HPV and Cervical Cancer

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March 2019

Topics

1. HPV and cervical cancer
2. The Human Papillomavirus: what is it?
3. Acquiring an HPV infection: How do you get it and get rid of it
4. Developing lesions: What happens if you don’t clear it?
5. Immunising against HPV: Preventing HPV infection

1. HPV and Cervical Cancer

HPV infection is necessary but not sufficient in causing cervical cancer

Cancer is a rare outcome of hrHPV infection.

- 80% of sexually active adults are likely to acquire an HPV infection during their lifetime

- 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

- age of first hrHPV infection usually between 15 and 25 years
- peak age of invasive cervical cancer is 35-50 years

HPV subtypes and cancer

- HPV 6 and 11 are common low-risk subtypes: cause genital warts frequently and rarely, can cause cervical cancer
- HPV 16 + 18 are the most common high-risk subtypes
  - cause about 70% of invasive cervical cancer cases
  - HPV 16 is the most common, the highest risk subtype and has 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% of invasive cancers, any type
  - HPV 16 is strongly associated with anal and oropharygngeal cancer
- The hrHPV test used in clinical practice, tests for 14 highrisk HPV subtypes

Worldwide subtype 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

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

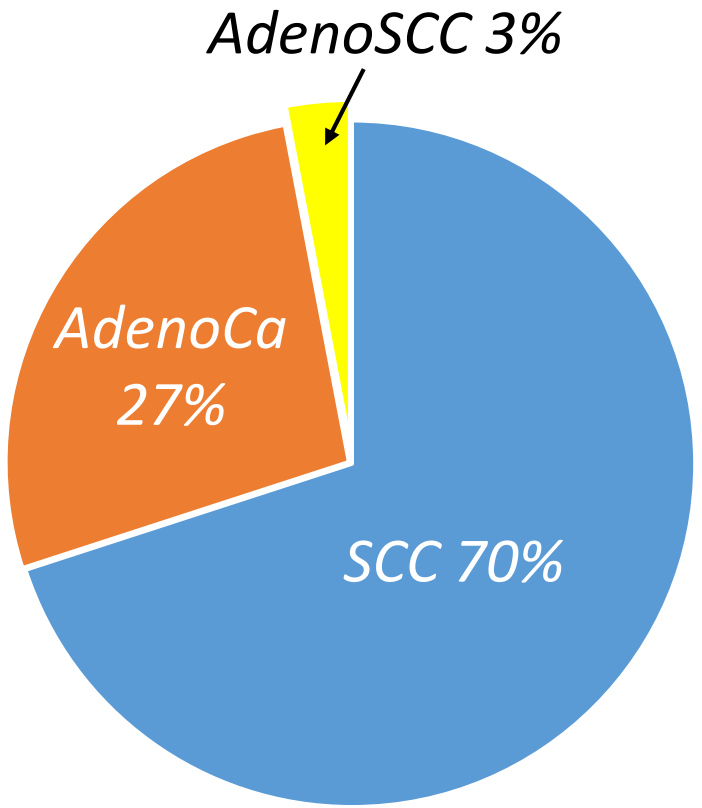
HPV Positivity rates

Invasive cancer cases n=227

HPV was detected in 88.5% overall

SCC = 93.1%

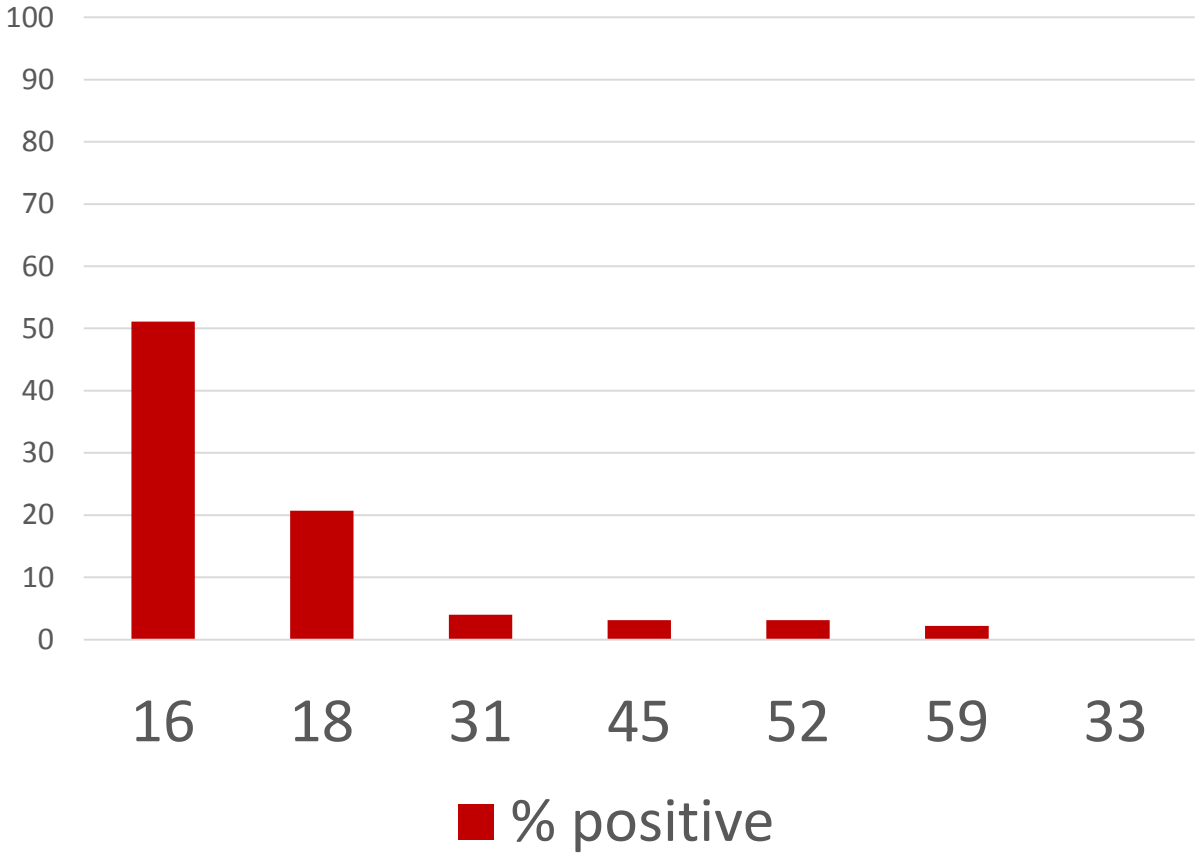
ADC/ASC = 77.9%



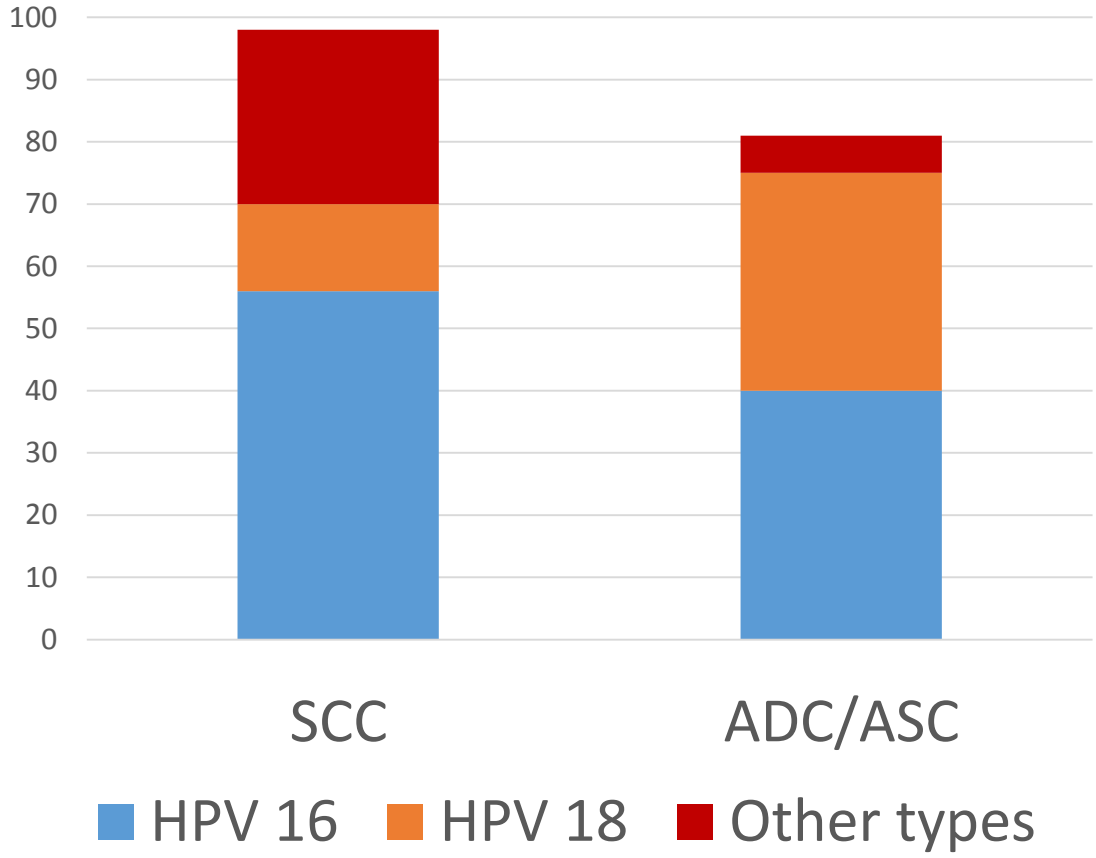
Invasive cervical cancers in New Zealand 2004-2010

Results: HPV subtypes

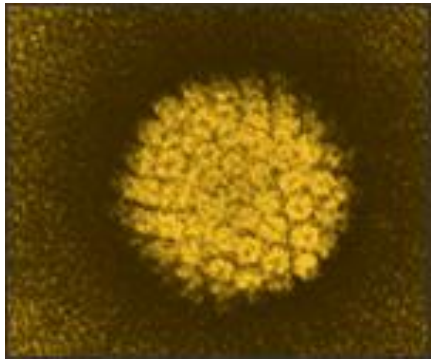
All cancers: HPV genotypes



Invasive cancers by type



2. Human Papillomaviruses (HPV)



HPV's: DNA viruses that infect human skin and mucosal epithelia
More than 200 different subtypes (genotypes)

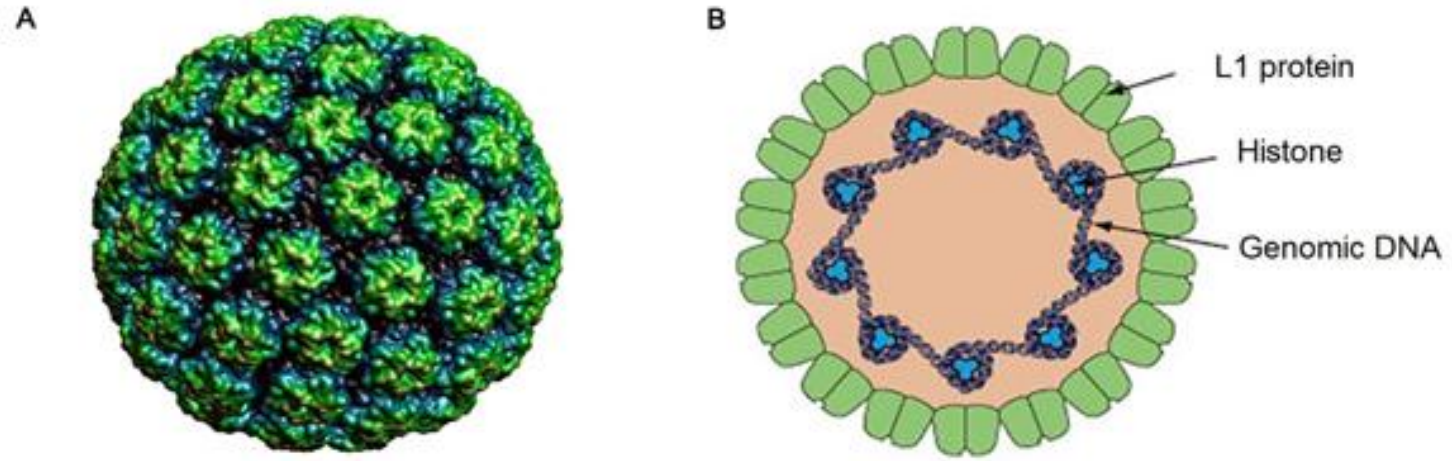
HPV subtypes are divided into cutaneous or **mucosal types:** at least 40 subtypes of HPV infect the genital tract/oropharynx

- genital warts are usually caused by low-risk HPV types
- high-risk HPV infections are predominantly caused by about 14 high-risk subtypes that 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.

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.



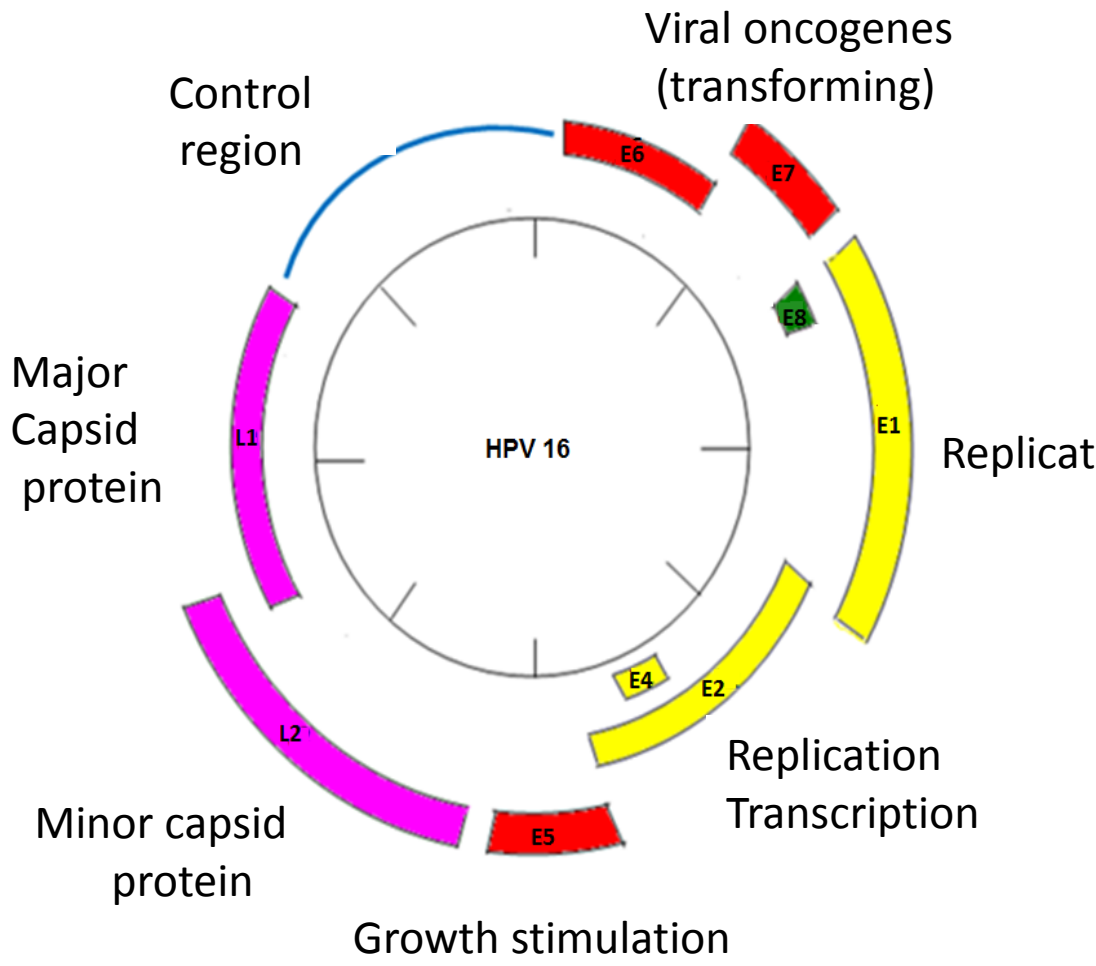
HPV Genome

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 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

Most women have one subtype, some have multiple subtypes

3. Acquiring HPV: Prevalence

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

HPV (any type) positive = 10.4% (CI 10.2-10.7) but geographic variation ++

- subtypes are the same as those with lesions, but the proportion who are HPV 16 or 18 positive is 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

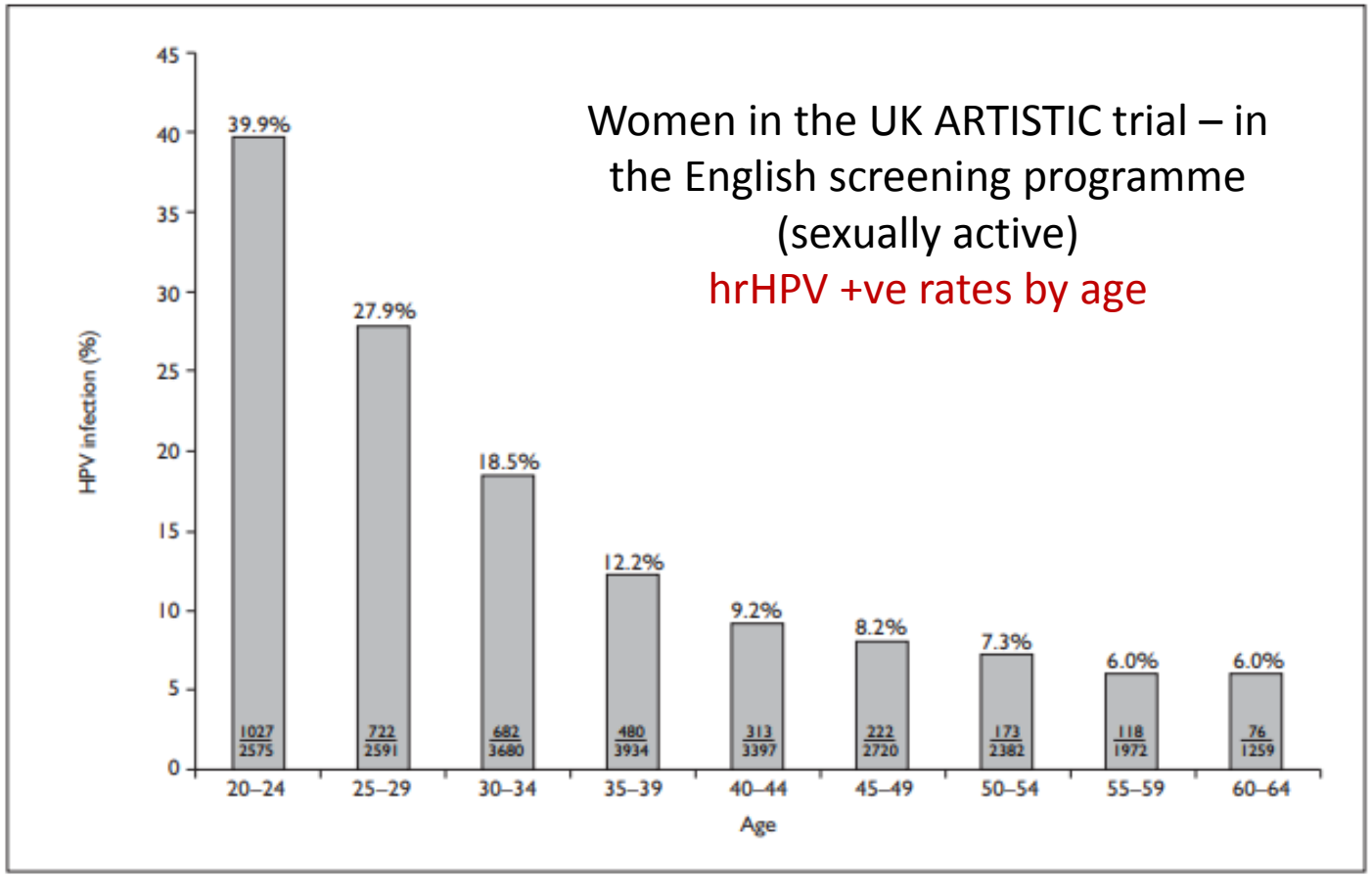
ARTISTIC trial data(UK):

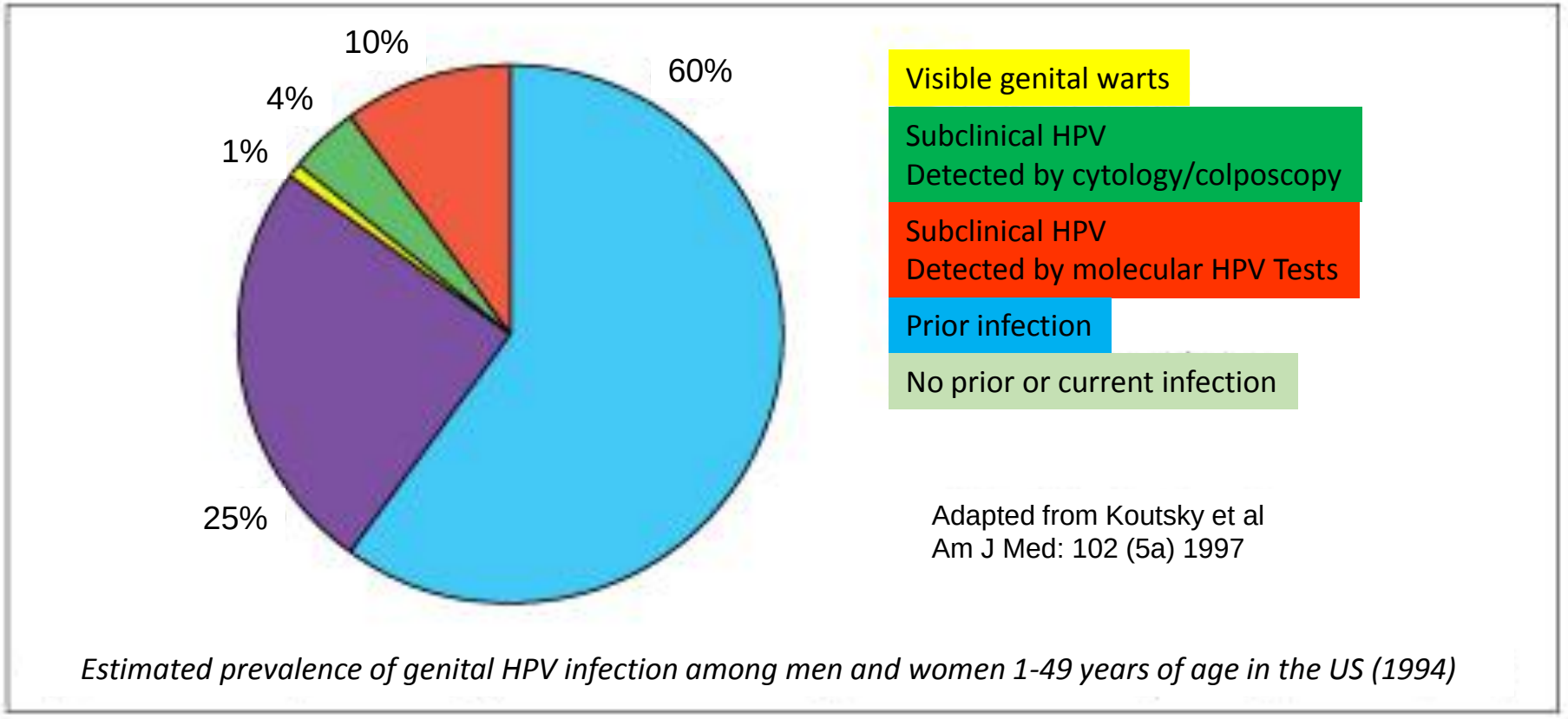
hrHPV +ve rates

20-24 years = 40%

35-39 years = 12%

50+ years = 7% or less





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***

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

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

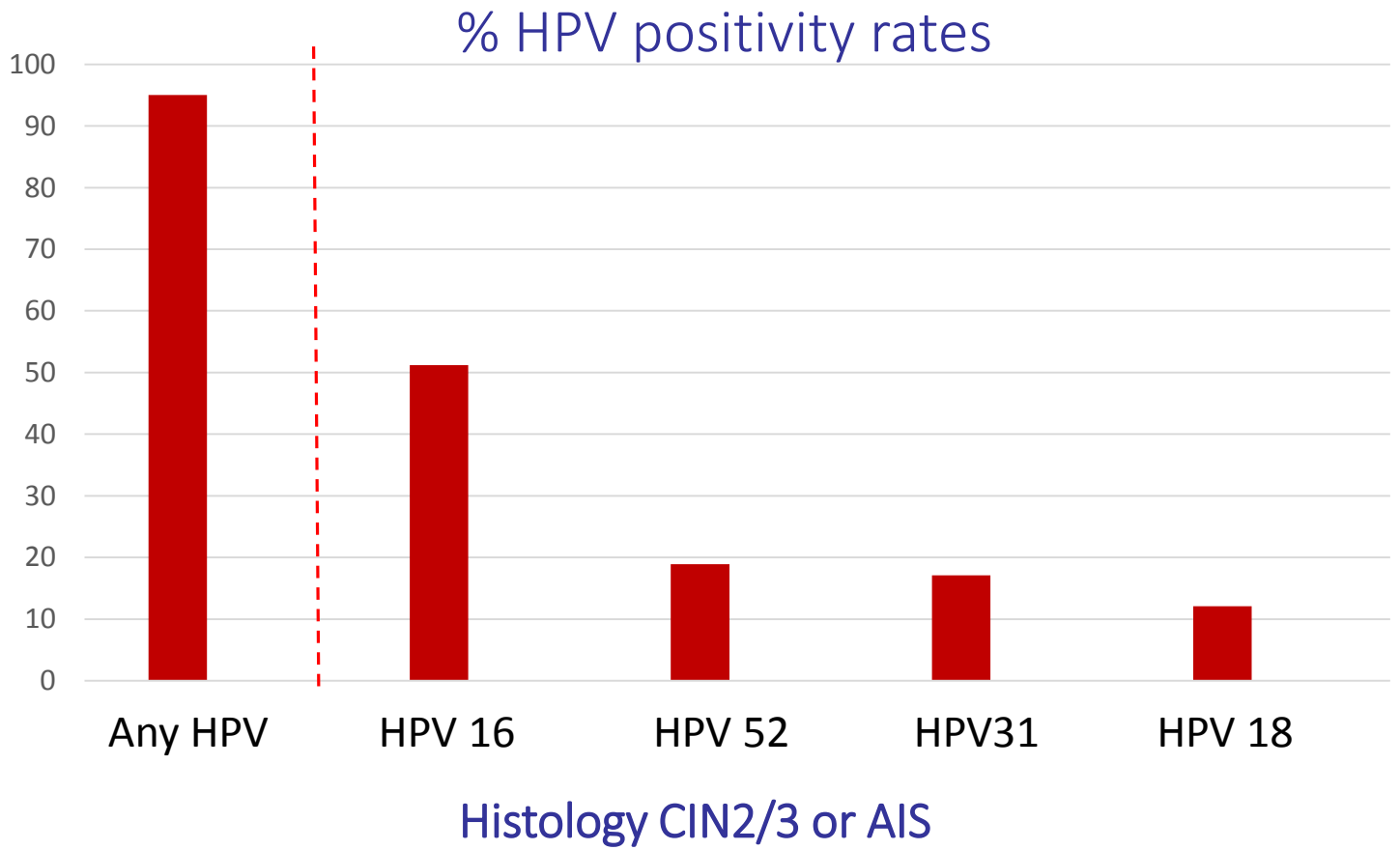
BMC Infectious Diseases 2013, 13:114

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 for histologically confirmed lesions



Histology

356 women = CIN2/3

6 women = AIS/glandular dysplasia

Any hrHPV infection: 95%

HPV 16/18: 60%

Most common subtypes:

16 (51.2%)

52 (18.8%)

31 (17.1%)

18 (12.1%)

Clearing the virus: becoming HPV negative

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. 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.

Clearing the virus: resulting immunity

Clearance may/may not leave 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
- 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

Clinical course

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

4. 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.
4. May progress if persists and become **symptomatic if invasive cancer**.
5. **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:

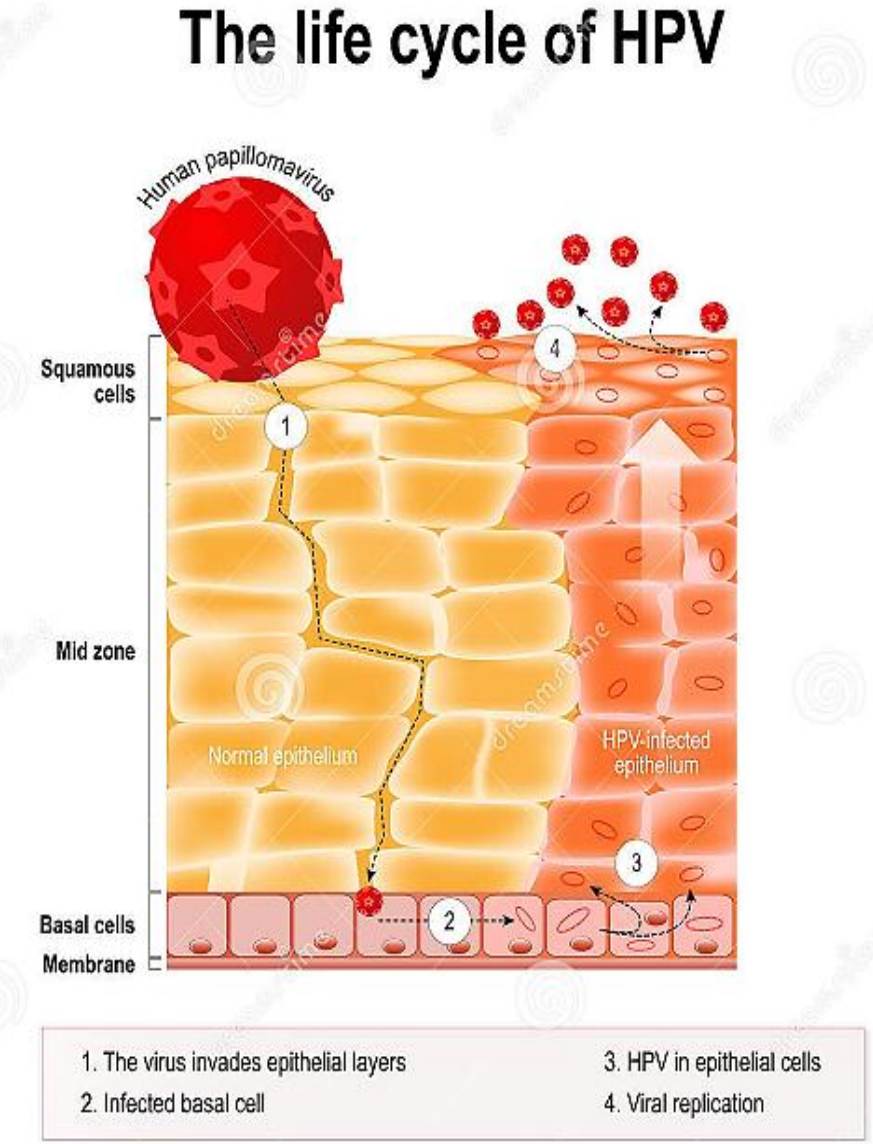
- Virus infects basal cells via minor abrasions
- Virus enters the cell by endocytosis
- Viral DNA migrates into the host cell nucleus

In the nucleus:

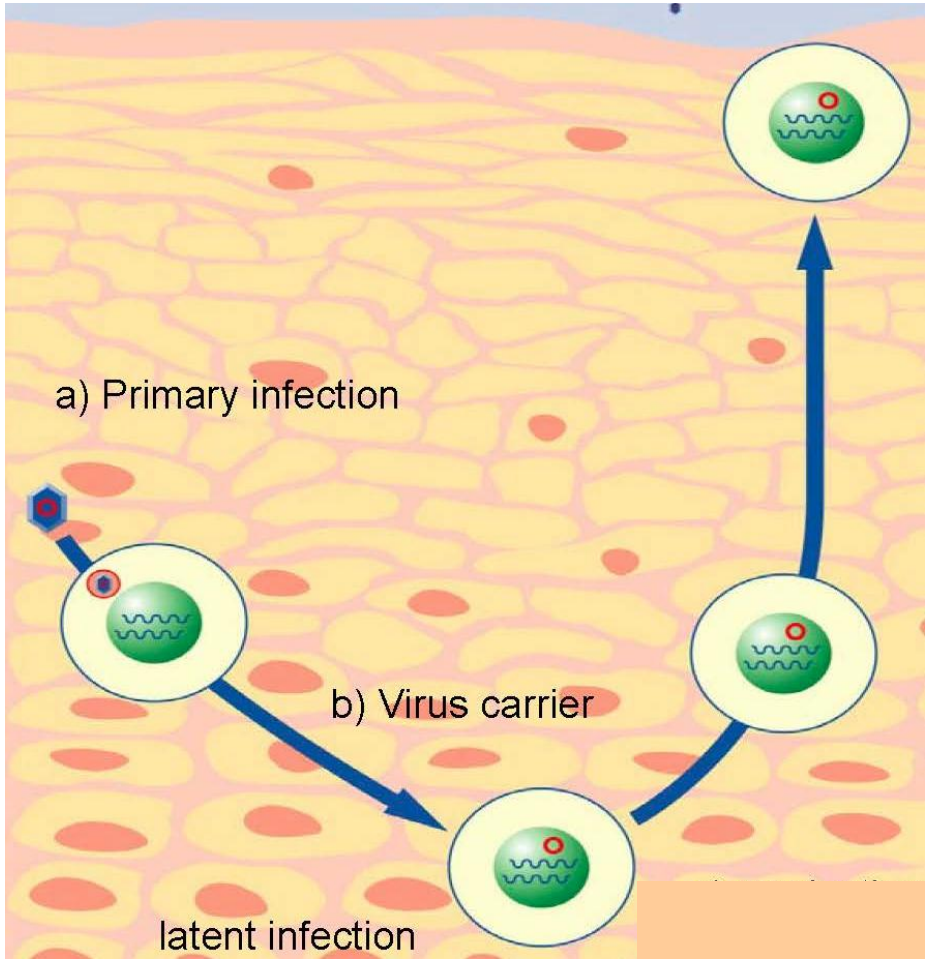
- Host cell factors regulate transcription
- Begins transcription of E6 and E7 genes
- Modifies the cellular environment to facilitate viral replication

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



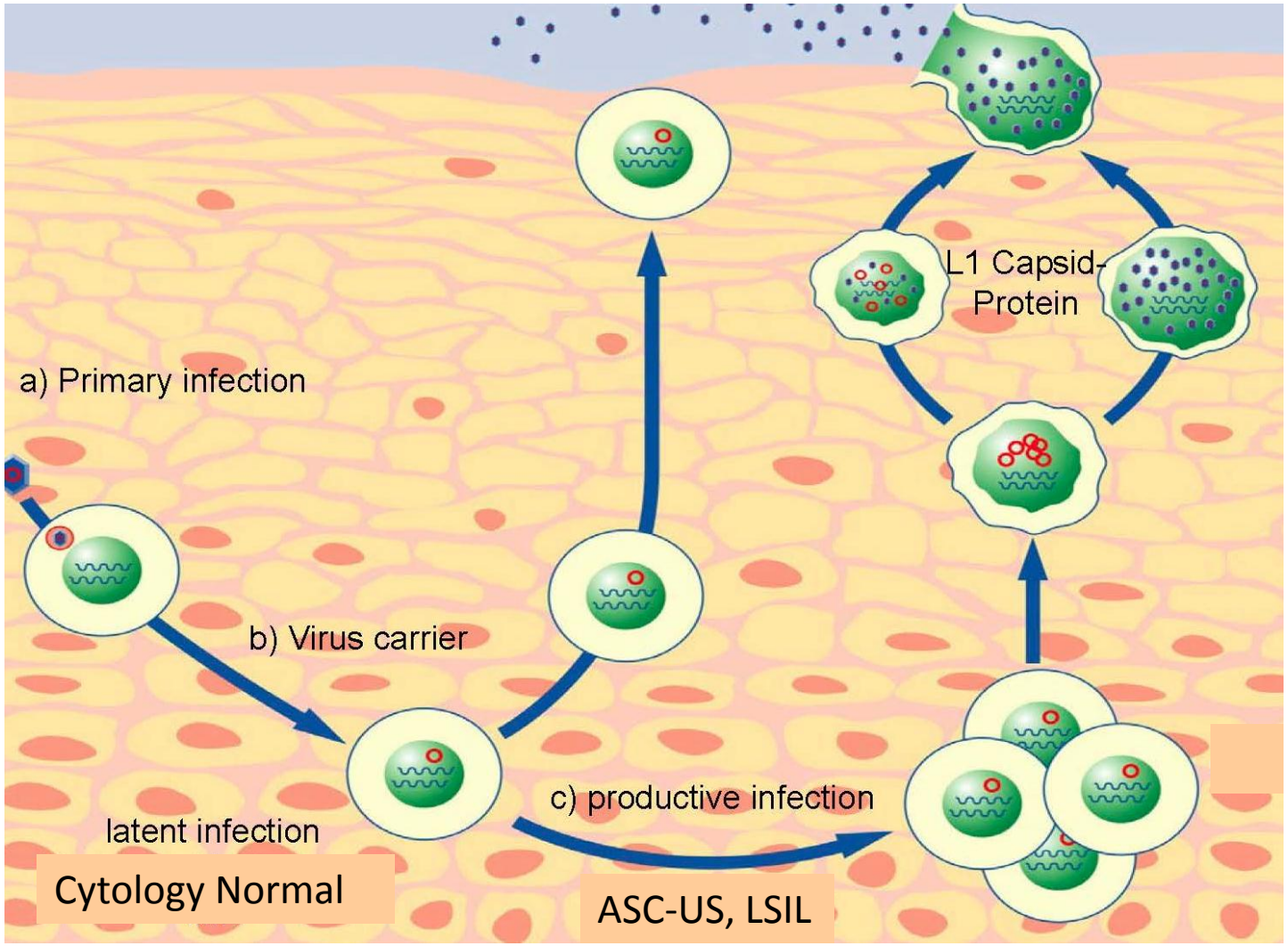
What is happening in the epithelium?



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

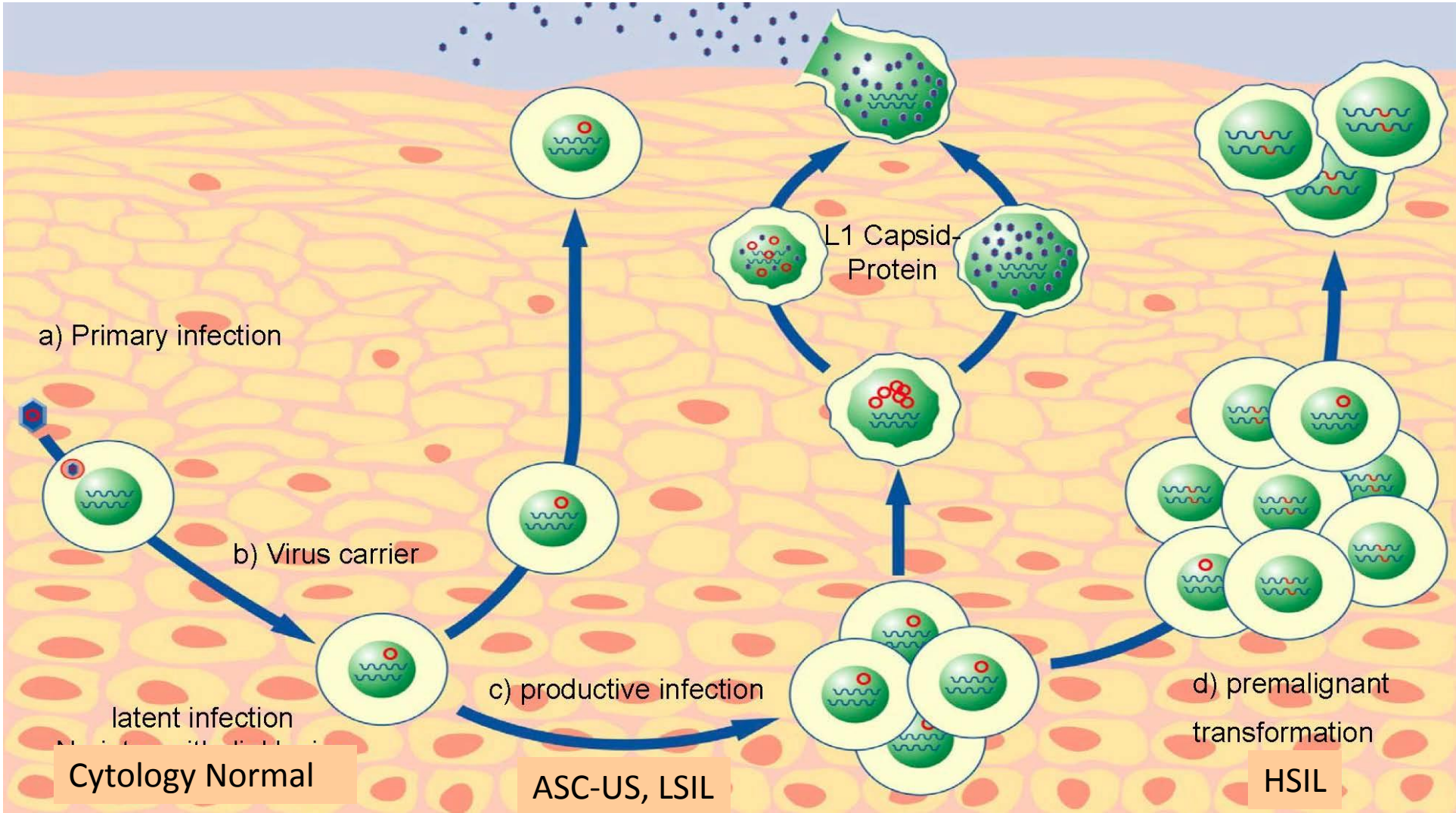
- Viral assembly occurs in the maturing squamous cell resulting in release of viral particles

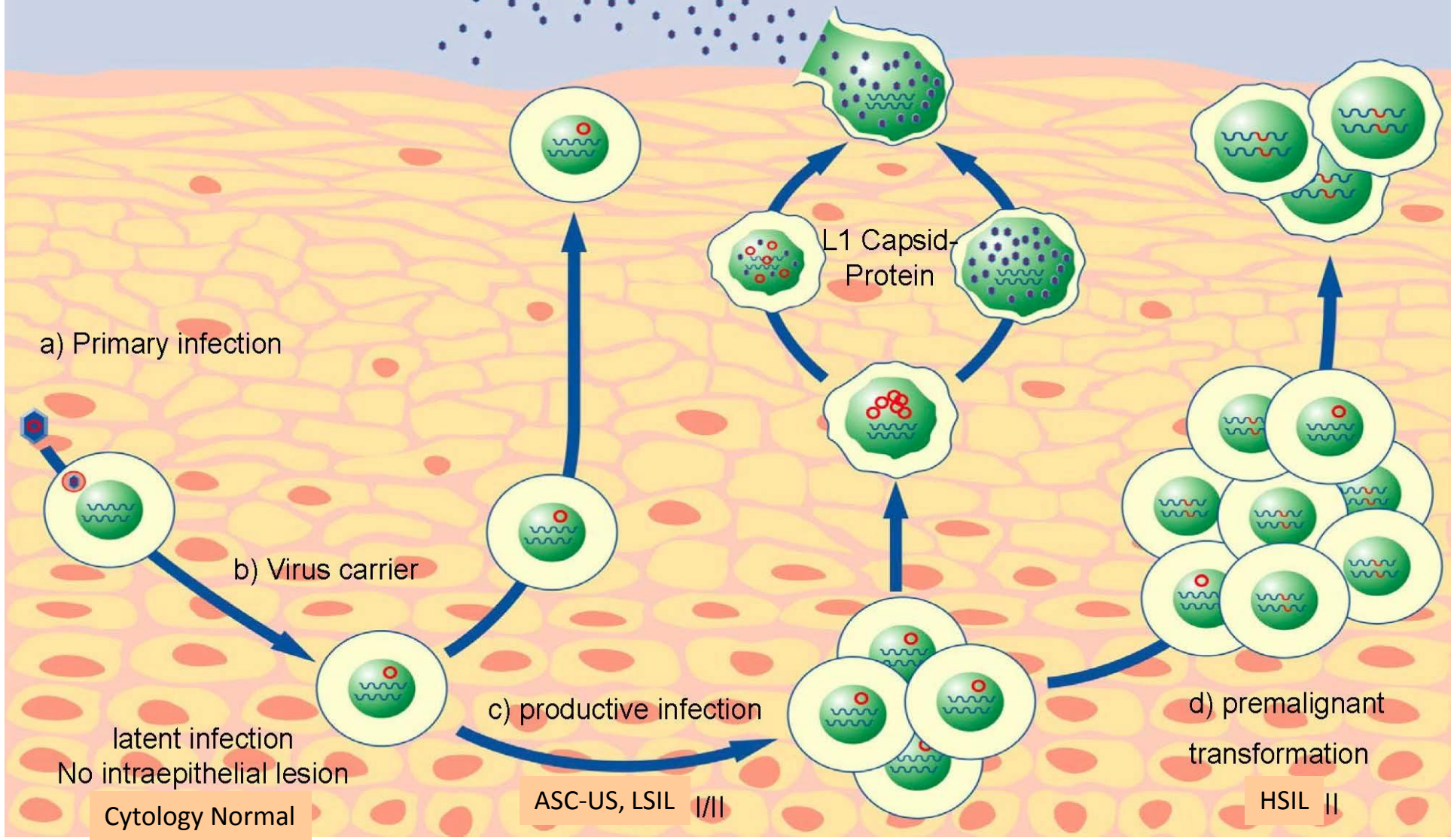
-Takes three weeks (time for basal cell- superficial cell maturation)

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

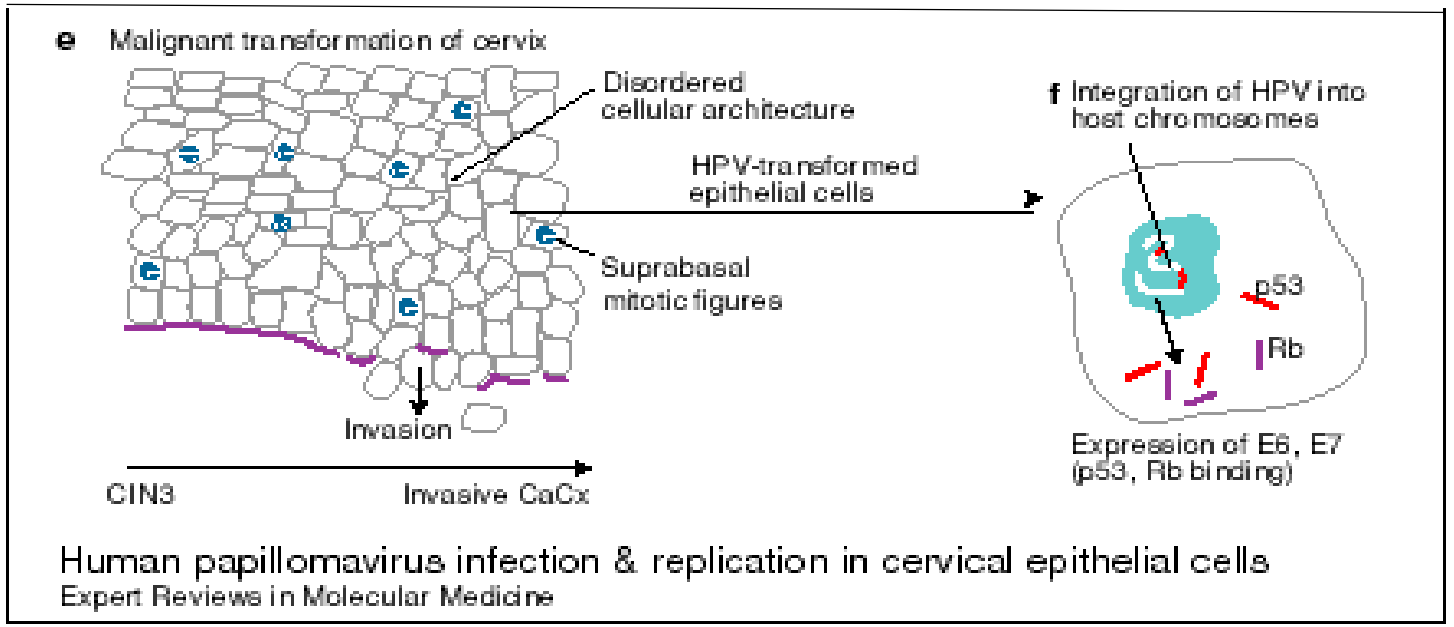




"Human Papillomavirus and Related Diseases From Bench to Bedside A Diagnostic and Preventive Perspective"
Ed: Davy Vanden Broeck, ISBN 978-953-51-1072-9, Published: April 30, 2013 © The Author(s).
Chpt 4:HPV L1 Detection as a Prognostic Marker for Management of HPV High Risk Positive Abnormal Pap Smears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

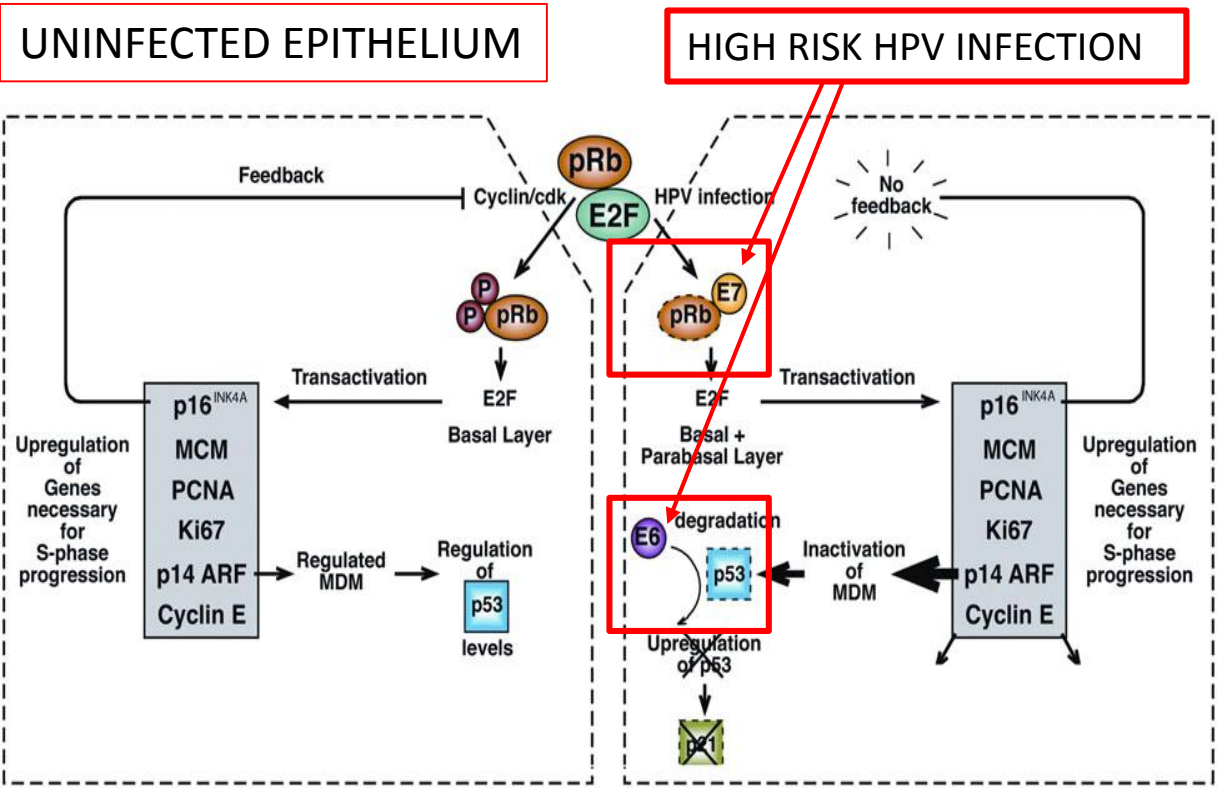
HPV Integration in high-grade lesions

- 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

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

HPV Vaccines

Gardasil 4 covers HPV 6,11,16,18. Used in NZ 2008-2017

Gardasil 9 covers HPV 6,11,16,18 plus 31,33,45,52 and 58

i.e. 7 of the 14 high-risk subtypes tested for using hrHPV testing

- Contain viral-like particles (VLPs) composed of the *L1* capsid protein of the virus.
- Vaccines don't contain viral DNA so can't cause HPV infection.

Cervical screening is still needed to protect against the 7 other hrHPV subtypes that aren't in the vaccine

HPV Immunisation in NZ

Since 1 Jan 2017:

Gardasil 9 is funded for both sexes aged 9-26 years (inclusive)

- 9-14 years: two-dose schedule: doses at 6-12 months apart
- 15-26 years: three-dose schedule: doses at 0,2 and 6 months

- is available (but not funded) up to (and including) age 45 years for females.

Vaccination should occur prior to commencement of sexual activity i.e. prior to HPV exposure.

- If sexual activity has already commenced, vaccination should still be administered as exposure to some or all of the hrHPV subtypes in the vaccine may not have occurred
- Vaccination is sometimes given after treatment of a high-grade lesion because it can still protect against reinfection, or infection with other HPV subtypes

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

HPV genotyping was performed on cervical tissue specimens for 227 cases of cervical cancer diagnosed in 2004 - 2010 from five NZ hospitals.

HPV was detected in 201 cases (88.5%) with multiple infections present in 11 cases (5.5%).

HPV Subtype	No. of cases
HPV-16	116
HPV-18	47
HPV-31	9
HPV-45	7
HPV-52	7
HPV-59	5
HPV-33	4
HPV-35	3
HPV-39	3
HPV-51	2
HPV-56	3
HPV-66	1
HPV-68	3
Unidentifiable subtype (technical reasons)	2
Low-risk HPV types (HPV-11,HPV-70)	2 (1)

Red highlighted HPV subtypes are those included in Gardasil-9

i.e. 191/212 infections could have been prevented by Gardasil-9

HPV Testing covers the red HPV types as well as the green types

Five Main Points

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