The Molecular Science of HPV

THE VIRUSES AND SUBTYPES

PATHOGENESIS

IMMUNIZATION

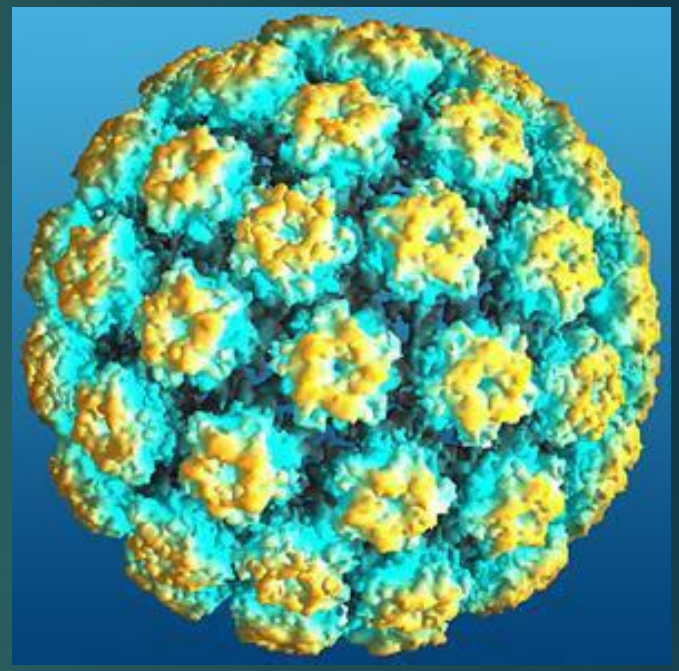
Developed by Melinda Paterson
Presented by *Christl Kirstein/ Ashika Bisson/ Margaret Sage*
NCPTS Training Team
August/September 2017

Topics

- 1. The HPV Virus and its Subtypes
- 2. The HPV Lifecycle
- 3. The Molecular Events in an HPV Infected Cell
- 4. HPV Test Technologies – overview
- 5. New Advances in Genotyping
- 6. Immunization

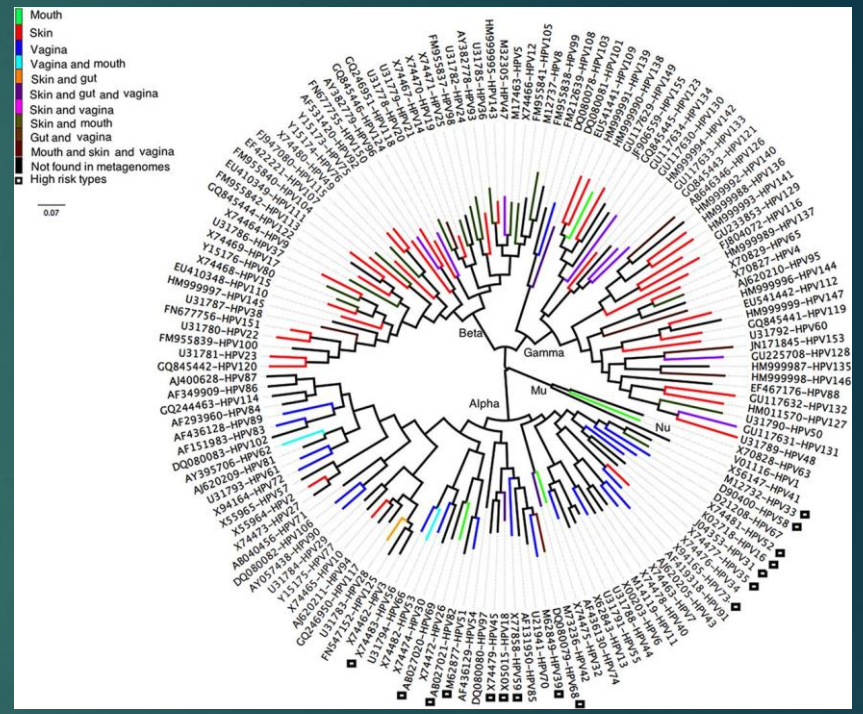
Papillomaviruses

- Ubiquitous DNA viruses that infecting the skin and mucosa of animal species: more than 200 HPV genotypes specifically infect humans
- Human types are divided into 5 genera based on differences in their DNA sequence; Alpha, Beta, Gamma, Mu and Nu groups
- More than 40 types infect moist anogenital tract epithelium of which 15 types cause most cervical cancers
- HPV also causes vulvar, vaginal, anal, penile and oropharyngeal cancers and anogenital warts



Alpha Genus

- Contains the types that cause important human disease
- High risk types are sexually transmitted and are controlled immunologically by the host
- Persistent hrHPV types can cause cancers of the cervix and other sites
- 15 high risk HPV types from the alpha genus were 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. HPV-66, HPV-26 and HPV-53 were also considered to be probably carcinogenic

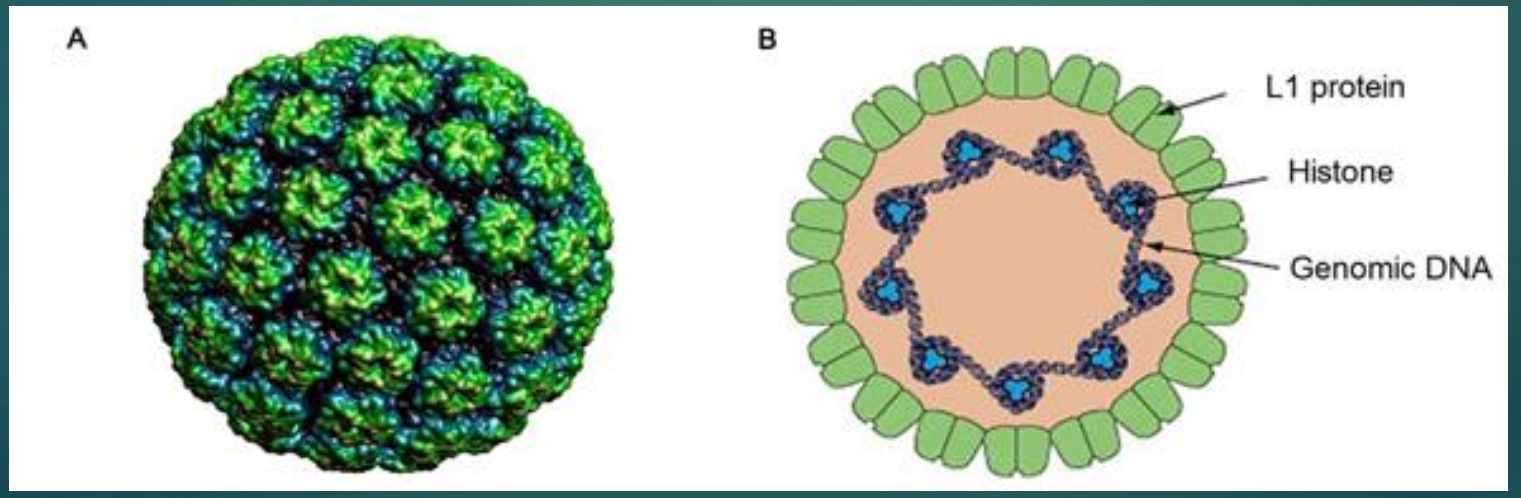


Doorbar J., Reviews in Medical Virology 2016

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

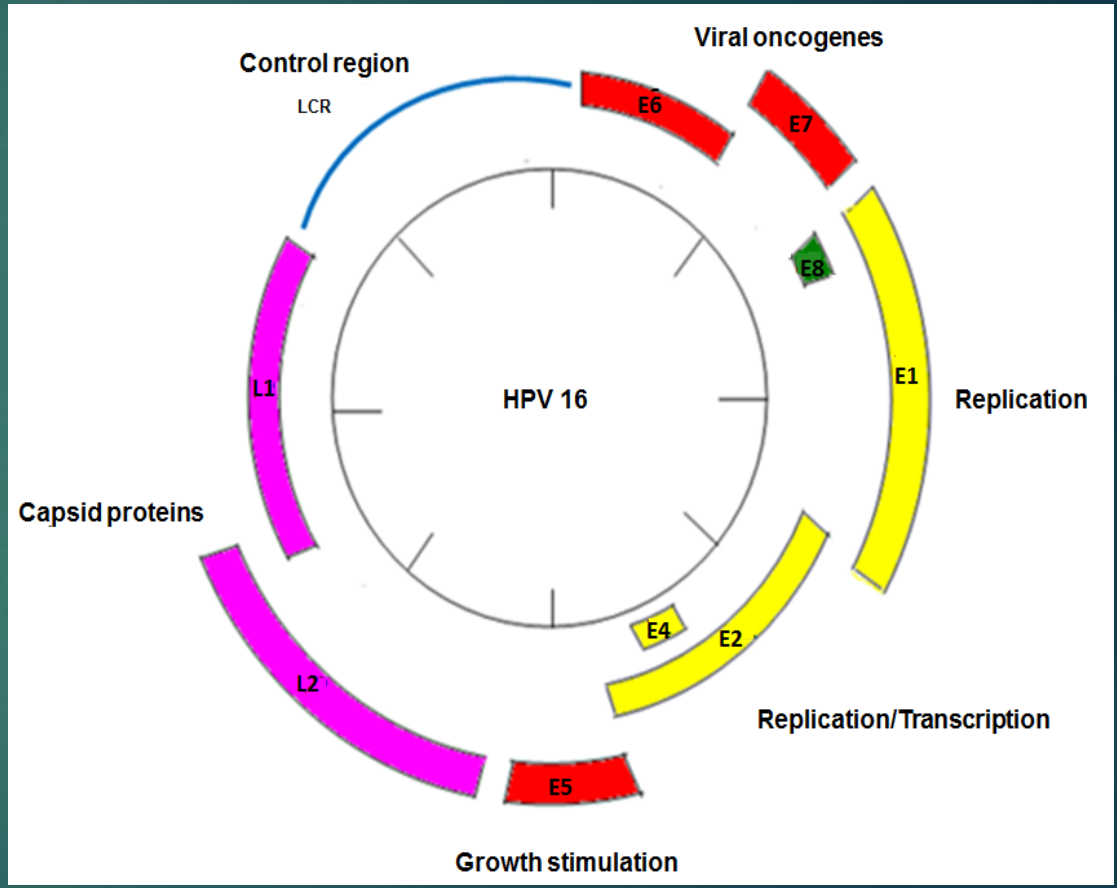
Virus Structure

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



Genome Organisation

- **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 and disrupts their structure causing virus release from the epithelial surface
  - 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 in all HPV types to allow genome amplification in the mid-layer of the epithelium



Human Papillomavirus Subtypes

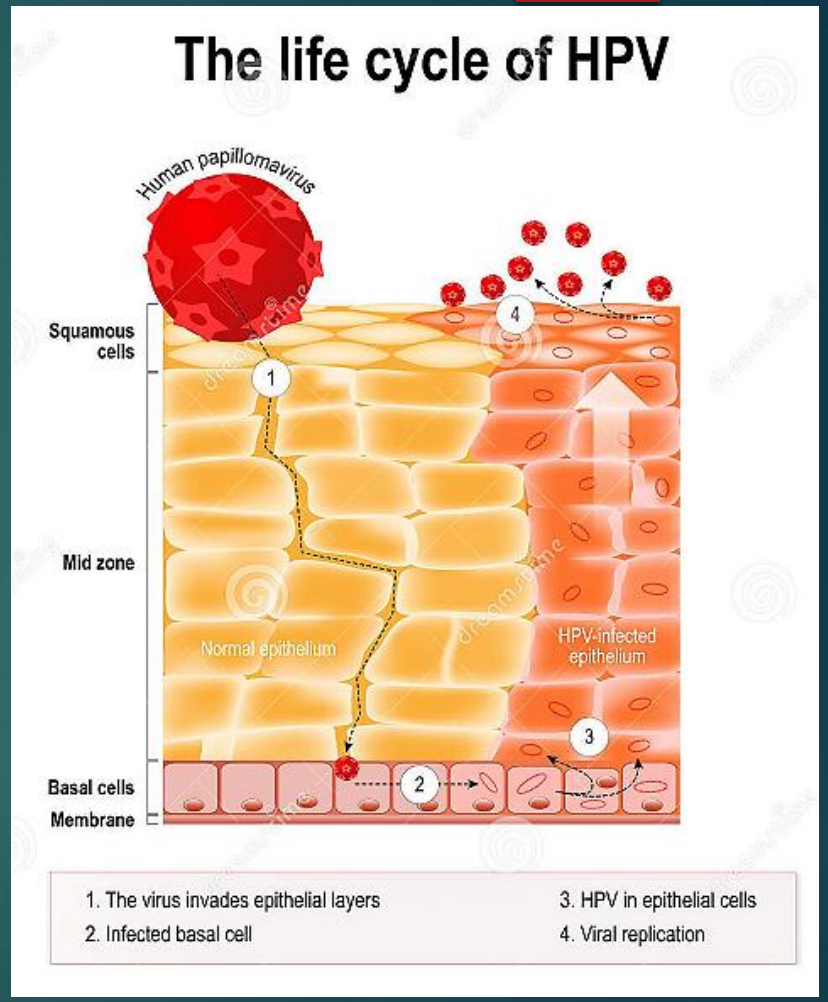
Disease	HPV Type
Cutaneous Warts	Non-carcinogenic: 1, 2, 3, 4, 7, 8, 10, 22, 63
Anogenital Warts	Non-carcinogenic: 6, 11, 42, 44
Genital Cancers	Carcinogenic: 16, 18, 31, 45 Very likely carcinogenic: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 Probably carcinogenic: 26, 53, 66, 68, 73, 72 Possibly carcinogenic: 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*

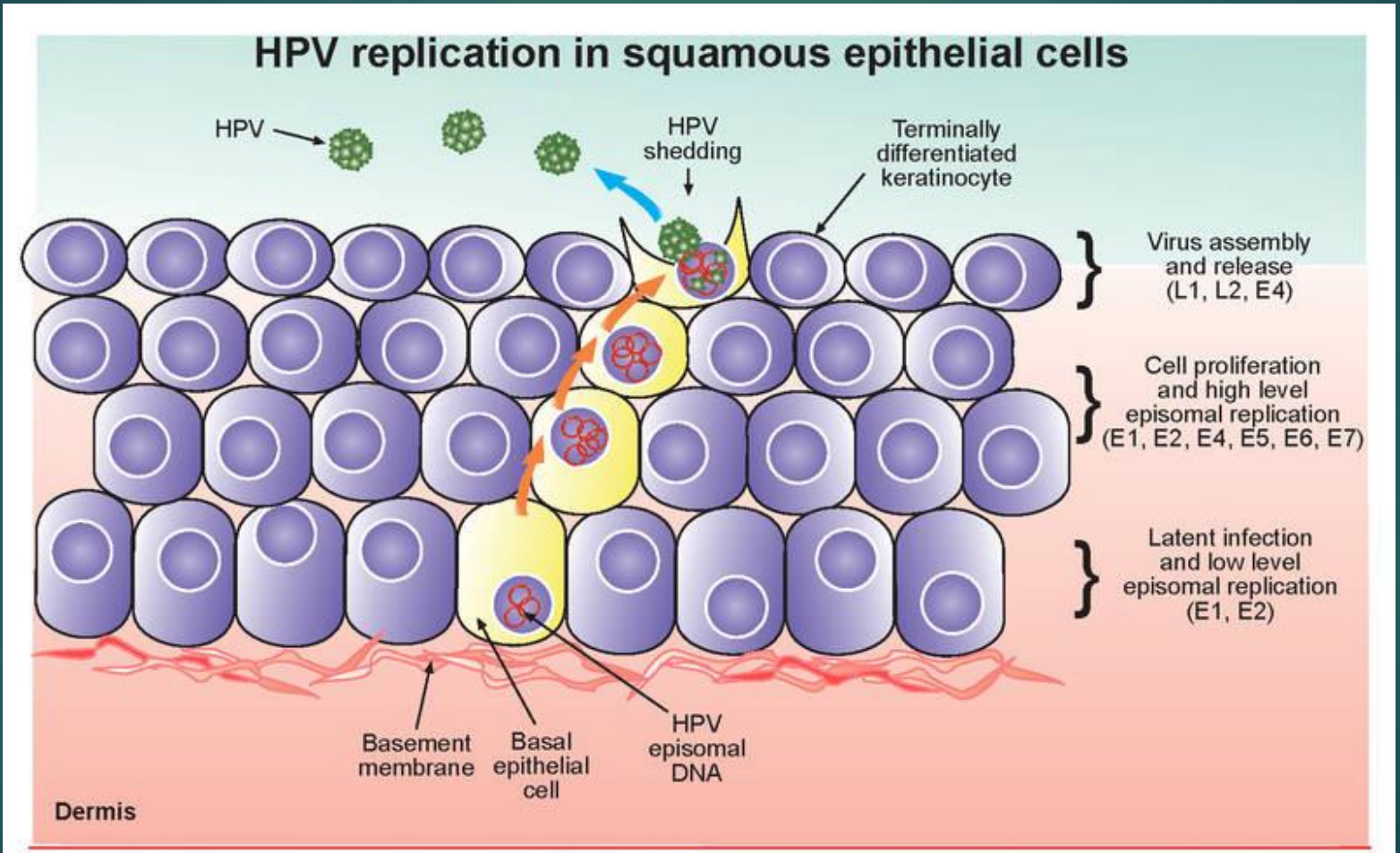
HPV Lifecycle

- 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



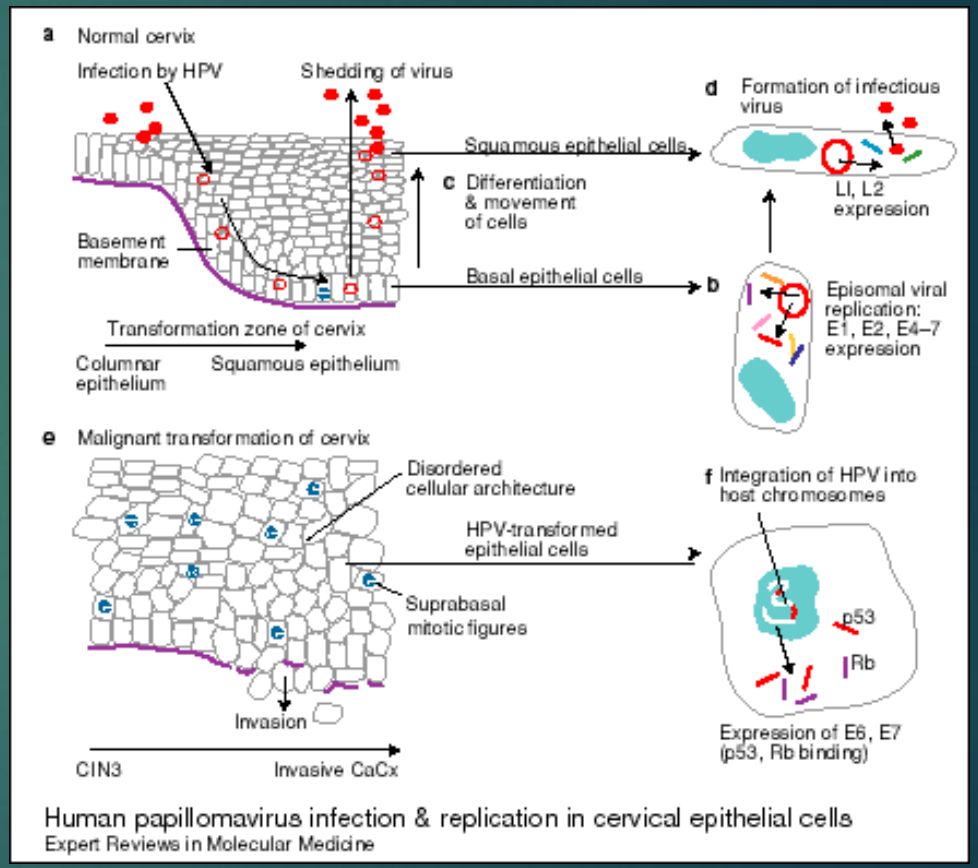
HPV Lifecycle: Low-grade lesions



http://immunopaedia.org.za/index.php?id=799

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



Gene Function

- hr-HPV E6 degrades p53 and hr-HPV E7 degrades pRB

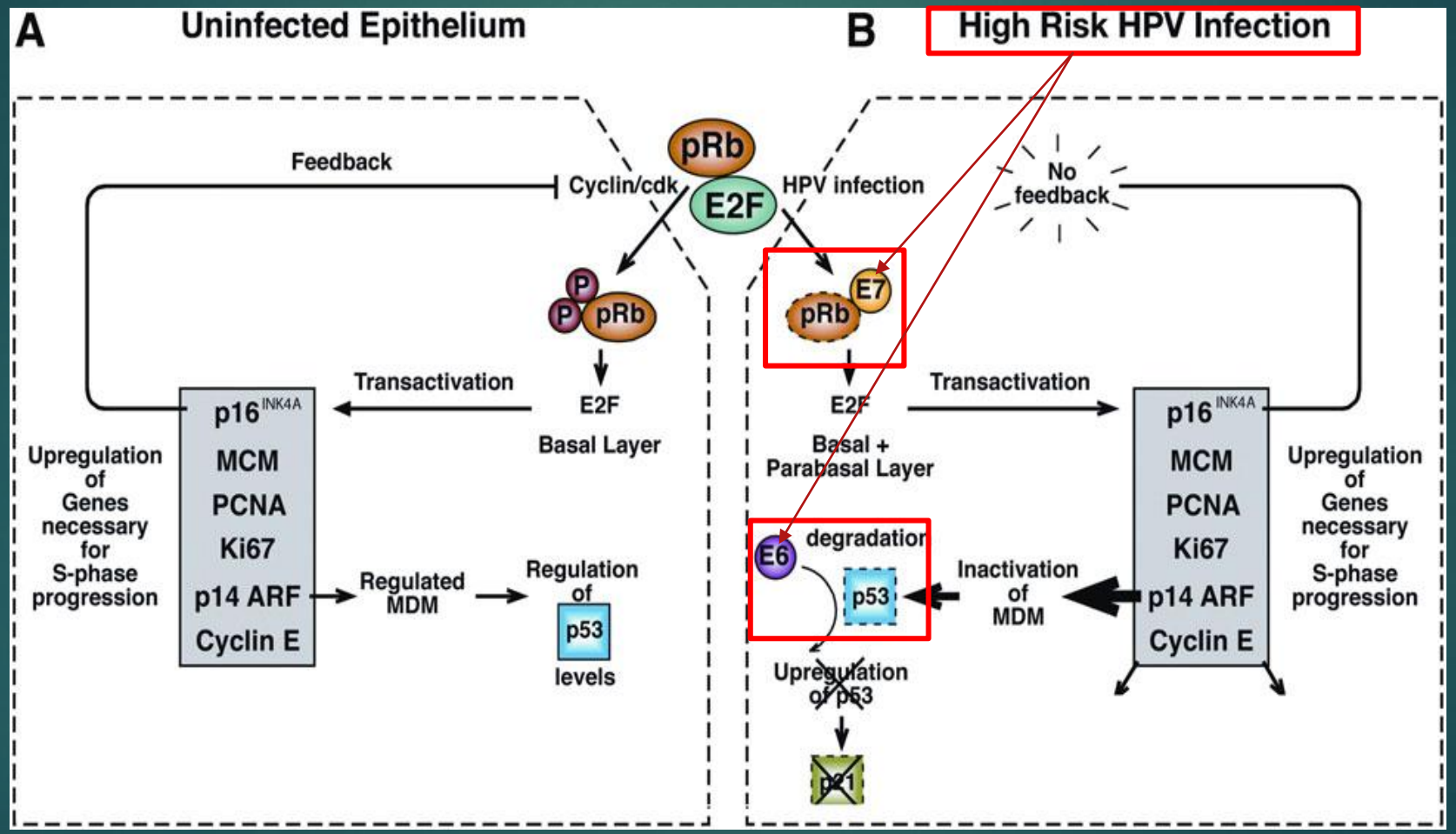
p53 protein:

- 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:

- also a tumour suppressor protein
- it is responsible for regulating cell cycle and prevents 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.

Integrated hrHPV cell mechanisms



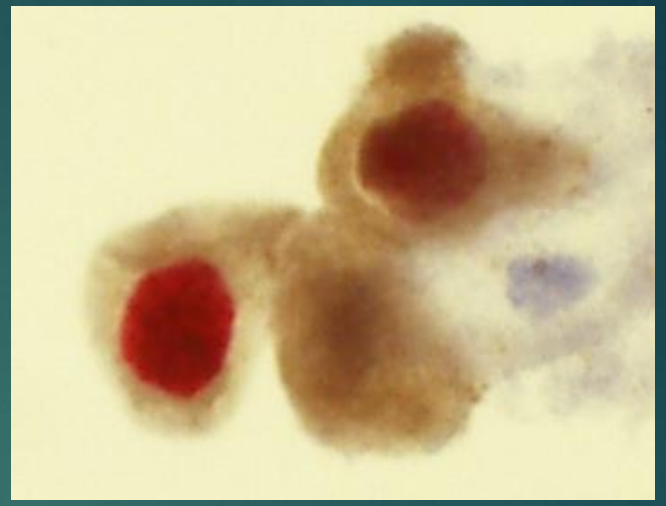
What is p16?

- 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.
- p16 stains both the nucleus and cytoplasm and is present in some normally proliferating epithelium as well as dysplastic epithelium

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").
- Present in the nucleus of normal basal and parabasal cells but not higher up in normal cervical epithelium.

Dual p16 and ki67 staining

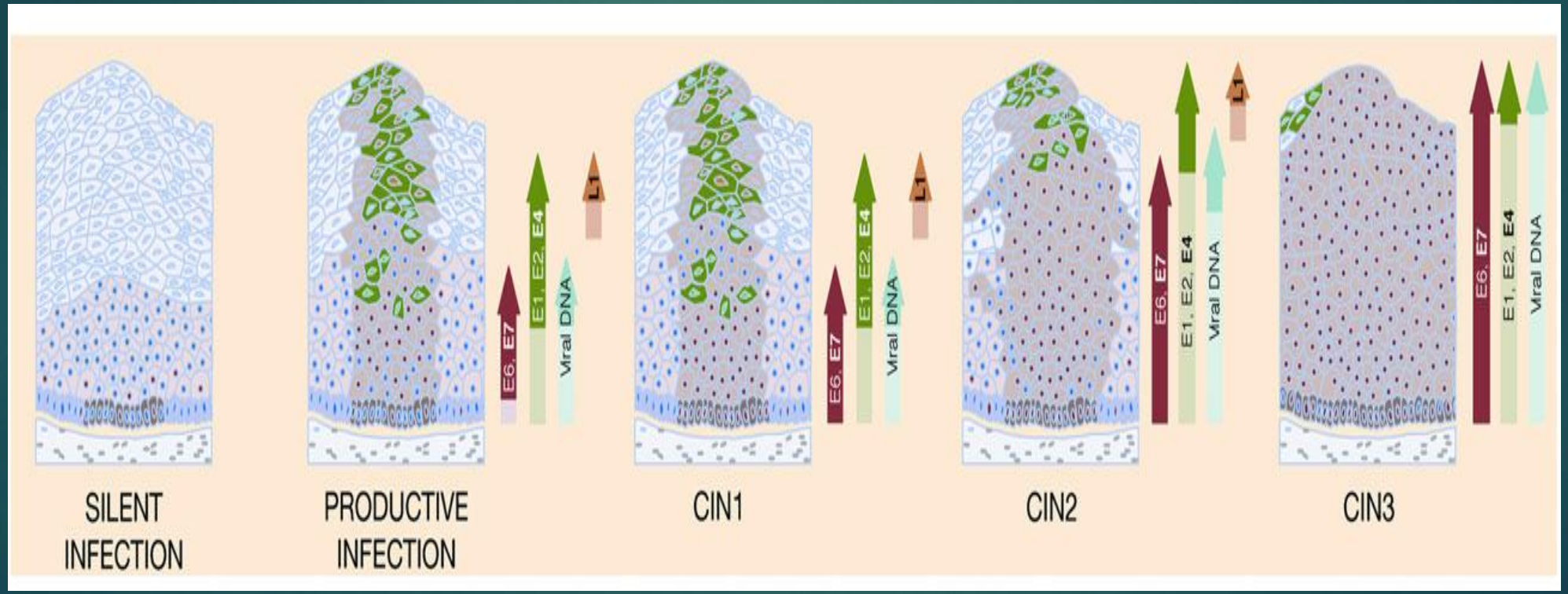


Positive when there is Ki67 nuclear staining (red) and p16 cytoplasmic staining (brown) in the same cell.

- 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

Gene Expression

- Gene expression varies in all the different states of HPV infection



Gene Function

- Different gene functions of E6 and E7 between high risk and low risk HPV

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

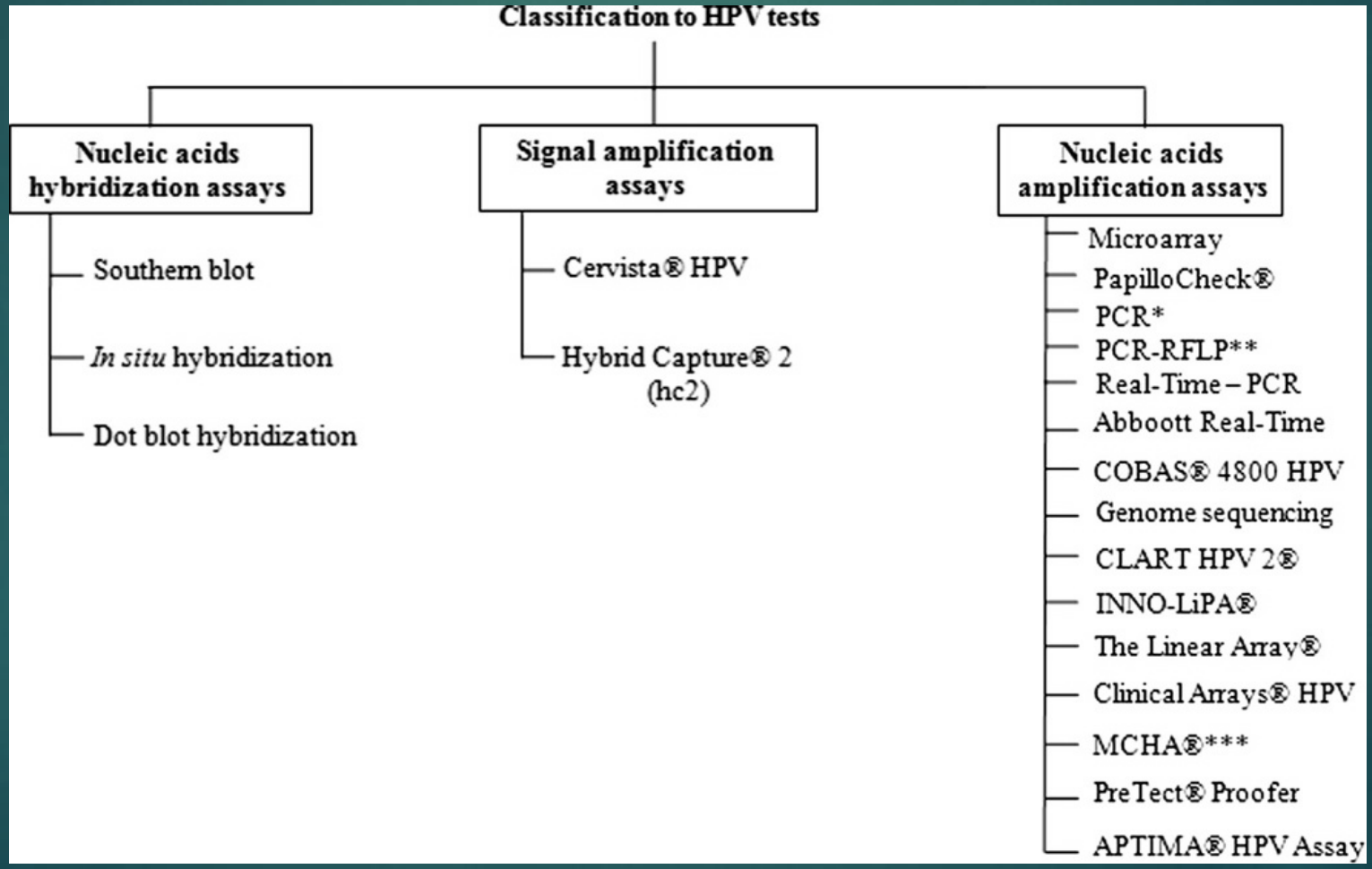
Weaker binding and no degradation of pRB

No stimulation of genome instability

Doorbar J., Reviews in Medical Virology 2016

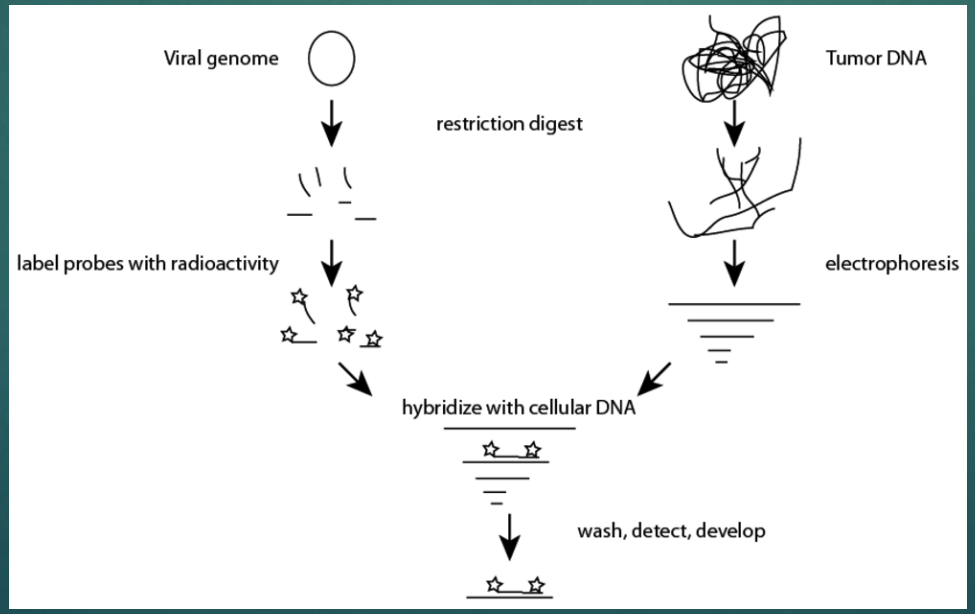
HPV Testing

- Three main HPV testing methodologies:



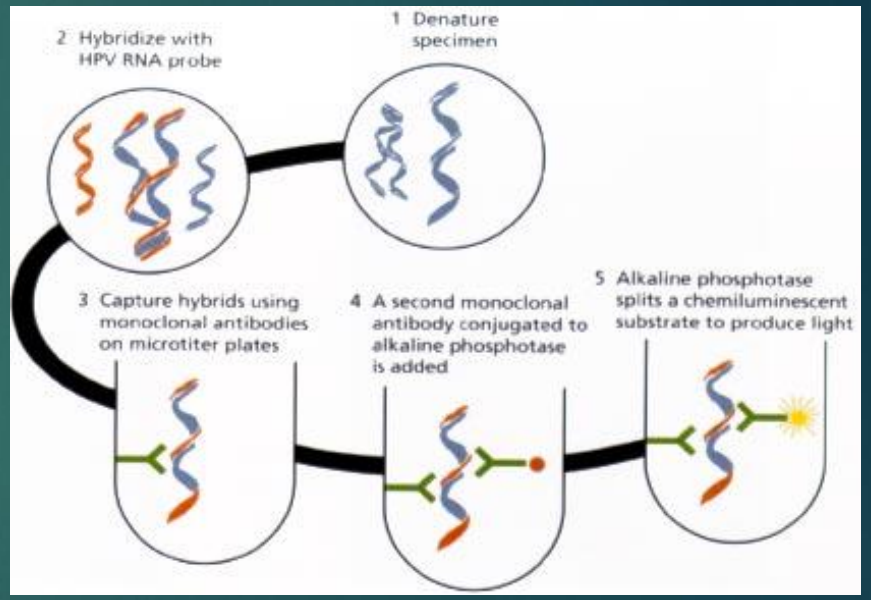
Nucleic Acid Hybridization Assays

- Techniques such as Southern blotting, *in situ* hybridization and dot-blot hybridization were previously used to detect HPV
- They all used radio-labelled nucleic acid hybridization assays
- They generated high quality results but had low sensitivity, needed large amounts of purified DNA and were time consuming procedures



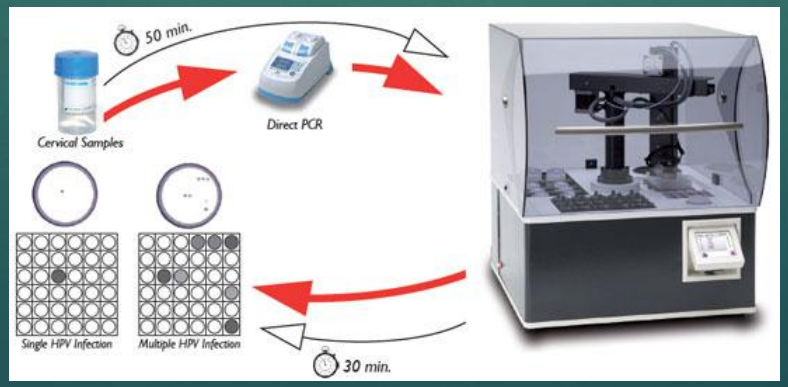
Signal Amplification Assays

- Qiagen Hybrid Capture 2 High-Risk HPV DNA Test
- Non-radioactive signal amplification method based on the hybridization of the target HPV-DNA to labelled RNA probes in solution
- This test detects 13 HR-HPV types or 5 LR-types
- This assay can distinguish between hr and lr groups but was not designed for individual genotyping
- Hologic Cervista HPV assay
- Detects 14 HR-HPV types
- This assay is more sensitive than hc2 and showed a lower false positive rate
- Able to individually genotype HPV-16/18



Nucleic Acid Amplification Assays

- Target amplification is the most flexible and sensitive of all DNA analysis techniques
- One of the most important in this group is the Polymerase Chain Reaction (PCR)
- PCR allows in vitro multiplication of unique regions of DNA so they can be detected within a large background
- This technology can be used for detection, viral load quantitation, DNA sequencing, and mutation analysis
- These assays can also be performed in multiplex, whereby multiple target DNA sequences can be analysed simultaneously



Nucleic Acid Amplification Methods

- Abbott Real Time High-Risk HPV Assay:
- Individual genotyping for HPV-16/18 and pooled detection of 12 HPV genotypes
- It is a qualitative *in vitro* PCR assay for the detection of high risk HPV DNA in cervical cells collected in liquid cytology media
- Amplification is performed using five different primers designed in the *L1* HPV genomic region
- Roche Cobas 4800 HPV Test:
- Automated sample preparation combined with Real-Time PCR technology to detect 14 hr-HPV subtypes with individual genotyping of HPV-16/18
- Primers in this test define a sequence of 200 nucleotides within the polymorphic *L1* region of the HPV genome

Nucleic Acid Amplification Methods

- Hologic Aptima HPV Assay:
- This assay targets high risk HPV messenger RNA from the *E6/E7* oncogenes in the 14 high risk subtypes
- A separate test (Aptima HPV 16 and 18/45 genotype assay) is available for partial genotyping of hrHPV-positive results for differentiation of HPV16 and HPV18/45
- BD Onclarity HPV Assay:
- A Real-Time PCR based HPV screening test which targets *E6/E7* DNA regions on the HPV genome
- It detects individual genotyping information for six hrHPV types (16, 18, 31, 45, 51, 52). The remaining eight high-risk genotypes are reported in three small groups: (33, 58), (35, 39, 68) and (56, 59, 66).

New Advances with HPV genotyping

- Over 200 different HPV subtypes have been described so far
- HPV subtypes can be further split into lineages and sub-lineages when complete genomes are considered
- This classification is recent and only some HPV subtypes have been classified
- Different lineages of HPV types may show differences in infection prognosis
- Next-generation sequencing is a recent technology that has been largely used to detect these known and novel subtype lineages

New Advances with HPV genotyping

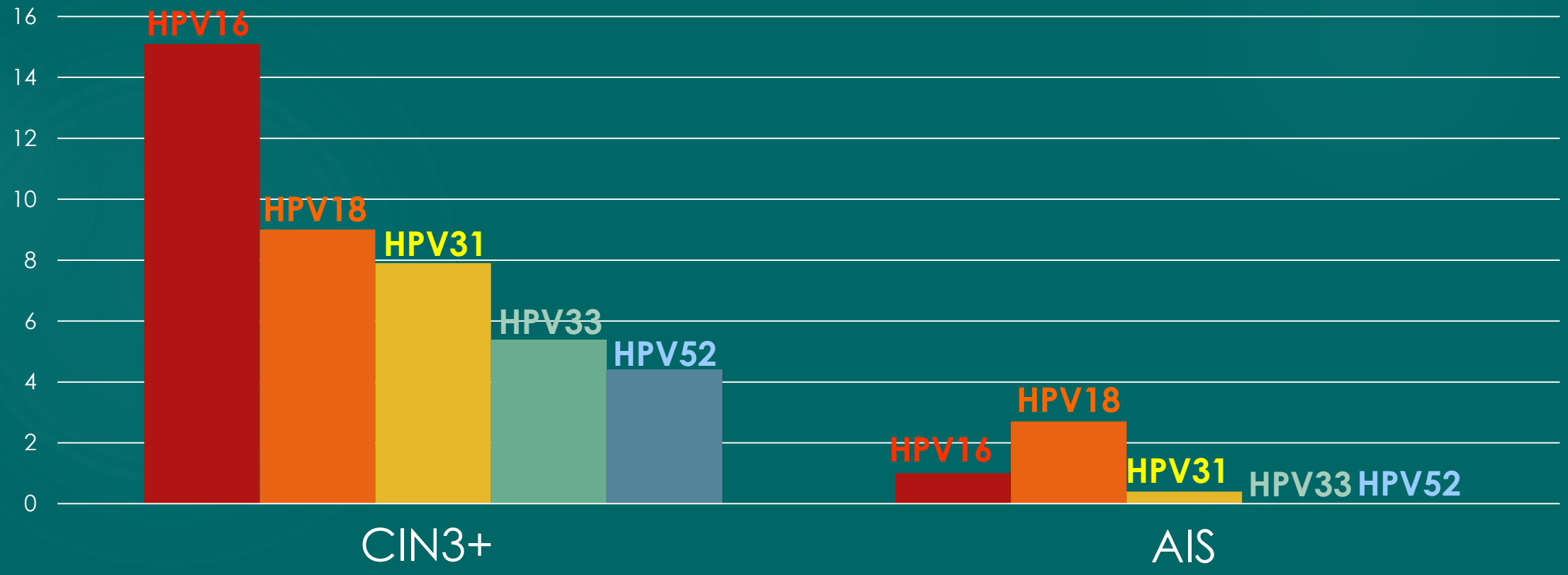
- Various recent studies have shown:
- HPV16 non-European lineages (A4, B, C and D) have a two-fold increased risk of infection persistence and a two- to four-fold greater risk for development of high or low-grade squamous intraepithelial lesion
- HPV31 lineage C is more persistent than A and B, however lineage B is associated with high-grade lesions
- HPV58 lineage A is associated with persistence and 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 control subjects

Extended HPV Genotyping

- Over the past 7 years, numerous studies have demonstrated differential risk for CIN2+, CIN3+, and cancer endpoints, for different HPV genotypes
- Partial genotyping (16, 18) is now established for triaging positive oncogenic HPV tests
- There is already strong interest in looking further at the "other" high risk HPV types which are likely to carry different risk profiles
- Would triaging the "other" oncogenic HPV subtypes into different management pathways be useful?

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*

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



Immunity

- HPV has an immune evasion mechanism which inhibits host detection of the virus
- The virus largely exists in an intracellular location where is it not visible to circulating immune cells
- During most of the duration of the HPV infectious cycle there is little or no release of cytokines to activate the immune response

HPV Vaccines

- Gardasil 4 and Gardasil 9 are approved for use in New Zealand
  - Gardasil 4 covers HPV 6,11,16,18
  - Gardasil 9 covers HPV 6,11,16,18 and also 31,33,45,52 and 58
  - i.e. 7 of the 14 high-risk subtypes tested for using hrHPV testing
  - Cervical screening is still needed to protect against other hrHPV subtypes
- Contain viral-like particles (VLPs) composed of the *L1* capsid protein of the virus.
- Vaccines don't contain viral DNA so cant cause HPV infection.

HPV Immunisation in NZ from 1 January 2017

Gardasil 9 (9-valent vaccine) can be used for females 9-45 years and males 9-26 years.

Funding:

- HPV9 is funded for both boys and girls 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
- HPV9 is available (but not funded) up to (and including) age 45 for females.

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

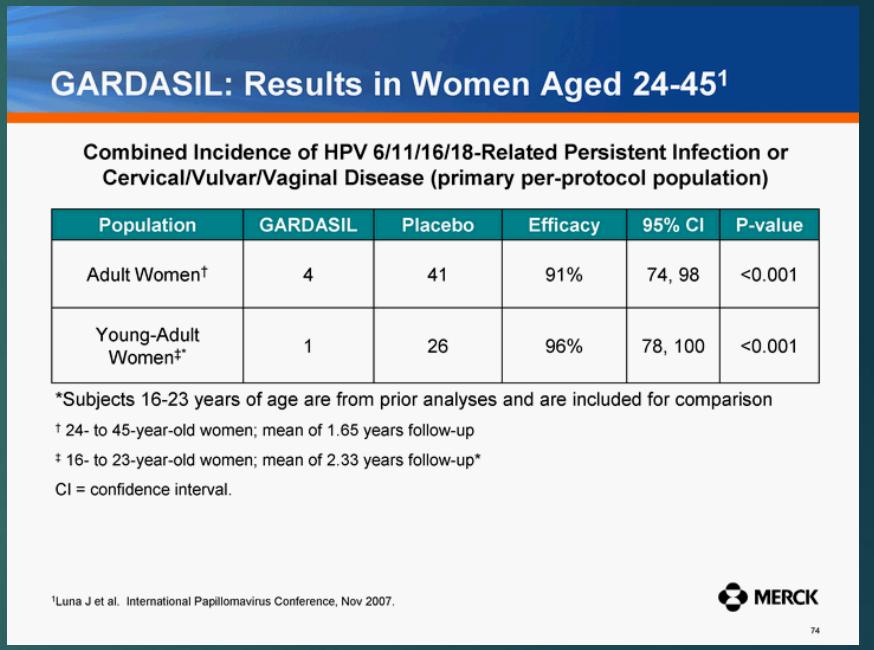
- If sexual activity has already commenced, vaccination should still be administered as exposure to some or all of the hrHPV subtypes in the vaccine may not have occurred
- Vaccination is sometimes given after treatment of a high-grade lesion because it can still protect against other HPV subtypes

Vaccine Safety

- Exemplary safety record
  - More than 200 million doses given worldwide
  - Have been repeated assessed as safe by expert vaccine safety bodies
- Injection site reactions occurs in some individuals
- Some individuals faint (a reaction to having an injection)
- Incidence of serious systemic reactions is no greater than the background rates in the population, despite contrary claims in the media in some countries

Vaccine Effectiveness

- HPV vaccines are highly immunogenic and induce cross protection against other subtypes
- Long-term effectiveness seems to be maintained: no sign that antibody levels are waning in women being followed from early clinical trials (10 years +)
- Gardasil 4: prevents 70% of invasive cervical cancers
- Gardasil 9: prevents 90% of invasive cervical cancers
- Very effective at reducing the incidence of genital warts (seen soon after vaccination) and cervical lesions (seen later). Impact on cancer rates will take longer
- Very effective at creating herd immunity i.e. un-immunised women gain appreciable benefit once about 70-80% of the population are immunised



GARDASIL: Results in Women Aged 24-45[1]

Combined Incidence of HPV 6/11/16/18-Related Persistent Infection or Cervical/Vulvar/Vaginal Disease (primary per-protocol population)

Population	GARDASIL	Placebo	Efficacy	95% CI	P-value
Adult Women†	4	41	91%	74, 98	<0.001
Young-Adult Women‡*	1	26	96%	78, 100	<0.001

*Subjects 16-23 years of age are from prior analyses and are included for comparison

† 24- to 45-year-old women; mean of 1.65 years follow-up

‡ 16- to 23-year-old women; mean of 2.33 years follow-up*

CI = confidence interval.

[1]Luna J et al. International Papillomavirus Conference, Nov 2007.

MERCK

74

Will other HPV subtypes replace those currently causing disease?

- Theoretically other HPV subtypes could fill the ecological niche vacated by say, HPV 16 and 18
- Not predicted to happen
- Will be closely monitored internationally to see if this does occur

Summary

- There has been an explosion of understanding about HPV infection, cellular events associated with HPV infection and mechanisms relating to cancer causation in recent decades
- New technologies resulting from this increased understanding is changing our approach to cervical cancer prevention
- We still don't understand why some women develop persisting hrHPV infections and why some but not others, progress to invasive cancer
- Immunisation will have an enormous impact on the rates of disease in future decades