HPV and pathogenesis of SIL

Behrooz Shokouhi, MD ACP

cancer of the cervix

- a burden of human suffering and mortality
  - is disproportionate to its size
- due to the susceptibility of the epithelium of the cervical transformation zone to infection by oncogenic HPV

➢ cervical screening programs

➢ Vaccination against HPV

- remains one of the most common cancers

HPV

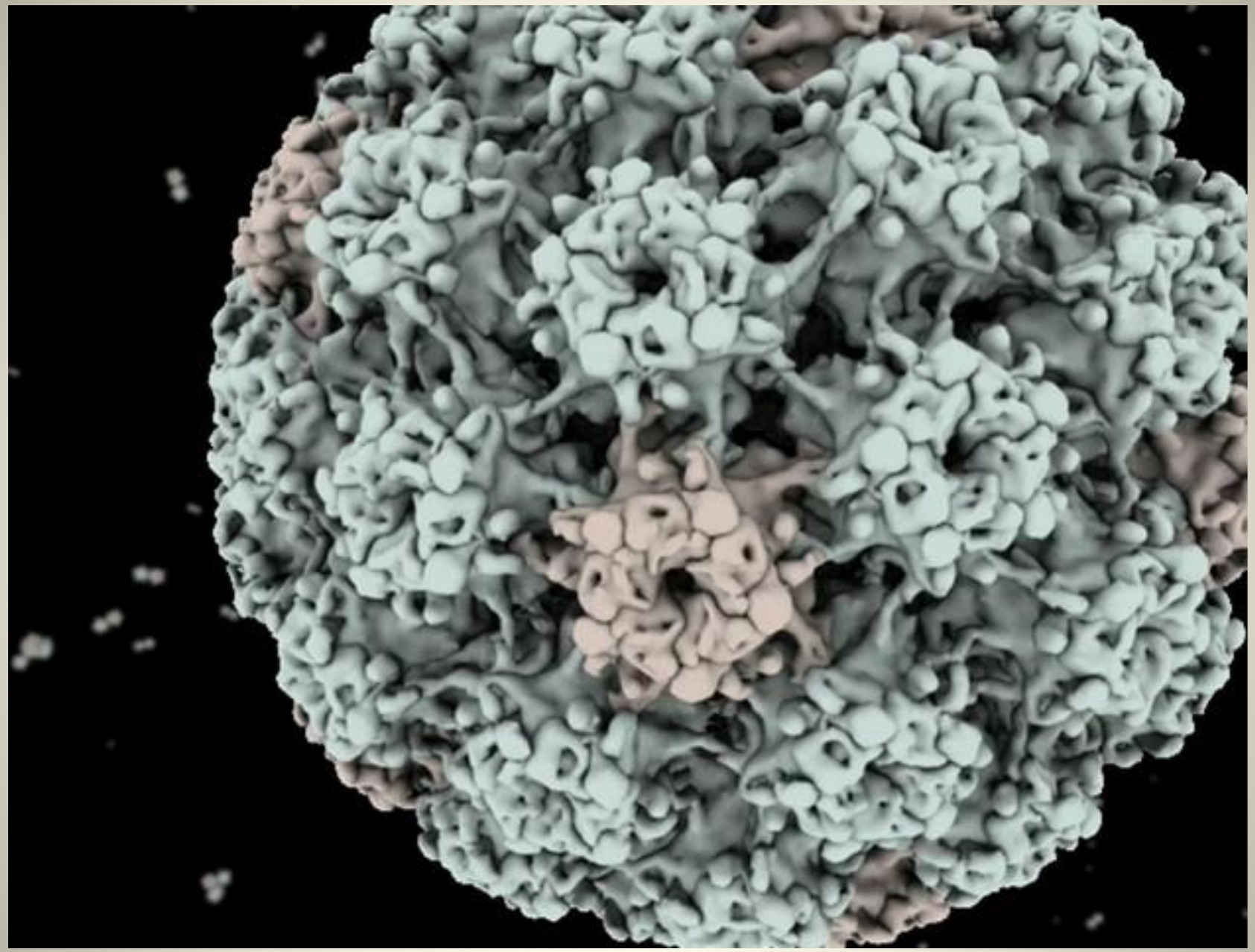
and

the Lower Female Genital Tract

a rapidly advancing field

The role of HPV in the pathogenesis

- of both condyloma and carcinoma
- **Relatively recently** was unsuspected
- **greater than 99% of cervical carcinomas** are associated with oncogenic HPV
- **the molecular mechanisms** of virus-mediated oncogenesis are understood in considerable detail



HPV, a DNA virus

- is classified on the viral genome into different genotypes
- HPV-2 is the most common cause of verruca vulgaris
- **the genital HPV genotypes**, transmitted by sexual contact
  - associated with condyloma, the low-risk HPV genotypes, 6 & 11
  - associated with cervical carcinoma, the high-risk genotypes (oncogenic), the risk varies, 16 & 18
  - possibly oncogenic, of uncertain oncogenic potential

Table 1 | **Papillomavirus types in genital lesions**

Type of genital lesion	HPV type	
	Less prevalent	More prevalent
Condylomata acuminata	42,44,51,53,83	6,11
Intraepithelial neoplasias	6,11,18,26,30,31,33,34,35,39,40,42,43, 45,51,52,53,54,55,56,57,58,59,61,62,64, 66,67,68,69,70,71,73,74,79,81,82,83,84	16
Cervical and other anogenital cancers	(6,11),18,31,33,35,39,45,51,52,54,56, 58,59,66,68,69	16

Human papillomavirus (HPV) types in brackets indicate extremely rare prevalence.

The genital HPVs are epitheliotropic

- the high-risk HPV types, in particular, have a tropism for **the metaplastic squamous cells at the squamocolumnar junction** of cervix
- This has very important consequences for cytologic screening of cervix
- Also for **native squamous epithelium** of other sites, in contrast to most cervical cases

cytologic screening of cervix

- The **most precursor lesions and carcinomas** arise at SCJ
- is **amenable to sampling** with a brush or spatula
- if the lesions of HPV-associated neoplasia of the genital tract were **randomly distributed** within the squamous mucosa, screening would have failed
  - as it has in the oral cavity, pharynx, esophagus, larynx, skin, vulva, vagina and ectocervix

Potentially infectious virus particles

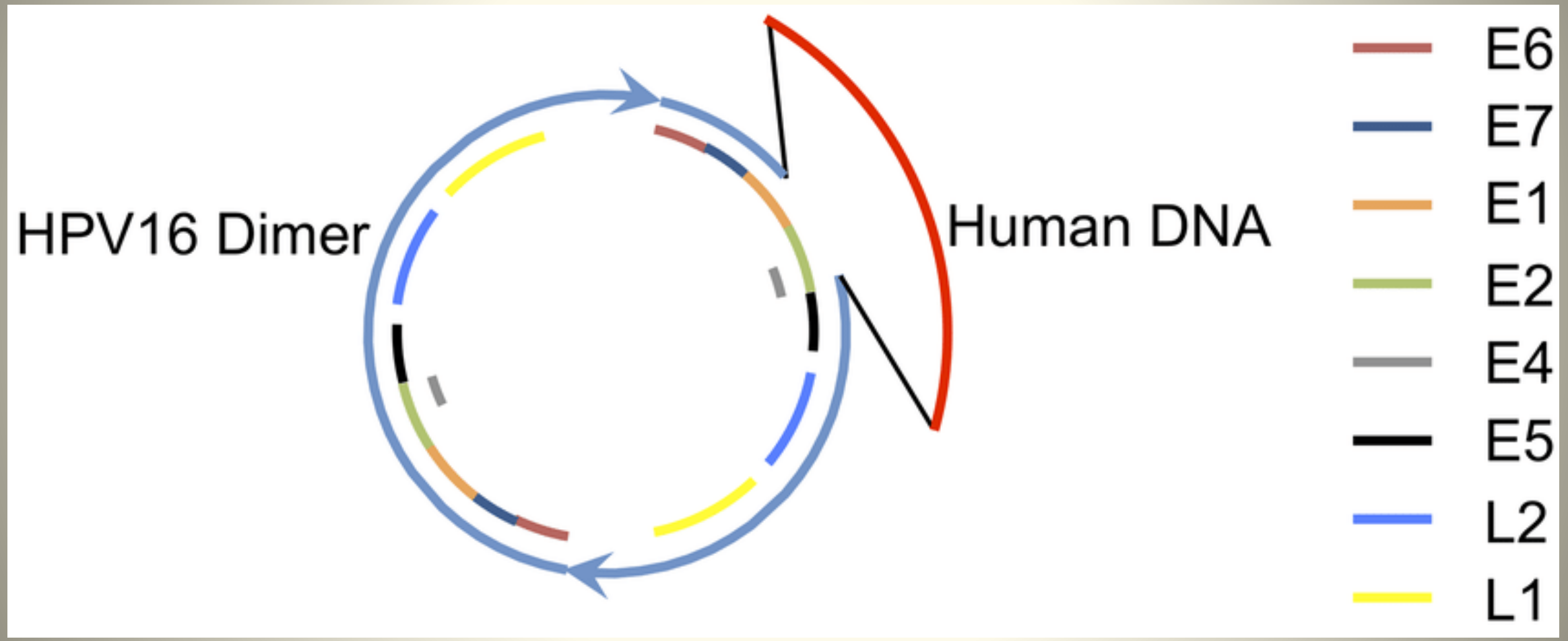
- must reach the **proliferating basal cells** for productive infection to take place
- **a micro-injury** to the squamous epithelium is required
- In the case of glandular epithelium
  - the stem cell compartment should be more accessible
  - infection of cervical glandular epithelial cells is less likely

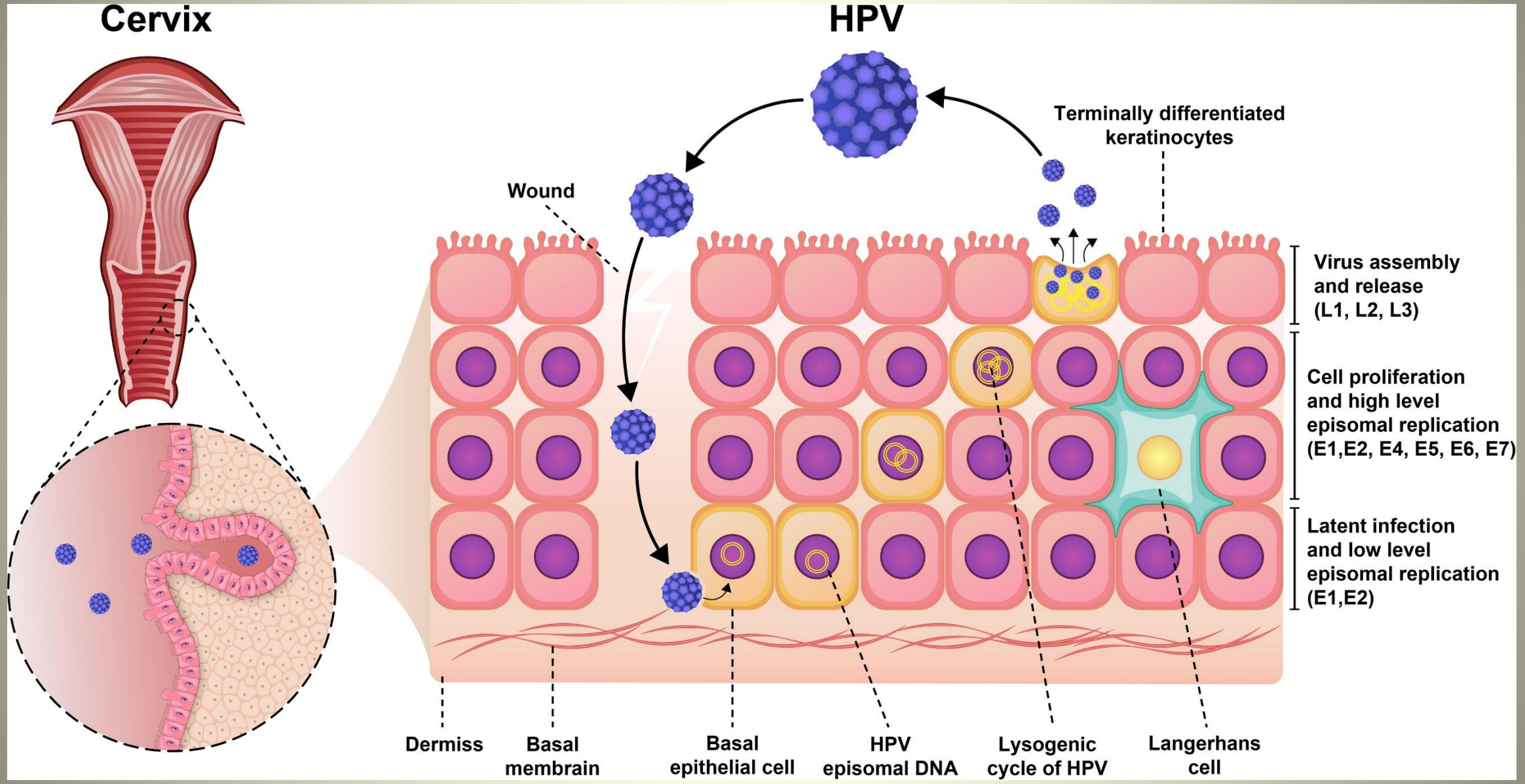
silent infection
productive infection

silent infection

- Once the virus particles are taken up by the basal epithelial cells
- methylation of the viral genome
- **Episomic viral DNA remains in the cell**
- but is not transcribed or translated

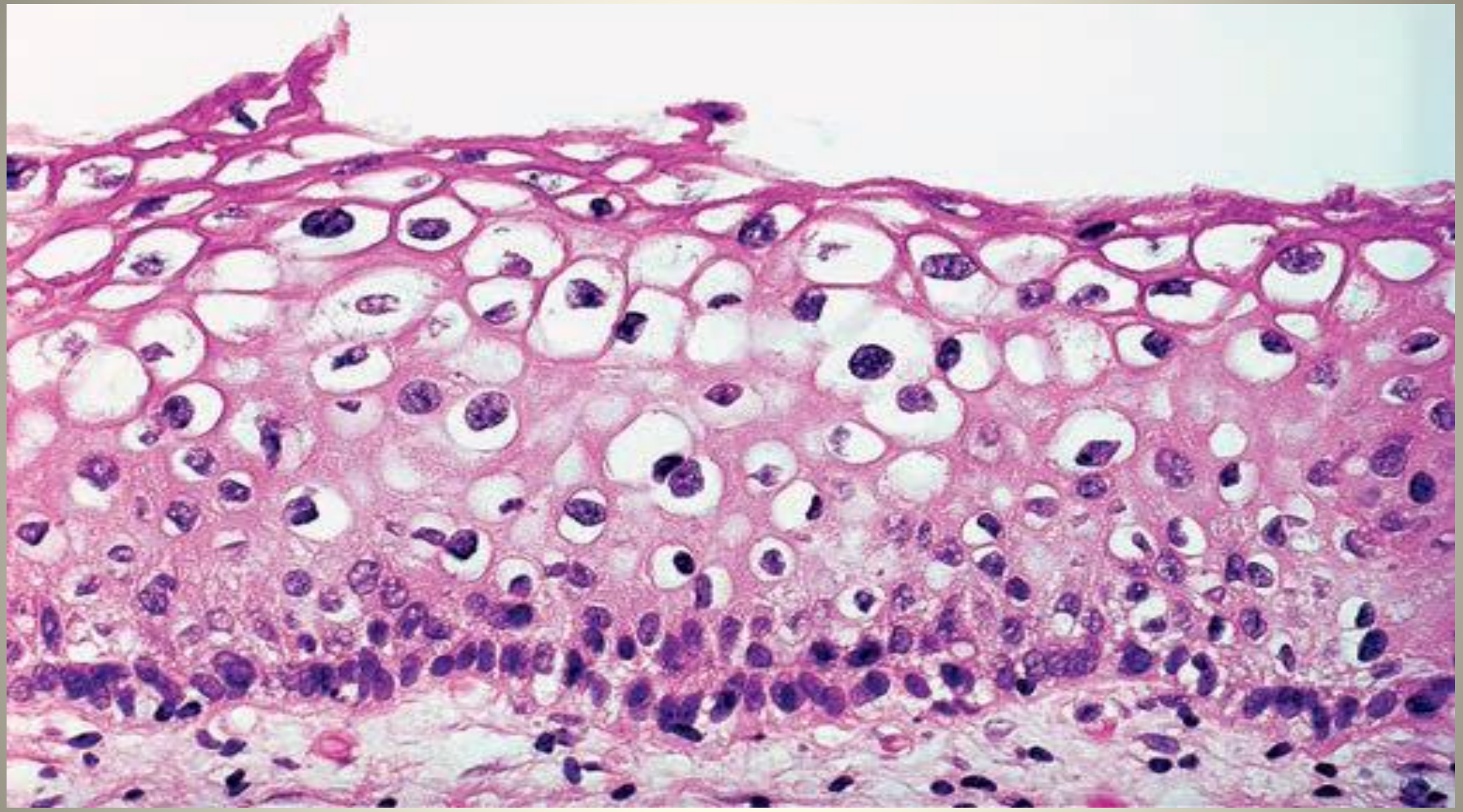
the HPV Dimer-Human DNA Hybrid Episome




Cervix
HPV
Terminally differentiated keratinocytes
Wound
Virus assembly and release (L1, L2, L3)
Cell proliferation and high level episomal replication (E1,E2, E4, E5, E6, E7)
Latent infection and low level episomal replication (E1,E2)
Dermiss
Basal membrain
Basal epithelial cell
HPV episomal DNA
Lysogenic cycle of HPV
Langerhans cell

productive infection may result

- may result in **orderly expression of viral genes**
- the host squamous cells mature
- assembly and release of infectious virus particles at the epithelial surface
- This is typically associated with **koilocytic change**
- a viral cytopathic effect: the E4 protein encoded by the viral genome causes disruption in the cytoplasmic keratin matrix

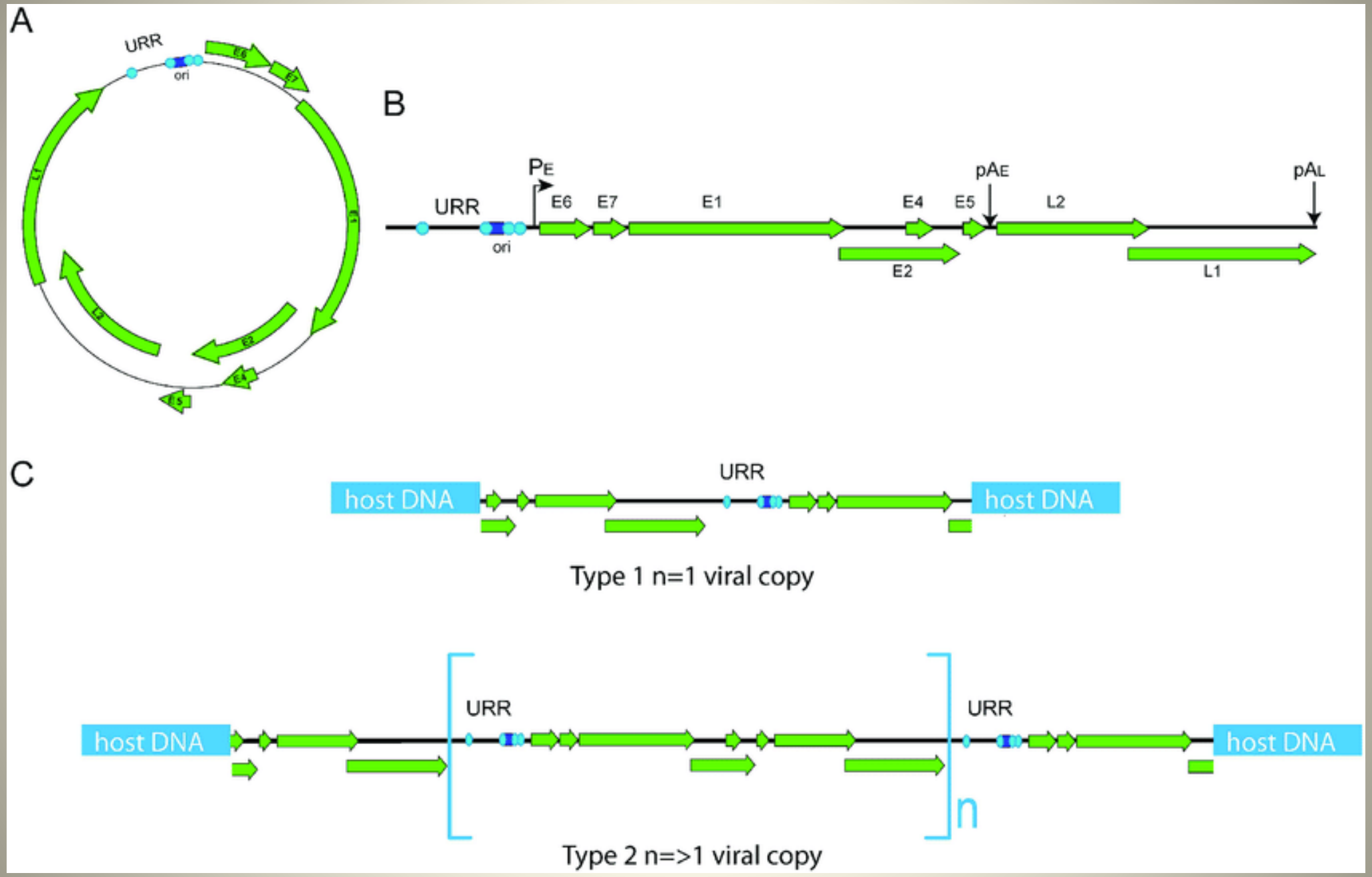


LSIL

- either a diploid or polyploidy nuclear DNA distribution
- orderly expression of the viral genome
- the squamous cells mature and move to the surface

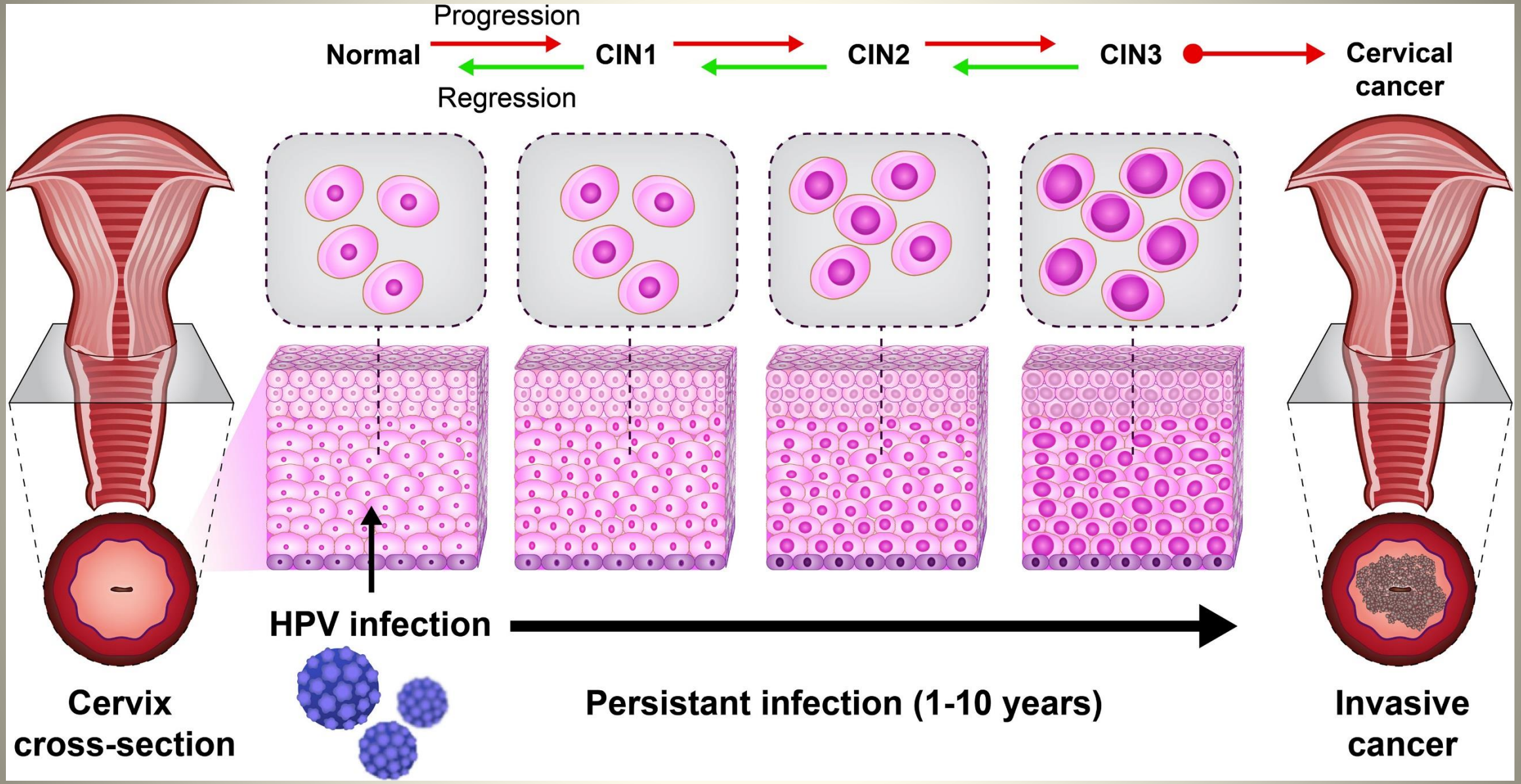
high-risk/oncogenic HPV infection

- deregulated expression of the viral genome
- The E6 and E7 proteins encoded by the genomes
- bind and inactivate the proteins encoded by the *TP53* and *RB* anti-oncogenes
- **unfettered proliferation of the host cell**
- decreased E4 expression and virion production
- decreased or absent koilocytic change


A
URR
ori
E6
E7
E1
E2
E4
E5
L2
L1
B
URR
ori
PE
E6
E7
E1
E4
E5
pAE
L2
pAL
E2
L1
C
host DNA
URR
host DNA
Type 1 n=1 viral copy
host DNA
URR
URR
host DNA
n
Type 2 n=>1 viral copy

increased likelihood of:

- **integration** of the viral genome into the host genome
- acquisition of **other genetic abnormalities** in the host cell
- **malignant transformation**


Progression
Normal
CIN1
CIN2
CIN3
Cervical cancer
Regression
HPV infection
Cervix cross-section
Persistant infection (1-10 years)
Invasive cancer

Not all viral infections lead to transformation

most are cleared by the immune system of the host

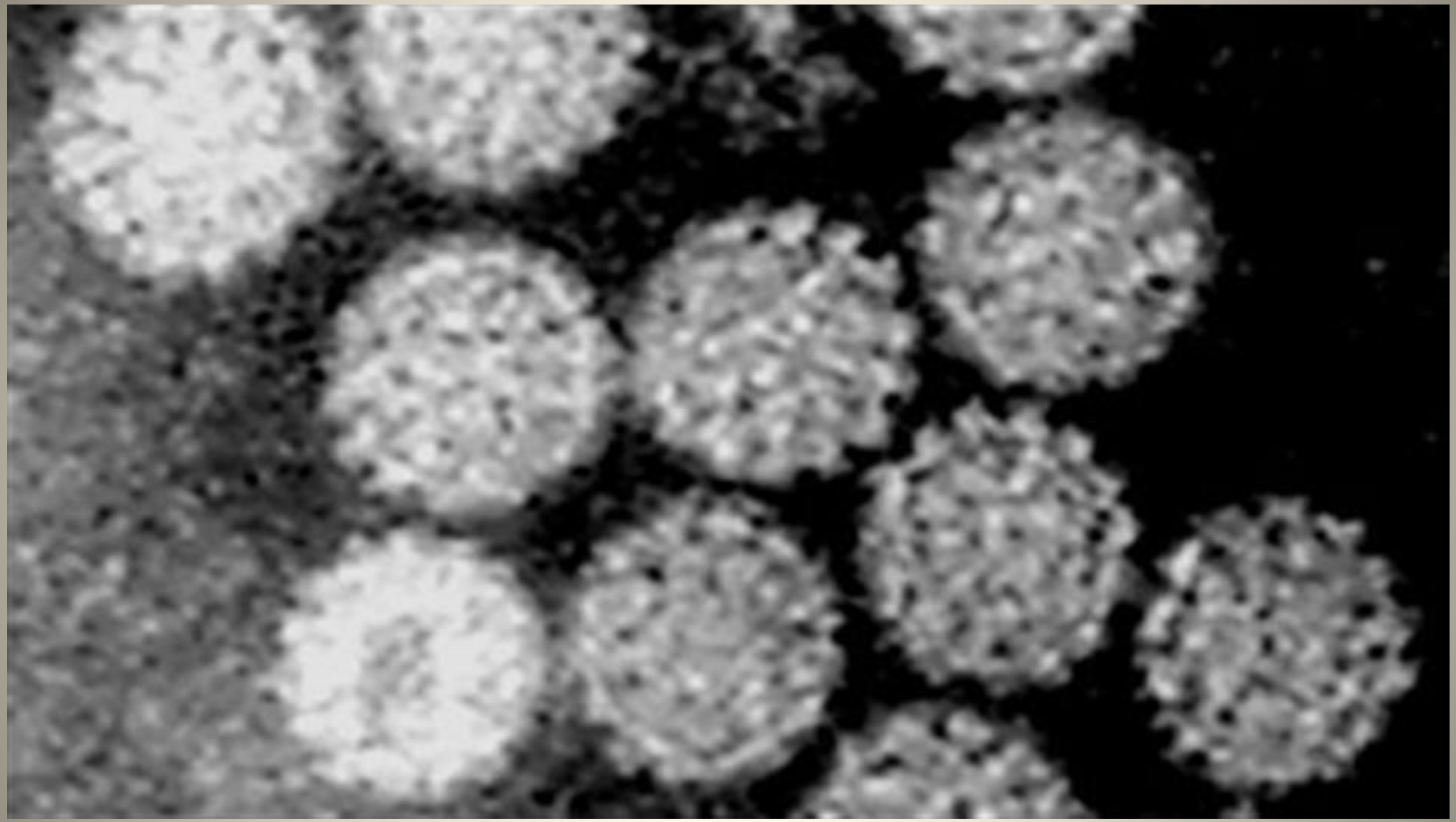
through cell-mediated immunity

productive infection due to

- **By low-risk HPV,** 6 or 11
  - LSIL/condyloma with viral cytopathic effects
- **By high-risk HPV**, most commonly 16 or 18
  - LSIL
  - progress to HSIL
  - invasive squamous cell carcinoma

HPV can be detected

- **electron microscopy**:
  - intranuclear crystalline occasionally filamentous inclusions
- specific identification: **nucleic acid hybridization**
  - with or without amplification
  - from DNA or RNA
  - either a liquid-based or *in situ* analysis



The p16 antioncogene

- in cells infected by high-risk HPV
- inactivation of the RB protein by the viral E7 protein
- **phosphorylated RB protein**
- **absence of the normal downregulation** of p16 expression
- **high-level expression of p16**
- **strong nuclear and cytoplasmic immunoreactivity**

Immunostaining for p16 (p16INK4a)

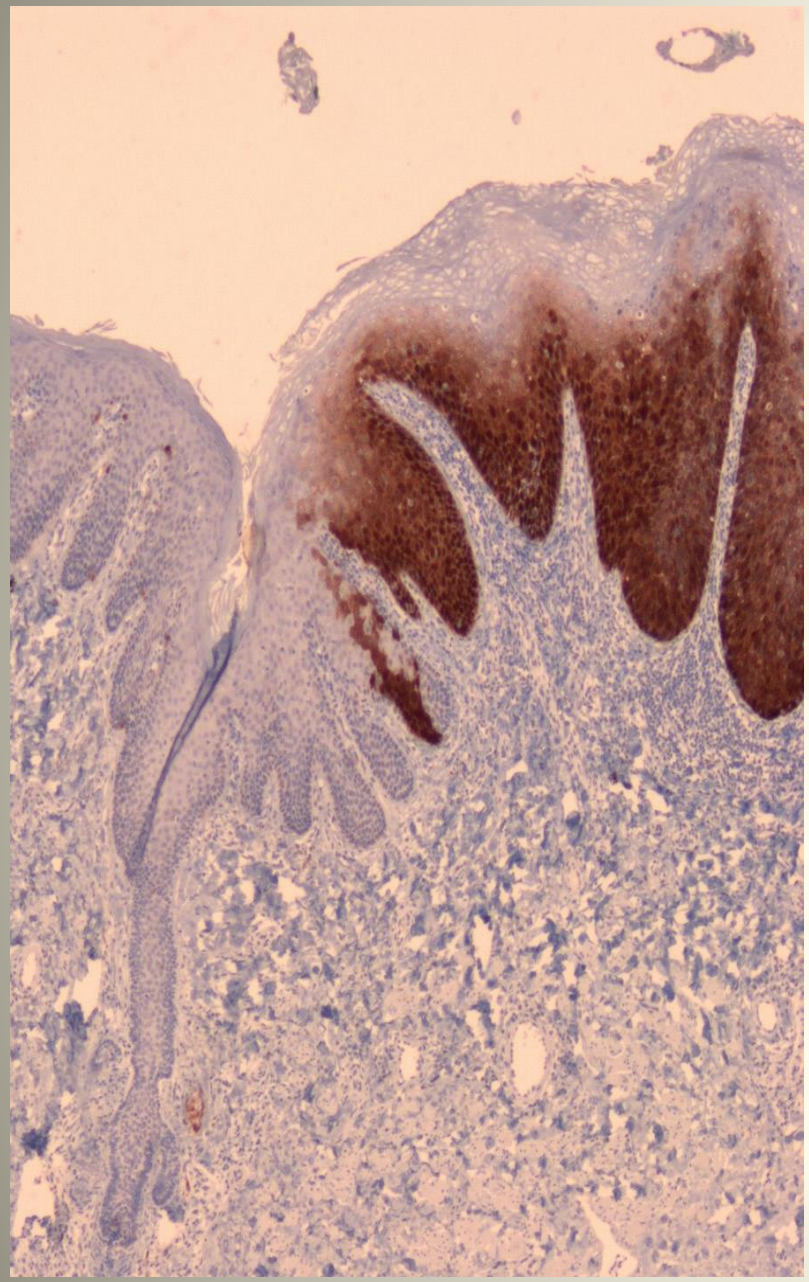
- a sensitive and specific surrogate for detection of HPV-associated versus HPV-independent vulvar squamous neoplasia
  - sensitivity of 100%
  - specificity of 98%–99%
- performed on intraepithelial lesions or early invasive carcinomas
  - p16 expression can be lost during tumor progression

Correct interpretation of p16 immunostaining

- the staining protocol **has been properly validated**
- appropriate **controls** run
- a number of antibody **clones** specific for p16
- it is incumbent on **each lab to optimize the staining** for the clone they have chosen
- a responsibility that is sometimes **neglected**

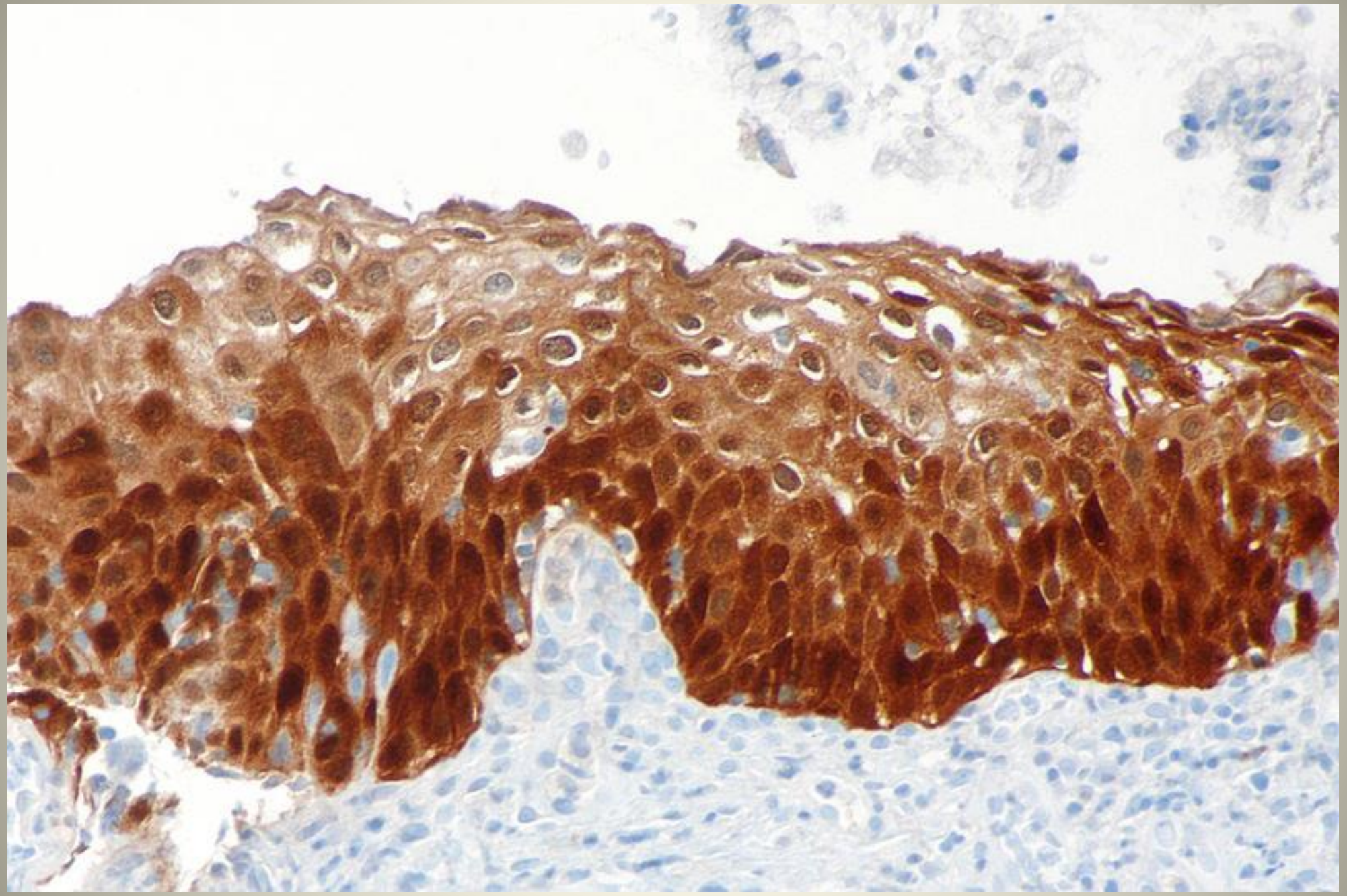
block positivity for p16

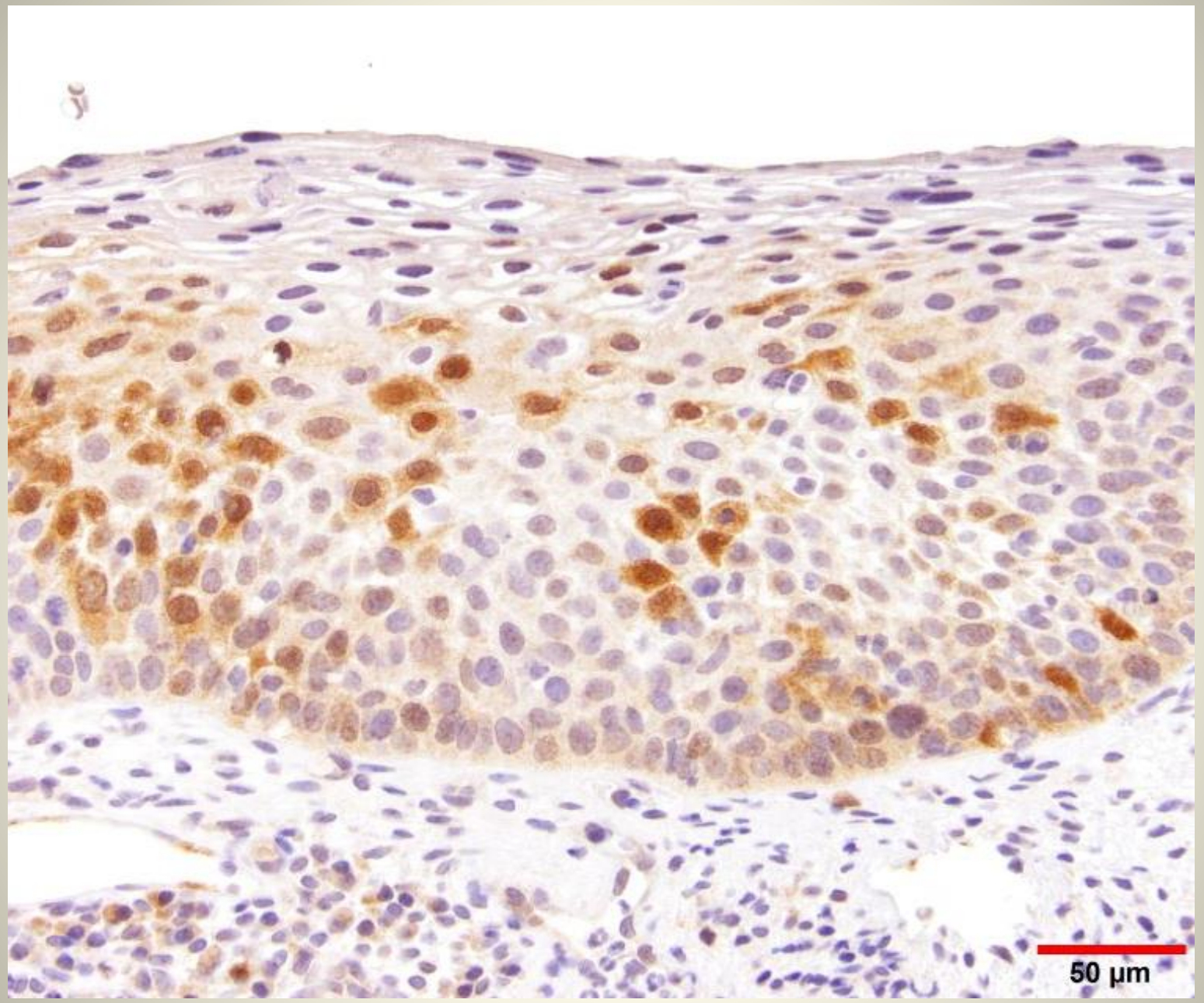
- indicative of high-risk HPV infection
- strong nuclear and cytoplasmic staining of every cell in the basal third of the epithelium
- typically extending into the middle and upper thirds



“Block” immunoreactivity in HSIL

a test for the presence of oncogenic HPV




50 µm

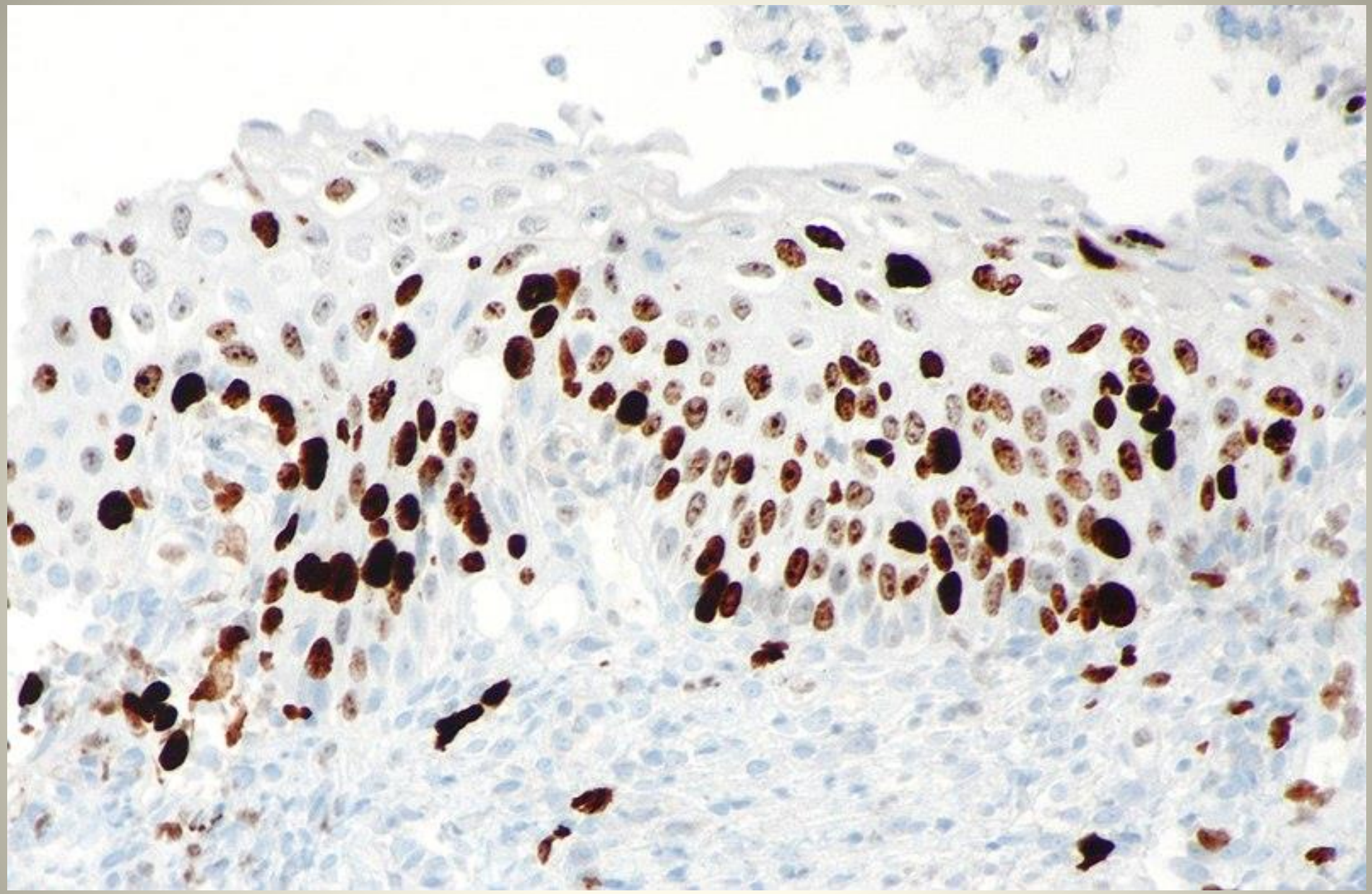
p16 immunostaining		HSIL n (%)	LSIL n (%)
Percentage of positive cells (%)	0(<5%)	5 (41.7%)	11(57.9%)
	1(5-49%)	4(33.3%)	6(31.6%)
	2 (50-80%)	3 (25.0%)	2(10.5%)
Intensity of reaction	0 (No reaction)	1 (8.3%)	3 (15.8%)
	1(Weak)	3 (25.0%)	10 (52.6%)
	2 (Variable)	4 (33.3%)	4 (21.1%)
	3 (Strong)	4 (33.3%)	2 (10.5%)
Cellular reaction pattern	0 (No reaction)	1 (8.3%)	3 (15.8%)
	1 (Focal)	10 (83.3%)	16 (84.2%)
	2 (Diffuse)	1 (8.3%)	0 (0%)
p16 Negative (0–3)	0	1 (8.3%)	3 (15.8%)
	2	3 (25.0%)	7 (36.8%)
	3	1 (8.3%)	4 (21.1%)
Positive (4–8)	4	4(33.3%)	2 (10.5%)
	5	0 (0%)	2 (10.5%)
	6	2 (16.7%)	1(5.3%)
	7	1 (8.3%)	0 (0%)

Immunoreactivity for p16

- is not specific for HPV
  - other tumor types, unrelated to HPV, dysregulation of RB:
  - high-grade serous carcinoma of tubo-ovarian or endometrial origin
- can be lost through mutation, as cervical cancer progresses
  - rare in the HSIL or in situ adenocarcinoma

Combined staining for Ki-67 and p16

- ✓a higher proliferative index
- ✓The strong, diffuse, block positivity for p16



le 3. Ki-67 grading in HSIL and LSIL

Ki67	HSIL n (%)	LSIL n (%)	Chronic Cx
0	0(0%)	0(0%)	16(80%)
1 (<5%)	6 (50.0%)	15 (78.9%)	4(20%)
2 (5-30%)	4 (33.3%)	4 (21.1%)	0(0%)
3 (>30%)	2 (16.7%)	0 (0%)	0(0%)

Risk factors for progression

- **the presence of high-risk HPV**

✓ high-risk HPV are present in most LSIL

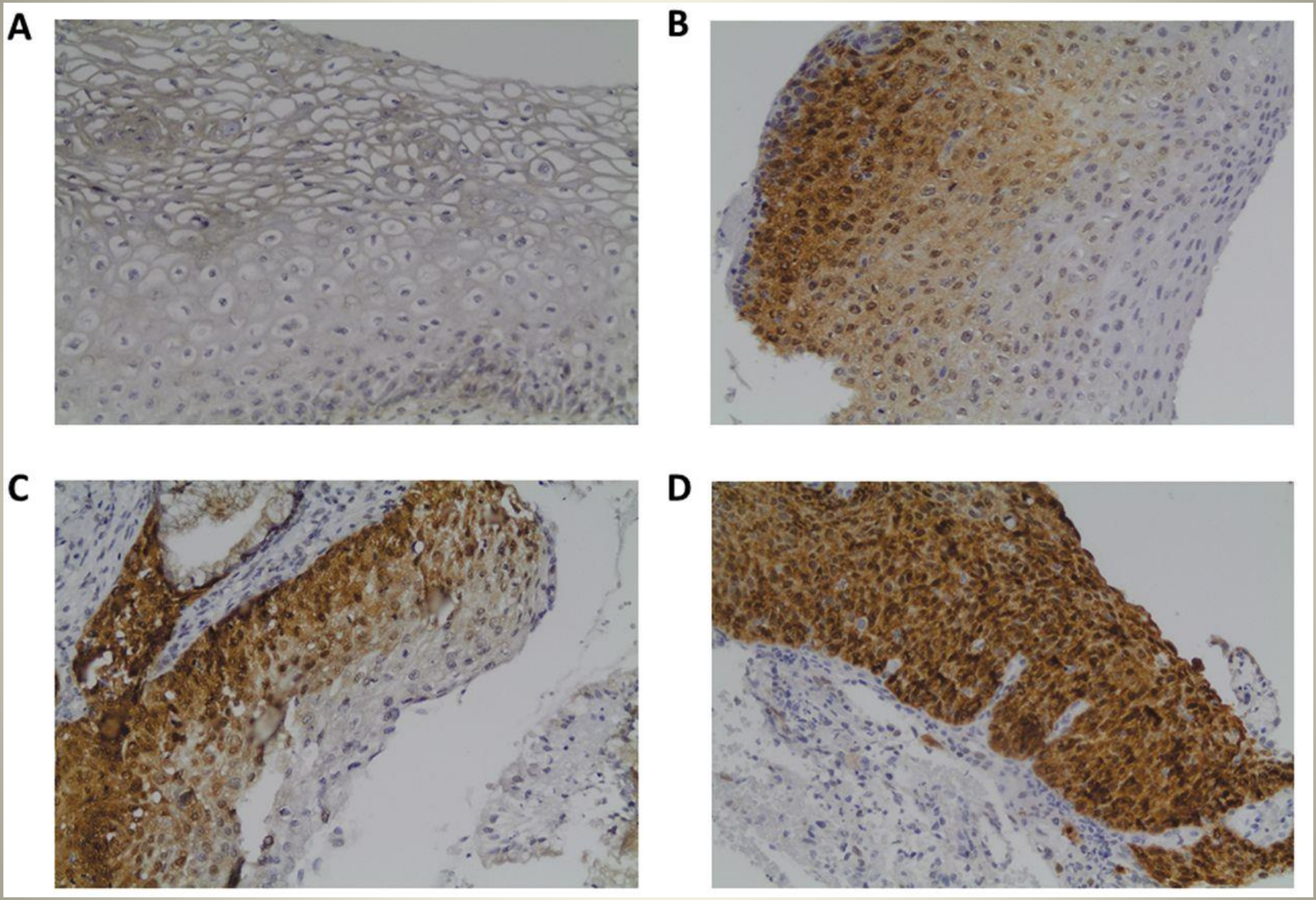
- **Cytokeratin 7**

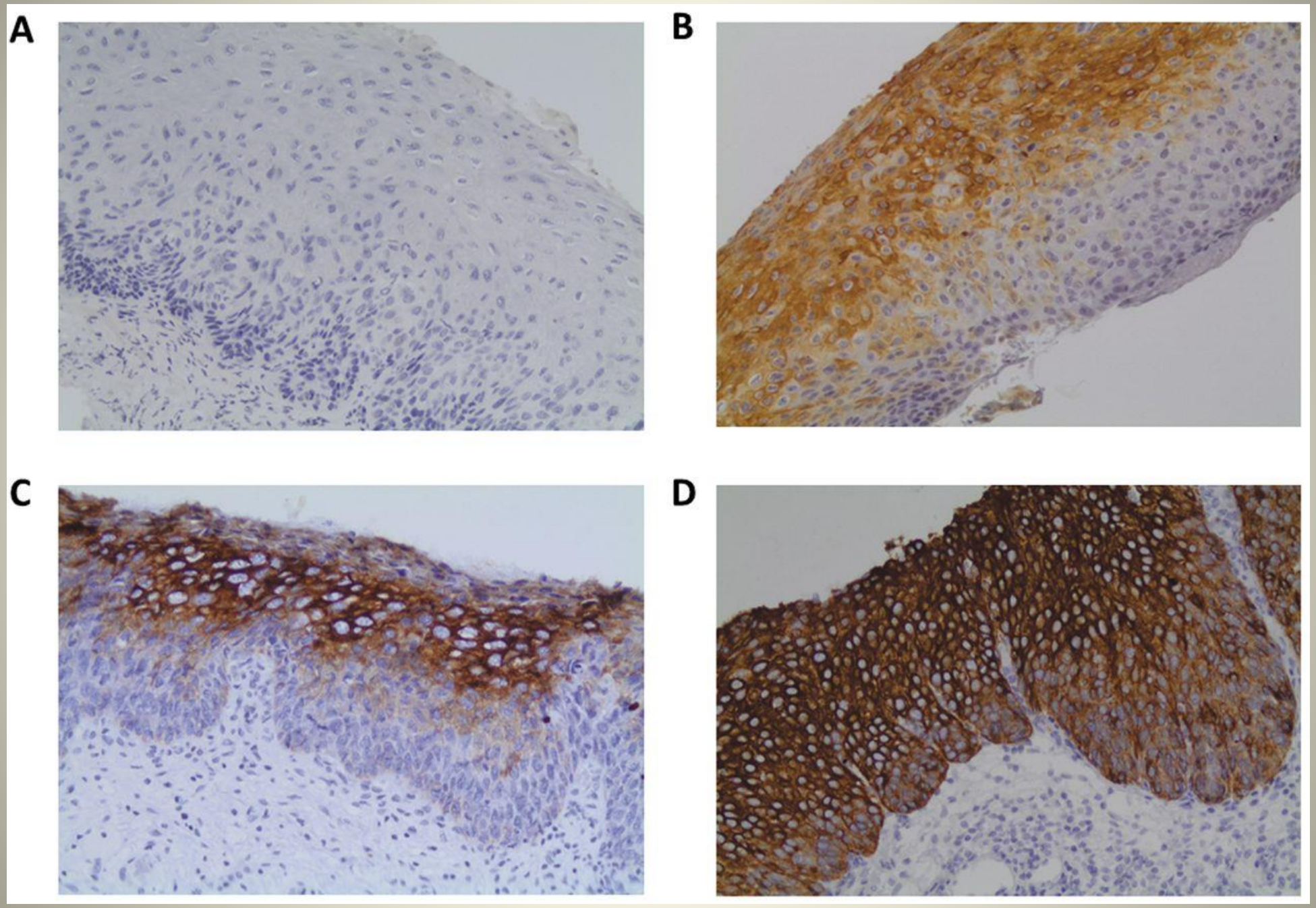
✓ a marker of metaplastic cells of the transformation zone

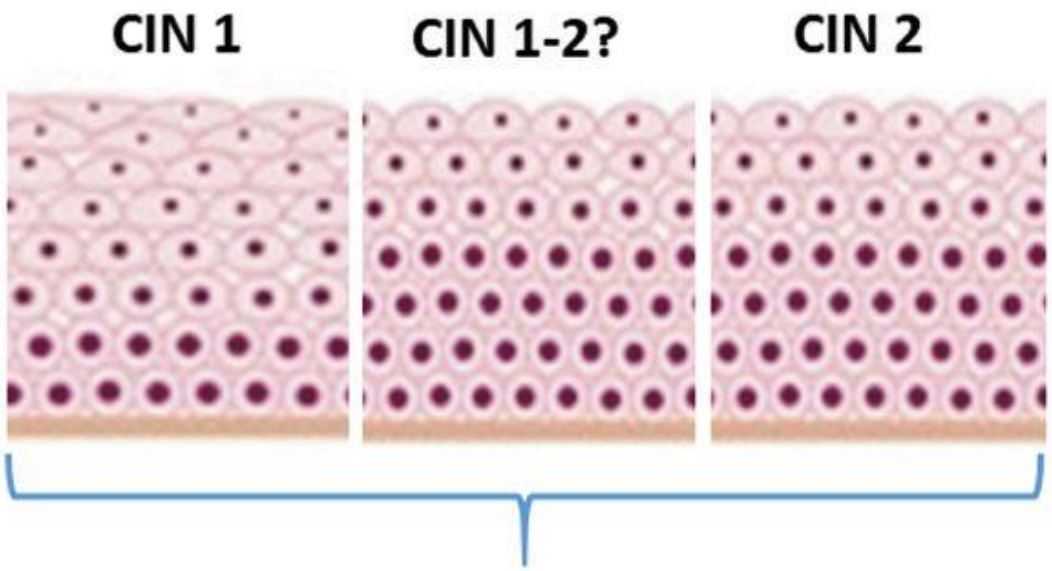
✓ a marker of LSIL that is more likely to progress

✓ LSIL/condyloma can arise on the ectocervix or elsewhere in the lower female genital tract: little risk of progression to HSIL

✓ further validation is required before it enters routine practice


A
B
C
D


A
B
C
D



CDKN2A and CK7 immunoexpression

CDKN2A- and CK7- : lower risk lesion (higher NPV)

CDKN2A+ and CK7+ : higher risk lesion

Other tests

- ✓ In addition to p16 immunostaining:
- ✓ can be valuable in establishing HPV status
- ✓ for cases where the clinical, histomorphologic, and p16 immunostaining data **are not concordant**

Other tests

- **PCR for HPV DNA**
  - **–viral genotyping**
- **The Hybrid Capture HPV test**
- **In situ hybridization for HPV**
- **viral E6/E7 oncoprotein mRNA**

PCR for HPV DNA

- **allows for viral genotyping**
- is highly sensitive
- lacks specificity
- may be present as a bystander if there is no transcription of the viral oncogenes

presence of HPV DNA may reflect

(1) virus particles on the surface of the epithelium secondary to **recent exposure**, without infection

(2) productive infection that **will be cleared completely** by the host

(3) a **latent** infection

(4) **oncogenic** HPV DNA that is integrated into the host genome

identification of the specific genotype of HPV

- is of import
- but **does not mean** premalignant change or malignancy
- Especially in the young, HPV **may be cleared** by the host

The Hybrid Capture HPV test

- a signal-amplified hybridization microplate-based array
- detect **multiple HPV genotypes**
- a **liquid based** nucleic acid detection technique
- more sensitivity than ISH techniques

In situ hybridization for HPV

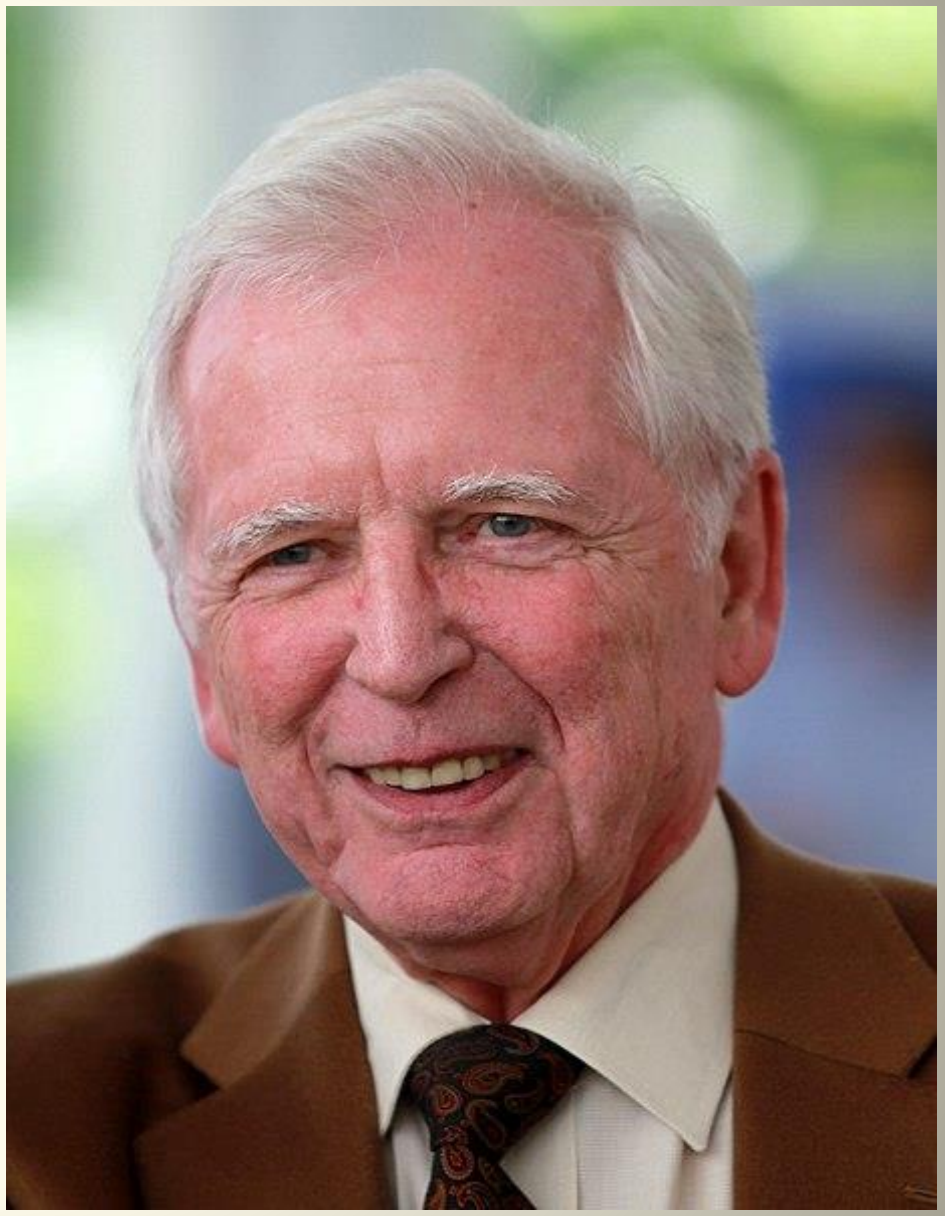
- **is highly specific**
- is more expensive than immunostaining
- has a longer turnaround time

Detection of mRNA of HPV oncogene E6/E7

- Expression of the oncoprotein mRNA
- the virus genome is being transcribed
- **a better marker of HPV**
- establish an etiologic role: a causative role in oncogenesis
- is currently **not widely available** in clinical laboratories

- **Harald zur Hausen**
  - هارالد تسور هاوزن

- deservedly won the **Nobel prize in 2008** for his work establishing the link between HPV and cervical cancer



role of HPV in cervical cancer

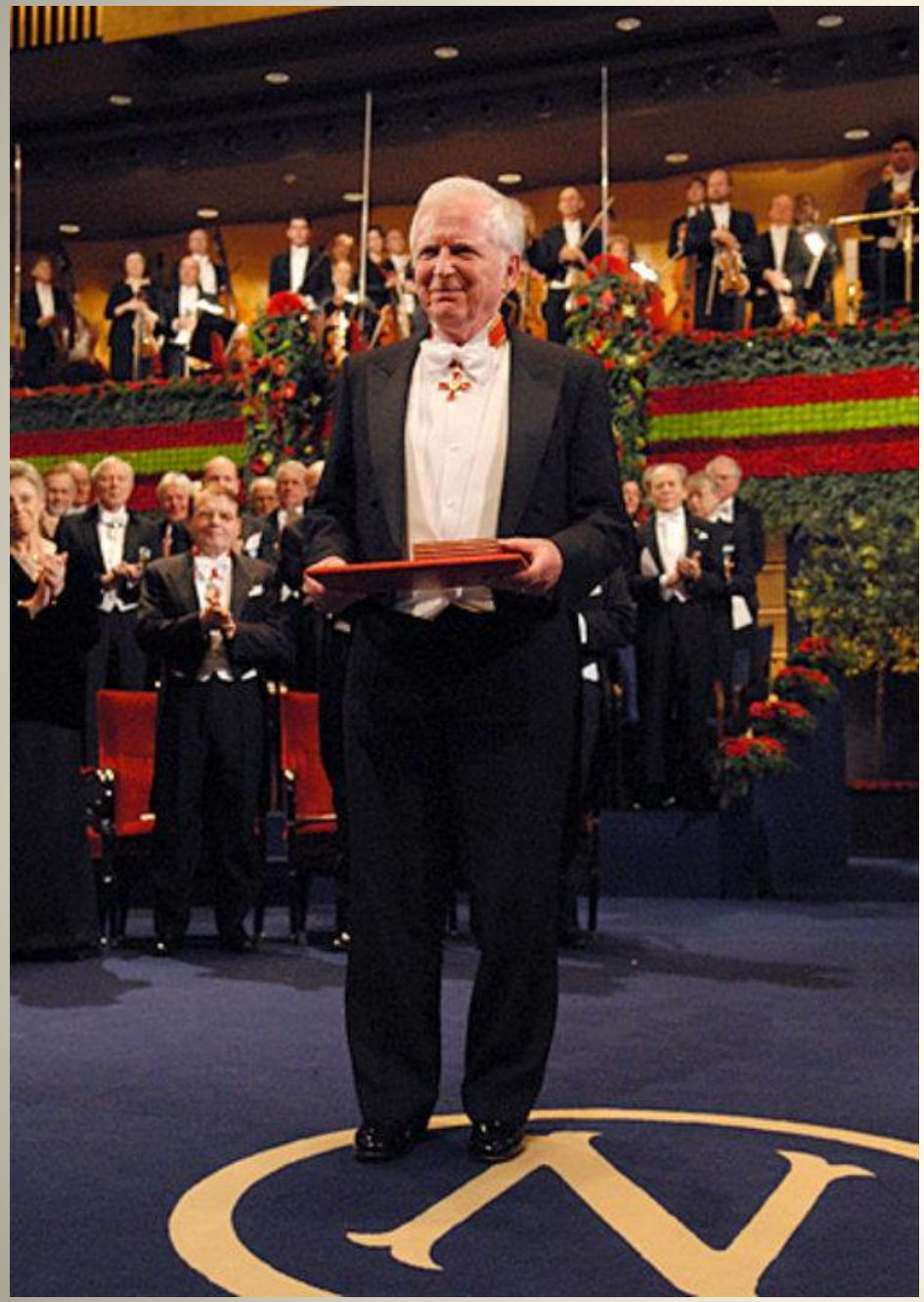
- researchers started to postulate and analyse a **possible role** of HPV in cervical cancer, 1974
- the appearance of **Koilocytes** in cervical smears indicates the presence of a papillomavirus infection, 1976
- The demonstration of **heterogeneity** within the papillomavirus family
- **HPV16 and HPV18** were cloned in 1983 and 1984

role of HPV in cervical cancer

- expression of specific viral genes (such as **E6 and E7**) was shown in cervical cancer biopsies, 1985
- viral ring molecule was shown for **integrated** genome copies
- the **immortalization** property of viral DNA, 1987
- **viral oncogene expression** for the maintenance of the malignant phenotype, 1992
- **E6-p53** and **E7-pRB** interactions, 1994

1994-2008

- a more detailed knowledge of the natural history of HPV infection
- epidemiological studies have also been performed
- **high-risk HPV** as the primary risk factor
- **persisting** HPV infections were the most significant risk factor



HPV pathogenesis

The papillomavirus life cycle

- differs from all other virus families
- the availability of epidermis or mucosal epithelium that are **still able to proliferate** (basal cells)
- **microlesions of skin or mucosa**

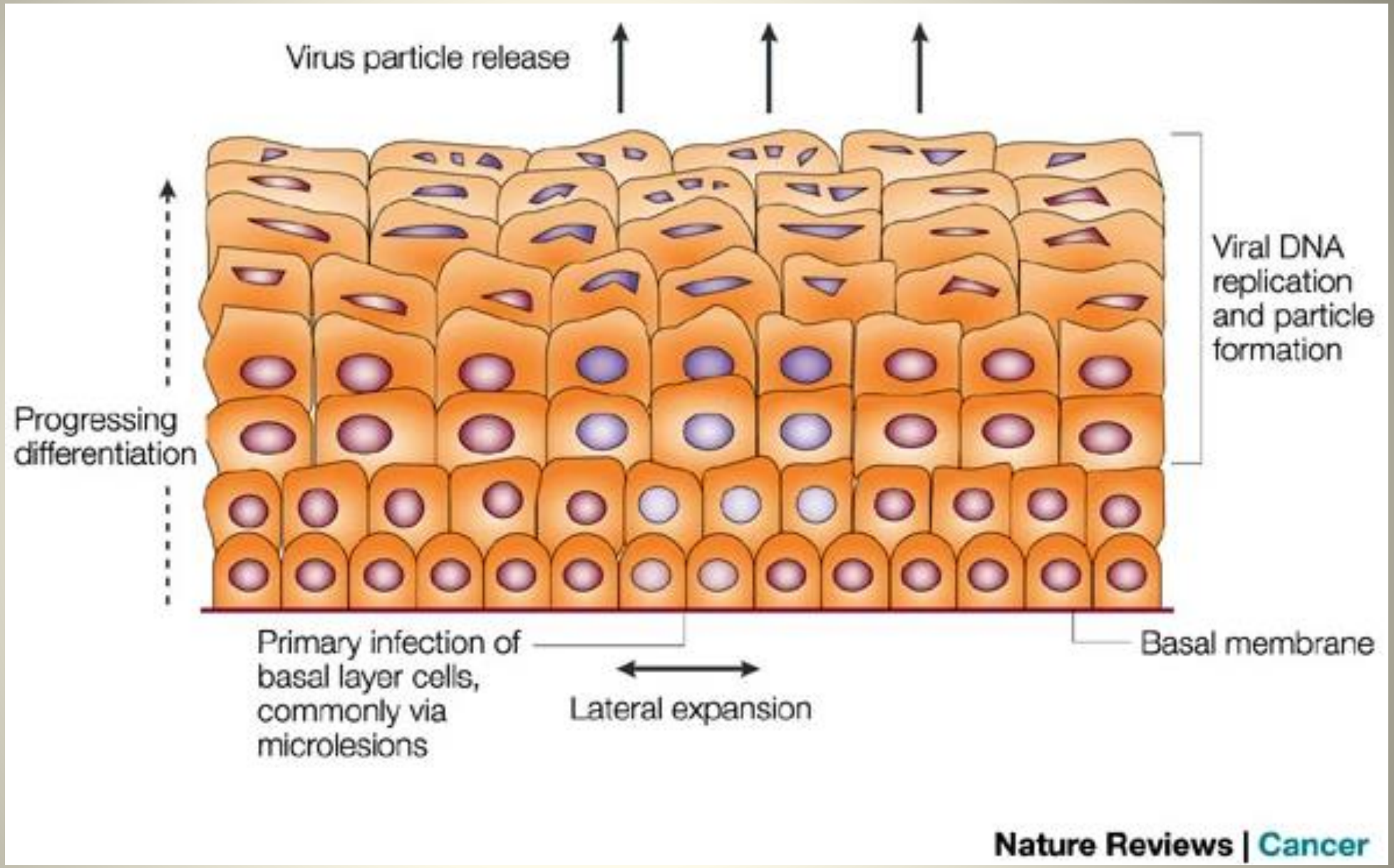
The papillomavirus life cycle

- viral gene expression is largely suppressed
- But limited expression of specific **'early' viral genes** (E5, E6 and E7) **results in enhanced proliferation** and their lateral expansion
- The infected cell divides
- **the population spreads laterally**
- **Some migrate into the upper layers**

the upper differentiating cells

- **'late' viral gene** expression is initiated
- the circular viral genome is then **replicated**
- **structural** capsid proteins are formed
- Viral particle formation ensues: complete viral particles are **assembled**
- **Particles are released at the surface** and might then infect additional tissues

HPV life cycle



HPV genome

- 6800-8000 base pairs
- eight open reading frames – E6, E7, E1, E2, E4, E5, L2 and L1 – coding for 'early' (E) or 'late' (L) functions
- Three genes possess **proliferation-stimulating activity**

✓**E5**

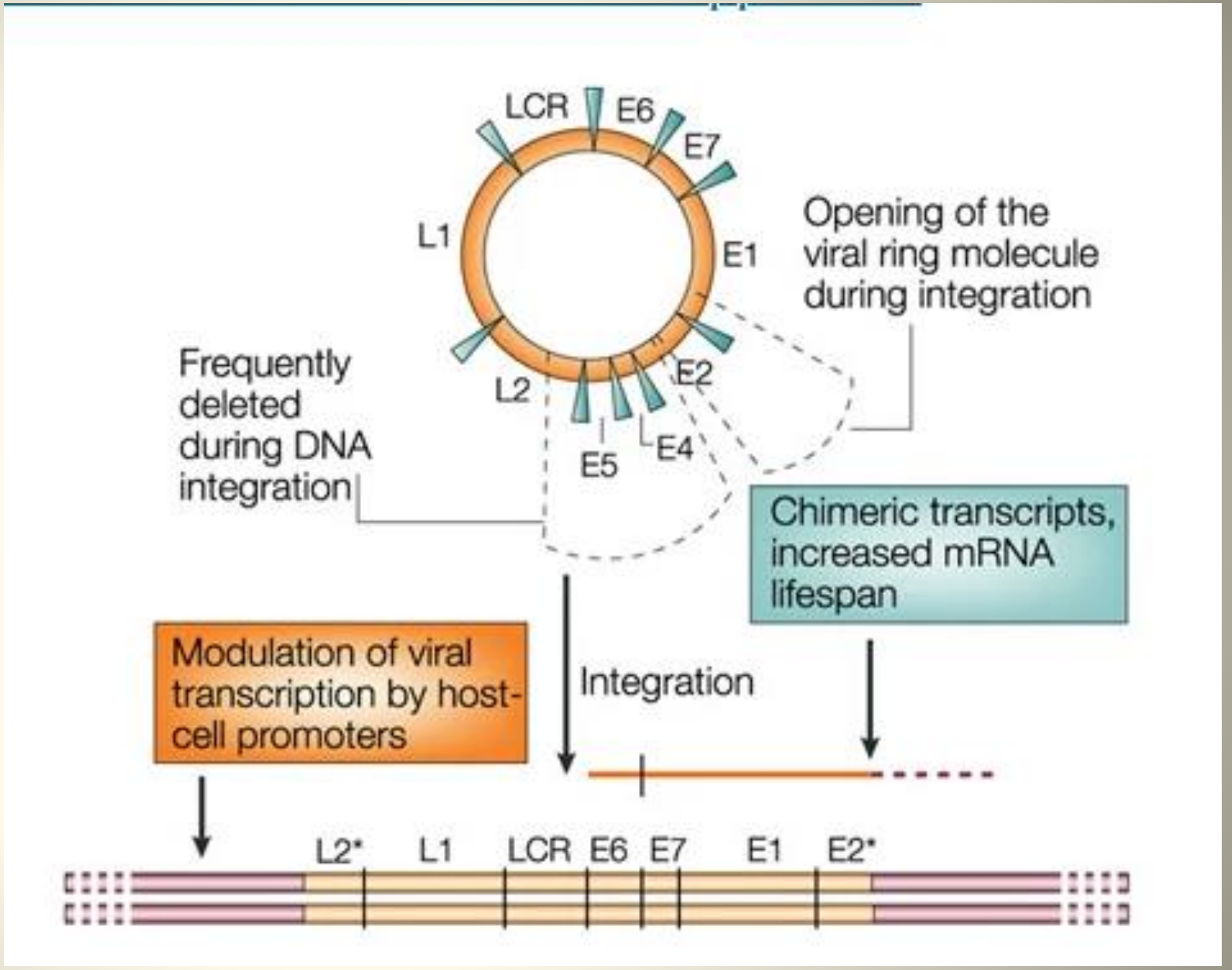
✓**E6**

✓**E7**

E5 is important in the early course of infection

- **stimulates cell growth** by forming a complex with the EGFR, PDGF-βR and the CSF-1R
- **prevent apoptosis** following DNA damage
- as HPV-infected lesions progress to cervical cancer, the episomal viral DNA frequently becomes integrated into host-cell DNA, and a substantial part of the genome, (including the E5 gene) is deleted
- **E5 is not obligatory** in late events of carcinogenesis

The circular HPV DNA and its integration



Integration of viral molecule into host-cell DNA

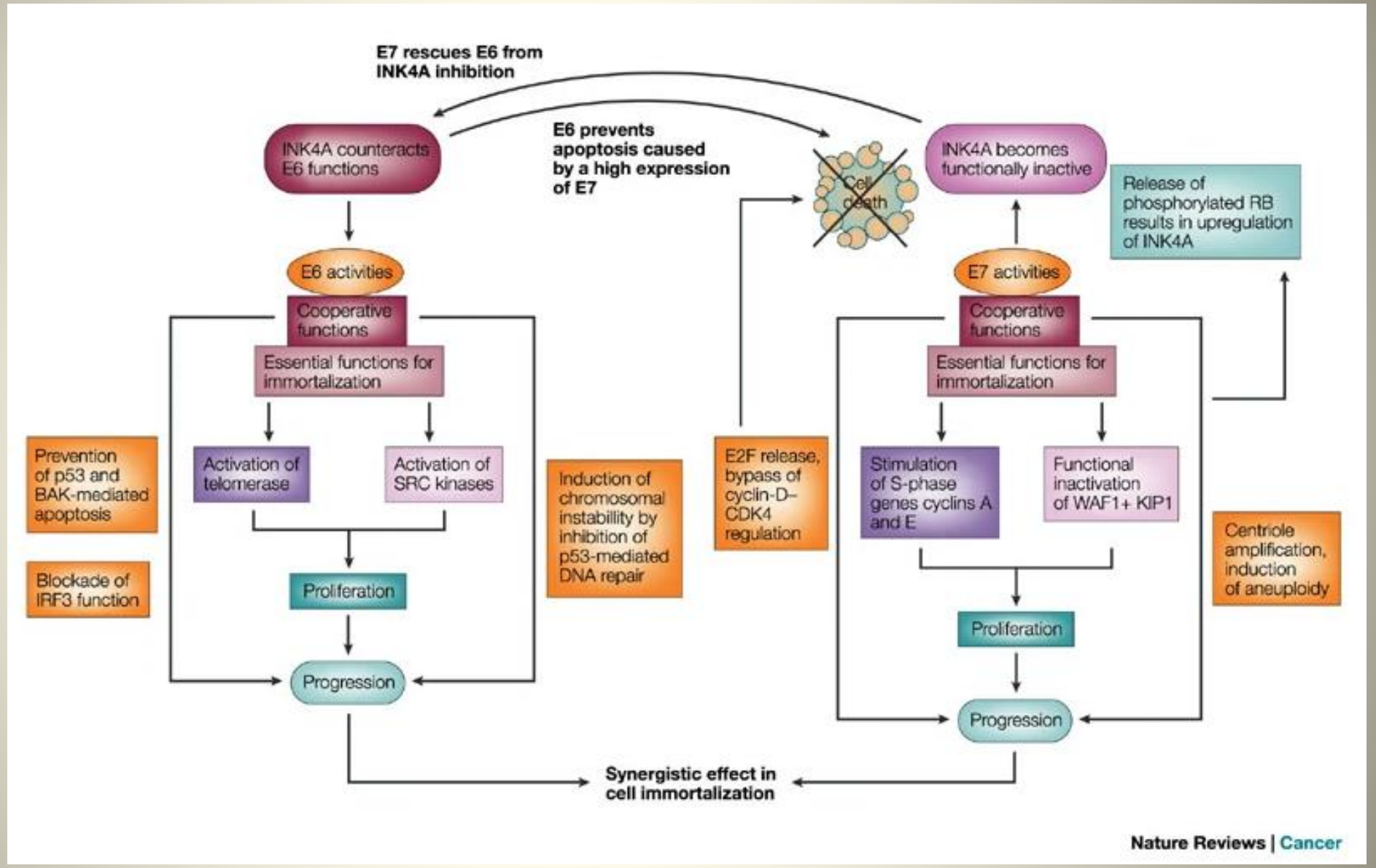
- The ring molecule is opened
  - Part of E2 and L2 and whole genes E4, E5 are deleted
- Viral transcripts uniformly span the **E6 and E7** region
- are often linked to **flanking cellular sequences**
  - transcription is enhanced by flanking host-cell promoters

E6 and E7 genes (their proteins)

- A more significant role for **malignant transformation**
- are **consistently expressed** in malignant tissue
  - inhibiting expression blocks the malignant phenotype
- They are **independently able to immortalize** various human cell types in tissue culture
- **efficiency is increased** when they are expressed **together**

Functions of the E6 and E7,

and their actions for cell immortalization



functions for E6 and E7

- **E6 interacts with p53**
- **E7 interacts with RB**
- **block the activity of these tumor suppressors**

✓**Immortalization**

E6 protein

- ✓**deregulated cellular growth** and **genomic instability**
  - both of which are characteristics of immortalized cells
- complex with **p53**
- resulting in the ubiquitination & degradation of p53
- **Loss of p53**

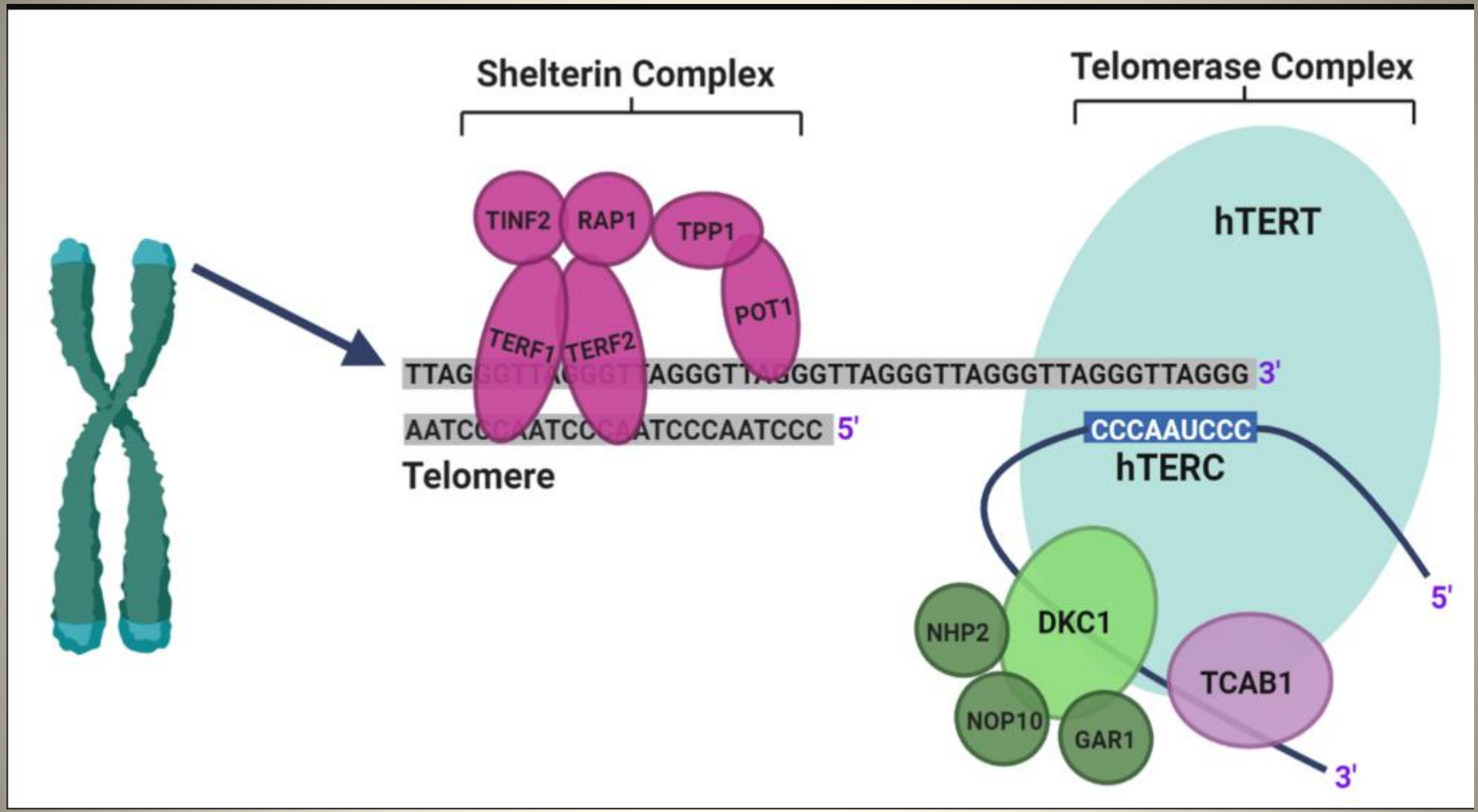
E6 protein

- ✓increased **chromosomal instability**
- Due to resistance to apoptosis
  - interacts with **p53**
  - interacts with pro-apoptotic protein **BAK**

E6 protein

✓growth stimulation

- the activation of **telomerase**
- inhibition of degradation of **activated SRC-family** kinases

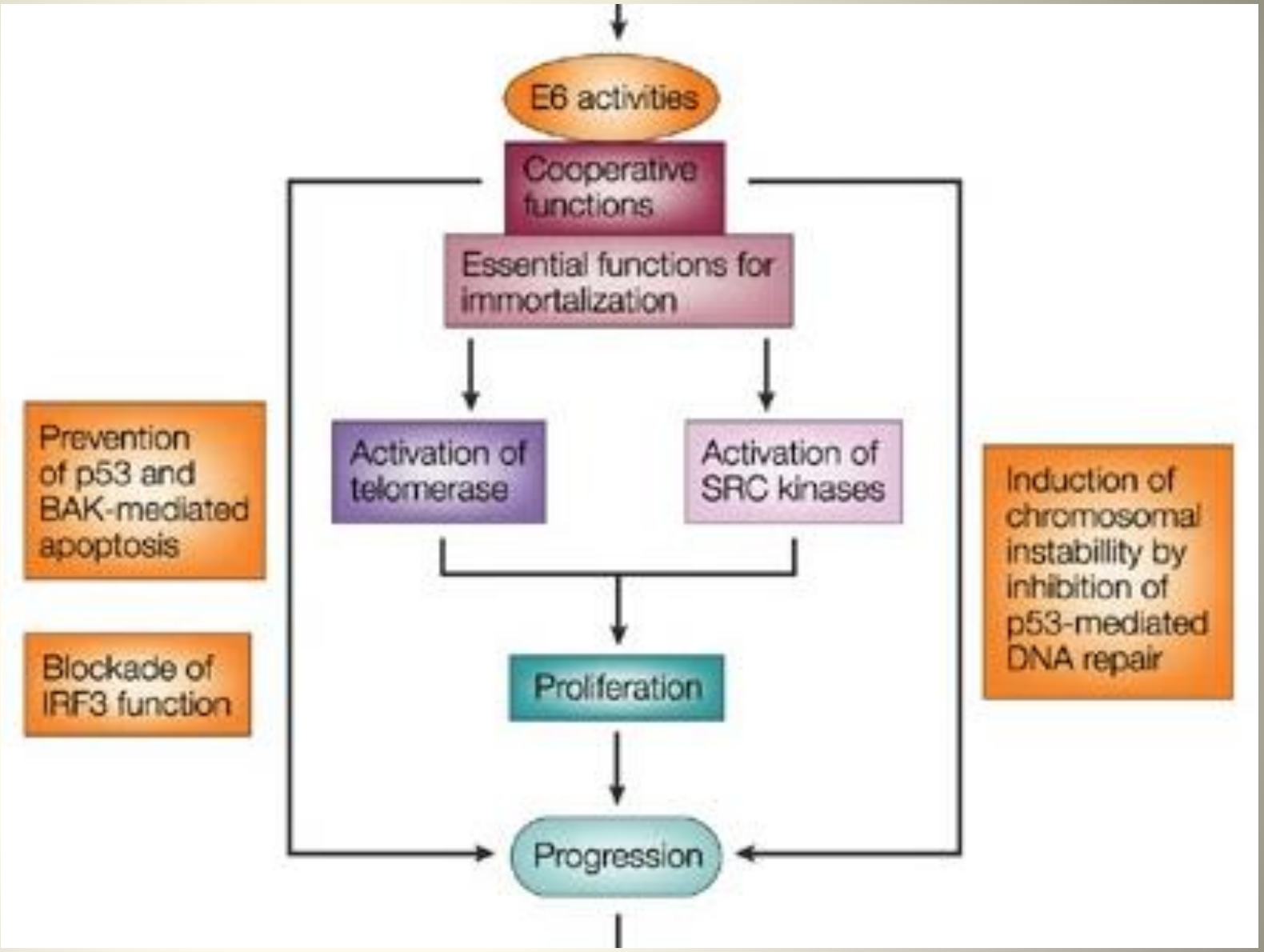

Shelterin Complex
Telomerase Complex
TINF2
RAP1
TPP1
POT1
TERF1
TERF2
hTERT
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG 3'
AATCCCAATCCCAATCCCAATCCC 5'
Telomere
CCCAAUCCC
hTERC
5'
NHP2
DKC1
TCAB1
NOP10
GAR1
3'

E6 protein

✓**disrupts antiviral response**

- binding to **IRF-3** (interferon regulatory factor-3)
- the **inhibition** of its transcriptional activity
- significantly dampens the induction of **IFNβ**

Functions of the E6



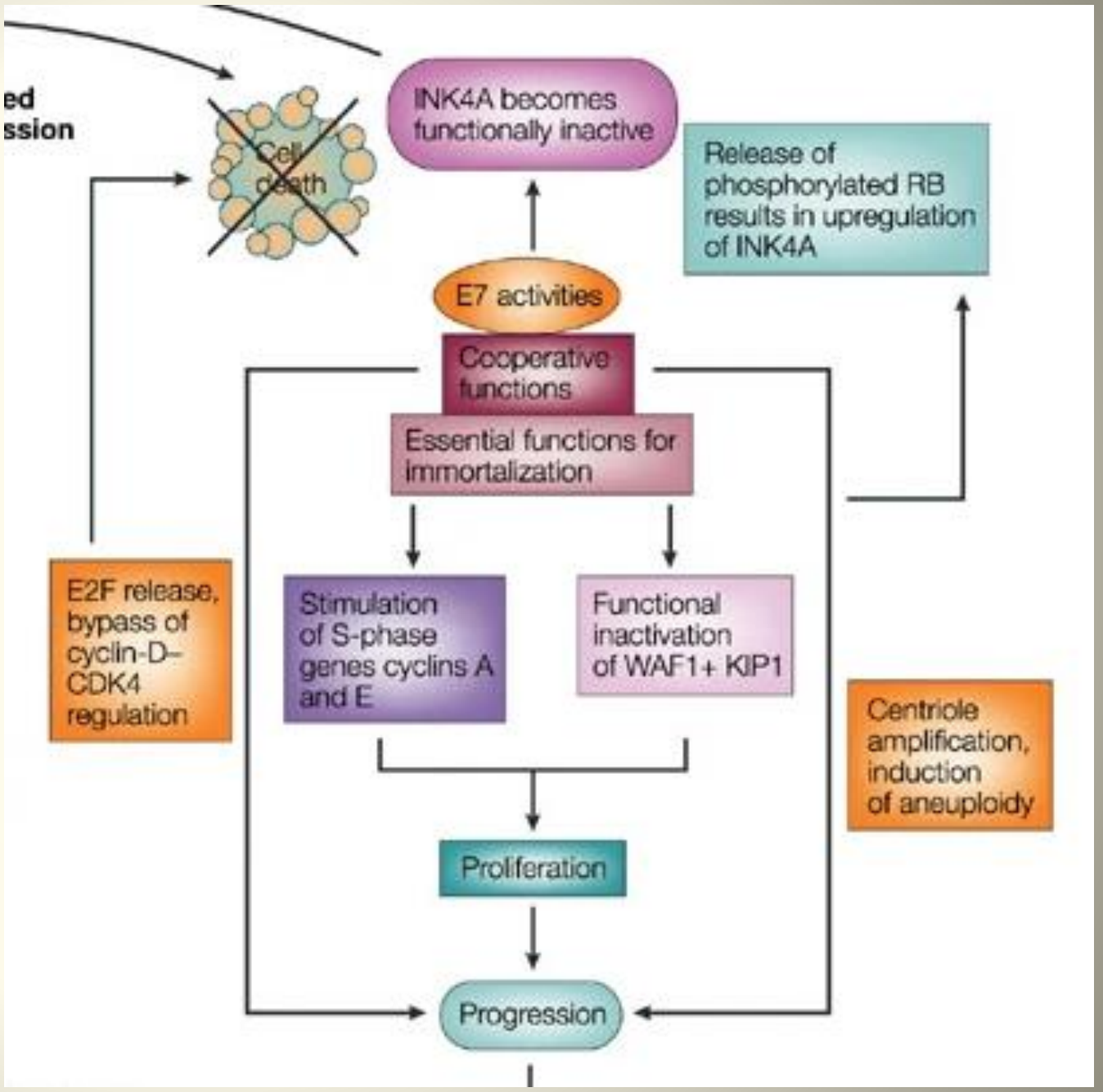
E7 protein

- interacts with and **degrades RB**
- releases the transcription factor E2F
  - might lead to apoptosis
  - upregulates cyclin-dependent kinase inhibitor INK4A
- **also inactivates INK4A (p16)**
- The INK4A (p16) counteract functions of **E6** protein

E7 protein

- stimulate **cyclins A** and **E** (s phase genes)
- inactivate the cyclin-dependent kinase inhibitors **WAF1** (CIP1 and p21) and **KIP1** (p27)
- inducing **centriole amplification**
  - –induces **aneuploidy**
  - –contributes to tumorigenesis

Functions of the E7

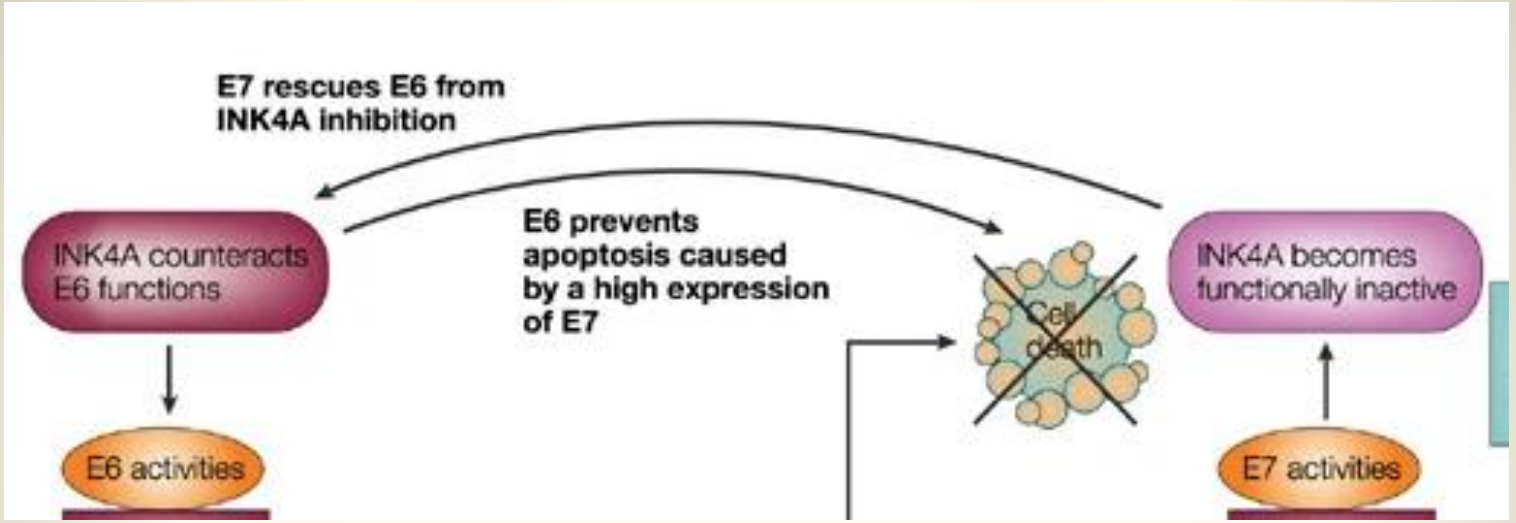


E6 and E7 **synergism**

- E6 prevents E7-induced apoptosis that is induced by E2F
  - By p53 and BAK
- E7 rescues E6 from inhibition by INK4A
  - inactivates INK4A E7
  - directly activating cyclins A and E

✓their joint function results in a marked increase in **immortalization & transformation**

E6 and E7 synergism



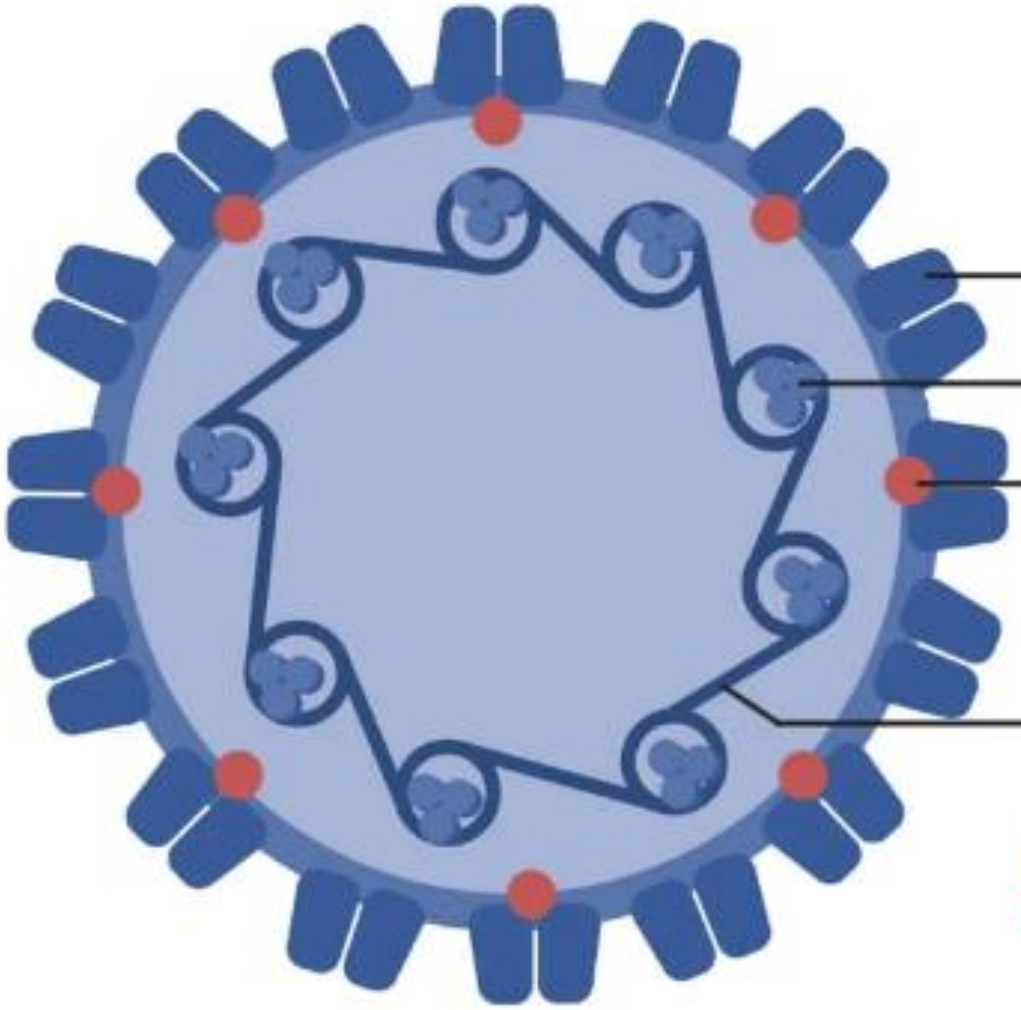
Prevention of p53 and BAK-mediated apoptosis

E2F release, bypass of cyclin-D–CDK4 regulation

two HPV early proteins

- structural proteins L1 and L2 are important for **vaccine development**

	Cervarix	Gardasil, Silgard	Gardasil-9
Valency	2-Valent	4-Valent	9-Valent
Types	HPV16/18	HPV6/11/16/18	HPV6/11/16/18/31/33/45/52/58
Adjuvant	ASO4 (0.5 mg aluminum hydroxide and 50 μg 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL))	0.225 mg aluminum hydroxyphosphate sulfate	0.5 mg aluminum hydroxyphosphate sulfate
Expression system	Baculovirus-insect cell	Yeast	Yeast



Capsid Protein (L1)

Histone

Capsid Protein (L2)

Genomic DNA

Human Papillomavirus (HPV)

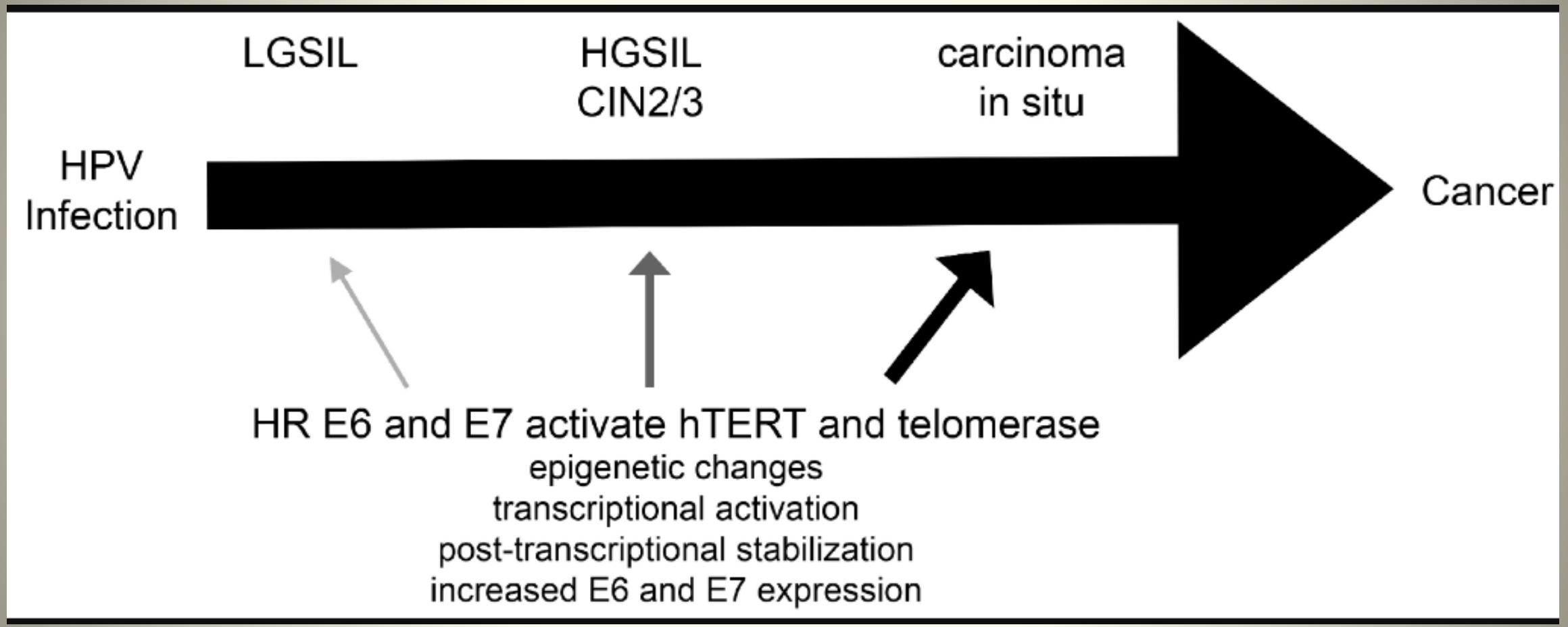
High- and low-risk HPV infections

- 'high-risk' types
  - are found preferentially in cervical and other anogenital cancers
- 'low-risk' types
  - found primarily in genital warts and non-malignant lesions

✓**only the E6 and E7 genes of high-risk types were able to immortalize human cells**

High-risk HPV types

- are widespread within **all human populations**
- Infection is commonly **transmitted** by sexual contact
- results initially in inconspicuous **squamous intraepithelial lesions (SIL)** in women
- **Most of these lesions are cleared** 6–12 months after appearance, probably due to immunological intervention


LGSIL
HGSIL
CIN2/3
carcinoma
in situ
HPV
Infection
Cancer
HR E6 and E7 activate hTERT and telomerase
epigenetic changes
transcriptional activation
post-transcriptional stabilization
increased E6 and E7 expression

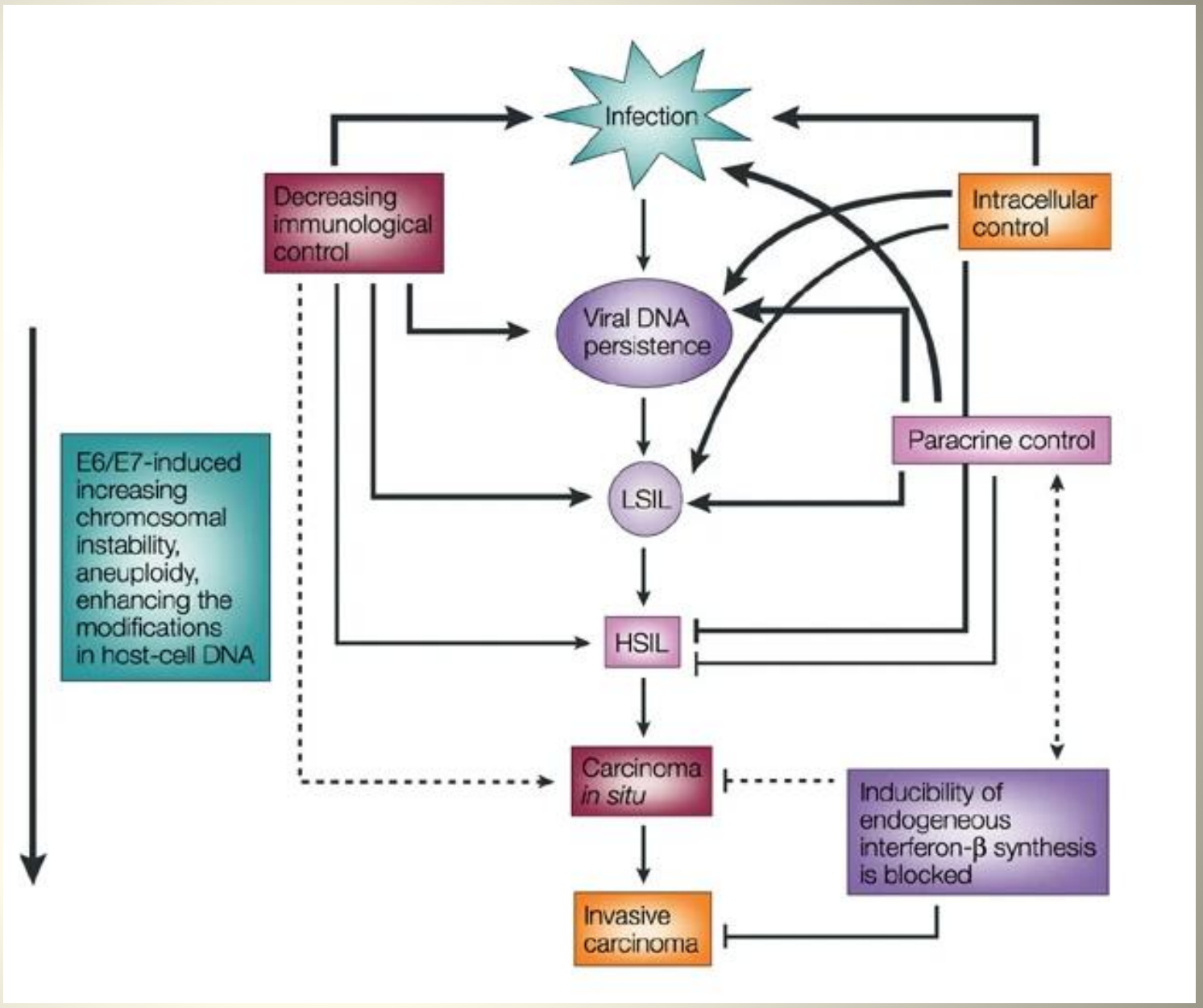
A small percentage

- Persists
- progresses to:

- **high-grade SIL**
- **carcinoma *in situ***
- **Squamous cell carcinoma**
- **adenocarcinoma**

HPV-induced progression:

failing control mechanisms



Host control of HPV infection

- an intracellular control
  - by cyclin-dependent kinase inhibitors
- a paracrine signaling cascade
  - by macrophages and cytokines
  - tumor necrosis factor-α
  - loss of synthesis of interferon-β
- a decreasing immunological control

Host immune response factors

- ✓ **Genes in the HLA region** of chromosome 6
  - – increased susceptibility to the transforming properties of high-risk HPV
- ✓ a detectable Humoral and Cellular immune response against HPV antigens during the course of **regression**
- ✓ The **escape** from immunological control is one important step in the progression of HPV-linked tumors

Impaired immune function

- increased incidence and prolonged persistence of SIL in **immunosuppressed** women

✓ a 5- to 10-fold increased risk

- immunosuppressant therapy in organ transplantation recipients
- HIV infection

High-risk HPV infections
progress to cervical cancer

in only a small percentage
after a long latency period

Factors that affect HPV malignancy

- modifications of cellular genes that influence antigen presentation
- signaling cascades that are engaged in viral oncogene transcription or viral oncoprotein function
- upregulating viral oncogene transcription
- modifying the viral promoter region
- amplifying the persisting viral genomes

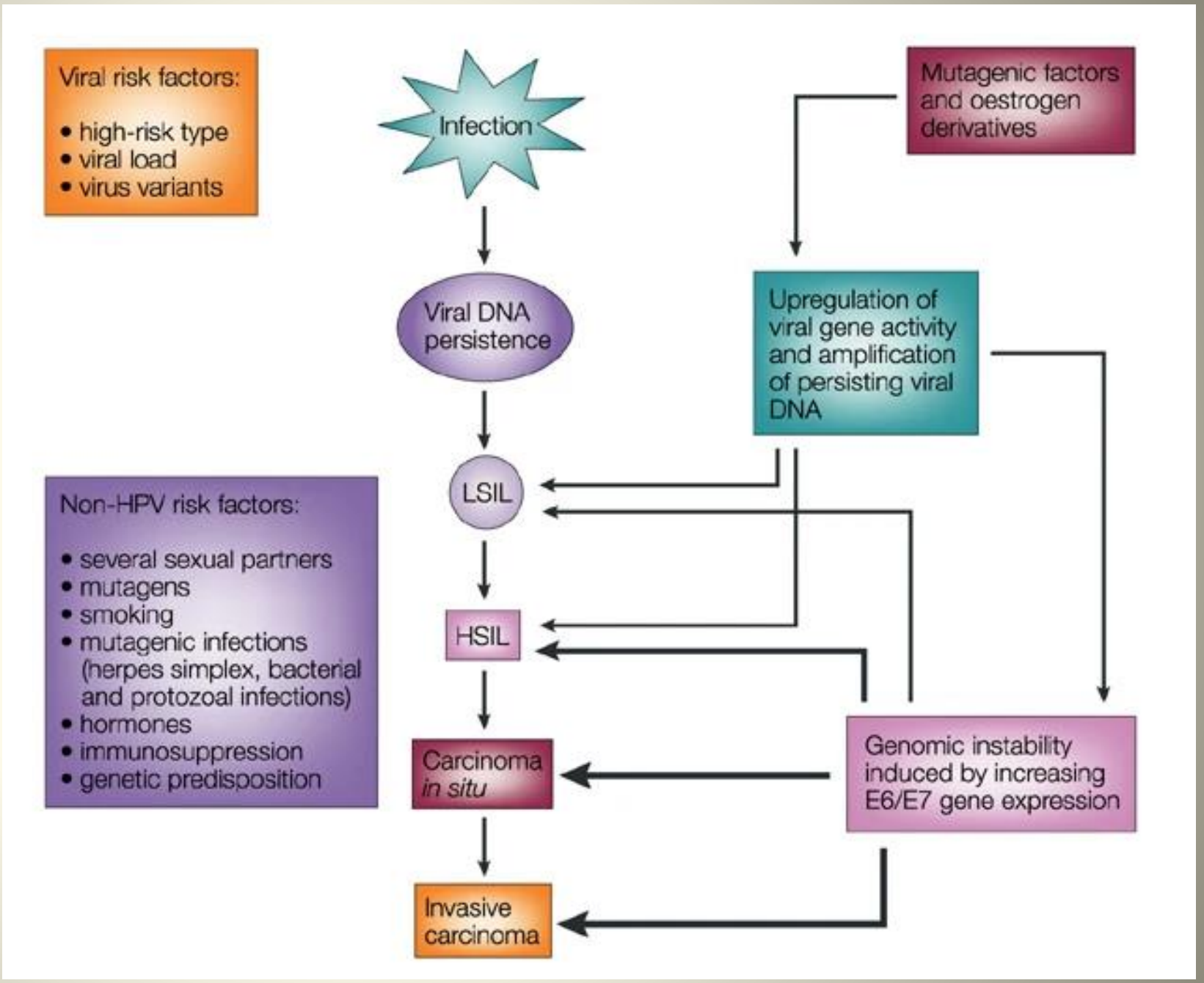
HPV and non-HPV factors

that contribute to HPV-induced malignant progression

- **Hormones** (estrogen) activate the HPV promoter and facilitate immortalization of HPV-infected cells
- **Mutagenic agents** amplify persisting HPV DNA
- A rising level of **E6 and E7 oncogene expression**, results in increasing genomic instability and facilitates progression towards invasive growth

HPV and non-HPV factors

that contribute to HPV-induced malignant progression



we now see the circle closing

- **primary HPV screening** to detect premalignant lesions
- **HPV immunization** to prevent such lesions increasingly being adopted

It is possible to envision

a world where the
burden of disease due to HPV is
dramatically reduced

SCC and Its Precursors

Squamous Intraepithelial Lesions/ Cervical Intraepithelial Neoplasia

SIL/CIN

- SIL terminology has gained **wide acceptance**, but there are still **holdouts** for the CIN terminology
- we will adopt the practice of providing **a two-part diagnosis**, with SIL first and the equivalent CIN in parenthesis thereafter
- LSIL (CIN1)
- HSIL (CIN2 and CIN3)

CIN2 and CIN3

- the distinction is arbitrary
- not clinically relevant
- Subtle differences in natural history have been reported
- **HSIL (CIN2/3)** for all high-grade lesions

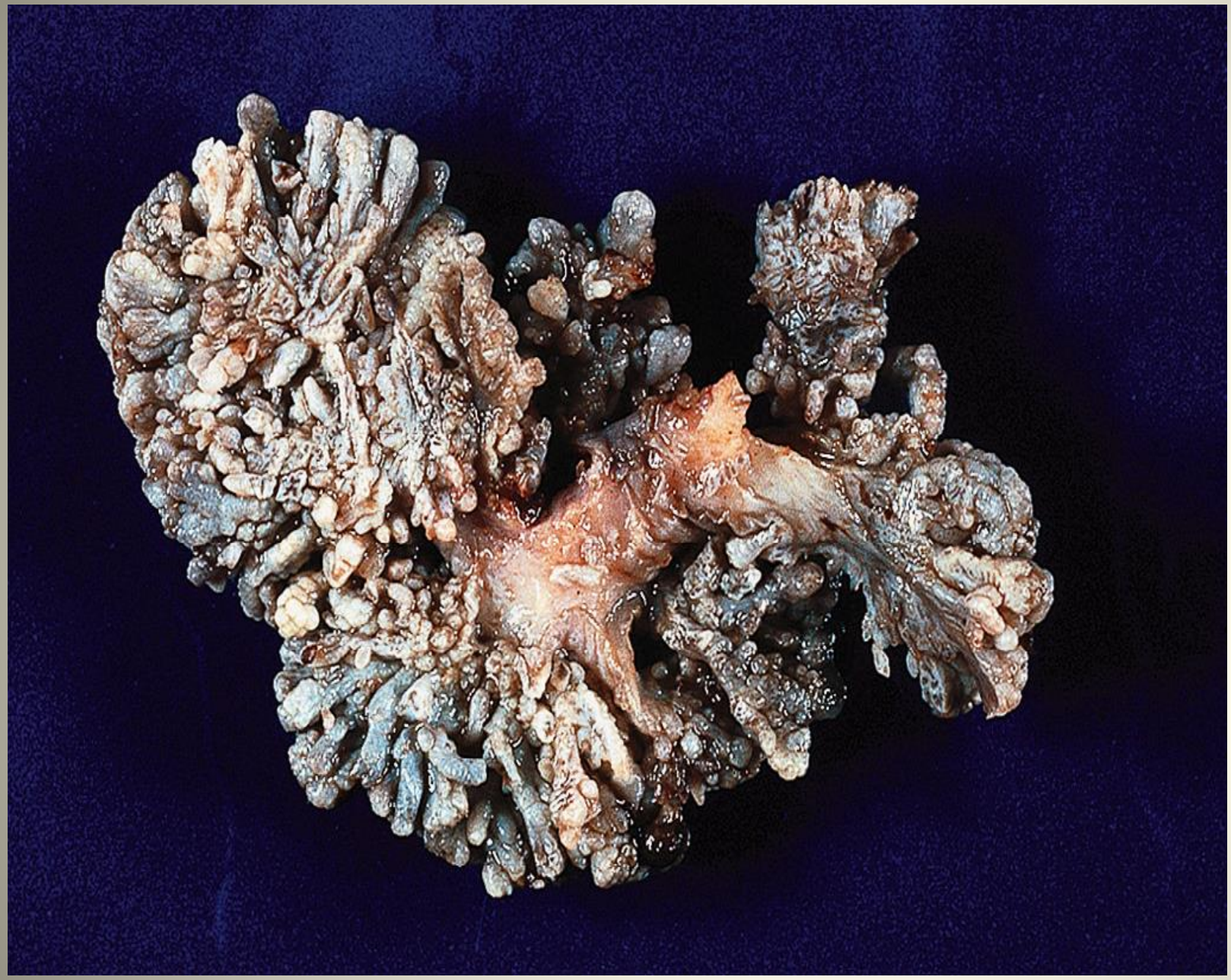
LSIL (CIN1)

- is inclusive of **condyloma** in the LAST criteria
- The former practice of attempting to determine whether there is or is not dysplasia within condyloma **has mercifully been brought to an end**.
- **condyloma ± CIN1** ???

Condyloma acuminatum

- one or several soft elevated masses of variable size
- is a venereal disease
- in vulva, vagina, cervix, bladder and penis
- is caused by HPV, usually type 6 (low-risk HPV infection)
- should be diagnosed as (VIN1, VaIN, PeIN)

✓ we do not recommend adopting LSIL, while we are able to endorse LSIL (CIN1) for cervical condyloma



Large condyloma of vulva

condyloma acuminatum

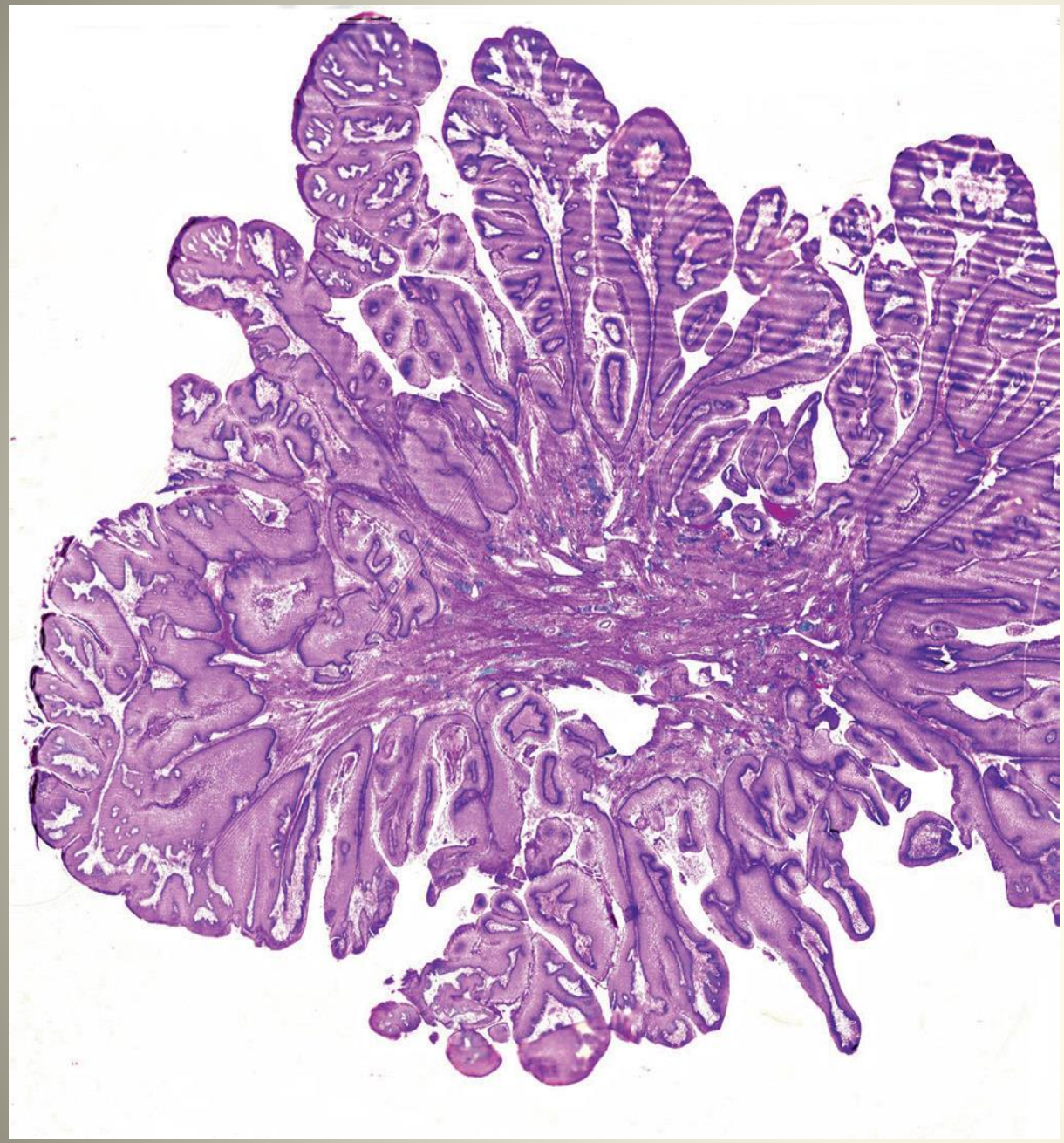
- **Exophytic** versus flat: grossly as a polypoid lesion
- considerably less common than the latter in cervix
- An **undulating** appearance of the epithelium on low-power
- **papillomatosis, acanthosis**
- expanded or hyperplastic parabasal cell layer

Microscopically

- **orderly maturation;** a smooth transition to intermediate and superficial cells
- **mitotic activity** confined to the lower third of the epithelium (but few or no abnormal mitoses)
- a variable degree of lymphocytic **inflammation** in the stroma

Condyloma acuminatum

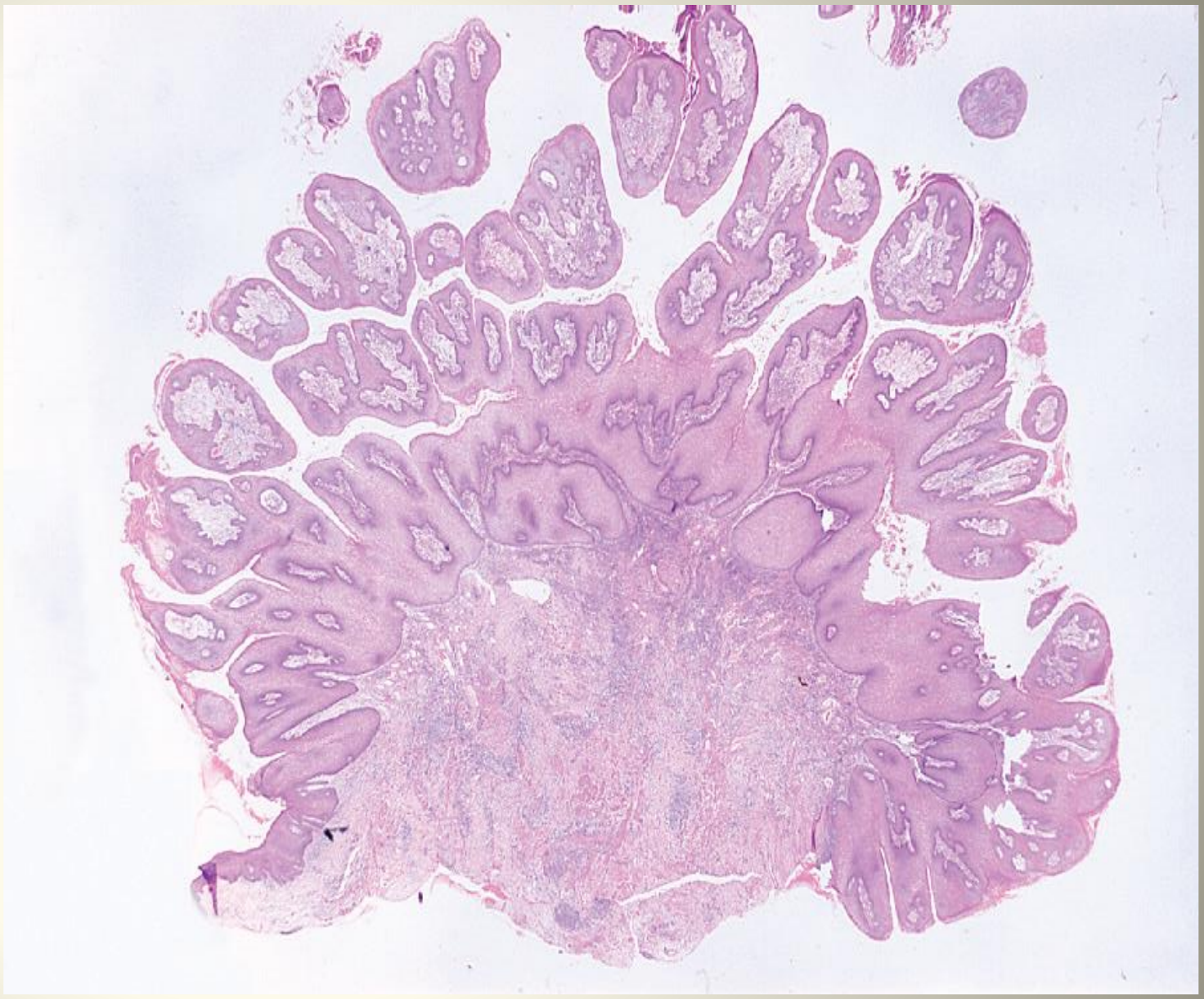
- A **mild degree of** basal or parabasal **atypia** is common
- if **more severe**, it should be evaluated and graded as for the flat SIL (is there HSIL [CIN2/3] present?)
- is associated with HPV-6 or HPV-11 in 70%–90% of the cases
- occasionally other types—such as HPV-16: high-grade cytologic atypia may be found

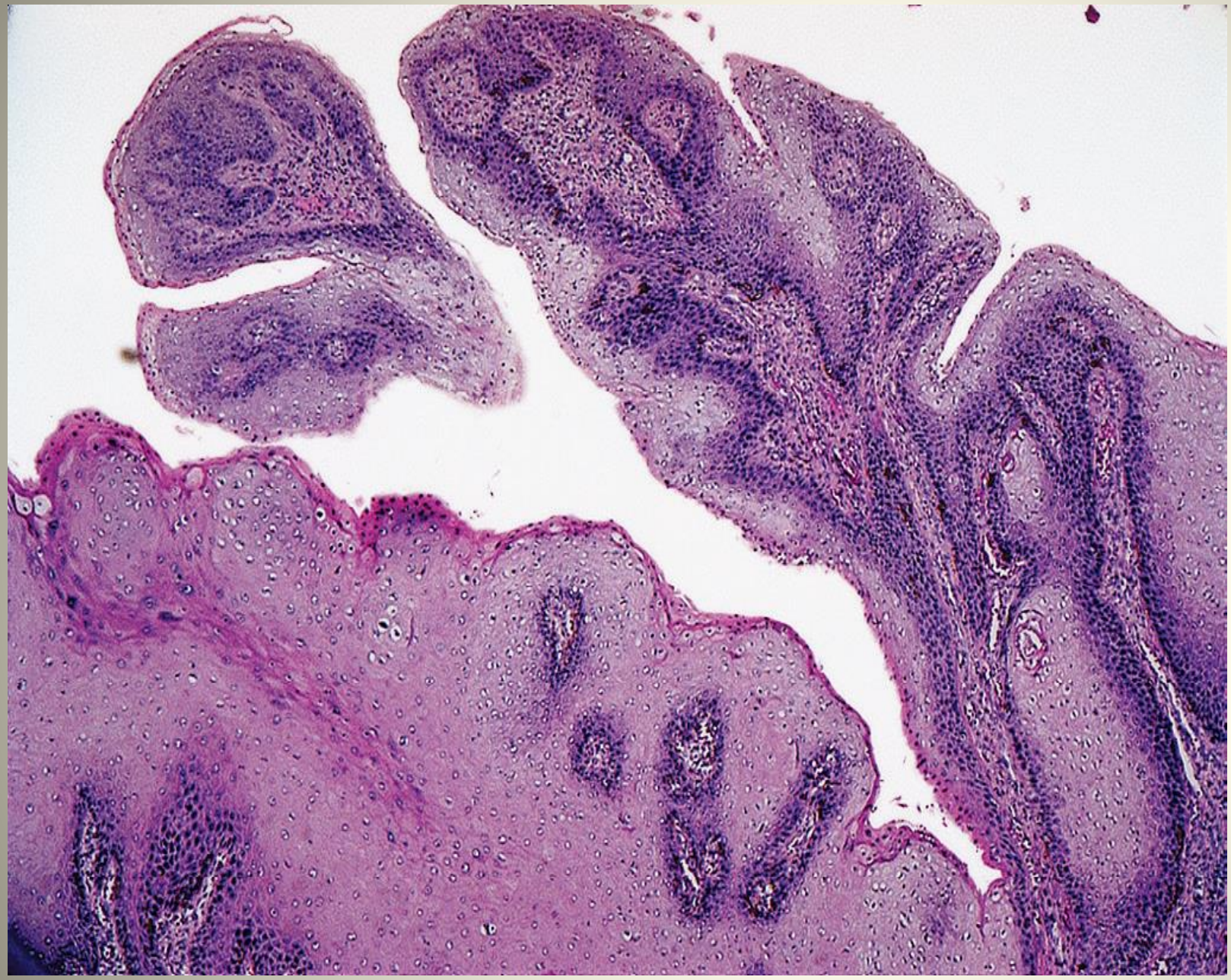


condyloma acuminatum

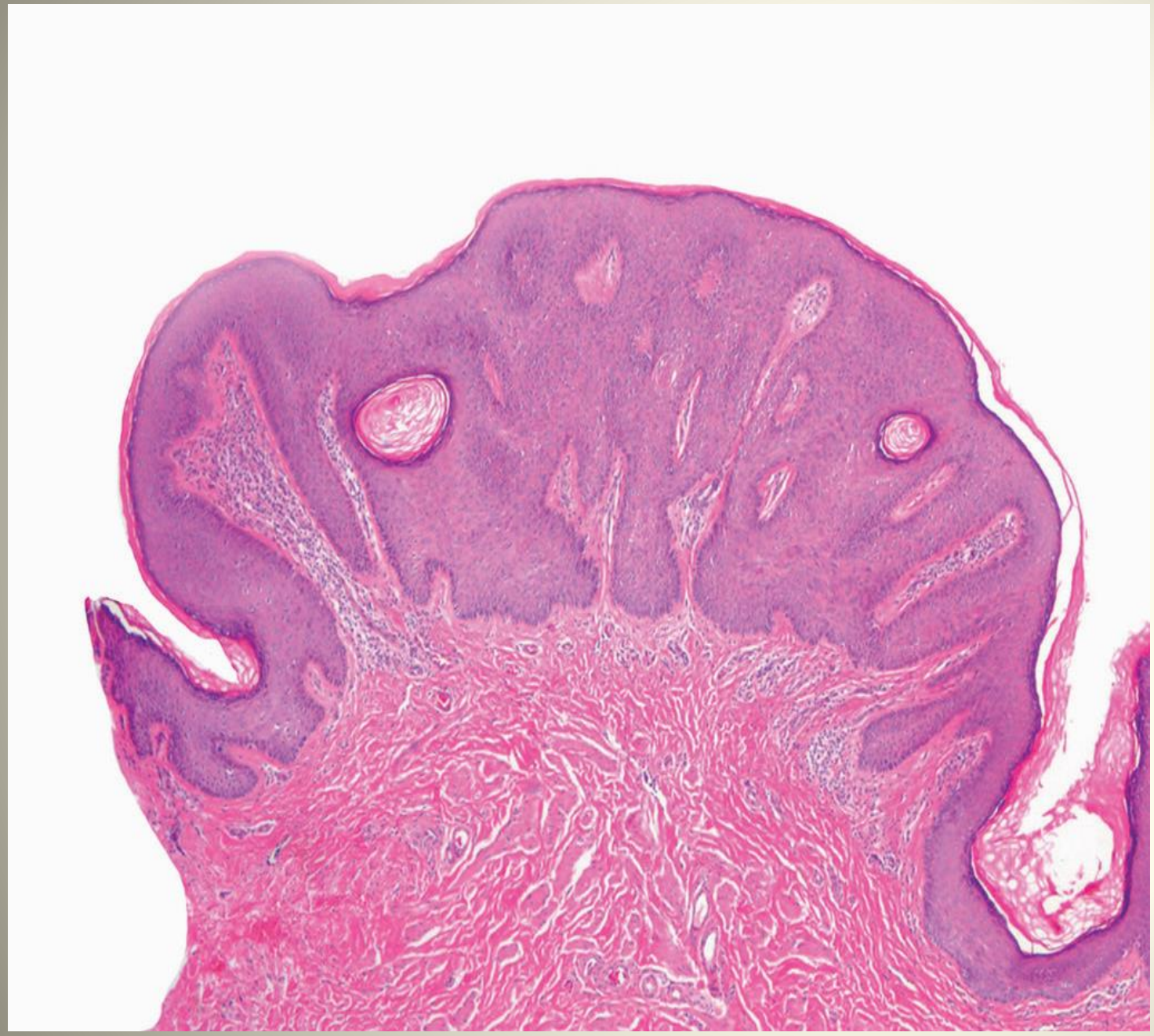
Condyloma Acuminatum

Complex papillary pattern composed of well-differentiated squamous epithelium





Papillomatous shape of vulvar condyloma



Some condylomas have a low-power appearance simulating seborrheic keratosis

The other form: **flat condyloma**

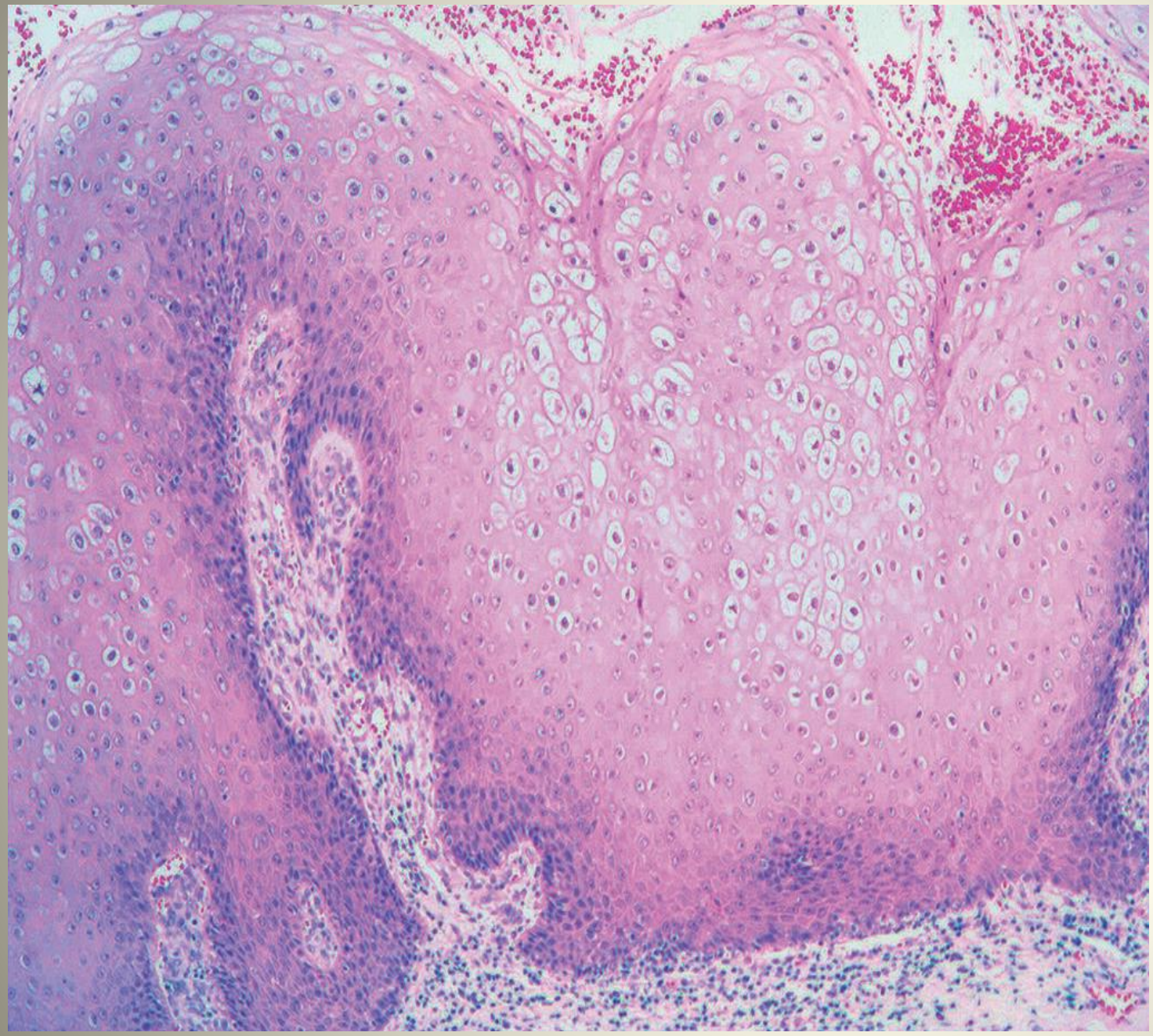
- **is more commonly encountered in cervix** than condyloma acuminatum
- The cytologic features are similar in both forms
- **the classic lesion of LSIL (CIN1)**
- It is typically not recognizable grossly

increased proliferative activity

- **mitoses** may be numerous, but are all typical
- with the **Ki-67 stain**
  - in contrast to fibroepithelial polyp and squamous papilloma
- The DNA content
  - Diploid
  - Polyploid (tetraploidy and octaploidy)

LSIL (CIN1)

- **Koilocytic viral cytopathic effect** is the pathognomonic
- it is **doubtful** whether LSIL (CIN1) can be diagnosed in the **absence of koilocytic change**
- koilocytic change (HPV effect) with or without dysplasia should not be distinguished

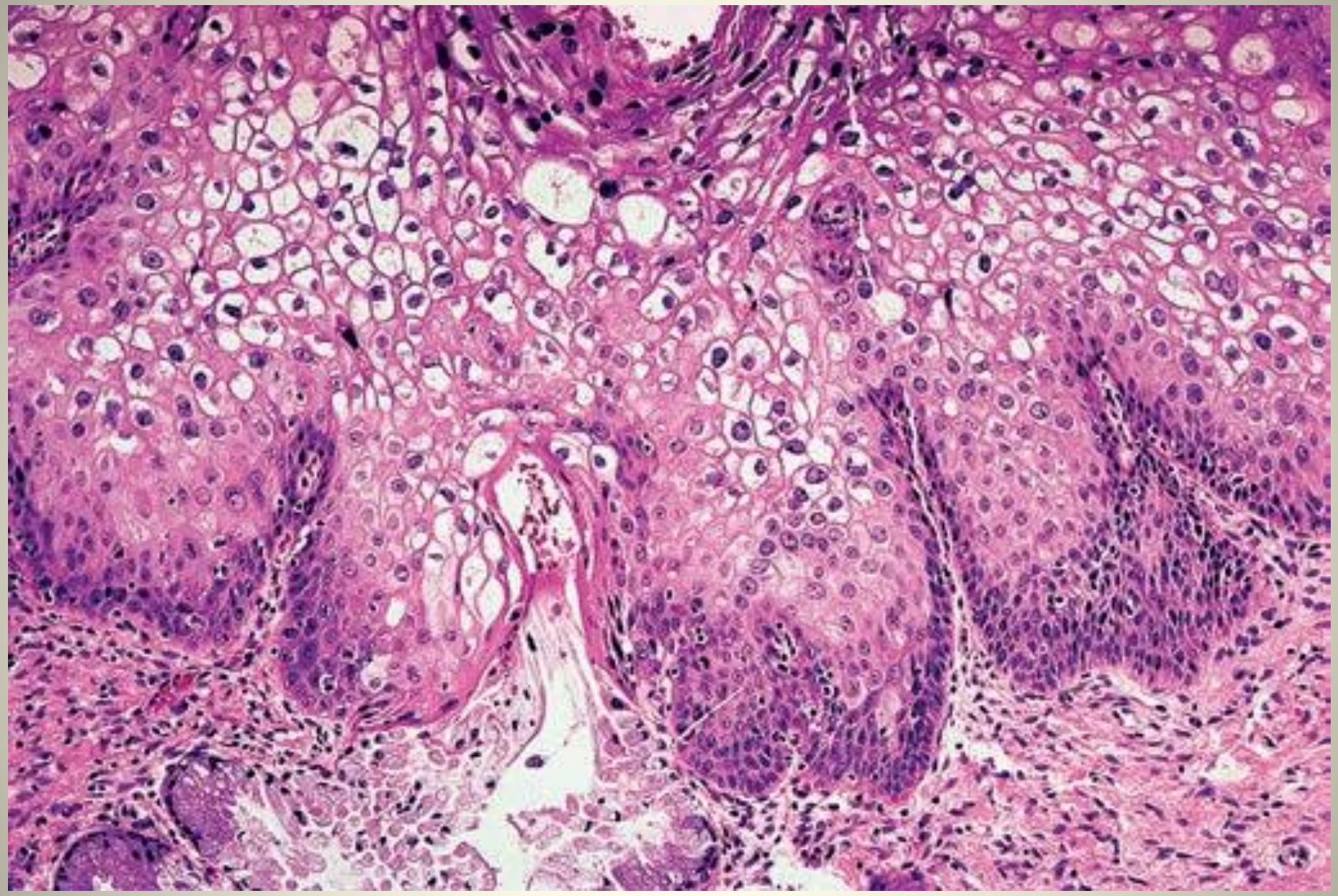


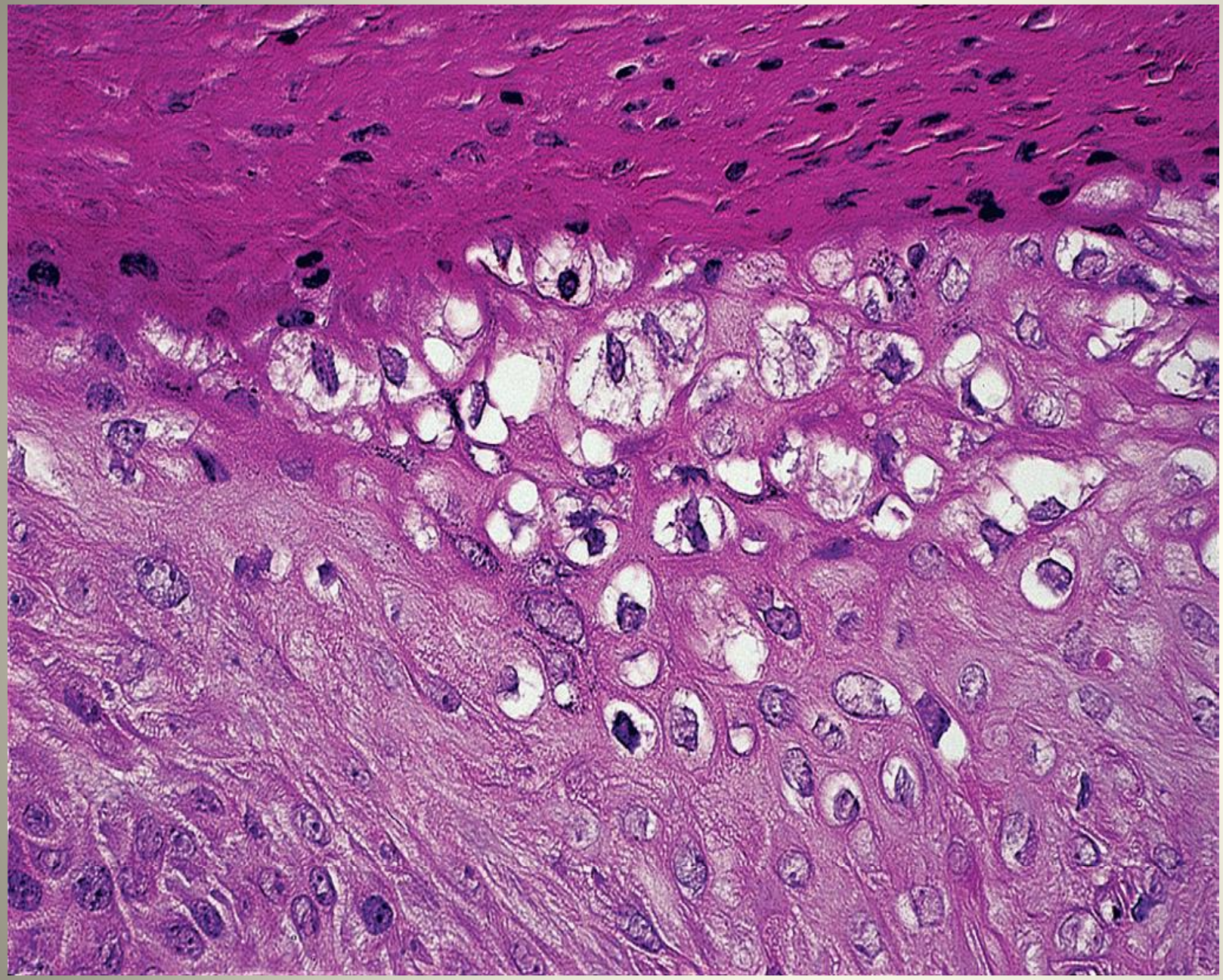
Virus induced cytopathic changes

Koilocytosis of the malpighian epithelium

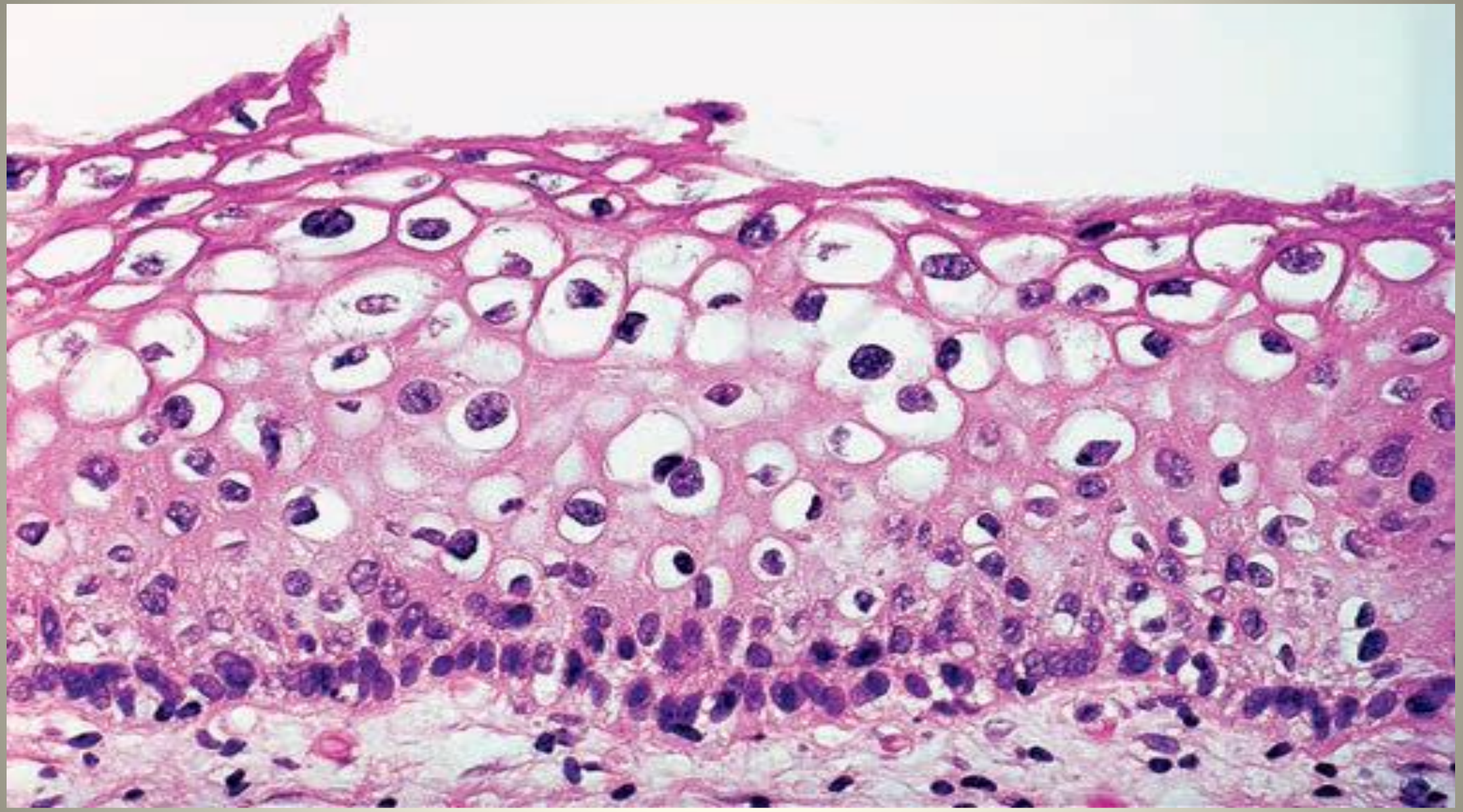
- a superficial or intermediate mature cell
- a sharply outlined perinuclear clear vacuolation
- an enlarged nucleus with an wrinkling or undulating nuclear membrane (prune-like or nuclear “**raisins**”)
- a rope-like chromatin pattern
- dense- and irregular-staining peripheral cytoplasm

- Binucleation and multinucleation
- **orderly maturation**; a smooth transition to koilocytotic intermediate and superficial cells
- Koilocytosis is not as florid as condylomas of the **cervix** in other areas





Prominent koilocytotic changes



LSIL

- dysplasia of the lower third of the epithelium
- the distinction between “dysplasia” and reactive expansion of the parabasal layer is **highly subjective**.

the old chestnut

should be relegated to the bin reserved for

medical trivia of historical interest only

- mild, moderate and severe cervical dysplasia (CIN1-3)
- dysplasia in the lower third, lower two thirds, and full thickness of the squamous epithelium

this is still repeated to **trainees** and has no underlying mechanistic basis

Bethesda classification

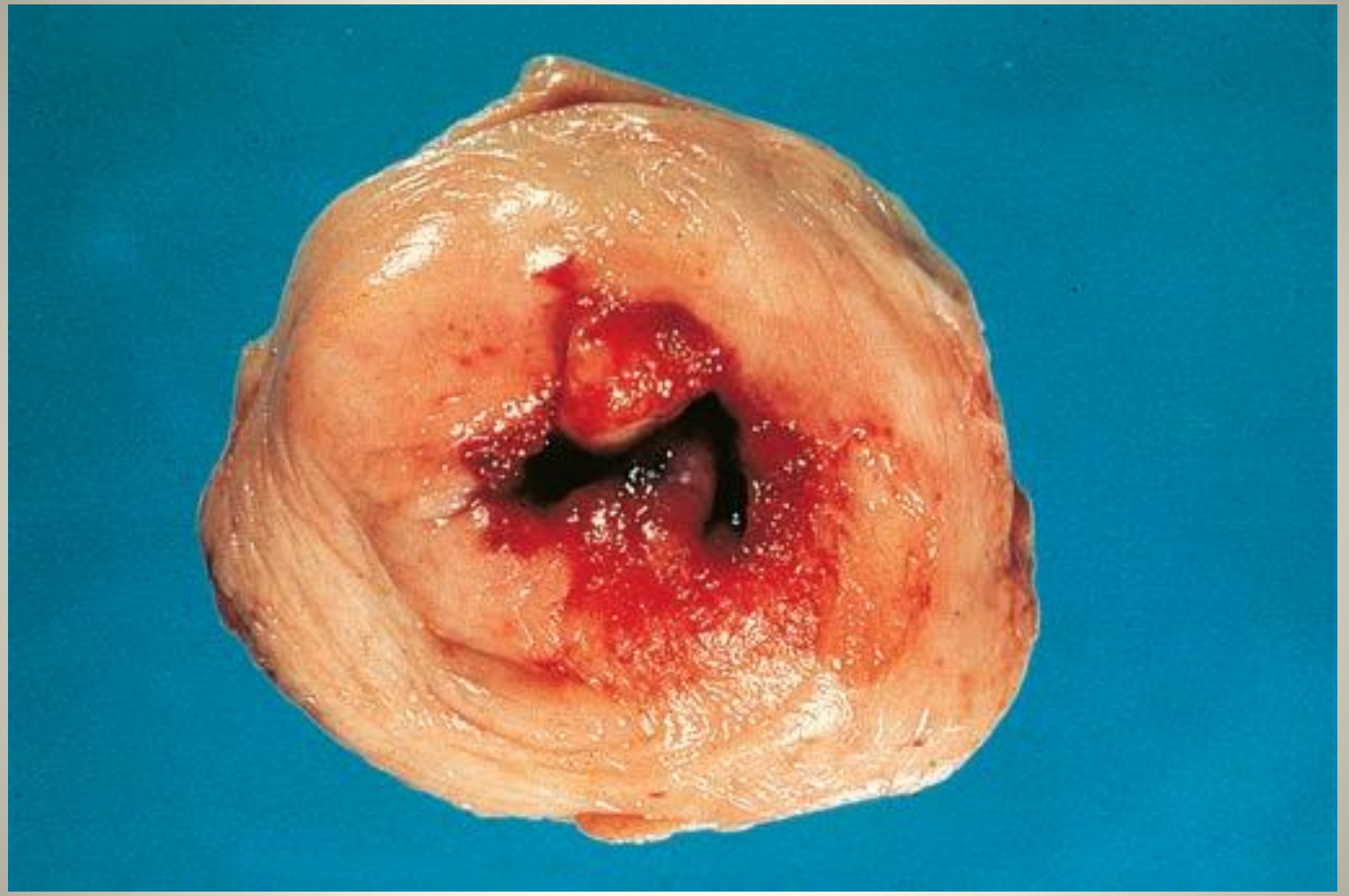
- **reflects the biology** of in situ squamous neoplasia of the cervix,
- especially as it relates to HPV
- a bipartite LSIL/HSIL system
- The proliferation rate of LSIL (CIN1) is higher than that of inflamed or metaplastic cervical squamous epithelium.

The natural history of LSIL

- most will **clear** spontaneously
- relatively few will **progress** to HSIL (CIN2/3)

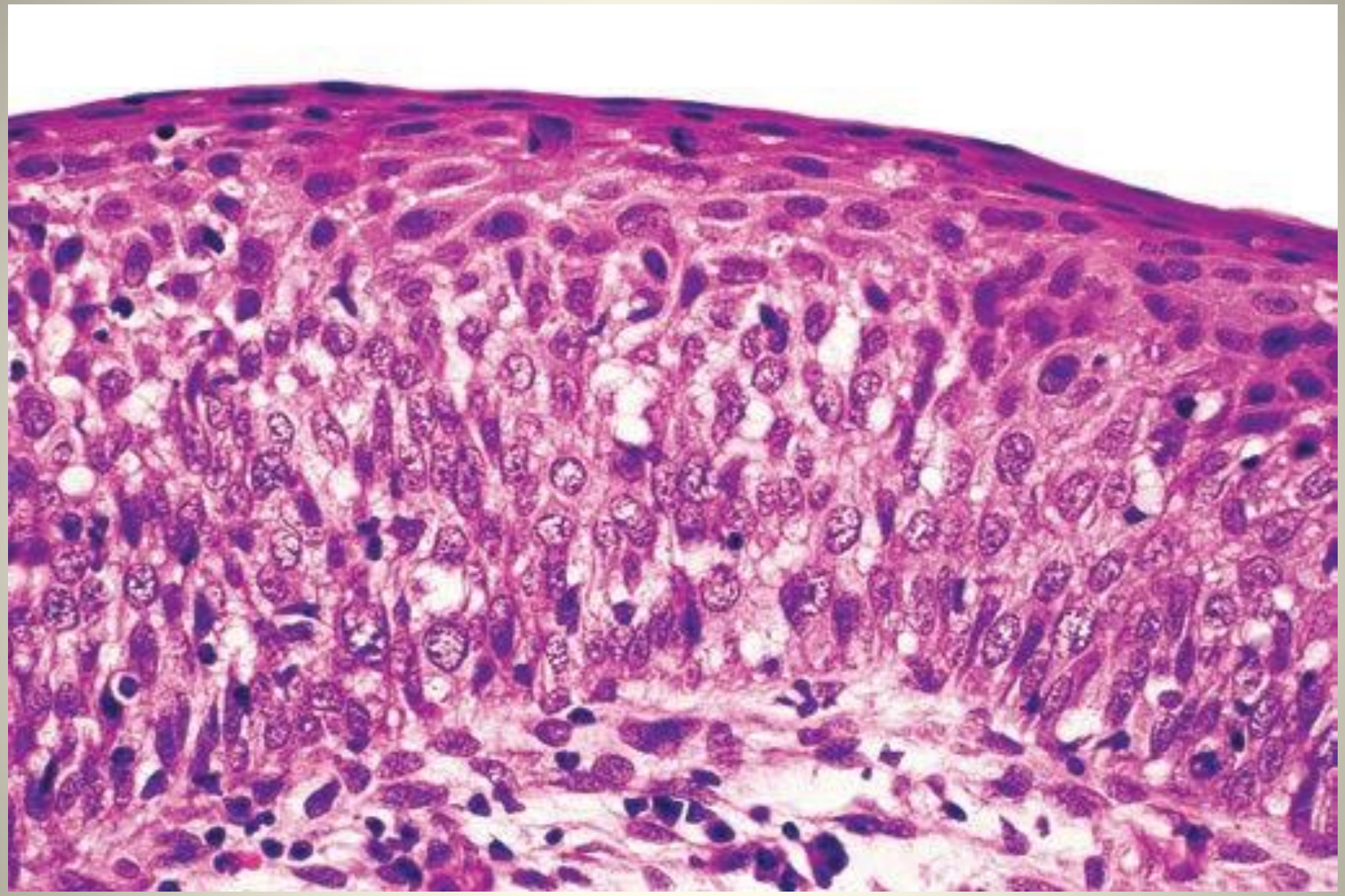
HSIL (CIN2/3)

- is best appreciated colposcopically
- may be seen **macroscopically**, when extensive



Mioscopically

- **high N:C ratio** in all layers of the epithelium
- Most importantly by **striking nuclear atypia**
  - nuclear pleomorphism, enlargement, and hyperchromasia
- Prominent **mitotic activity**, with atypical mitoses
  - in the upper layers of the epithelium



the differential diagnosis between LSIL and HSIL

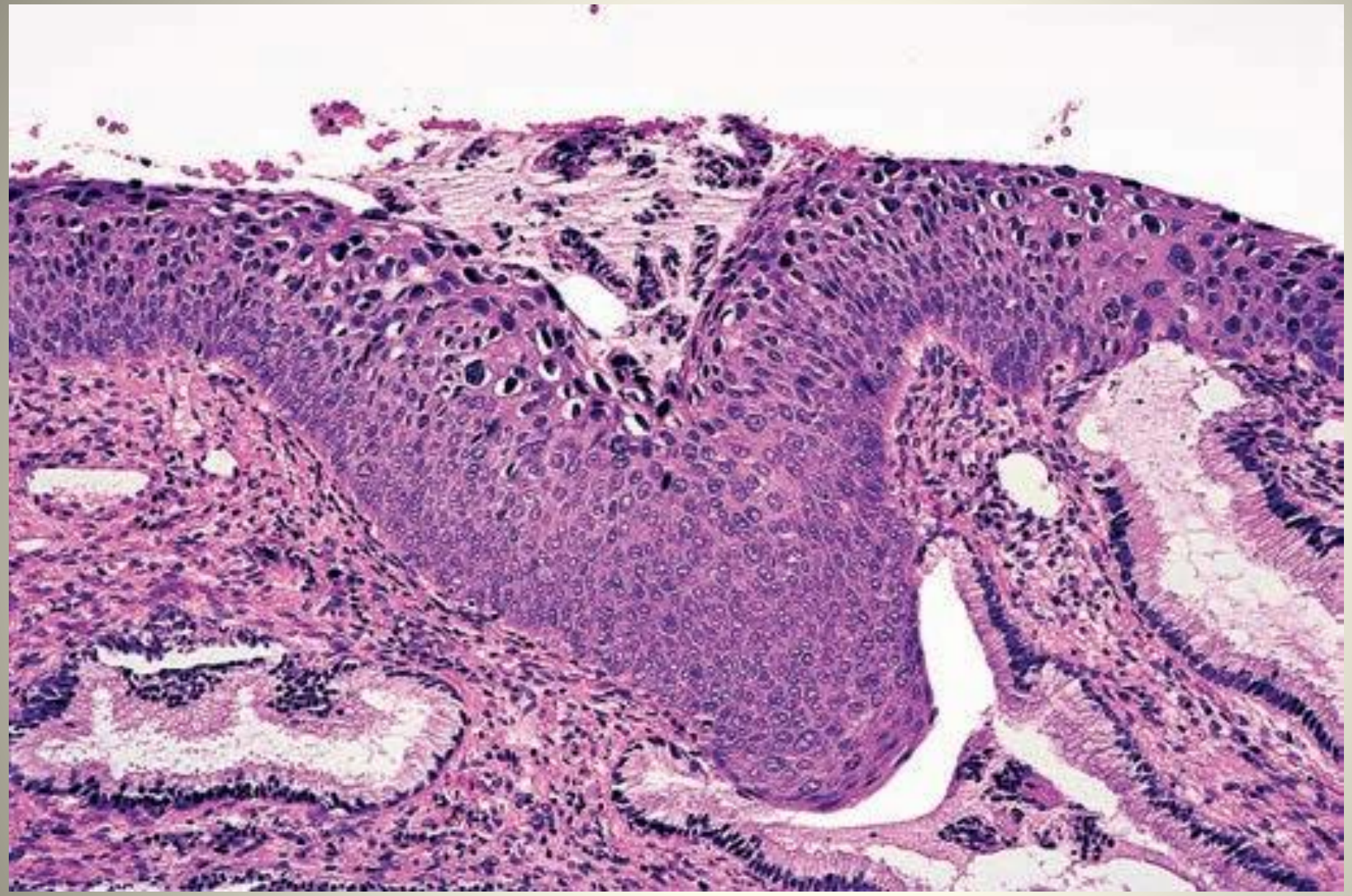
- is made primarily based on H&E
- Only in selected borderline cases:

✓**p16 immunostaining** **strong and diffuse block positivity**

✓**Ki-67 stain**: increased proliferative activity

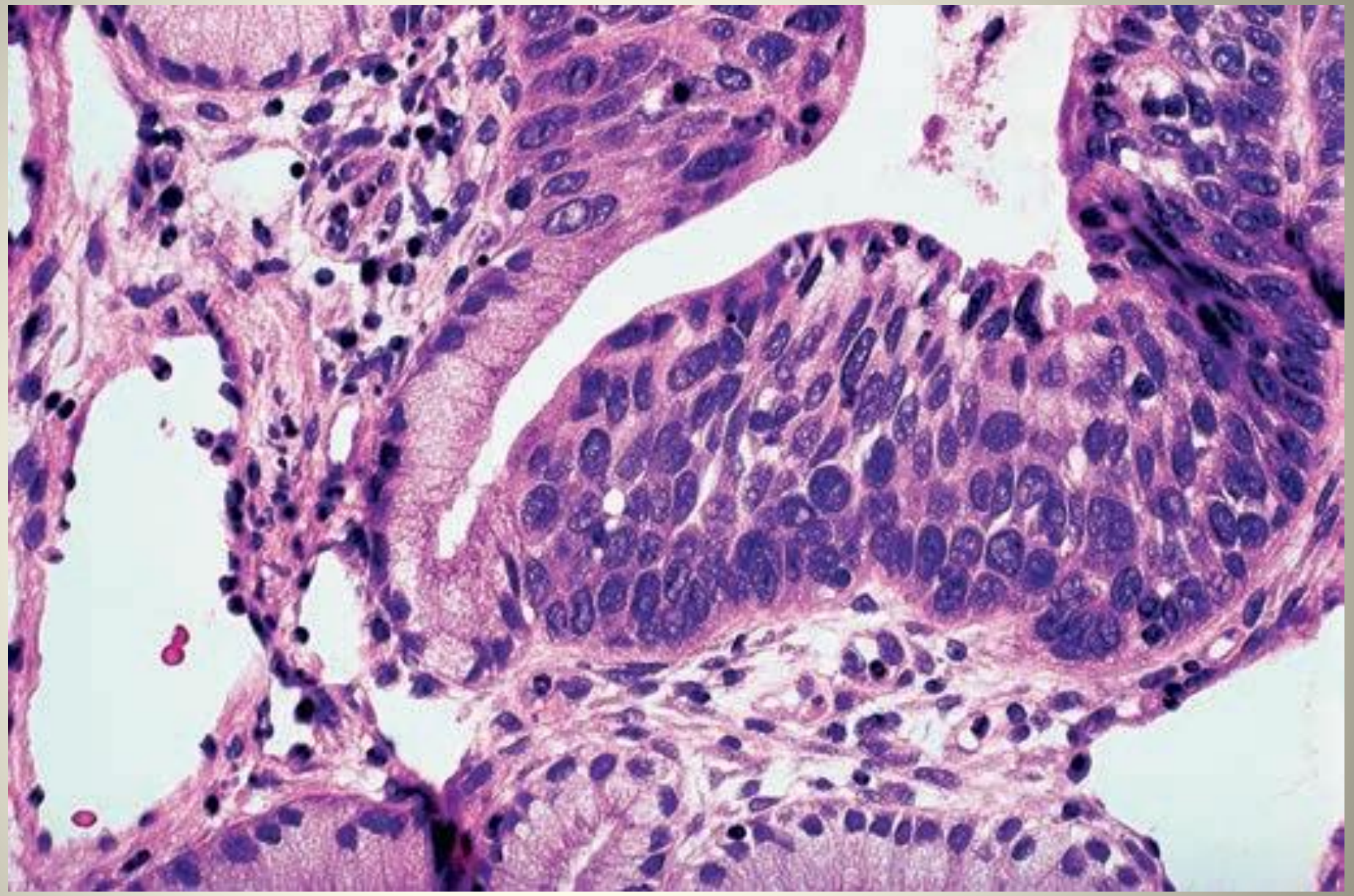
these have to be taken into account

- the surface
- Margins
- the possible depth of involvement
- extension into endocervical glands



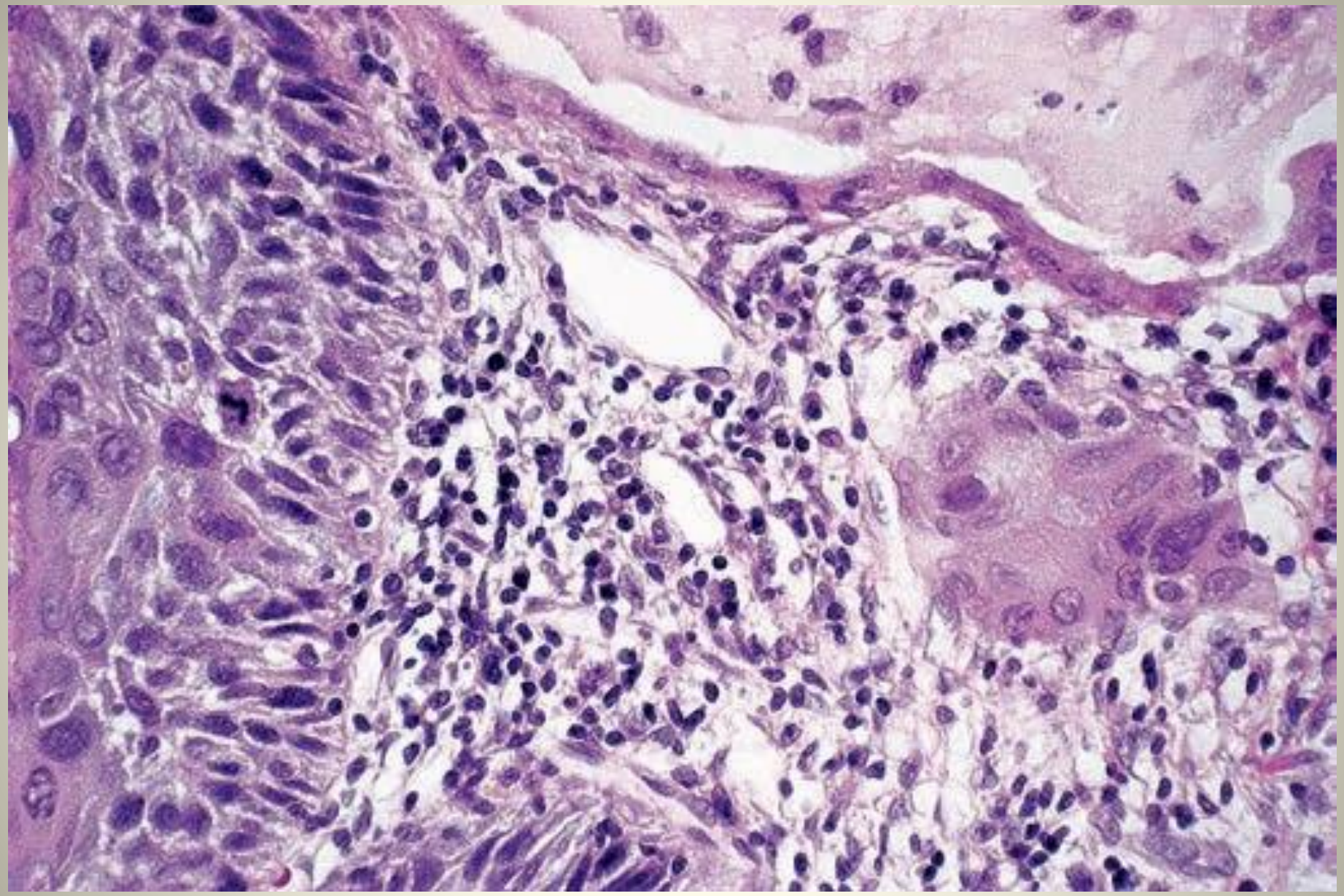
Extensive involvement by HSIL (CIN2/3) of surface epithelium and glands of endocervix

Partial replacement of endocervical glandular epithelium by HSIL (CIN2/3)



the presence or absence of invasive carcinoma

- **Thorough cervical sampling**
- **the proper sectioning of tissue**
- continuing abnormal **cytology** after initial treatment of HSIL (CIN2/3) were found to be 25 times more likely to develop invasive carcinomas than women with normal follow-up cytology



VIN2/3

- abnormal mitoses and nuclear pleomorphism, enlargement, and hyperchromasia **in the basal and parabasal cell layers**
- “block positivity” on p16 immunostaining,
  - at least the basal third of the epithelium
  - usually extending into the upper half
- The increased proliferative activity with the Ki-67 stain
- The DNA content is aneuploid pattern in most cases
- **a significant number of HPV-independent VIN**

VaIN2/3

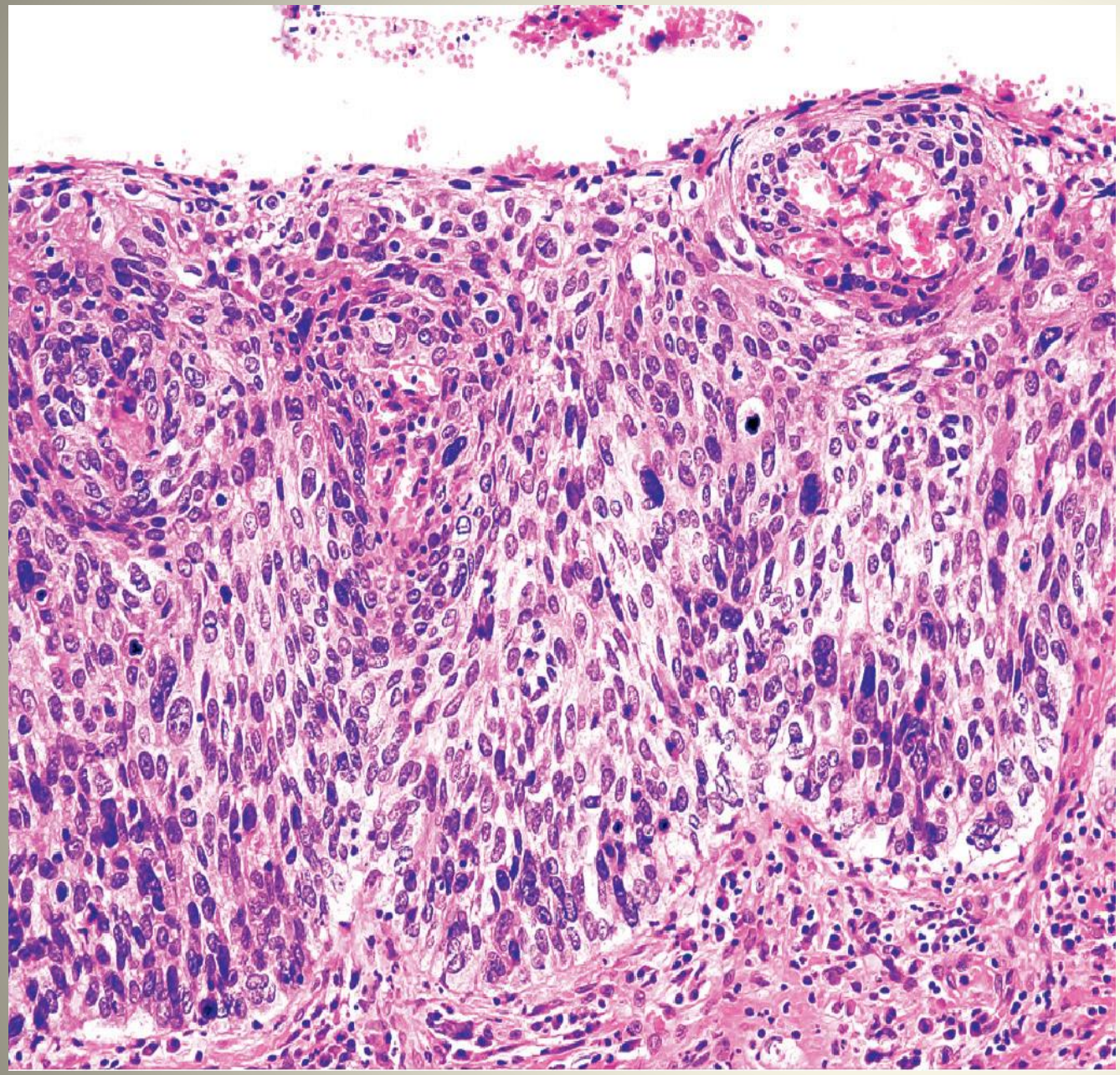
- The upper third of the vagina is the most common site, the vaginal and cervical lesions may be **confluent**
- arise from **native** squamous epithelium, in contrast to most cervical cases, which originate from *metaplastic* epithelium
- is **multifocal** in about half of cases
- very frequently associated with **concomitant, subsequent, or prior** (in situ or invasive) neoplasms elsewhere in the lower genital tract, especially the cervix

Penile Intraepithelial Neoplasia (PeIN)

- Human Papilloma Virus Related PeIN
  - **Basaloid (undifferentiated) PeIN**
  - **Warty (Bowenoid) PeIN**
  - **Warty-basaloid PeIN**, shows an admixture
- HPV-unrelated PeIN
  - **Differentiated PeIN** has been associated with lichen sclerosus or other chronic inflammatory conditions

Basaloid (undifferentiated) PeIN

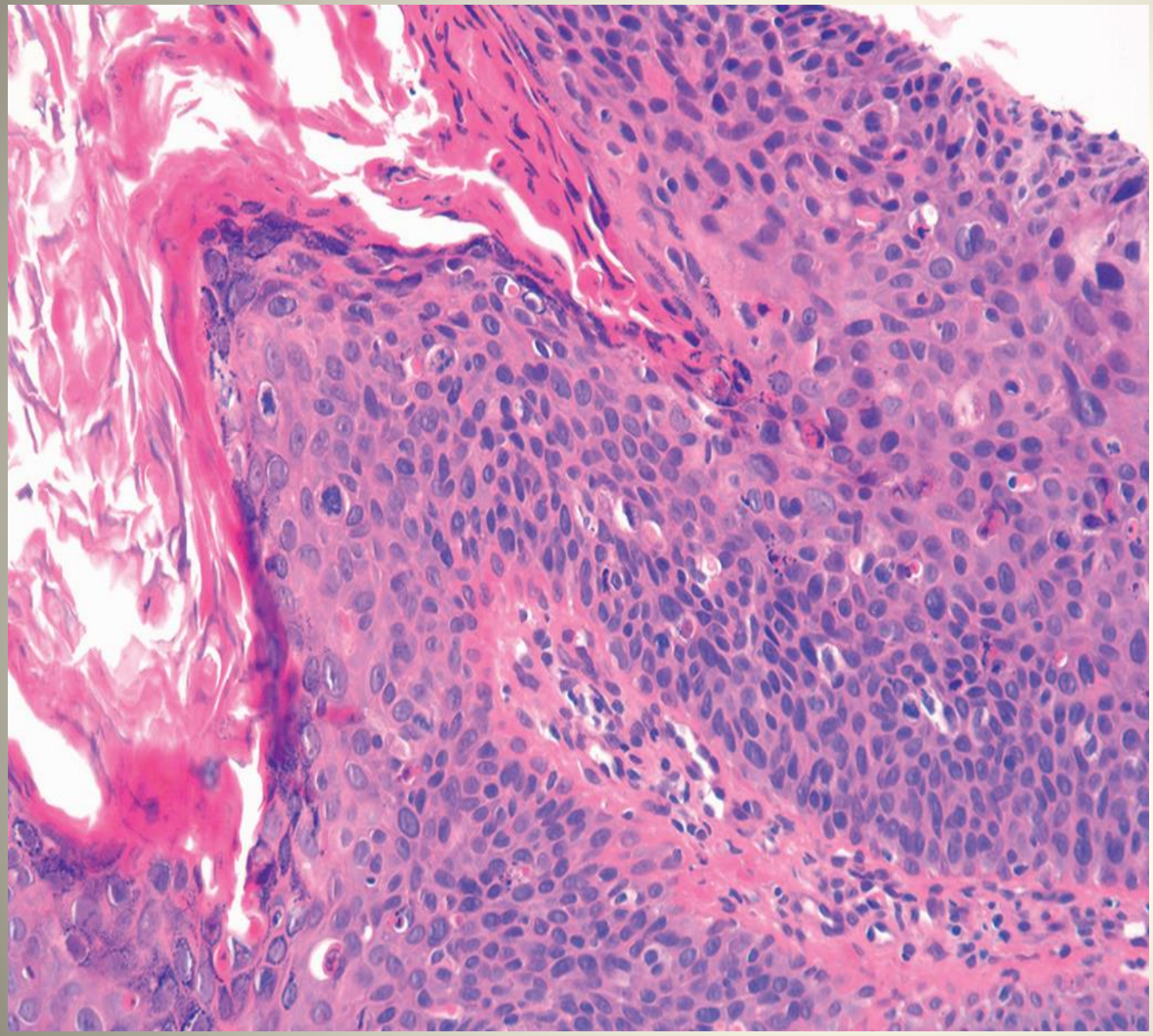
- typically involves the glans penis in young men
- **similar to HSIL of the cervix** with full-thickness involvement by small monotonous immature cells with high nuclearto-cytoplasmic ratio
- Mitotic activity and apoptotic bodies may be frequent, but squamous maturation is not characteristic
- show diffuse staining for p16
- a common association with HPV type 16



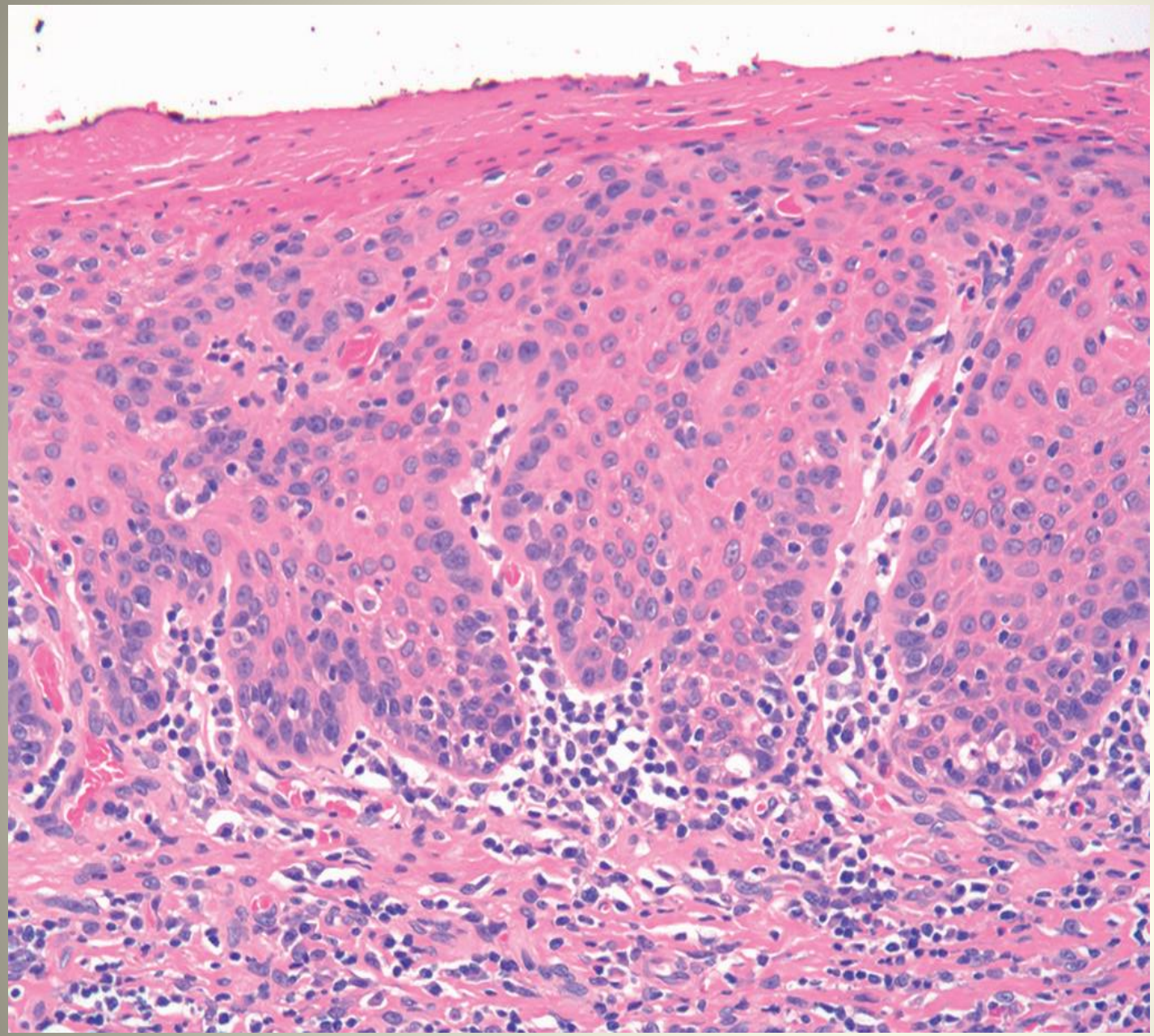
Basaloid PeIN

Warty (Bowenoid) PeIN

- has more complex architecture than basaloid PeIN
- frequent squamous maturation, a papillomatous surface and more abundant surface keratin, a contrasting point with basaloid PeIN
- more nuclear pleomorphism, and well-developed koilocytotic atypia
- expresses strong p16
- the associated HPV type is more variable



Warty-basaloid PeIN



Differentiated PeIN