Clinical and Molecular aspects of HPV Infection

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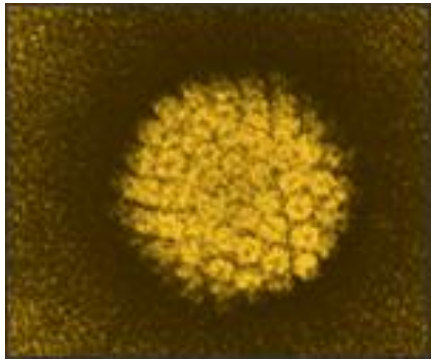
Topics

1. HPV and its subtypes
2. Acquiring an HPV infection: How common is it?
   How do you get it?
3. Developing lesions: What can happen if you have an HPV infection?
   The HPV lifecycle
   The molecular events in an HPV infected cell
4. Resolving an HPV infection: Clearance and immunity
5. HPV and cervical cancer

Human Papillomaviruses (HPV)



HPV's are DNA viruses that infect human skin and mucosal epithelia
More than 200 different subtypes (genotypes) cause different manifestations of infection.

HPV subtypes are divided into cutaneous or **mucosal types:** at least 40 subtypes of HPV infect the genital tract/oropharynx

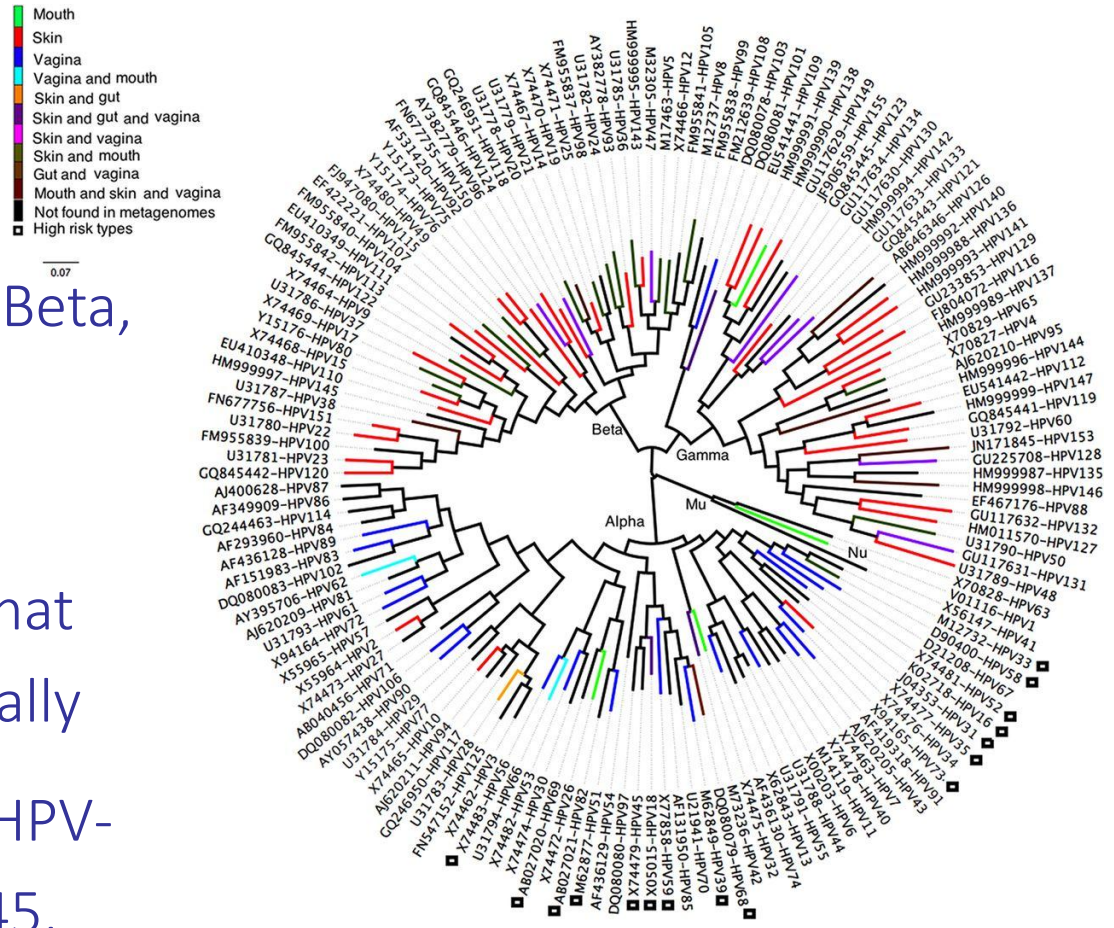
- genital warts are usually caused by low-risk HPV types
- high-risk HPV infections are predominantly caused by about 14 high-risk subtypes and cause cancer in a very small proportion of those infected

Persistent hrHPV infection causes almost all cancers of the cervix as well as most cancers of the vagina, vulva, penis and anus.

Alpha Genus

HPV's: 5 genera based on their DNA sequence - Alpha, Beta, Gamma, Mu and Nu

- the Alpha genus contains those that cause important human disease including the high-risk subtypes are that are sexually transmitted and controlled immunologically
- 15 high risk HPV types identified as carcinogenic(1); HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68, HPV-73 and HPV-82. Probably also 66,26 and 53



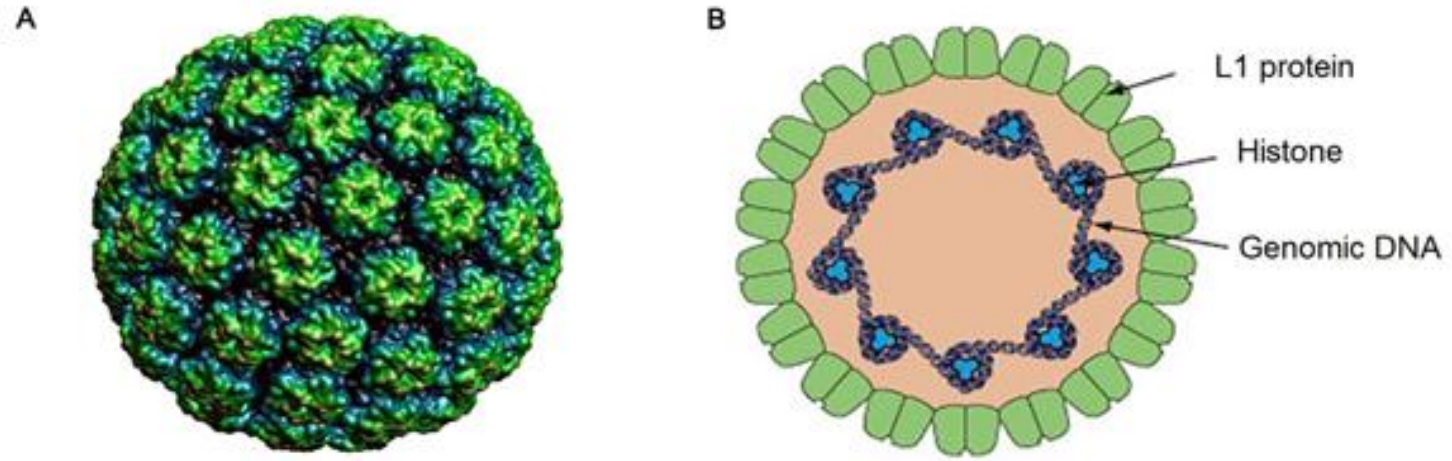
Doorbar J., Reviews in Medical Virology 2016

HPV: Phylogenetic tree

1. Ref: Munoz N et al Epidemiologic Classification of Human Papillomavirus Types associated with Cervical Cancer. N Engl J Med 2003;27:472-80

Virus Structure

- Papillomaviruses share a common non-enveloped icosahedral structure (50-60nm diameter)
- Their genomes are comprised of circular double-stranded DNA of almost 8000 base pairs
- The virus coat contains 360 molecules of L1 protein arranged into 72 capsomeres which have a beta-jellyroll core.



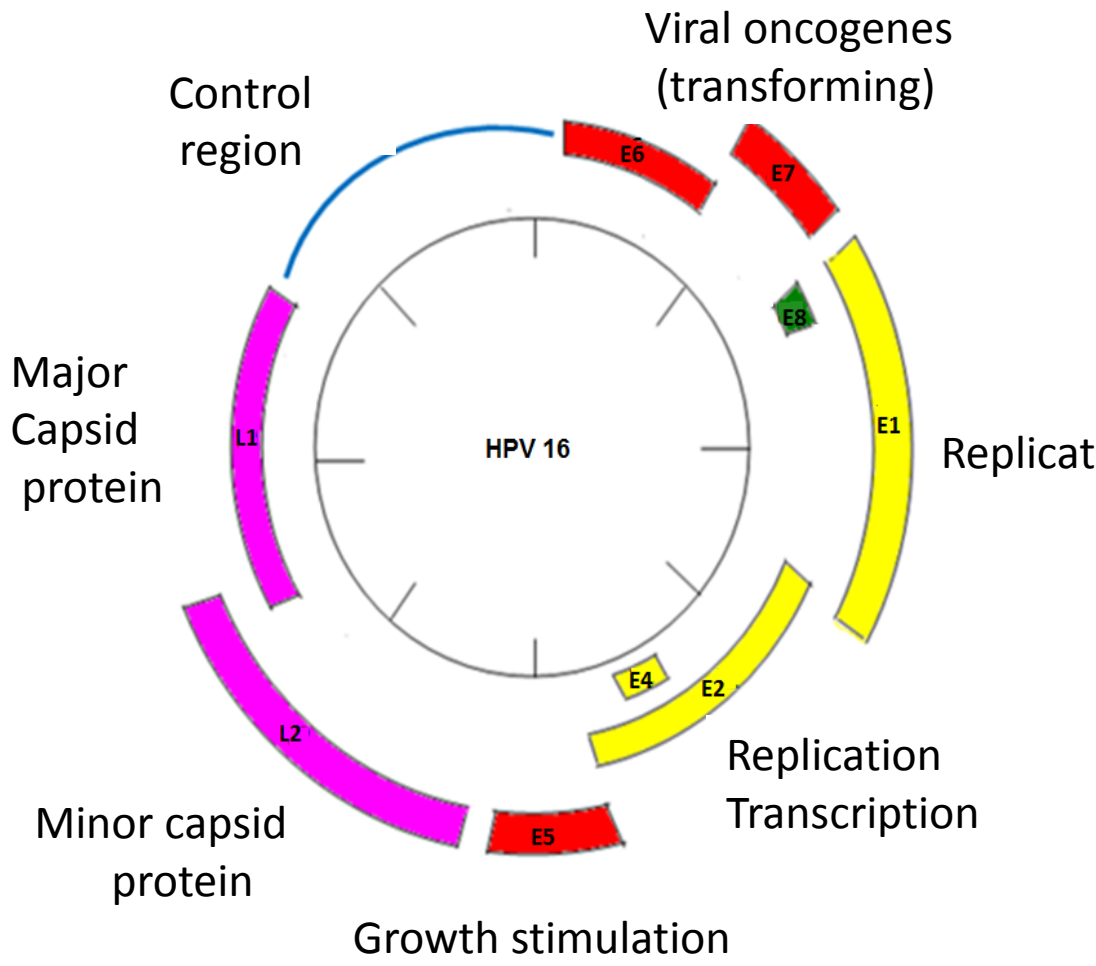
HPV Genome

Core proteins are common to all HPVs

- E1 & E2 regions – code for proteins related to viral genome replication and amplification
- E4 region – codes for a protein which binds to host cytokeratin filaments disrupting their structure causing release of the virus from surface epithelium
- L1 & L2 regions – code for capsid proteins which assemble DNA and package it into the virion

Accessory proteins are variably expressed between different HPV subtypes

- E6 & E7 – Cell cycle entry allows genome amplification in the mid-layer of the epithelium



THE HPV GENOME
is divided into Early and Late regions

HPV subtypes

Disease	HPV subtype
Cutaneous Warts	Non-carcinogenic: 1, 2, 3, 4, 7, 8, 10, 22, 63
Anogenital Warts	Non-carcinogenic: 6, 11, 42, 44
Genital Cancers	Carcinogenic: 16, 18, 31, 45 Very likely carcinogenic: 33, 35, 39, 51, 52, 56, 58, 59 Probably carcinogenic: 26, 53, 66, 68, 73, 72 Possibly carcinogenic: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81
Oral Papillomas	Carcinogenic: 16 Possibly carcinogenic: 6, 11 Non-carcinogenic: 7, 32
Oropharyngeal Cancer	Carcinogenic: 16
Laryngeal Papillomatosis	Possibly carcinogenic: 6, 11

** Subtypes present in Gardasil-9*

HPV subtypes and pre-invasive lesions

Genital warts: 90% = HPV 6 and 11

LSIL: 10% = HPV 6 and 11

Up to 35% = HPV 16 and 18: more likely to progress to HSIL

Remainder = a mix of other high-risk and low-risk viral types

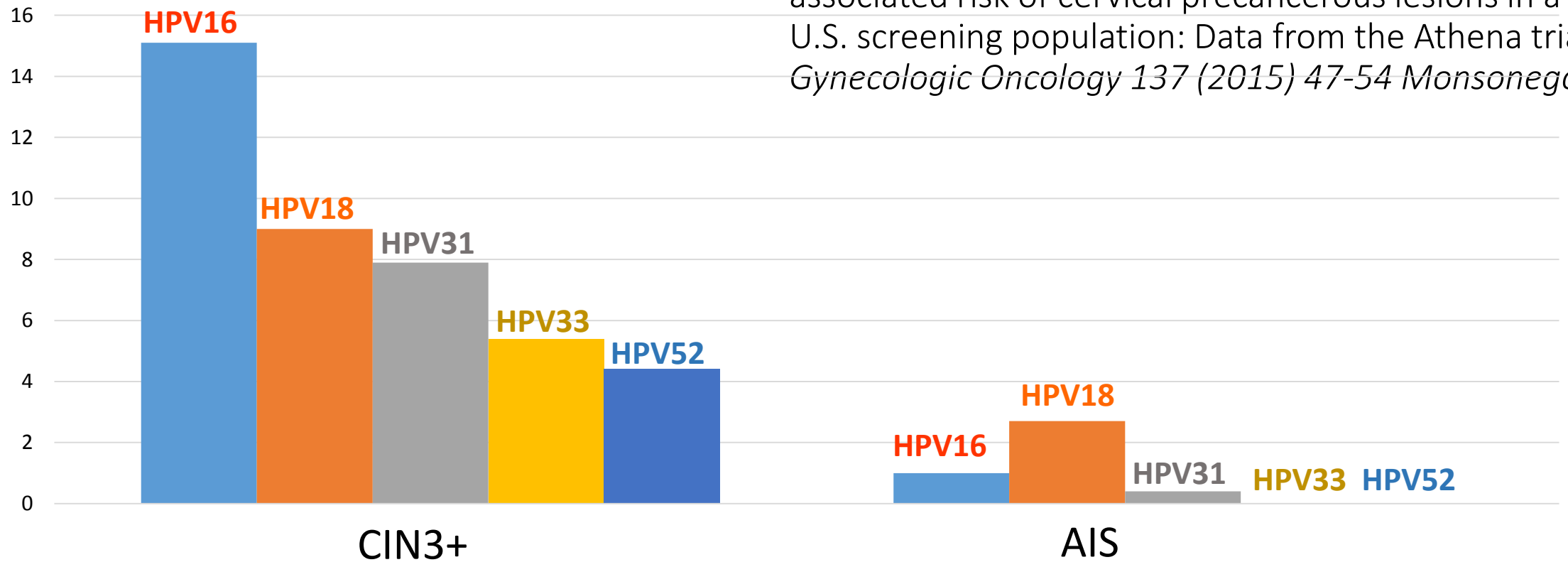
HSIL: Majority have HPV 16, 18. Also 52,31,33 in NZ

Remainder = mainly other hrHPV subtypes

Extended HPV Genotyping: would triaging the “other” oncogenic HPV subtypes be helpful?

Risk of CIN3+ and AIS by HPV subtype, women 30+ years

Prevalence of high-risk human papilloma genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: Data from the Athena trial. *Gynecologic Oncology 137 (2015) 47-54 Monsonego et al*



New Advances with HPV genotyping

Some HPV subtypes have recently been split into lineages and sub-lineages by examining their complete genomes with next-generation sequencing

Different lineages of HPV types may show differences in infection prognosis:

- HPV16 non-European lineages (A4, B, C and D) have a 2x increased risk of infection persistence and a 2-4x greater risk for development of HSIL or LSIL
- HPV31 lineage C is more persistent than A and B; lineage B is associated with high-grade lesions
- In HPV33, the A1 sub-lineage was more prevalent in cases of cervical cancer
- The B2 sub-lineage of HPV45 was significantly more prevalent in cancer cases than in controls

Acquiring HPV: Prevalence in women

Worldwide [1] : 157,879 women with normal cytology:

(any type) HPV positive = 10.4% (CI 10.2-10.7)

By region: Africa = 22.1%

Central America and Mexico = 20.4%

North America = 11.3%

Europe = 8.1%

Asia = 8.0%

Estimated that 291 million women worldwide are carriers of HPV DNA

- subtypes are the same as those with lesions, but proportions with HPV 16 and 18 are lower

Artistic trial (UK) [2] : 20,697 women 20-64 years in the English screening programme, (normal or abnormal cytology): 15.6% were hrHPV +ve

Refs:

1. *Worldwide prevalence and genotype distribution of cervical HPV in women with normal cytology: a meta-analysis. de Sanjose S et al Lancet Infect Dis 2007 Jul;7(7):453-9*
2. *HPV testing in routine cervical screening: cross sectional data from the ARTISTIC trial. Kitchener HC et al British Journal of Cancer 2006 95, 56-61*

HPV Prevalence: Age variation

Prevalence is high in women under 30 years of age then declines rapidly

- Point prevalence in sexually active young women of about 25%
- ARTISTIC (UK) Data: 20-24 years = 40% hrHPV +ve
  35-39 years = 12%
  50+ years = 7% or less
- 80% of sexually active adults are likely to be infected at some point during their life.

"Greatest behavioural risk for acquisition of mucosal HPV is sexual contact, specifically the greater number of lifetime sexual partners"

Carter et al. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 2011;51:103-08

HPV is commonly acquired when sexual activity commences

- UK Study [1] : 242 women with one sexual partner: risk of acquiring an HPV infection was about 45% after three years
- USA [2] : 603 university students in Washington State followed. At 2 years, cumulative incidence of first-time HPV infection was 32.3%. Smoking, oral contraceptive use and a new male partner were predictive of incident infection
- hrHPV infection occurs in about 50% of women within 6 months after sexual debut (Kitchener, UK)[3]

1. *High incidence of cervical human papillomavirus infection in women during their first sexual relationship. Collins A et al BJOG Jan 2002, Vol 109,pp96-98*
2. *Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Winer RL et al Am J Epidemiology 2003 Feb 1;157(3):218-26*
3. *Report to the National Steering Committee (UK). Professor HC Kitchener, Chair Advisory Committee for Cervical Screening June 2015*

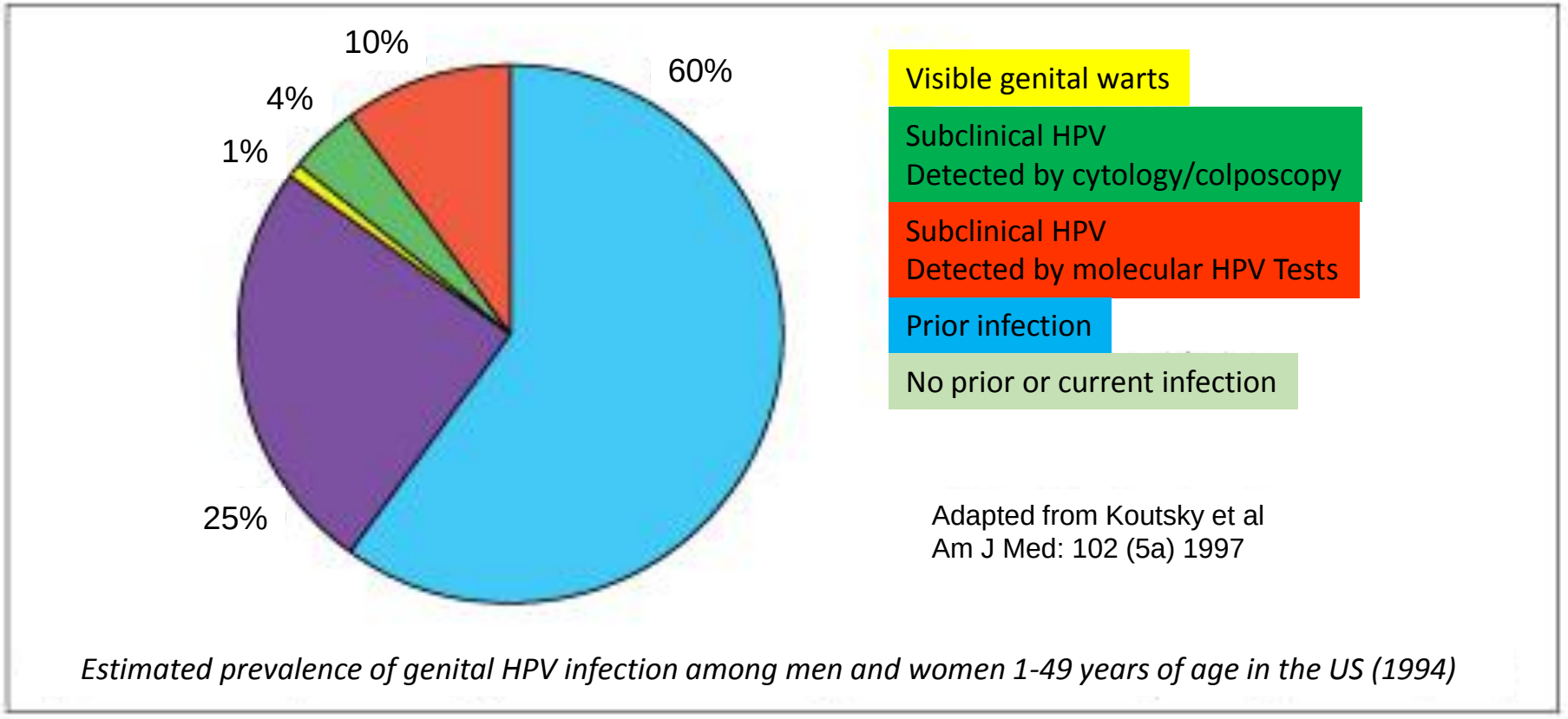
HPV Prevalence: Men

- Prevalence of anogenital HPV not as well studied
- Men who have Sex with Men (MSM) have high rates of anogenital HPV

Refs:

1. Giuliano AR et al Incidence and clearance of genital human papillomavirus infection in Men (HIM): a cohort study. The Lancet 2011;377(9769):932-402.

2. Goldstone S et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. Journal of Infectious Diseases,2011 Jan 1;203(1):66-74



Estimated prevalence of genital HPV infection among men and women 1-49 years of age in the US (1994)

Note: "Subclinical" means not visible to the naked eye

Acknowledgement: The New Zealand HPV project ***www.hpv.org.nz***

Type-specific oncogenic human papillomavirus infection in high grade cervical disease in New Zealand

Leonardo Simonella, Hazel Lewis, Megan Smith, Harold Neal, Collette Bromhead and Karen Canfell

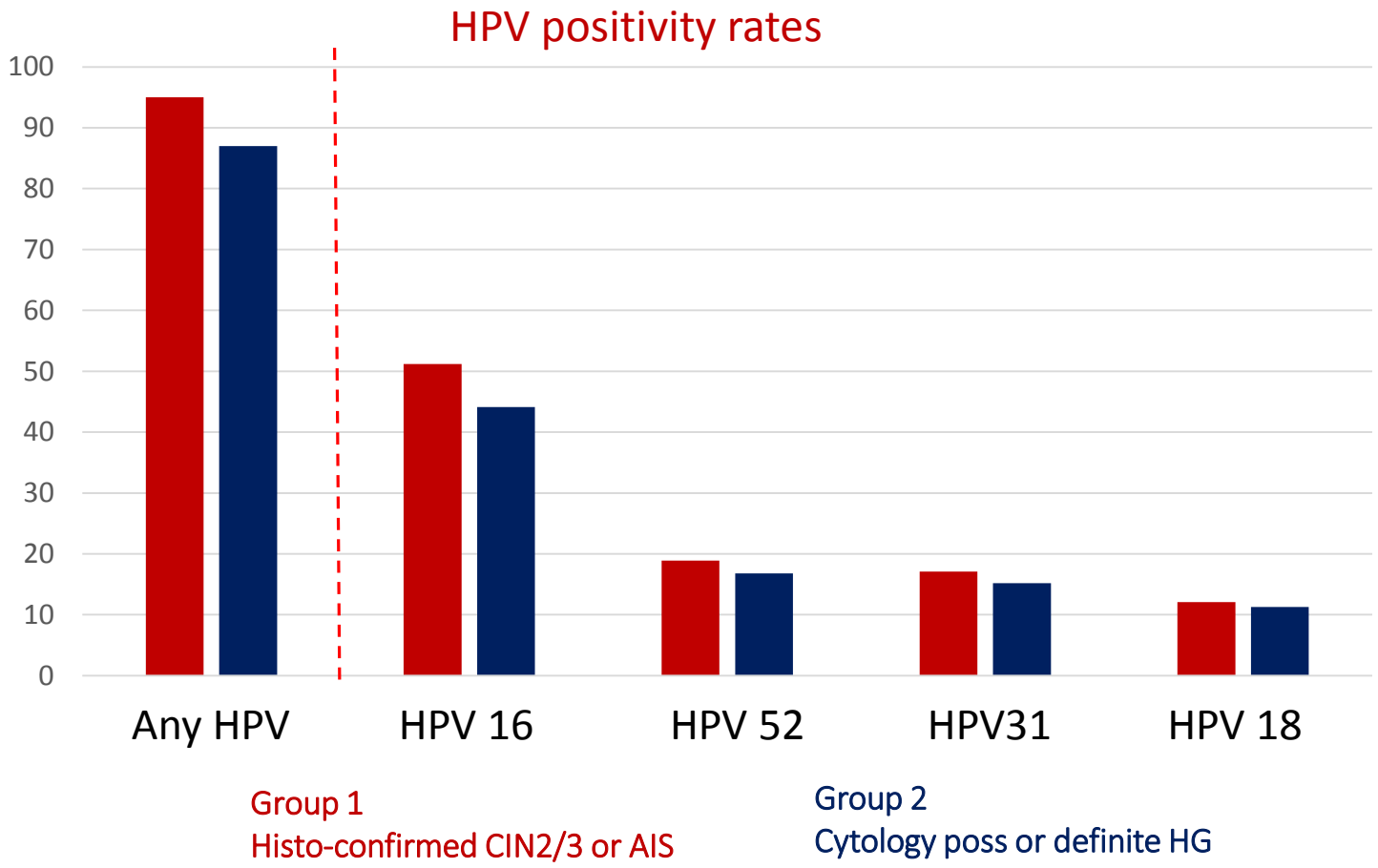
BMC Infectious Diseases 2013, 13:114

Aim: to measure the pre-vaccination prevalence of hrHPV in 20-69 yrs women with high-grade squamous and glandular lesions

594 women 20-69 years from NCSP-Register 2009-2011

LBC specimens were genotyped for 37 HPV subtypes

Results



Group 1: Histology confirmed

356 women = CIN2/3

6 women = AIS/glandular dysplasia

Any hrHPV infection: 95%

HPV 16/18: 60%

Most common types: **16** (51.2%)

52 (18.8%)

31 (17.1%)

18 (12.1%)

Group 2: Possible or definite HG cyto

594 Women

Any hrHPV infection: 87%

HPV 16/18: 53%

Most common types: **16** (44.1%)

52 (16.8%)

31 (15.2%)

33 (11.8%)

18 (11.3%)

Acquiring HPV: How do you get it?

Almost always sexually transmitted

Probability of transmission is very high: greater than 50% following unprotected sexual intercourse with a person with a productive anogenital infection

Greatest behavioural risk of HPV infection is sexual contact

- HPV infection rates increase in proportion to the number of lifetime sexual partners
- can get low-risk HPV transmitted to the vulva/vagina via fingers, tampons. Transmission by non-penetrative sexual intercourse is described
- vertical transmission in Juvenile Respiratory Papillomatosis
- condoms may reduce risk but are not fully protective

What happens next?

Acute infection generally occurs 6-12 weeks after exposure but the latency period is very variable

- Warts often appear after 3-6 months but can be much later
- HPV can be detected by molecular techniques before the acute infection is apparent by cytology/colposcopy

- Women who have been sexually inactive for many years can suddenly develop genital warts or cervical abnormalities
  - Important that women know that developing warts or cervical lesions many years into a long-term sexual relationship does not necessarily imply other recent sexual contacts

Developing lesions: what can happen after acquiring an HPV Infection?

1. **Asymptomatic** infection with no cytologic/histologic lesion, which resolves
2. **Asymptomatic** infection with a low-grade lesion detectable by cytology/histology
3. **Asymptomatic** infection with a high-grade lesion detectable by cytology/histology. May progress if persists and become **symptomatic if invasive**.
4. **Symptomatic** warts: usually low-risk HPV

Whether hrHPV Testing is positive will depend on the HPV subtype and the viral load

HPV Lifecycle

Entry to the cell:

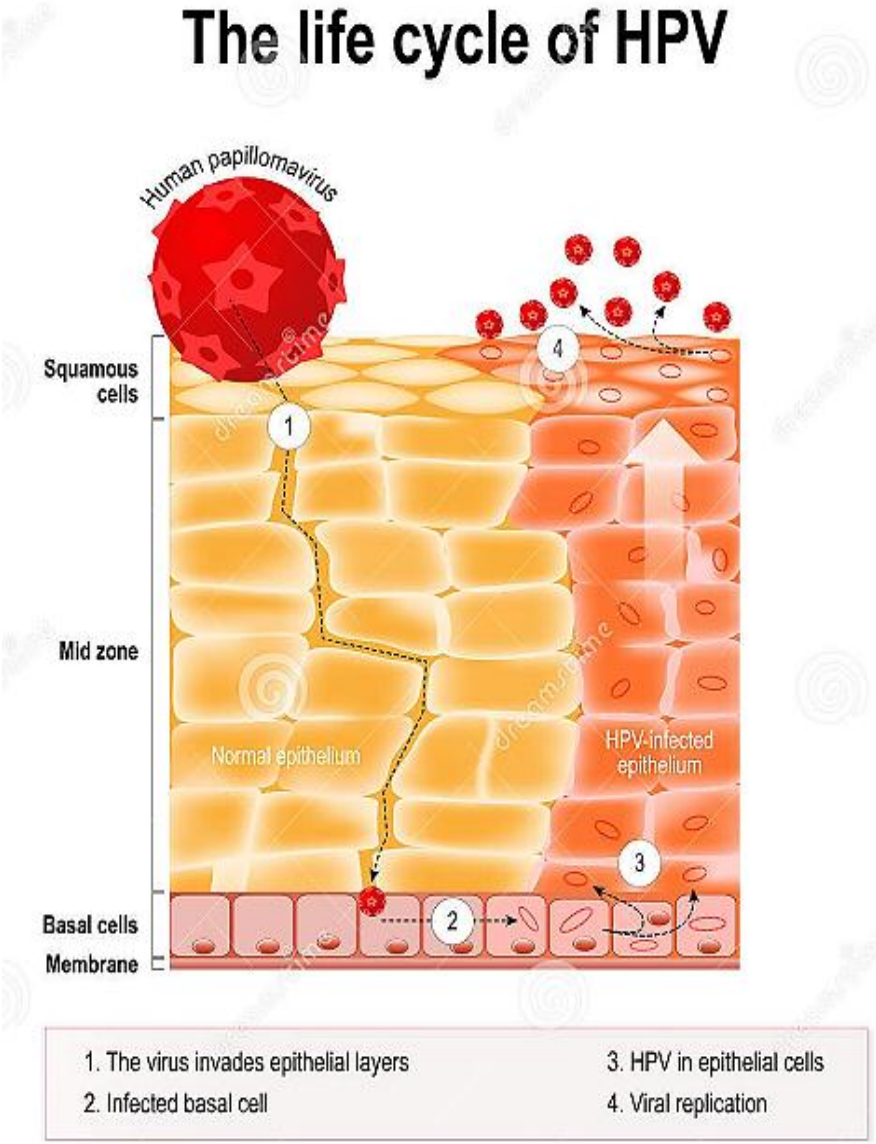
- Virus infects basal cells via minor abrasions
- Virus enters the cell by endocytosis
- Viral DNA migrates into the host cell nucleus

In the nucleus:

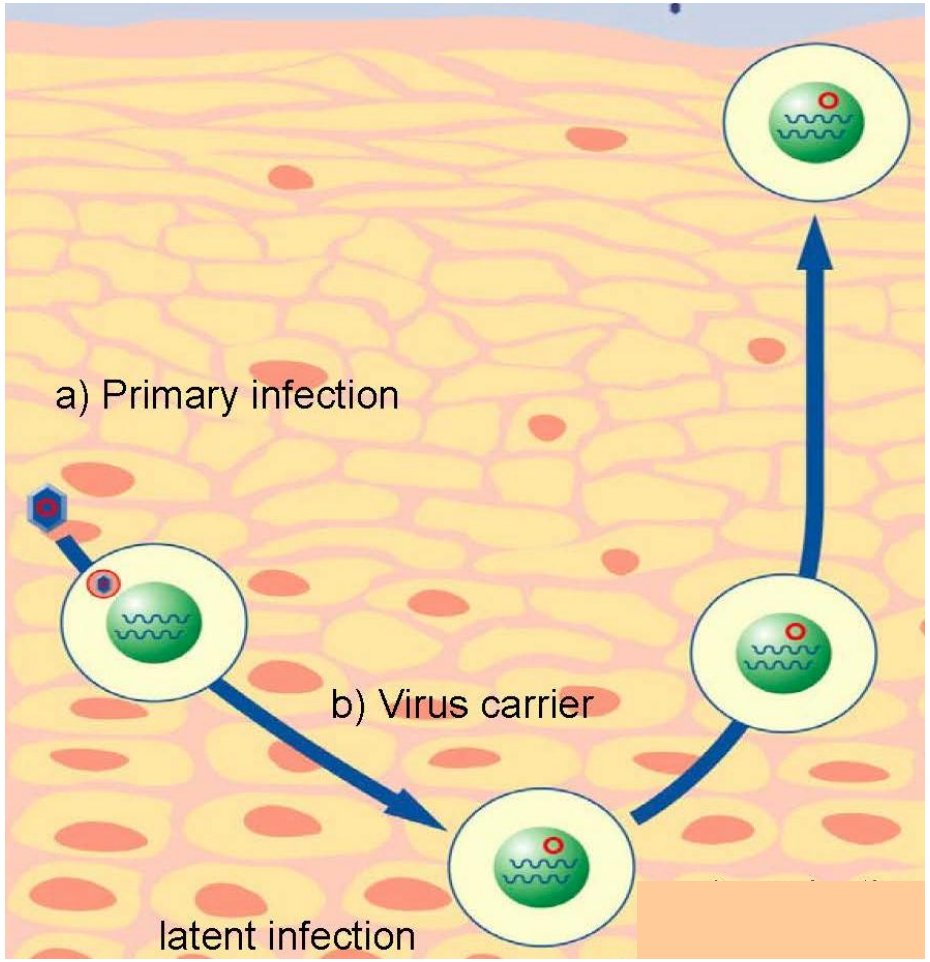
- Host cell factors regulate transcription
- Begins transcription of E6 and E7 genes
- Modifies the cellular environment to facilitate viral replication

Papillomaviruses must infect a dividing cell to become established

For a persistent lesion to develop the initial infected cell is likely to be a long-lived epithelial stem or stem-like cell



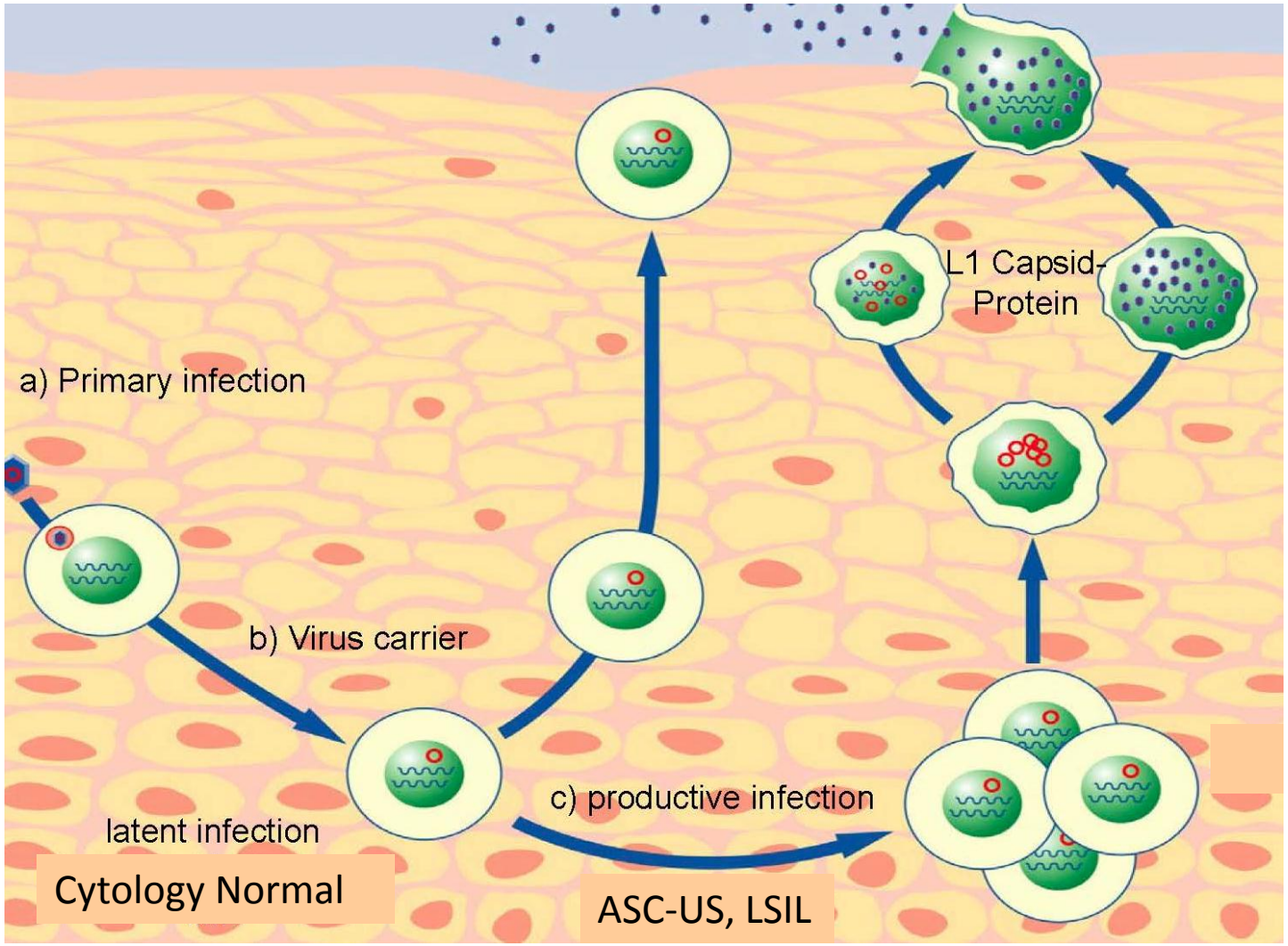
What is happening in the epithelium?



low viral load; no cytologic abnormality

- HPV infects epithelial cells
- the virus enters through microabrasions in the epithelium which allow it to be deposited on the basement membrane where it binds
- vial particles enter basal cells over the next 2-4 hours
- as soon as they are taken up, the envelope protein is decomposed and the viral DNA enters the cell nucleus where it exists as episomes within the nucleus but separate from the host DNA

What if a productive viral infection develops?



Episomal HPV DNA remains in the nucleus

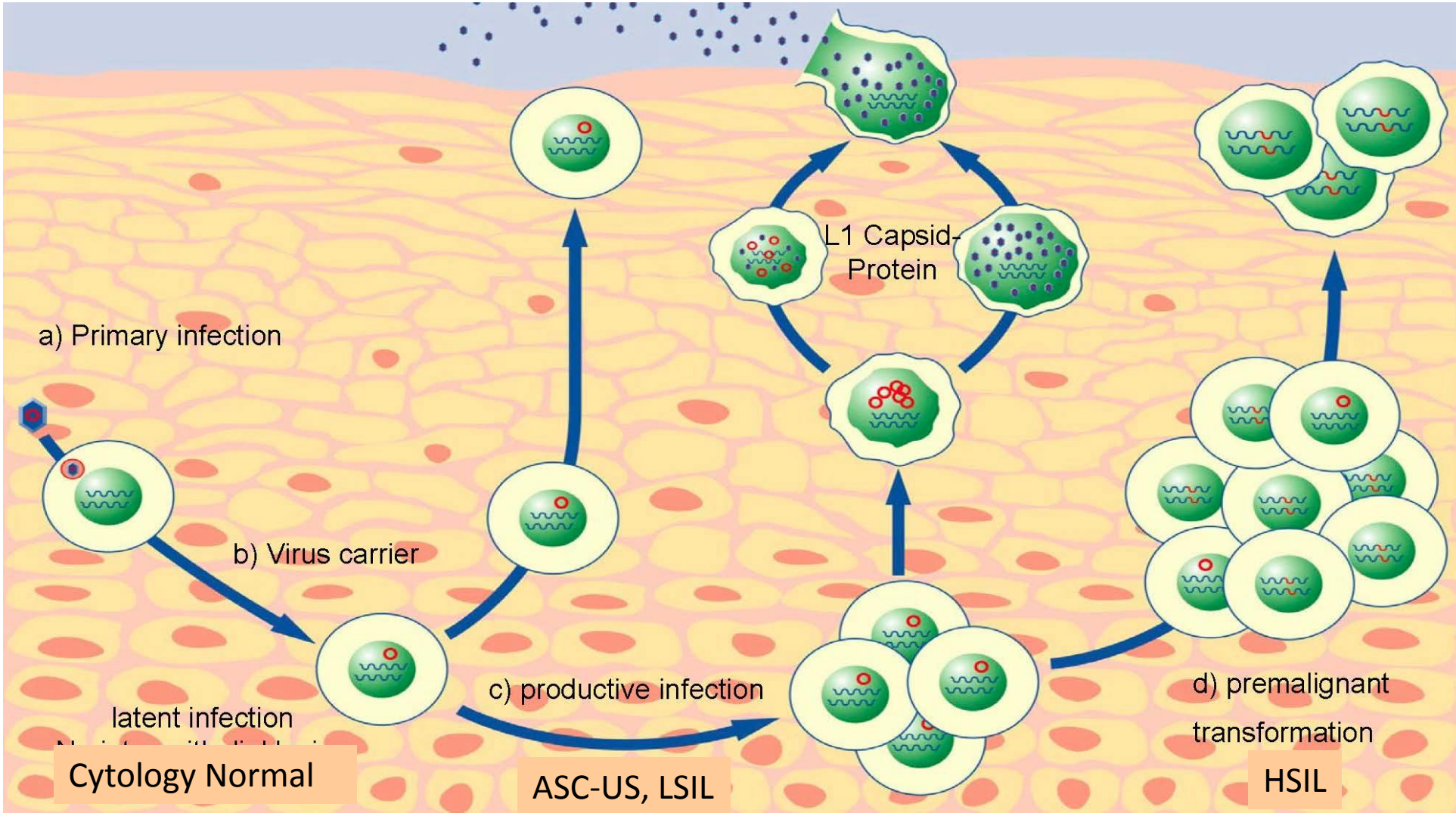
- Viral assembly occurs in the maturing squamous cell resulting in release of viral particles

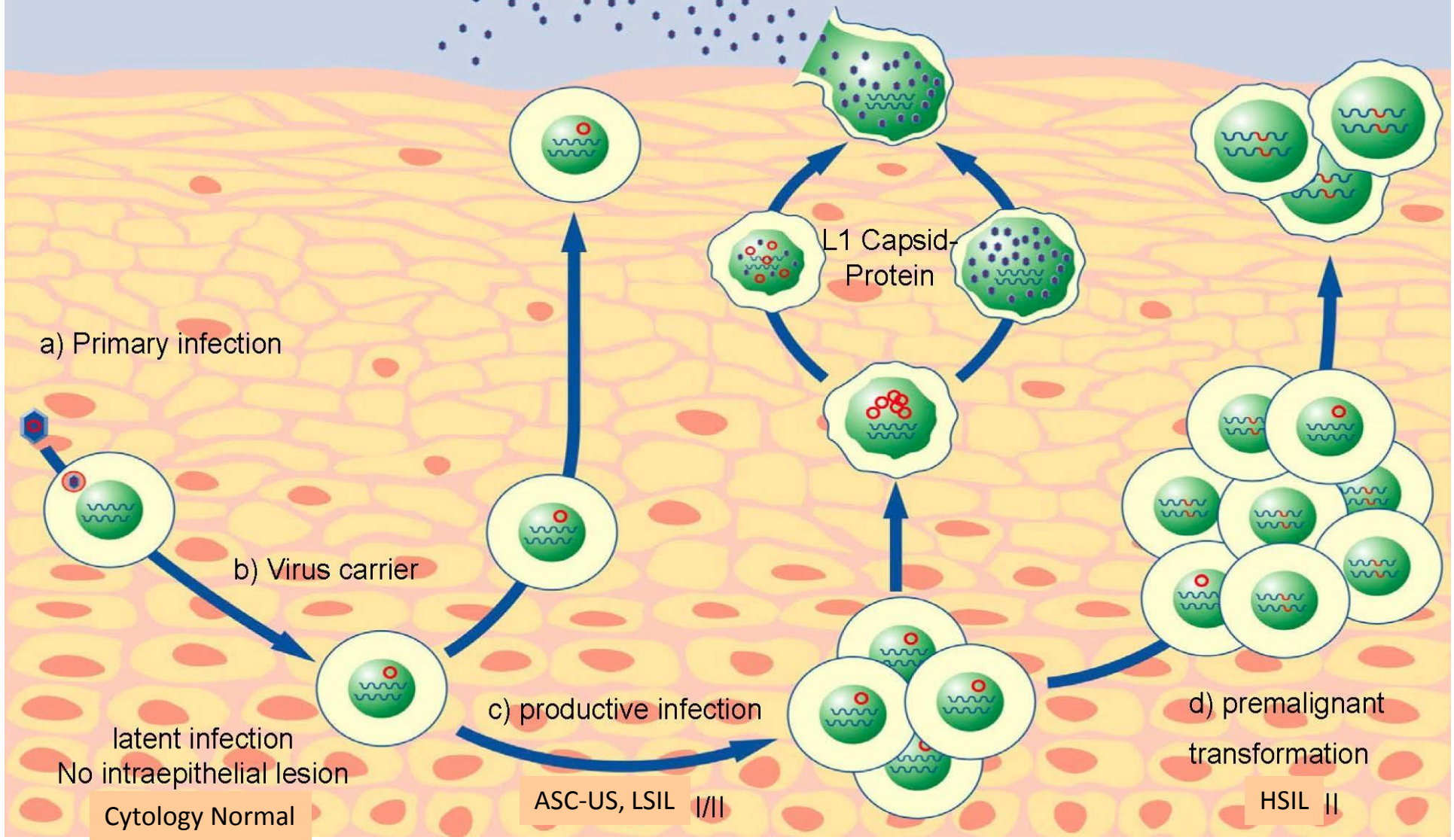
-Takes three weeks (time for basal cell- superficial cell maturation)

high viral load; low-grade cytologic/histologic abnormality

What about high-grade lesions?

- HPV DNA becomes linear and integrates into host DNA
- results in cellular effects leading to uncontrolled cell proliferation

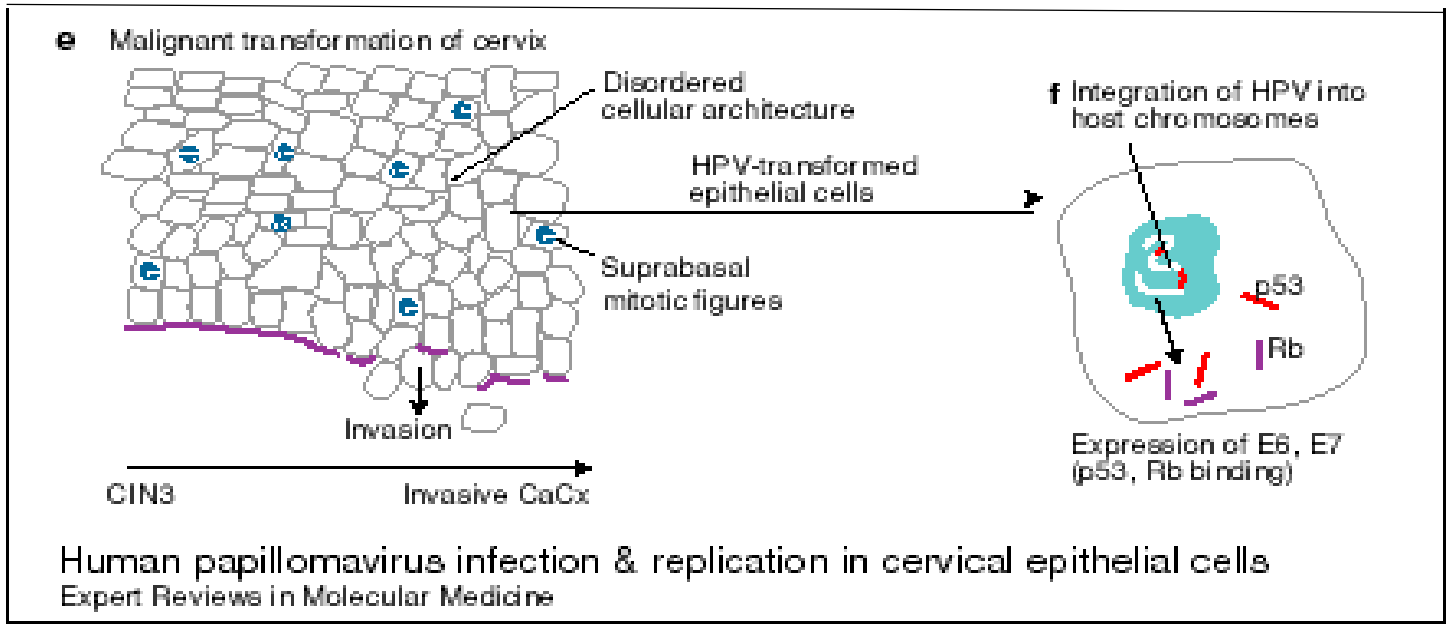




"Human Papillomavirus and Related Diseases From Bench to Bedside A Diagnostic and Preventive Perspective"
Ed: Davy Vanden Broeck, ISBN 978-953-51-1072-9, Published: April 30, 2013 © The Author(s).
Chpt 4:HPV L1 Detection as a Prognostic Marker for Management of HPV High Risk Positive Abnormal Pap Smears (Ralf Hilfrich)

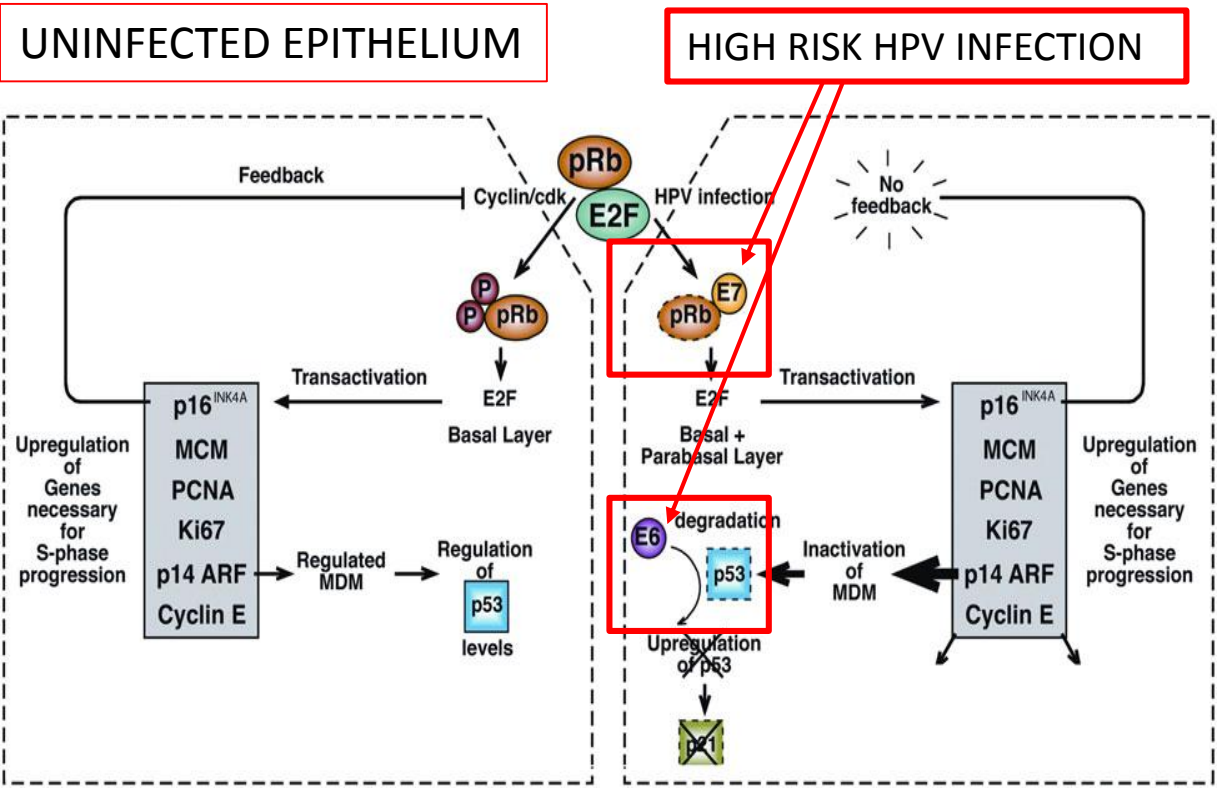
HPV Integration in high-grade lesions

- The viral DNA becomes linear instead of circular enabling integration with host DNA
- The break occurs in the E2 region
- E6 and E7 bind with p53 and pRB which causes increased proliferation and genomic instability
- The host cell accumulates more and more damaged DNA which cannot be repaired
- Mutations accumulate leading to fully transformed malignant cells



Human papillomavirus infection & replication in cervical epithelial cells
Expert Reviews in Molecular Medicine

E6 and E7 effects



p53 protein: degraded by HPV E6

- is a tumour suppressor protein.
- has been described as “the guardian of the genome" because of its role in conserving stability by preventing genome mutation.
- initiates DNA repair, cell cycle check points and apoptosis

pRb protein: degraded by HPV E7

- also a tumour suppressor protein
- responsible for regulating cell cycle and preventing replication of damaged DNA in the cell

Degradation of these proteins results in unscheduled cell replication and cell division causing genomic errors that are not repaired leading to increasing accumulation of genetic errors.

Gene Function: Different functions of E6 and E7 in high risk and low risk HPV infections

Binds and degrades p53

Bypass of growth arrest following DNA damage

Binding and degradation of pRB

Induction of genome instability

	HIGH RISK HPV	LOW RISK HPV
E6	encodes E6* products	no E6* products
	binding and degradation of… •p53 •specific PDZ-domain proteins (e.g. Dlg, MAGI-1, Scribble)	weaker binding (no degradation) of… •p53 •no binding of PDZ-domain proteins
	interact with the E6AP ubiquitin ligase inhibition of p53 transactivation and acetylation	
	inhibition of apoptosis	unknown
	bypass of growth arrest following DNA damage	normal growth arrest following DNA damage
	inhibition of keratinocyte differentiation	unknown
	inhibition of interferon response	weaker inhibition of interferon response
	activation of signaling pathways… •Akt •Wnt •Notch •mTORC1	unknown
	telomerase activation	no activation
	c-myc activation	no activation
E7	binding and degradation of… •pRb •p107 •p130	weaker binding (no degradation) of… •pRb •p107 •E2F1
	binding (no degradation) of… •E2F1 •Cullin2 •HDAC	binding of… •p130
	binding of regulatory proteins including E2F6, p600, HAT, PP2A induction of cell cycle entry and DNA synthesis role in genome amplification	
	induction of genome instability	no stimulation of instability
	suppression of STAT-1 function	no suppression
	immortalization and transformation functions	no such functions
	activation of signaling pathways… •Akt	unknown

Weaker binding and no degradation of p53

Normal growth arrest following DNA damage

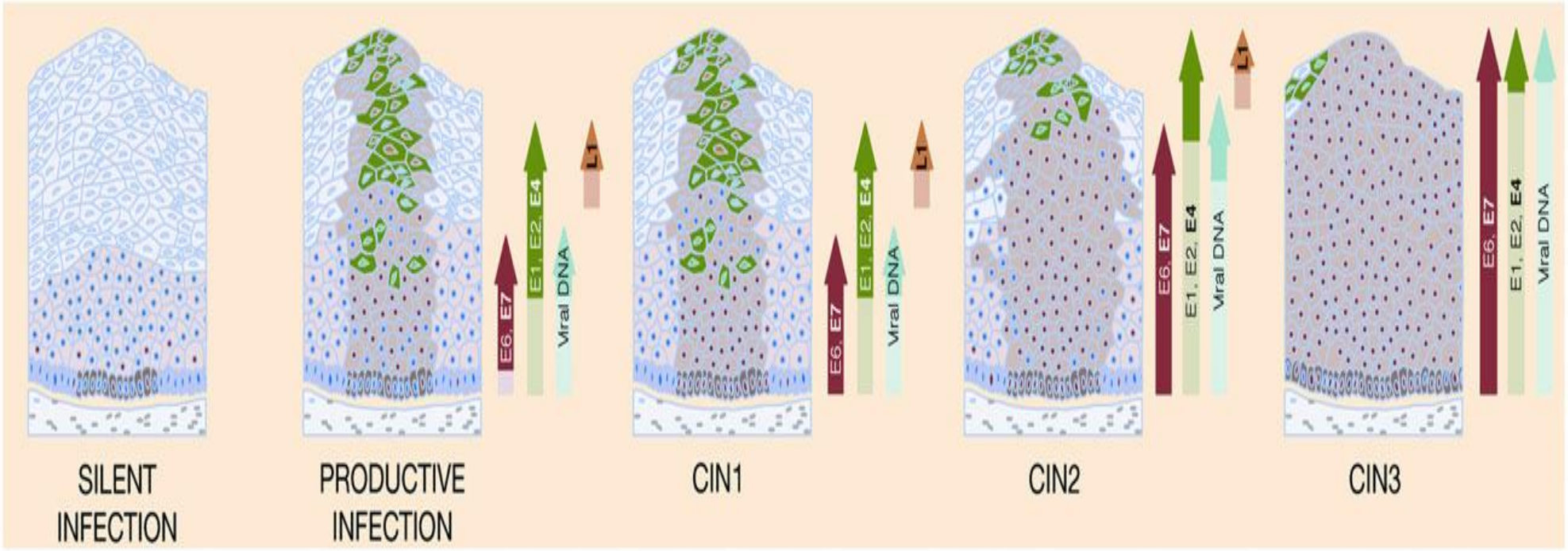
Weaker binding and no degradation of pRB

No stimulation of genome instability

Doorbar J., Reviews in Medical Virology 2016

Gene Expression

Gene expression varies in all the different states of HPV infection



What is p16?

- a normal cell protein that regulates the cell cycle by turning off cell proliferation.
- Retinoblastoma protein (rPb) binding to E2F normally controls cell proliferation. When hrHPV infects the cell, the viral oncogene E7 disrupts the binding of rPb to E2F.
- This allows the cell to proliferate at an abnormally high rate: p16 levels increase dramatically.

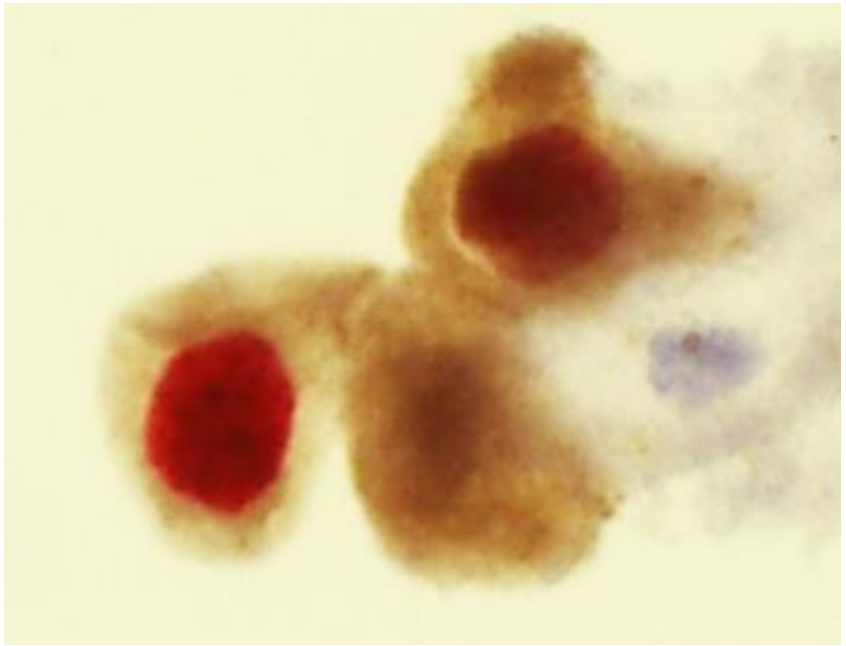
What is Ki67?

- A nuclear and nucleolar protein that is expressed in cells that are proliferating, but is not expressed when cells are not (“resting phase”)

Dual p16 and ki67 staining

Positive when there is Ki67 nuclear staining (red) and p16 cytoplasmic staining (brown) in the same cell.

- Dual staining indicates concurrent cell proliferation (ki67) and deregulation of the cell cycle (p16 overexpression)
- Not used in NZ currently but an area of active research



Progression: what happens over time?

- Old idea of CIN1 ⟶ CIN2 ⟶ CIN3 now regarded as an oversimplification: many CIN3 lesions probably arise de novo

- Factors associated with progression: ongoing HPV exposure, hrHPV genotype, HPV viral load, immune status

- Smoking is an independent risk cofactor which may increase progression rates

Clearing the virus: becoming HPV negative

Resolution = the virus is eradicated or suppressed to non-detectable levels

Most HPV infections are rapidly cleared by cell-mediated immune mechanisms, usually in 6-12 months.

- 80-90% become HPV negative on HPV testing by 2 years.
- 98% of infections resolve spontaneously in 3-5 years
- hrHPV subtypes take longer to clear. Type-specific viral persistence may be seen in around 30% of women with hrHPV subtypes after 2 years.

The 2% of women in whom high-risk HPV infection persists after 5 years, are the group at significant risk for developing cervical cancer. Persistent HPV infection is associated with increasing risk of high-grade disease.

What role does immunity play?

The HPV life cycle is within squamous cells so is hidden from host immune systems

- no blood-borne viraemic phase
- there is little or no release of cytokines to activate an immune response
- infected epithelial cells undergo natural death, without invoking an inflammatory response
- if an immune response does occur after natural infection, there are LI neutralising and binding IgG and IgA antibodies throughout the epithelium which bind to HPV before it is able to bind to the basement membrane and infect basal epithelial cells

Clearing the virus: resulting immunity

Clearance leaves a woman partly or fully immune to that particular genotype

- after acute infection with hrHPV with HPV 16, only about 50% of women will seroconvert i.e. many women do not have antibodies to HPV 16 or 18 despite having had an infection
- an insufficient immune response leaves these women at risk of future infection with the same HPV subtype

Immunisation helps a lot here because the antibody titres induced by the vaccines are considerably higher than those achieved by natural infection

HPV and Cervical Cancer

HPV infection is necessary but not sufficient in causing cervical cancer

Cancer is a rare outcome of hrHPV infection. Women worldwide:

- Estimated prevalence of genital HPV infection = 326 million
- Annual incidence of new cases of cervical cancer = 530,000

Progression to cancer is generally slow

- Modal age of first hrHPV infection between 15 and 25 years.
- Modal age of invasive cervical cancer is 35-50 years (in unscreened women, some differences geographically)

HPV subtypes and cancer

- HPV 6 and 11 rarely cause cervical cancer
- HPV 16 + 18 cause about 70% of invasive cervical cancer cases
  - HPV 16 is the most common and highest risk subtype with the highest progression rate. Present in 55% of SCC
    48% endocx AdenoCa
  - HPV 18 present in 12% of SCC, 36% endocx AdenoCa
  - HPV 45, 31, 33 in 4-5% for invasive cancer, any type
- HPV 16 is strongly associated with anal and oropharygngeal cancer

Worldwide prevalence

Same HPV subtypes cause cervical cancer with little regional variation

- no population-based genetic predisposition
- risk is directly related to amount and time of HPV exposure
- are some identified cofactors such as smoking

We don’t know (yet) why some women with HPV infections don’t clear the virus and progress to invasive cancer. Likely to be related to host immunity in some way.

Type distribution of human papillomavirus among adult women diagnosed with invasive cervical cancer (stage 1b or higher) in New Zealand

Peter Sykes, Kusuma Gopala, Ai Ling Tan, Diane Kenwright, Simone Petrich, Arico Molijn, and Jing Chen
BMC Infectious Diseases 2014,14:374

- Women ≥18 years of age with ICC FIGO stage 1b or higher diagnosed 2004 - 2010 from five NZ hospitals
- Stored paraffin embedded cervical specimens were used with HPV genotyping performed on malignant tissue

Results: HPV Positivity rates

Invasive cancer cases n=227

Squamous cell carcinoma (SCC) = 70% (n=159)

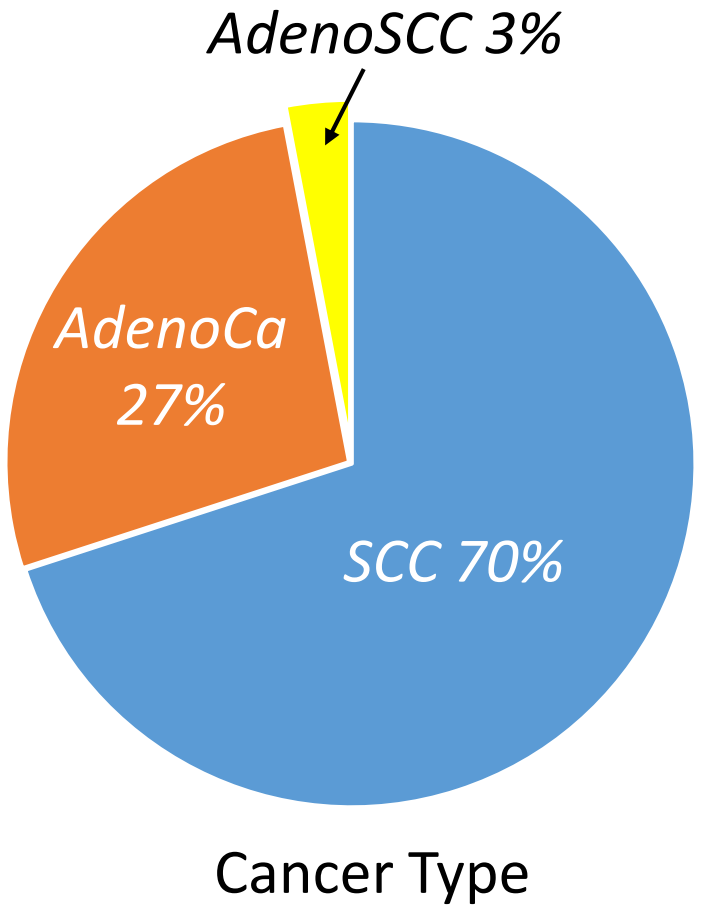
Adenocarcinoma (ADC) = 27% (n=61)

Adenosquamous carcinoma (ASC) = 3% (n=7)

HPV was detected in 88.5%

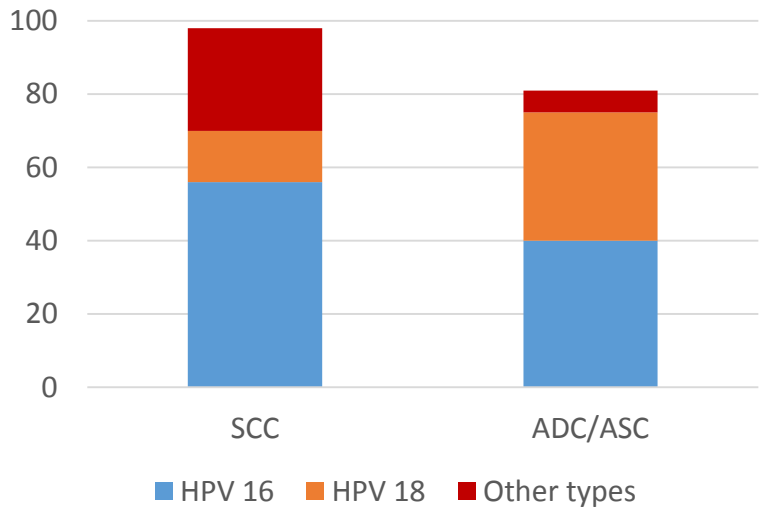
SCC = 93.1%

ADC/ASC = 77.9%



Results: HPV subtypes

Invasive cancers by type

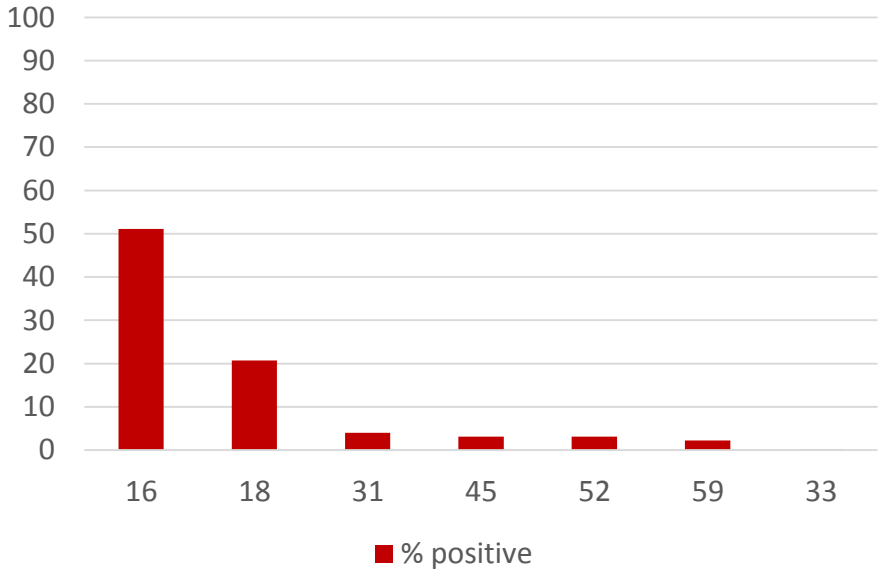


For the 227 ICC cases, HPV 16 and 18 were the most frequent

HPV 16 = 51% : 56% SCC
40% ADC/ASC

HPV 18 = 21% : 15% SCC
35% ADC/ASC

All cancers: genotypes



For the 201 HPV positive cases

HPV 16 + 18 = 81.1%

Frequent non-16/18 types: 31, 45, 52, 59,33

Two low-risk HPV types (11,70) both SCC

Multiple subtypes

5.5% had multiple subtypes present (Both SCC and ADC/ASC)

Five Main Points

1. HPV infection is sexually transmitted, common and usually asymptomatic and transient. High prevalence of infection in women under 30 years of age
2. High-grade lesions occur when HPV DNA is integrated into host DNA, resulting in a cascade of cellular events that result in uncontrolled cell proliferation
3. Clearance can mean eradication of viral DNA or suppression to undetectable levels: latent infection can be reactivated years later
4. Immunity following natural infection is variable whereas immunisation achieves high antibody titres
5. Cancer is a rare outcome of HPV infection