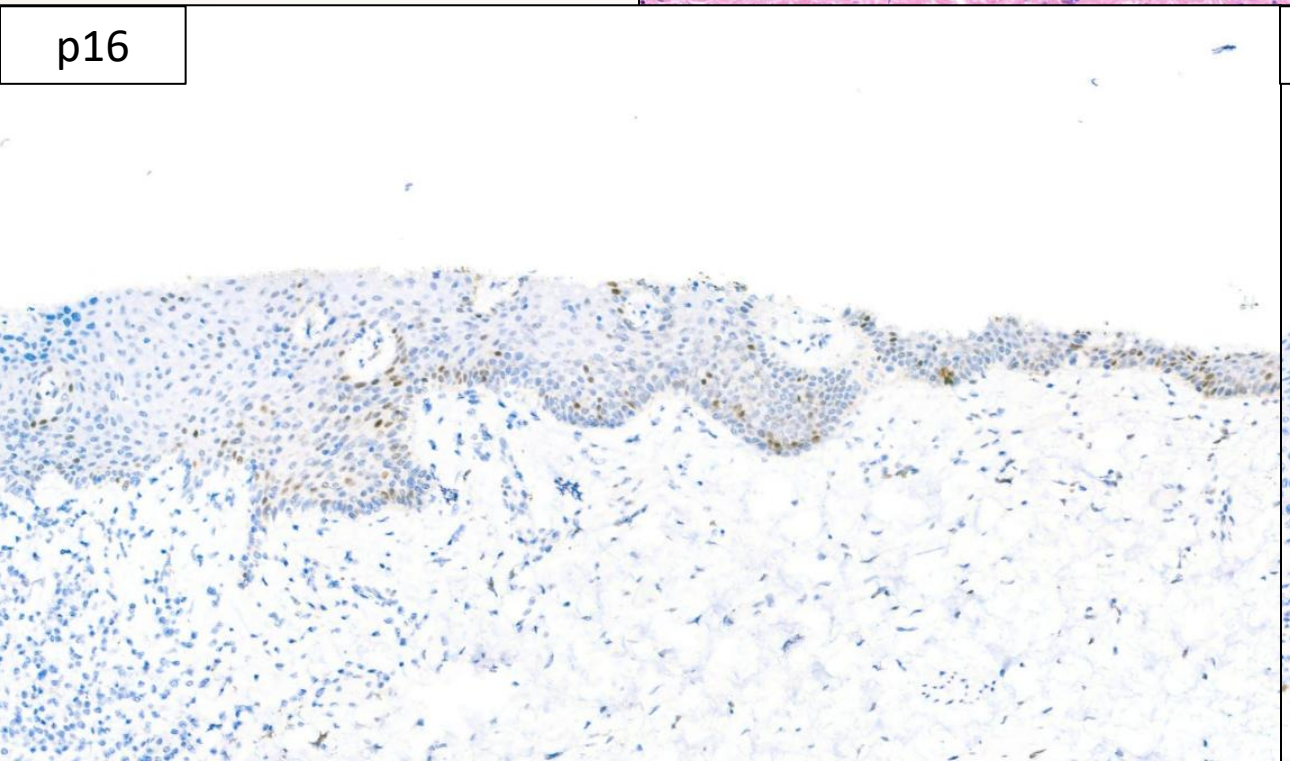
dVIN Mimicking HSIL



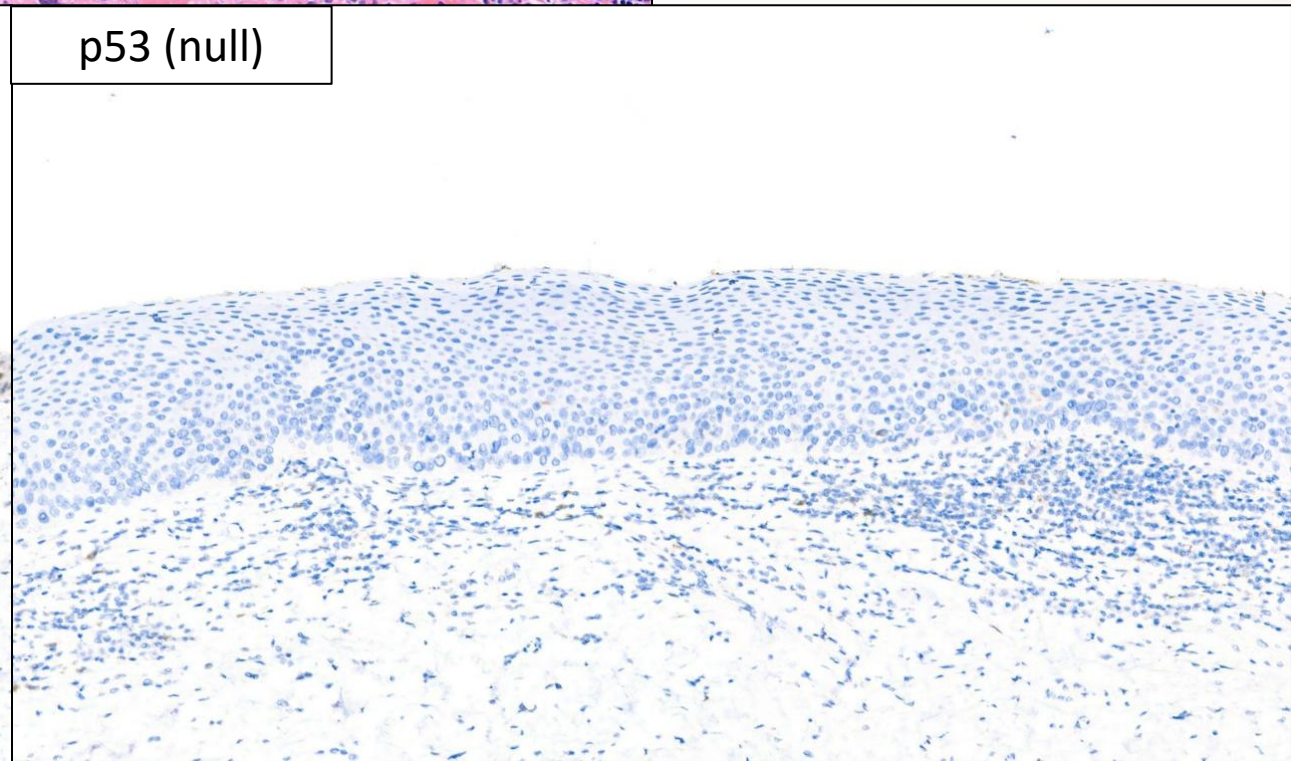
dVIN Mimicking HSIL



p16

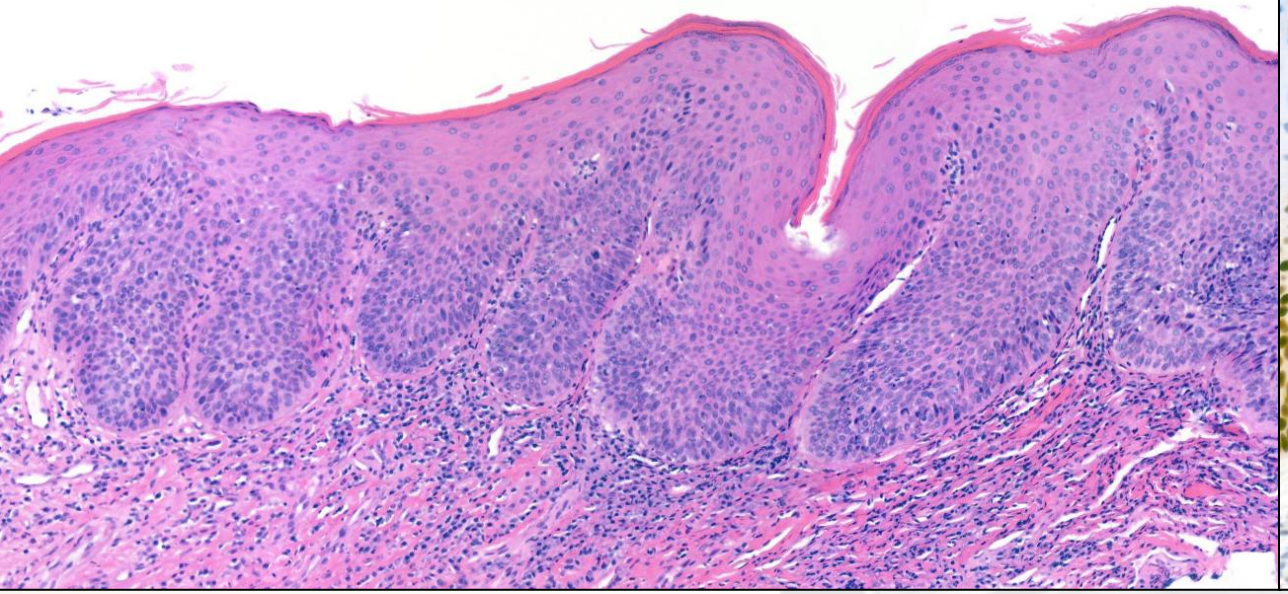


p53 (null)

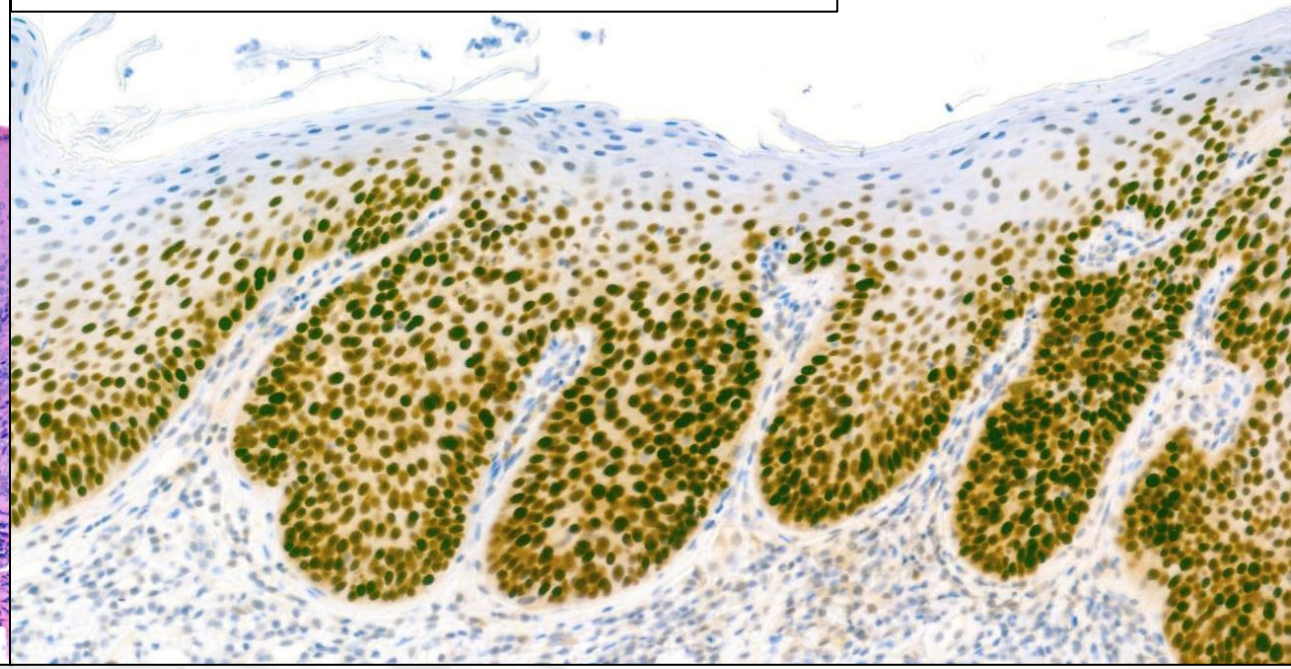


dVIN Mimicking HSIL

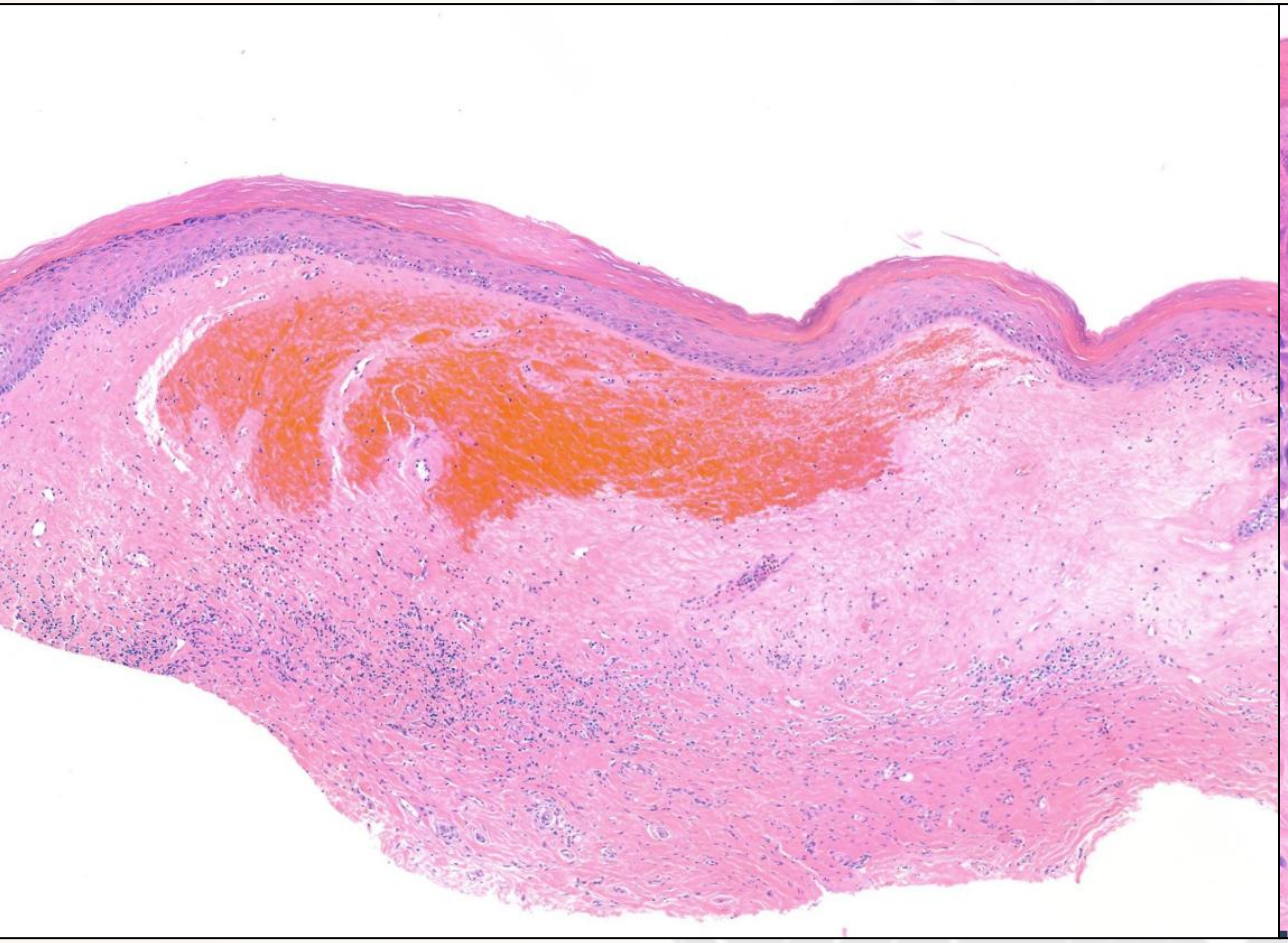
p16

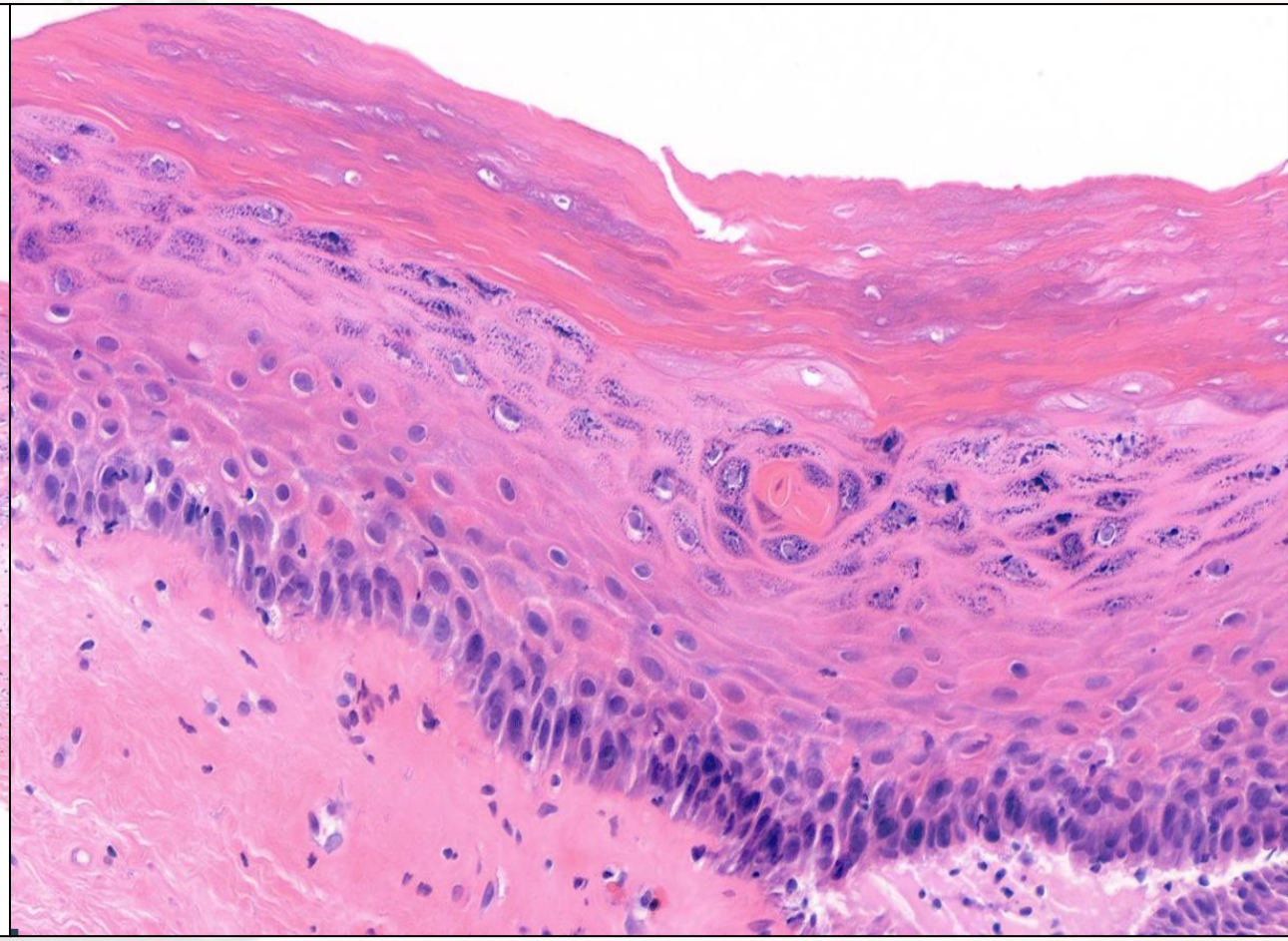


p53 (basal + parabasal overexpression)

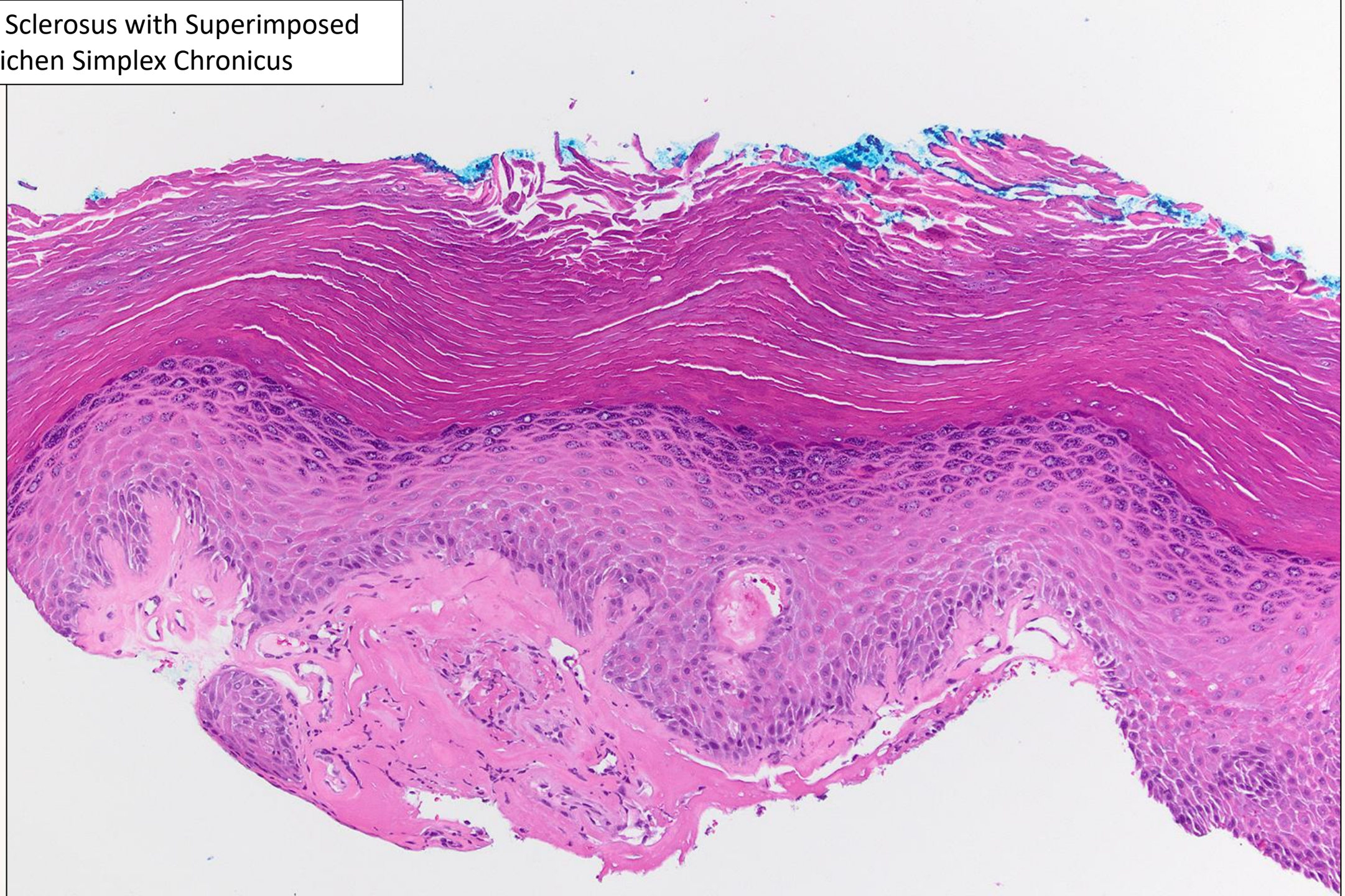


Lichen Sclerosus



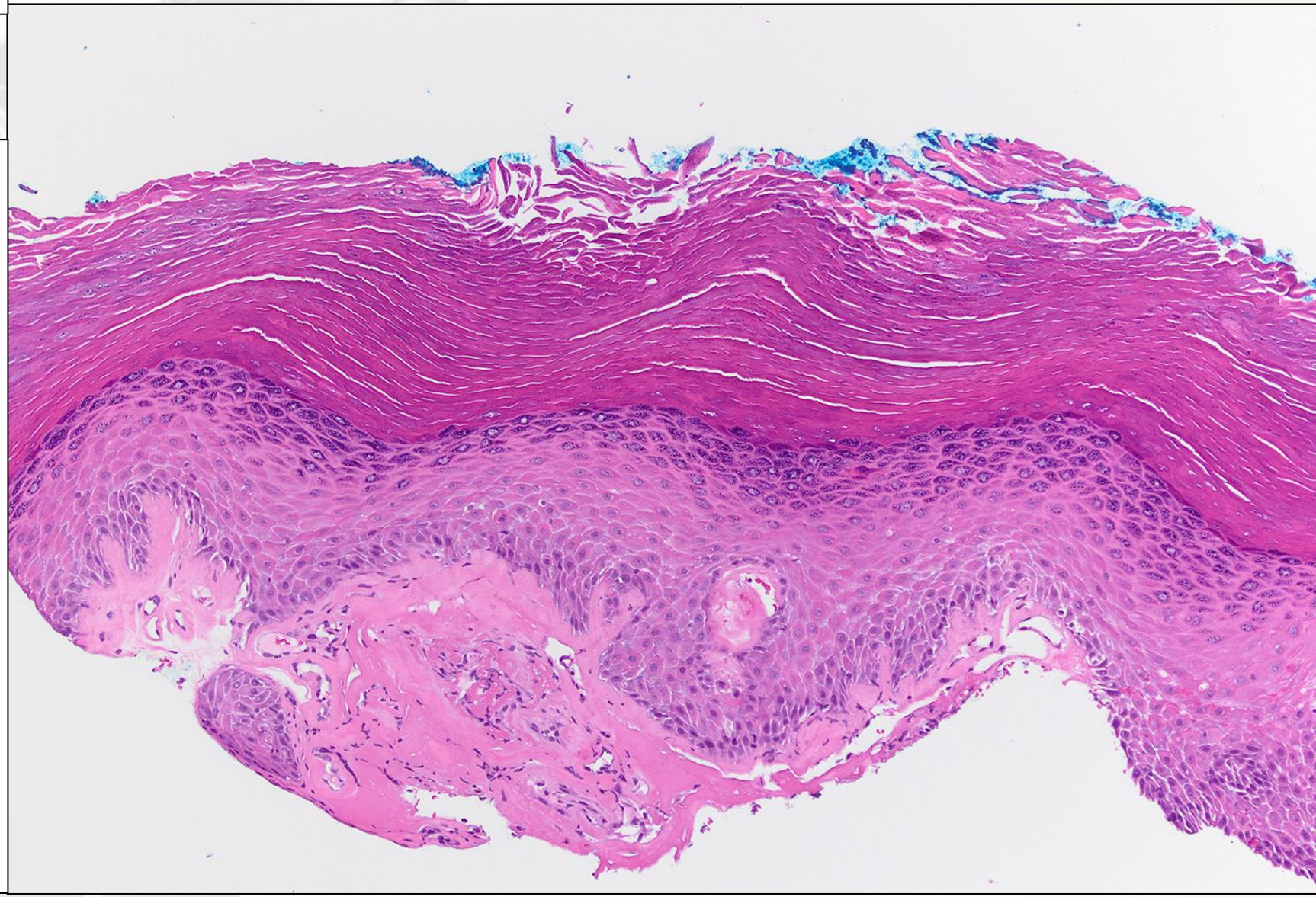


Lichen Sclerosus with Superimposed Lichen Simplex Chronicus

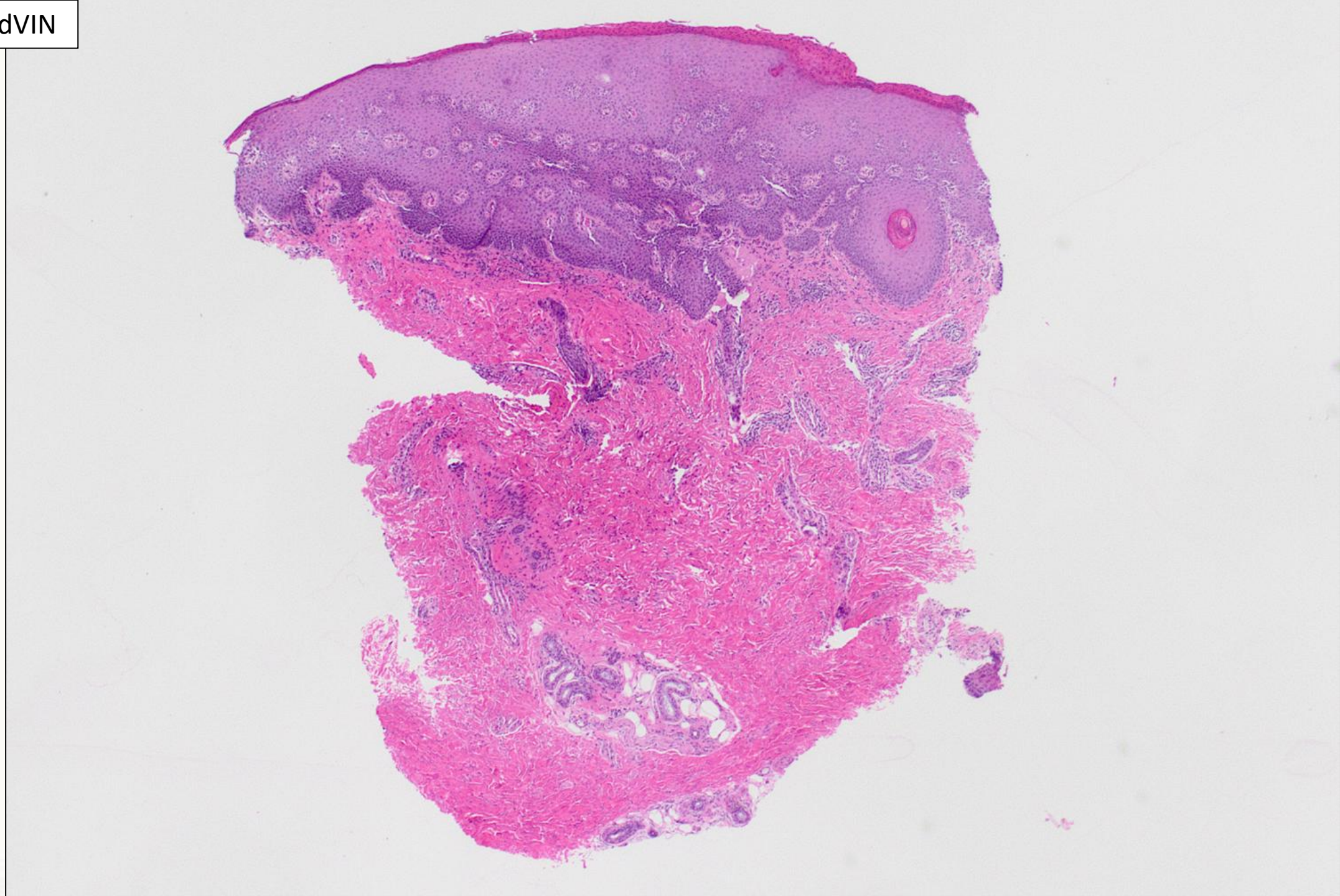


Lichen Sclerosus with Superimposed Lichen Simplex Chronicus

- Acanthosis and hyperkeratosis
- Scattered dyskeratotic cells
- Spongiosis
- May lack inflammation in late stages with advanced sclerosis
- Artifactual separation from underlying sclerotic stroma
- Lacks significant basal atypia
- Wild-type p53

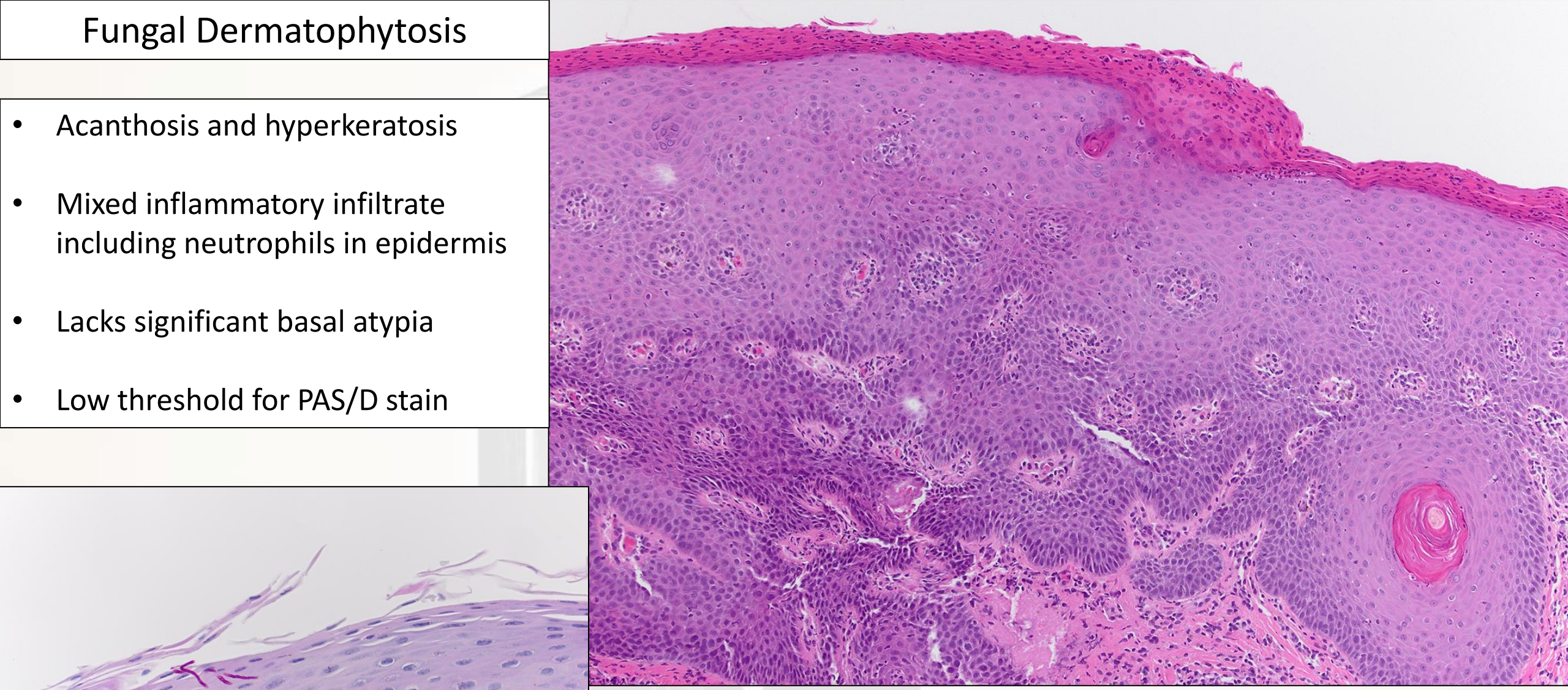


Potential Mimic of dVIN

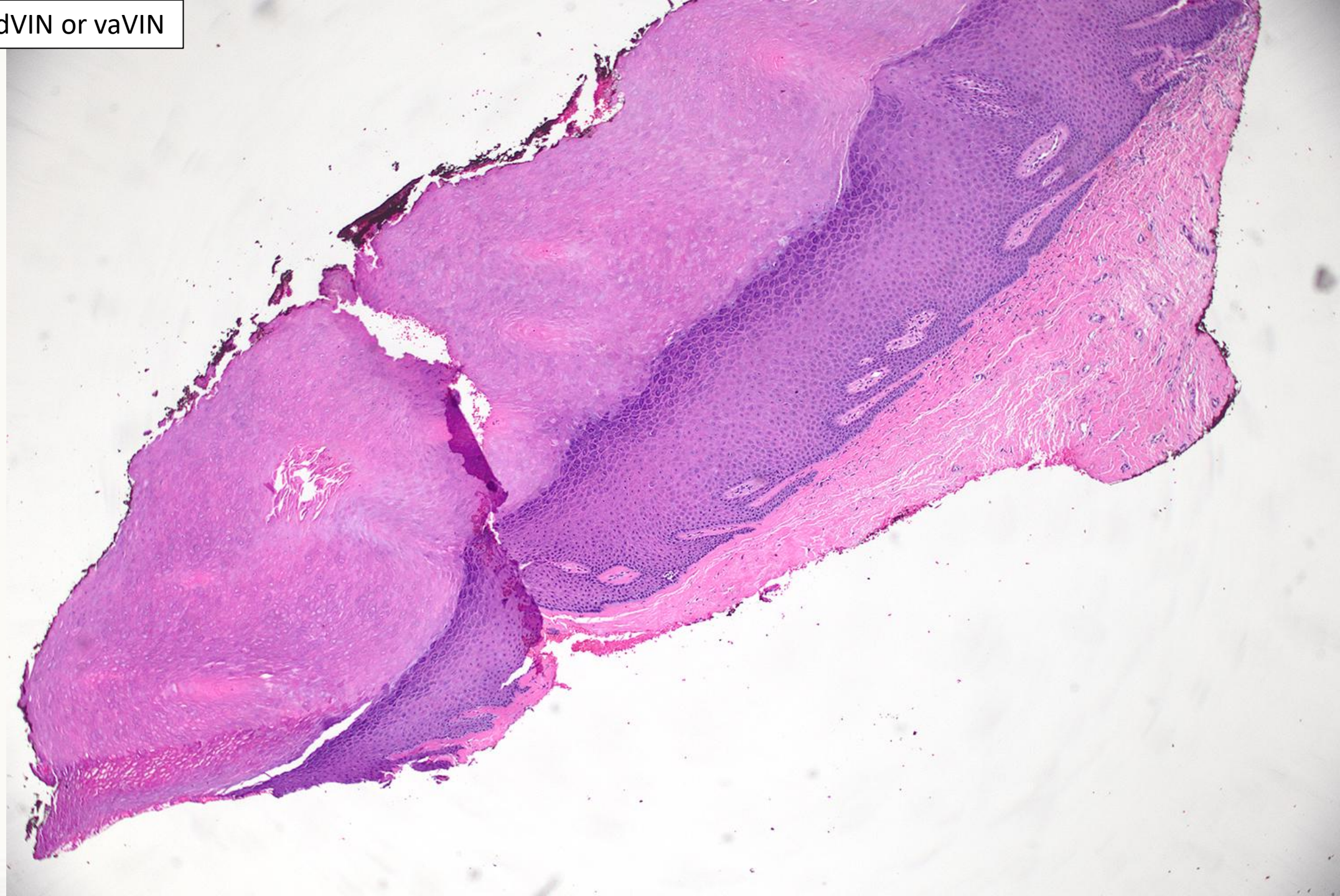


Fungal Dermatophytosis

- Acanthosis and hyperkeratosis
- Mixed inflammatory infiltrate including neutrophils in epidermis
- Lacks significant basal atypia
- Low threshold for PAS/D stain



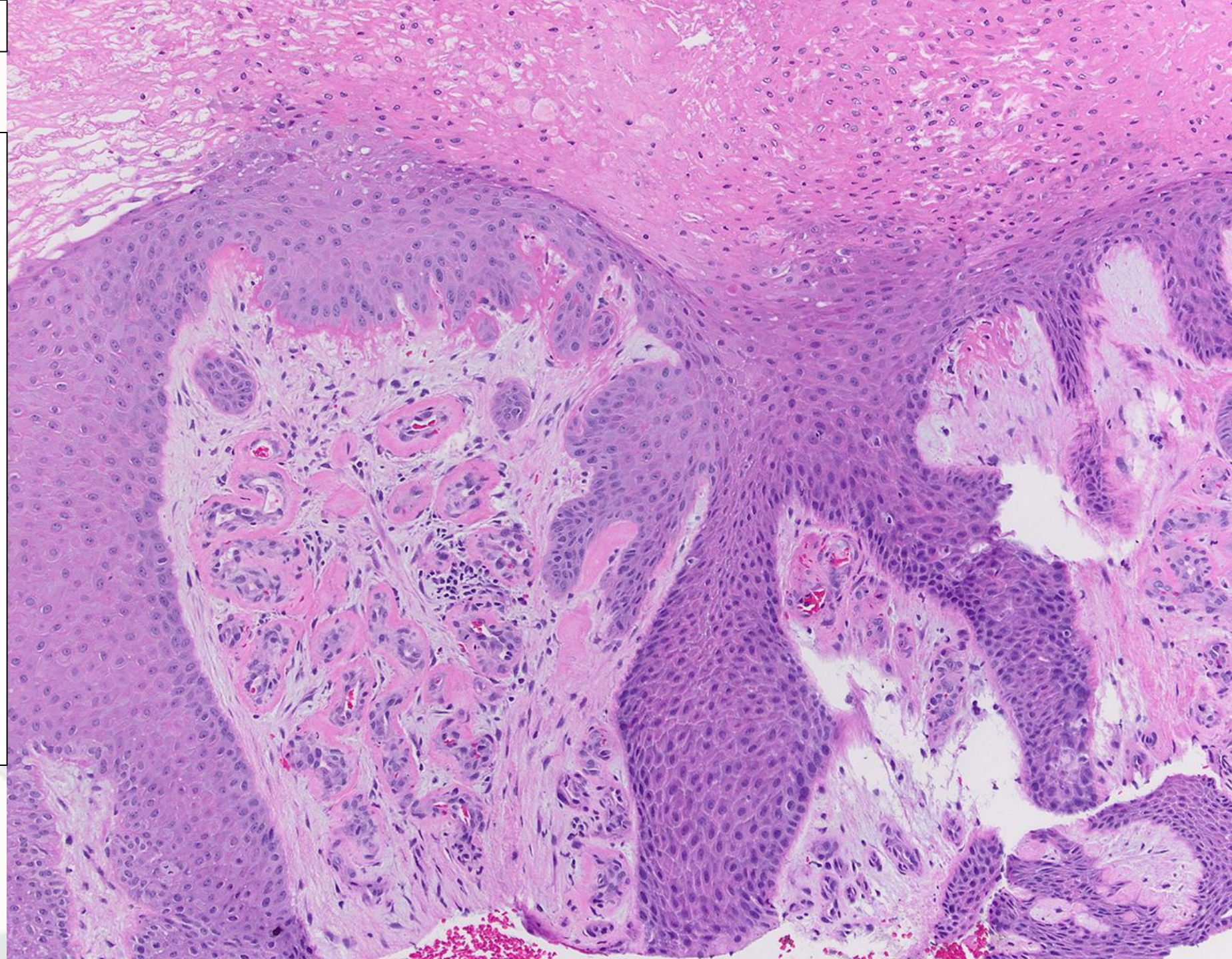
Potential Mimic of dVIN or vaVIN



Radiation Dermatitis

- Occurs 3-20 years after radiation exposure
- Epidermal necrosis, spongiosis, and/or basal keratinocyte degeneration
- Dermal edema with or without inflammatory infiltrate
- Capillary vascular proliferation, hyalinization, and/or thrombosis in superficial dermis
- Scattered degenerative atypia in dermal fibroblasts

- **Often a diffuse process
  - Excludes vaVIN

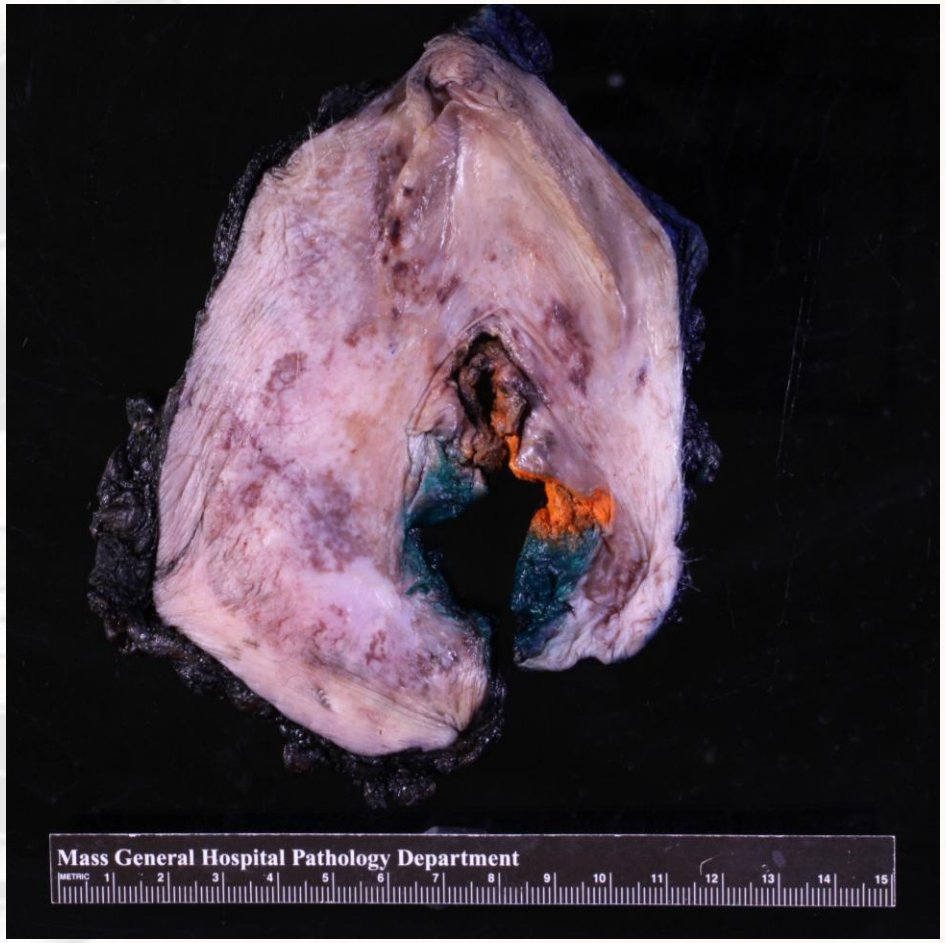


Summary: Vulvar Squamous Precursors

- Interpret morphology in context of p16 and p53 stains
  - Do not diagnose dVIN without an aberrant p53
  - If cytologic atypia is present without abnormal p16 or p53
    - Consider HPV-ISH
    - Descriptive diagnosis if uncertain
- Exclude benign mimics including inflammatory etiologies
  - Low threshold for fungal stains with hyperkeratosis
- Essential features for vaVIN
  - Discrete exophytic lesion
  - Lack of cytologic atypia
  - Very thick, aberrant maturation

Mutant p53 at Resection Margins

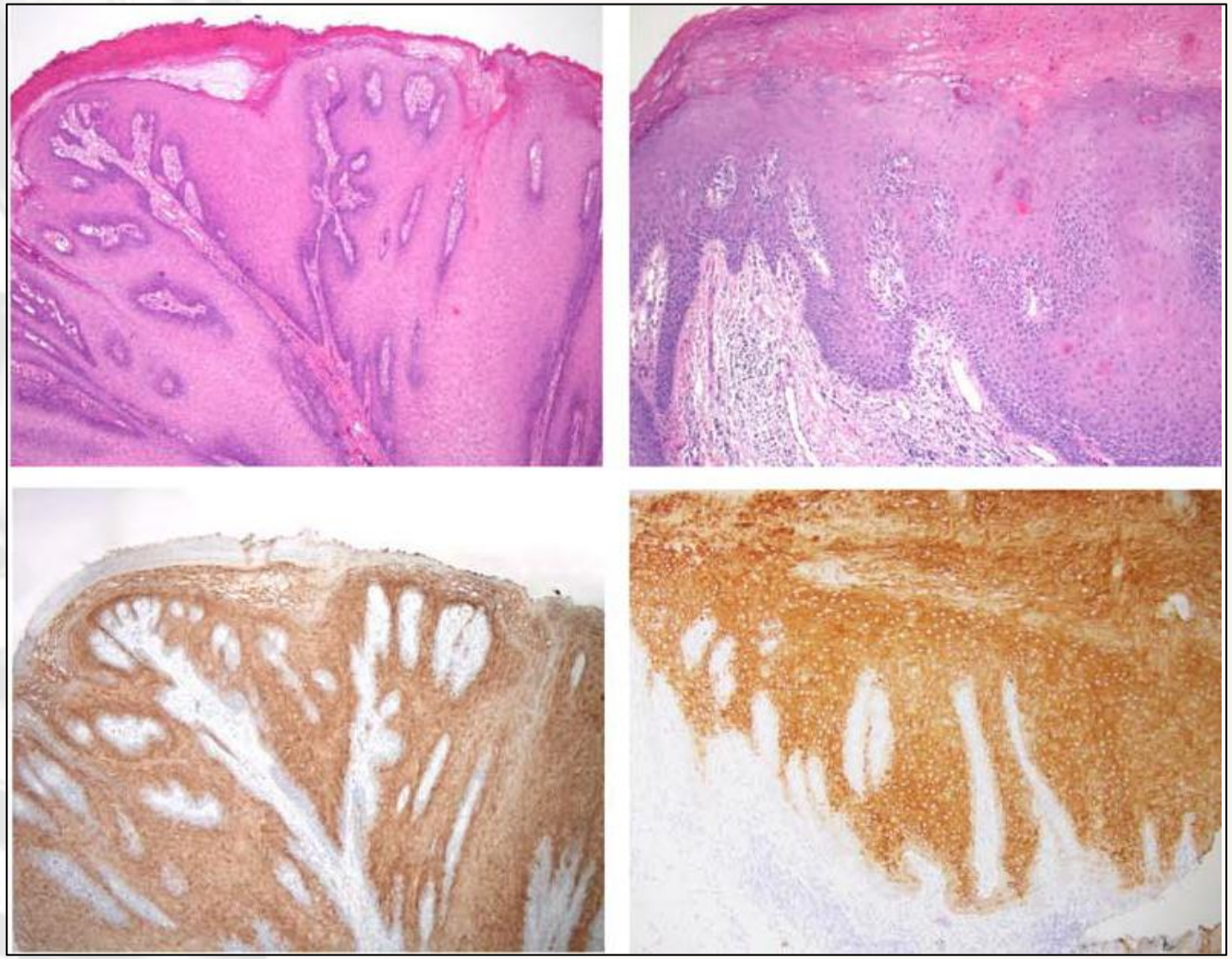
- Mutant p53 staining without morphologic dVIN
  - Recurrence rates similar to dVIN at margin (75% each compared to 33% with negative margins)
- Implications for practice?
  - We do not routinely stain all margins for p53
  - Note presence of mutant pattern at margin if stained for other indications



Thompson EF, et al. Significance of p53 and presence of differentiated vulvar intra-epithelial neoplasia (dVIN) at resection margin in early stage human papillomavirus-independent vulvar squamous cell carcinoma. Int J Gynecol Cancer. 2022 Sep 13:ijgc-2022-003763. doi: 10.1136/ijgc-2022-003763.

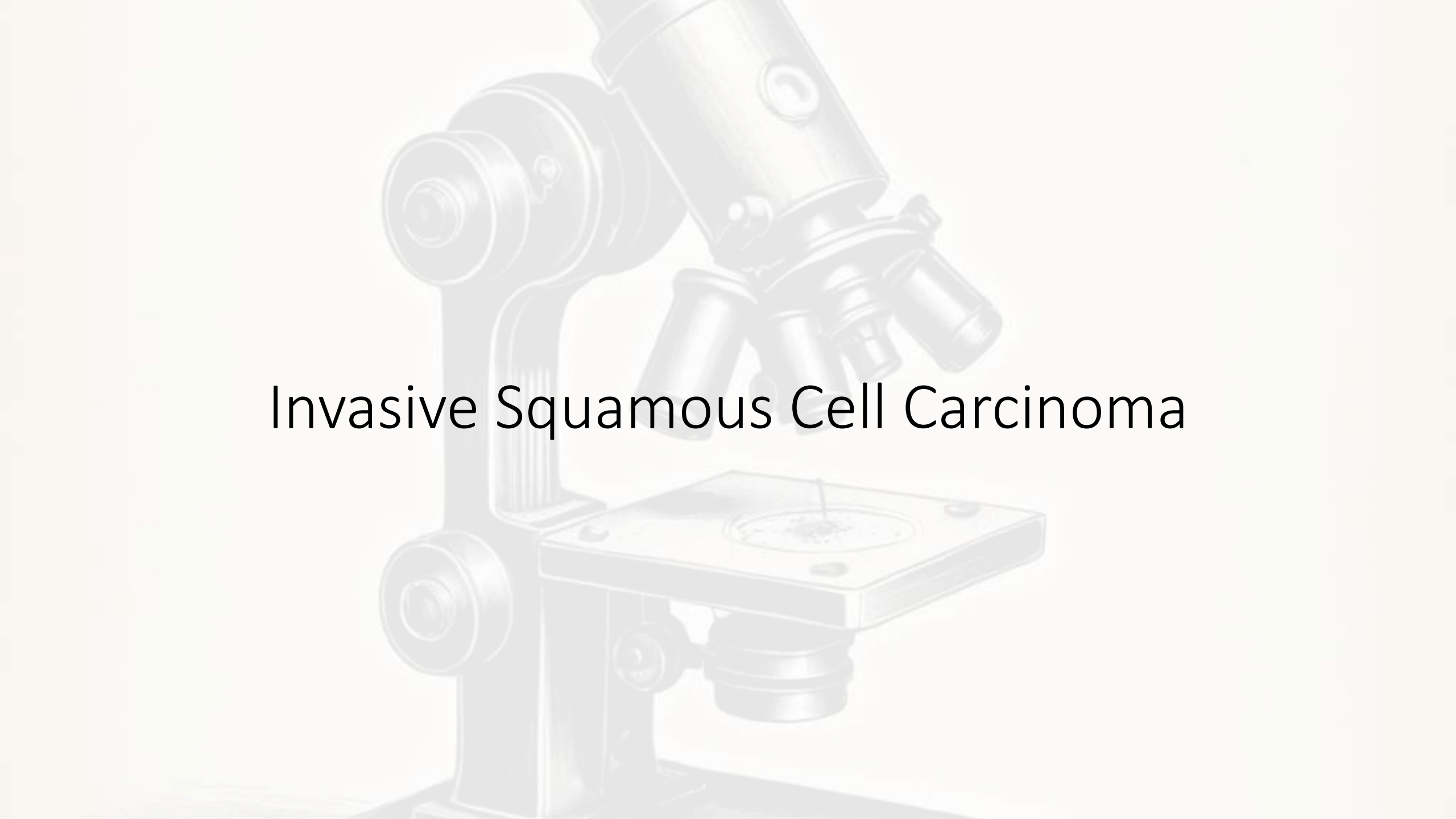
Cytokeratin 17

- Normally expressed in basal layer and suprabasal cells of hair follicles
- Overexpressed in dVIN, vaVIN, and vulvar SCC
- May be used as diagnostic adjunct in difficult cases



Podoll MB, Singh N, Gilks CB, Moghadamfalahi M, Sanders MA. Assessment of CK17 as a Marker for the Diagnosis of Differentiated Vulvar Intraepithelial Neoplasia. Int J Gynecol Pathol. 2017 May;36(3):273-280.

Hartsough EM, Nazarian RM, Watkins JC. CK17 Immunohistochemistry Is a Useful Adjunct in the Diagnosis of HPV-independent, TP53-wild-type Verruciform/Acanthotic Vulvar Intraepithelial Neoplasia (vaVIN). Am J Surg Pathol. 2025 Jul 1;49(7):658-662.



Invasive Squamous Cell Carcinoma

Vulvar Squamous Cell Carcinoma

WHO Classification of Female Genital Tumours 5th Edition

HPV-Associated	HPV-Independent

HPV-Associated	HPV-Independent *TP53* Mutant	HPV-Independent *TP53* Wild-Type

Kortekaas KE, et al. Vulvar cancer subclassification by HPV and p53 status results in three clinically distinct subtypes. Gynecol Oncol. 2020 Dec;159(3):649-656.

HPV-associated

- Incidence varies widely geographically (20%-75%)
- Relatively favorable prognosis
- Various histologic patterns reported, none are specific to this subtype

HPV-independent, *TP53* mutant

- Frequency of HPVI (vs. HPVA) varies widely based on testing method (25-80%)
  - ~80% of HPVI SCC is *TP53* mutant
- Poor prognosis compared to HPVA SCC
- Most are keratinizing, although no morphologic features are specific for this subtype

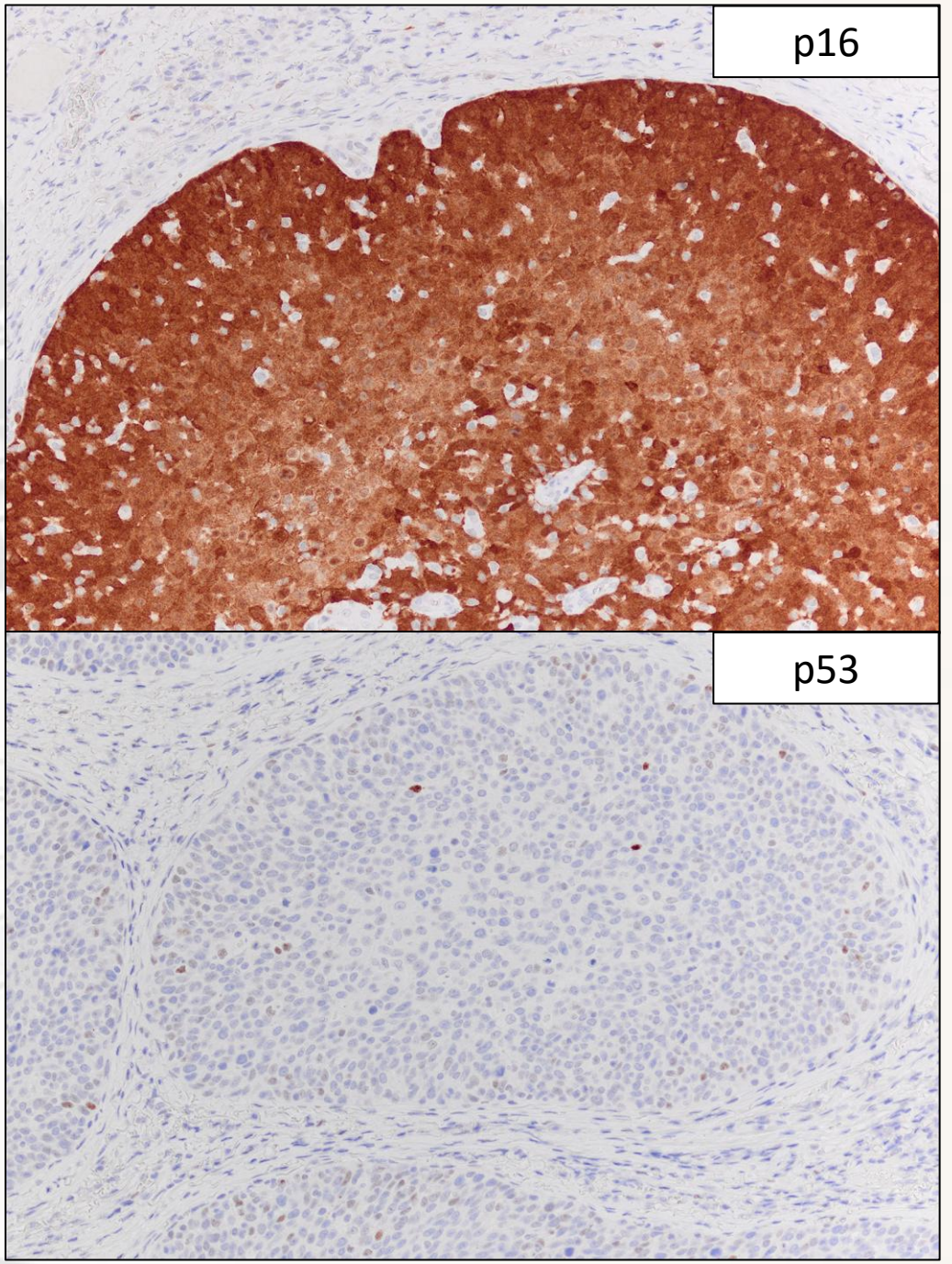
HPV-independent, *TP53* wild-type

- Frequency of HPVI (vs. HPVA) varies widely based on testing method (25-80%)
  - ~20% ofHPVI SCC is *TP53* wild-type
- Intermediate prognosis, although historic series do not always differentiate from p53 mutant SCC
- Most low-grade carcinomas with pushing patterns of invasion (“verrucous carcinoma”) fall in this subtype, although no morphologic features are specific

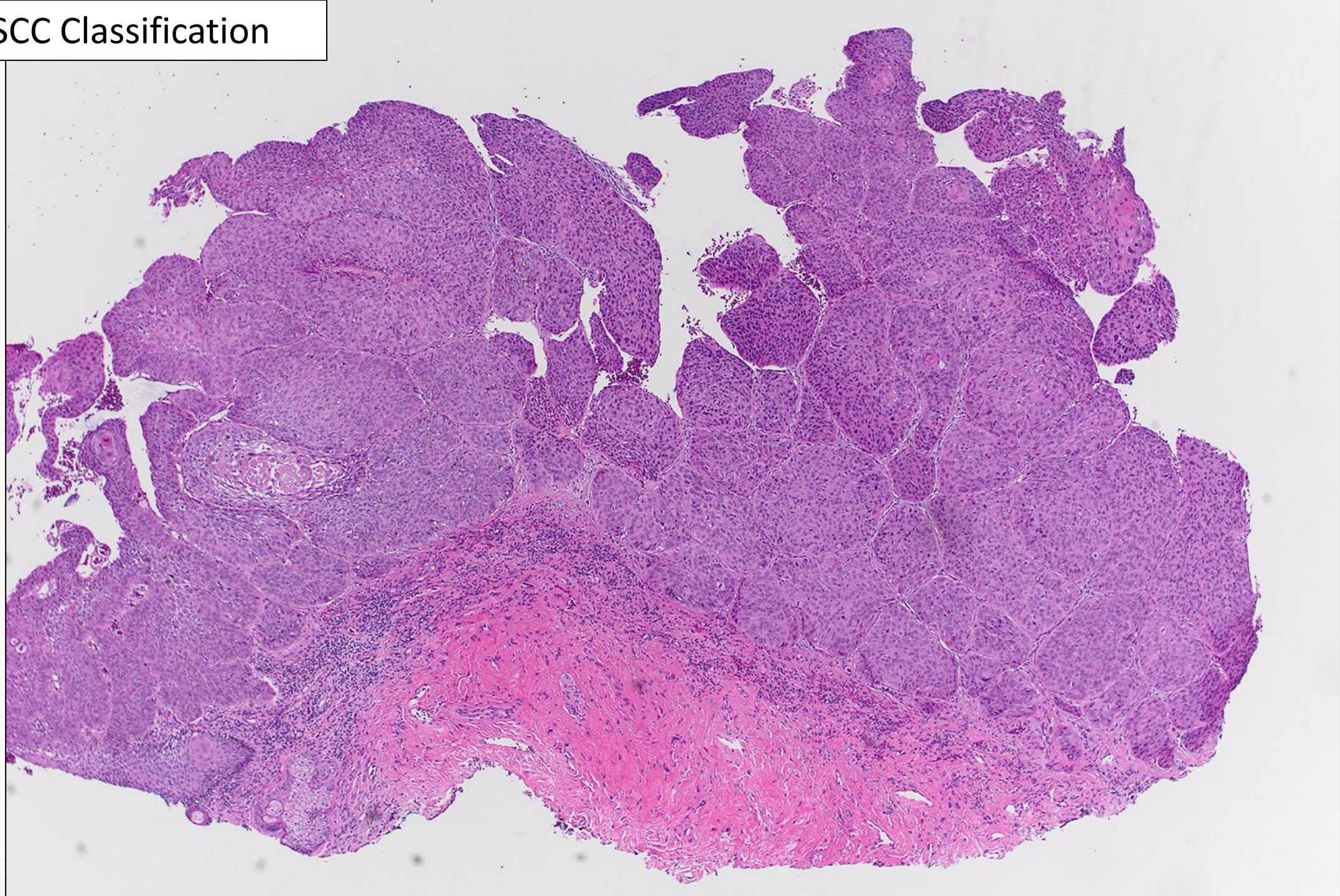
Histologic grading has no impact on prognosis

Identifying HPV-Association

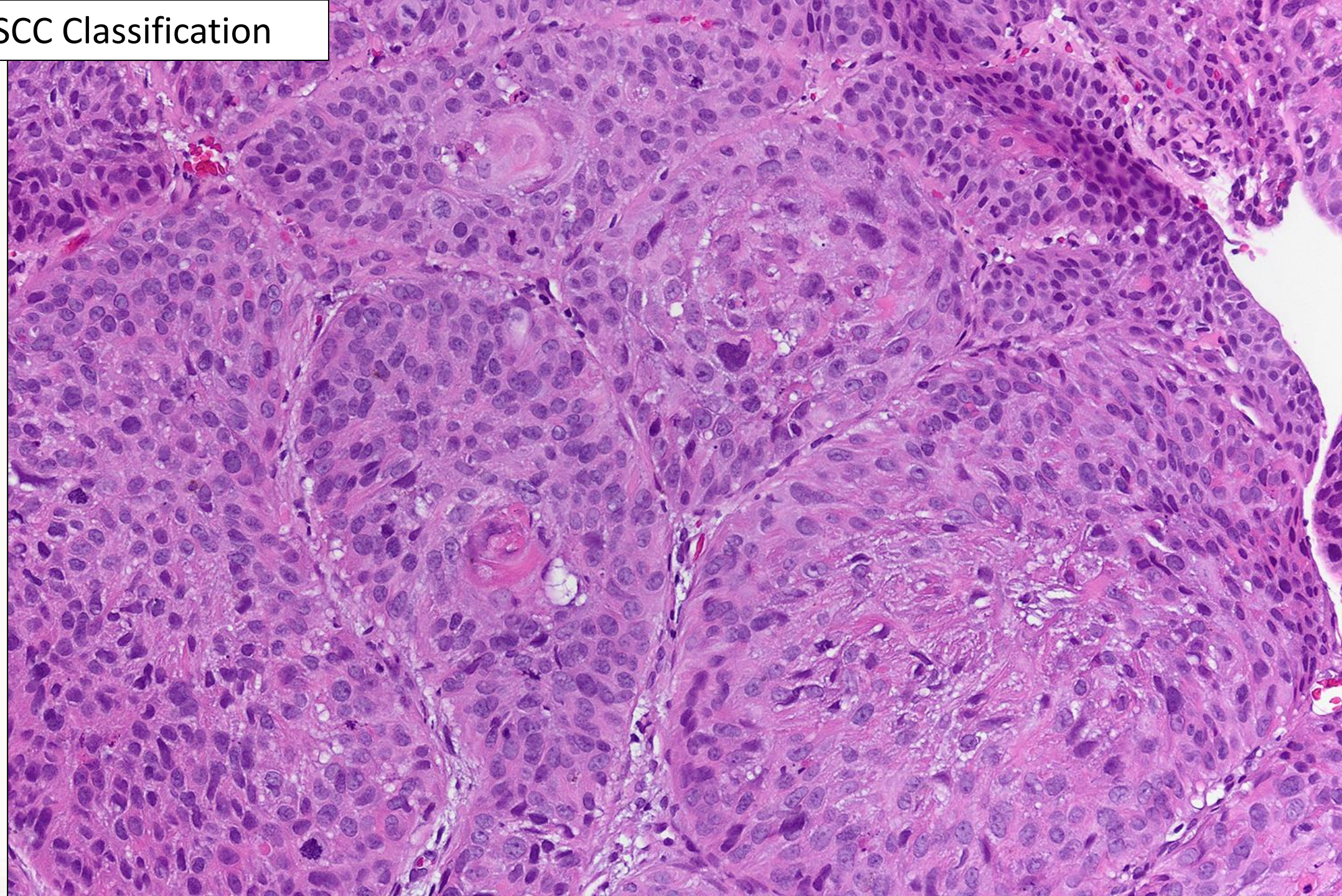
- p16 and p53 IHC for every invasive squamous cell carcinoma
- Associated precursor lesions
- HPV testing – when p16/p53 are indeterminate
  - In situ hybridization
  - PCR
- *TP53* mutations rare in HPV-A SCC
  - Inactivation of p53 and Rb by E6 and E7 proteins
  - No selective advantage for *TP53* mutation



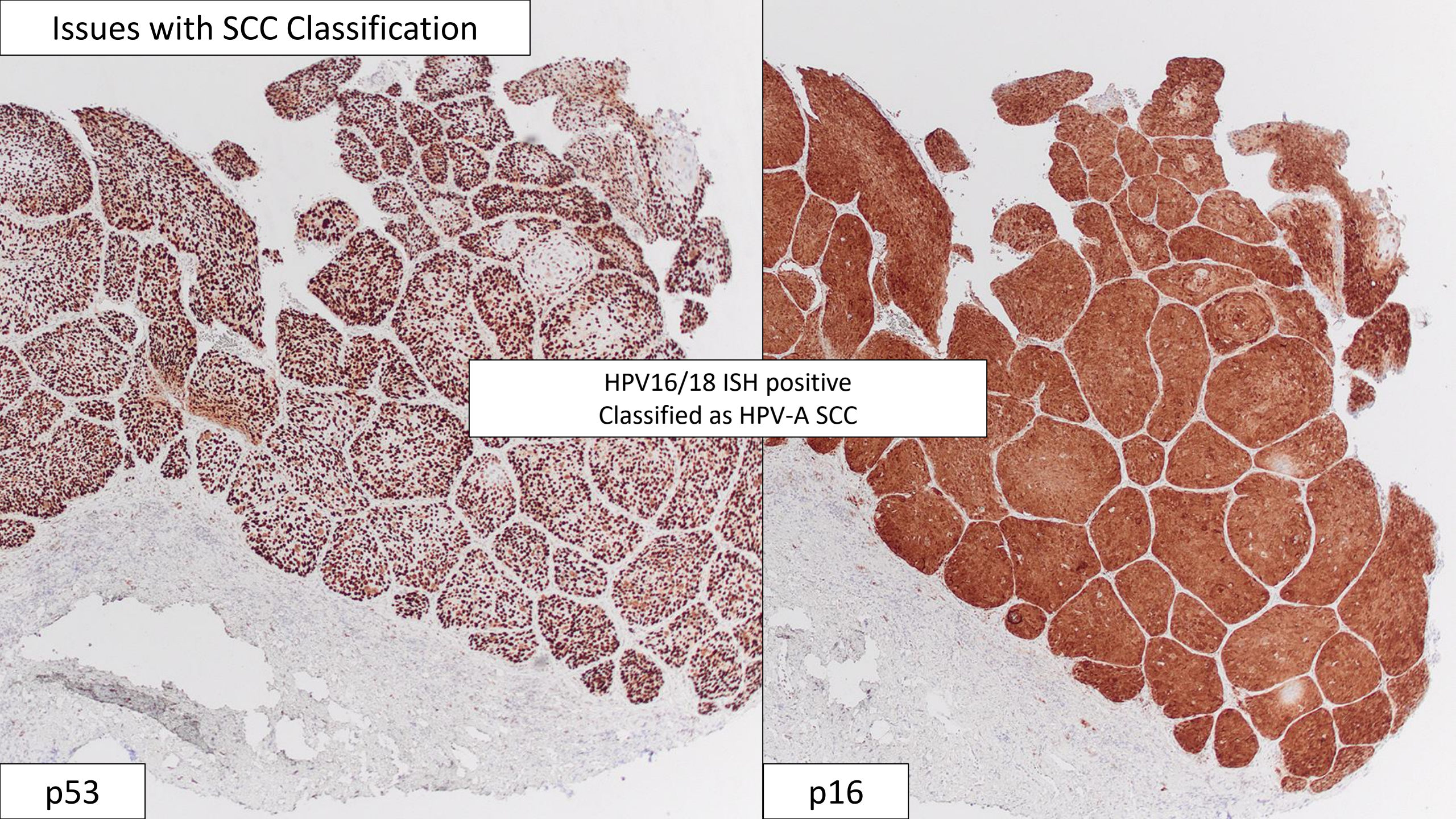
Issues with SCC Classification



Issues with SCC Classification



Issues with SCC Classification

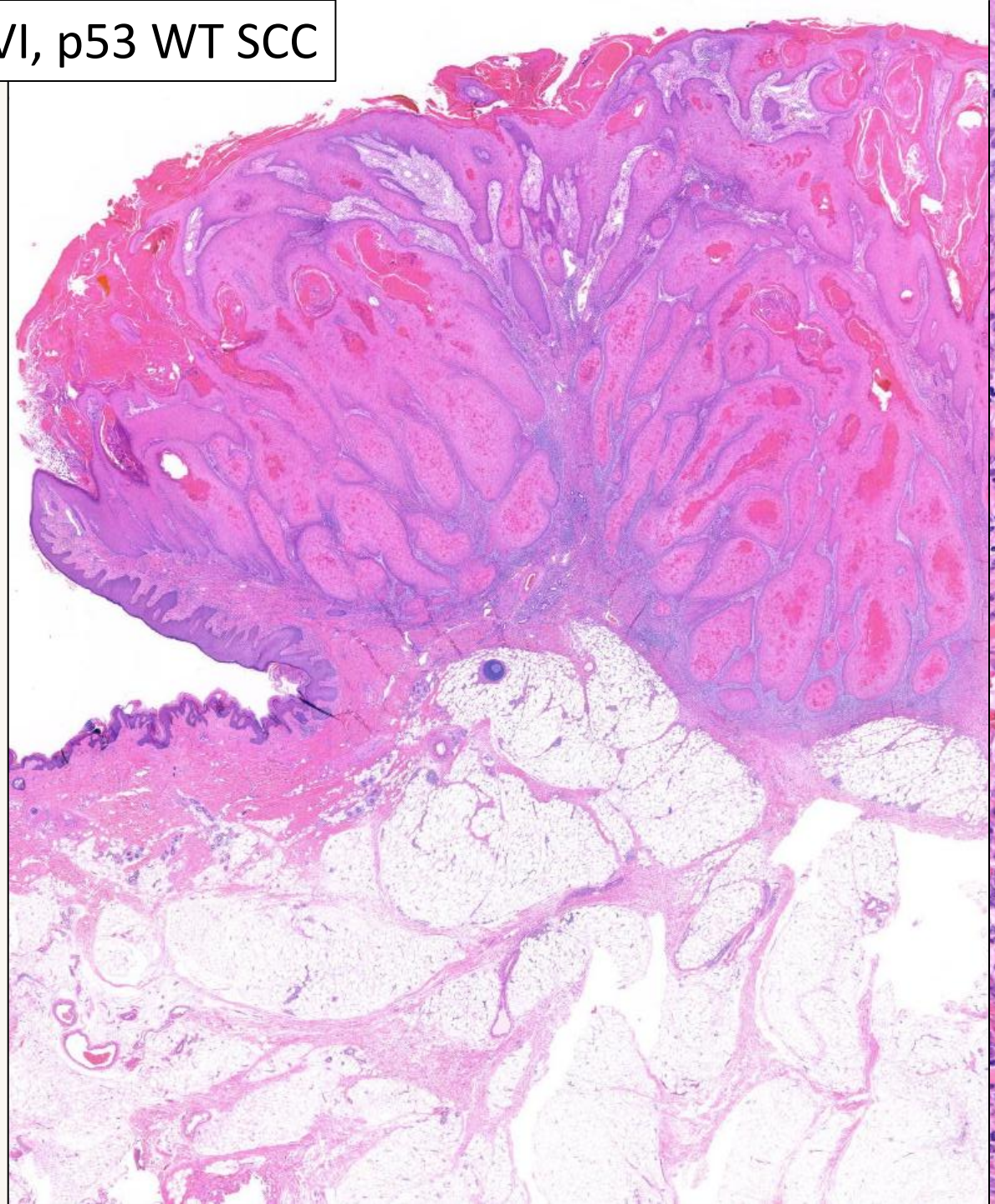


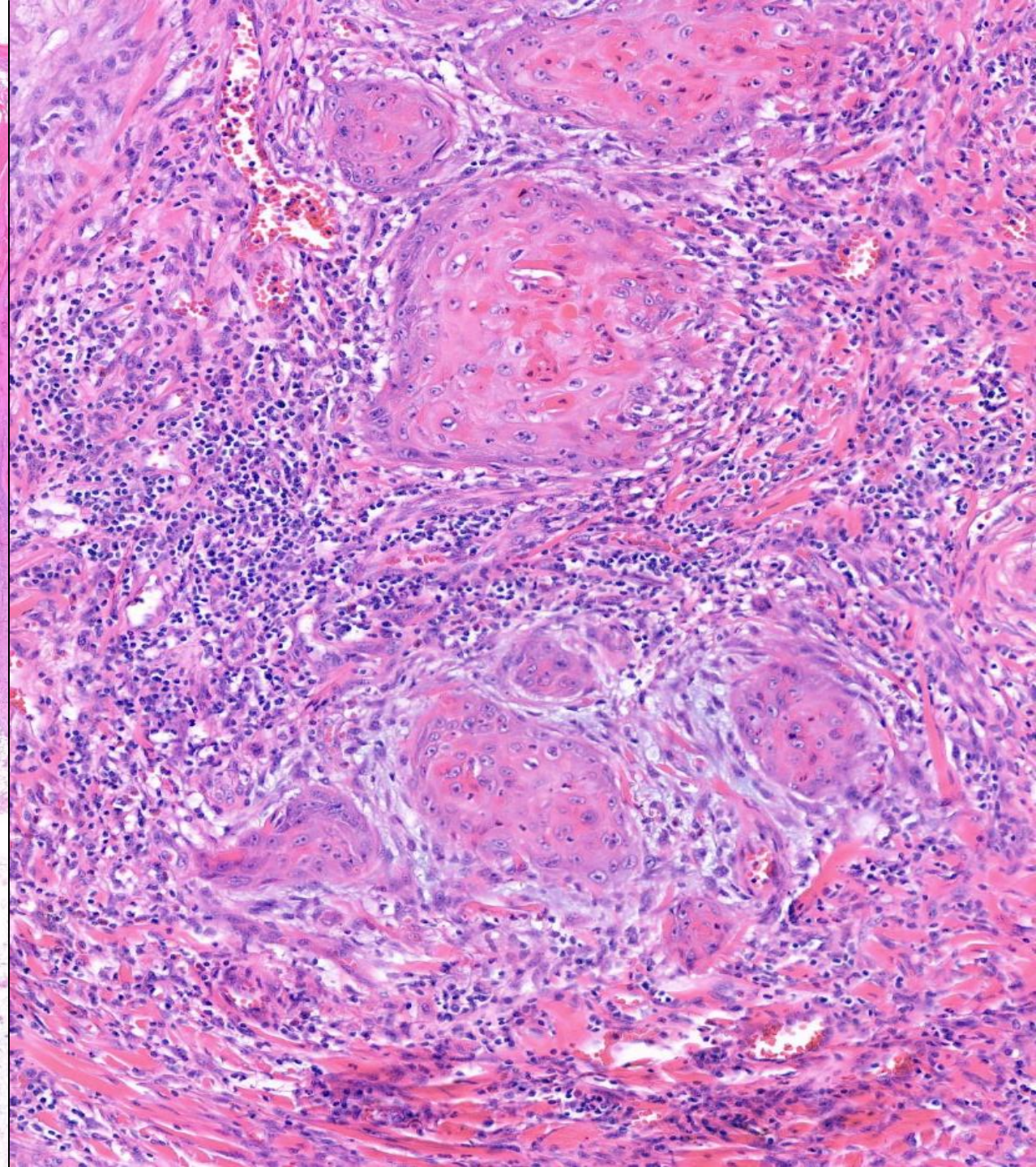
HPV16/18 ISH positive
Classified as HPV-A SCC

p53

p16

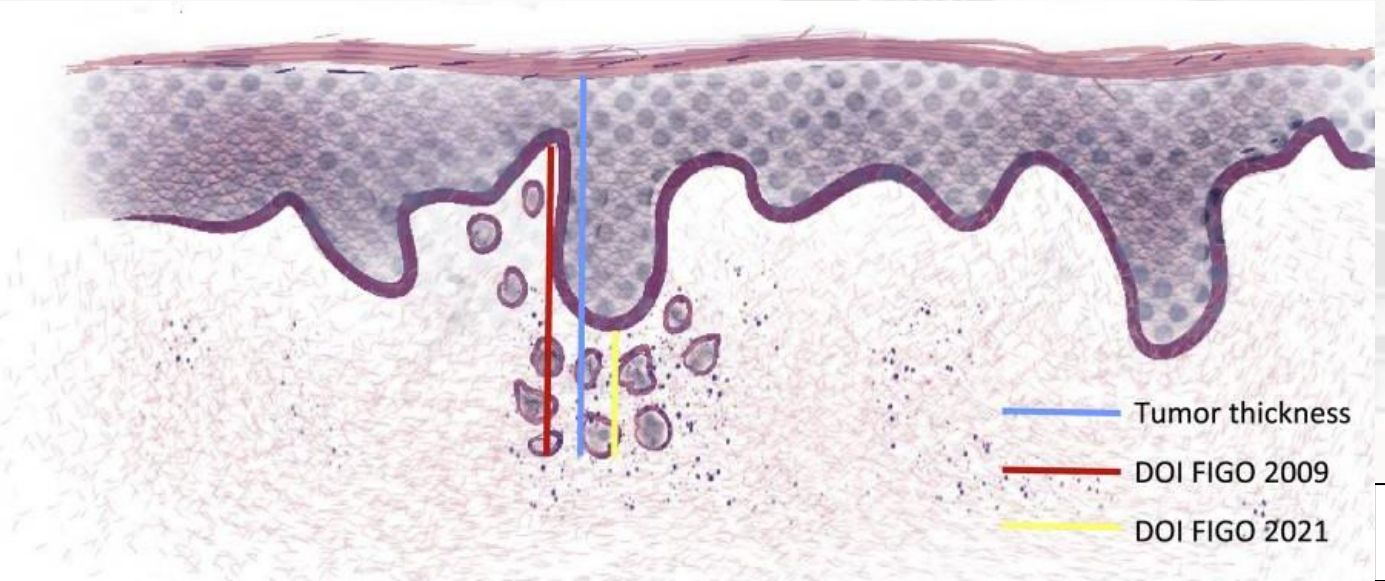
HPVI, p53 WT SCC





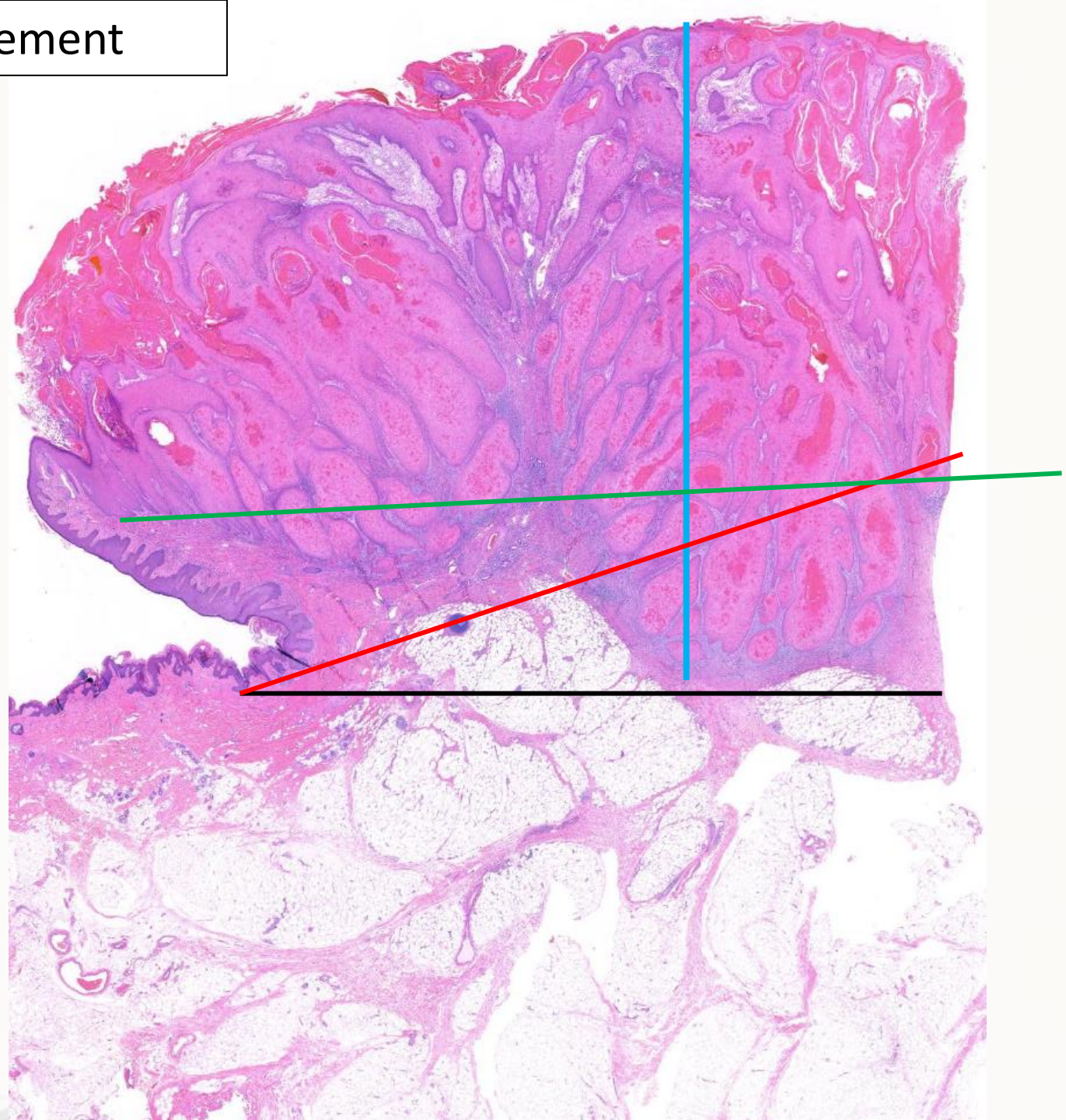
Depth of Invasion

- FIGO 2009
  - Measure from most superficial adjacent stromal papillae
- FIGO 2021
  - Measure from the basement membrane of the deepest adjacent (tumor-free) rete ridge to the deepest point of invasion

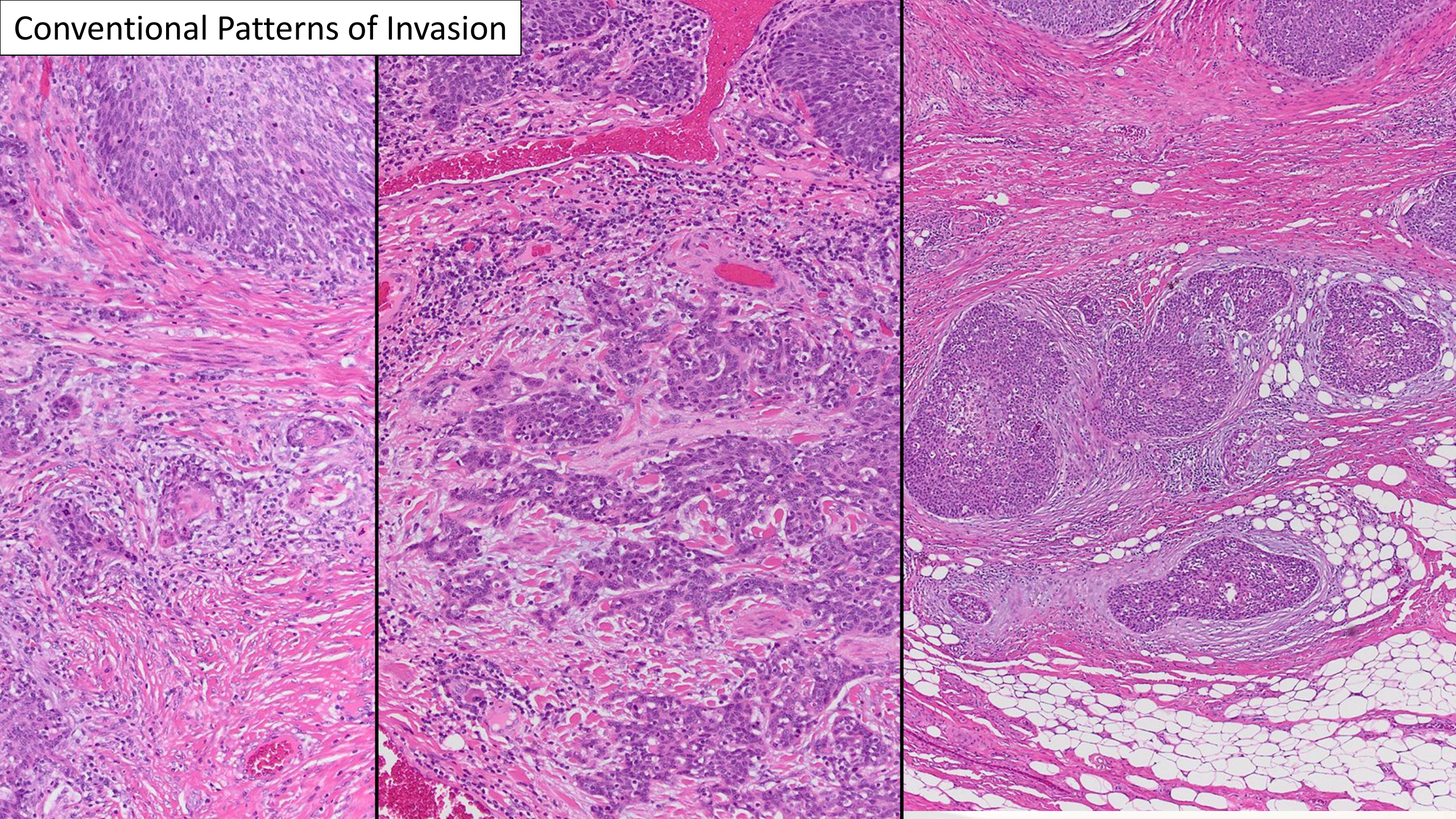


Bleeker MCG, et al. Does "One Size Fits All"? Rethinking FIGO Depth of Invasion Measurements in Vulvar Cancer. Int J Gynecol Pathol. 2024 Sep 1;43(5):457-463.

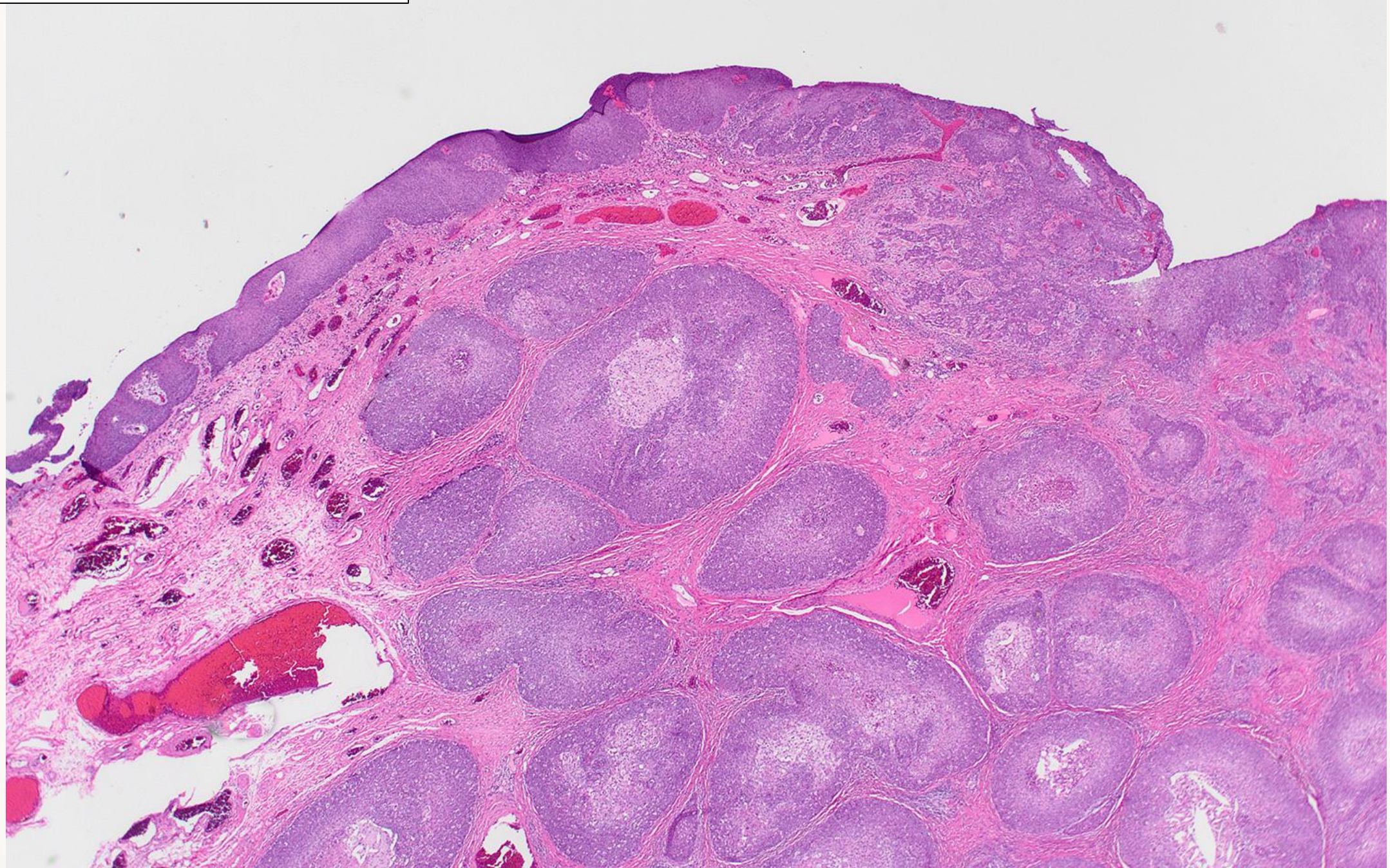
Issues with Measurement



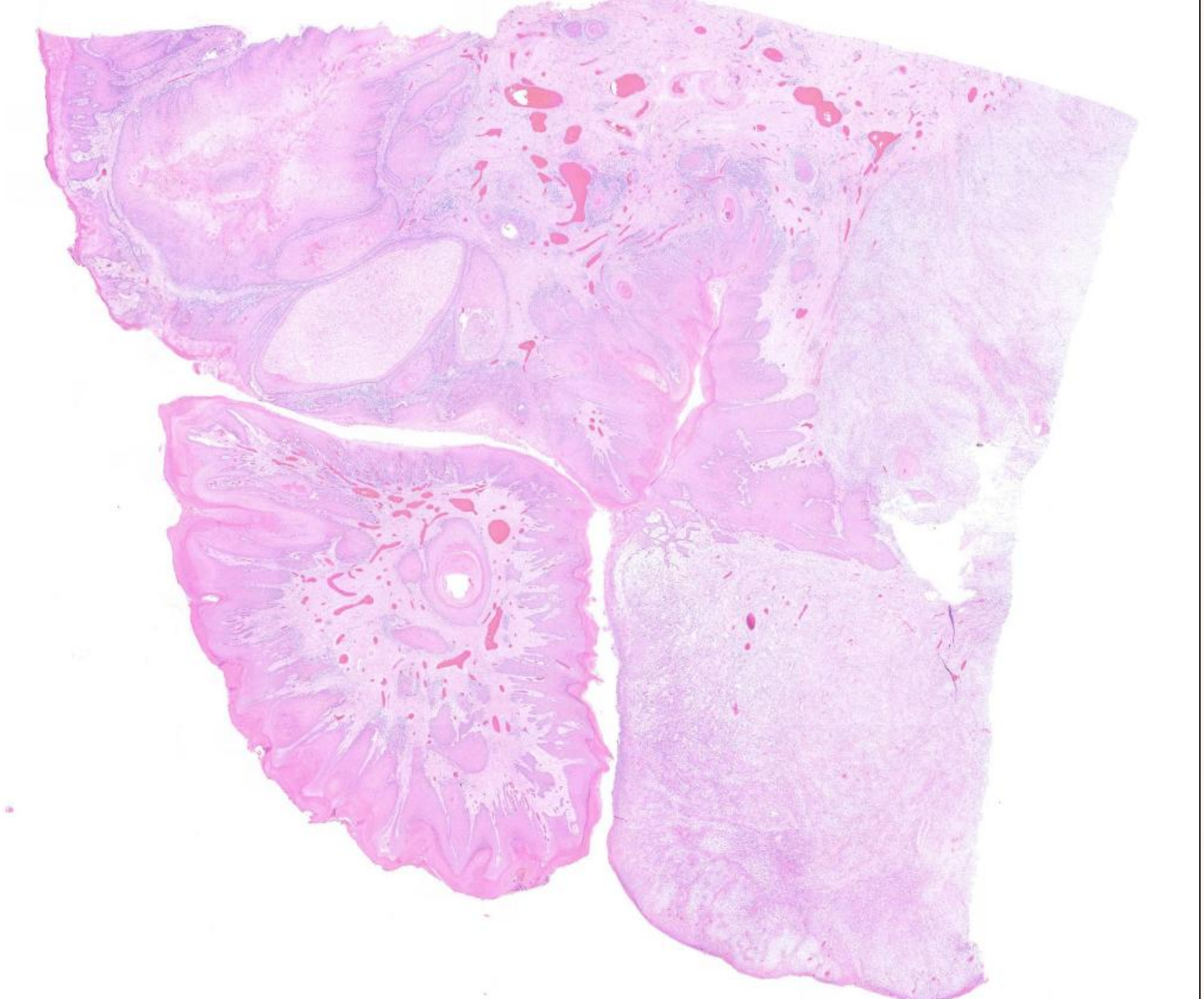
Conventional Patterns of Invasion



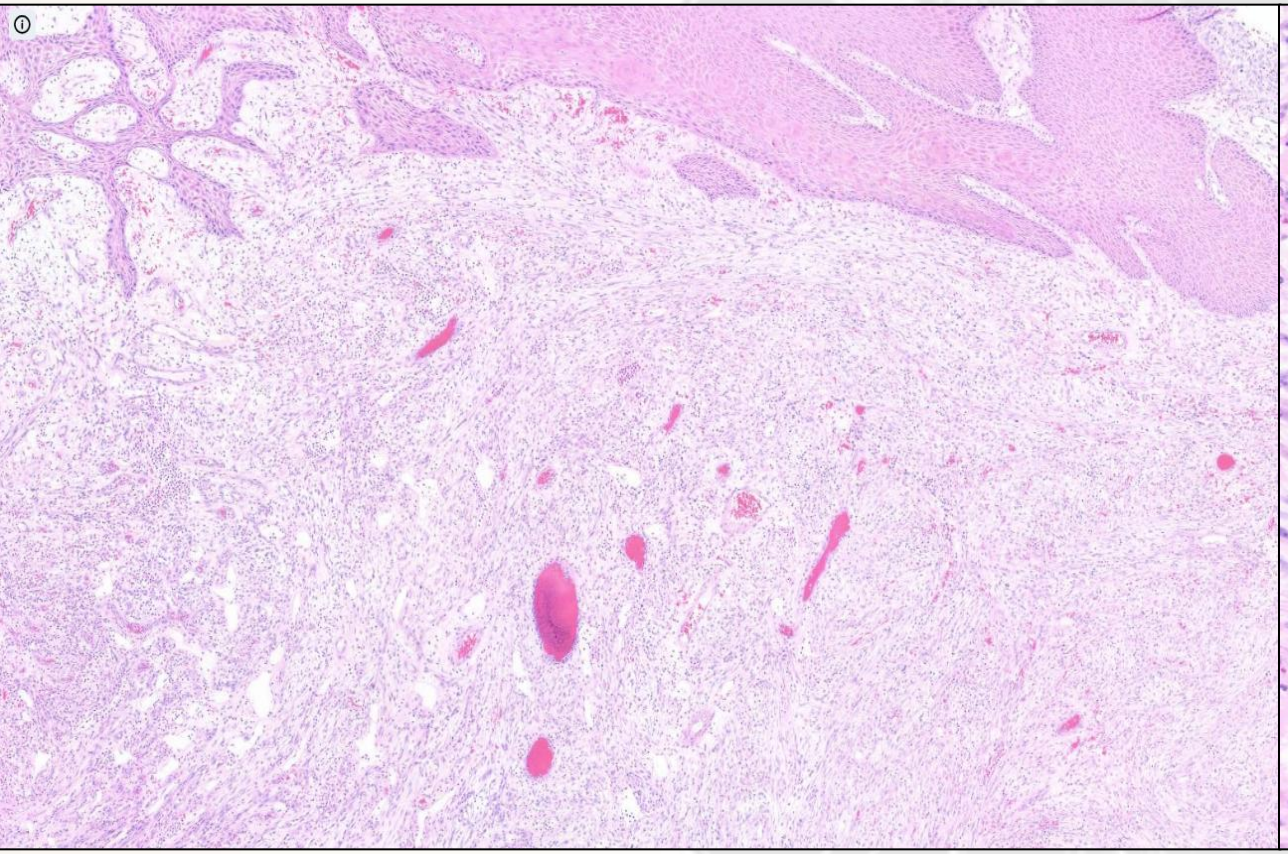
Deceptive Patterns of Invasion

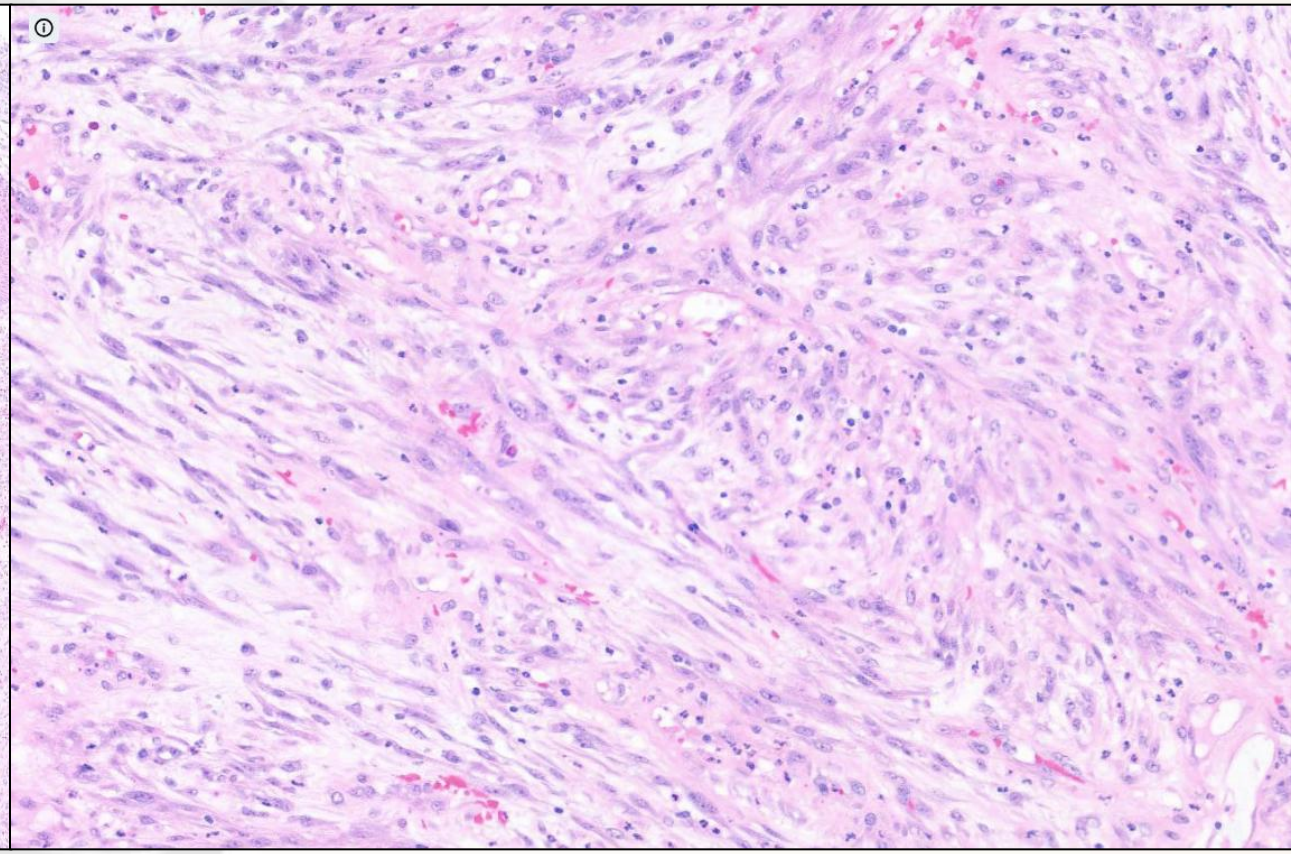


Sarcomatoid SCC – HPV Independent p53 mutant

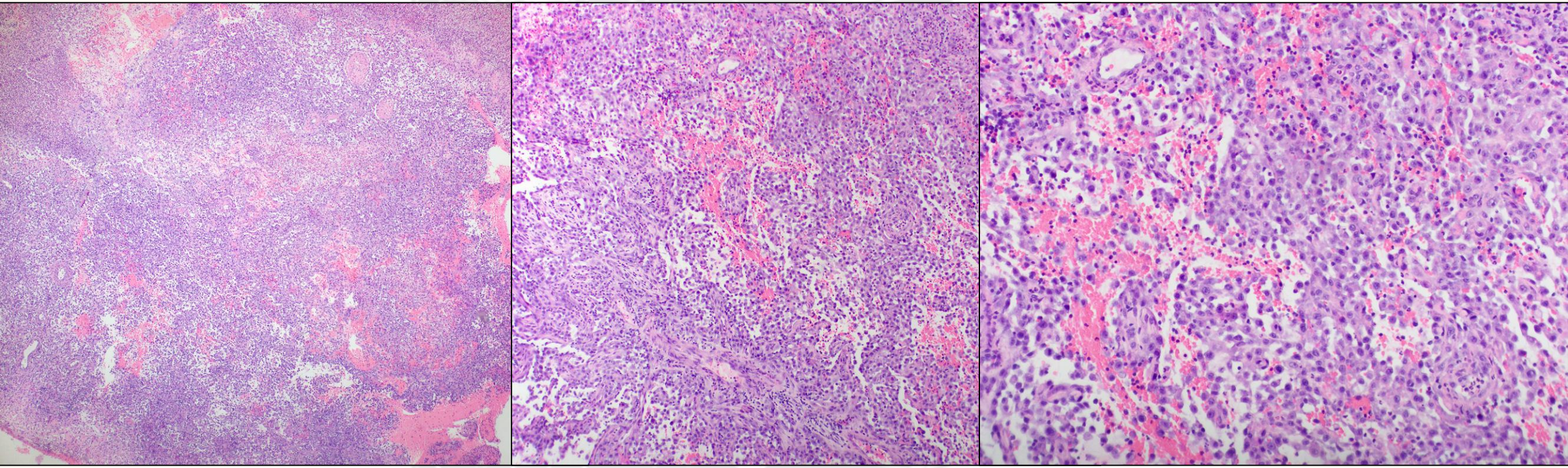


Sarcomatoid SCC – HPV Independent p53 mutant





Acantholytic SCC – HPV-A



Summary

- Etiologic classification of vulvar squamous cell carcinoma is most informative for treatment/prognostication
  - HPV-associated
  - HPV-independent
    - p53 Mutant
    - p53 Wild-type
- Precursors and invasive lesions are best interpreted in the context of p16 and p53 stains
- vaVIN should be diagnosed sparingly and only after excluding inflammatory and other neoplastic mimics