Junctional Epithelial Phenotypes and Gynecologic Neoplasia: The Concept and its Application

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Potential Conflicts

- Patent holder on p63 for diagnostic use
- Received honoraria from UpToDate

Volume 10 November 1957 Number 5

Cancer of the Uterine Cervix

A preventable disease

PAUL A. YOUNGE, M.D.

patients. The late Paul Gustafson of the Boston Lying-In Hospital began routine postpartum cauterization of the cervix over 30 years ago. Seven years ago shortly before his death he told me that he had cauterized over 6000 cervices. In the Boston area to date we know of only one of his patients who later developed cervical cancer and that was an in situ lesion treated this past year. These and other similar impressions are not statistically valid because of incomplete and too short a follow-up period, but the impressions are very strong. The fact that over 90

Outline

- The question
- Defining the SCJ and transformation zone
- Parallels between metaplasia and neoplasia
- **Differential risk**
- Grading schemes in mature and immature metaplastic epithelium
- The good and bad of p16
- Targeted prevention

The Question

Why does cervical carcinoma develop near the squamo-columnar (SC) junction?

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Molecular Cell, Vol. 2, 305–316, September, 1998, Copyright ©1998 by Cell Press

p63, a *p53* Homolog at 3q27–29, Encodes Multiple Products with Transactivating, Death-Inducing, and Dominant-Negative Activities

Annie Yang,[1] Mourad Kaghad,[2] Yunmei Wang,[1] Emily Gillett,[1] Mark D. Fleming,[3,4,5] Volker Dötsch,[6] Nancy C. Andrews,[3,4] Daniel Caput,[2] and Frank McKeon[1,7]

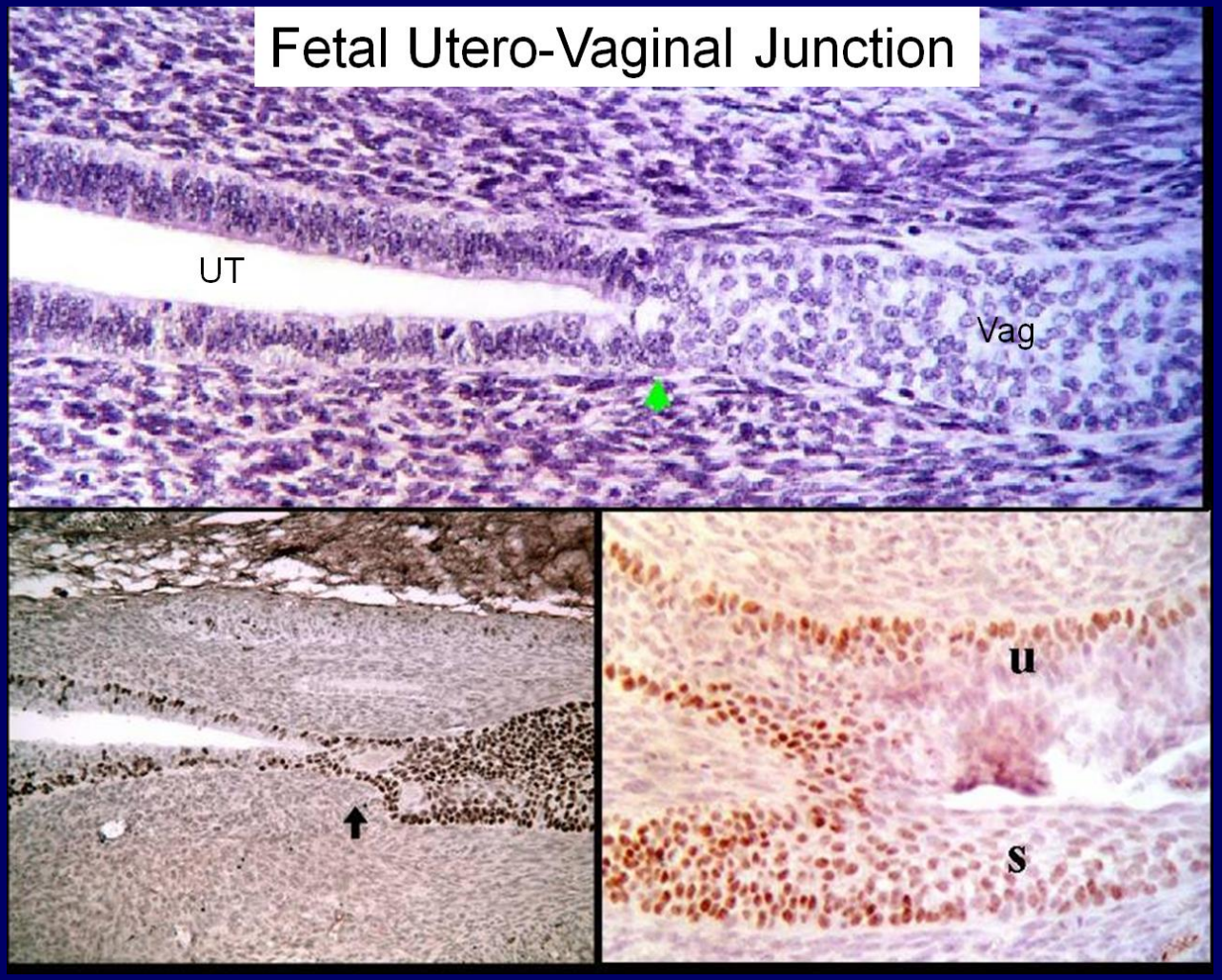
al., 1993; Cross et al., 1995; Graeber et al., 199
et al., 1997). Supporting this restricted and co
role for *p53* is the observation that *p53* nullizygo
as well as humans bearing constitutional *p53* m

Frank McKeon

- p63 is a stem cell marker in squamous epithelium
- p63 highlights basal squamous cells and reserve cells (RC) in the cervix
- Where do RCs come from?

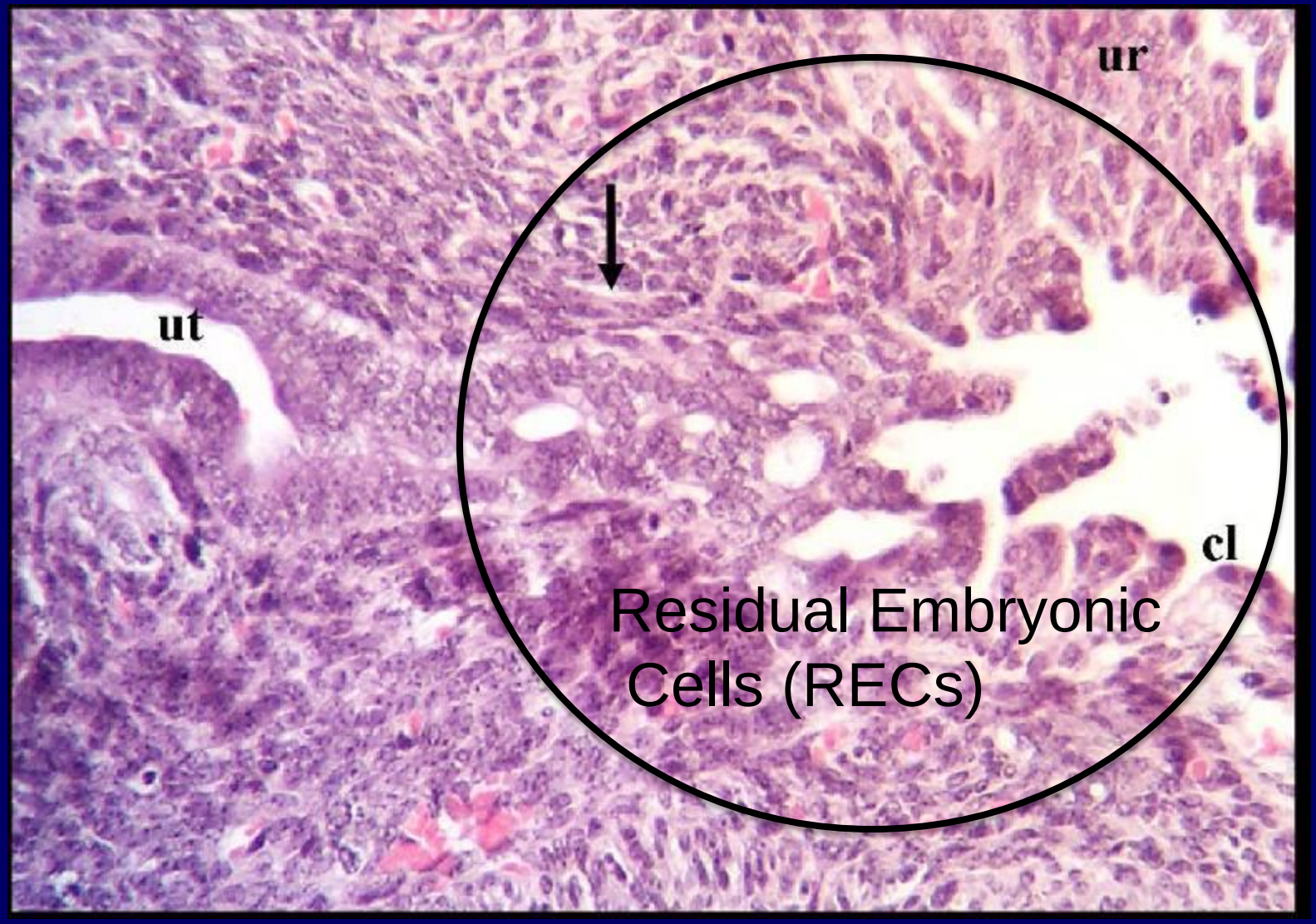
Yang, Cancer Cell 1998

p63 and the Urogenital Tract



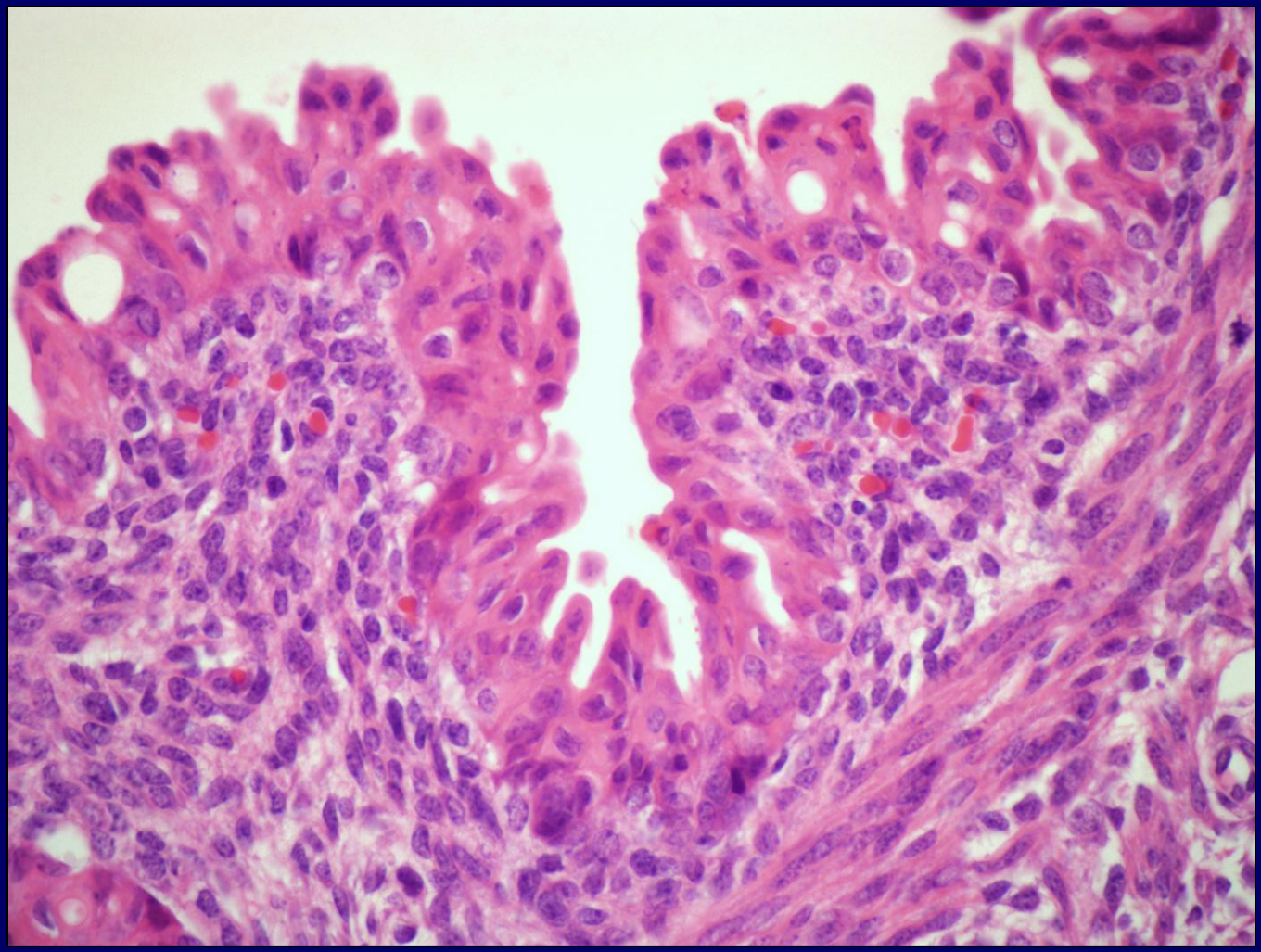
Ince, Am J Pathol 2002

Absence of Cervical Basal/Reserve Cell Induction (p63-/-)

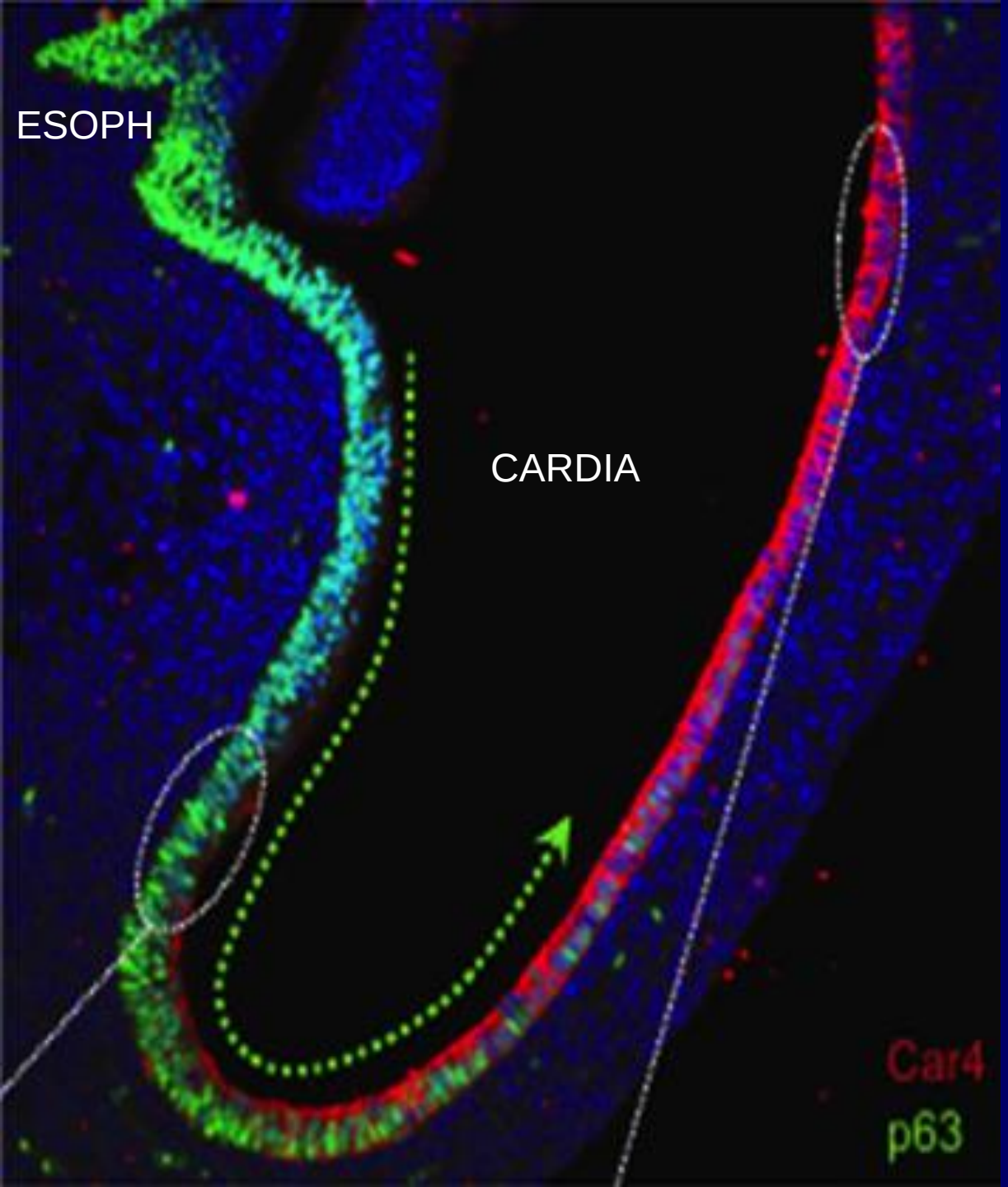


Ince Am J Pathol 2002

RECs at the Esophago-Gastric Junction



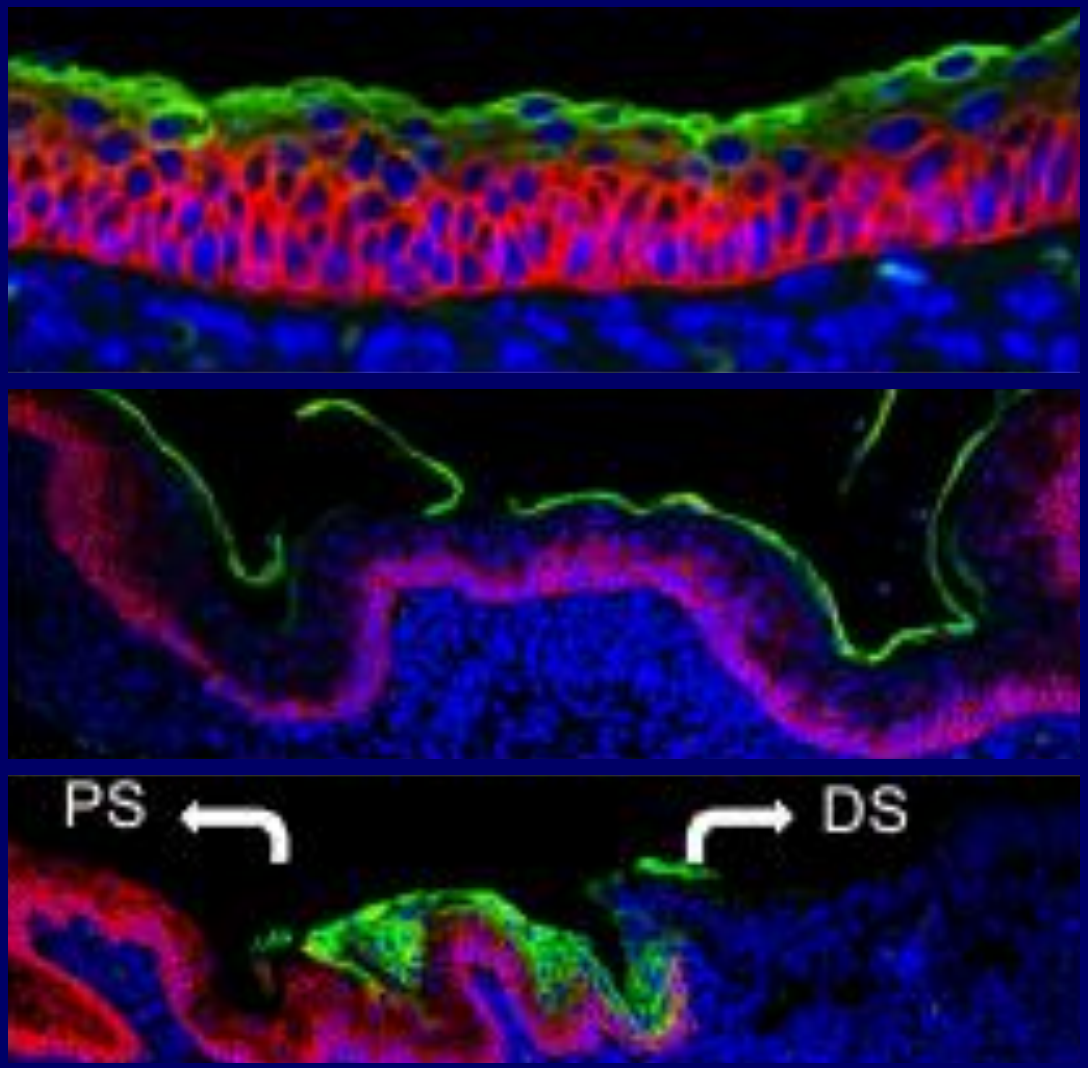
Yang, Nature 1999, Stairs 2008



Replacement of embryonic gastric epithelium by squamous basal cells

Wang et al , Cell 2011

Mouse



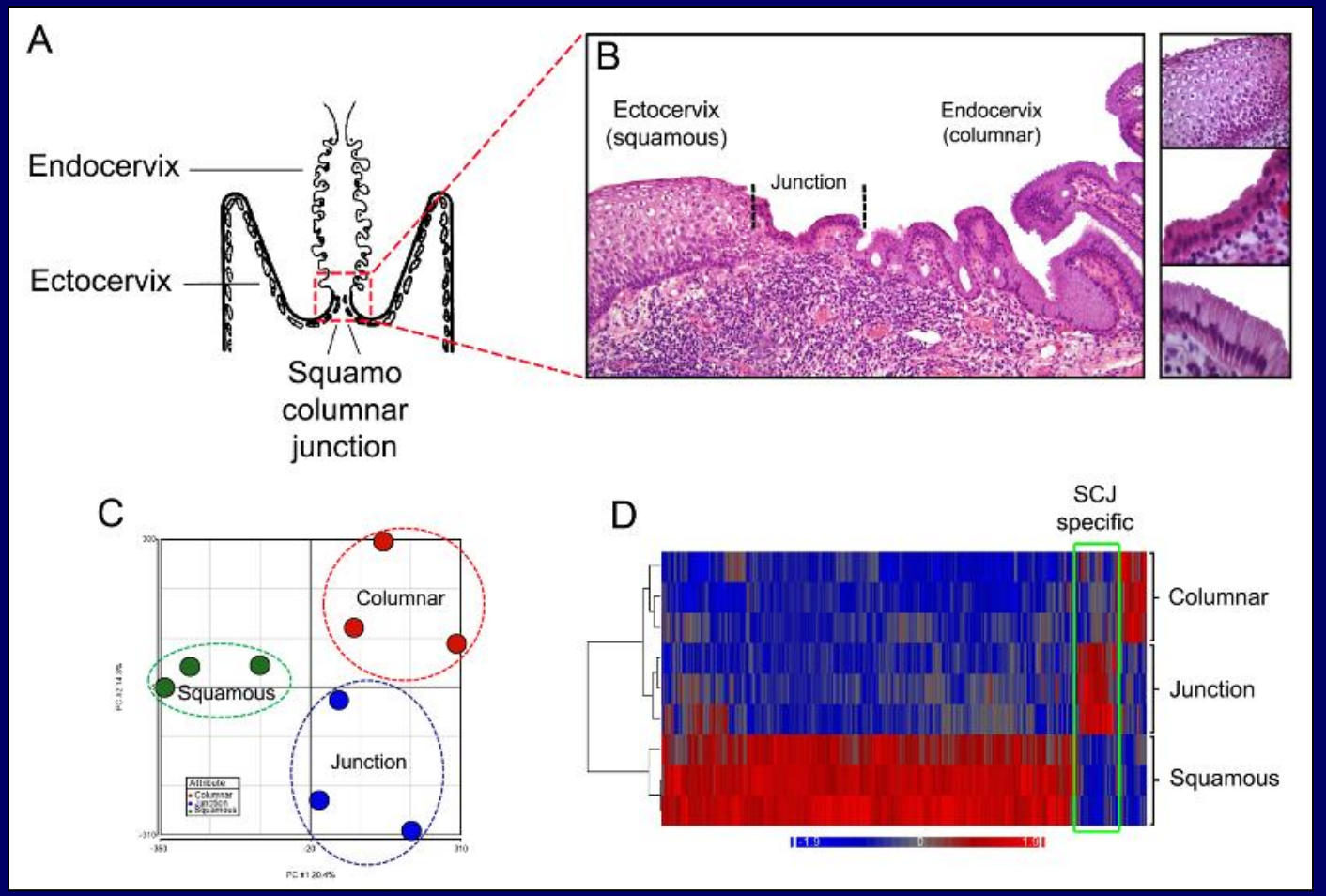
Undermining of the Embryonic epithelium

Dislodgement of the embryonic cells

Focal persistence of embryonic cells at the SC junction!

Wang, Cell 2011

The Uterine Cervix

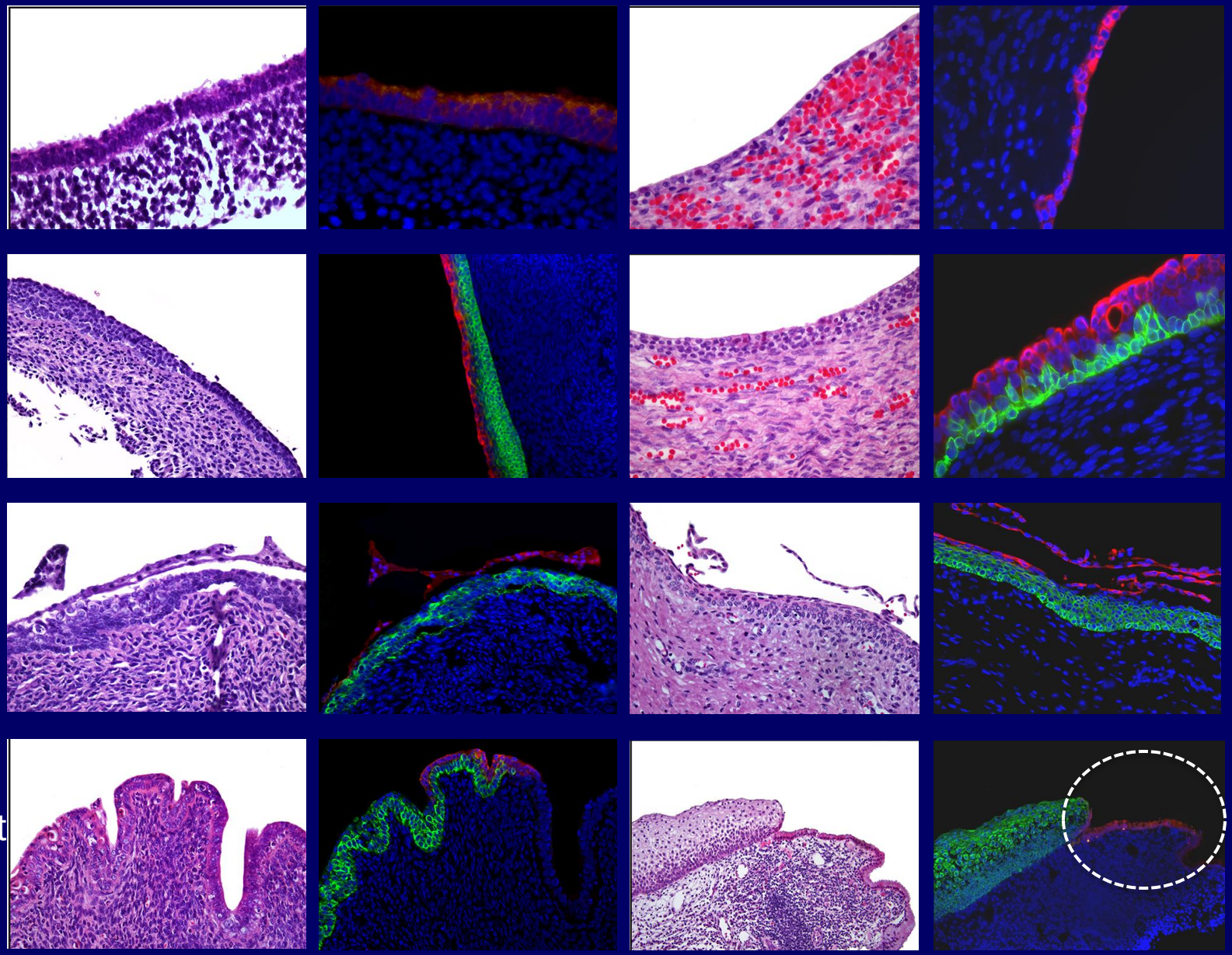


Herfs, PNAS 2012

Cervix

Mouse

Human



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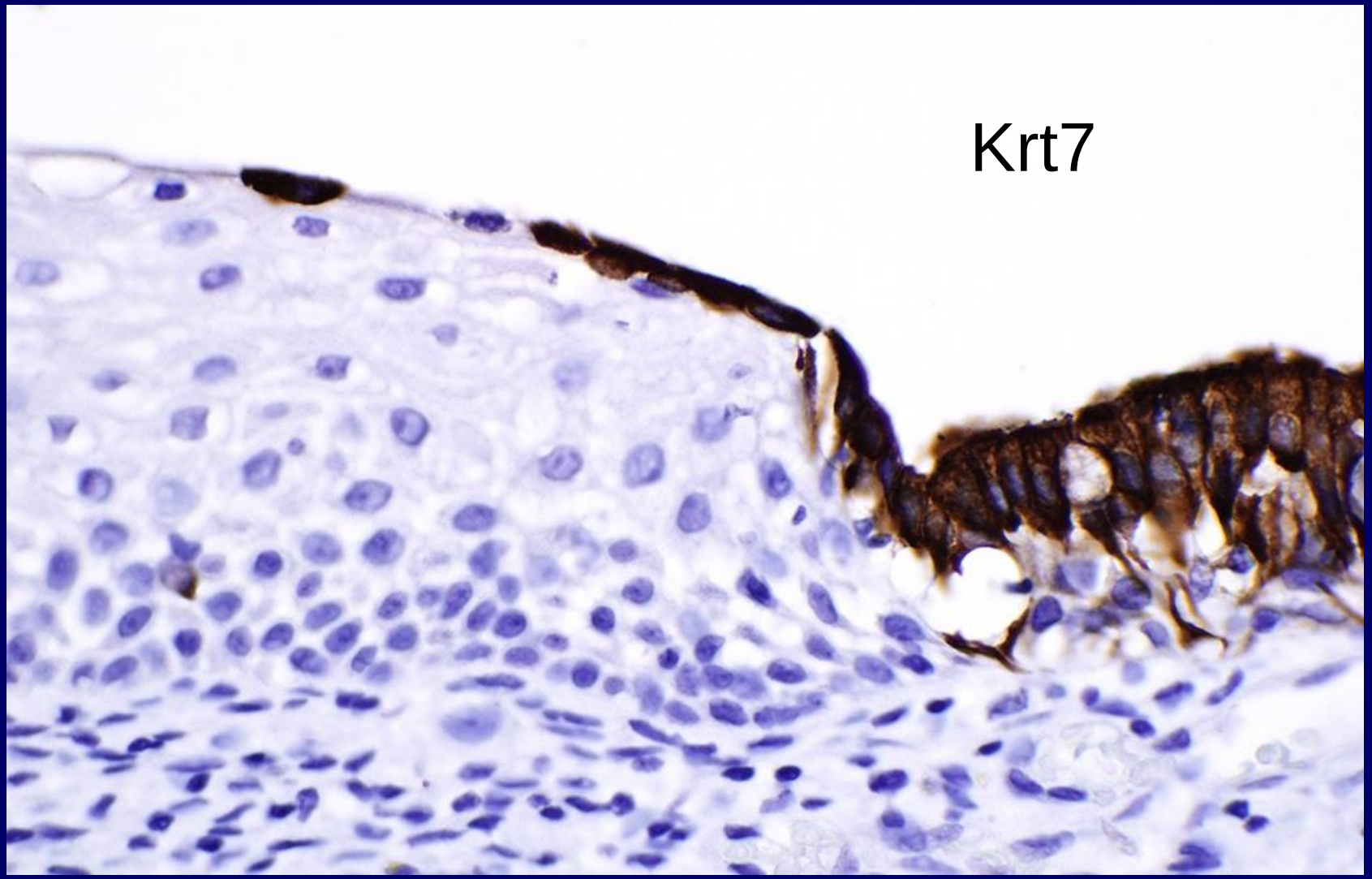
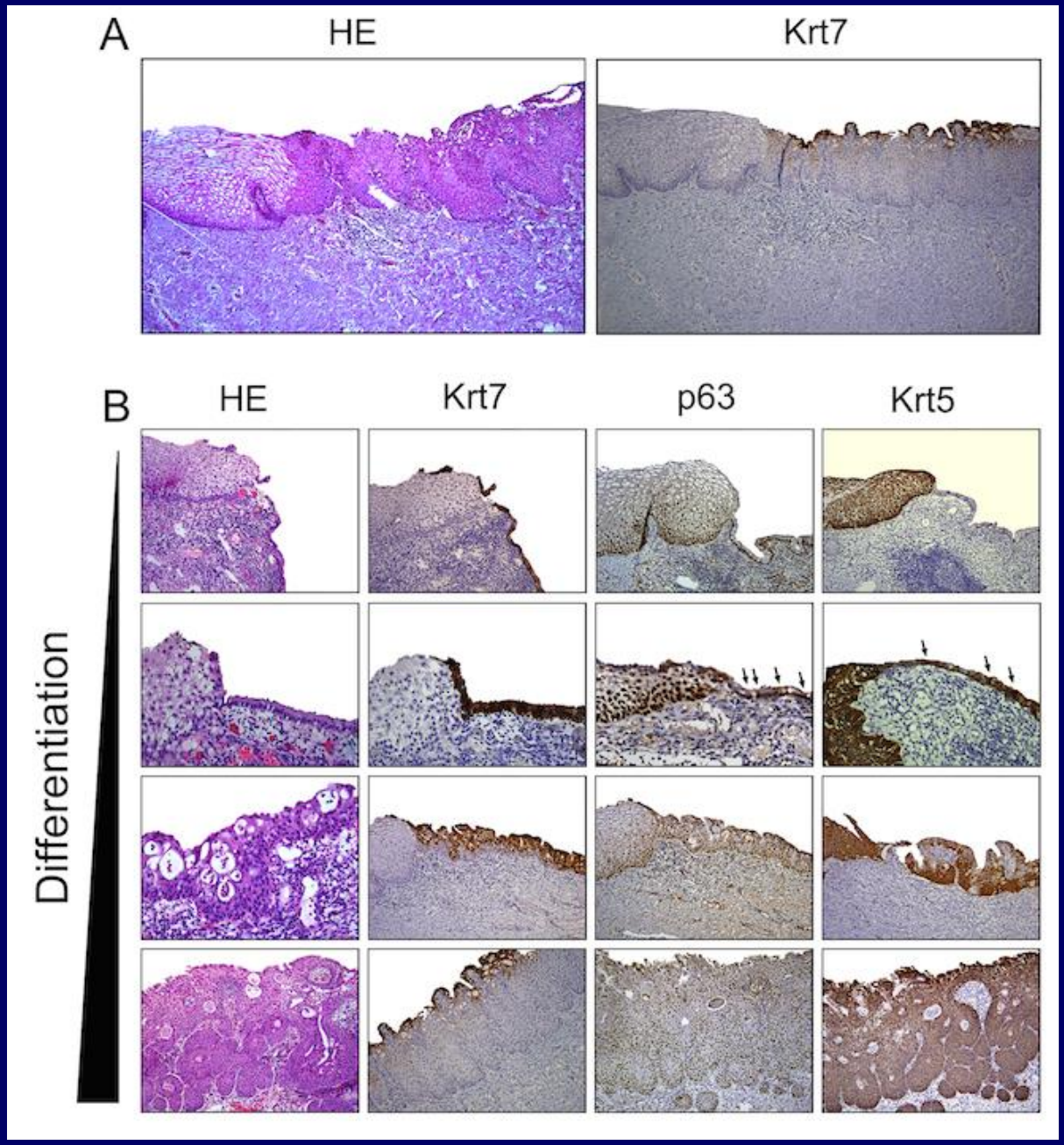
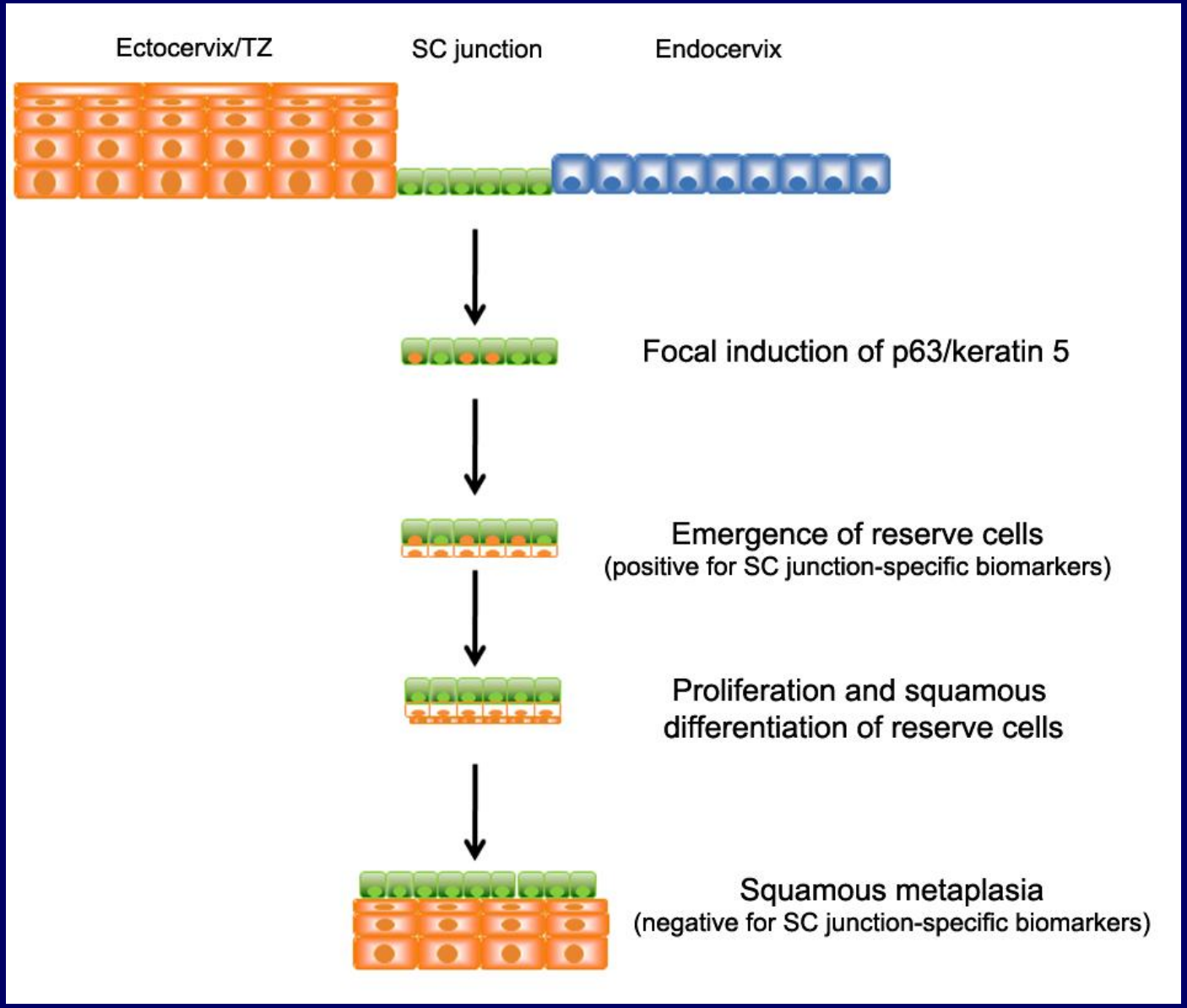
The SC Junction Cell



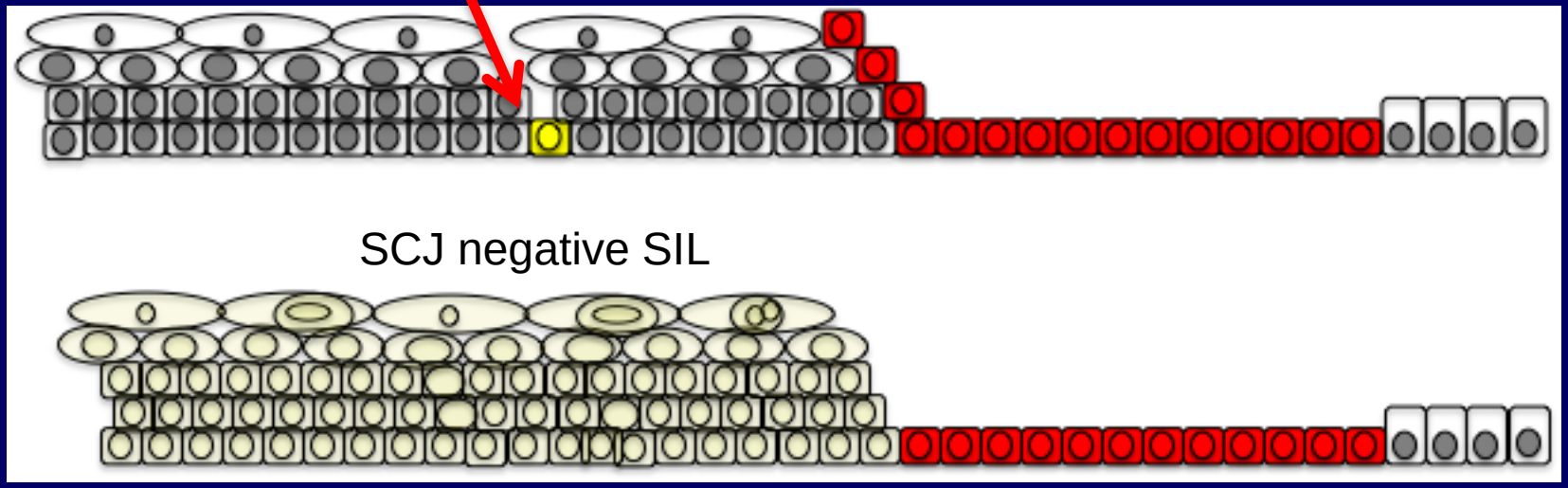

A
HE
Krt7
B
HE
Krt7
p63
Krt5
Differentiation

“Top-down Differentiation”



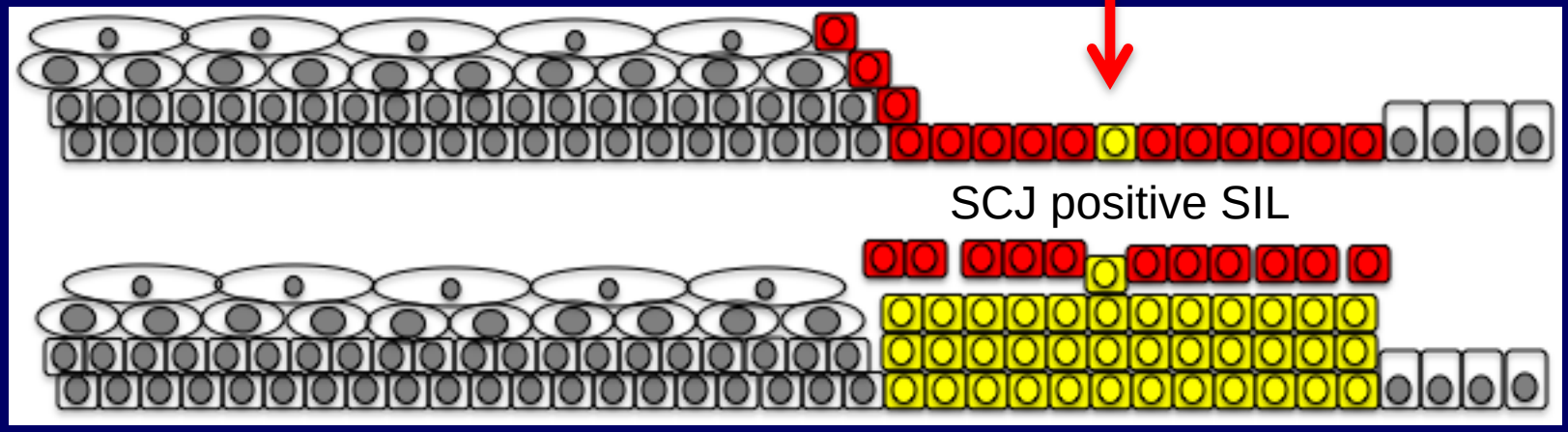
HPV

Conventional “bottom-up” HPV infection/differentiation



HPV

Novel “top-down” HPV infection/differentiation



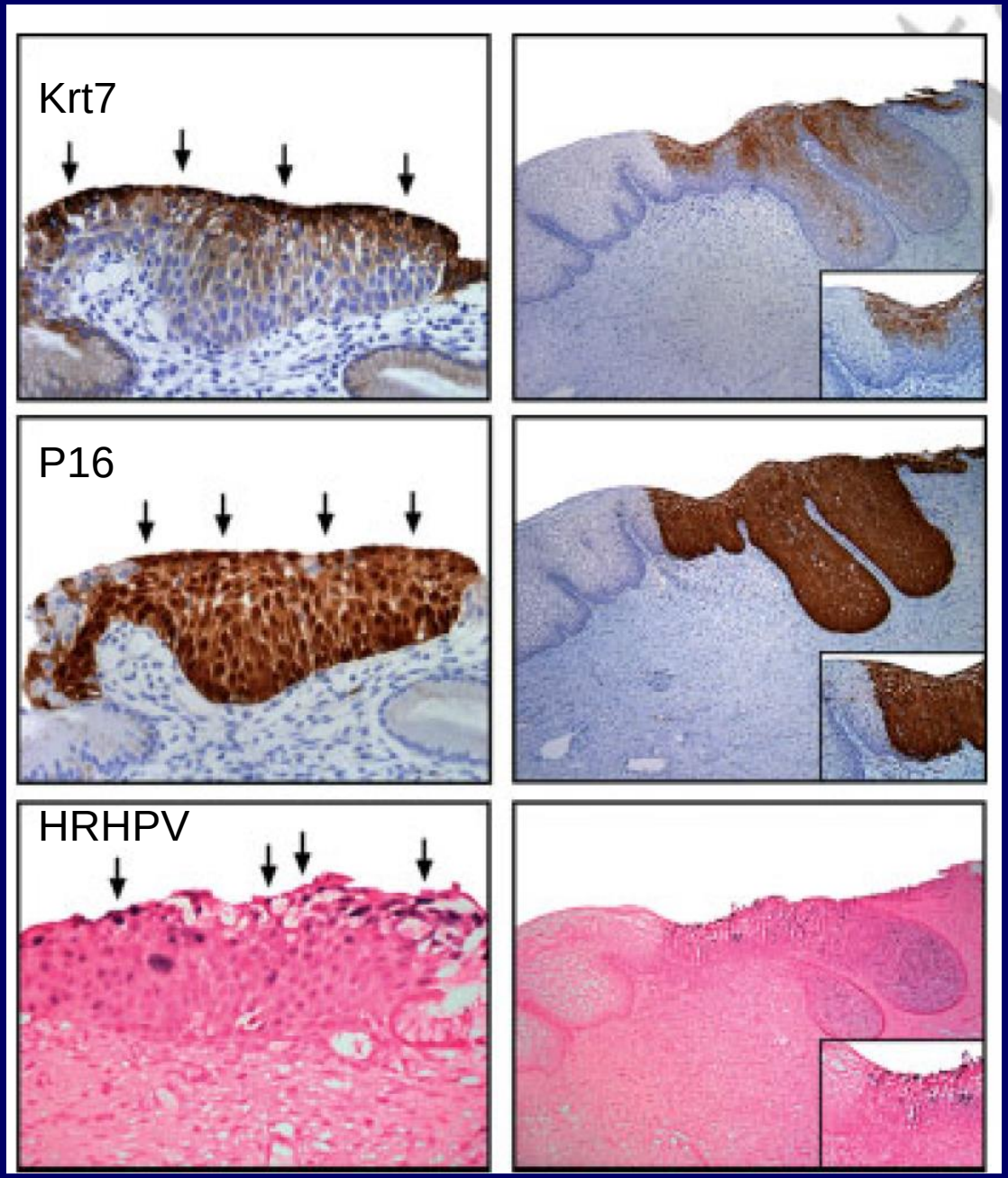
“Top-down” Differentiation in Preinvasive Disease

<u>Simultaneous transformation and trans-differentiation</u>

During embryogenesis

During remodelling

During early neoplasia



Herfs, J Pathol 2012, Abstracts 1157, 1158

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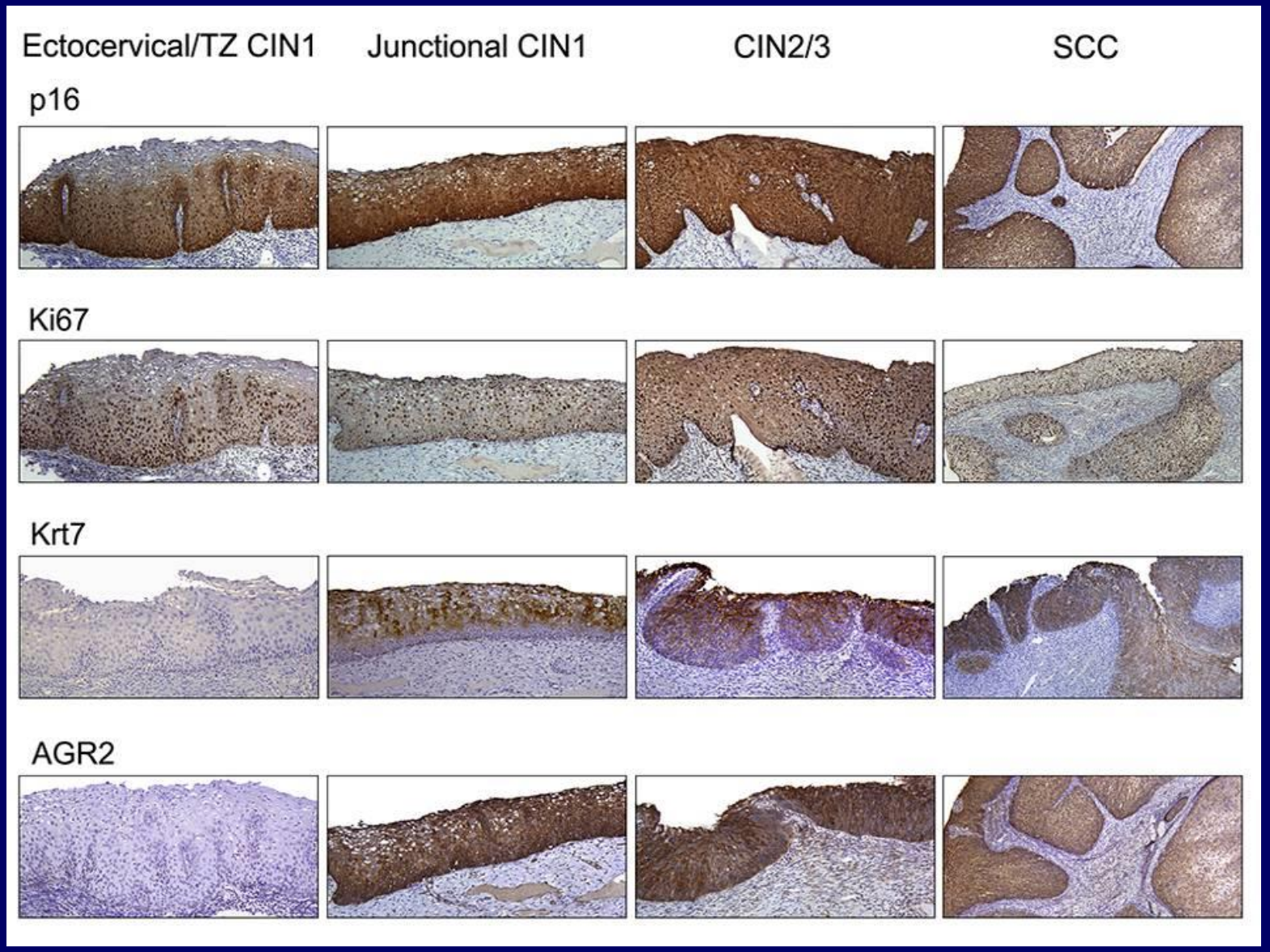
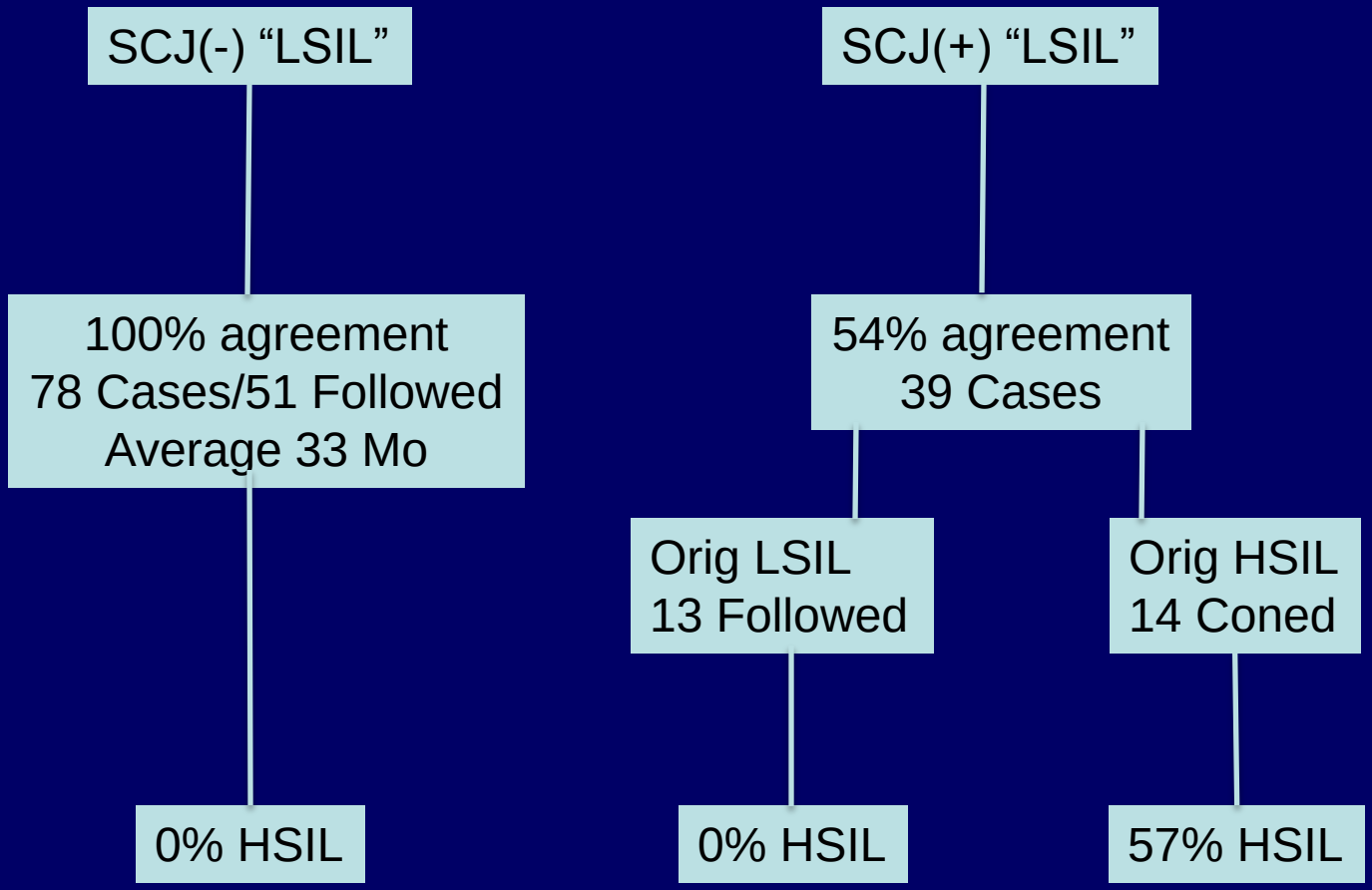
SCJ and TZ Derived Precursors are Different



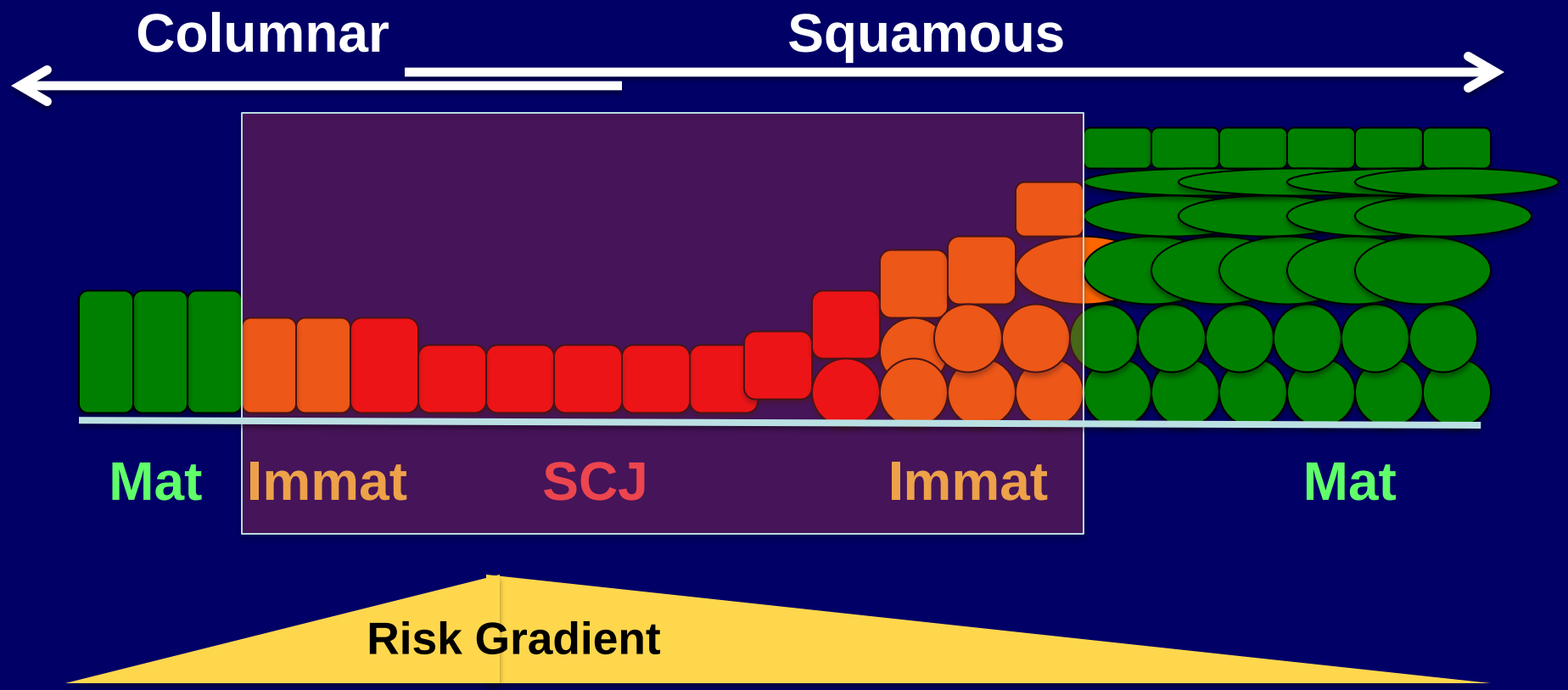
Herfs PNAS 2012, J Pathol 2012; Am J Surg Pathol 2013

“LSIL” Dx/Outcome



Herfs AJSP 2013

A Risk Model for Cervical Cancer



Incidence Rates (1975)

Anus 1.0

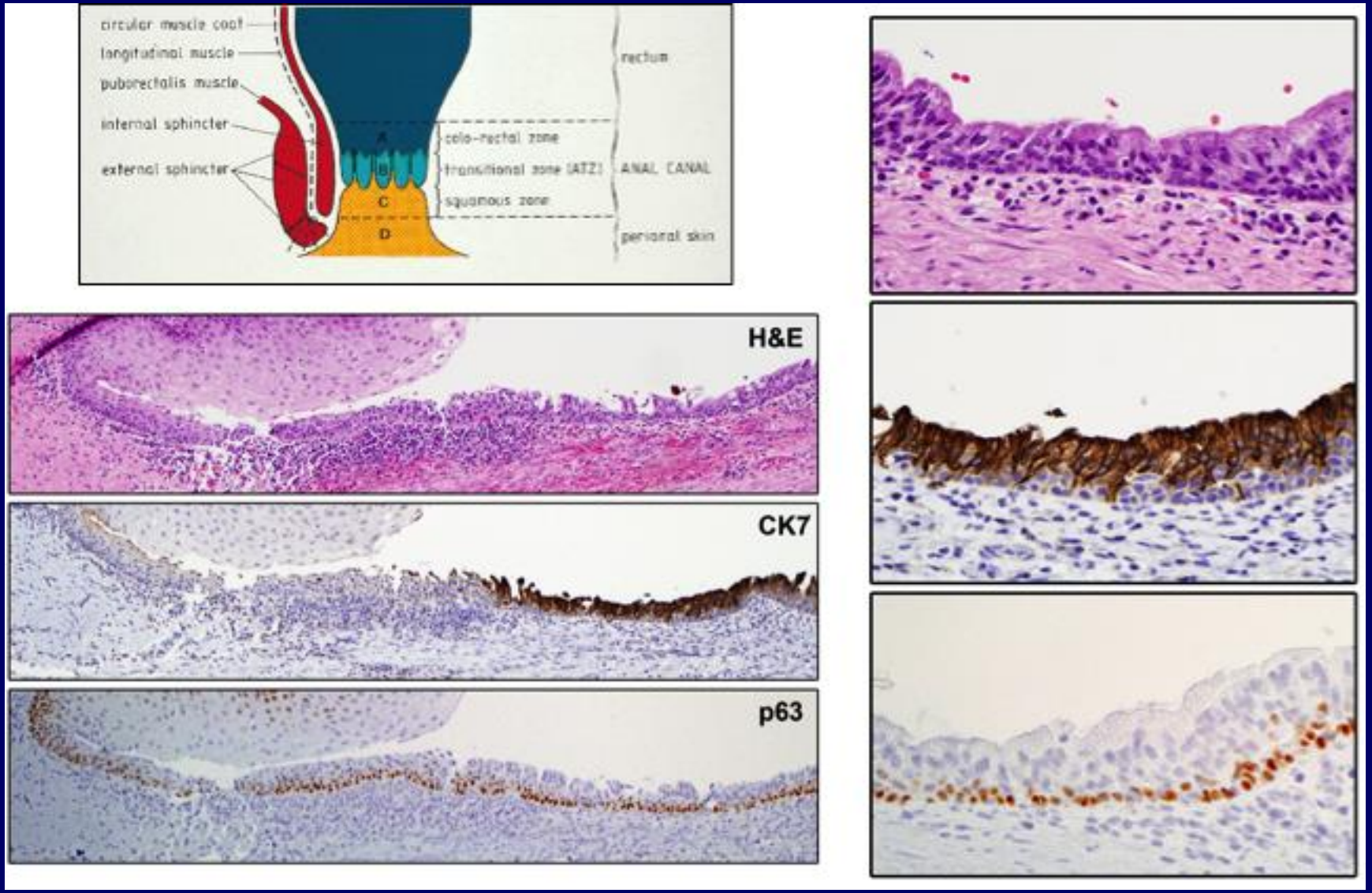
Vagina 0.9

Vulva 1.0

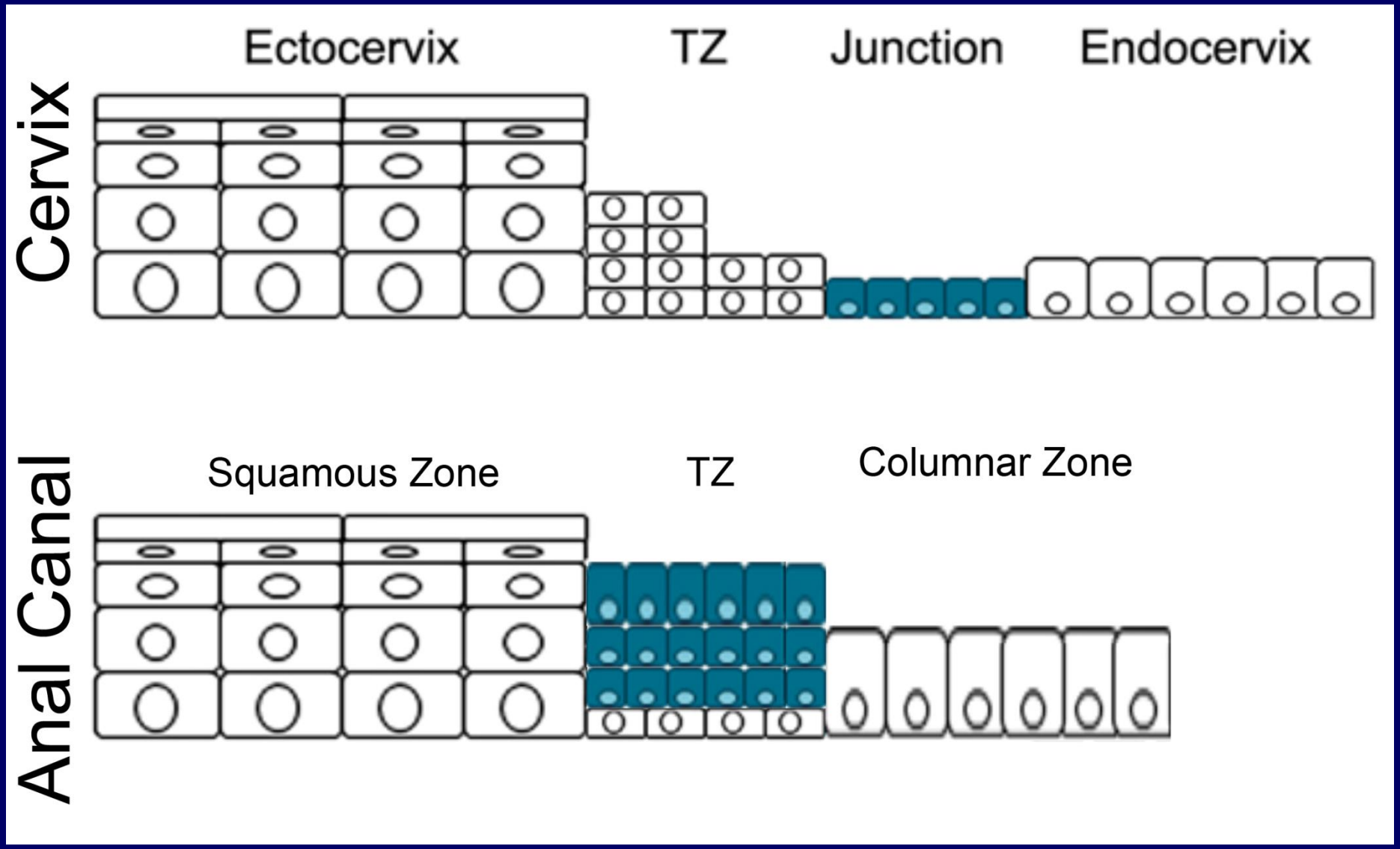
Cervix 15.0

http://seer.cancer.gov/statfacts/html

Absence of SCJ cells in the Anal TZ



E. Yang, C. Crum and M. Herfs 2014



Adapted from: *Am J Surg Pathol* • Volume 37, Number 9, September 2013

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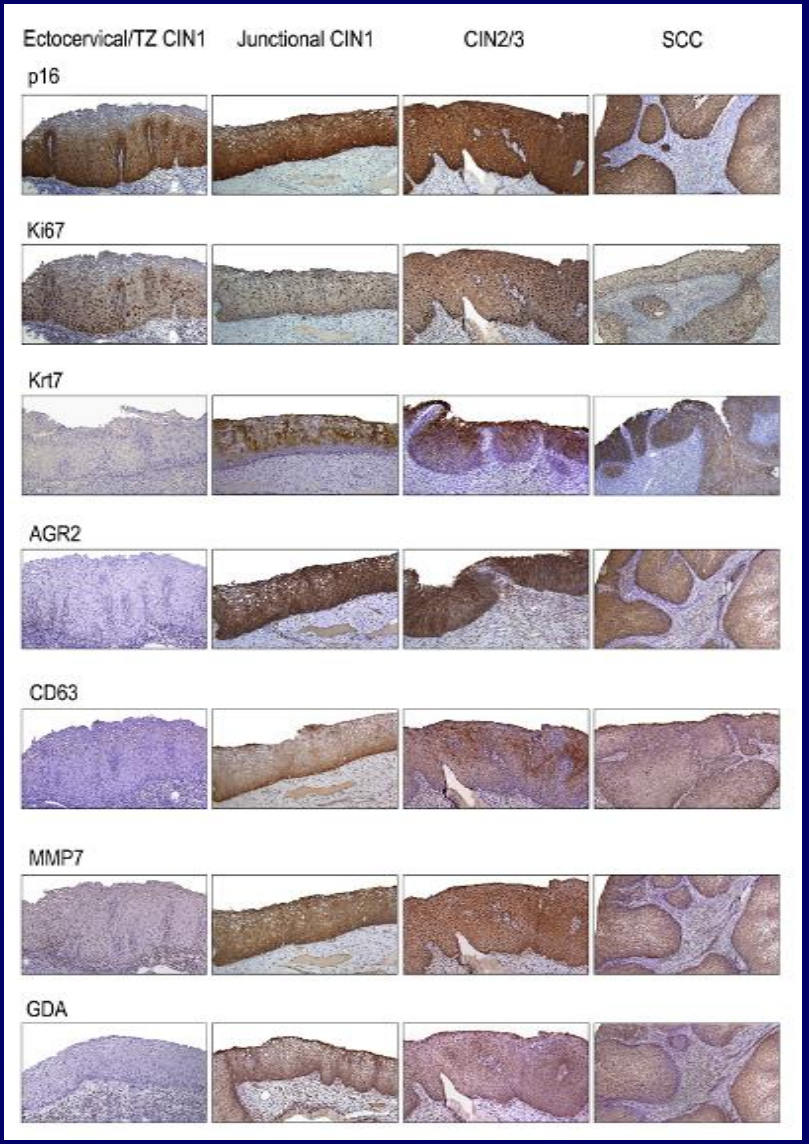
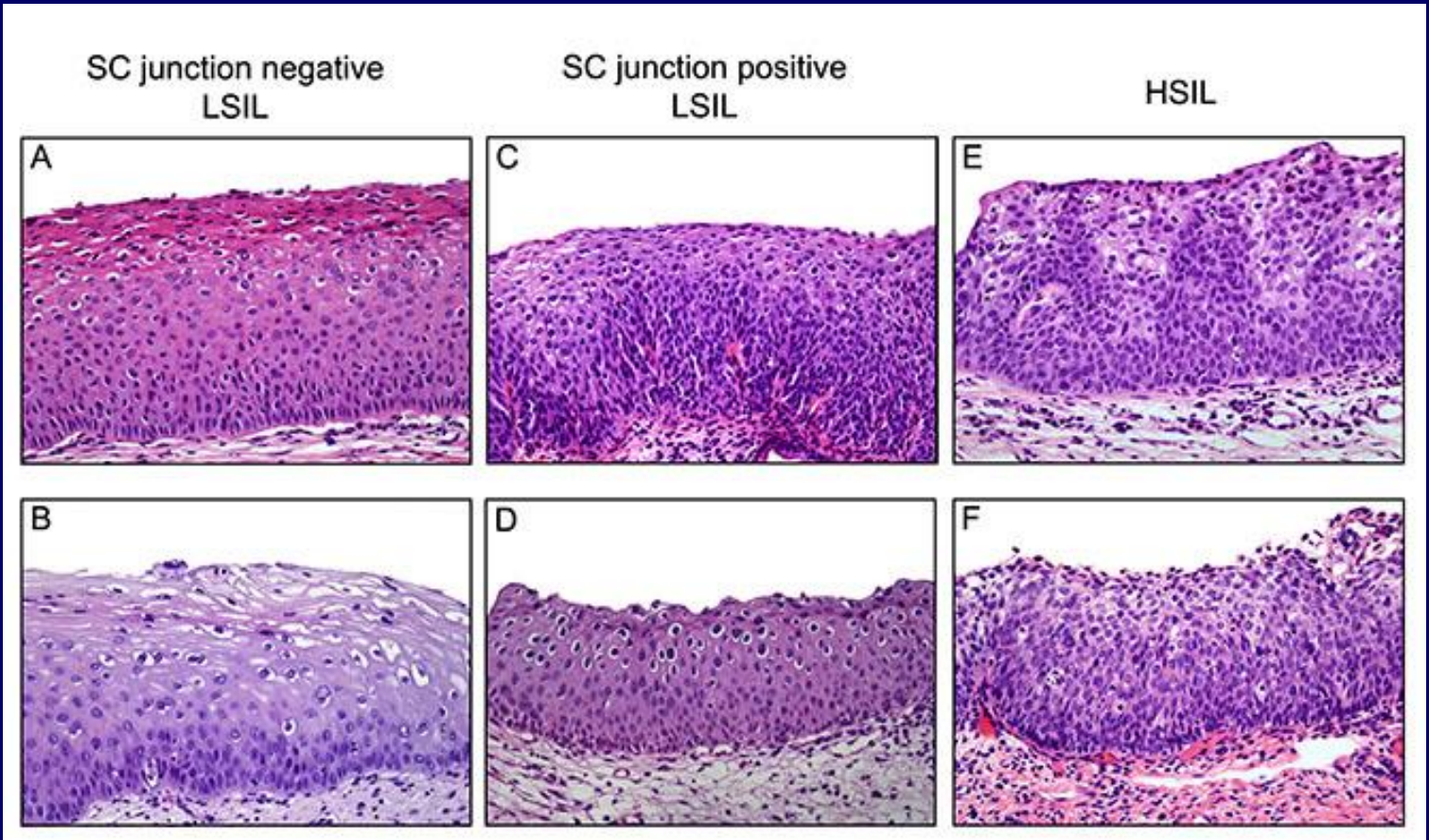
Most HSILs Come from the SC Junction

- Three major types of SIL
  - Ectocervical/meta-plastic LSILs (CK7-) considered very low risk
  - SC junction LSILs (CK7+) higher risk of dx disagreement and HSIL outcome
  - SC junction HSILs (CK7) considered high risk

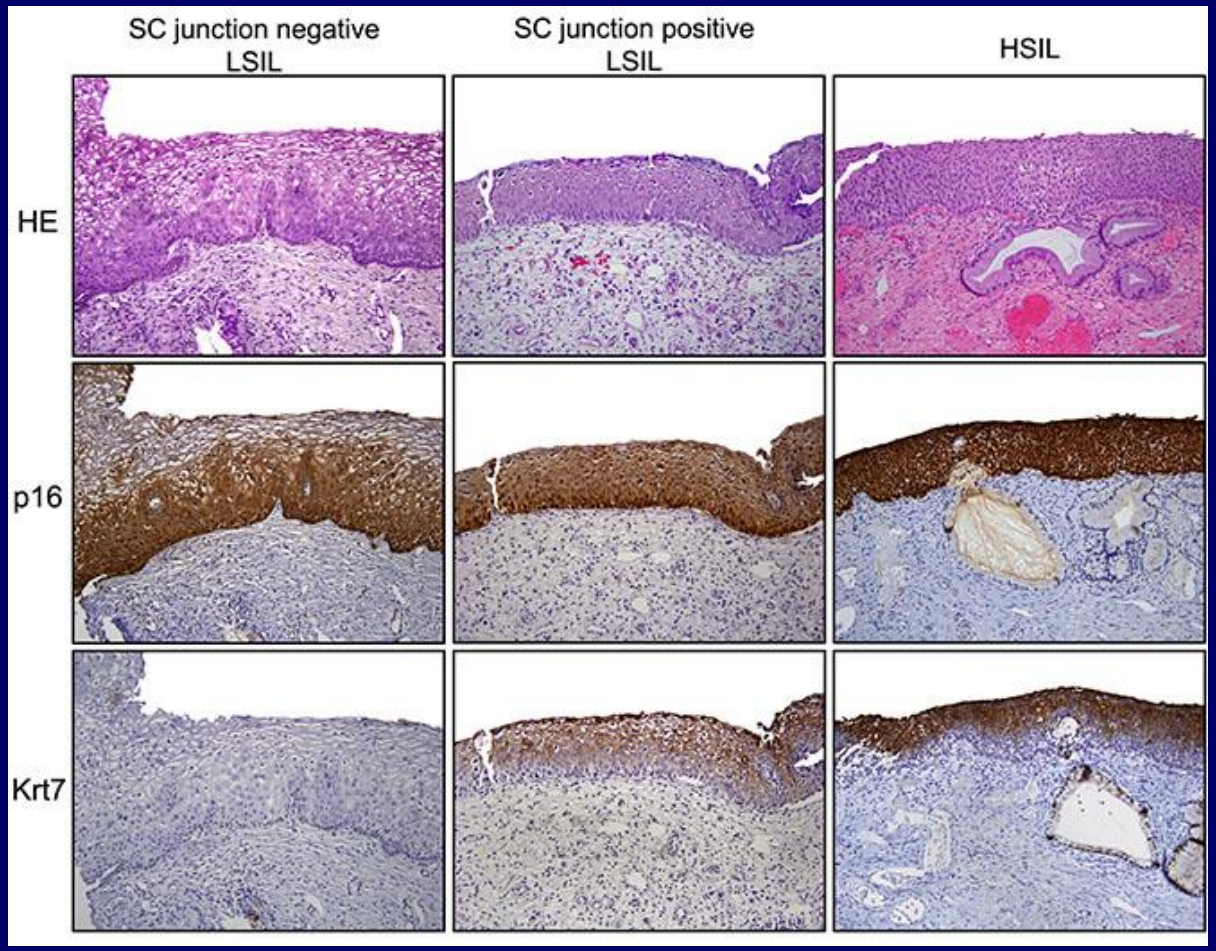


Three types of SIL



SC junction negative LSIL	SC junction positive LSIL	HSIL
90% agree	50% agree	90% agree
50% HRHPV	100% HRHPV	100% HRHPV
10% HPV16	60% HPV16	60% HPV16
CK7(-)	CK7+	CK7+

Diffuse $p16^{ink4}$ staining

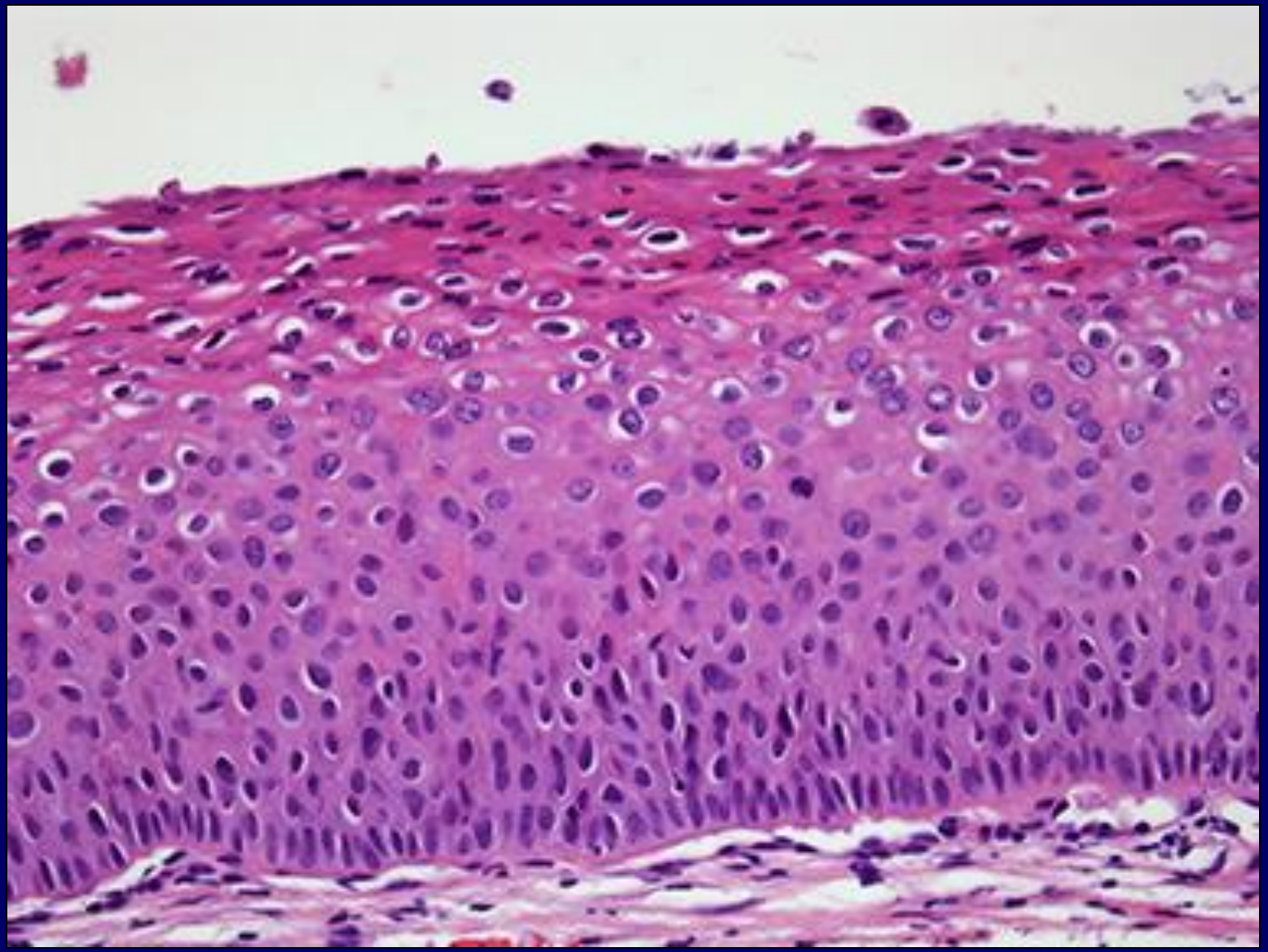


23% 74% 96%

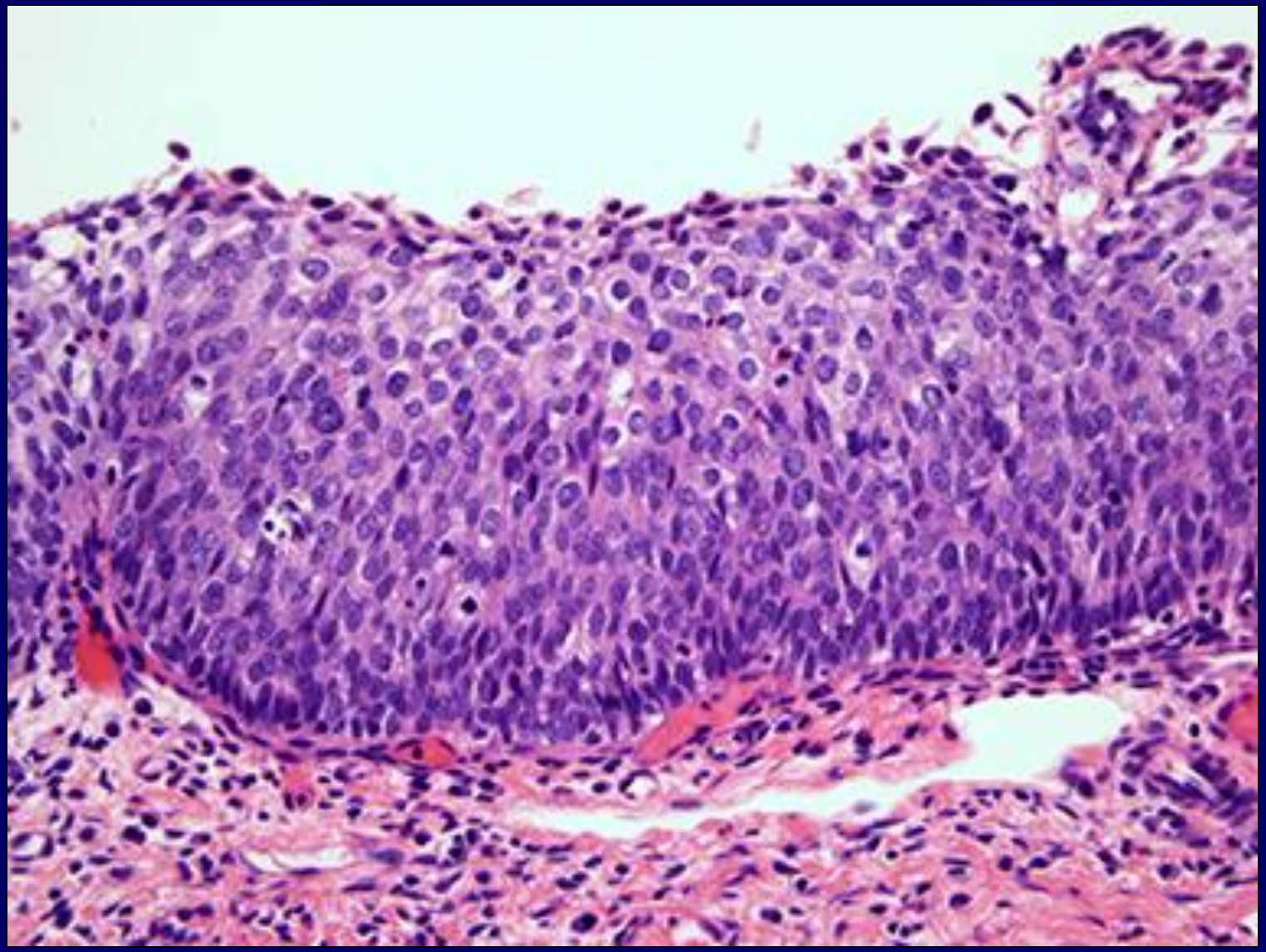
A different perspective

- Using this model it is possible to envision three types of SIL, easy HSIL, easy LSIL and problematic (L)SILs or QSILs, which arise in the SC junction.
- The diagnostic algorithm includes LSIL, HSIL and QSIL.
- With a little practice you can spot a QSIL
- P16 is of virtually no value in making this distinction

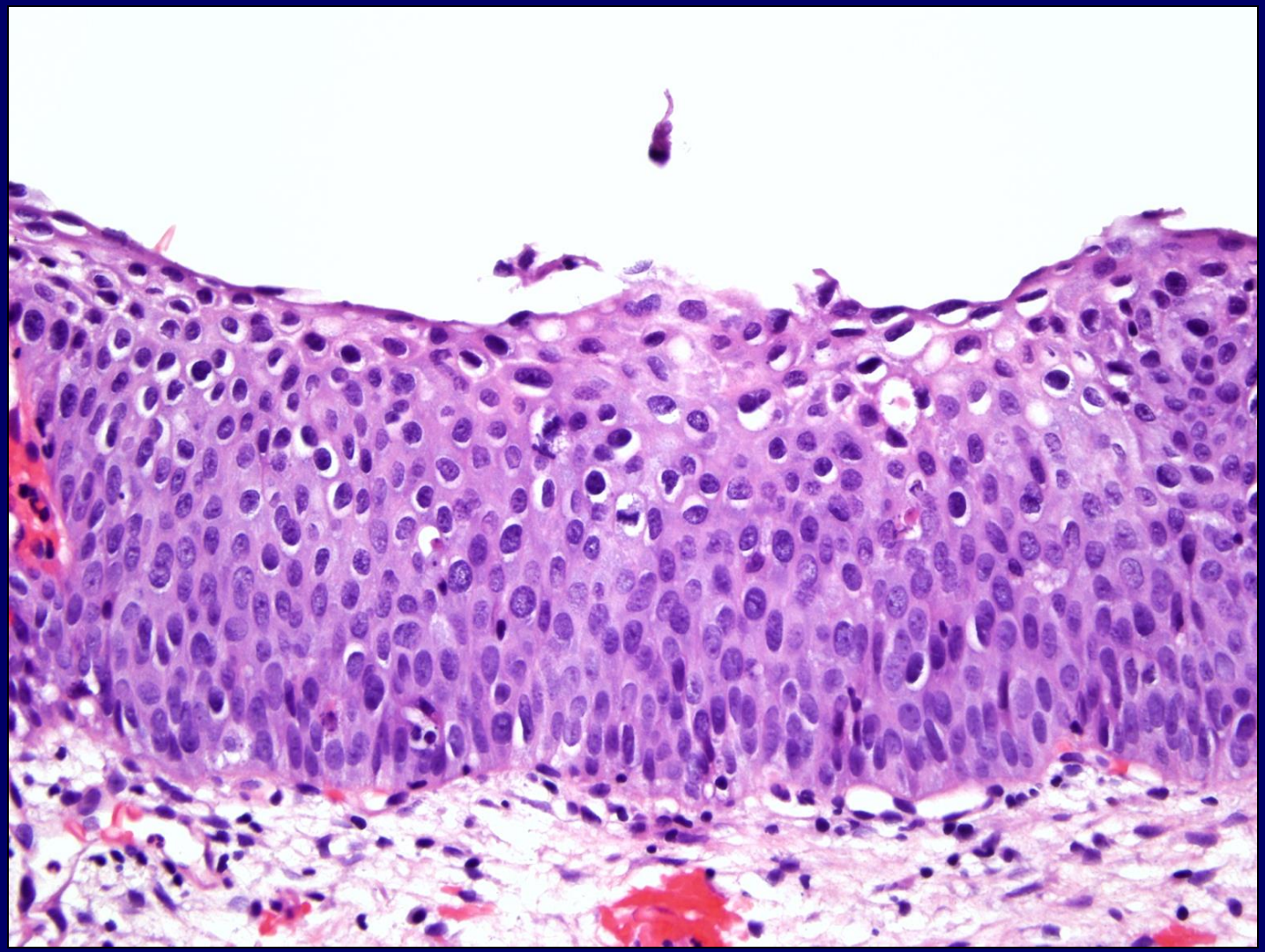
Diagnosis?



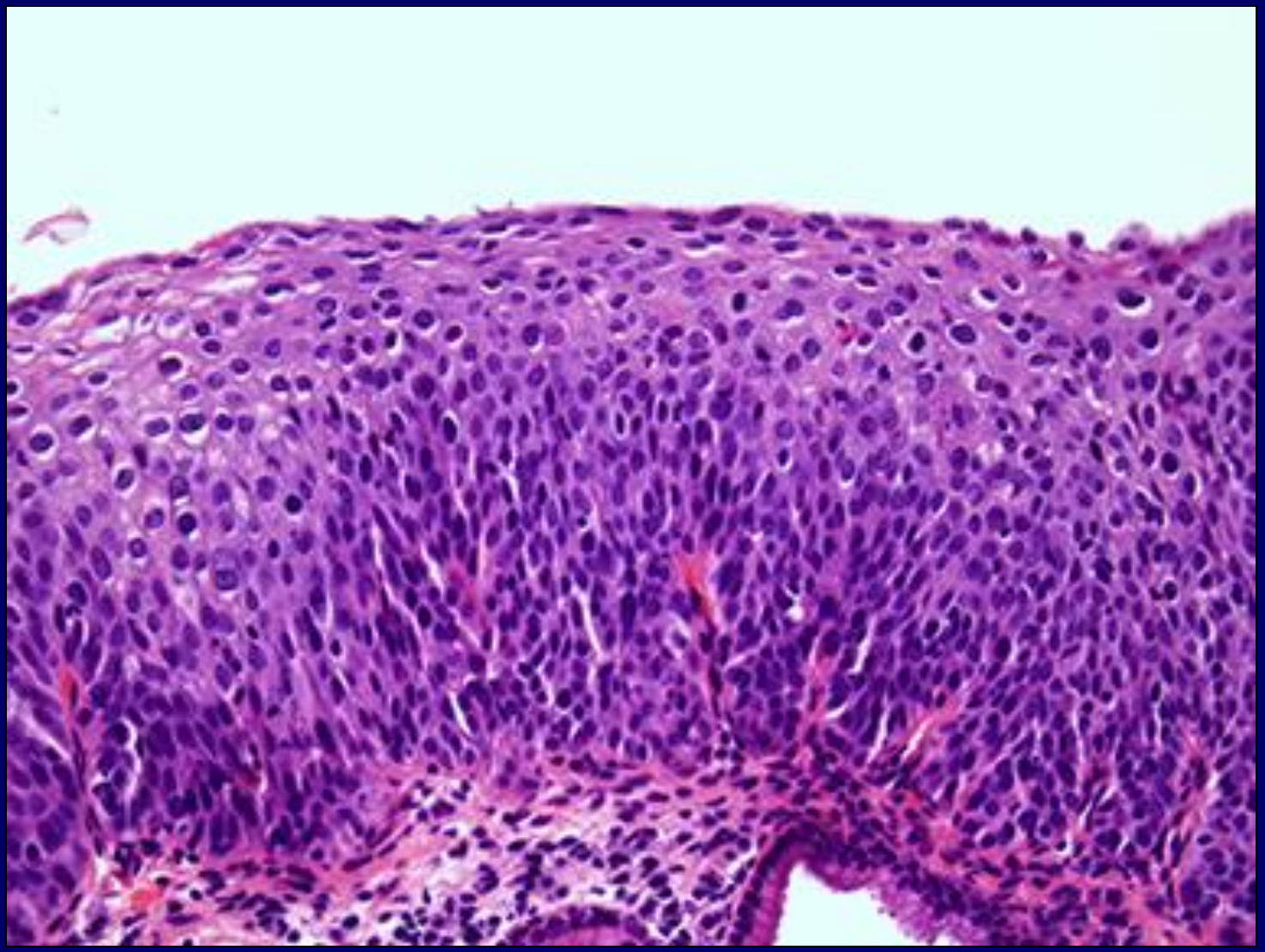
Diagnosis?



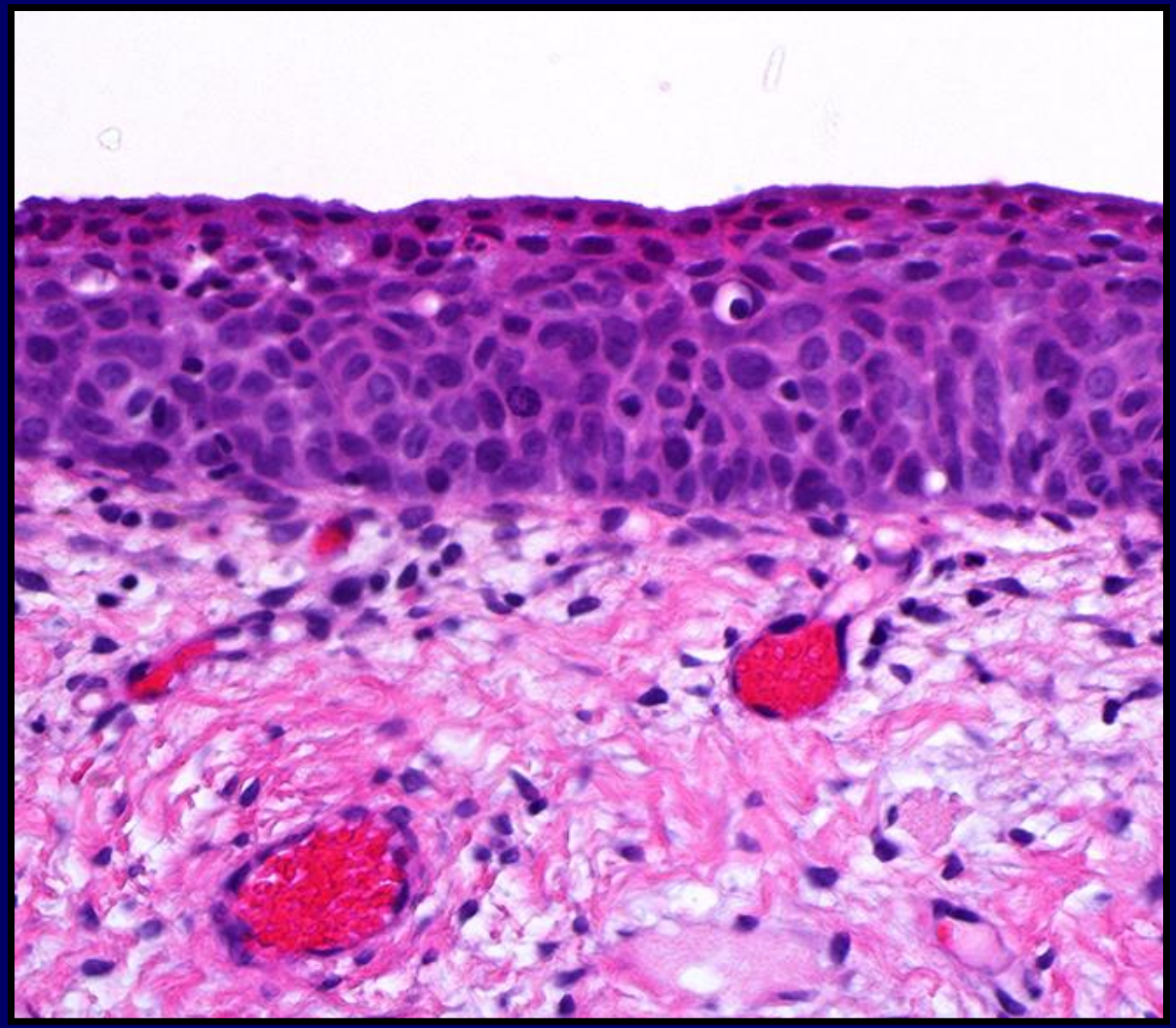
Diagnosis?



Diagnosis?



Diagnosis?



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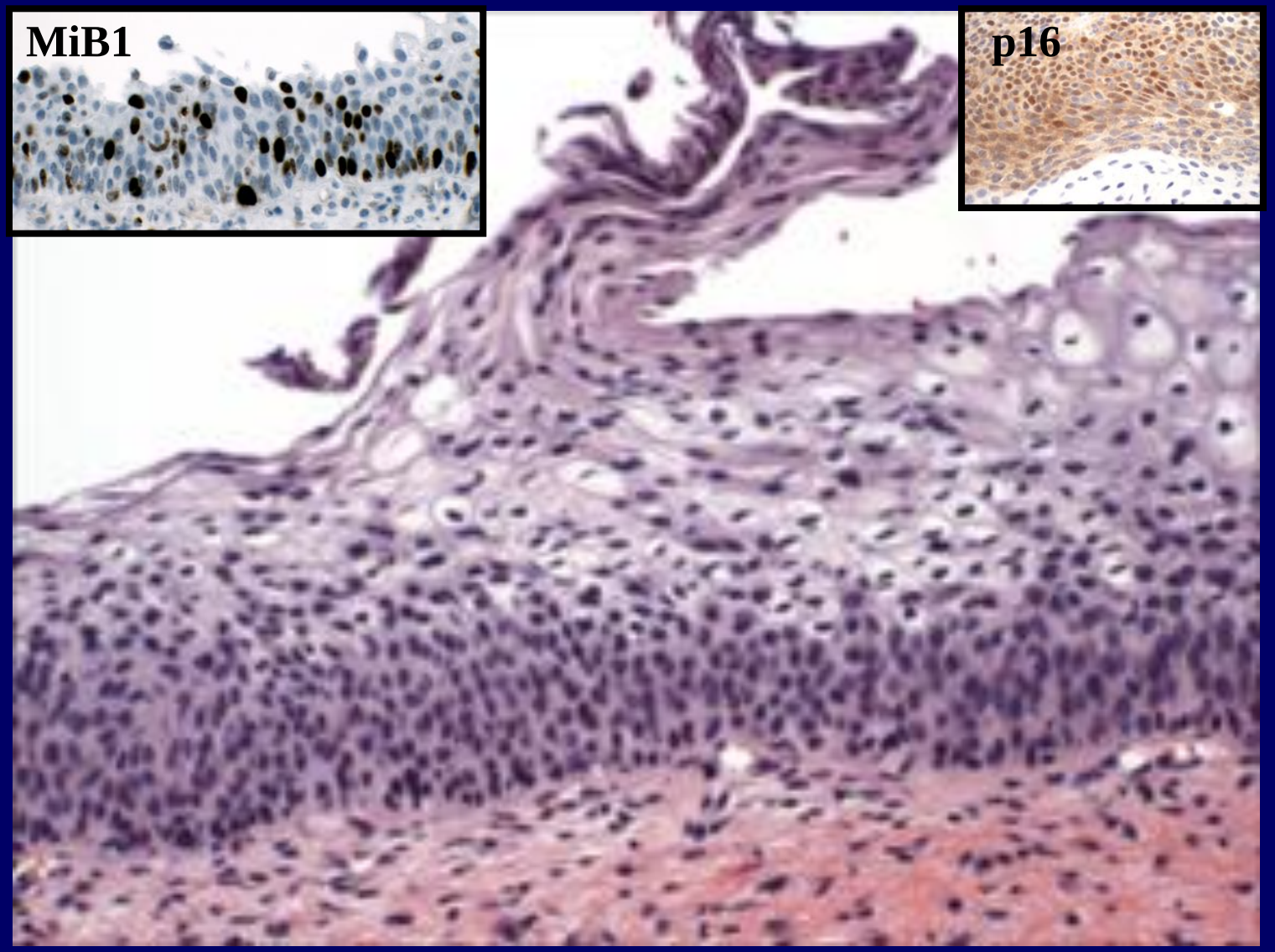
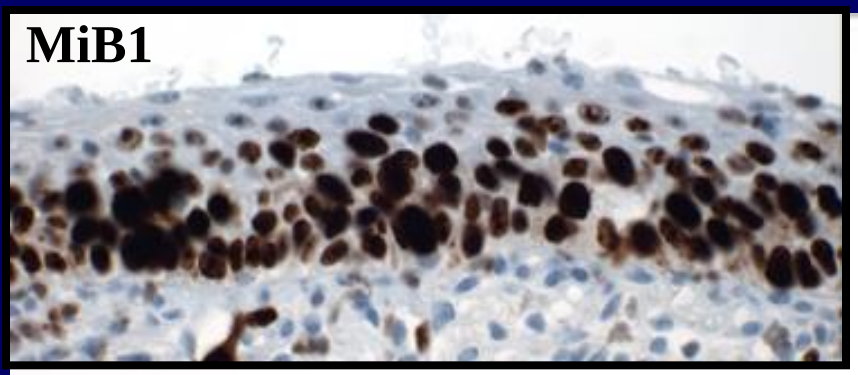
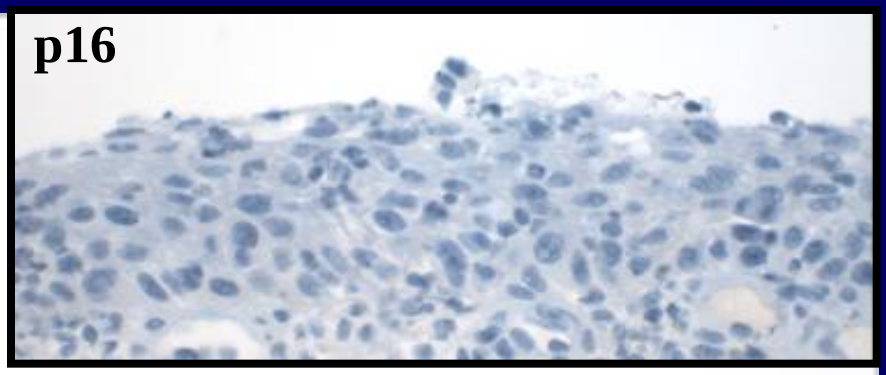
Biomarker Staining

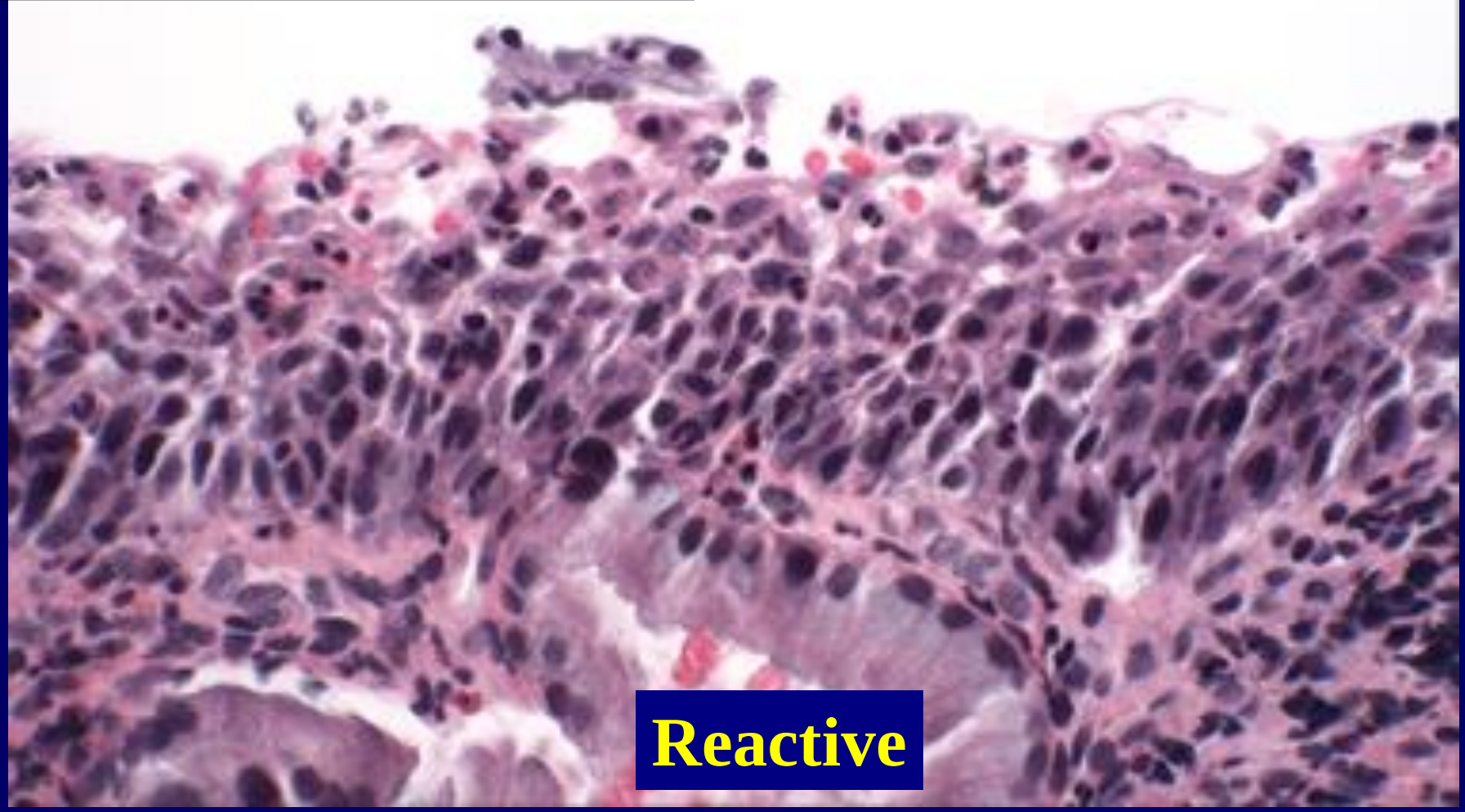
- P16 – Particularly useful for immature epithelia in reproductive age women
- MiB-1 – Atrophic background
- We use neither when the differential diagnosis is LSIL vs Normal
- P16 immunostaining will not discriminate LSIL from HSIL.

Atrophy +HSIL




MiB1

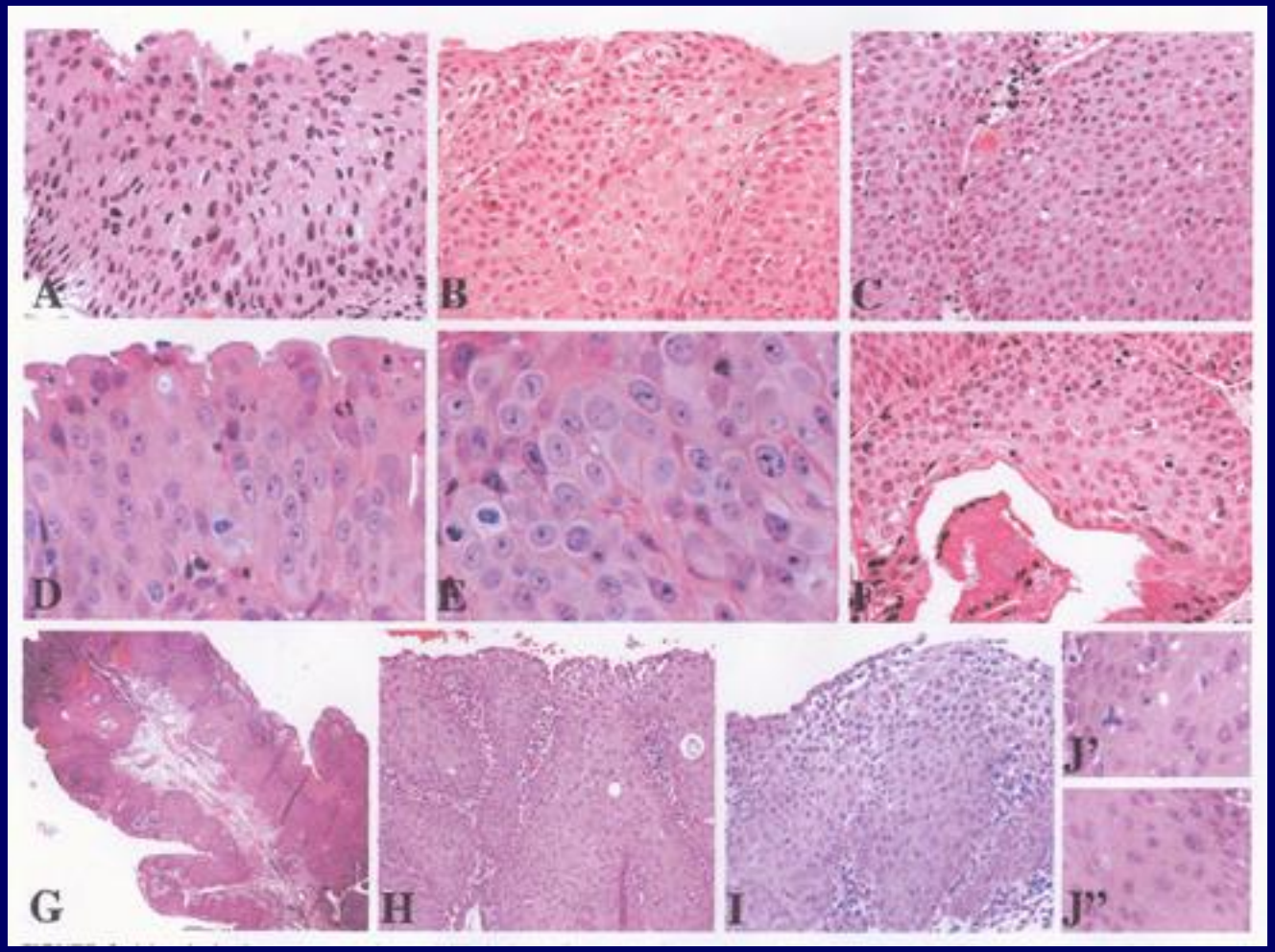

p16


Reactive

Issues

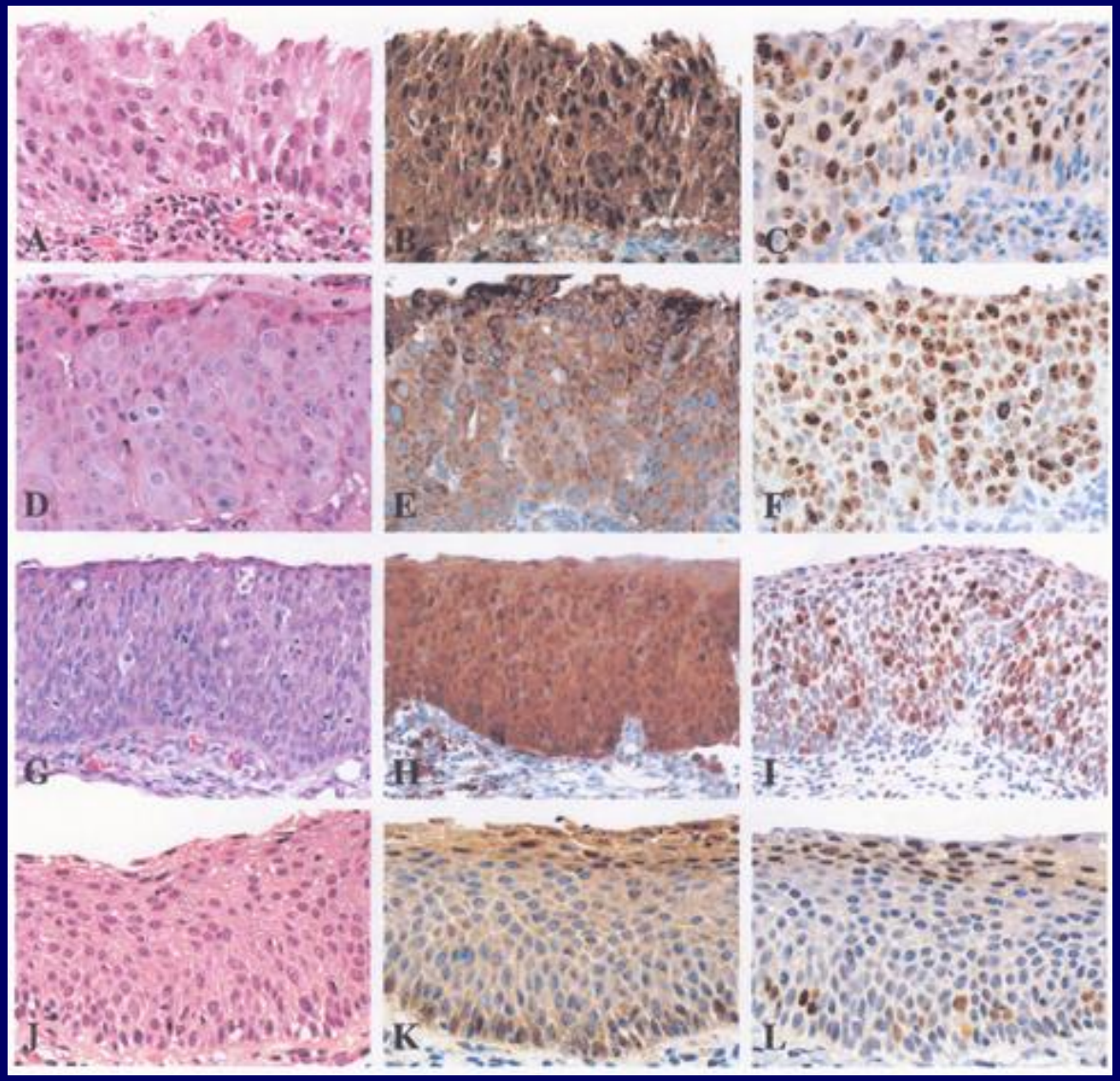
- What p16 staining pattern signifies a high-risk HPV?
- How do we confirm “progression” from LSIL to HSIL?
- Does p16 identify CIN2 lesions more likely to persist?
- Is high-risk HPV infection sufficient to produce progression?

Milder metaplastic atypias



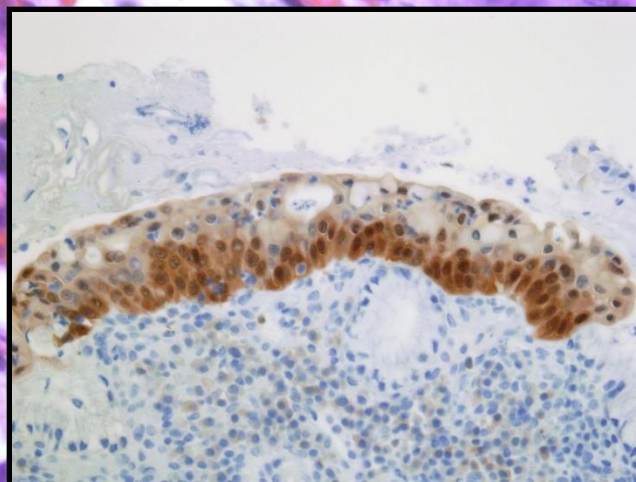
Zheng et al, 2004

Milder metaplastic atypias (p16)



Zheng et al, 2004

LSIL (immature met phenotype)



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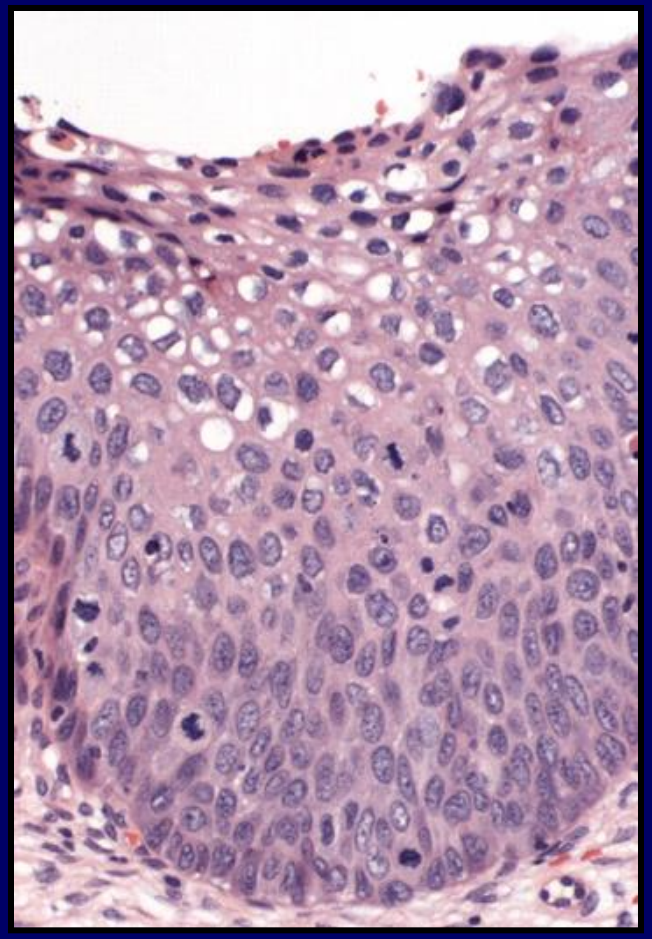
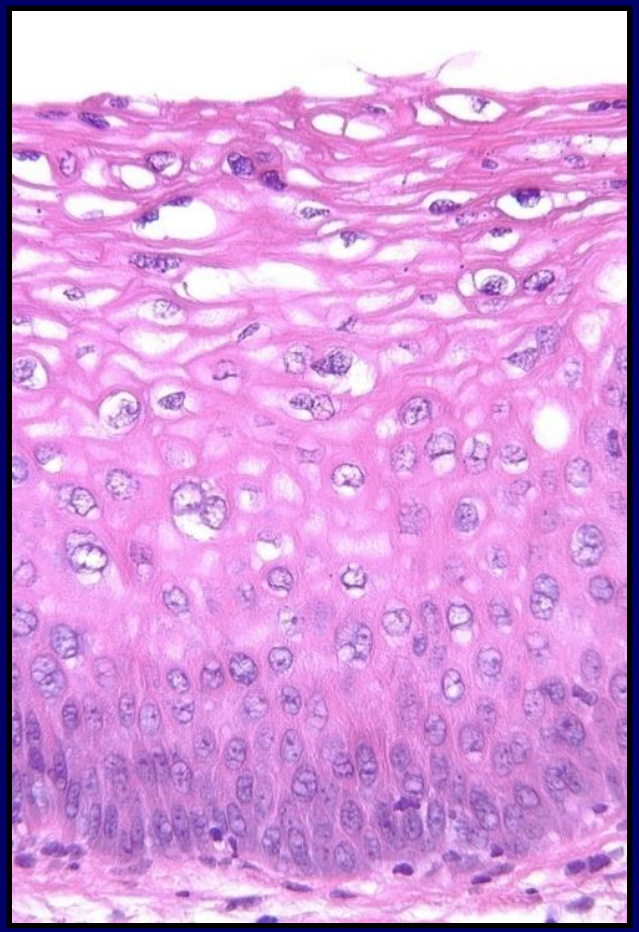
Ascertaining Outcome Risk

- Most high risk HPVs (85%) will not result in an HSIL (CIN3) outcome (Kahn)
- 40-60% or more of confirmed CIN2 biopsies will be followed by regression in women under age 25 (Crum, Mosckicki)
- The risk of HSIL in women with mild abnormalities and negative colpo or a biopsy of CIN1 is 11% (Cox)

Defining CIN1



1970



2000

Frequency of True Progression

- 12% of LSILs are followed by an HSIL at 2 years (Cox et al).
- 1% of LSILs (CIN1) progress to carcinomas (Östor's review)
- What percent are true progressions from LSIL to HSIL (CIN2)?

What We Found in our Practice

- 10% of biopsy proven LSILs (by report) will be followed by HSIL (CIN2+) in followup.
- About half on review will be re-classified as LSIL
- Overall, about 5% of LSILs are followed by HSIL
- Any LSIL followed by HSIL should be viewed with suspicion and all slides reviewed.

Issues

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Predicting Regression/Progression

- Guedes et al
  - 45 women followed with CIN2
  - 42% regressed
  - 11% persisted
  - 22% progressed to CIN3
  - 20% partial regression
  - No relationship to p16 status

International Journal of Gynecologic Cancer 2007

Issues

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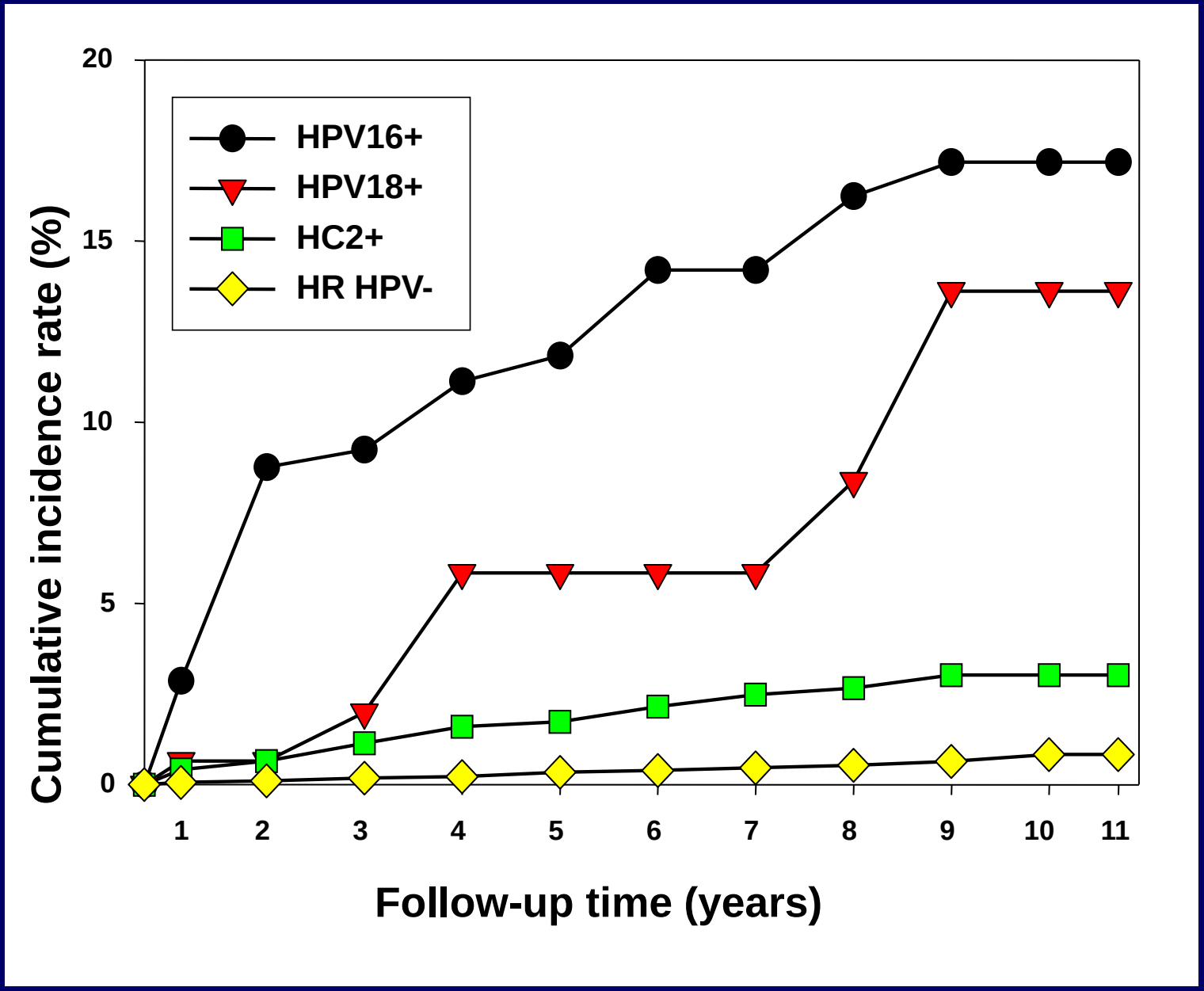
Prospective Risk of ≥CIN3 with mild atyp cytology



Khan *et al.*, JNCI

Summary

- The range of cervical histologic abnormalities that represent HPV infections is expanding
- “Progression” of LSIL (as currently defined) to HSIL is enriched for error and should be reviewed.
- 40+% of CIN2 regress in 6 months.
- Depending on the clinician it may be more prudent to define a histologically “indeterminate” group and follow than to employ p16 and arbitrarily classify as CIN2.

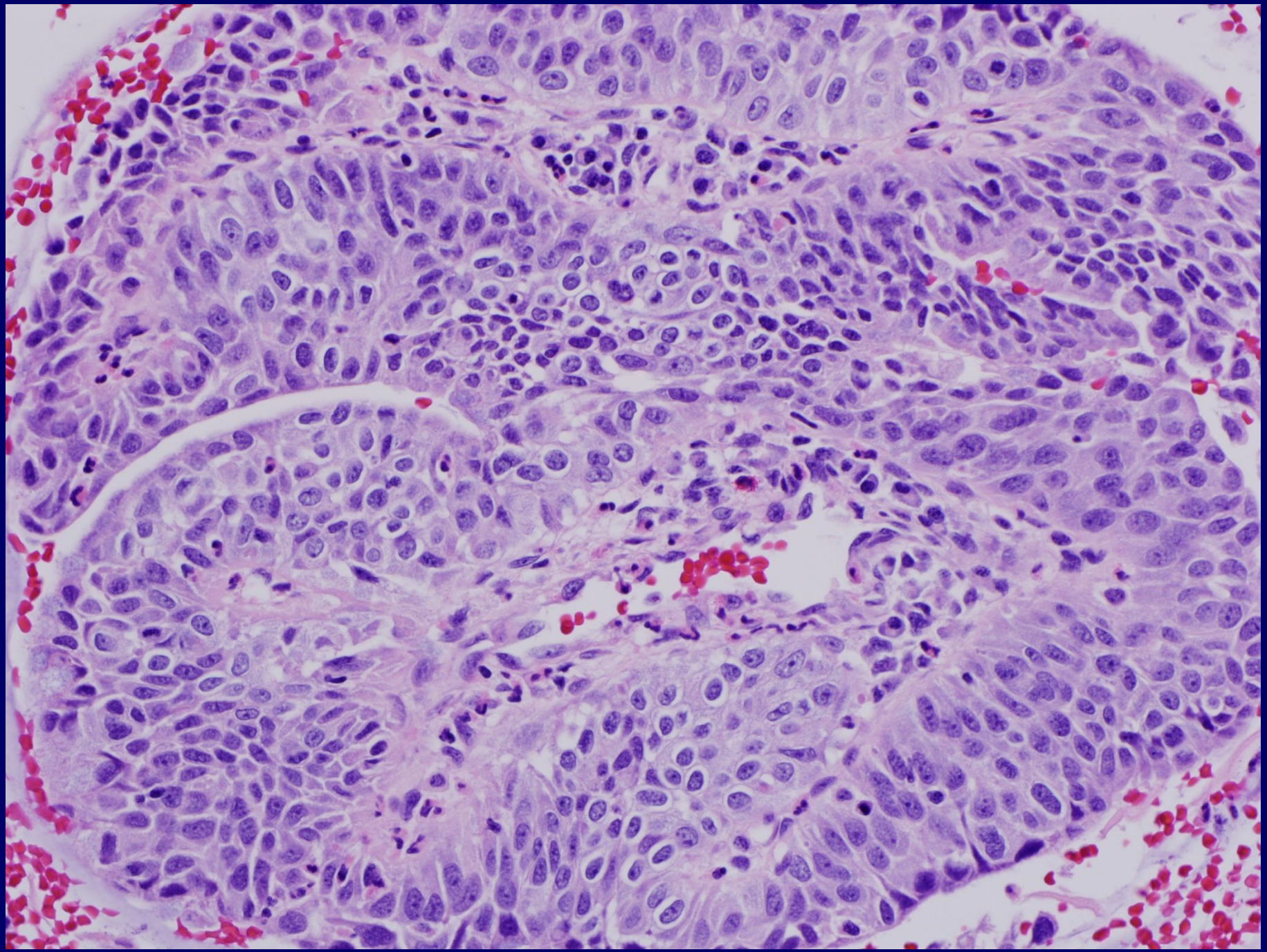
Three Approaches

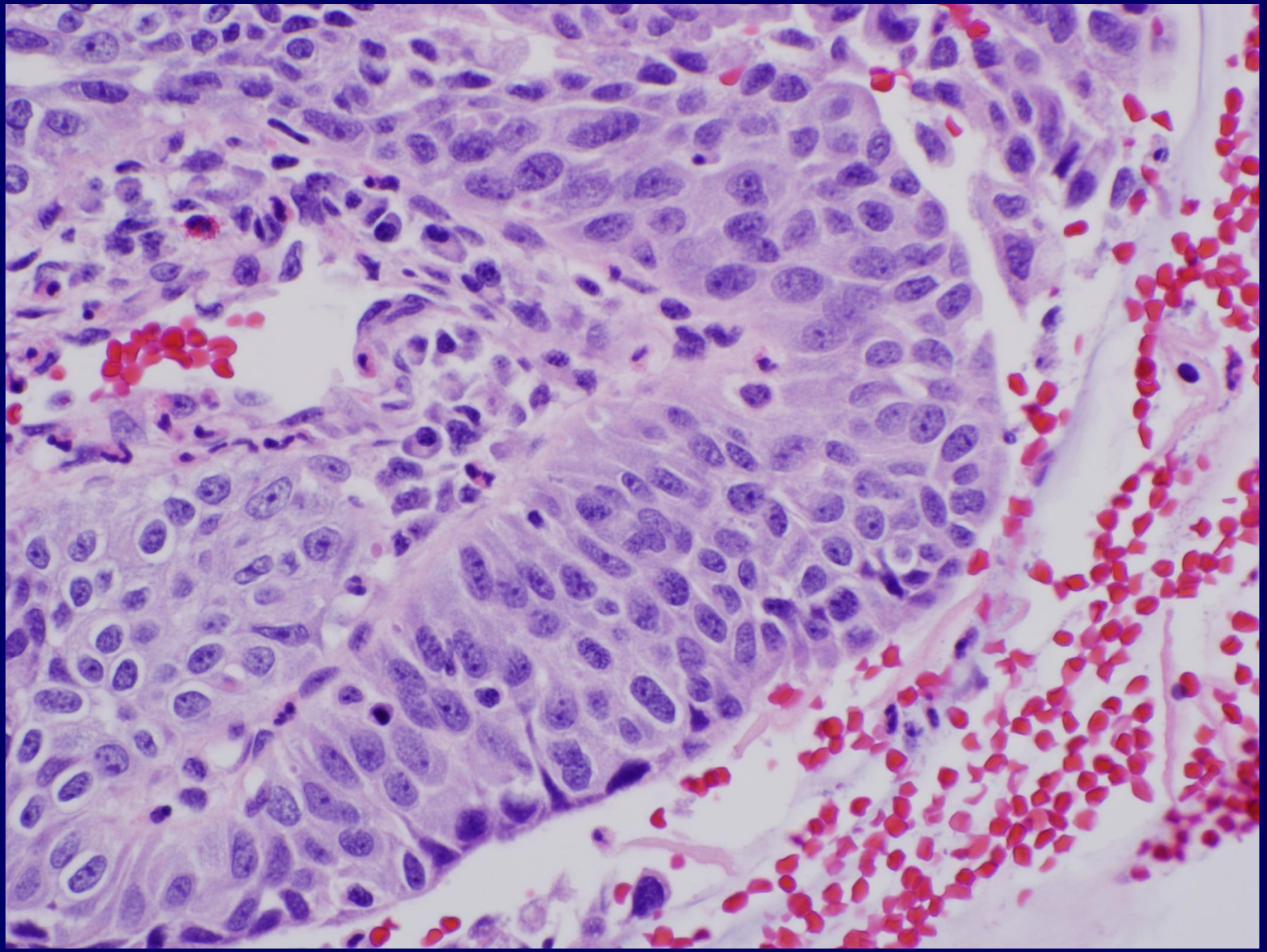
- Triage borderline with p16 (LAST recommendations)
  - Everyone's borderline is different
  - Potential over-reliance on the p16 immunostain
- Triage borderline with a second observer, p16 optional
- Apply an intuitive grading system (LSIL, HSIL, QSIL) and triage QSIL to a six month repeat.

Two Cases

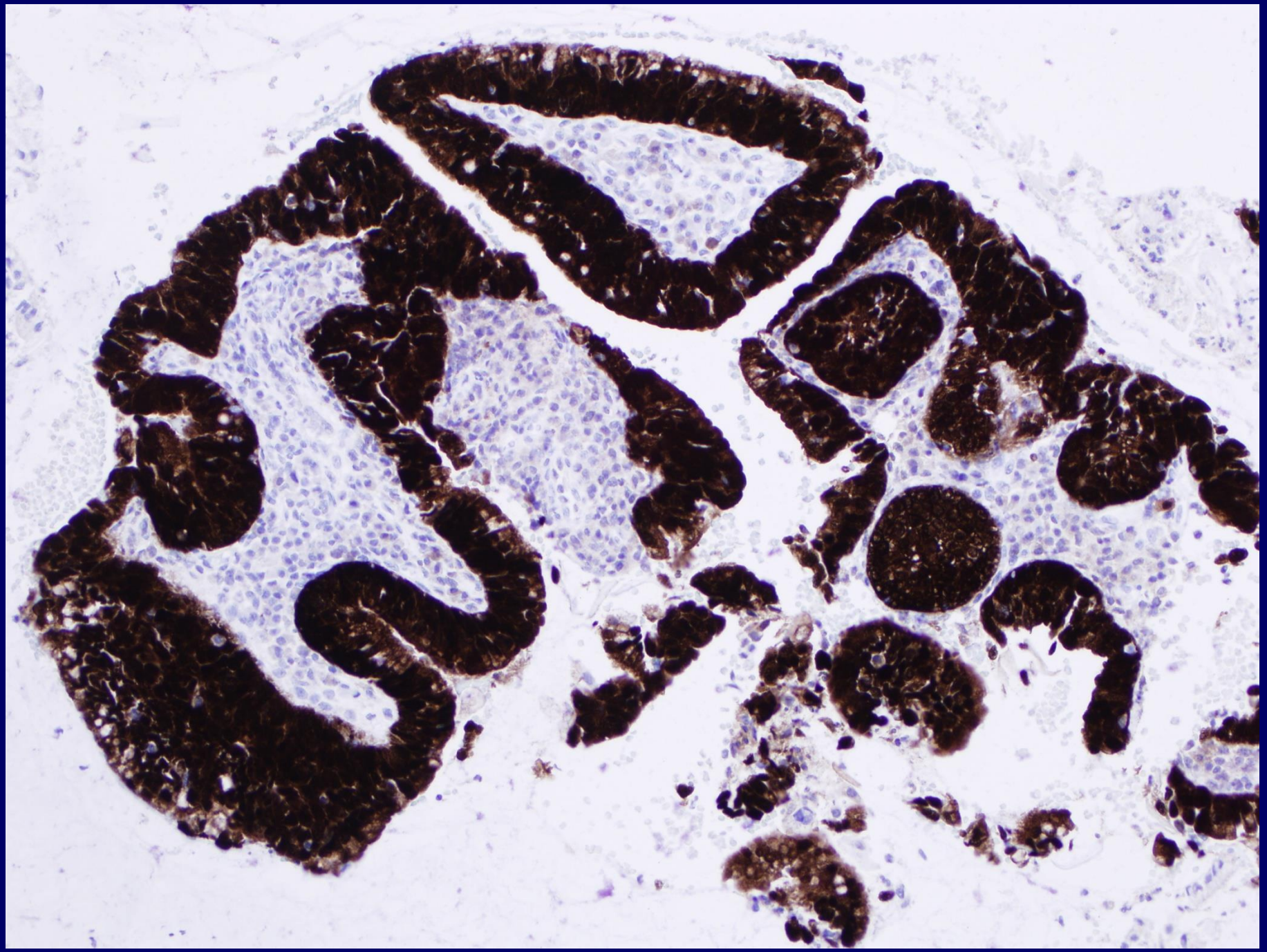
History

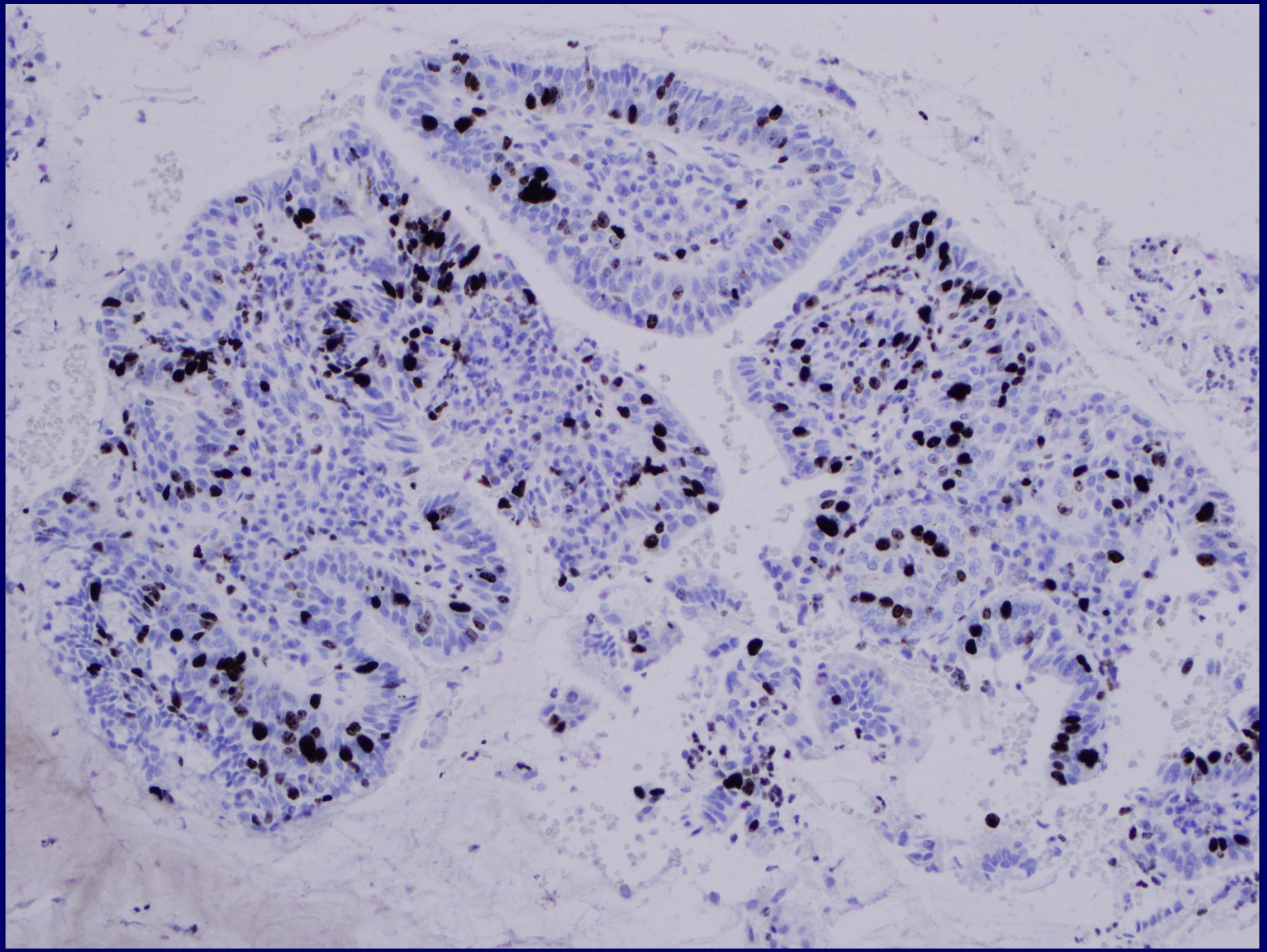
- Reproductive age woman with an abnormal cervical cytology (ASCUS)
- This is a cervical biopsy



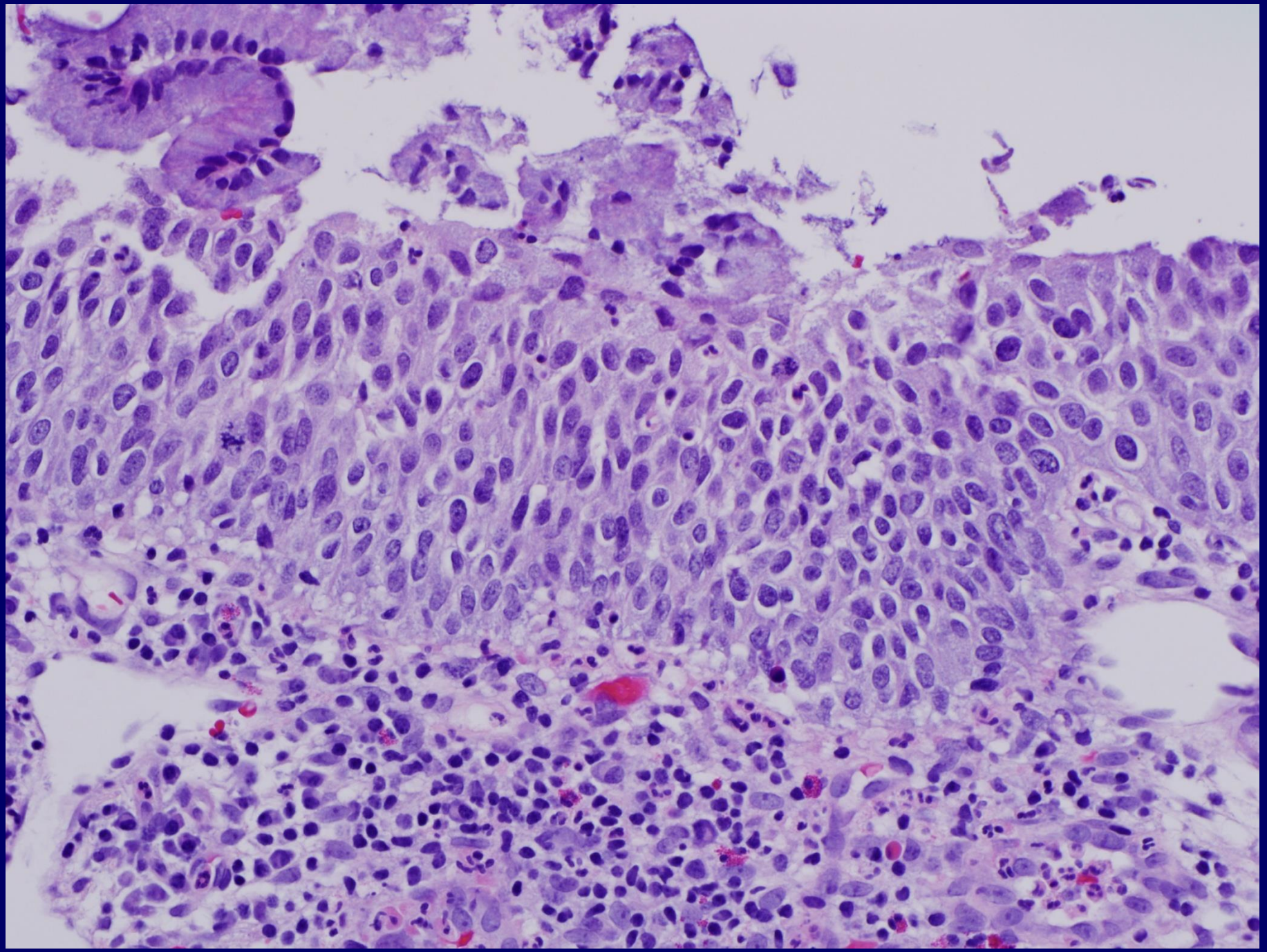


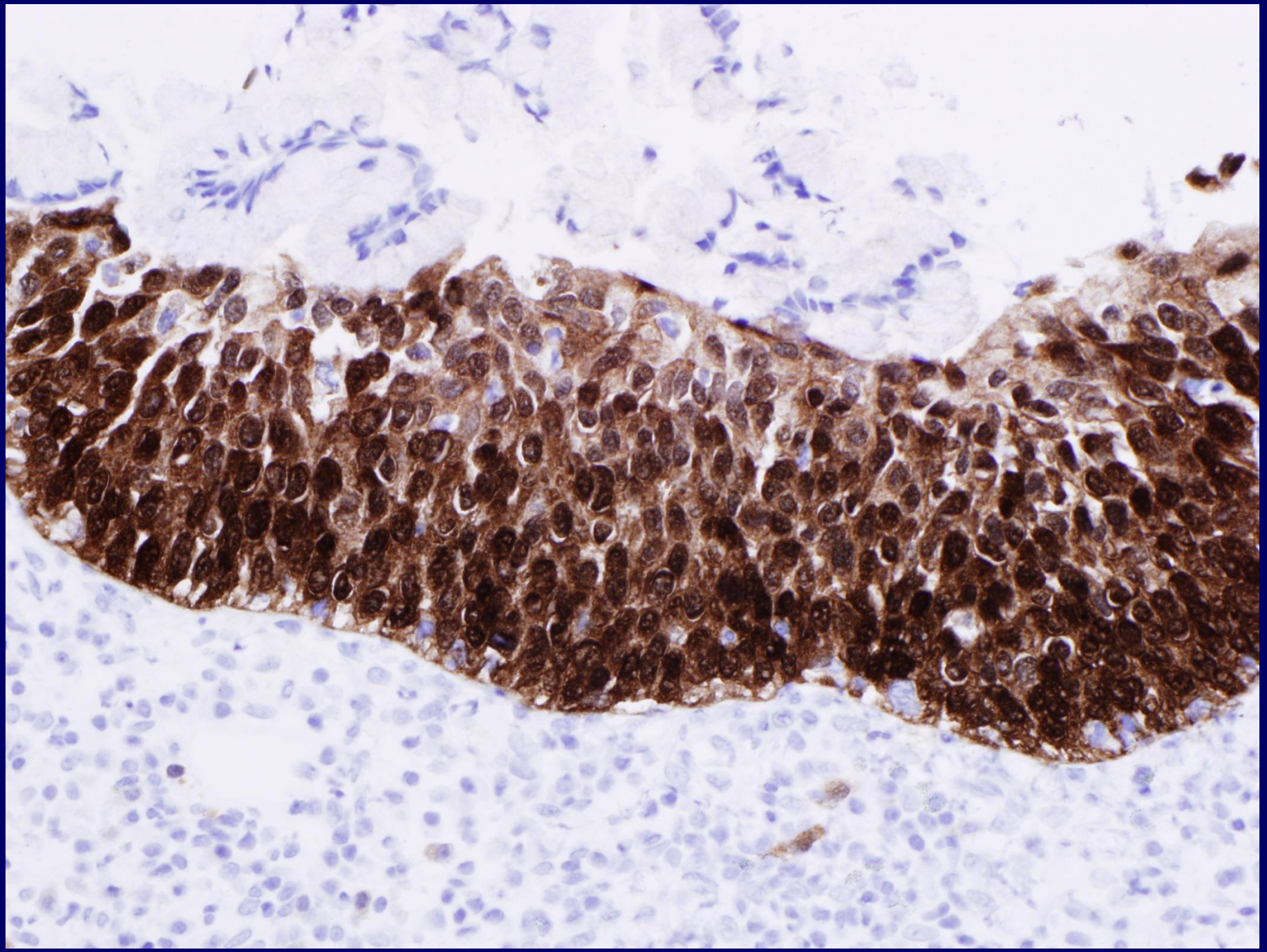
Diagnosis?

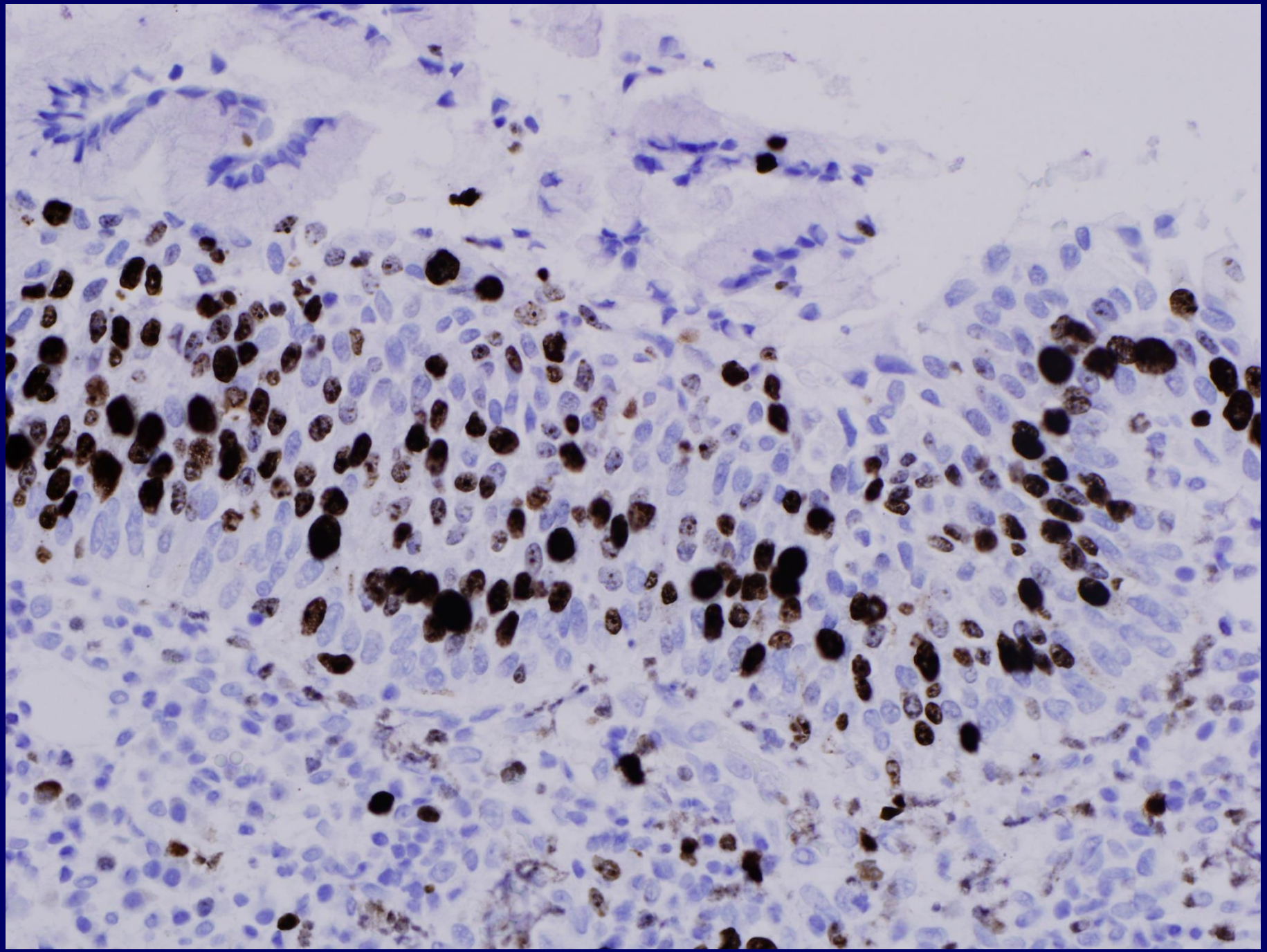


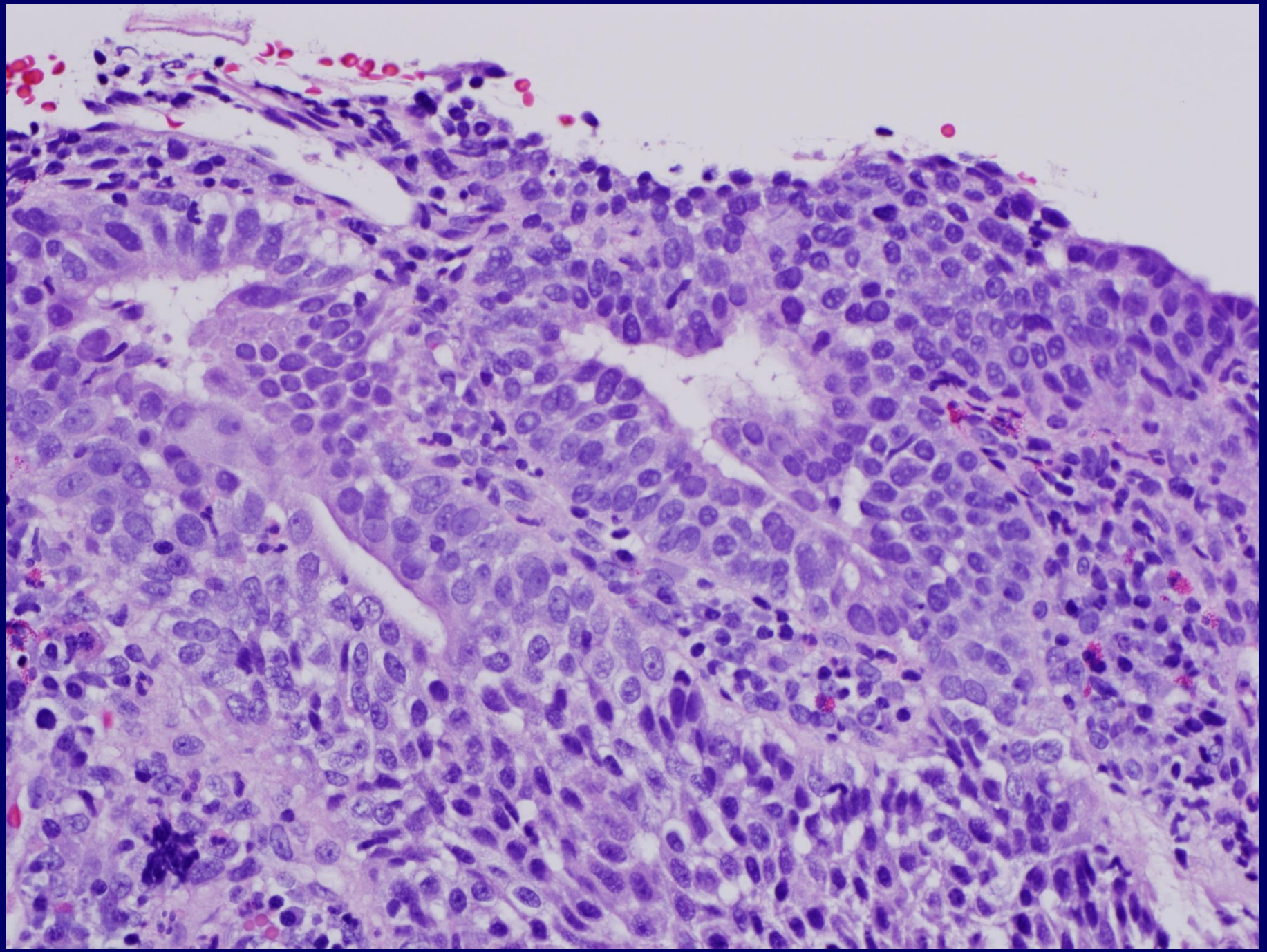


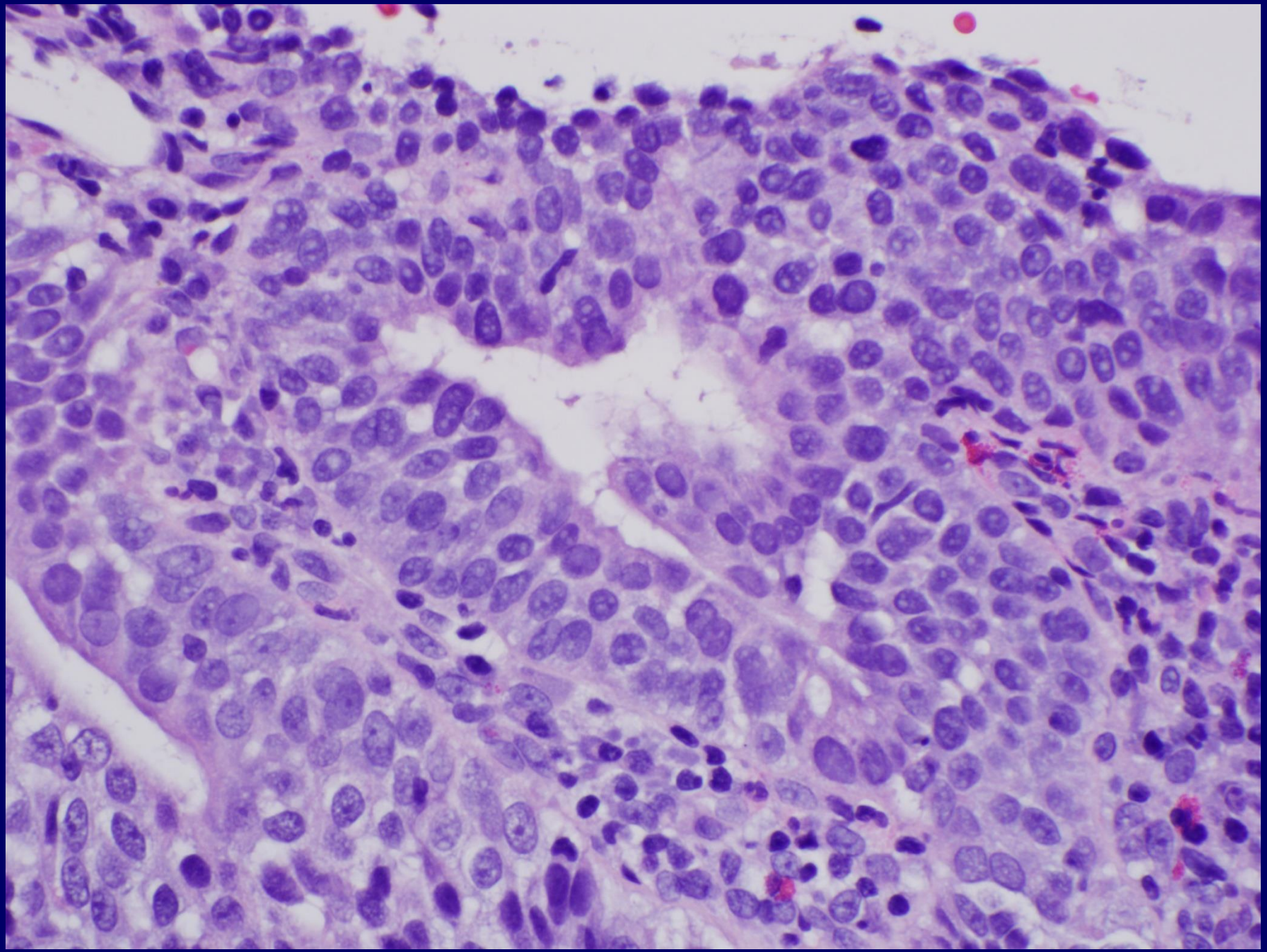
Additional Biopsy

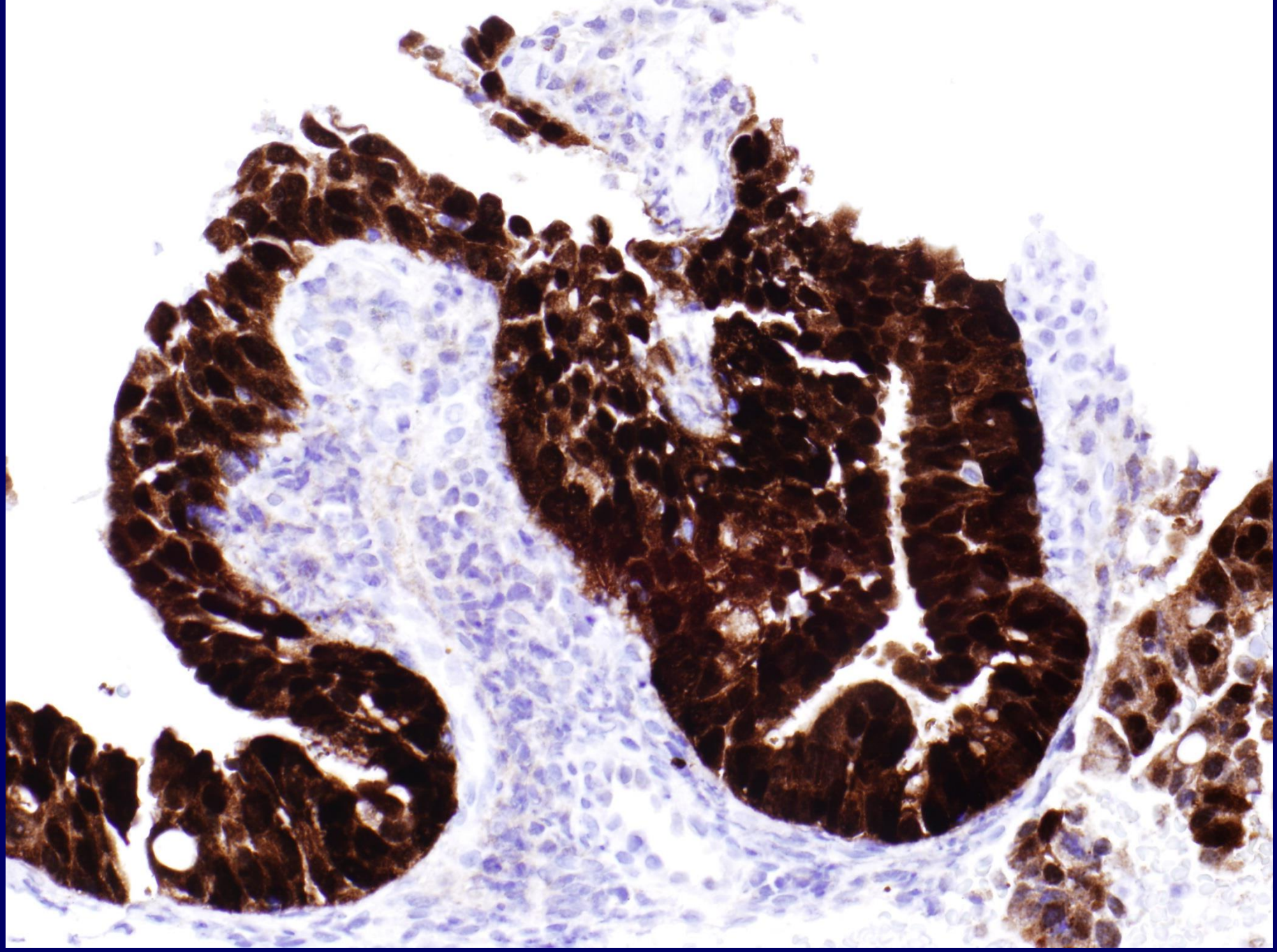


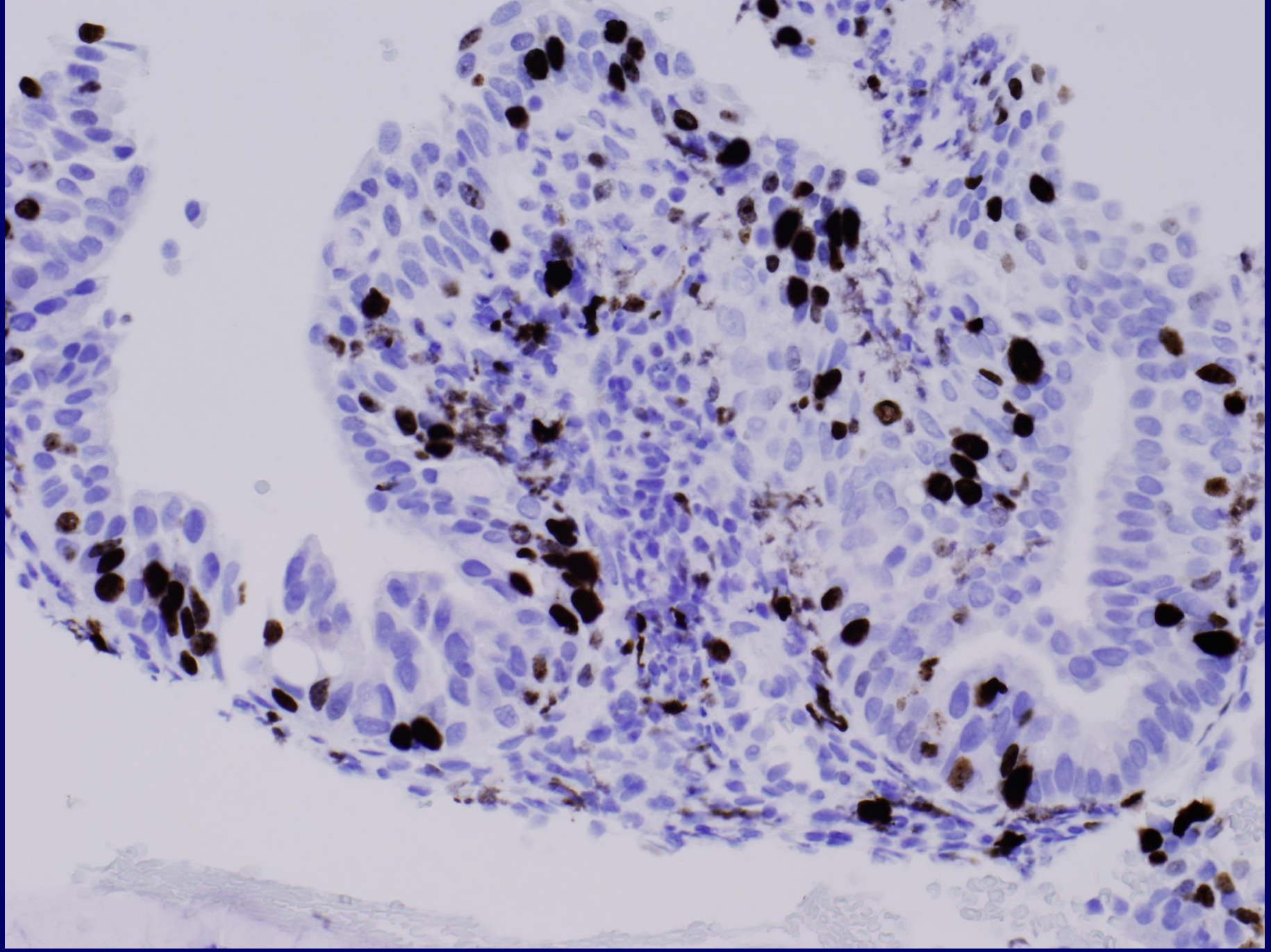












Diagnosis?

Diagnosis

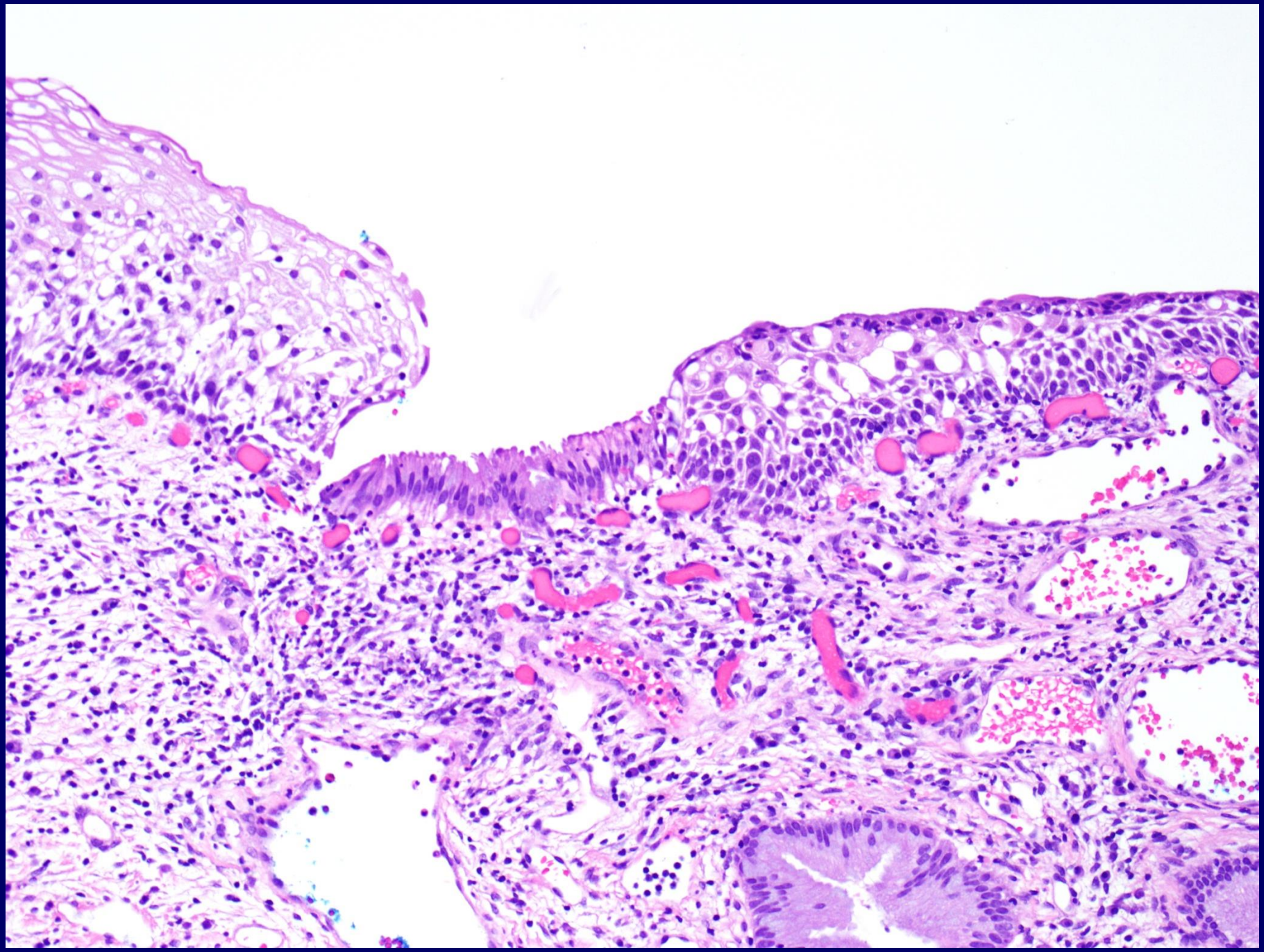
SPECIMEN DESIGNATED “Cervix BIOPSY":

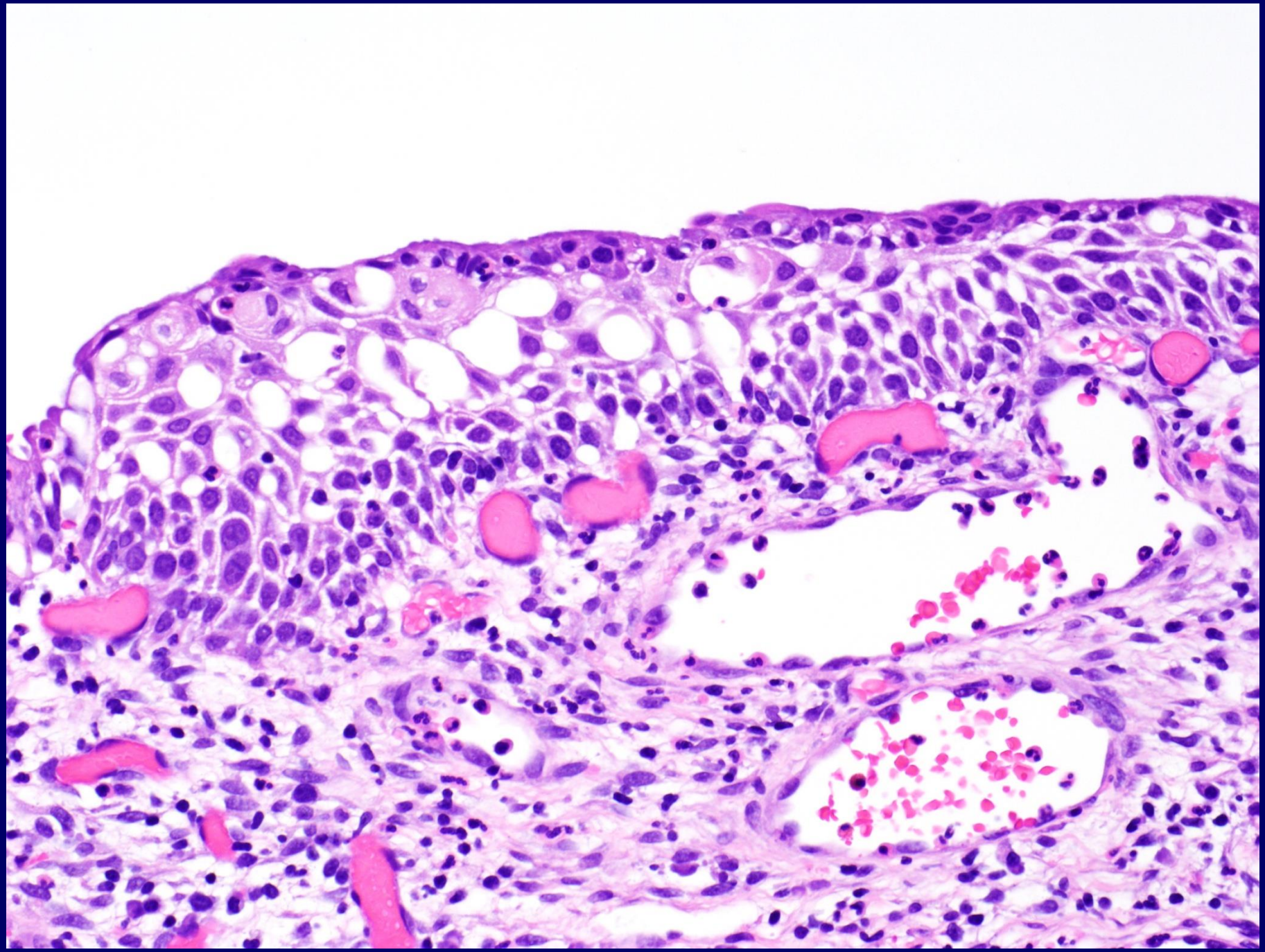
High grade squamous intraepithelial lesion (CIN 2).

Note: This lesion exhibits a spectrum of changes ranging from CINI to CINII. It exhibits a metaplastic phenotype.

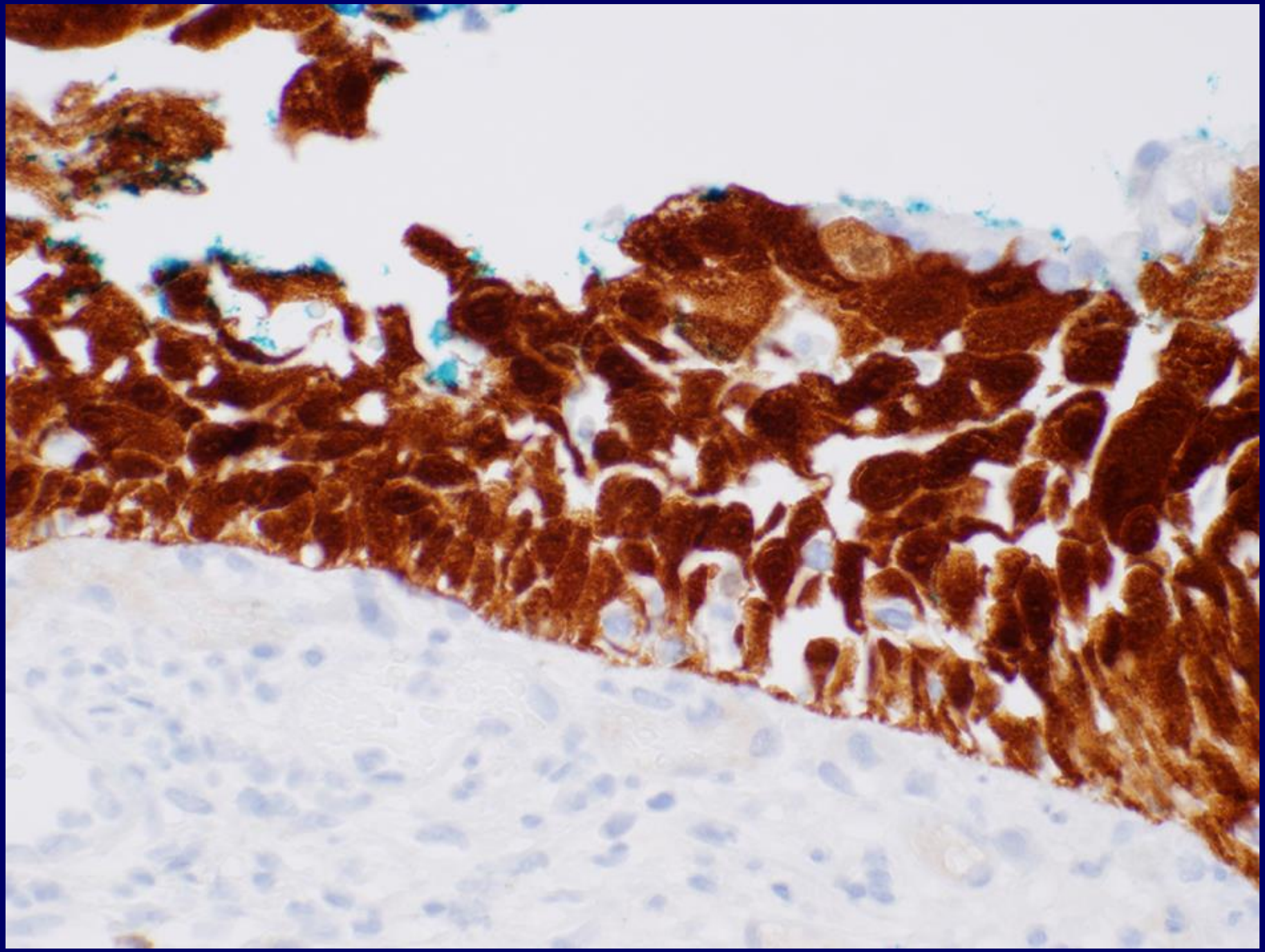
History

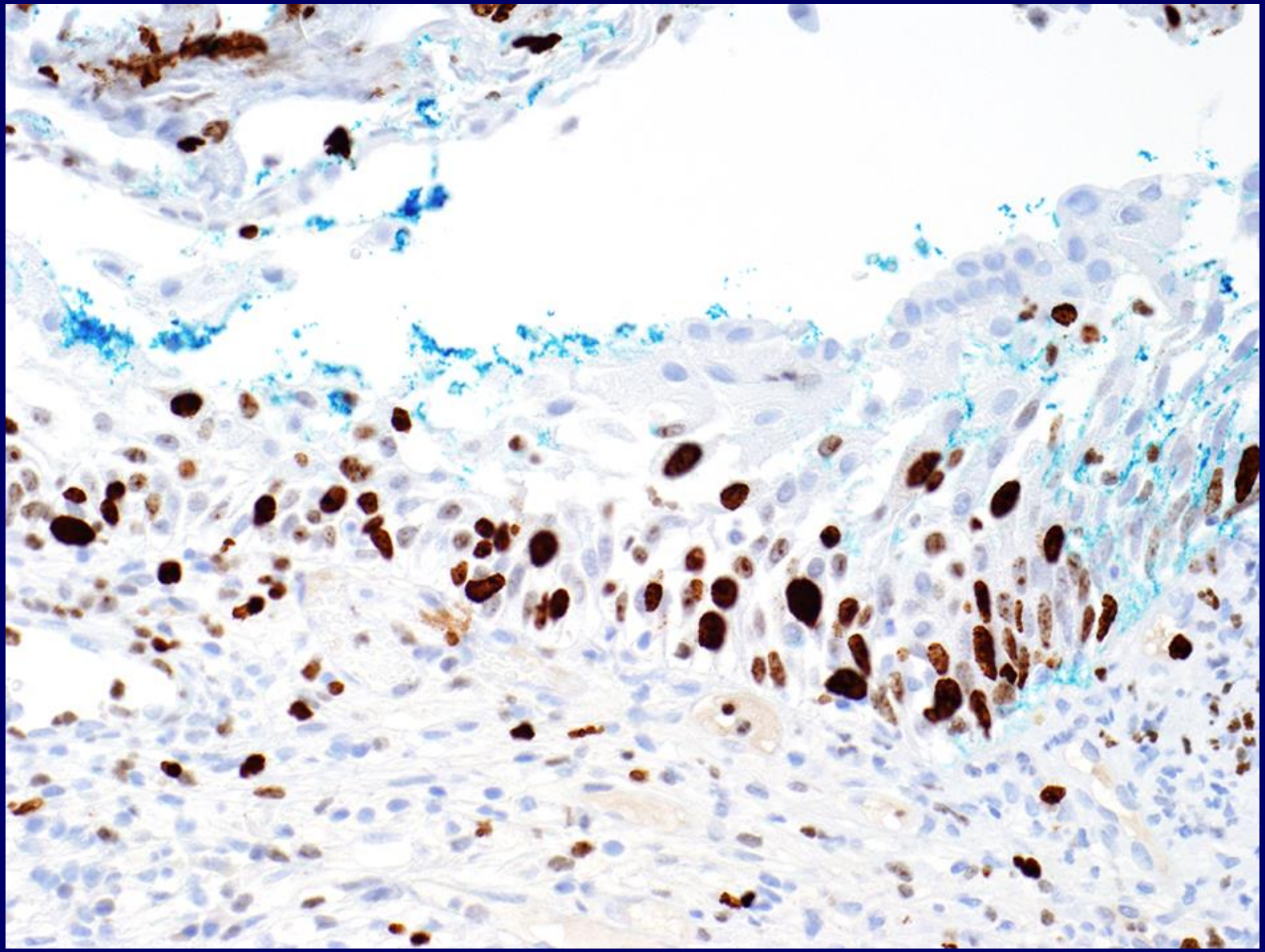
- Reproductive age woman with an abnormal cervical cytology (ASCUS)
- This is a cervical biopsy





Diagnosis?





Diagnosis

SPECIMEN DESIGNATED “Cervix BIOPSY":

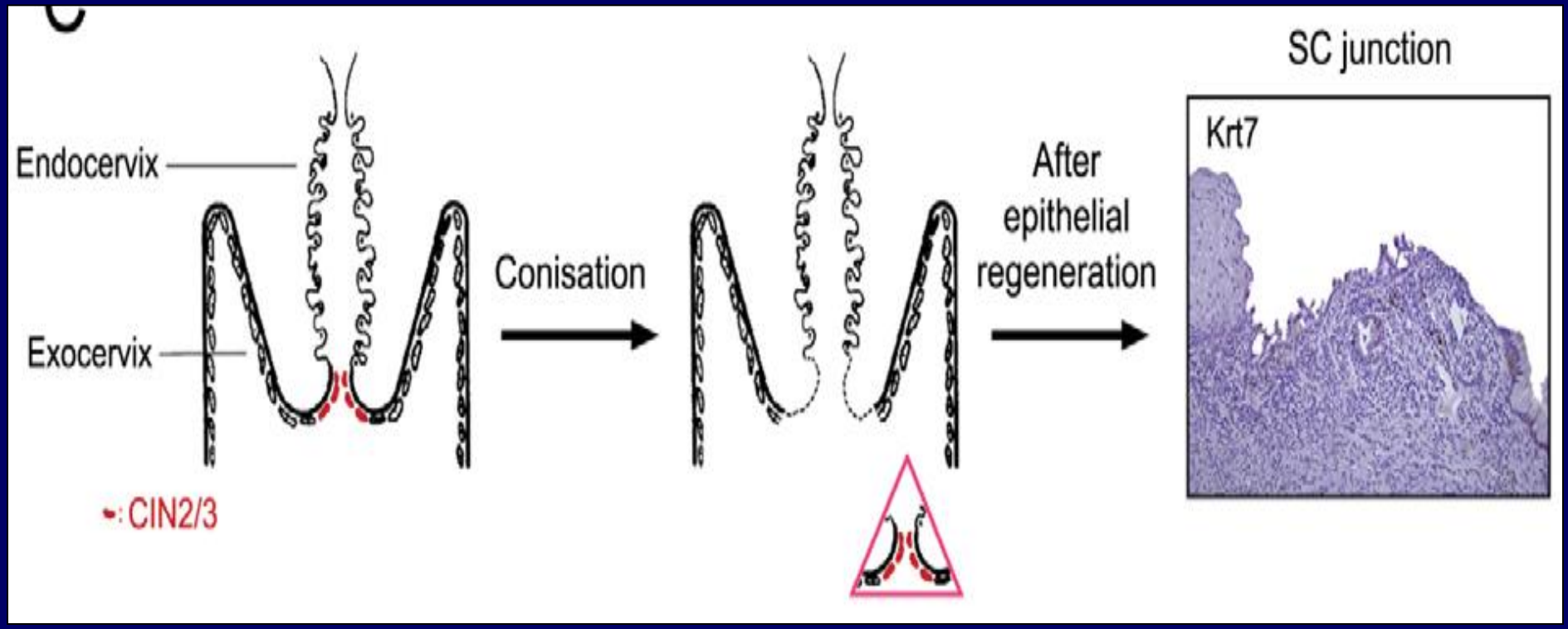
Atypical metaplasia consistent with squamous intraepithelial lesion, but not amenable to precise grading (“QSIL”)

Note: This lesion is p16 positive and exhibits a metaplastic phenotype with mild atypia. It is consistent with a squamous intraepithelial lesion but cannot be graded. Followup is advised.

Outline

- The question
- Defining the SCJ and transformation zone
- Parallels between metaplasia and neoplasia
- Differential risk
- Grading schemes in mature and immature metaplastic epithelium
- The good and bad of p16
- Targeted prevention

SC junction cells are not replenished after excision (LEEP)



Herfs, PNAS 2012

Evidence that SCJ ablation alters recurrence patterns

- Disparity between pre and post ablation HPV-related lesions (Herfs et al)
  - Infrequent
  - Ectocervical
  - Lower grade
  - Low progression rate

Herfs et al 2014, Int J Cancer

Does excision of the SCJ prevent cervical cancer?

- Recurrence rates of SIL exceedingly low in the cryotherapy era, when both LSIL and HSIL were treated.
- Most recurrences are LSIL and do not appear to originate from the SC junction
- Cryotherapy reduces subsequent HPV infection by 50% in HIV infected women (Taylor et al 2010).
- Anecdotal evidence (Gustafson and others)

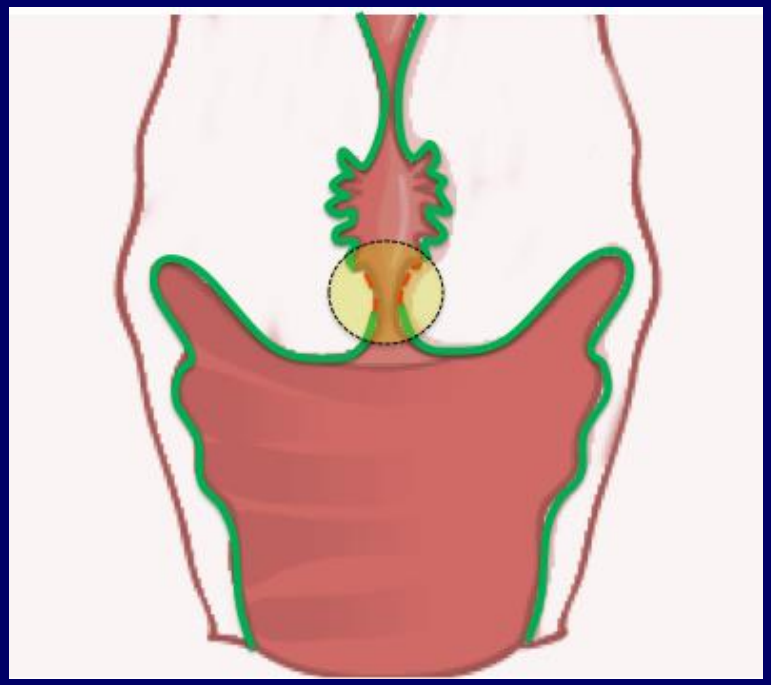
Risk of Recurrent CIN3 Post LEEP

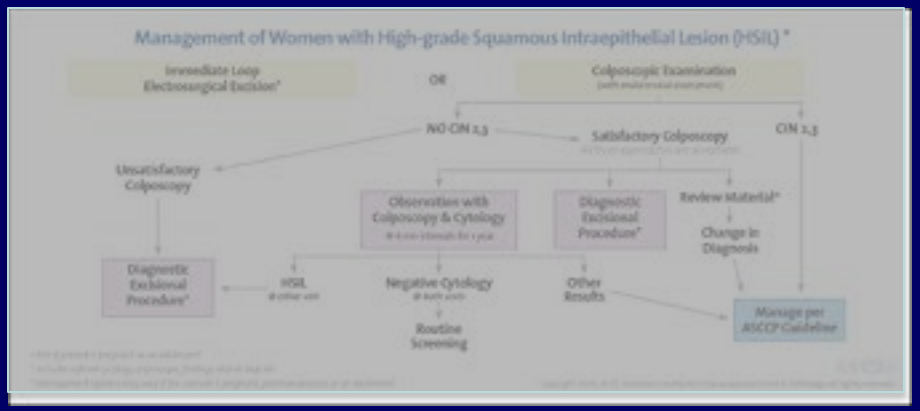
435 Women

Parameter	Percent Recurring			
	CIN II+ (5yr)	CIN II+ (10 yr)	CIN3 (5yr)	CIN3 (10yr)
All Cases	16.5	18.3	8.6	9.2
3 Neg Paps	2.9	5.2	0.7	0.7
Neg co-testing at 6/24 mo	1.0	3.6	0.0	0.0

Kocken et al. Lancet Oncol 2011

Preventing Cervical Cancer in a Sexually Active Vulnerable Population





? OR ?

Summary

- Tread lightly when you are not certain of whether a lesion is CIN1 or CIN2
- In such cases expect p16 to almost always be positive; if you know how to separate CIN from reactive changes you will not get much help from p16.
- Post excisional “recurrences” should be critically evaluated.
- Low impact pre-emptive SCJ ablation should be explored in populations where sexually active women are at high risk due to inadequate resources to guarantee regular screening.

Acknowledgements

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www.womenspath.org
Education/lecture files