HUMAN PAPILLOMAVIRUSES

Introduction

- Papillomaviruses have been detected in variety of vertebrates
- Are highly species specific and cross-species infections do not occur even under experimental conditions
- Are widespread producing benign and malignant diseases
  - Hand, plantar and anogenital warts (benign)
  - Genital tract malignancies
    - cervical and penile cancer
  - Epithelial tumors of the skin (squamous cell carcinoma) and respiratory tract

- **Infectious nature of human warts was first recognized late 19th century – inoculation to uninfected individuals**

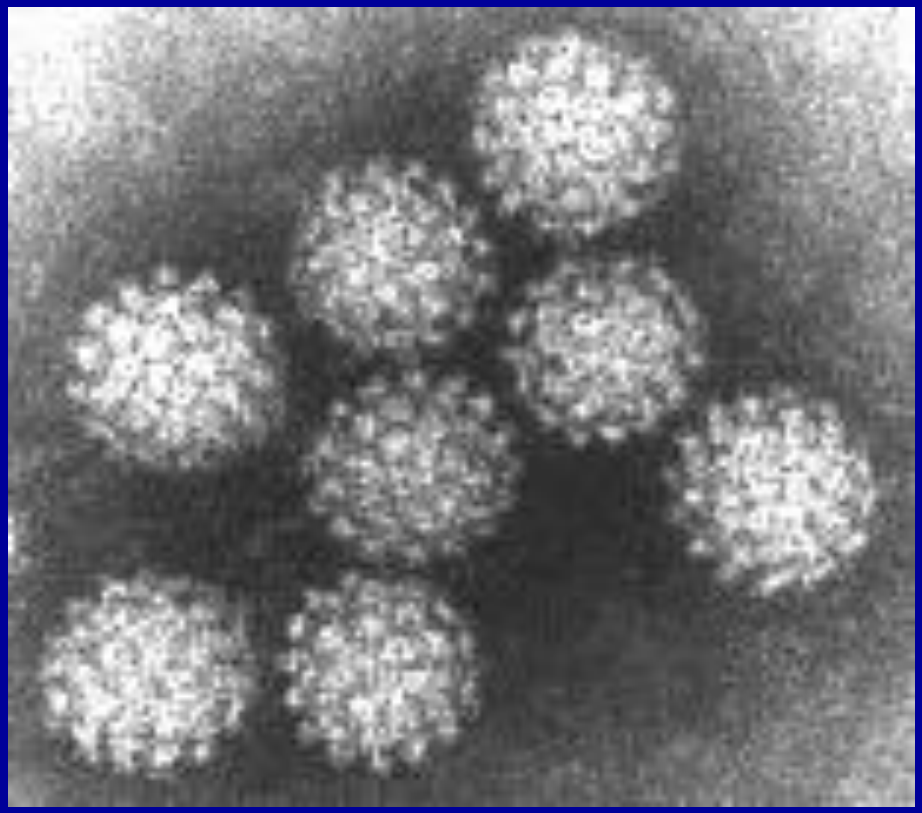
- **There are so many variations of HPV that we make the following rule: Types are distinct if their genomes have less than 90% homology in their L1 major capsid protein), otherwise subtype**

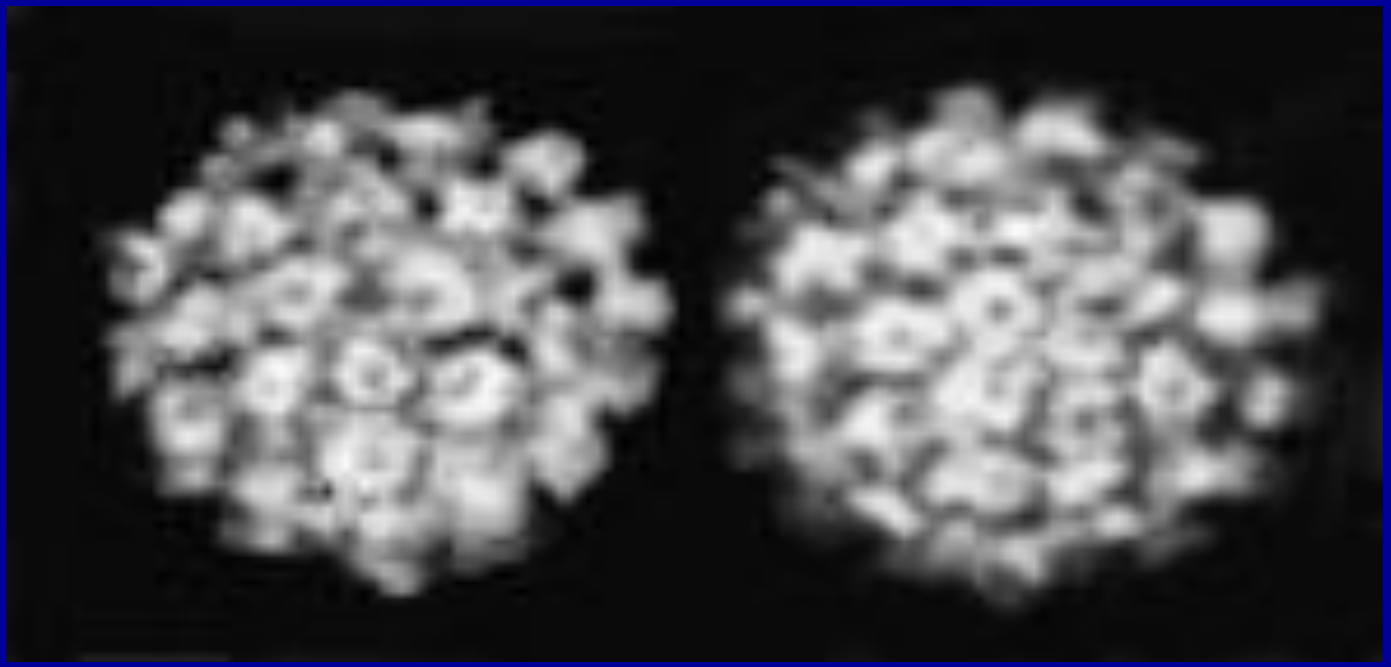
- **There are over 100 different genotypes with over 220 types at various stages of characterization**
  - **Low-risk HPV types – warts e.g. 6 and 11**
  - **High-risk HPV types – malignancies e.g. 16 and 18**

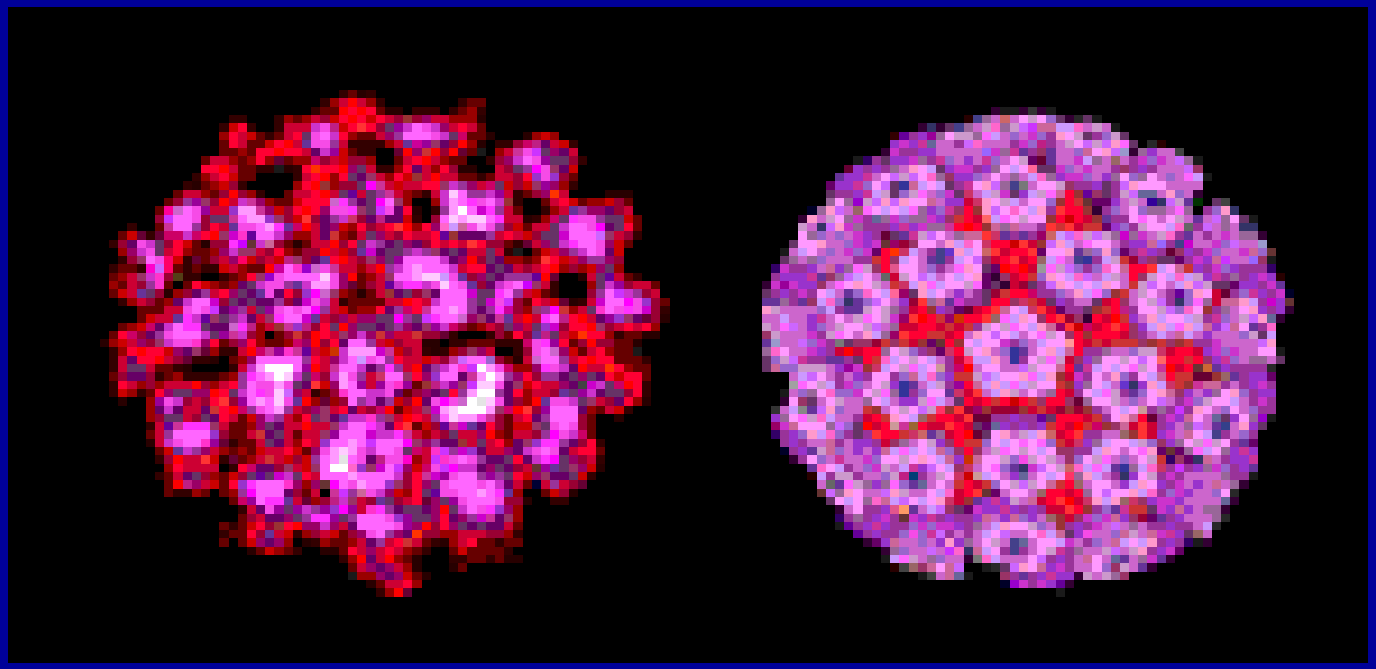
Virology

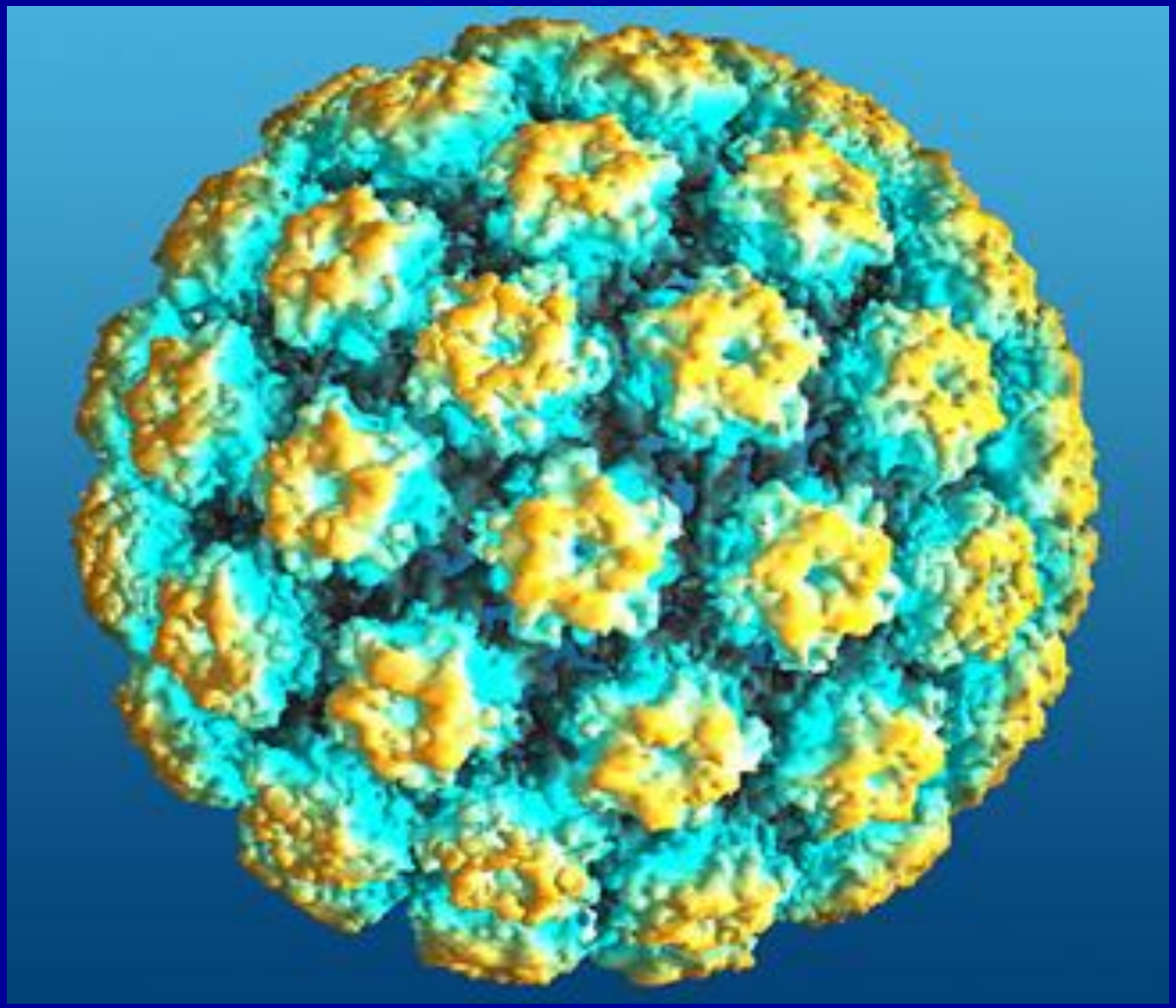
- Papillomaviruses have the same structure
- Non-enveloped (naked) icosahedral capsid
- Capsid consists of 72 pentamers
  - Each pentamer is composed of 5 major capsid proteins (L1), with the minor capsid protein at the center (L2)
- Virions are ~55-60 nm in diameter, small, even for a virus.

HPV CAPSID









Howard Hughes Medical Institute investigator Stephen C. Harrison

- **The viral genome is double-stranded circular, supercoiled DNA**

- **Papillomavirus genomes have same general organization which consists of eight open reading frames (ORFs)**

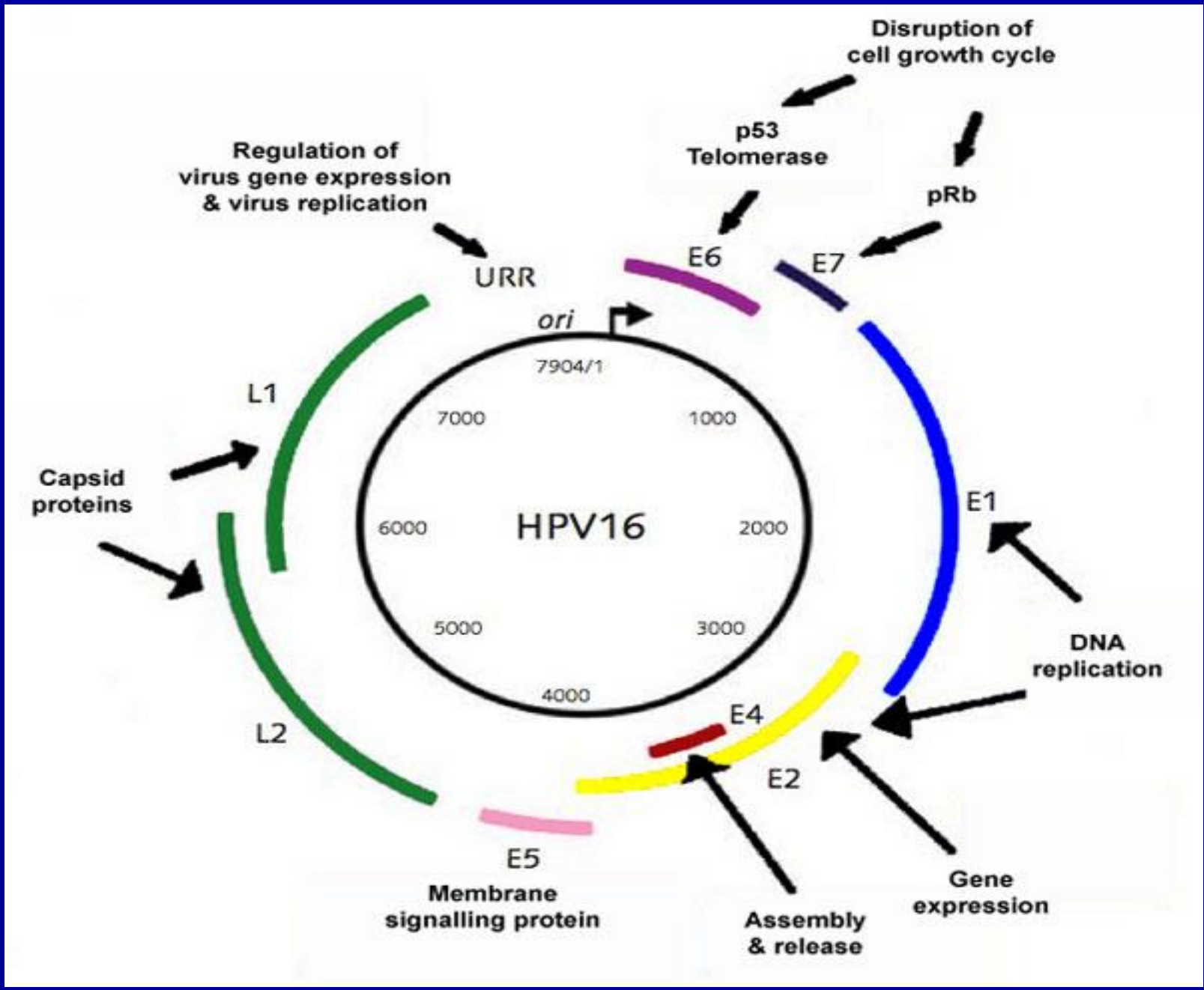
- **ORFs are designated either E (early) or L (Late)**
  - **Early genes encode for regulatory proteins = E1, E2, E4, E5, E6, E7**
  - **The late genes encodes capsid proteins = L1 & L2**

Papilloma virus gene function

ORF	Function
L1	L1 protein, major capsid protein
L2	L2 protein, minor capsid protein
E1	Initiation of viral DNA replication
E2	Transcriptional regulatory protein with an auxillary role in viral DNA replication
E3	No known function
E4	Late protein; disrupts cytokeratins
E5	Membrane-transforming protein; interacts with growth factor receptors - inhibits endosome acidification, EGF
E6	Transforming protein of HPVs; targets degradation of p53
E7	Transforming protein of HPVs; binds to the retinoblastoma protein
E8	No known function

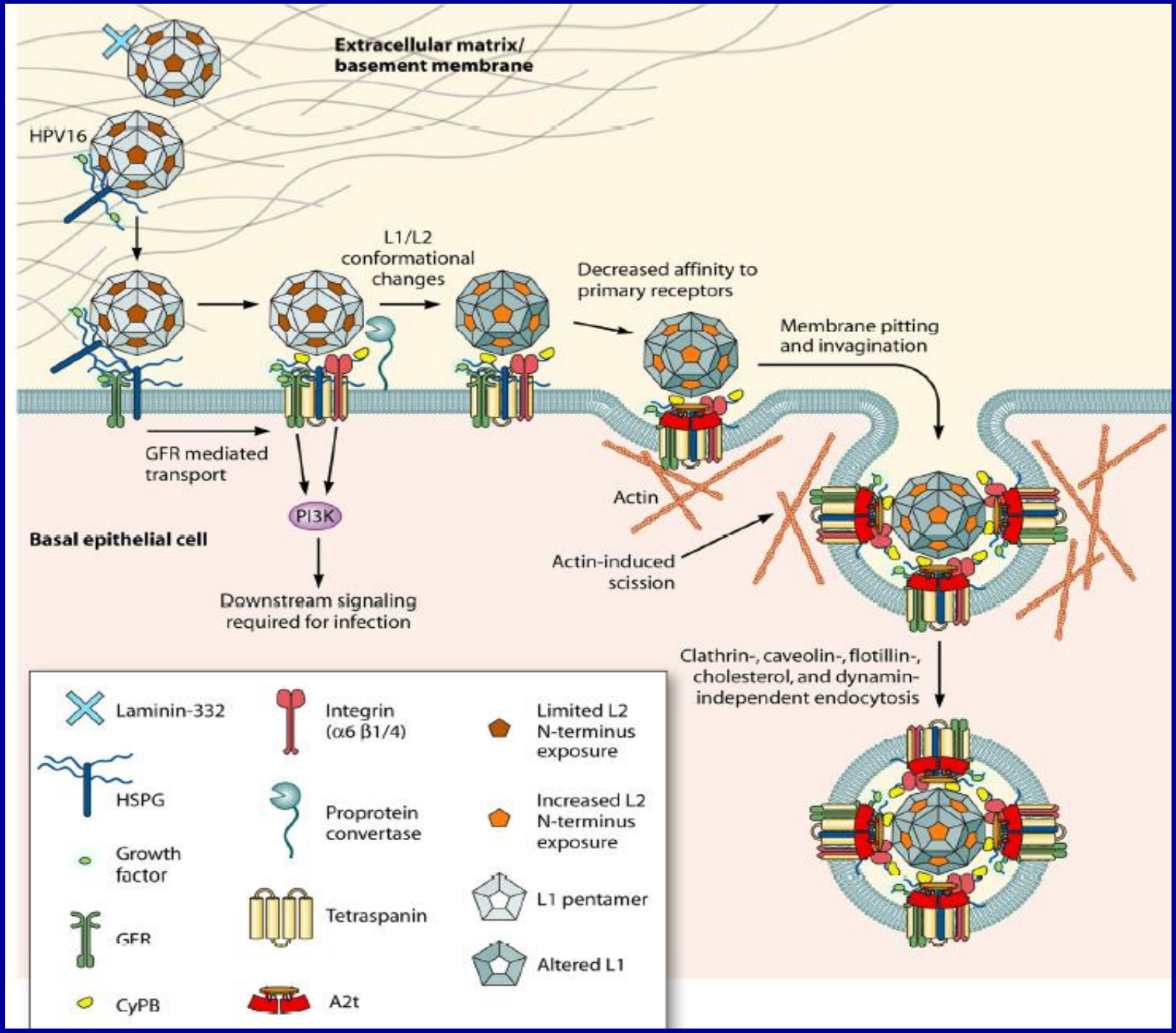
ORF, open reading frame; HPV, human papillomavirus.

(From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 66-1)

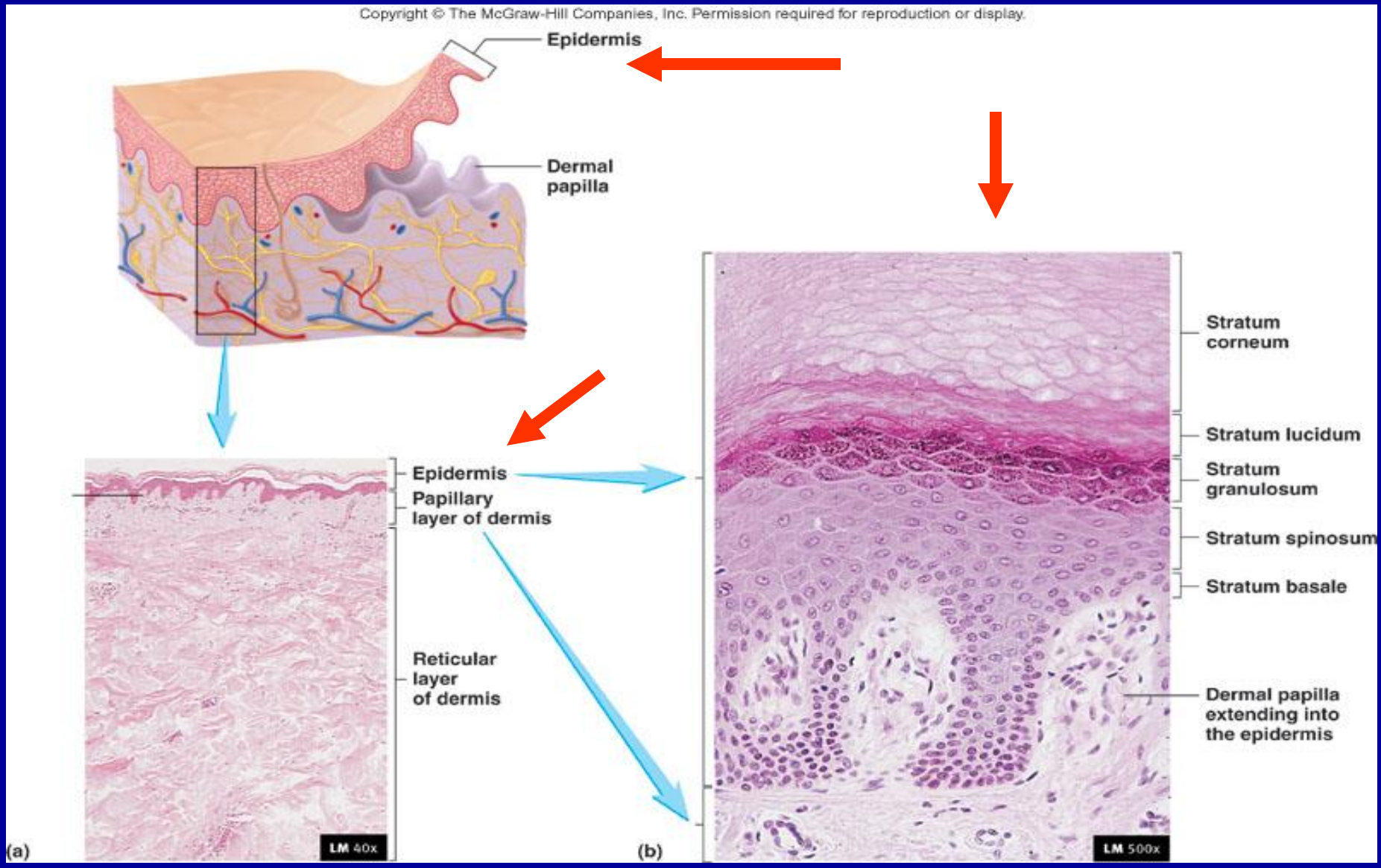

Disruption of
cell growth cycle
p53
Telomerase
pRb
Regulation of
virus gene expression
& virus replication
URR
E6
E7
ori
7904/1
1000
2000
3000
4000
5000
6000
7000
HPV16
L1
L2
Capsid
proteins
E1
DNA
replication
E4
E2
E5
Membrane
signalling protein
Assembly
& release
Gene
expression

REPLICATION

- Knowledge of replication is very limited. How does it get to the stratum basal? Microruptures/abrasions suspected.
- Putative receptors are integrin and heparin sulfate
- Virus initiates its replication as an episome (DNA that can reproduce in the cytoplasm or integrate into a chromosome) after entering a basal cell generating less than 50 copies
- Replication begins by displacement of cellular histones from viral DNA and unwinding the supercoiled DNA
- The virus creates growth factors so the cells reproduce more, (hence the bump) in some strains causing cancer
- After that it is packaged and the release of viral particles is presumably passive


Extracellular matrix/
basement membrane
HPV16
L1/L2
conformational
changes
Decreased affinity to
primary receptors
Membrane pitting
and invagination
GFR mediated
transport
PI3K
Basal epithelial cell
Downstream signaling
required for infection
Actin
Actin-induced
scission
Clathrin-, caveolin-, flotillin-,
cholesterol, and dynamin-
independent endocytosis
Laminin-332
HSPG
Growth
factor
GFR
CyPB
Integrin
(α6 β1/4)
Proprotein
convertase
Tetraspanin
A2t
Limited L2
N-terminus
exposure
Increased L2
N-terminus
exposure
L1 pentamer
Altered L1

Epidermis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Epidermis
Dermal papilla
Epidermis
Papillary layer of dermis
Reticular layer of dermis
LM 40x
(a)
Stratum corneum
Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale
Dermal papilla extending into the epidermis
LM 500x
(b)

EPIDERMAL STRATA

30-40 days for the cell to reach epidermal surface

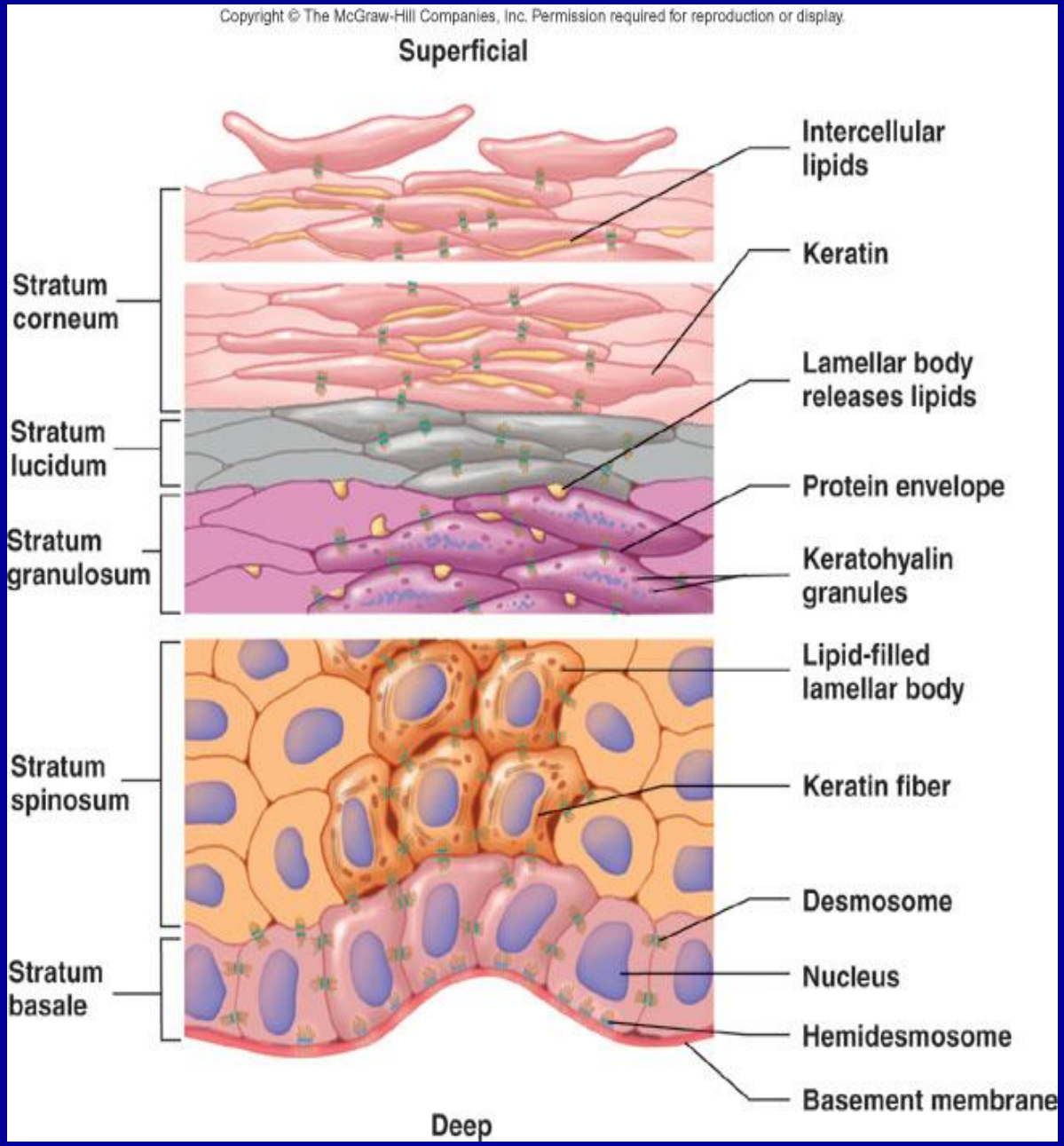
Dead cells contain keratin and Are surrounded by lipids
25 or more layers of dead cells

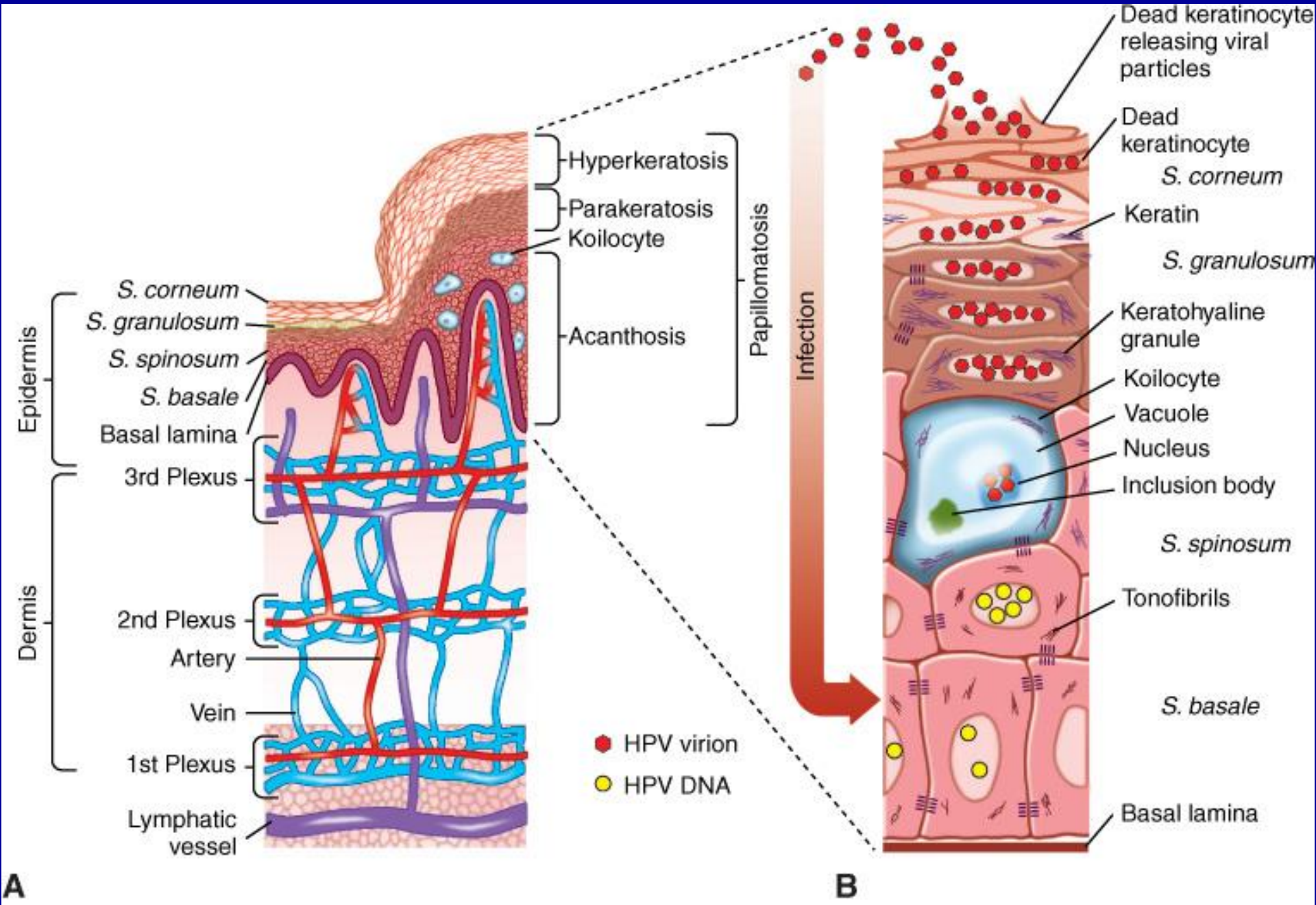
Cells are dead
Contain keratohyalin

Keratohyalin granules accumulate
Hard protein envelope forms
Lamellar bodies release lipids
Cells die

Keratin fibers and lamellar bodies accumulate

Cells divide by mitosis
Cells become keratinized
Contain melanocytes that secrete melanin





Pathogenesis

- The clinical outcome of infection is associated with the type of the HPV
  - Oncogenic types HPV-16, -18, -31, -41
  - Other types induces warts
- The duration of incubation for HPV disease is poorly known
- For cutaneous warts, experimental inoculation leads to the development of lesions within 3 to 4 months on average
- It can occur as early as 6 weeks or as late as 2 years
- Similar incubation periods have been obtained from natural history studies where sexual partners know the date of exposure

- In addition to keratinized skin cells, HPV have been shown to infect mouth, upper airways, vagina, cervix and anal canal
- It also infects the conjunctiva, lacrimal sac, nasal passage, bronchi, esophagus, bladder
- The malignant transformation is associated with latency
- The duration of development of malignancy is unknown – may be several years to decades
- Unlike herpes, in latency, histopathological changes are absent and no viral particles are produced
- The factors that induce, maintain or abrogate the latency is unknown

HPV and skin warts

Clinical type	Location and characteristics	Associated HPV(s)[a]
Deep plantar wart	Bottom surface of feet; generally single	HPV-1
Common wart	Mostly on hands; generally multiple	HPV-2, -4
Mosaic wart (superficial spreading wart)	Feet and hands; resistant to treatment	HPV-2
Flat wart	Arms, face, around knees; multiple	HPV-3, -10, -28, -41
Reddish-brown (macular) plaques of EV	Potential for malignancy in light-exposed areas	HPV-5, -8, -9, -12, -14, -15, -17, -19, -20, -21, -22, -23, -24, -25, -36, -37, -38, -47, -49
Butcher's warts	Common warts on hands of butchers and meat handlers	HPV-7

[a]HPV-27, -29, -37, -38, -46, -48, and -49 are also recovered from skin warts.
EV, epidermodysplasia verruciformis.

(From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 66-3.)

Clinical genital tract and mucosal HPV's

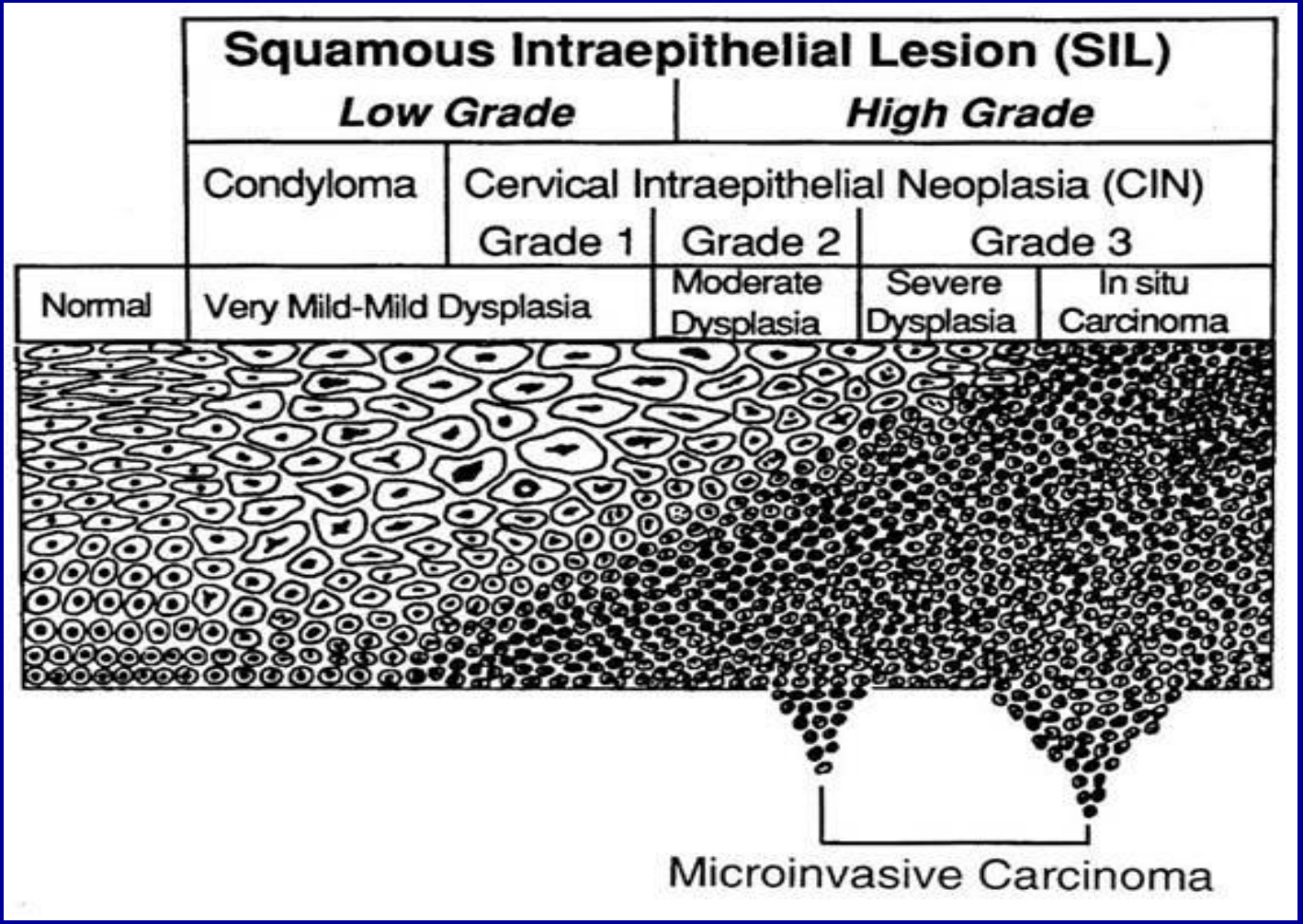
Clinical association	Viral type(s)
Genital tract	
Subclinical infection	All genital HPVs
Exophytic condyloma (any site)	HPV-6, –11
Flat condyloma (especially cervix)	HPV-6, -11, -16, -18, -31, others
Bowenoid papulosis	HPV-16
Giant condyloma (Bushke-Lowenstein tumor)	HPV-6, -11
Cervical cancer	
Strong association	HPV-16, -18, -31, -45
Moderate association	HPV-33, -35, -39, -51, -52, -56, -58, -59, -68
Weak or no association	HPV-6, -11, -26, -42, -43, -44, -53, -54, -55, -62, -66
Vulvar cancer	HPV-16
Penile cancer	HPV-16
Respiratory papillomas	HPV-6, -11
Conjunctival papillomas	HPV-6, -11
Oral cavity	
Focal epithelial hyperplasia	HPV-13, -32
Infection with genital tract HPVs	HPV-6, -11, -16
Lesions on lip	HPV-2

(From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 66-4.)

Transformation

- Malignant transformation appears to be the result of complex series of events that are independent from viral particle production
- Integration of HPV genome seems to be associated with the progression from neoplasia to cancer
- Integration occurs in the majority of invasive cervical carcinomas, but is rare in benign and pre –malignant lesions
- Both episomal and integrated HPV genomes may exist in cell

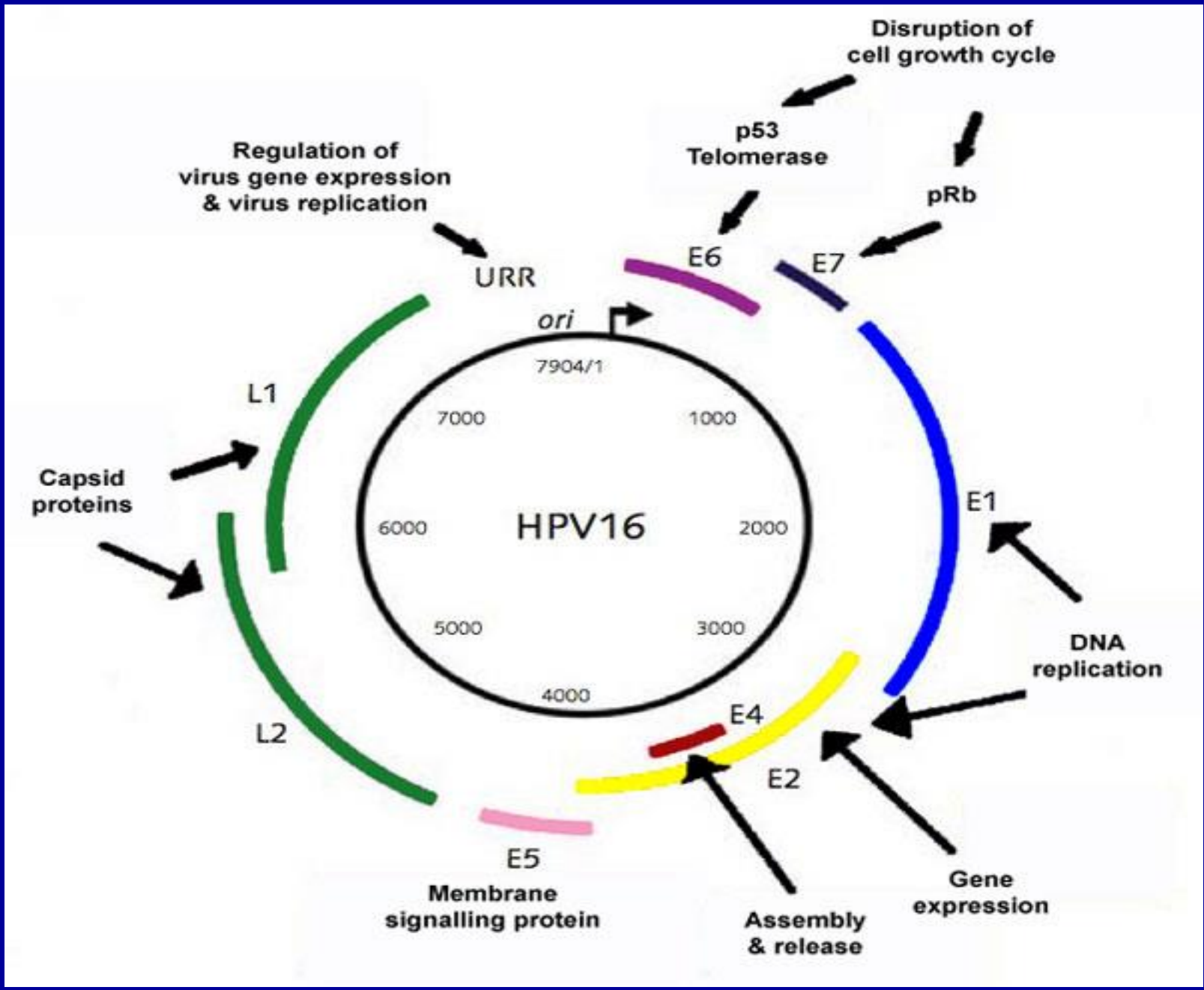
Cervical dysplasia



Cervical squamous carcinoma precursors. Schematic representation of cervical cancer precursors and the different terminologies that have been used to refer to them. The risk for microinvasion from different states of squamous intraepithelial lesions (cervical intraepithelial neoplasia) is arbitrarily represented and is not necessarily proportional to that illustrated in this scheme. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 66-4.)

- Integration usually occurs so that E2 is deleted. E2 represses the activity of E6 and E7

- E6 and E7 make regulatory proteins that disrupt cellular defense against cancer (p53 and RB.)

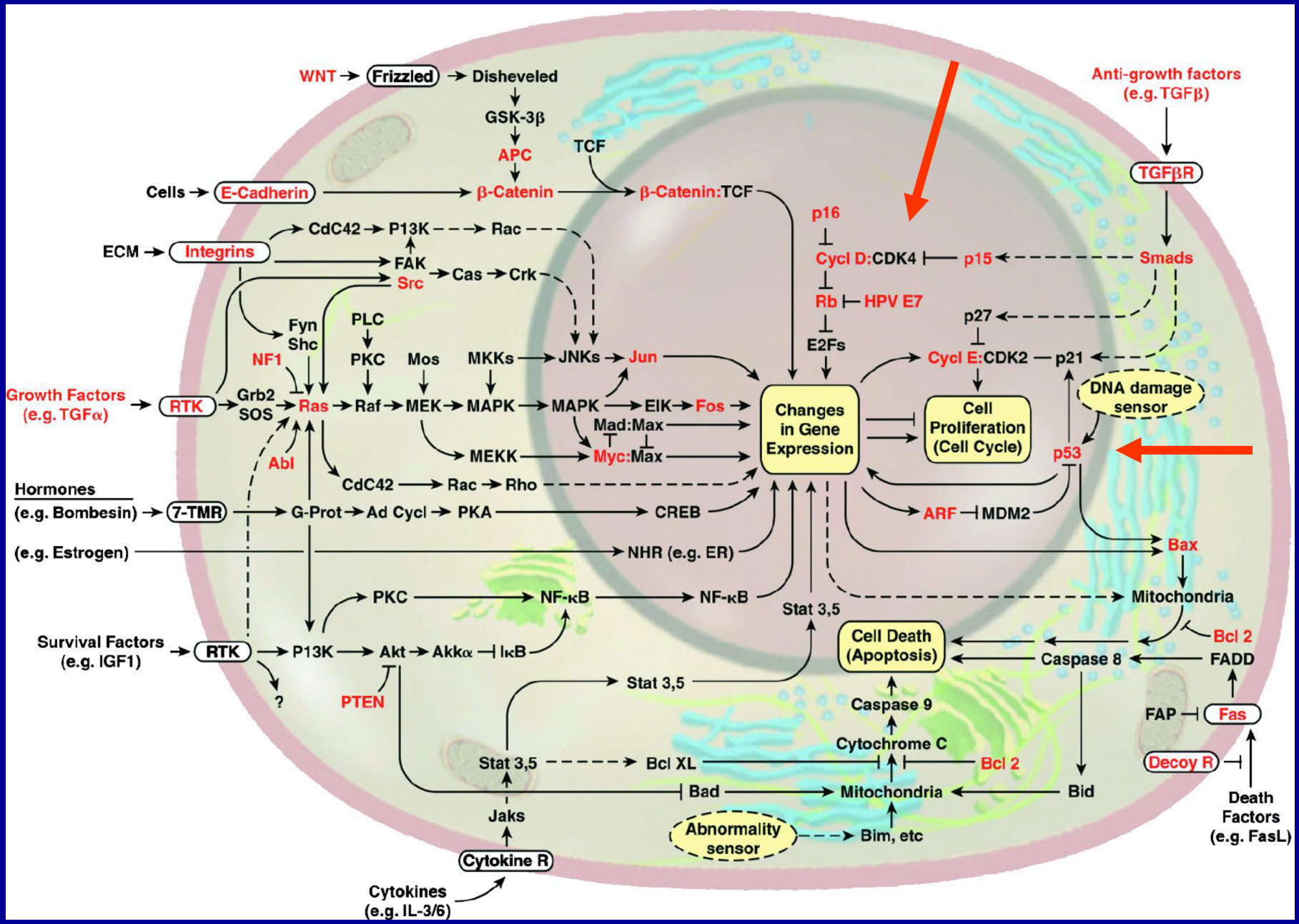

Disruption of
cell growth cycle
p53
Telomerase
pRb
Regulation of
virus gene expression
& virus replication
URR
E6
E7
ori
7904/1
1000
2000
3000
4000
5000
6000
7000
HPV16
L1
L2
Capsid
proteins
E1
DNA
replication
E4
E2
E5
Membrane
signalling protein
Assembly
& release
Gene
expression

- E2 decreases expression of E6/E7. **Loss of E2 is the first stage in transformation** (Integration to chromosome begins)

- **P53** is made by a tumor suppressor gene. It stops reproduction of a cell if there is genetic damage. E6 binds to p53 via a cellular protein ("p100") and targets it for degradation.

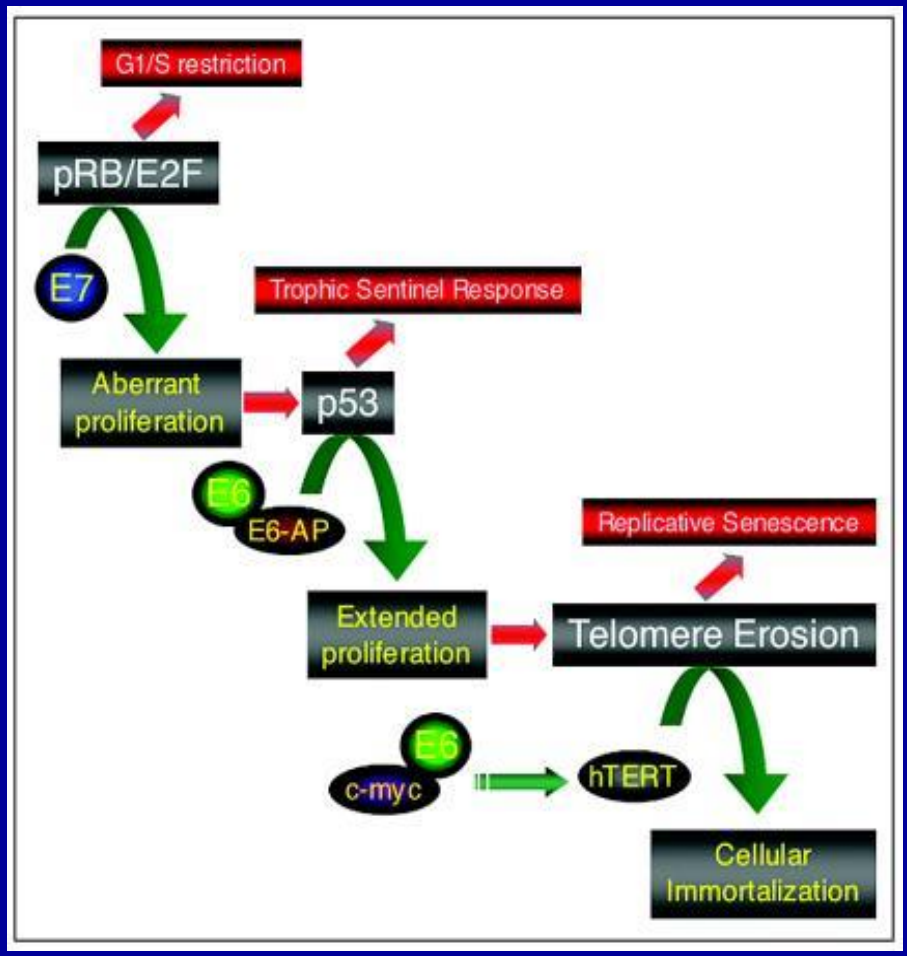
- **RB (retinoblastoma)** usually stops excess cellular reproduction. E7 binds and inactivates the pRB and abrogates the inhibitory effect of RB on cell proliferation:

Contact inhibition is partially controlled by P53 and RB


WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
β-Catenin
β-Catenin:TCF
Cells
E-Cadherin
Anti-growth factors
(e.g. TGFβ)
TGFβR
Smads
p16
Cycl D:CDK4
p15
Rb
HPV E7
E2Fs
p27
Cycl E:CDK2
p21
ECM
Integrins
CdC42
P13K
Rac
FAK
Src
Cas
Crk
Fyn
Shc
PLC
PKC
NF1
Mos
MKKs
JNKs
Jun
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
MAPK
ElK
Fos
Mad:Max
Myc:Max
MEKK
Changes
in Gene
Expression
Cell
Proliferation
(Cell Cycle)
DNA damage
sensor
p53
Abl
CdC42
Rac
Rho
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
ARF
MDM2
(e.g. Estrogen)
NHR (e.g. ER)
Bax
PKC
NF-κB
NF-κB
Stat 3,5
Mitochondria
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
?
PTEN
Cell Death
(Apoptosis)
Bcl 2
Caspase 8
FADD
Stat 3,5
Caspase 9
FAP
Fas
Cytochrome C
Stat 3,5
Bcl XL
Bcl 2
Decoy R
Bad
Mitochondria
Bid
Jaks
Abnormality
sensor
Bim, etc
Death
Factors
(e.g. FasL)
Cytokine R
Cytokines
(e.g. IL-3/6)

Transformation Mechanism

- Protein products of E6 and E7 interfere with the normal function of tumor suppressor genes
  - E6
    - interacts with p53, impairing its ability to repress the cell cycle when DNA errors occur
    - Activates cellular telomerase, maintaining telomerase length, and thus preventing apoptosis. Cell immortalized.
  - E7
    - Binds to pRb and activates genes that initiate cell cycle, leading to cell proliferation
  - E5
    - Enhances activity of epidermal growth factor (EGF)



J Virol. 2004 November; 78(21): 11451–11460.
doi: 10.1128/JVI.78.21.11451-11460.2004 **FIG. 3.**

The mammalian cell cycle

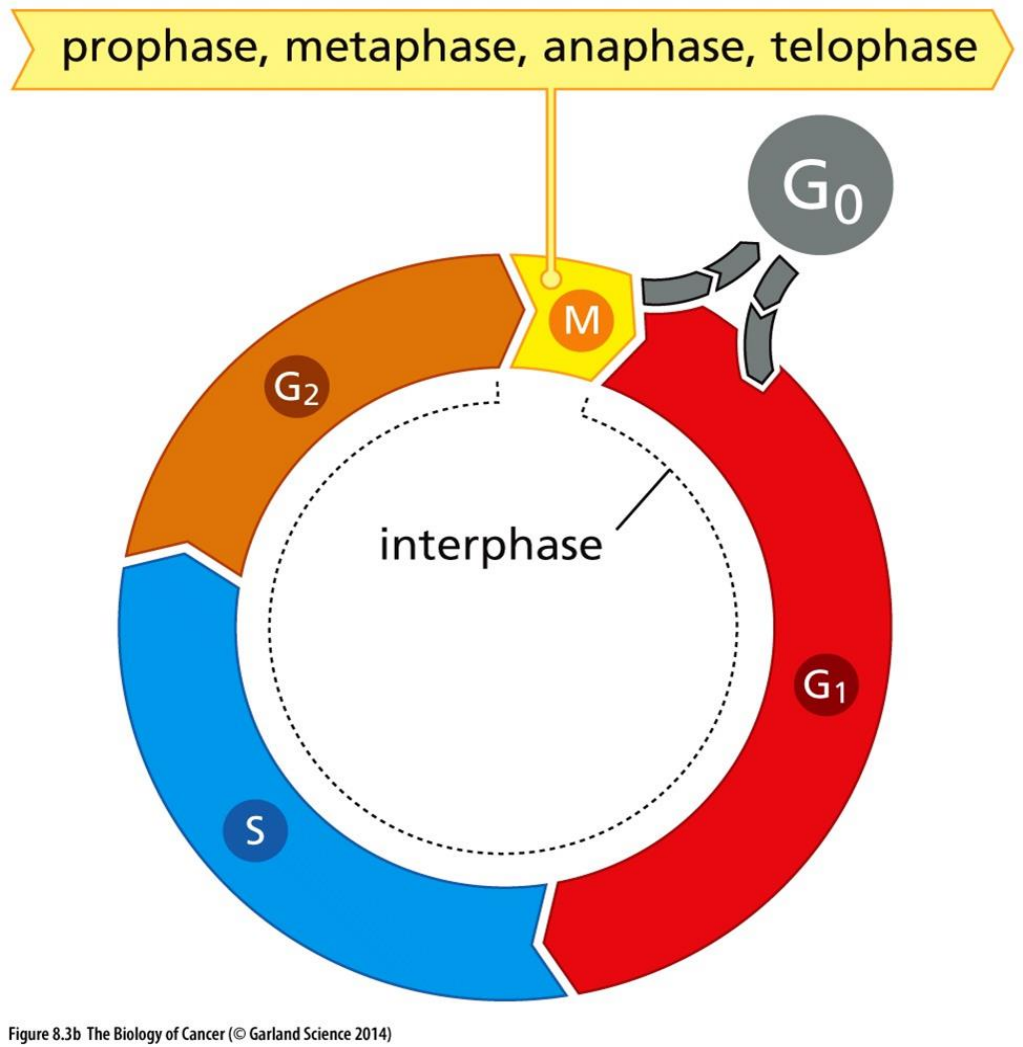


Figure 8.3b The Biology of Cancer (© Garland Science 2014)

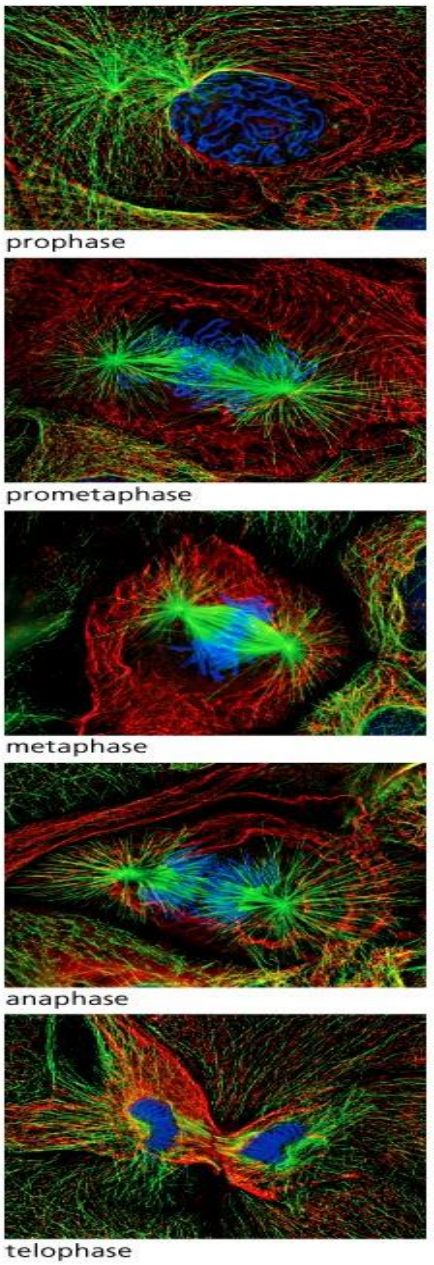


Figure 8.3a The Biology of Cancer (© Ga

Checkpoint controls

Decatenation checkpoint: In S, circular chromosome reproduce. They will looks like 2 links in a chain. Separation of these links is decatenation. Decatenation must be complete in G2 before M stage.

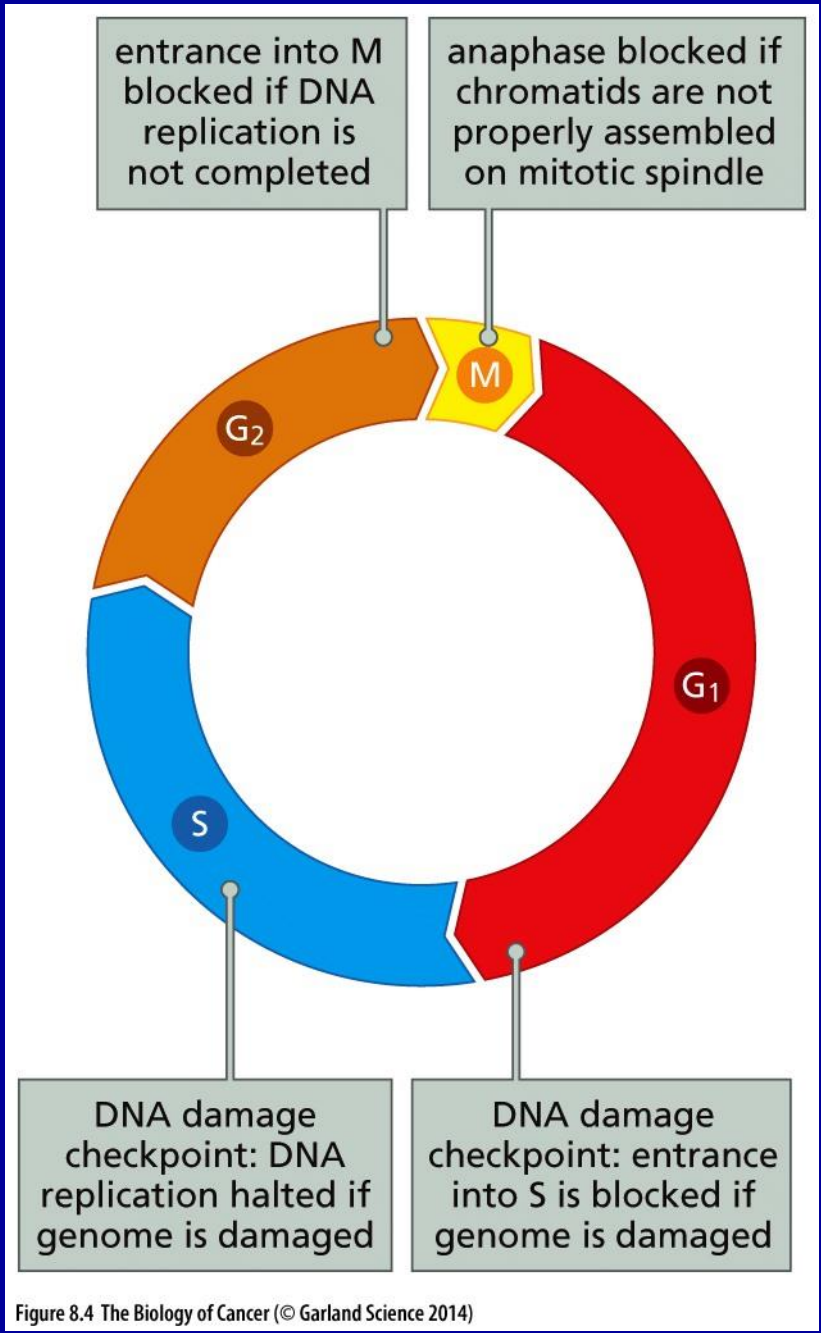


Figure 8.4 The Biology of Cancer (© Garland Science 2014)

Responsiveness to extracellular signals

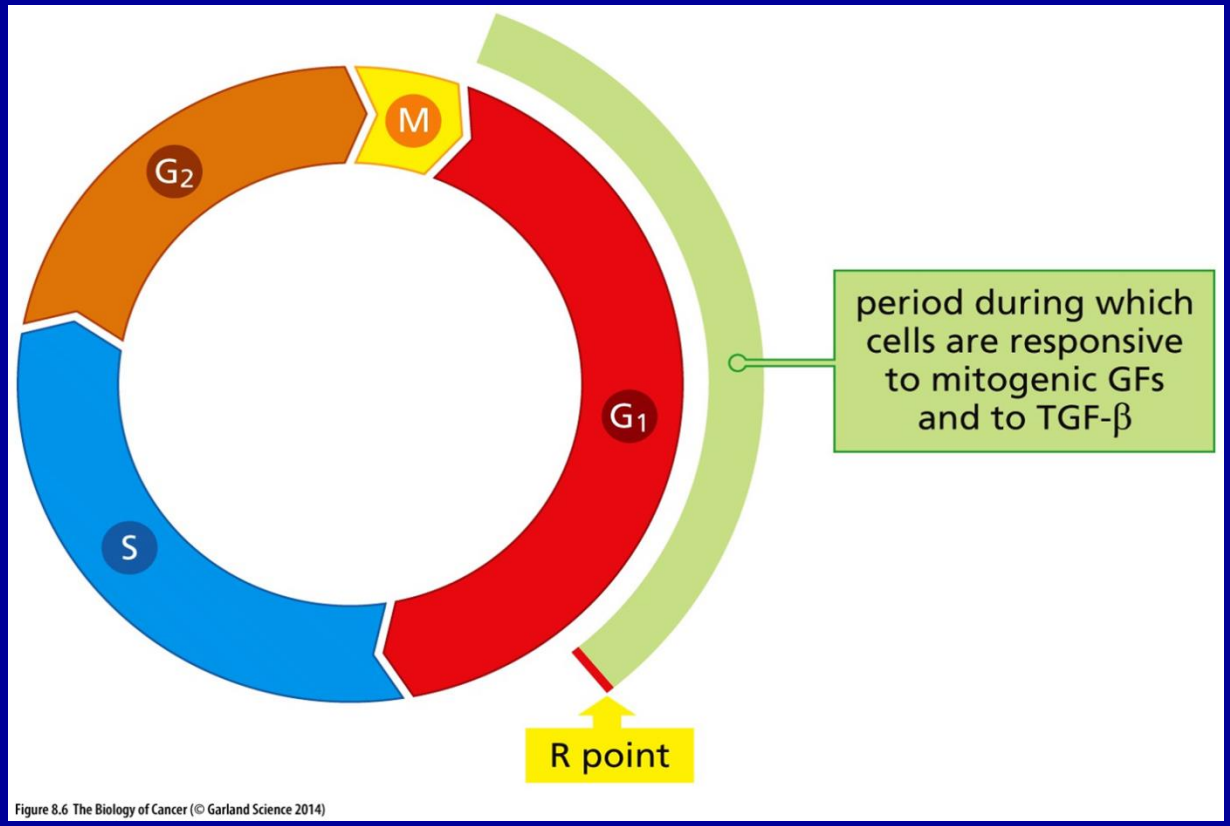


Figure 8.6 The Biology of Cancer (© Garland Science 2014)

- R point = restriction point
- Occur several hours before the G1/S phase transition
- Once crossed, cell commits to division in ordered manner
- Between R point and G1/S transition exits another checkpoint for adequate ECM tethering. If it does not exist integrins mediate the halt in transition or if completely absent drives the cell into apoptosis

Cyclin/CDK (cyclin-dependent kinase) pairing and cell cycle

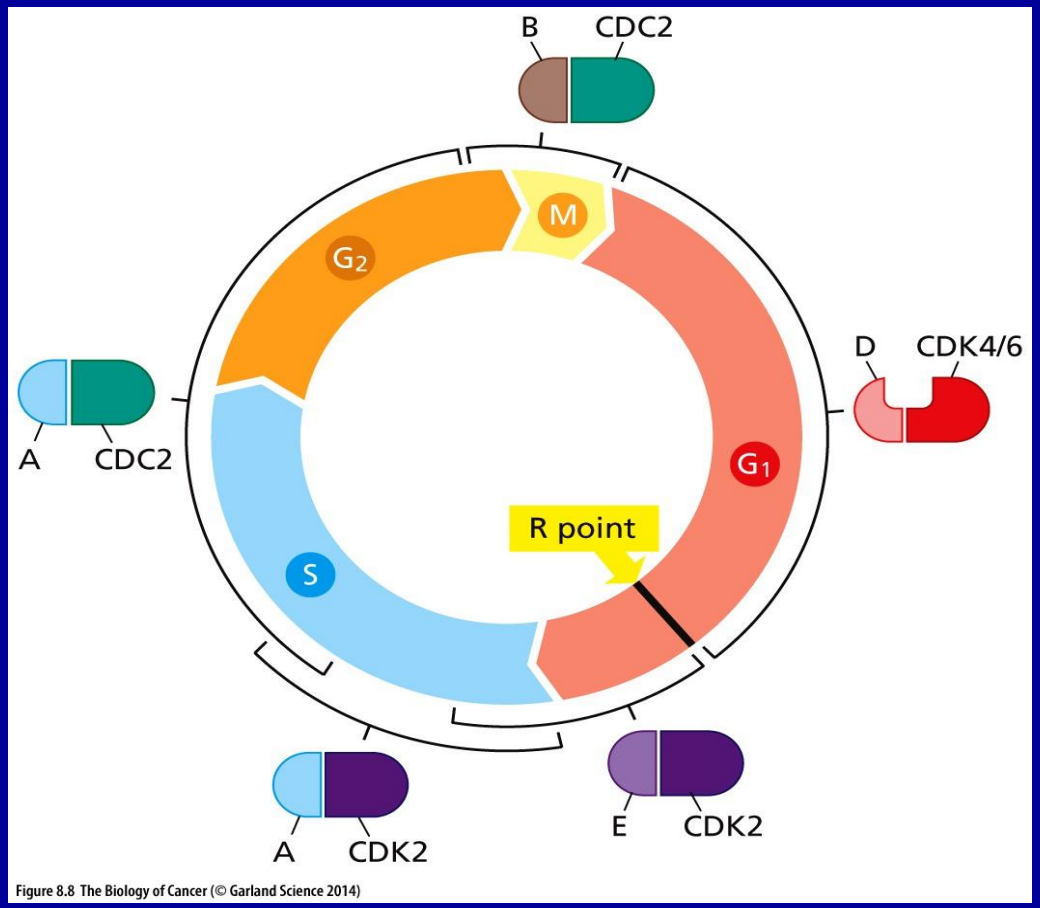


Figure 8.8 The Biology of Cancer (© Garland Science 2014)

- Cyclins are protein signals that bind to cdk molecules at different parts of the cell cycle. They make sure everything is occurring in the right order and the right processes are being done.

Control of cyclin levels

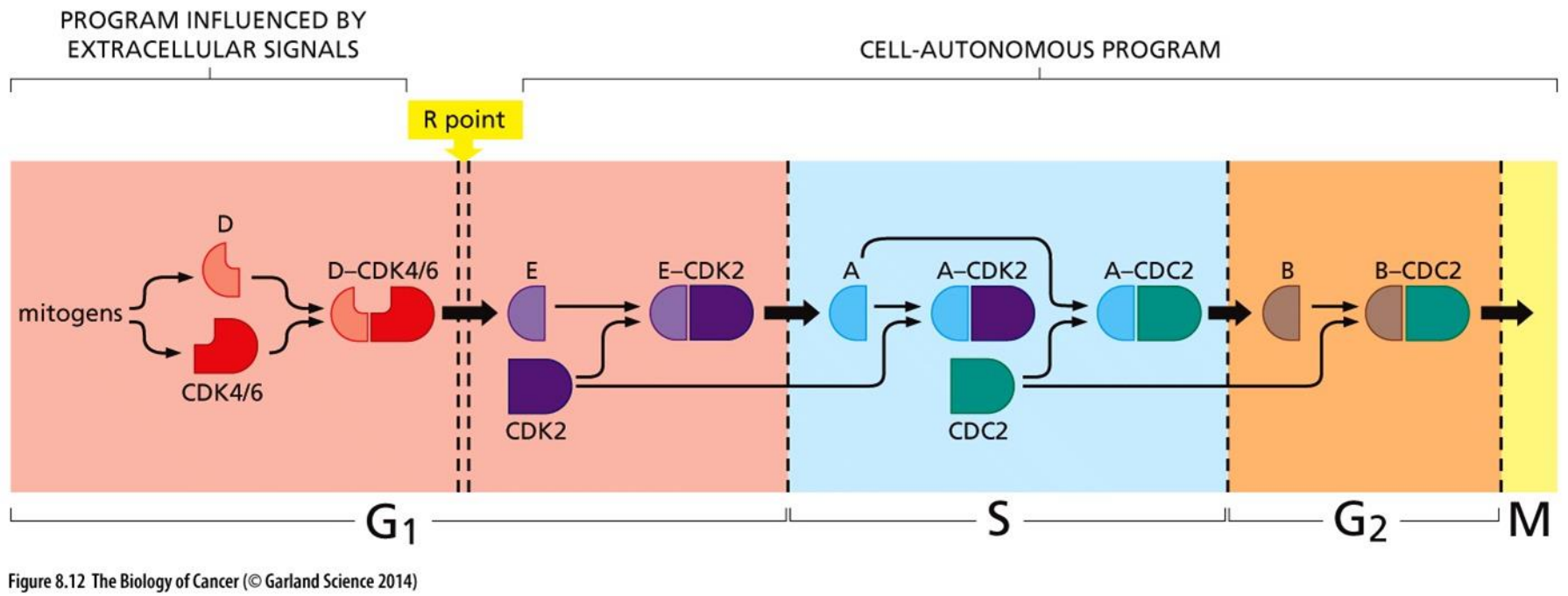
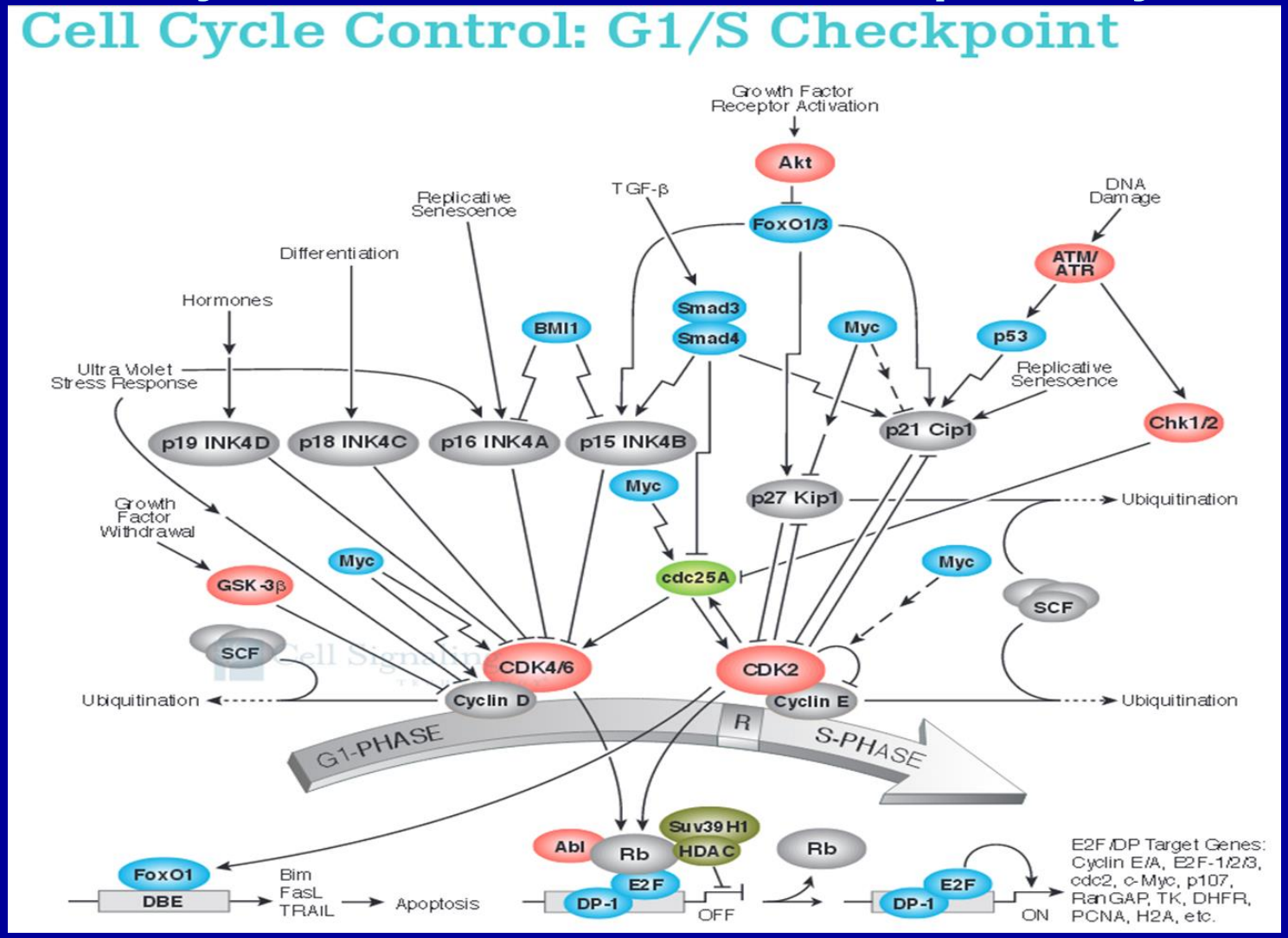


Figure 8.12 The Biology of Cancer (© Garland Science 2014)

- **Cell-autonomous program tightly regulated independently from extracellular signals**
- **Partly, coordination achieved because cyclin/CDK complex from one phase activates those in following phase**
- **Also, cyclin/CDK complexes in a later phases are able to suppress the activities of earlier complexes in earlier phases**

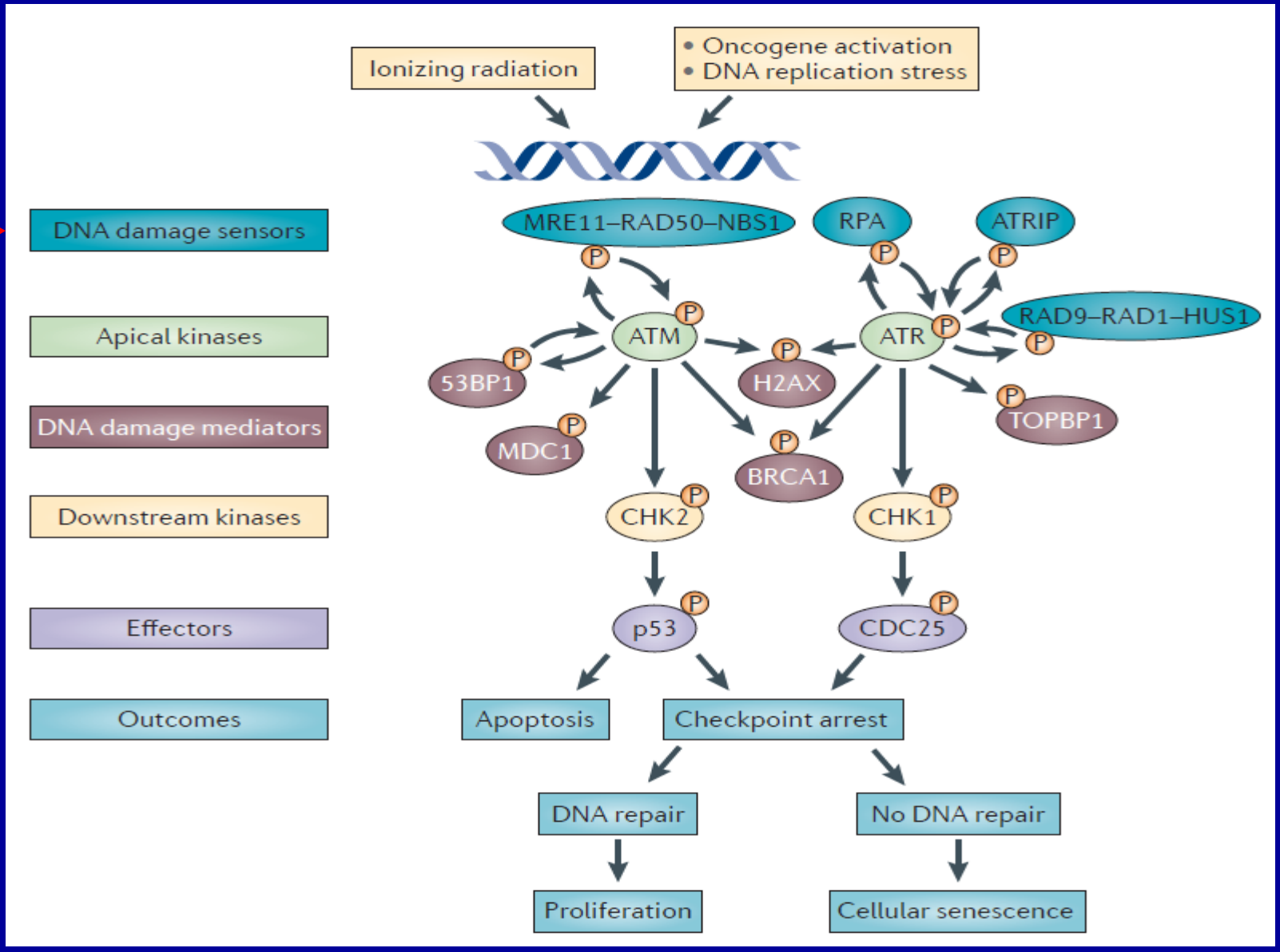
Cyclin/CDK/Retinoblastoma pathway



In brief, if a cell reproduces too much, there will be too much cyclin activity which will free up too many E2f's and they will start to accumulate. This triggers retinoblastoma to halt reproduction.

p53 – Tumor suppressor gene

- If p53 receives information of metabolic disorder/genetic damage it can arrest cell cycle.
- It will attempt to correct the damage.
- If the damage is too severe it may force the cell into apoptosis

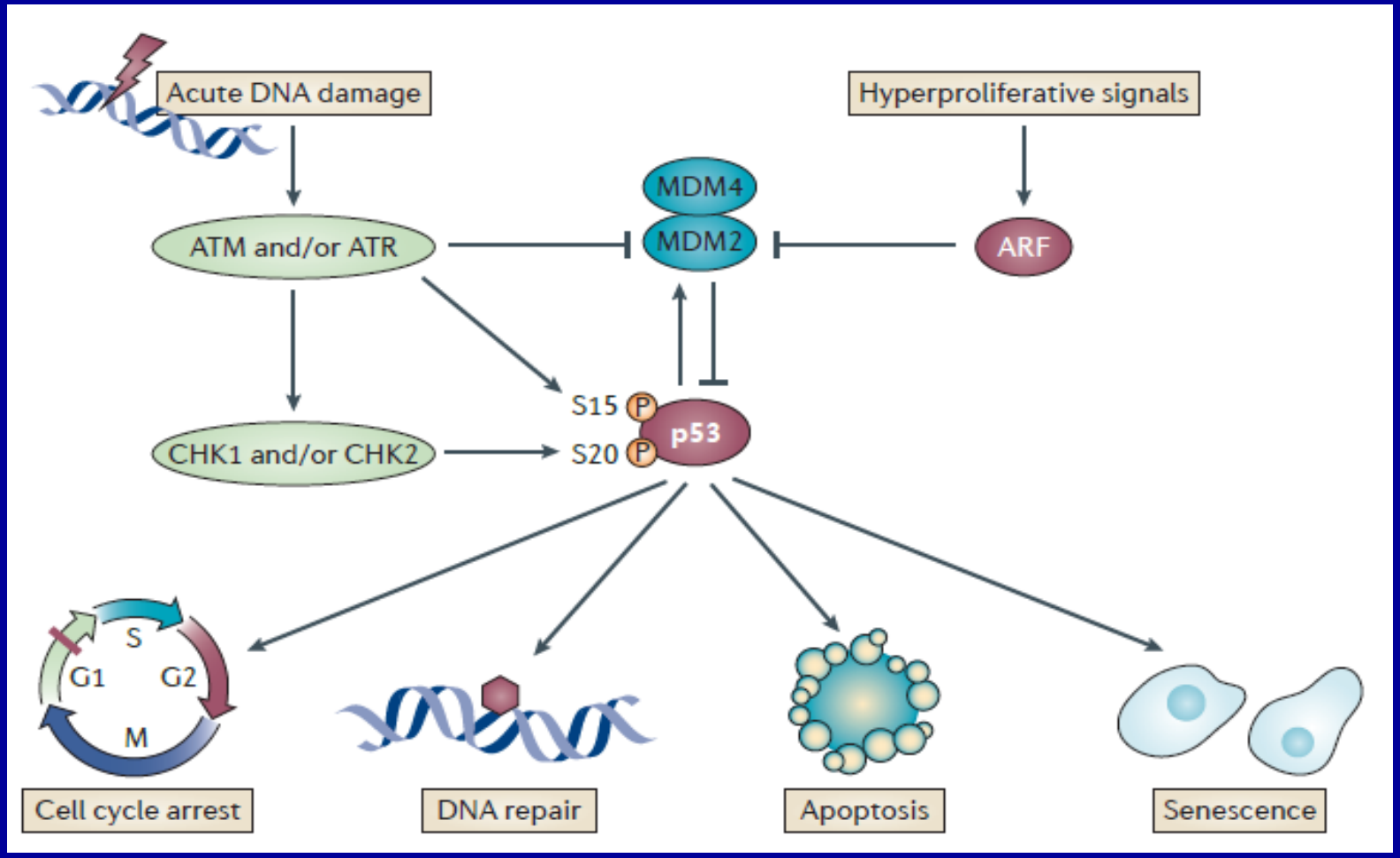


The DNA damage response (DDR) pathway is composed of two main DNA damage sensors: MRN complex that detects DNA double-strand breaks (DSBs); and replication protein A (RPA) complex that detects exposed regions of single-stranded DNA.

DNA damage causes p53 activation

- If all is well p53 is destroyed
- Single strand DNA sensors (stalled replication fork) or dsDNA breaks phosphorylate p53 (protects it)
- Aberrant growth signals activate p53

“Classical” TP53 Pathway

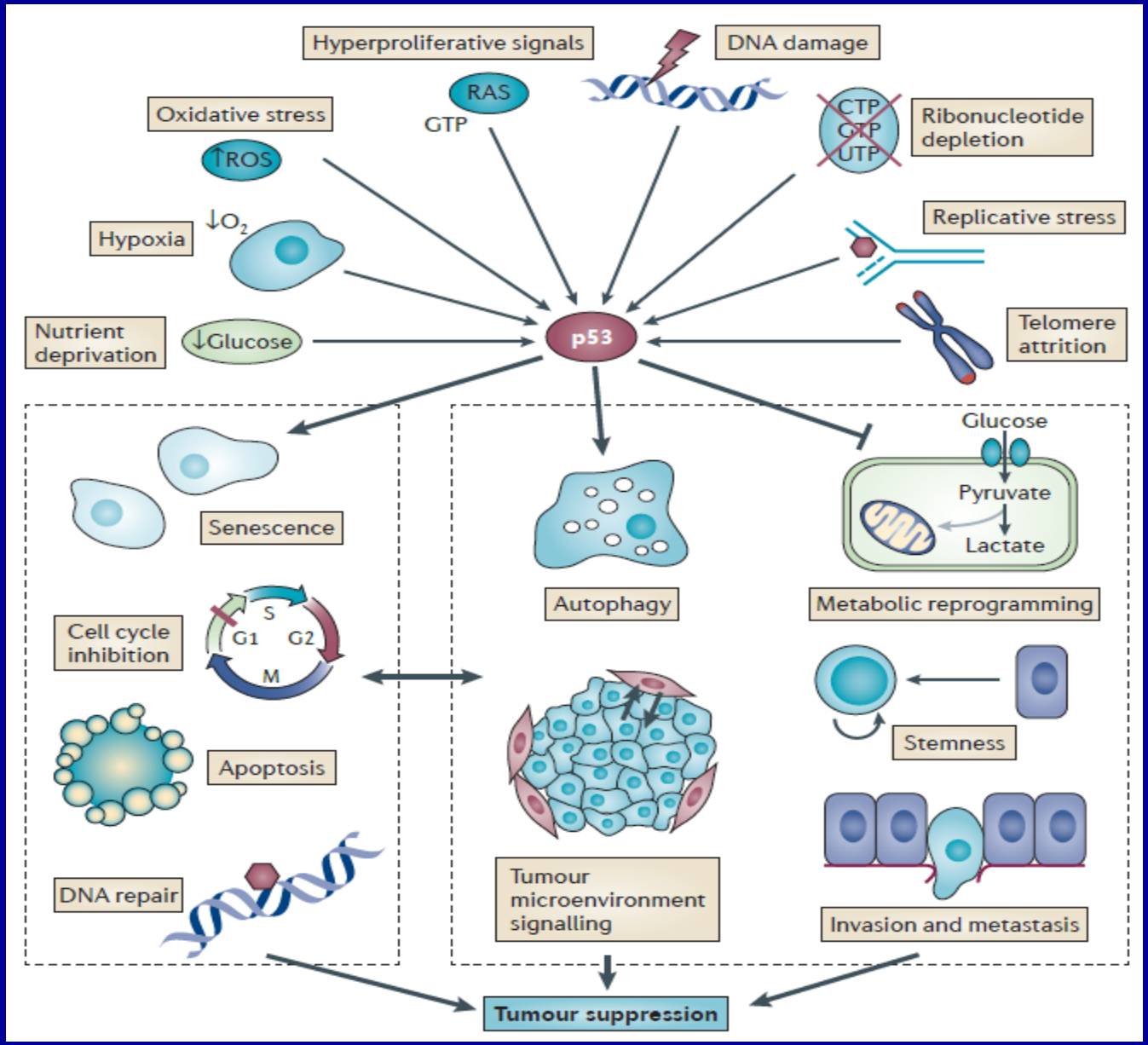


MDM2 and MDM4 inhibits p53 function by binding to it
If there is damage or too much proliferation ARF deactivates MDM2 and P53 will stop proliferation of the cell.

ARF and p53-mediated apoptosis protect against cancer by monitoring intracellular signaling

- Cell monitors the activity level of E2Fs as an indicator of whether its pRb circuitry is functional.
- Excessive high levels of E2Fs provide information that something is wrong with pRb signaling – pRB not working for some reason.
- Runaway E2Fs drives expression of multiple genes including p73 (cousin of p53) which drives the apoptotic program
- Whenever E2Fs deregulation occurs cells are driven to apoptosis
- Whenever pRb circuitry is damaged potentially forming cancer cells, cells are effectively driven into apoptosis by P53.

TP53 Pathway



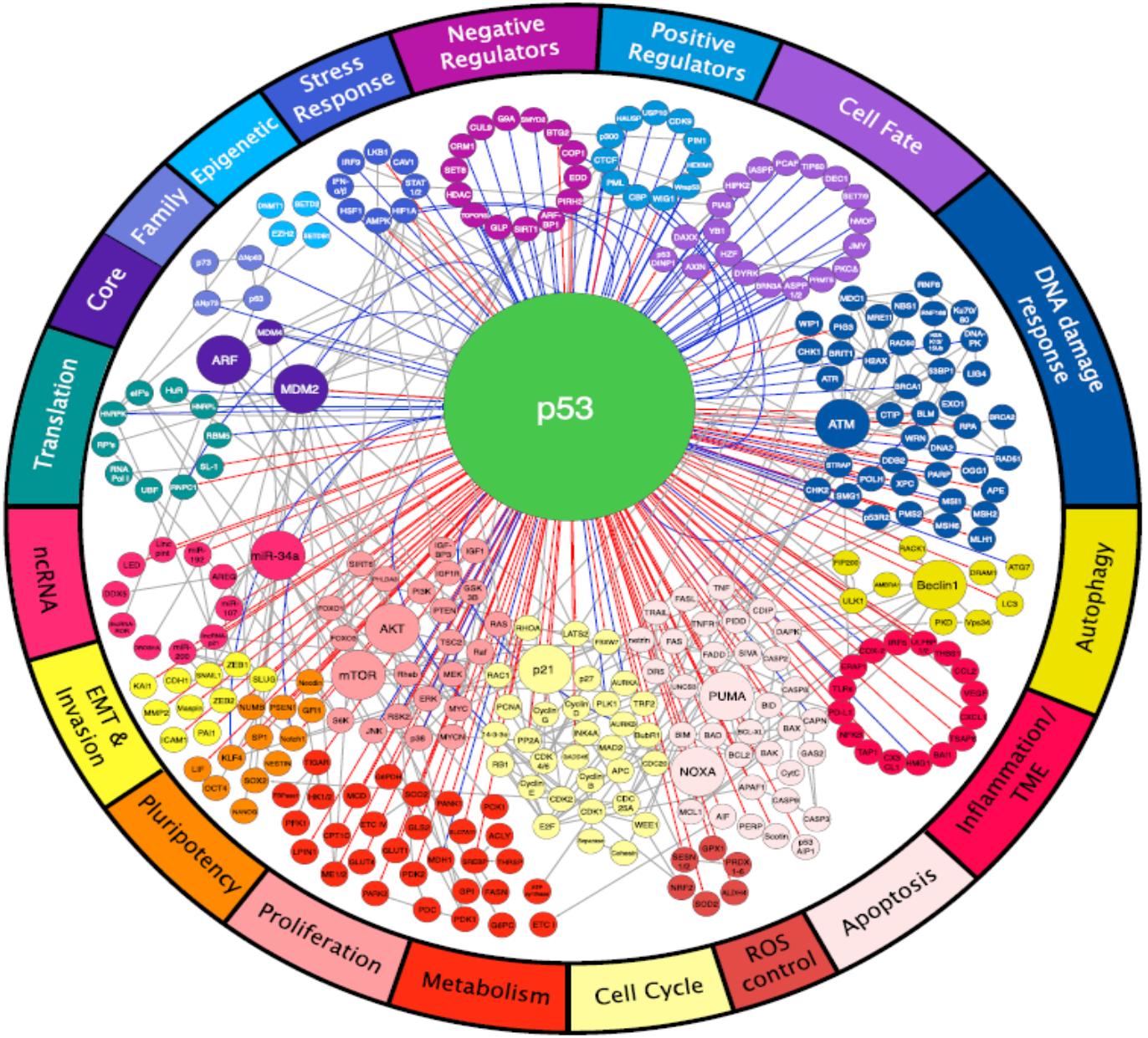
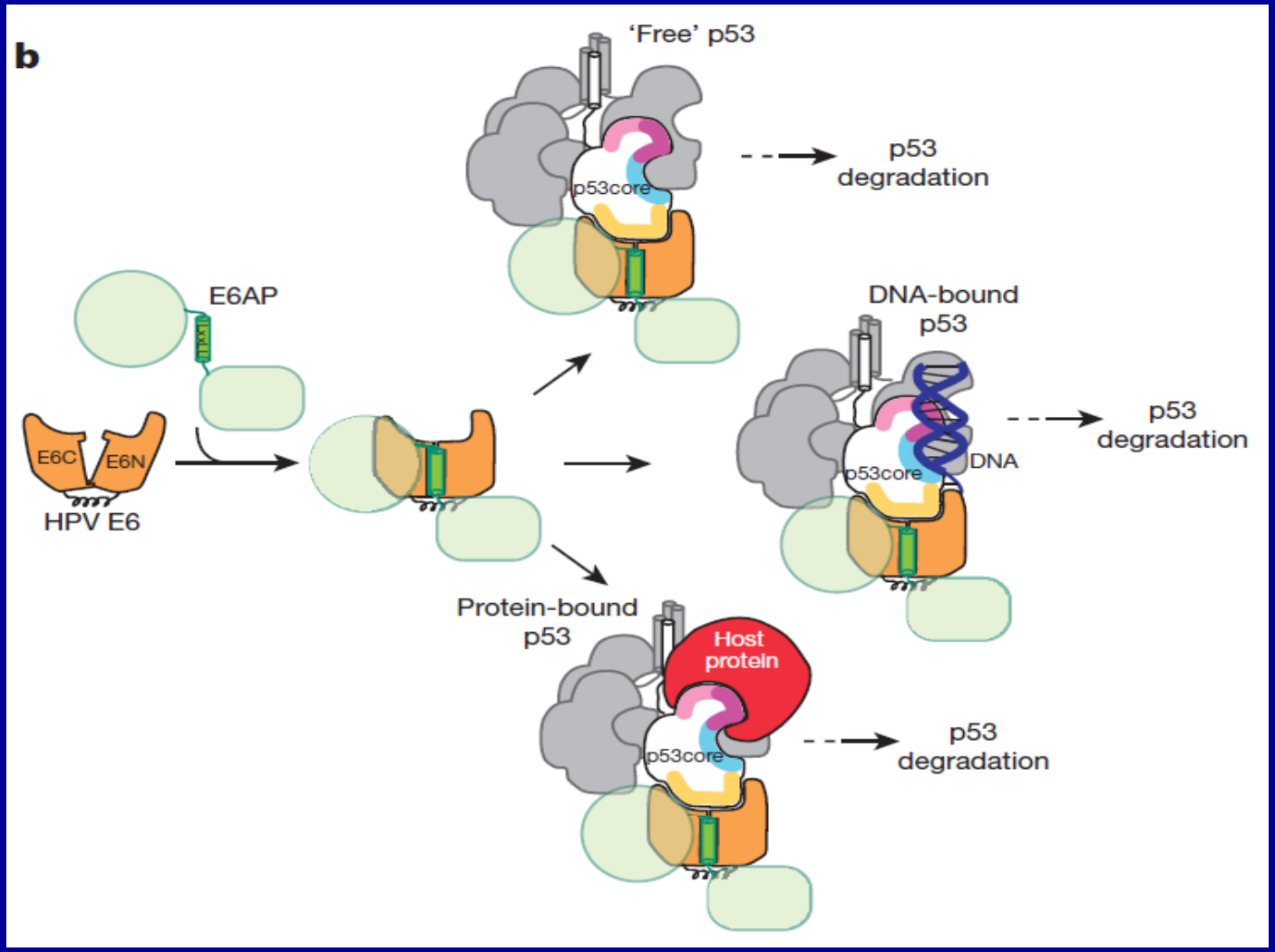


Figure 1. The p53 Network

A wide variety of regulators govern the activity of p53 (top), which, in turn, controls many distinct biological processes (bottom). Each node represents a gene and each line represents an interaction. Direct p53 inputs are indicated as blue lines and direct p53 outputs are indicated as red lines. Noticeably, p53 controls effector processes by activating multiple target genes. Downstream pathways are highly interconnected (gray lines). Interactions are annotated as positive (arrow), negative (T-bar), or modifying (solid circle).



The p53 pro-apoptotic tumor suppressor is mutated or functionally altered in most cancers, including HPV cervical carcinoma and a growing number of head-and-neck cancers.

E6 binds to and degrades P53 in all 3 of it's forms; free floating, protein bound or DNA bound.

Oncogenesis

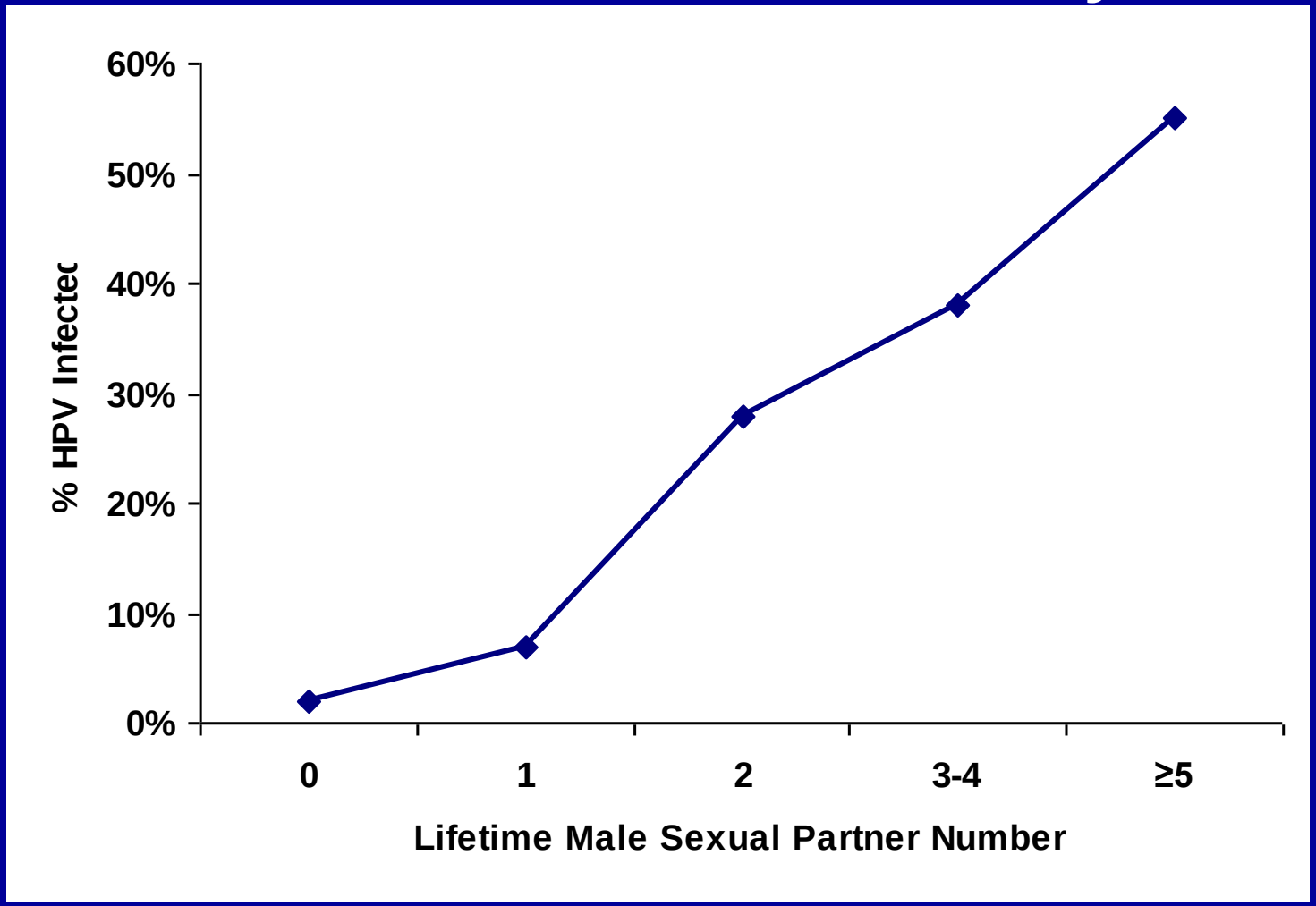
- To recap:
- Without retinoblastoma the cell undergoes uncontrolled proliferation.
- Without P53 the cells can reproduce even though they have genetic damage.
- With telomerase the cells are immortal.

TRANSMISSION

- Close personal contact is assumed to be important for the transmission of most cutaneous warts
  - Minor trauma at the site of inoculation may be required (butcher's warts)

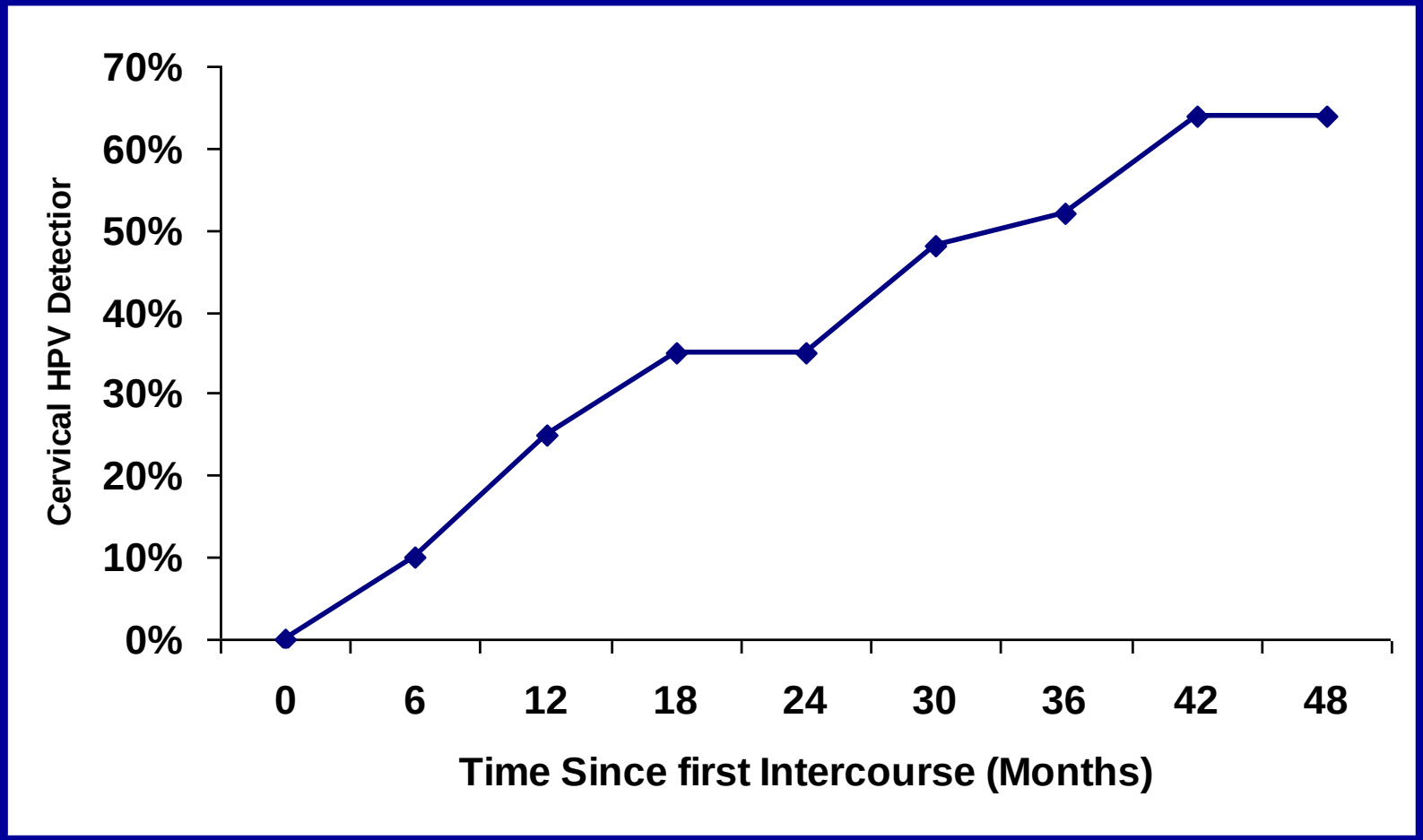
- Anogenital warts are sexually transmitted
  - Only certain types associated with lesions in this region
  - These types are rarely found in other sites
  - Having a large number of sexual partners is associated with greater risk

Incidence of HPV Infection is Strongly Tied to Sexual Activity



Burk RD *J Inf Dis* 1996; 174:679

Incidence of Cervical HPV Detection in Women from the Time of Onset of their First Sexual Relationship



Collins et al. *Brit J Obstet Gynecol* 2002; 109:96-98

- Recurrent respiratory papillomatosis
  - In young children – acquire by passage through an infected birth canal
  - Median age of onset ~3 years
  - Often upper respiratory tract
    - Sometimes need to be removed surgically
  - Adult-onset respiratory papillomatosis associated with oral sex
    - Less aggressive than in children
    - May undergo malignant transformation

- Nosocomial infections
  - Infectious virus can be recovered from fumes of laser therapy
  - Is resistant to heat
  - Laser surgeons appear to have higher incidence of hand and nasopharyngeal warts
  - Now they wear gowns, gloves and masks and use smoke evacuation equipment.

Epidemiology

- **Although clinical HPV infections are most recognizable – subclinical and asymptomatic (latent) infections are most common.**

- **9 out of ten HPV infections, including genital, resolve within 2 years without complications.**

Genital HPV infections

- Annual incidence of newly infections is 14 million in USA
- Half of those cases are in the 15 -24 year group
- MOST COMMONLY ACQUIRED STD IN USA!
- Overall in the United States, an estimated 79 million persons are infected
- At least 3 quarters of the population may have been infected in their lifetime, but it usually resolves.
- 95% of genital warts are due to HPV 6 and 11 (non cancerous)

Clinical testing

- **HPV cannot be cultured directly from patient specimens**

- **Epidemiologic studies of HPV typically use nucleic acid amplification methods or Polymerase chain reaction (PCR) .**

- **The Food and Drug Administration (FDA) has approved clinical HPV tests for detecting clinically significant levels of any of 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) from cervical specimens.**
- **HPV tests are not recommended or approved for use in men or adolescents, for detection of HPV in partners, or at anatomic sites other than the cervix**
- **Screening is expensive, so it is restricted to the most endangered population where screening is most likely to be effective**

- HPV infections are largely shielded from the host immune response because they are nonlytic and restricted to the epithelium
- Humoral and cellular immune responses have been documented, but correlates of immunity have not been established
- Not all infected persons develop detectable antibody; in one study, 54%–69% of women with incident HPV 6, 16, or 18 infections had type-specific antibody
- Among newly infected men, 4%–36% developed type-specific antibody to one of seven types
- Only 13% developed antibody after infection with HPV 16
- Screening by looking for antibodies is therefore ineffective.

Figure 45. Human Papillomavirus — Prevalence of High-risk and Low-risk Types Among Females Aged 14 – 59 Years, National Health and Nutrition Examination Survey, 2003 – 2006
CDC data

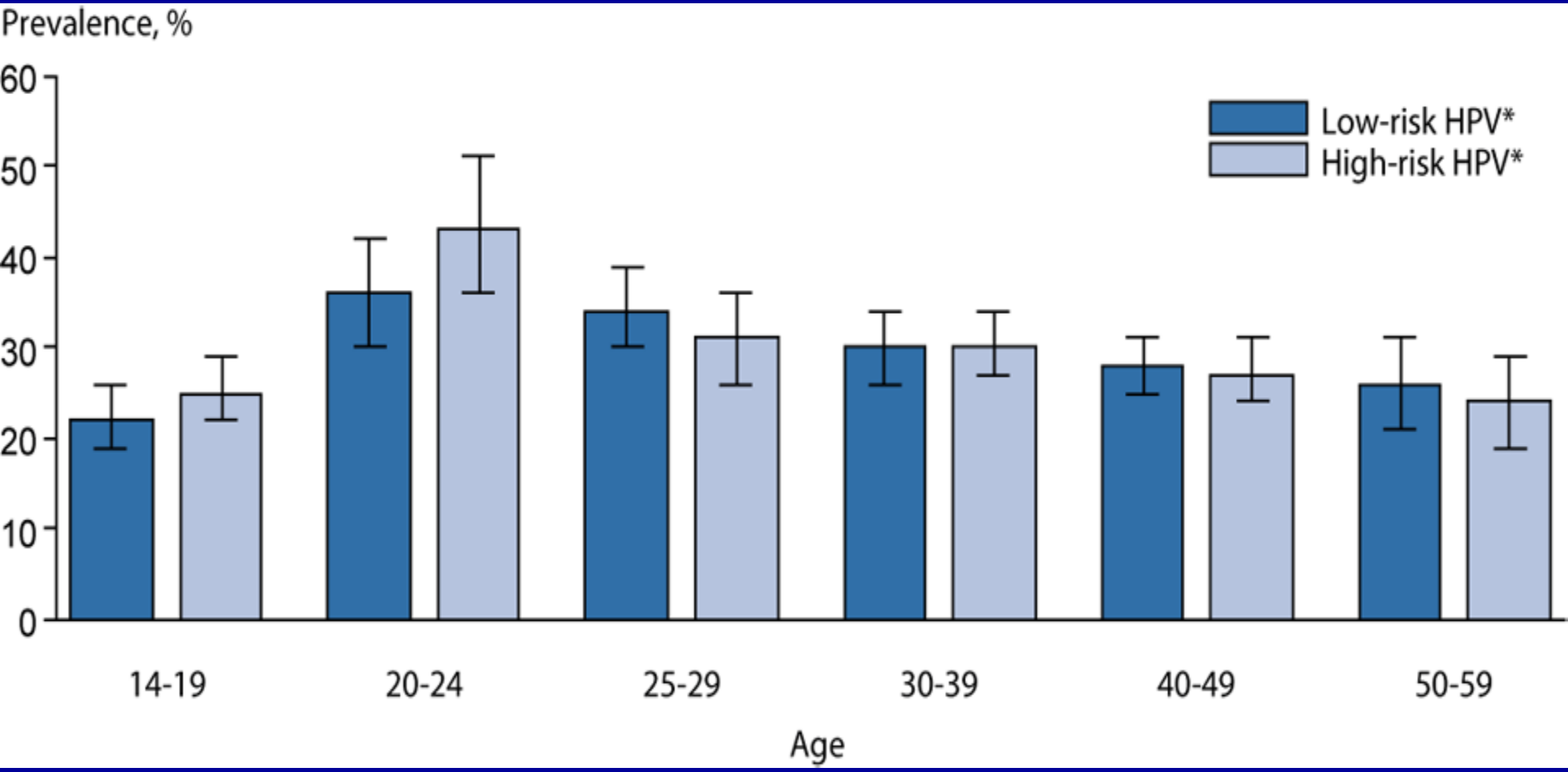
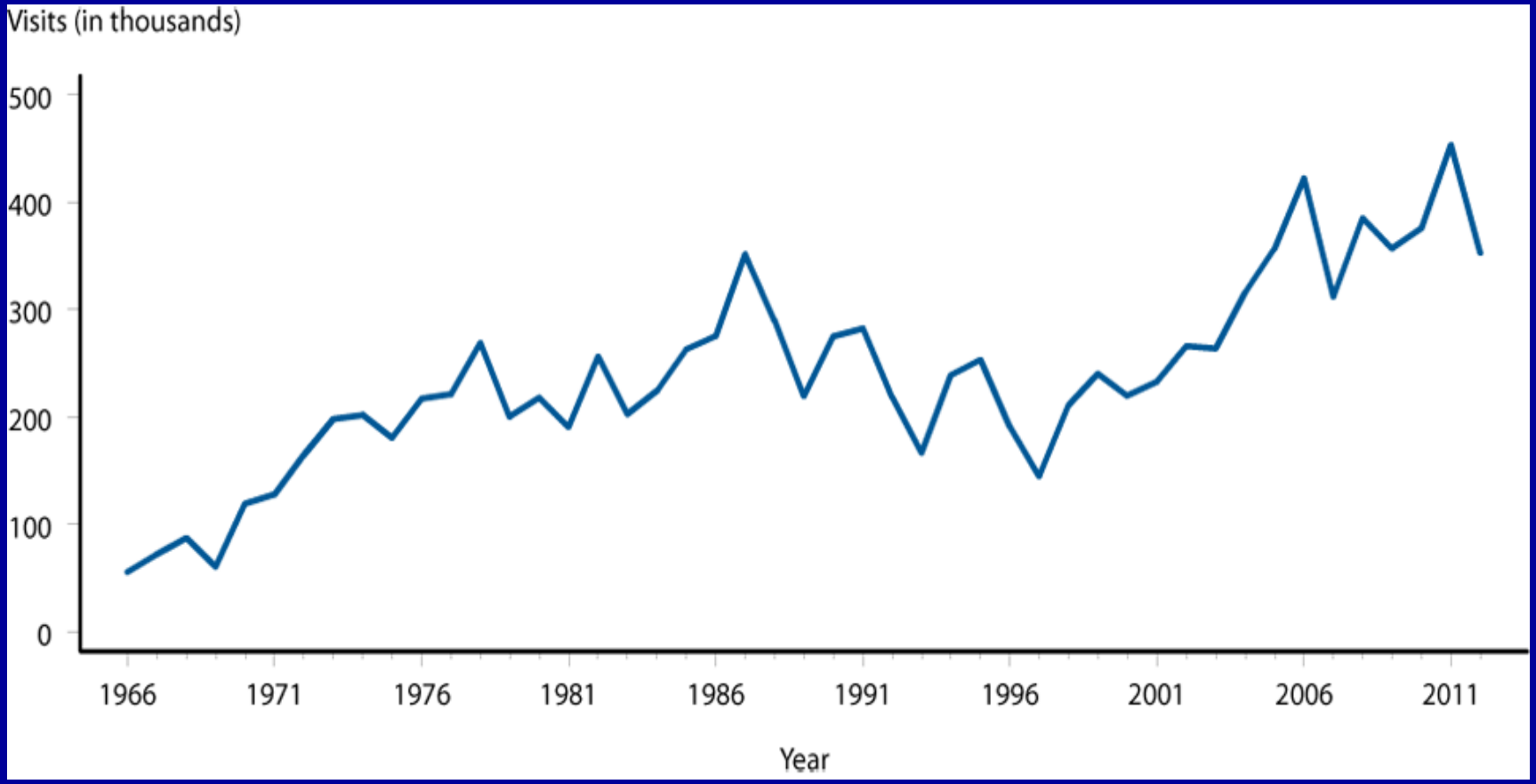


Figure 46. Genital Warts — Initial Visits to Physicians' Offices, United States, 1966 – 2012
CDC data

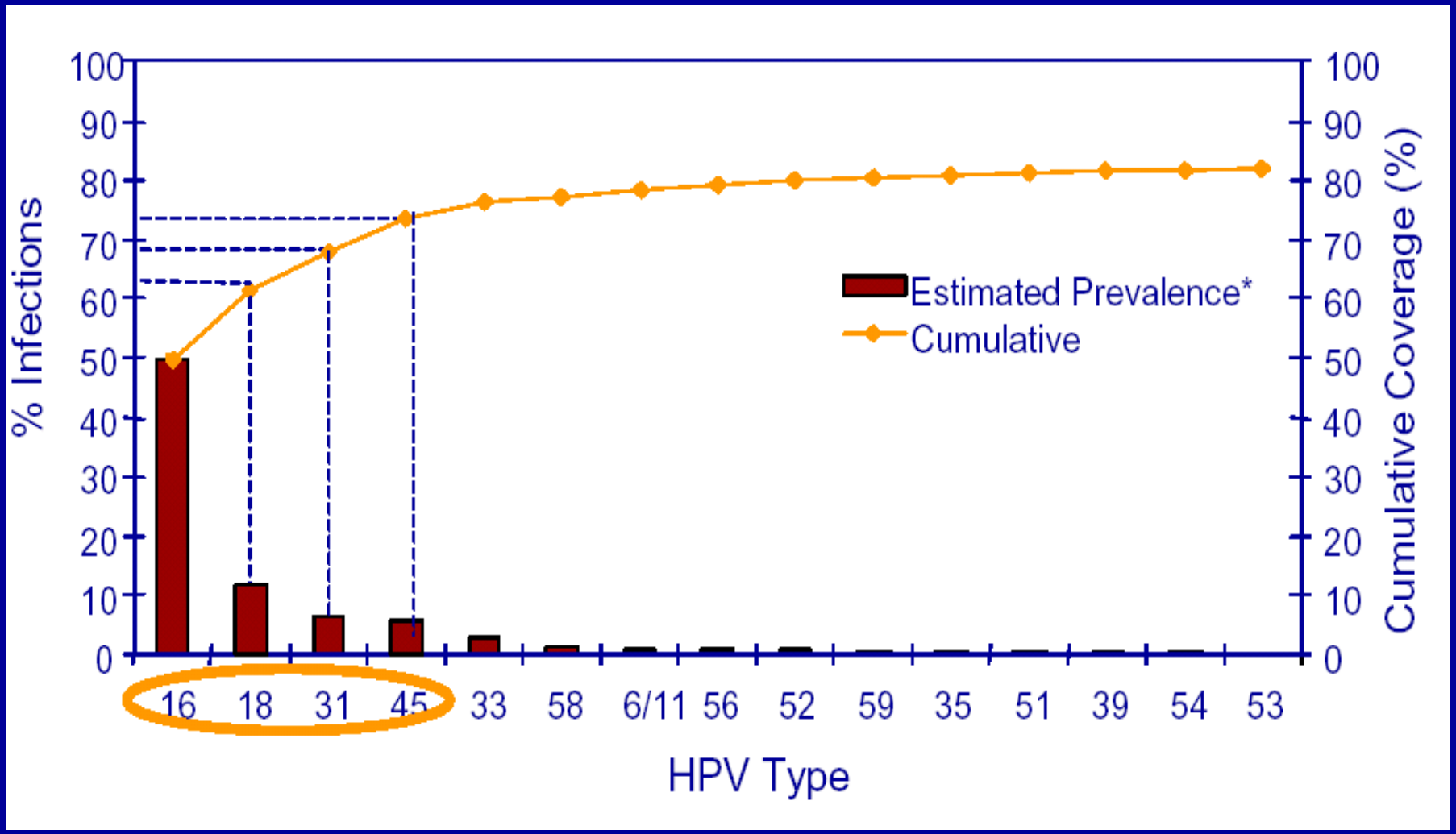


At least 70% of sexually active persons will be infected with genital HPV at some time in their lives.

- **90% of cases resolve by themselves or through treatment**

- HPV infection causes vast majority of cervical cancers (>85%)
- Low prevalence of cervical cancer in Catholic nuns
- Direct association with the number of sexual partners
- Increased risk of having male sexual partner whose previous consort had developed cervical cancer
- Every year in USA ~ 30,000 cases of cervical cancer and 4 000 deaths.
- Types 16 and 18 are the cause of ~70% of the cases, but 16 and 18 don’t always cause cancer.

Prevalence of HPV Types in HPV cervical Infections

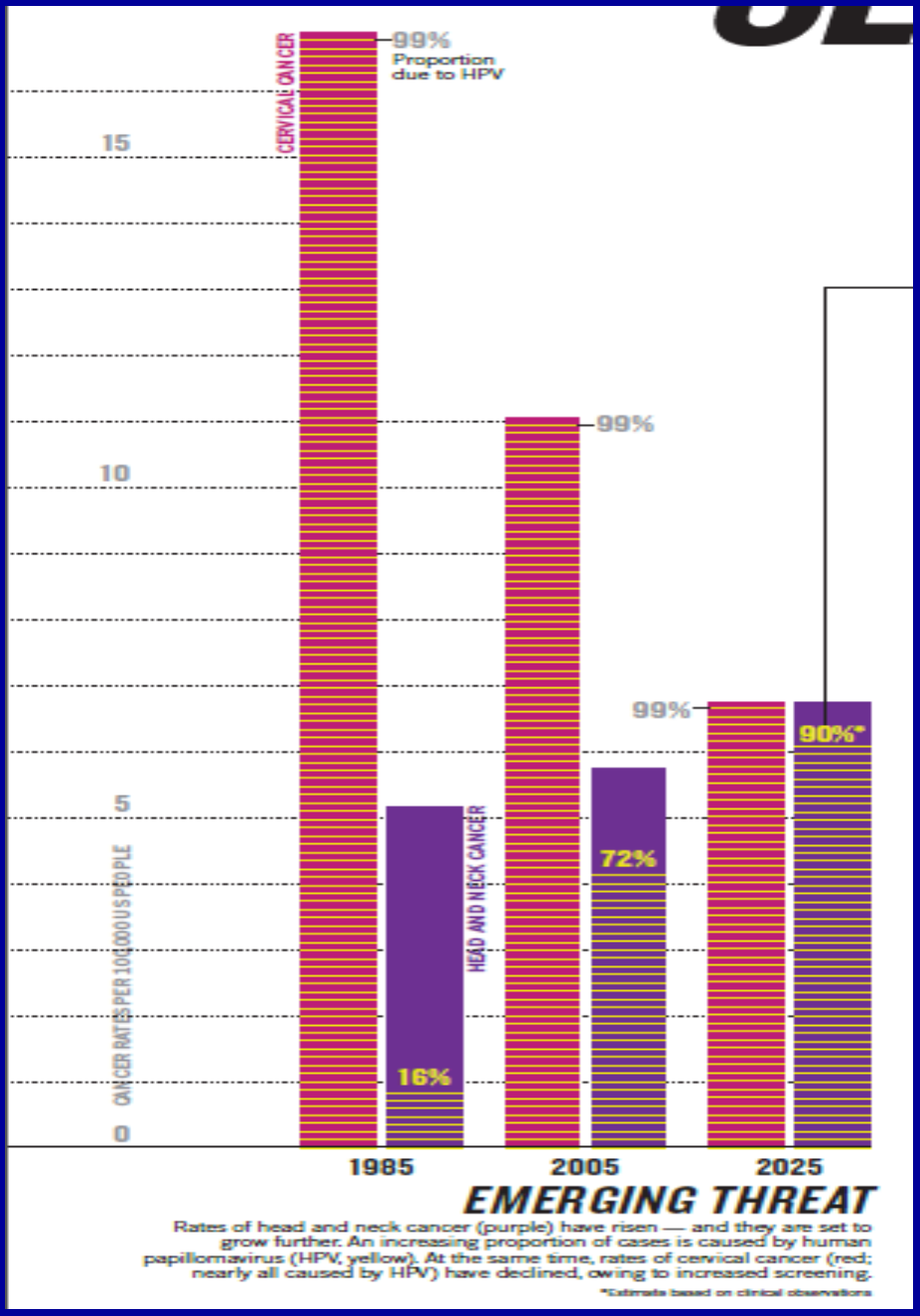


Schiffman, et al. J Nat Canc Inst 1993. 85:958 and Liaw, et al. J Nat Cancer Inst. 1999. 91:954

- **Men who have sex with men (MSM) and persons who have HIV infection are at higher risk for anal precancer and cancer**
-
- **Both long- and short-term trends indicate that invasive anal cancer has increased at a steady rate among both males and females and among persons in almost every racial/ethnic group**

- **A recent U.S. study reported that approximately 72% of oropharyngeal cancers were positive for HPV; 61% had HPV 16.**

-



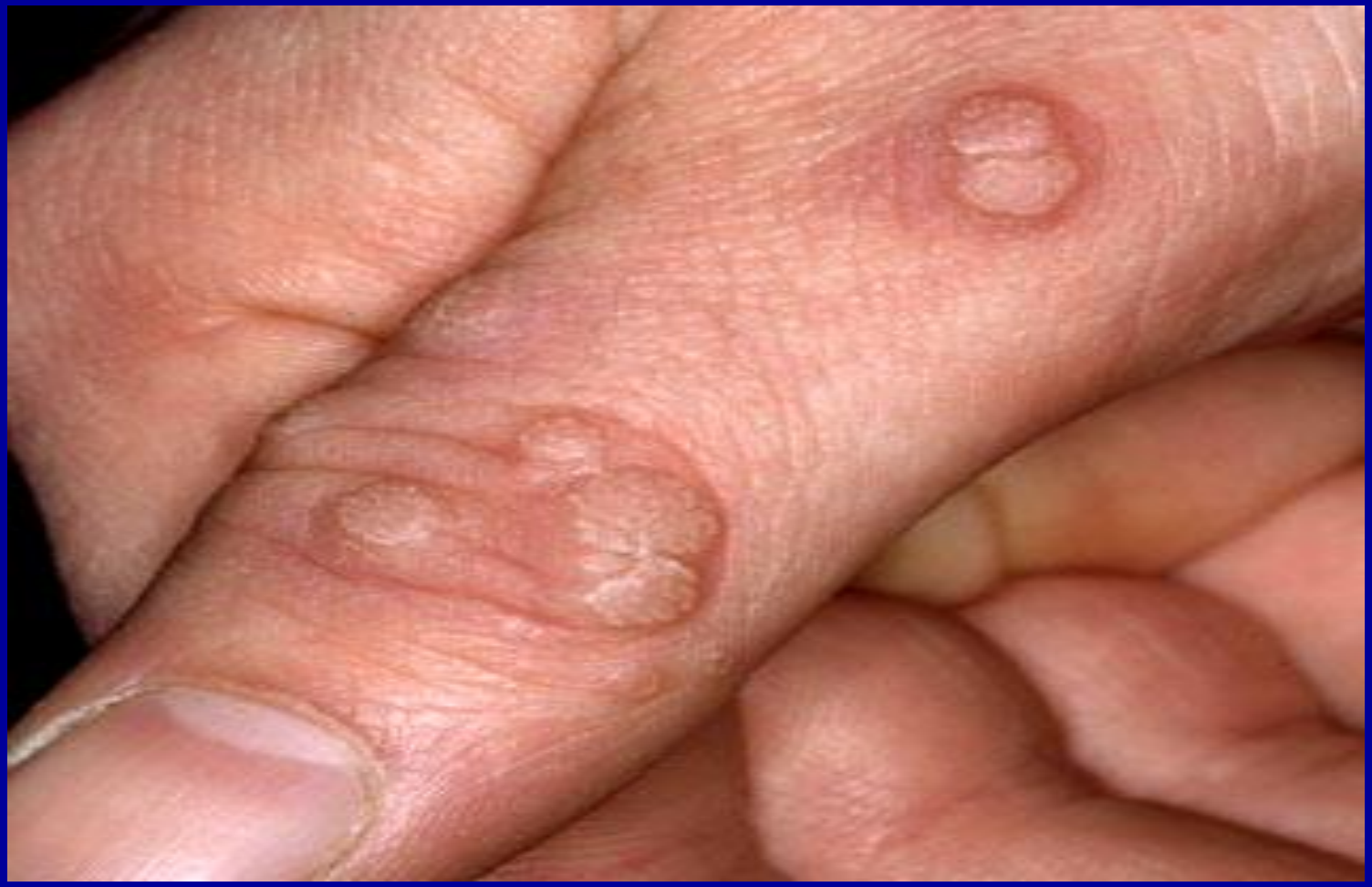
EMERGING THREAT

Rates of head and neck cancer (purple) have risen — and they are set to grow further. An increasing proportion of cases is caused by human papillomavirus (HPV, yellow). At the same time, rates of cervical cancer (red; nearly all caused by HPV) have declined, owing to increased screening.

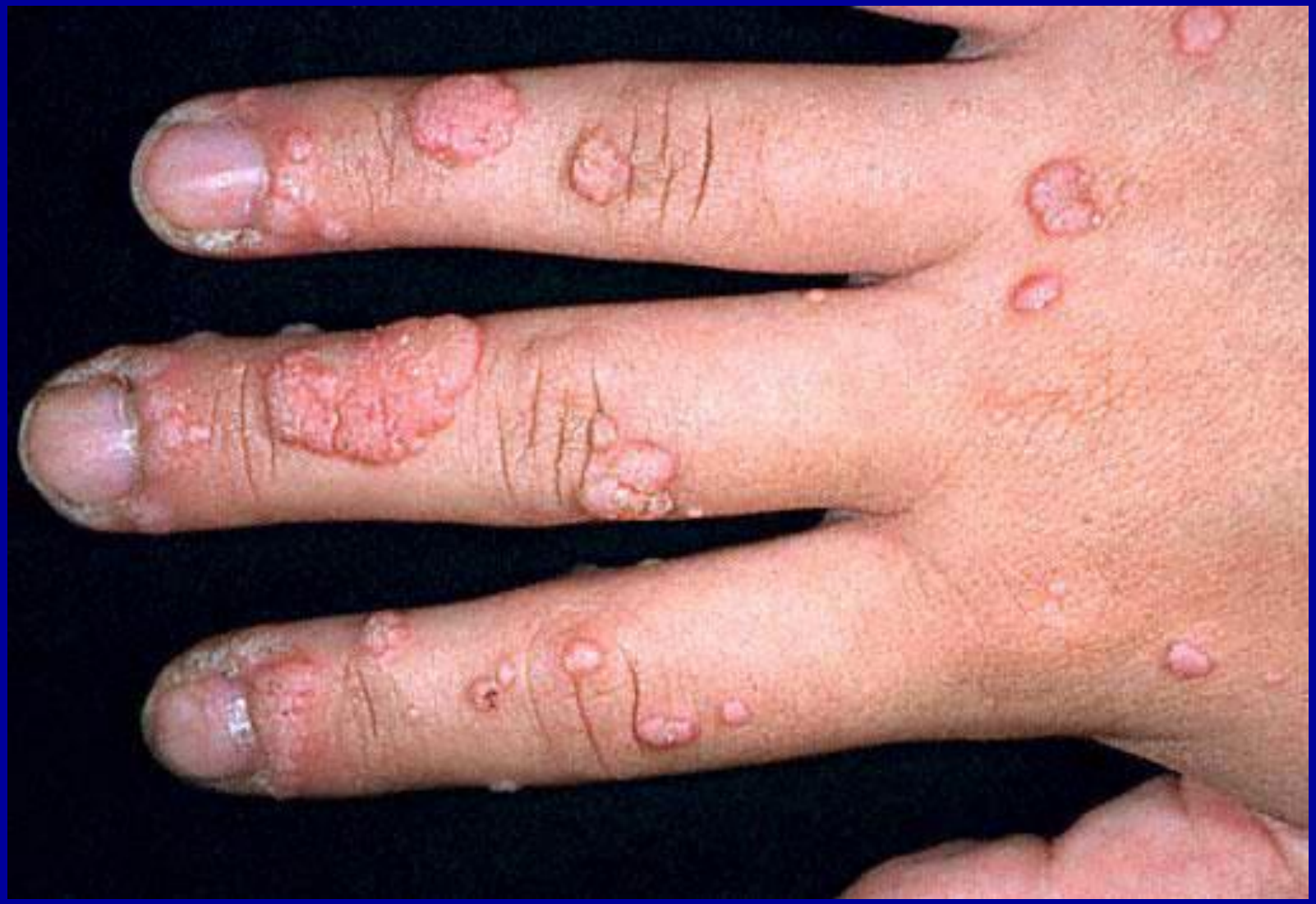
*Estimate based on clinical observations

Clinical pictures

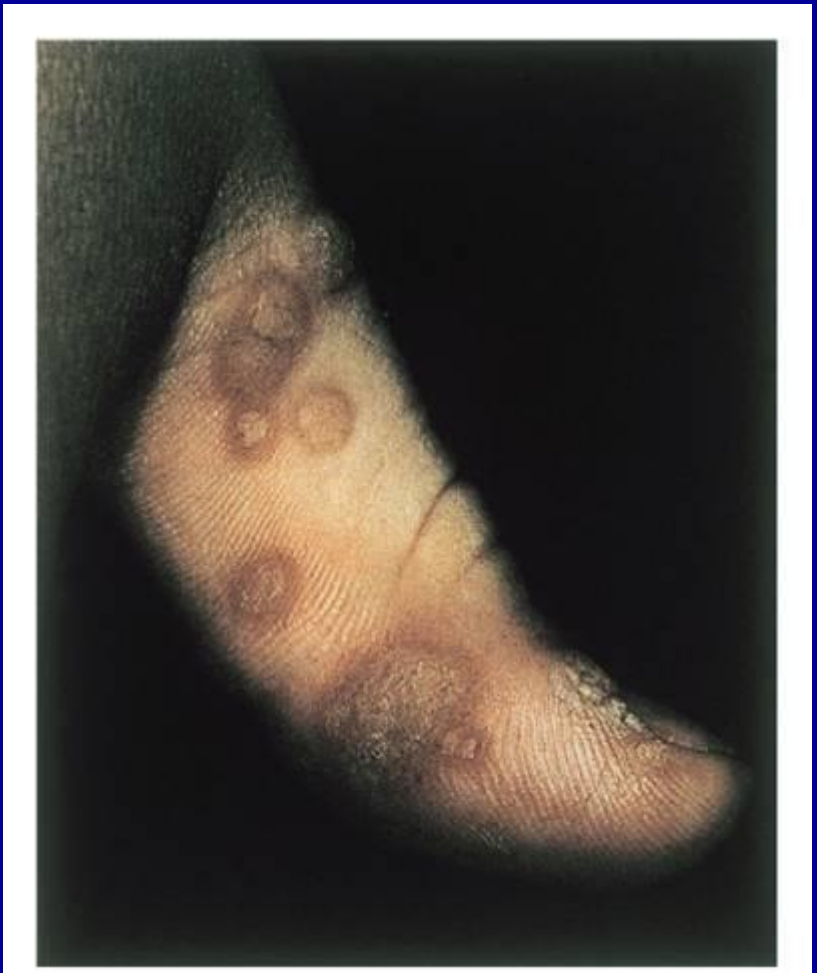
Common warts



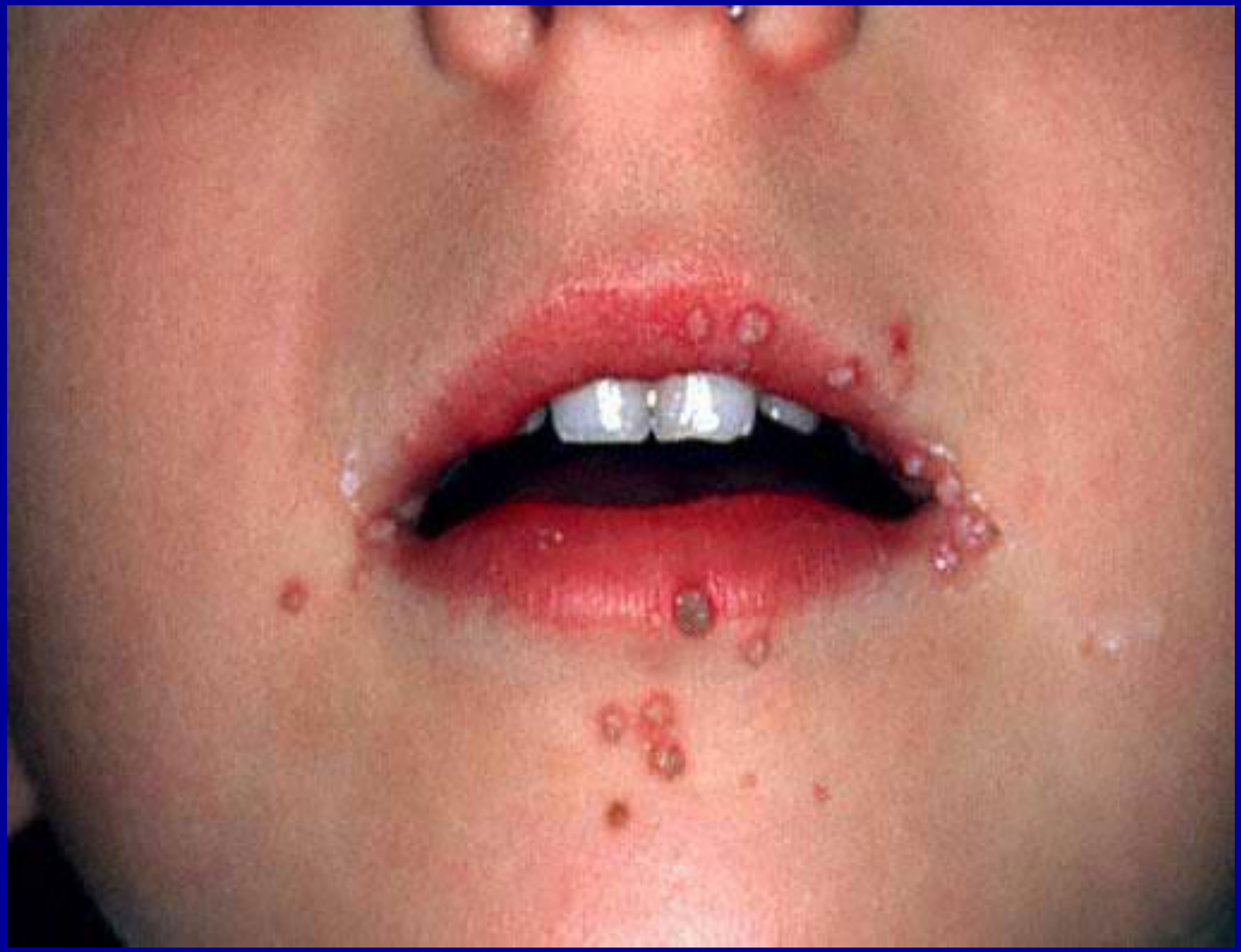
Courtesy of Dermnet.com, Interactive Medical Media LLC



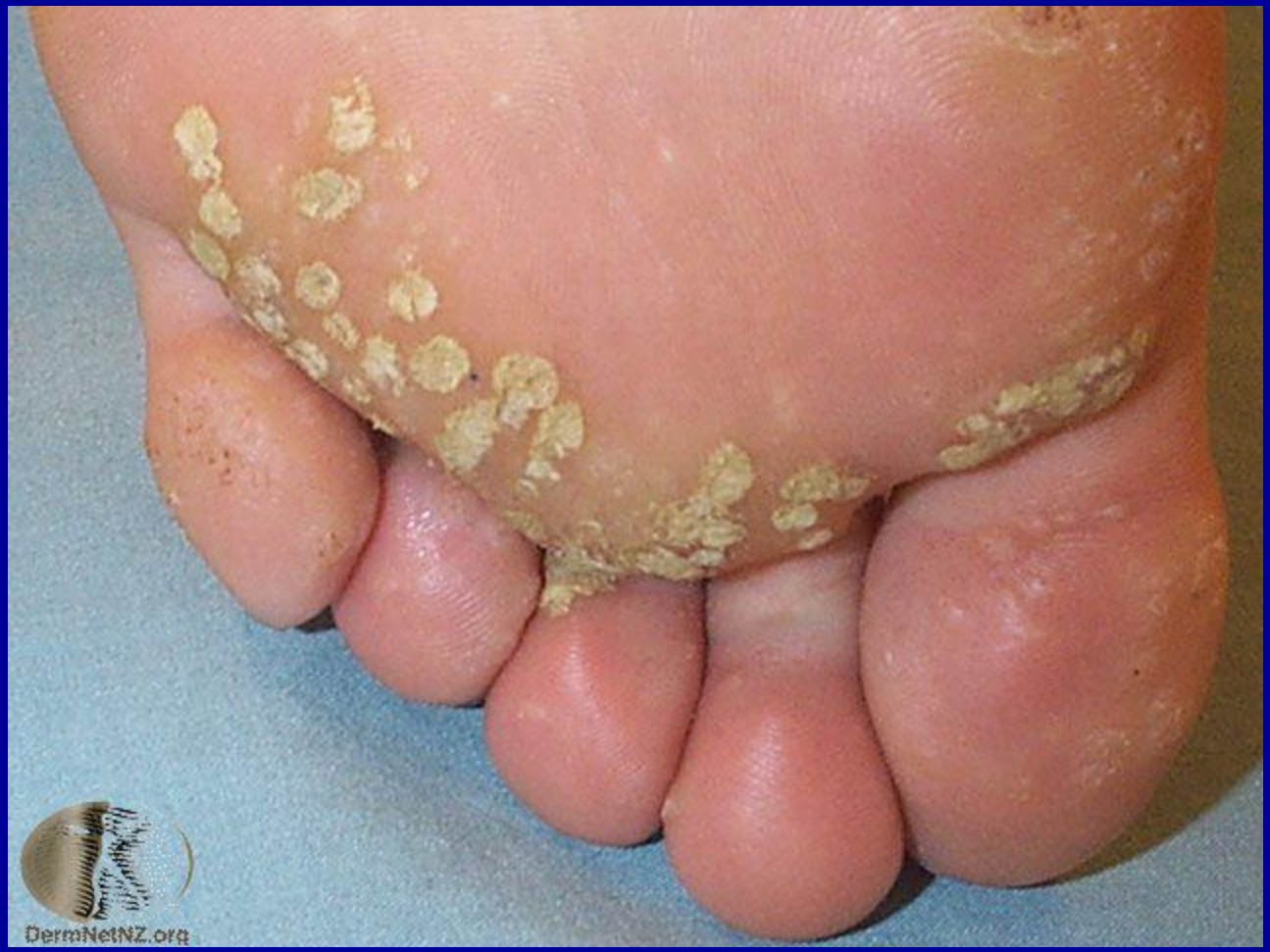
Common warts



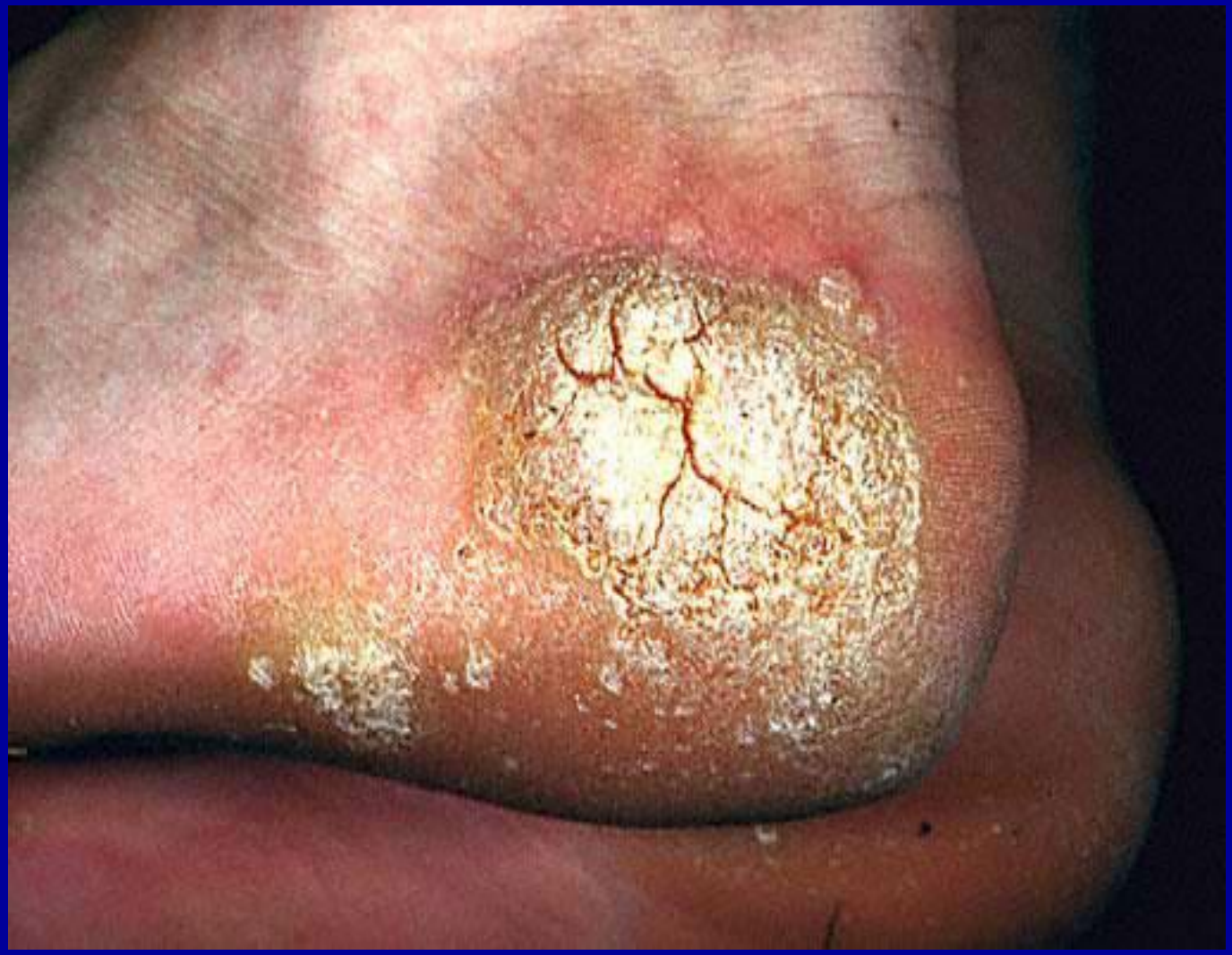
Common warts with thrombosed vessels (black dots). (From Medical Microbiology, 4th ed., Murray, Rosenthal, Kobayashi & Pfaller, Mosby Inc., 2002, Fig. 49-4.



Common warts



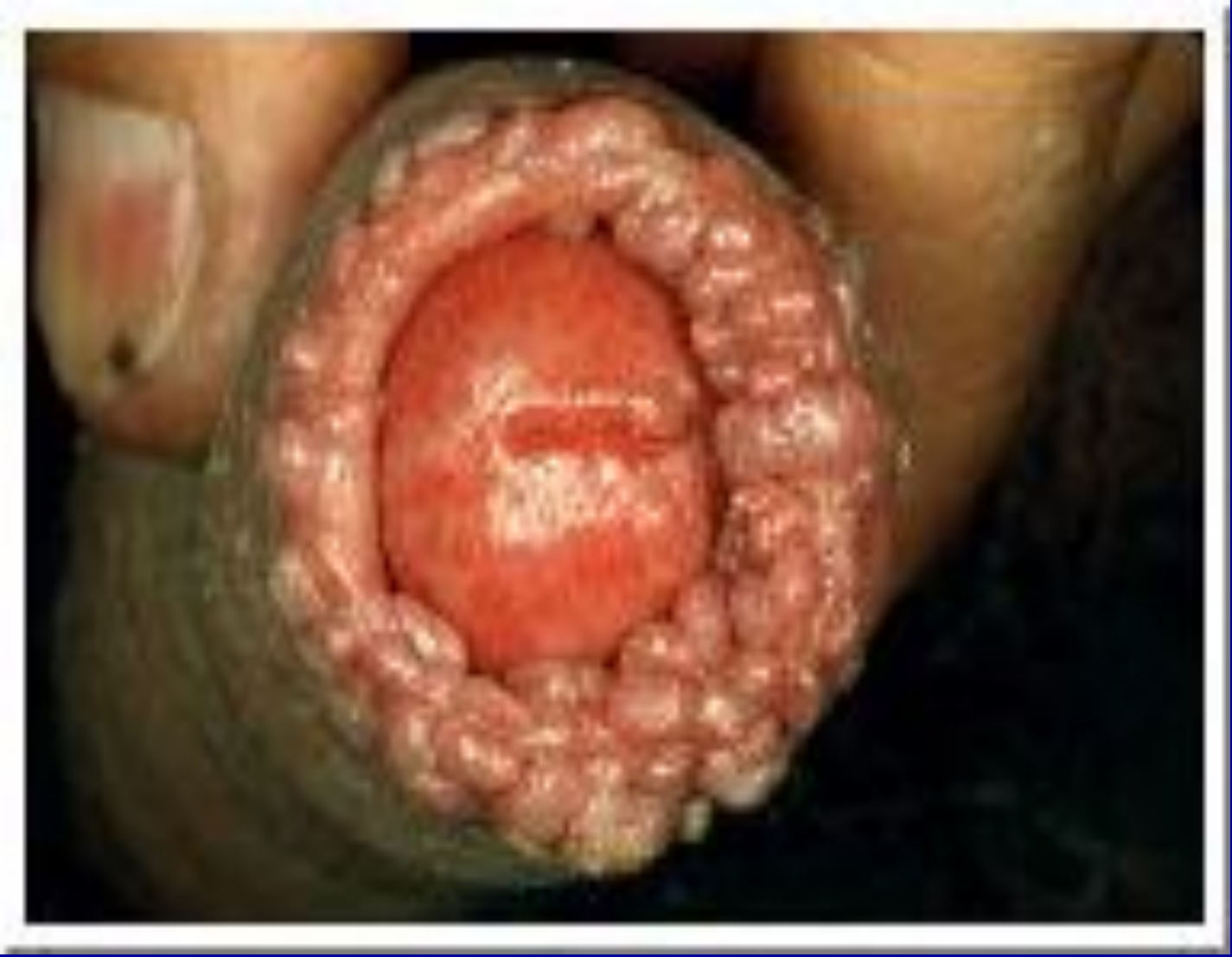
Courtesy of Dermnet.com, Interactive Medical Media LLC



Genital warts slides

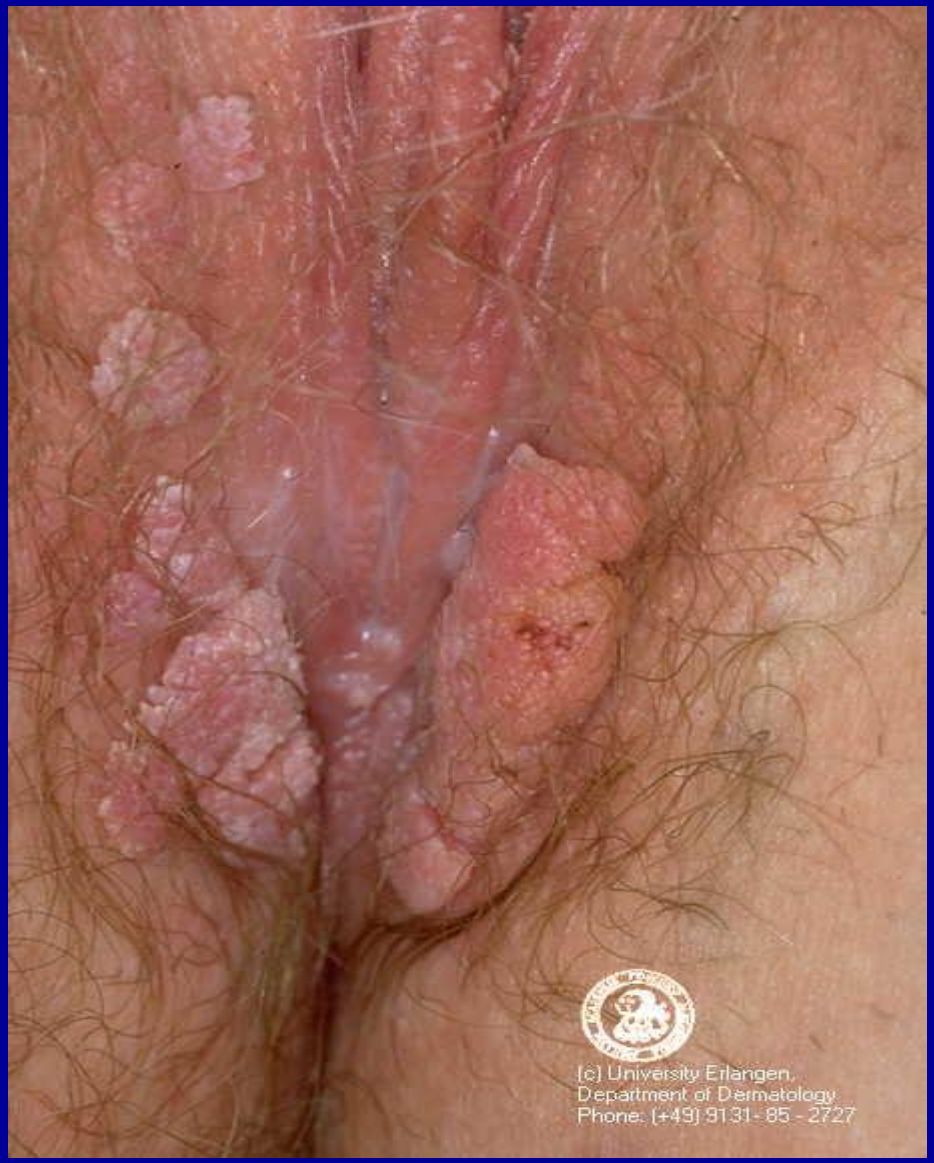
Genital warts in penis

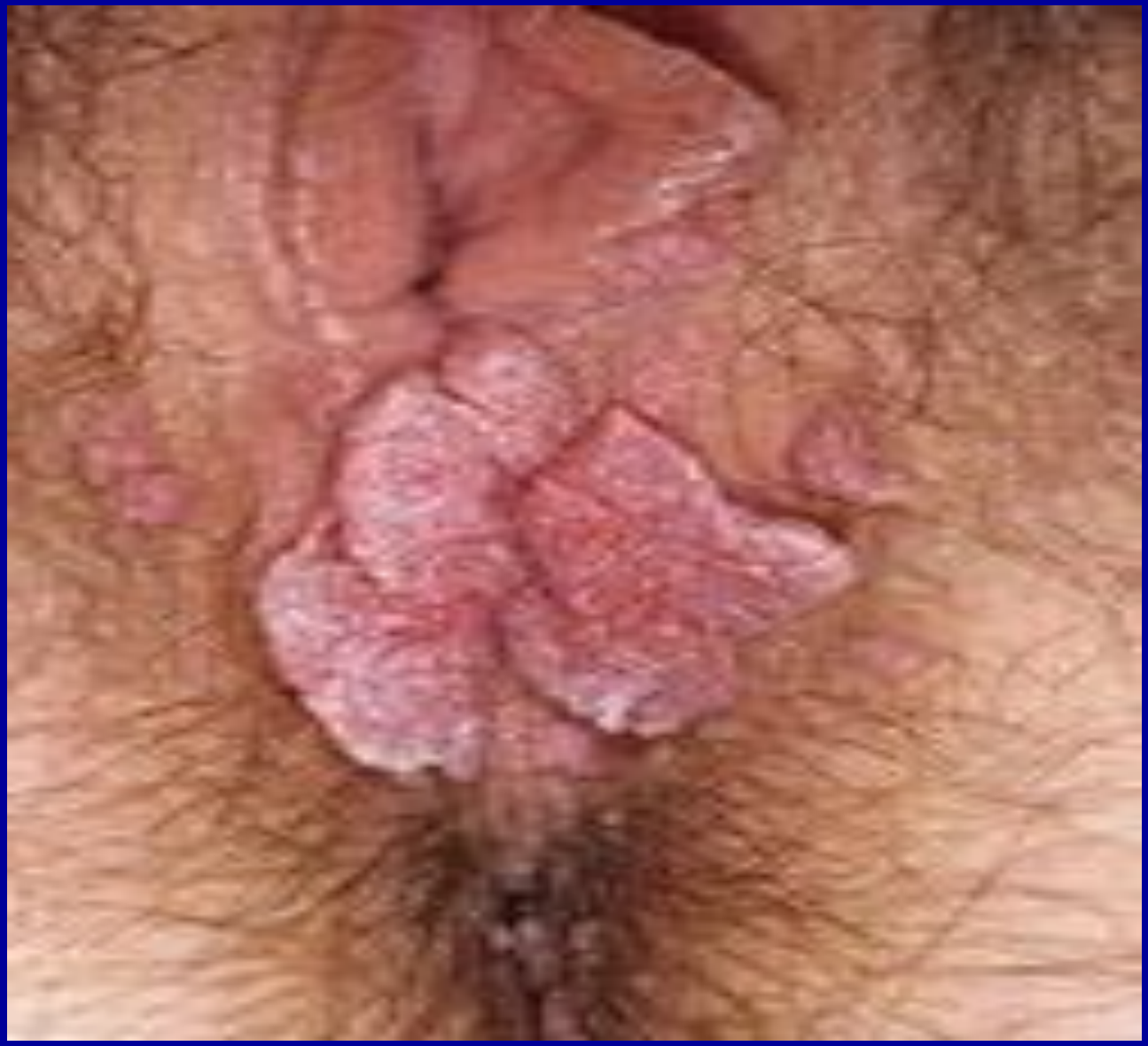
Genital warts in penis



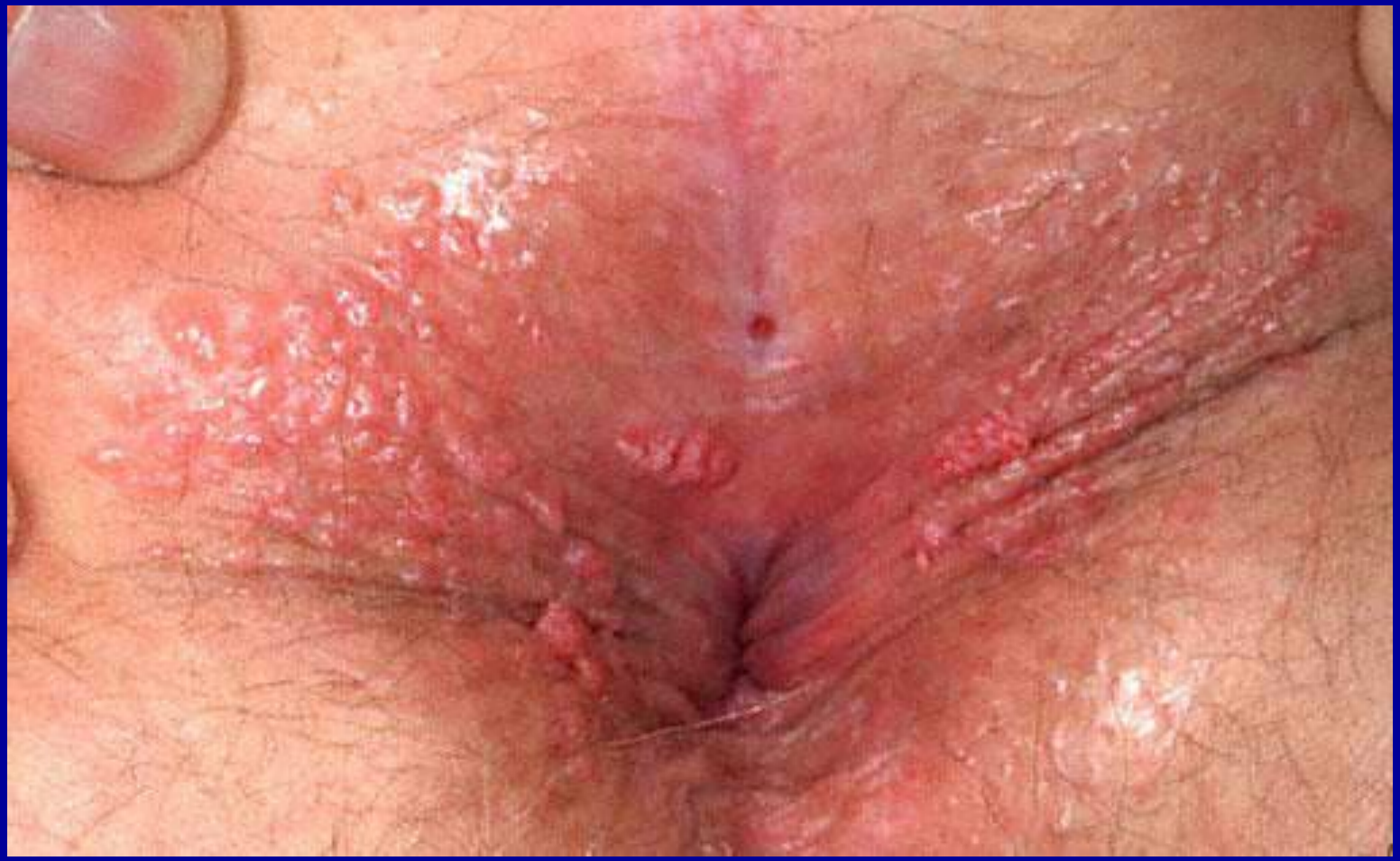
Courtesy of Dermnet.com, Interactive Medical Media LLC

Genital warts in vulva



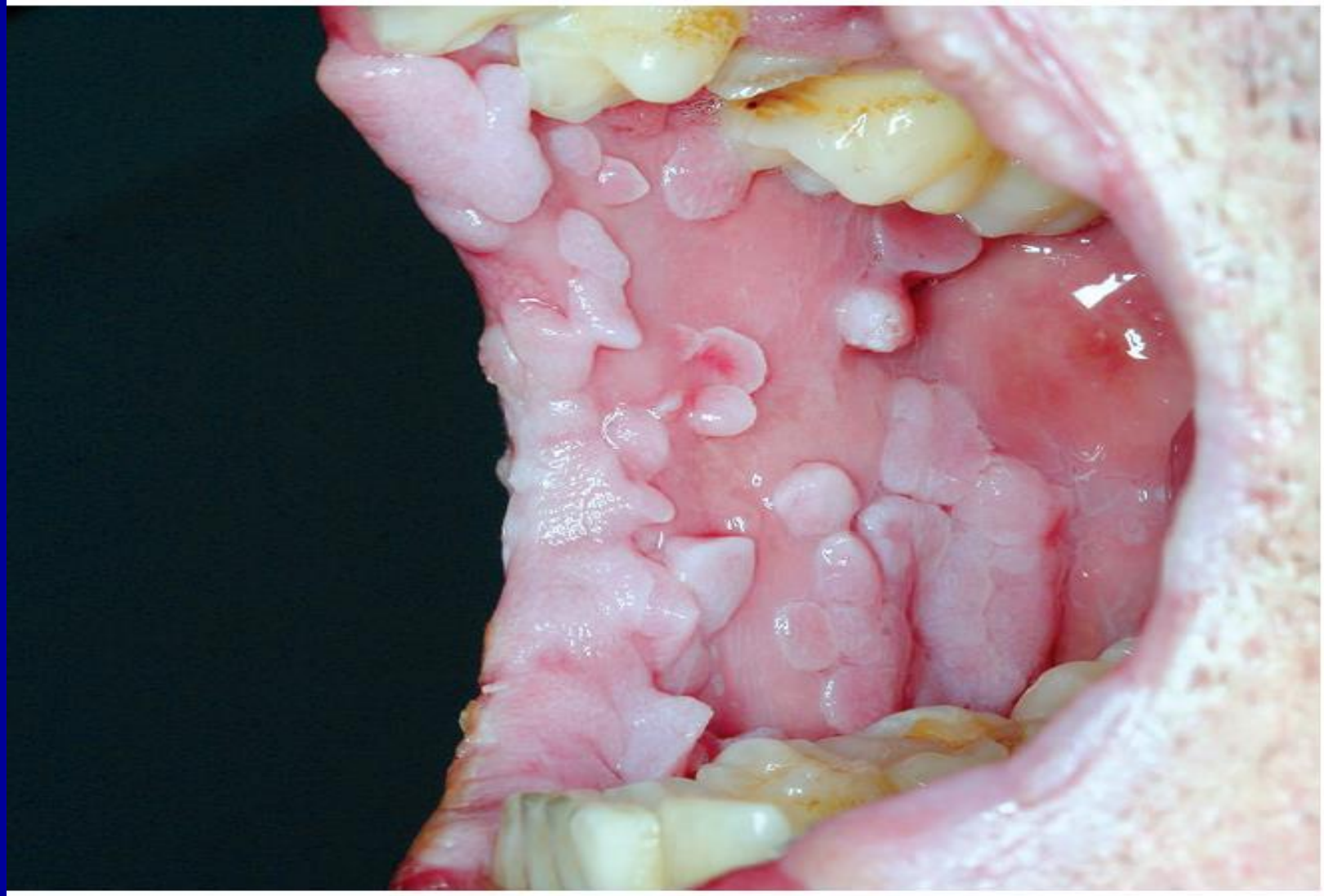


Genital Warts anus



Courtesy of Dermnet.com, Interactive Medical Media LLC

Oral HPV-Associated Papillomatosis in AIDS



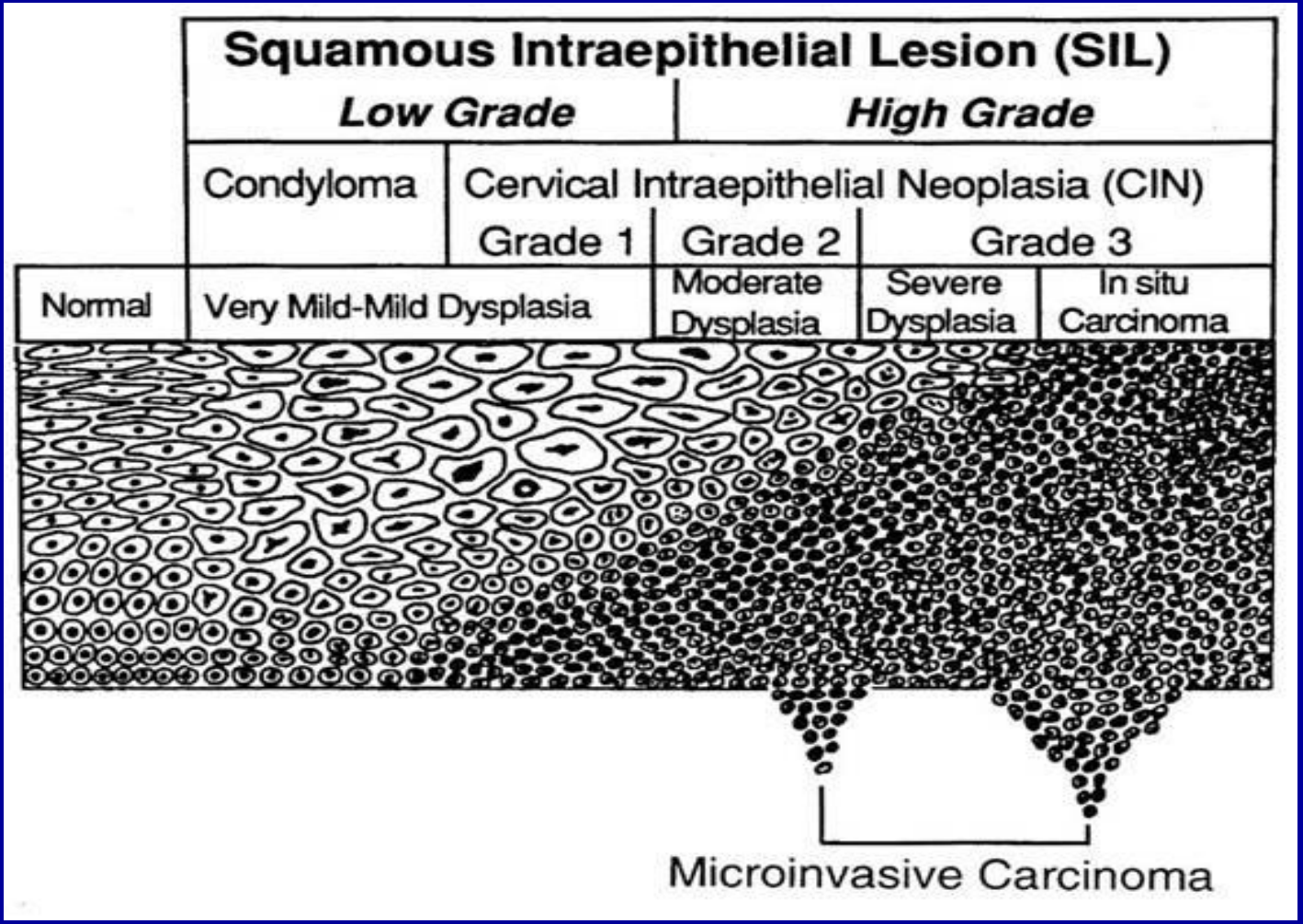
TREATMENT

- Highly effective and safe treatments for HPV diseases are not available

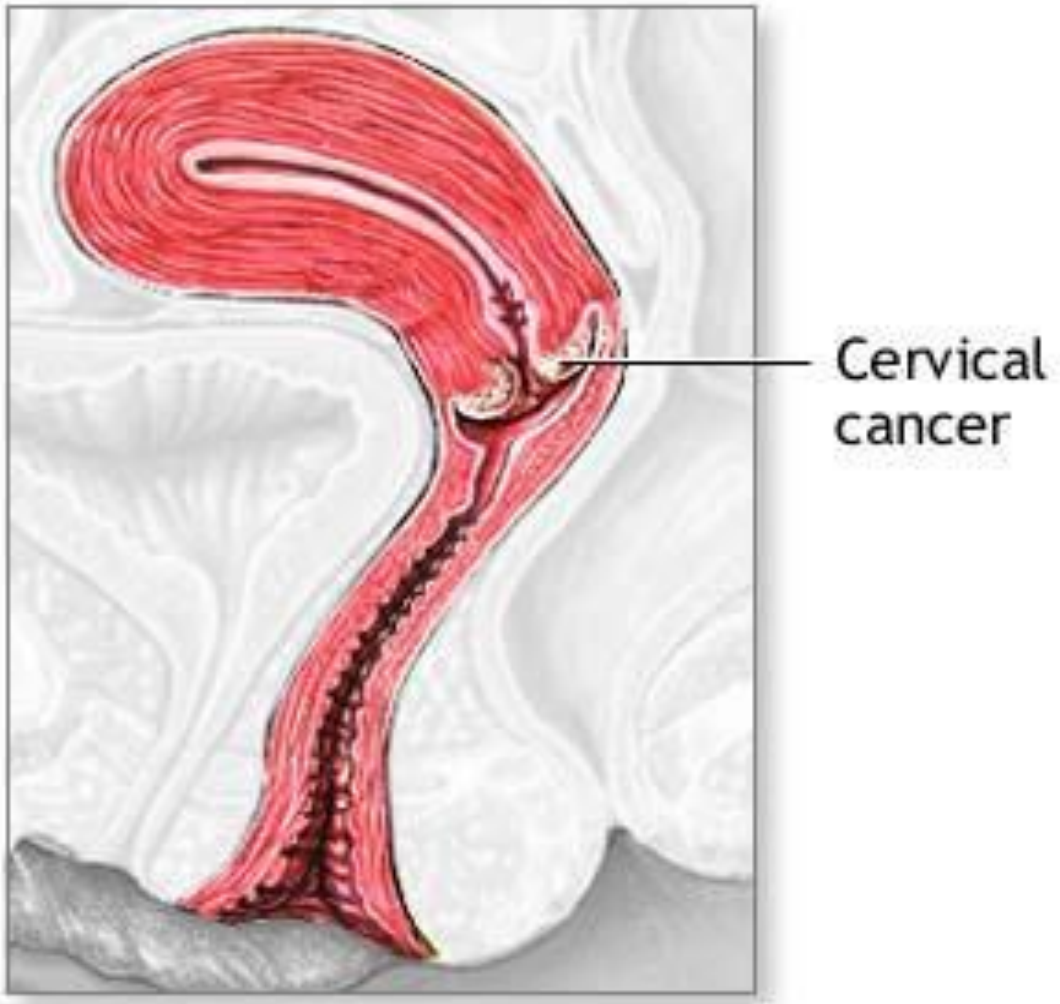
- Cutaneous warts
  - Topical application containing salicylic acid – response rate 75%
  - Cryotherapy – response rate 75%

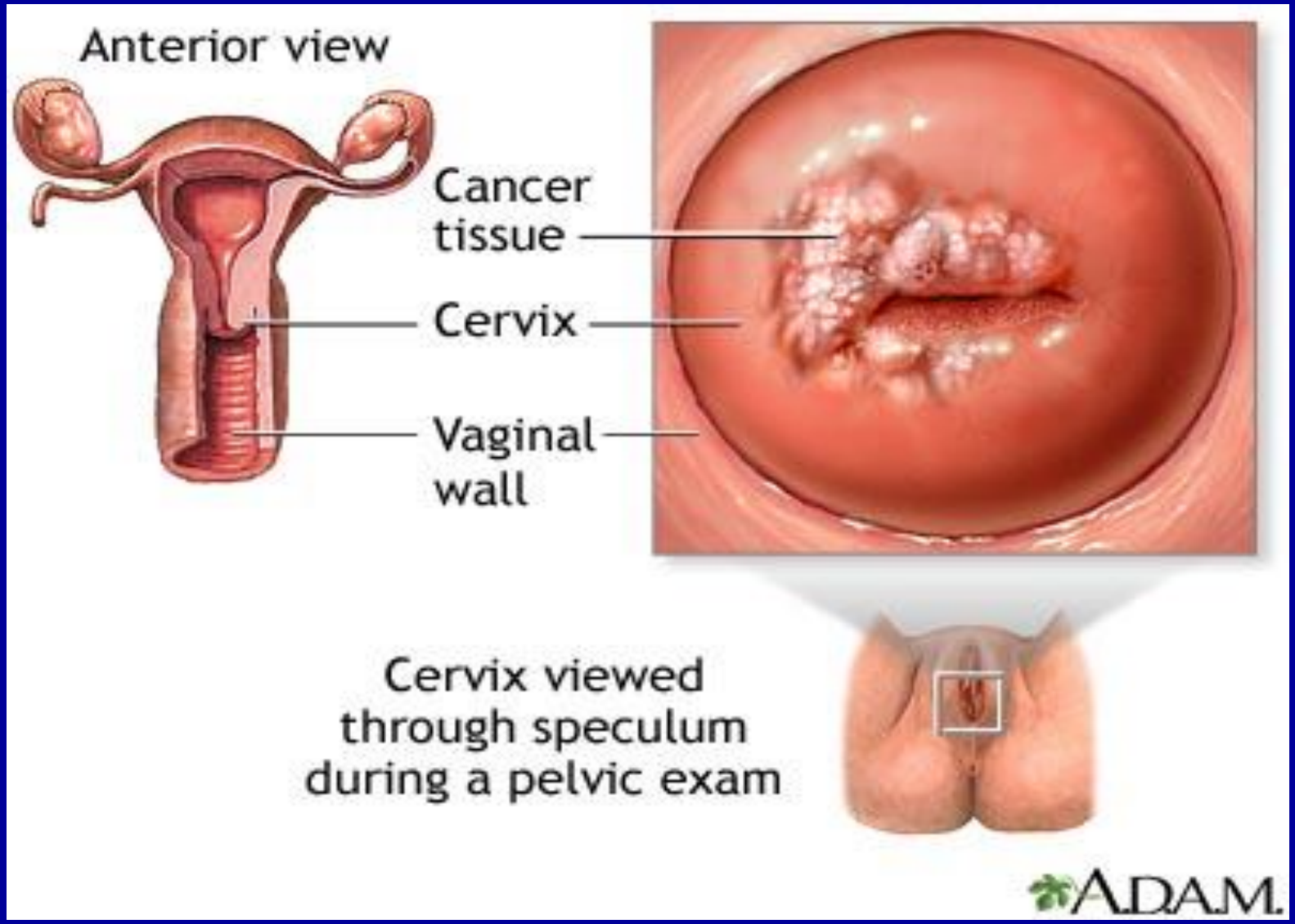
- Anogenital warts
  - 10-30% of patients have spontaneous resolution of disease
  - Podophyllin (antimitotic – disrupt mitotic spindle)
    - Efficacy 20-40%
    - Chemical burns (Do not leave over 24 hours)
    - Neurologic and hematologic complications
  - Interferon therapy – response rate ~50%
  - Cryotherapy – response rate 50-100%
    - Safe during the pregnancy
    - Side effects: burning and ulcerations

Cervical dysplasia



Cervical squamous carcinoma precursors. Schematic representation of cervical cancer precursors and the different terminologies that have been used to refer to them. The risk for microinvasion from different states of squamous intraepithelial lesions (cervical intraepithelial neoplasia) is arbitrarily represented and is not necessarily proportional to that illustrated in this scheme. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 66-4.)


Cervical cancer
A.D.A.M.


Anterior view
Cancer tissue
Cervix
Vaginal wall
Cervix viewed through speculum during a pelvic exam
A.D.A.M.

PREVENTION OF SEXUAL TRANSMISSION

- There is a direct proportion between number of sexual partners and likelyhood of getting genital warts. Limiting the number of partners and choosing partners who also haven't had a lot of partners is safest. Boring = safe.

- Consistent and correct condom use can reduce the risk for genital warts and cervical cancer

- One study among newly sexually active women attending university demonstrated a 70% reduction in HPV infection when their partners used condoms consistently and correctly

- And there is the vaccine.

Confirmation that cervical cancer is caused by HPV, led to the development of HPV vaccines to fight the disease

Vaccine

- There are currently 50 different vaccine products directed against HPV in preclinical and clinical trials
- Vaccines currently in Phase III trial are based on virus-like particles produced using recombinant DNA technology and incorporating L1 capsid protein of the relevant type

Rationale for an HPV Vaccine

- Organized screening programs have reduced cervical cancer
  - 75% reduction in well-screened population
  - High infrastructure and costs
  - Most positive results are false-positives
- In developing countries, where 80% cervical cancer cases occur, screening is not feasible
- HPV vaccine offer a better way of preventing cervical cancer
  - Developed countries – fewer cancers and pre-cancers, lower costs, less surgery
  - Developing countries – simpler, cheaper, more effective

HPV VACCINES

- Two alternatives to consider for effective vaccine development:

Prevent infection?

OR

Clear an established infection ?

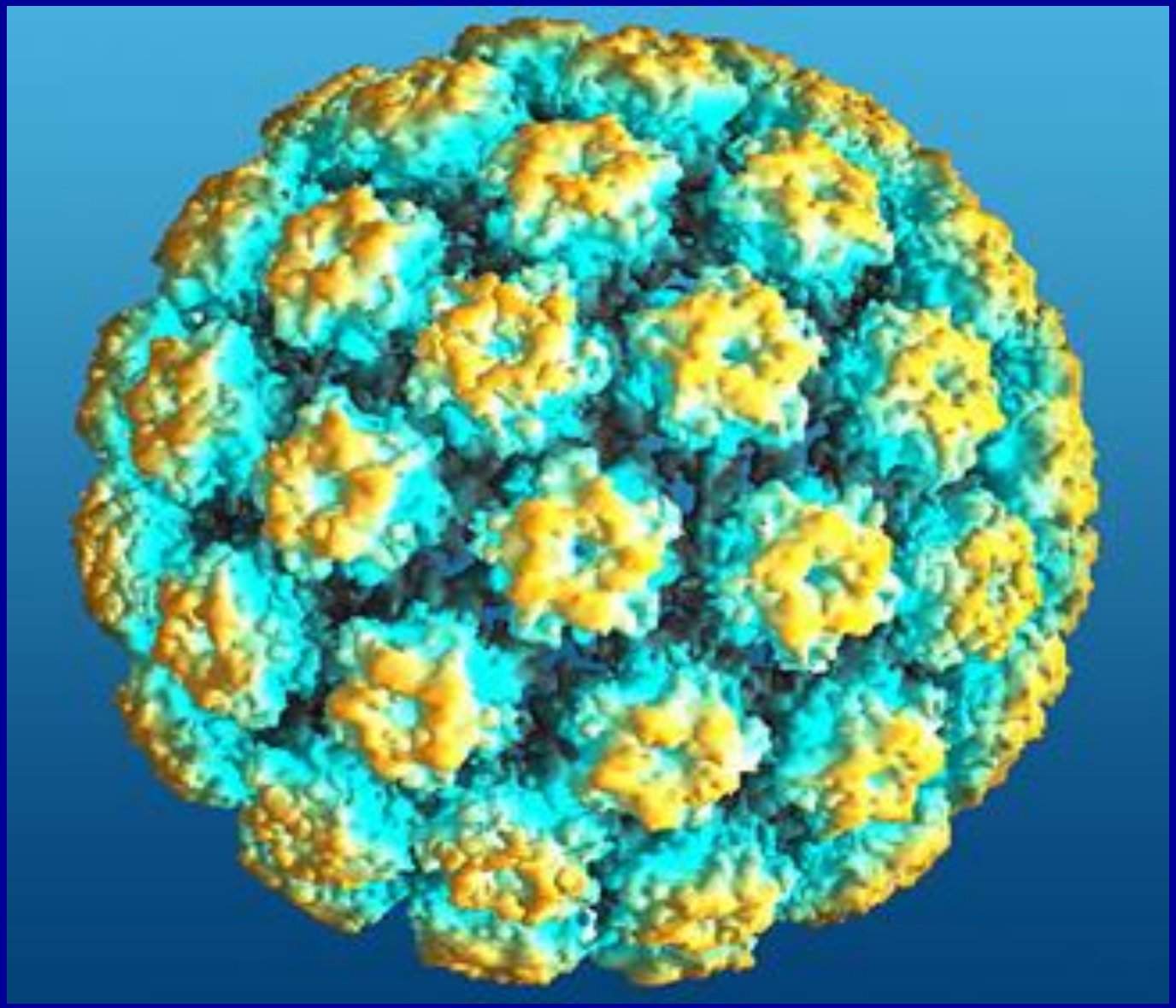
- Depending on the approach, the choice of viral antigens to use as immunogens AND the choice of delivery system could be quite different

Types of HPV Vaccines

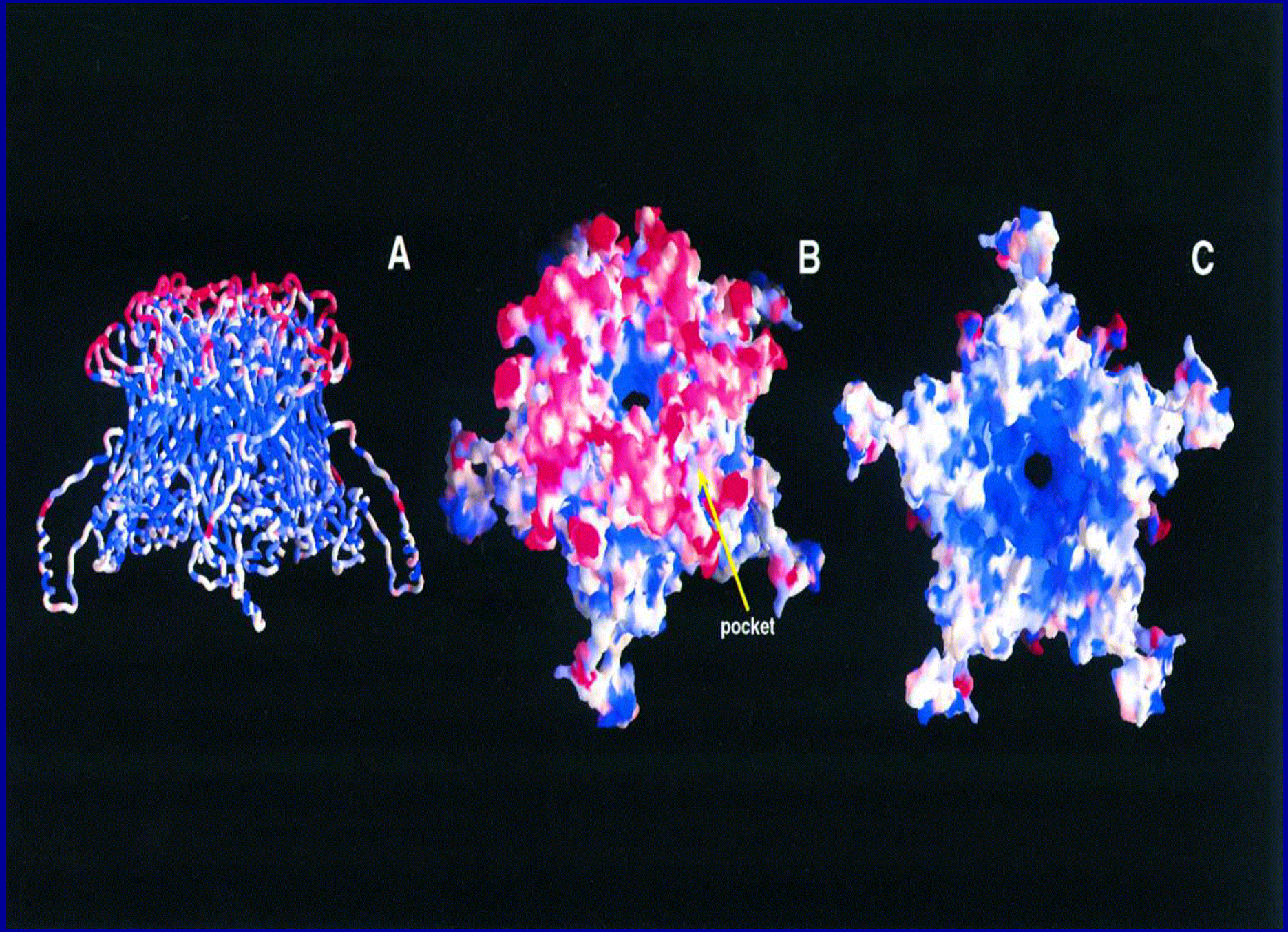
- Therapeutic Vaccine
  - Clears or “keeps in check” established infection
  - Must elicit immune response that the immune system failed to do during the primary infection; “has to do better than nature”
  - Vaccine antigens must be expressed in every infected cell so immune system will attack
    - target antigens are E6 and E7, and E1 and E2
  - Induce immune response that initiates the cell-mediated arm of the immune system to destroy virus-infected cells.

Types of HPV Vaccines

- Preventative Vaccine
  - Simpler since it only has to initiate an immune response sufficient to limit infection and prevent disease
  - Target antigens are capsid proteins b/c only they will induce a strong, long lasting, virus-neutralizing antibody response

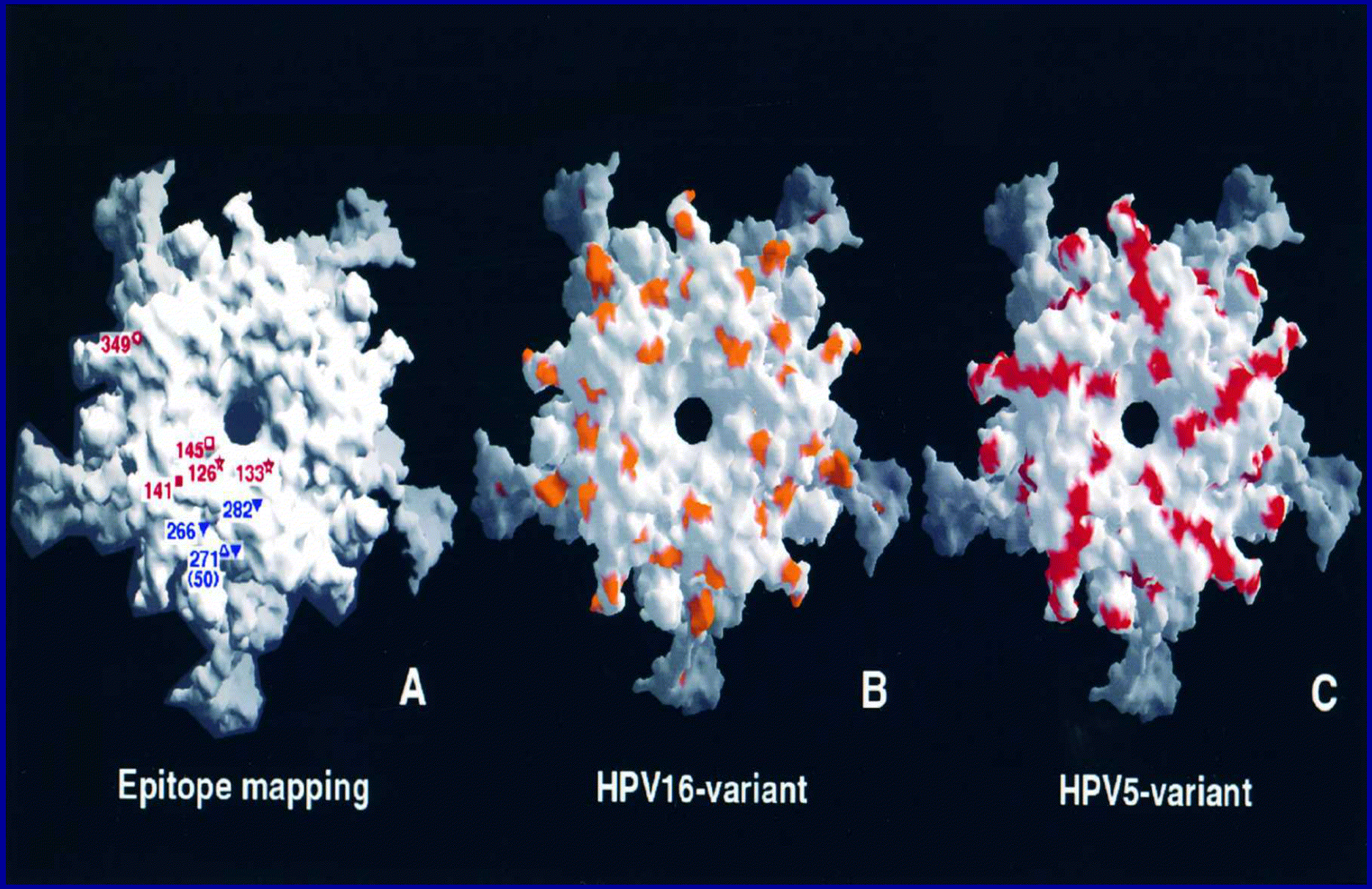


Howard Hughes Medical Institute investigator Stephen C. Harrison



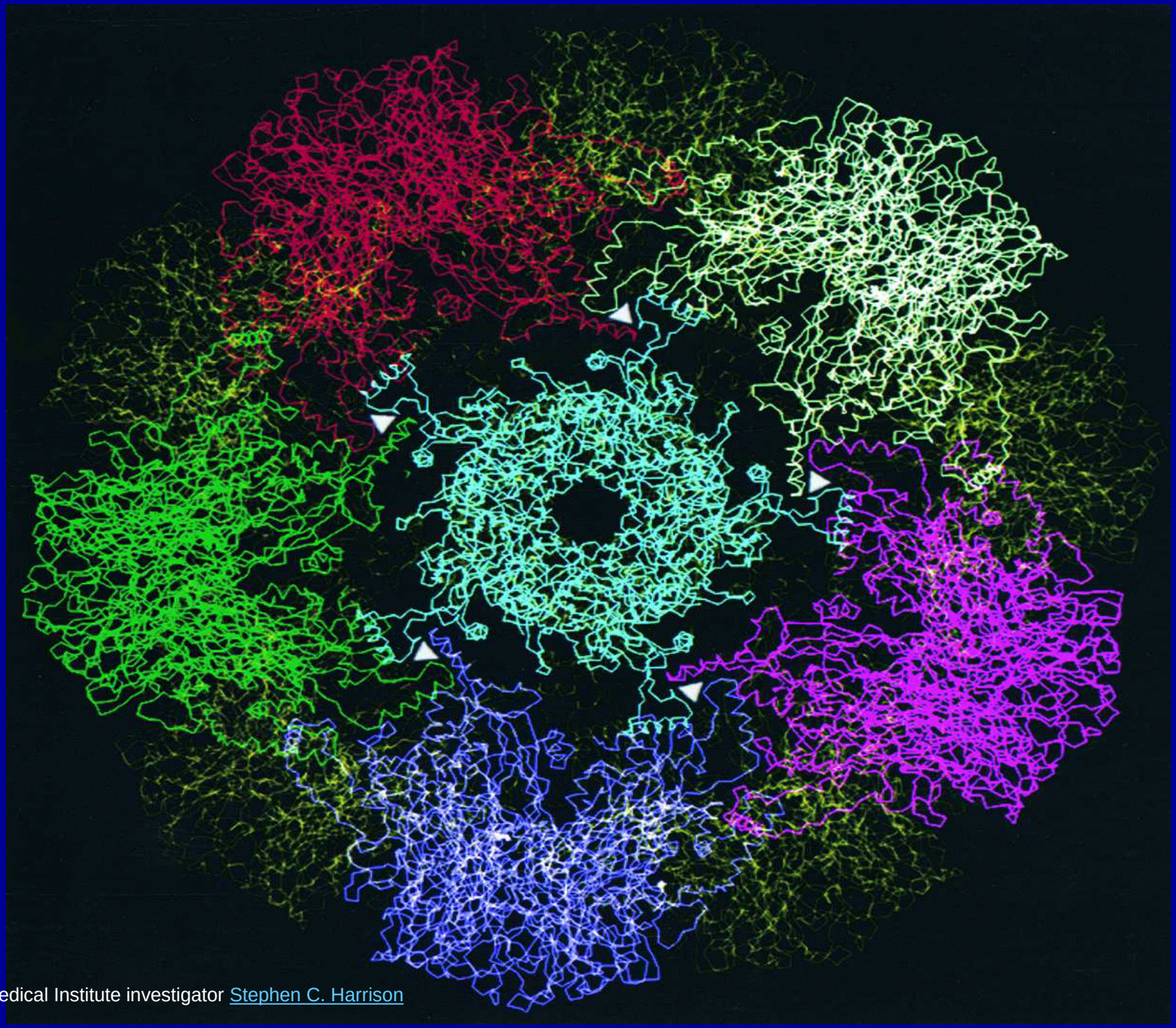
Howard Hughes Medical Institute investigator Stephen C. Harrison

Neutralizing epitopes



Howard Hughes Medical Institute investigator Stephen C. Harrison

L1-VLP



Howard Hughes Medical Institute investigator Stephen C. Harrison

HPV 6, 11, 16 and 18 vaccine trials

- **Phase II trials already have shown high efficacy (89%)**

- **Phase III trial:**

- **Total of 12,167 women aged 16 to 23**

- **Analysis showed that after 17 months of follow-up, vaccinated group did not have any cases of HPV infection (out of 5301)**

- **In placebo arm there were 21 cases (out of 5258)**

- **Response is humoral response – inhibiting infections.**

- **Vaccine does not inhibit already infected patients – need cellular mediated immunity vaccine which we don't have.**

- **The U.S. Food and Drug Administration (FDA) approved the first vaccine that can prevent cervical cancer on June 8, 2006 = FIRST CANCER VACCINE!**

- **Merck's Gardasil® vaccine approved for women between the ages of 9 to 26**

- **IT IS ALSO APPROVED FOR BOYS (same age category)**

- **Bivalent vaccine Cervarix from Glaxo-Smith Kline approved 2009**
  - **Types 16 and 18 L1 VLP vaccine**

Vaccine schedule

- Cost: ~330 USD
- Three dose schedule
- Second after 1-2 months
- Third after 6 months (of first vaccination)
- Pregnant women should not take vaccine
  - Discontimue vaccination schedule if initiated

TABLE 5. Per-protocol efficacy of quadrivalent human papillomavirus vaccine for prevention of HPV 6-, 11-, 16-, and 18-related disease among males aged 16–26 years*

	Vaccine		Control		Vaccine efficacy	
Endpoint	No.	Cases	No.	Cases	%	(95% CI)
Genital warts†	1,397	3	1,408	28	89.4	(65.5–97.9)
PIN†	1,397	0	1,408	3	100.0	(-141.2–100.0)
AIN 1/2/3§	194	5	208	24	77.5	(39.6–93.3)
AIN2/3§	194	3	208	13	74.9	(8.8–95.4)

Abbreviations: AIN = anal intraepithelial neoplasia; CI = confidence interval; HPV = human papillomavirus; PIN = penile/perineal/perianal intraepithelial neoplasias.

* Per-protocol population included males who received all 3 vaccine doses, were seronegative at day 1 and DNA negative at day 1 through month 7 to the respective HPV type, with case counting started after month 7. Median duration of follow-up was 2.9 years.

† **Source:** Giuliano A, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med 2011;364:401–11.

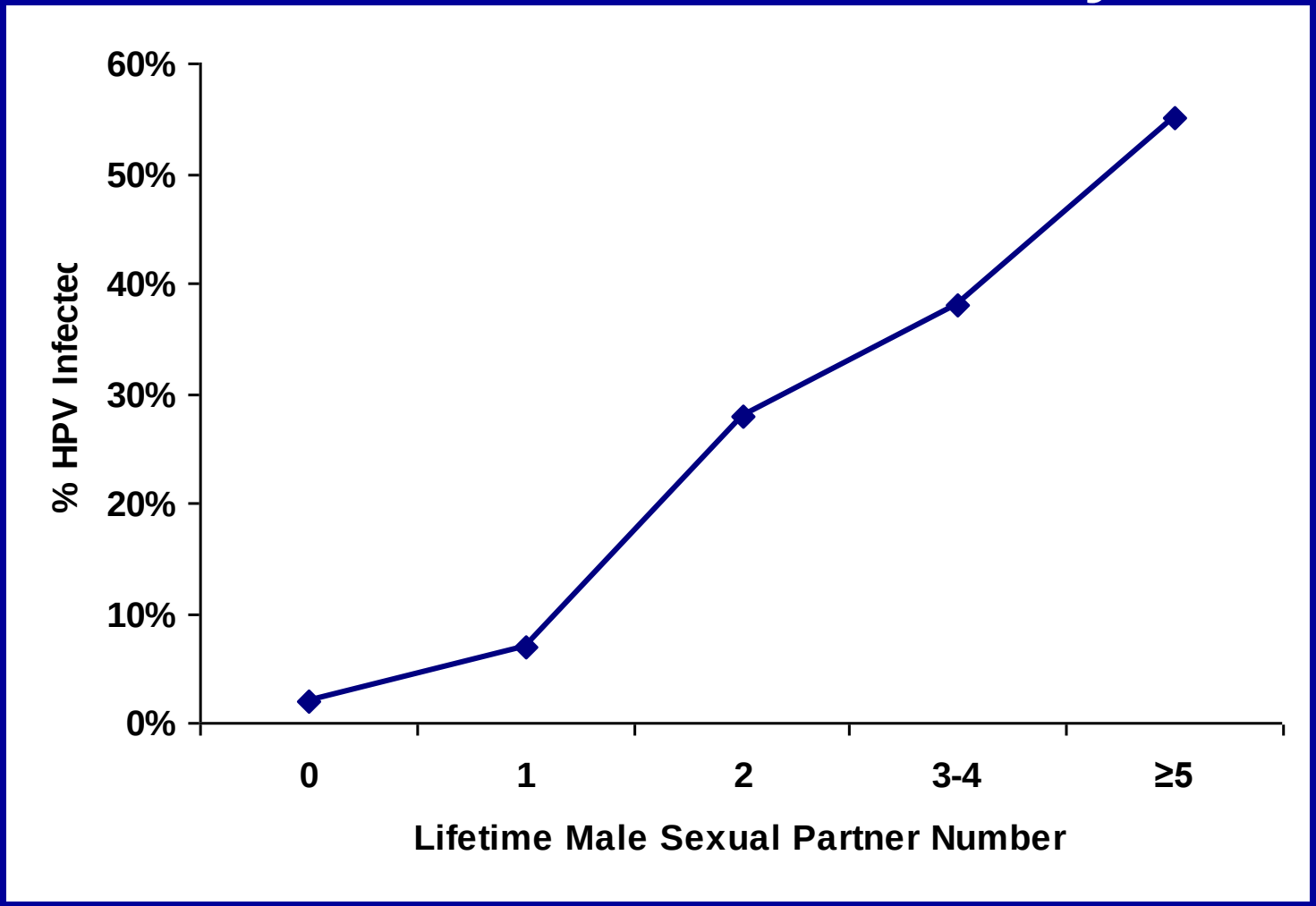
§ Efficacy for AIN studied in men who have sex with men. **Source:** Palefsky J, Giuliano AR, Goldstone S, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. N Engl J Med 2011;365:1576–85.

SAFETY AND EFFICACY

Public Health Aspects

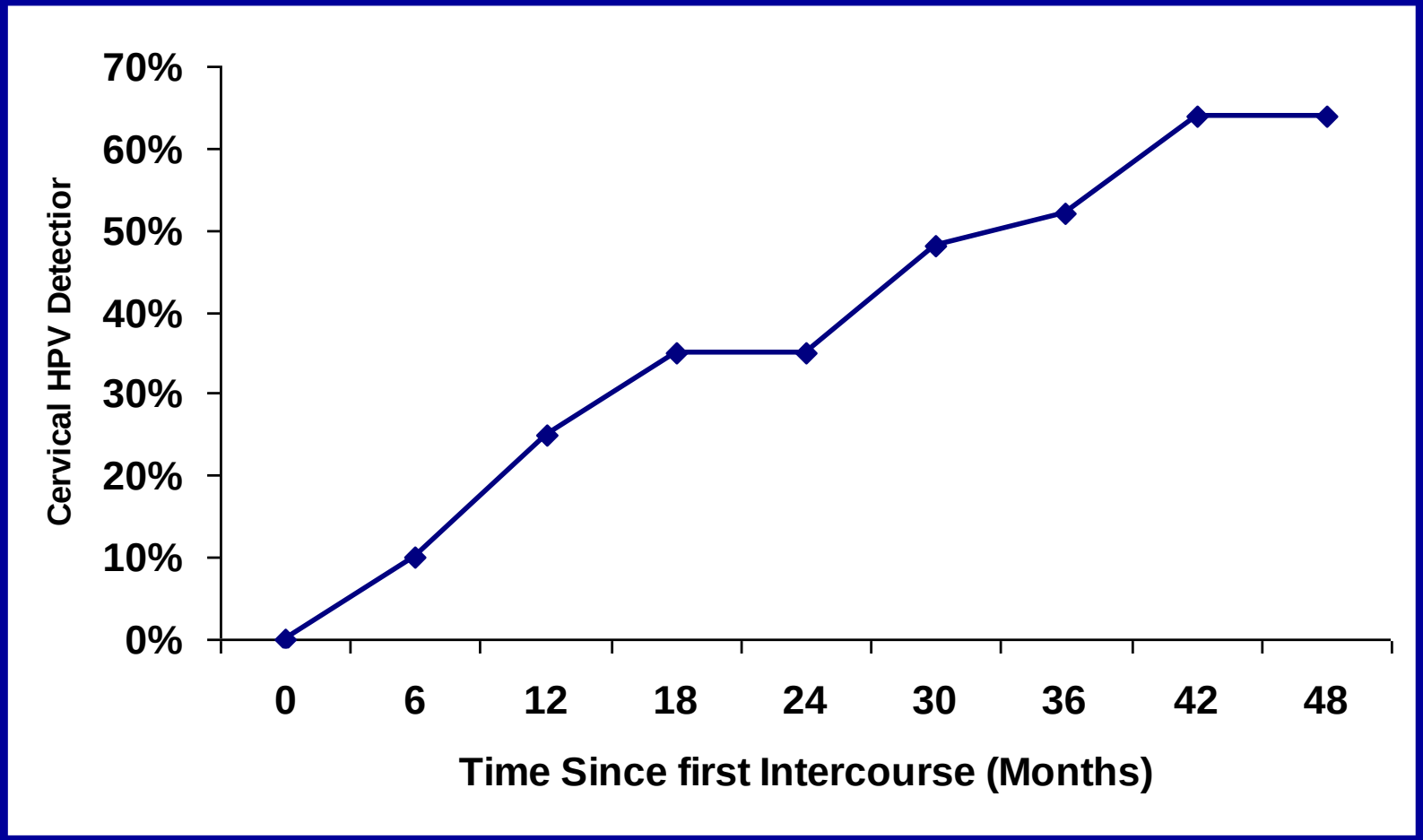
- When to Vaccinate? How young is too young?
  - it must be given early in life since inoculation at that time provides the best chance of lifetime protection (we do not know if booster shots will be necessary later in life).
  - As of 2019 booster shots not seen as necessary.

Incidence of HPV Infection is Strongly Tied to Sexual Activity



Burk RD *J Inf Dis* 1996; 174:679

Incidence of Cervical HPV Detection in Women from the Time of Onset of their First Sexual Relationship



Collins et al. *Brit J Obstet Gynecol* 2002; 109:96-98

The Vaccine Adverse Event Reporting System (VAERS) (CDC)

- To date, VAERS has not received any adverse event reports occurring in the U.S. following Cervarix.
- As of January 1, 2010, approximately 28 million doses of Gardasil were distributed in the United States.
- As of January 31, 2010, there were 15,829 VAERS reports of adverse events following Gardasil vaccination in the United States. Of these reports, 92% were reports of events considered to be non-serious, and 8% (1266) were reports of events considered serious.

Non-serious adverse event reports

- VAERS defines non-serious adverse events as those other than hospitalization, death, permanent disability, and life threatening illness.

- 92% of the adverse events reports following Gardasil vaccination have included fainting, pain, and swelling at the injection site (the arm), headache, nausea, and fever.

- Fainting is common after injections and vaccinations, especially in adolescents. Falls after fainting may sometimes cause serious injuries, such as head injuries, which can be prevented by closely observing the vaccinated person for 15 minutes after vaccination

Serious adverse event reports

- VAERS defines serious adverse events as adverse events that involve hospitalization, permanent disability, life-threatening illness, and death.

- As with all VAERS reports, serious events may or may not have been caused by the vaccine.

Guillain-Barré Syndrome (GBS)

- Guillain-Barré Syndrome (GBS) has been reported after vaccination with Gardasil.

- GBS is a rare disorder that causes muscle weakness. It occurs in 1-2 out of every 100,000 people in their teens. A number of infections can cause GBS.

- There has been no indication that Gardasil increases the rate of GBS in girls and women above the rate expected in the general population, whether or not they were vaccinated.

Blood Clots

- There have been some reports of blood clots after receiving Gardasil.
- These clots have occurred in the heart, lungs, and legs.
- Most of these people had a risk of getting blood clots, such as taking oral contraceptives (the birth control pill), smoking, obesity, and other risk factors.

Deaths

- As of January 31, 2010, there have been 49 U.S. reports of death among females who have received Gardasil.

- Twenty eight of these reports have been confirmed and 21 remain unconfirmed due to no identifiable patient information in the report such as a name and contact information to confirm the report.

- Confirmed reports are those that scientists have followed up on and have verified the claim. In the 28 reports confirmed, there was no unusual pattern or clustering to the deaths that would suggest that they were caused by the vaccine.

TABLE 7. Injection-site reactions within 5 days after receipt of quadrivalent human papillomavirus vaccine in females and males aged 9–26 years

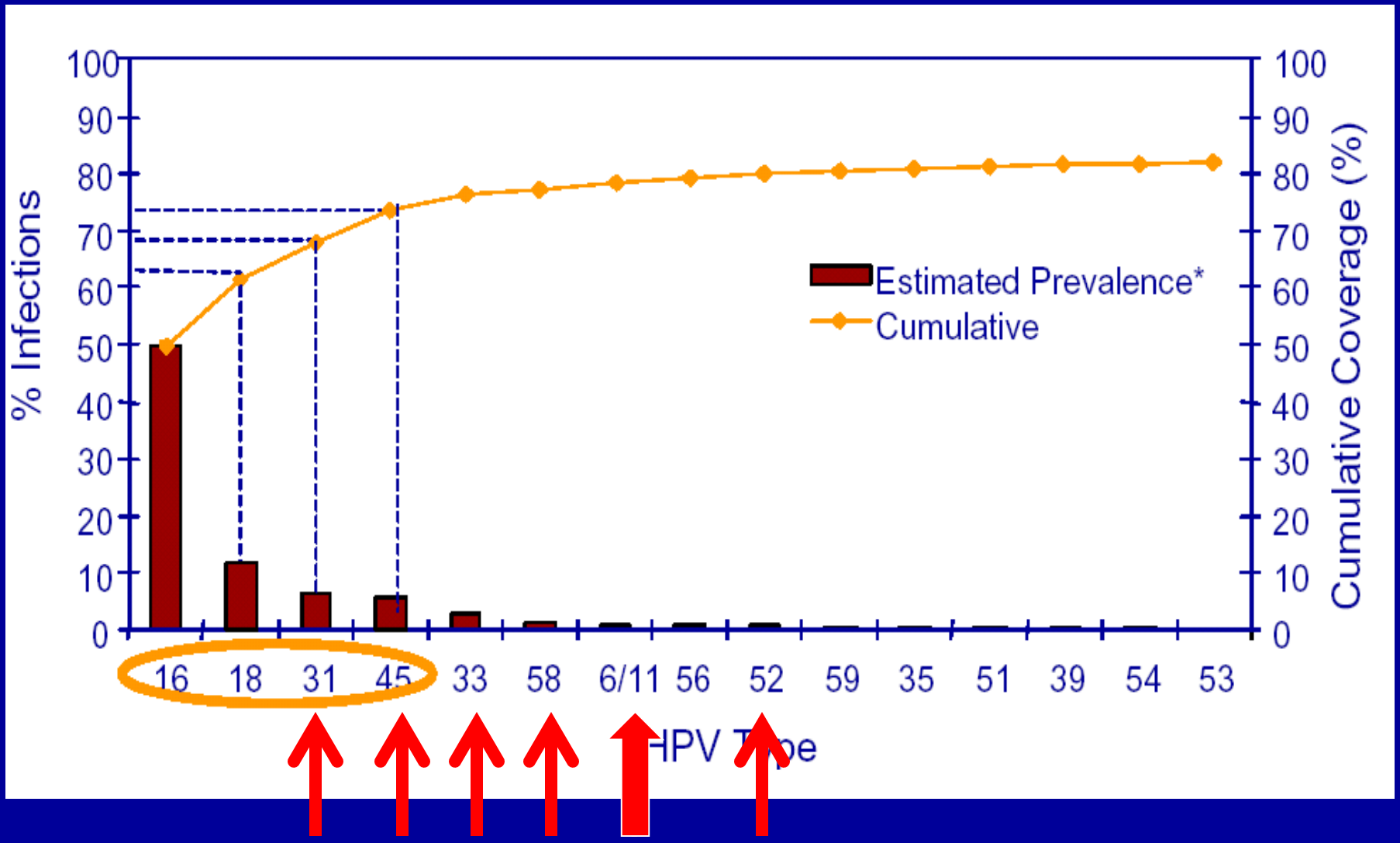
Adverse event	Quadrivalent vaccine %	AAHS control %	Saline control %
Females	N = 5,088	N = 3,470	N = 320
Pain	83.9	75.4	48.6
Swelling	25.4	15.8	7.3
Erythema	24.7	18.4	12.1
Males	N = 3,093	N = 2,029	N = 274
Pain	61.4	50.8	41.6
Swelling	13.9	9.6	8.2
Erythema	16.7	14.1	14.5

Abbreviation: AAHS = Amorphous aluminum hydroxyphosphate sulfate.
Source: Food and Drug Administration. Product approval-prescribing information [Package insert]. Gardasil [human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine, recombinant], Merck & Co, Inc. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration; 2014. Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM111263.pdf.

- WHITEHOUSE STATION, N.J.--(BUSINESS WIRE)--Merck announced that 9-valent HPV vaccine (V503) prevented approximately 97 percent of cervical, vaginal and vulvar pre-cancers caused by HPV types 31, 33, 45, 52, and 58. V503 also generated immune responses to HPV types 6, 11, 16, and 18 that as good as those generated by GARDASIL® [Quadrivalent (Types 6, 11, 16, and 18) Vaccine].

- It was licensed in 2015.

- It is now the standard vaccine and is recommended for girls and boys 9 – 26 years.



Schiffman, et al. J Nat Canc Inst 1993. 85:958 and Liaw, et al. J Nat Cancer Inst. 1999. 91:954

PUBLIC HEALTH ASPECTS

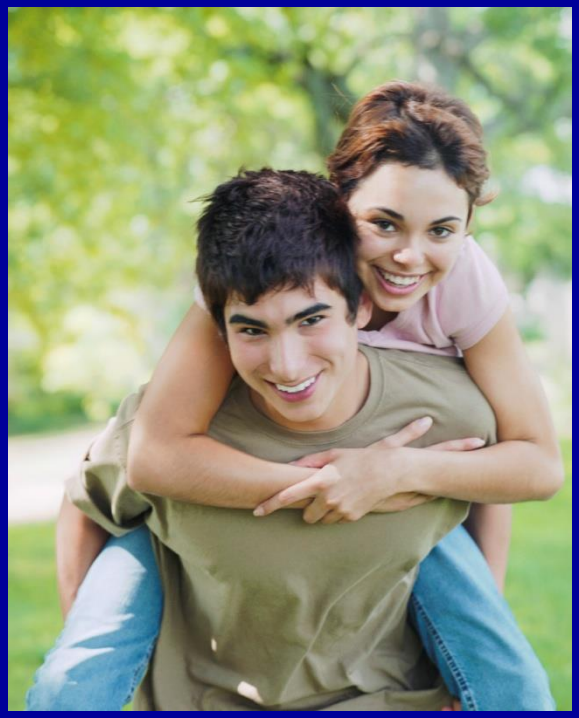
Should schools make the vaccine compulsory?

- Or should parents decide?
- On Feb 5, 2007 Texas was the first state to mandate the vaccine for girls ages 11-12 for entry into the 6th grade. They immediately reversed due to protests from the right.

Public Health Aspects

- Vaccinate Males?
  - Men can pass the infection on to women, who then go on to develop cervical cancer
  - It is now recommended for males and females at 11-12 years to 26 (can start as early as 9)
  - FDA has approved it for males and females up to 45.

Public Health Aspects



- Educate MDs.
- It is now recommended for everyone under 26 with a few exceptions like the immunocompromised.
- Parental resistance – Abstinence vs Protection
  - Vaccine encourages promiscuity among teens?

Public Health Aspects

- Implementation in 3rd World Countries
  - Must raise awareness for need of vaccine where HPV knowledge is low
  - Must ensure acceptability in regions where teenage sexuality is a sensitive subject
  - Deliver a series of 3 injections to population that usually has minimal contact with health care facilities?
  - $360/subject too expensive

    in extremely low-resource settings