NCPTS

HPV Technologies update

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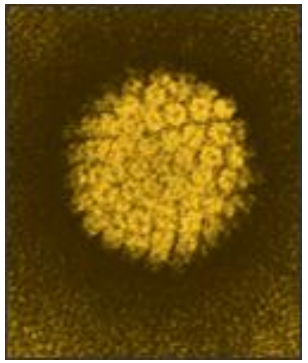
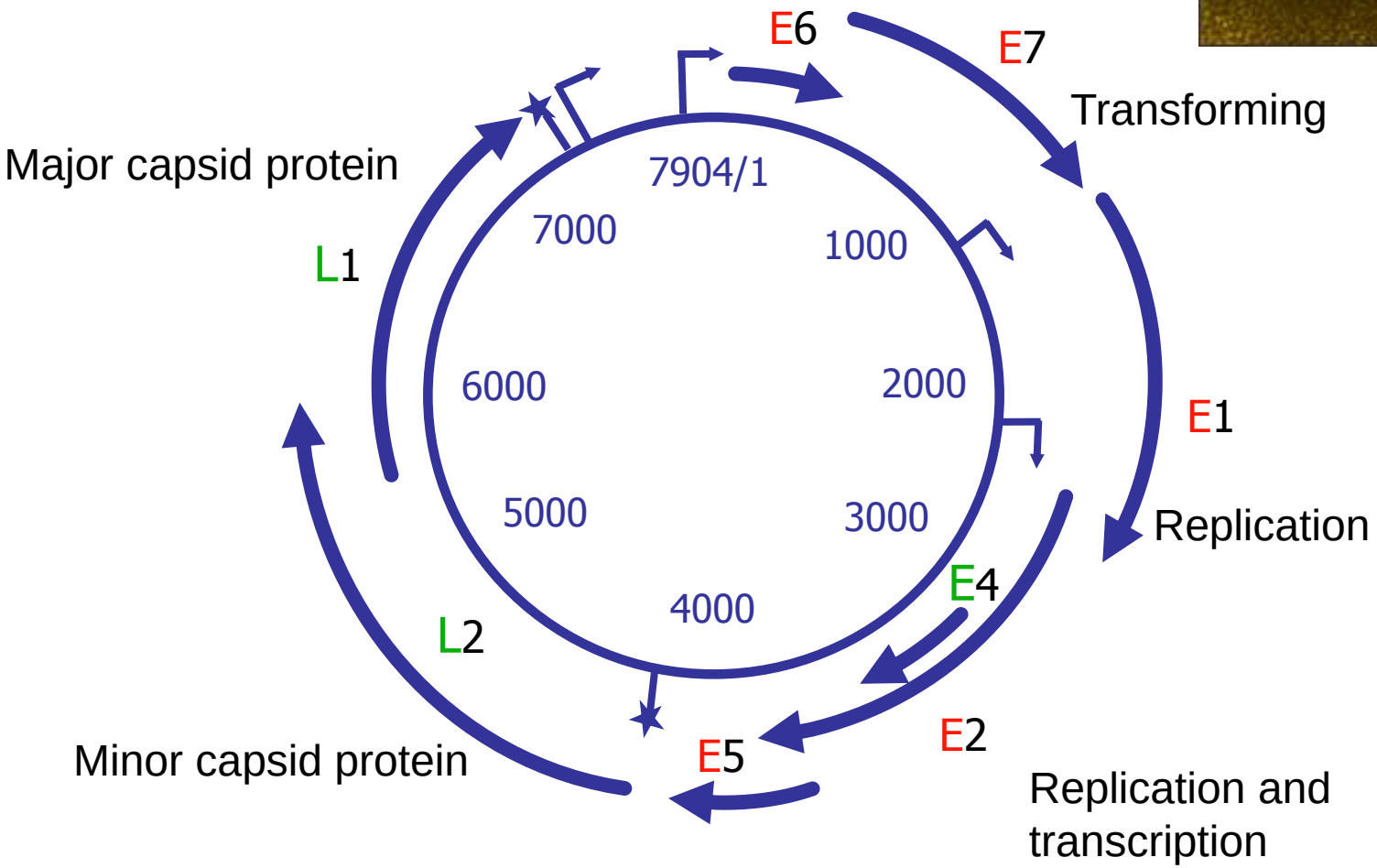
October 2016

The HPV genome

is divided into Late and Early regions





hrHPV Testing
Detecting high-risk HPV subtypes

- Test is positive if any of the selected high-risk HPV subtypes are present

  c.f. specific genotyping - identifies specific subtype(s) present

- Two PCR-based amplification methods in use in NZ:

  Cobas 4800 System (Roche)

  Abbott Realtime High risk HPV Assay

  - based on detecting the L1 region of the HPV genome
    - both detect 14 high-risk HPV genotypes
    - identify HPV 16, HPV 18 and 12 "Other" HrHPV subtypes: 31,33,35,39,45,51,52,56,58,59,66,68)
    - can both be used with either ThinPrep or SurePath

Other hrHPV test methodologies

Hybrid-Capture 2 (Digene): was initially the standard test used in research-based clinical trials. Uses a DNA-RNA hybrid capture technique for 13 subtypes: HPV 16, 18,31,33,35,39,45,51,52,56,58,59 and 68

Onclarity™ HPV assay (BD): targets E6/E7 DNA oncogenes for 14 HPV subtypes. Will identify HPV 16,18. Can also provide additional discrete genotyping for four other genotypes (45, 31, 51 and 52) and stratify the remaining eight high-risk types into three groups (33, 58), (56, 59, 66) and (35, 39 and 68).

Aptima® HPV assay (Gen-Probe)
Identifies 14 high-risk genital HPV types. Detects E6 and E7 messenger RNA and so differs from all other currently approved HPV assays, which detect HPV DNA. The test is claimed to be more specific at identifying cervical lesions *cf* the presence of HPV per se.

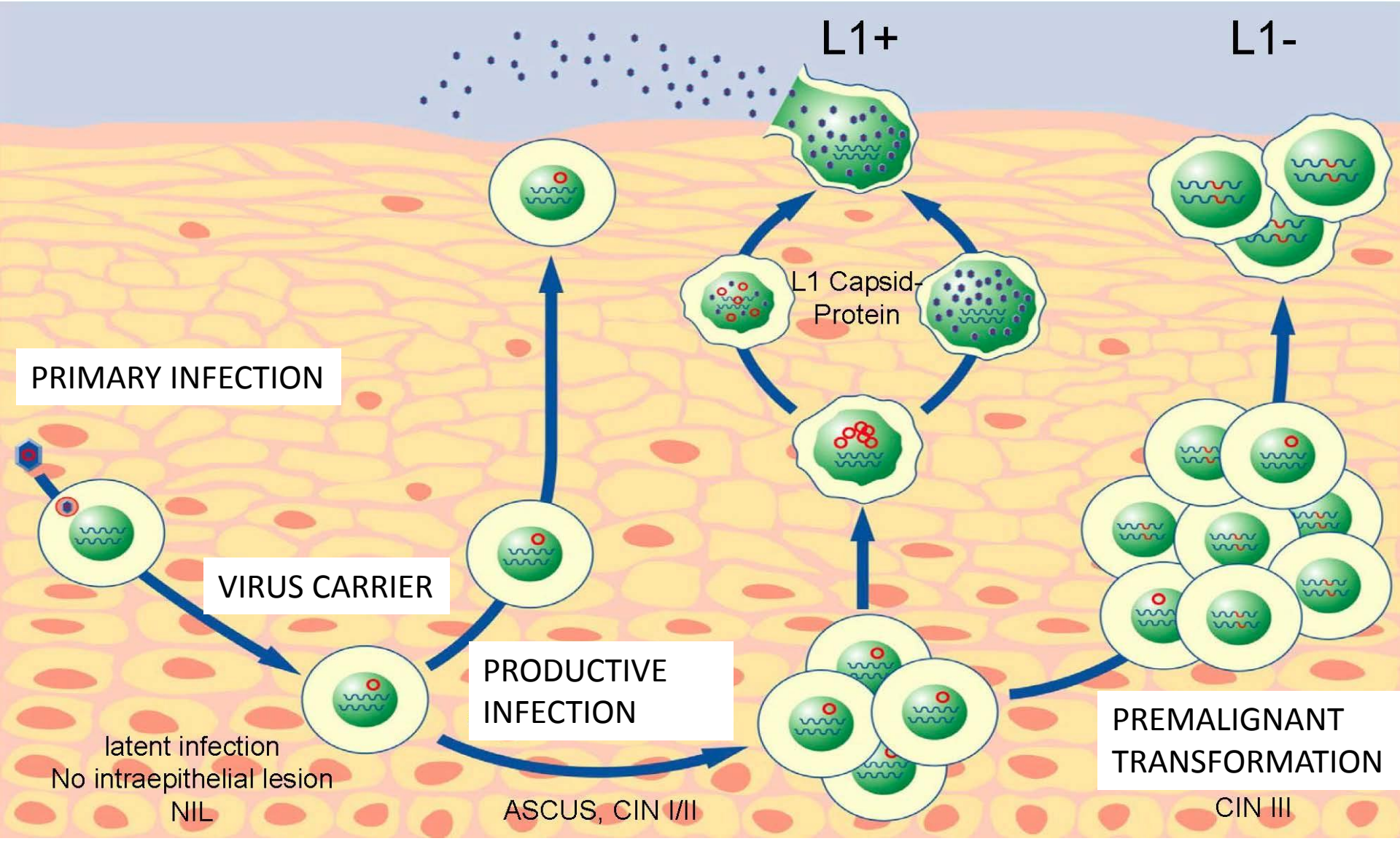
Cervista (Hologic)
Isolated DNA is mixed with probes that specifically recognise HPV DNA for 14 high-risk subtypes. This reaction is detected by another substance that produces light, which measured to determine the presence of HPV.

HPV Genotyping techniques

Genotyping is performed by amplifying each genotype using primers and probes specific for each HPV type. Differences in nucleotide sequence between HPV genotypes make it possible to amplify and detect each HPV type individually by PCR.

Sequencing is another approach for genotyping. With this method, a woman's HPV DNA is amplified and sequenced. The sequence of the amplified product can be compared with a reference sequences with known genotypes using various data bases.

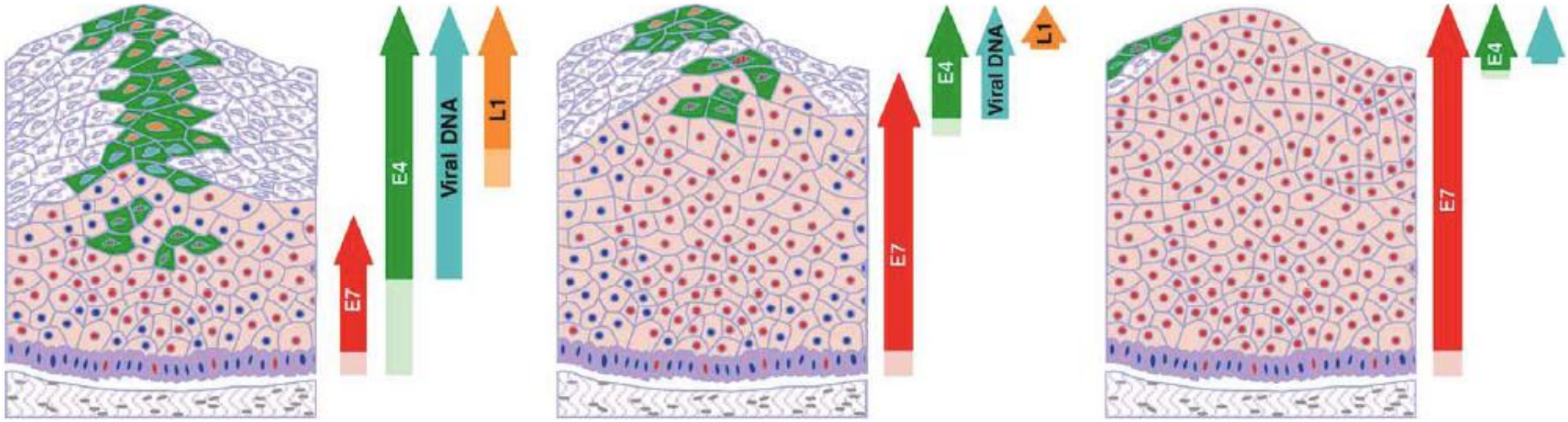
- Role of genotyping likely to become increasingly important in clinical pathways and to monitor prevalence of HPV subtypes as immunisation rates increase.



"Human Papillomavirus and Related Diseases From Bench to Bedside A Diagnostic and Preventive Perspective"
Ed: Davy Van den 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)

Progression to cancer is associated with deregulation of viral gene expression

CIN 1 CIN 2 CIN 3



High-grade lesions:

- Viral genome integrates into host cellular DNA
- increased E6/E7 expression
- Loss of L1, L2 expression. Therefore, current vaccine can't clear pre-cancerous lesions.

Doorbar, J Clin Virol 32:7-15, 2005

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

- Group 1: histologically confirmed CIN2/3 or AIS
- Group 2: possible or definite high-grade squamous or glandular cytology reports

LBC specimens were genotypes for 37 HPV subtypes

Results

Group 1: Histologically confirmed lesions

356 women = CIN2/3 6 women = AIS/glandular dysplasia

Any hrHPV infection: 95% HPV 16/18: 60%

Most common subtypes: 16 (51.2%) 52 (18.9%) 31 (17.1%) 18 (12.1%)

Group 2: 594 Women with possible or definite HG cytology

Any hrHPV infection: 87% HPV 16/18: 53%

Most common subtypes: 16 (44.1%) 52 (16.8%) 31 (15.2%)

Highest relative prevalence of HPV 16/18 in confirmed CIN3 was seen in women 20-29 years of age

International comparisons

- The prevalence of HPV 16 in CIN2/3 was broadly consistent with that in Australia and Europe (about 50%) but higher than that reported for North America, Asia and South/Central America (about 40%)

- The prevalence of HPV 18 in CIN2/3 was broadly consistent with Australia and North America but higher as that reported for Asia, Europe and South/Central America

- The prevalence of HPV 52 was higher than that reported from other regions

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 consent.
- Cervical specimens underwent histopathological review and assays were performed for HPV genotyping: 227 Invasive cancer cases

  159 (70.0%) = squamous cell carcinoma (SCC)

  61 (26.9%) = adenocarcinoma (ADC)

  7 (3.1%) = adenosquamous carcinoma (ASC)

Results: HPV was detected in 88.5%

93.1% = SCC

77.9% = ADC/ASC

Results: HPV Subtypes

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

HPV 16 = 51% : 56% of SCC 40% of ADC/ASC
HPV 18 = 21% : 15% of SCC 35% of ADC/ASC

For the 201 HPV positive cases:

HPV 16 + 18 = 81.1%

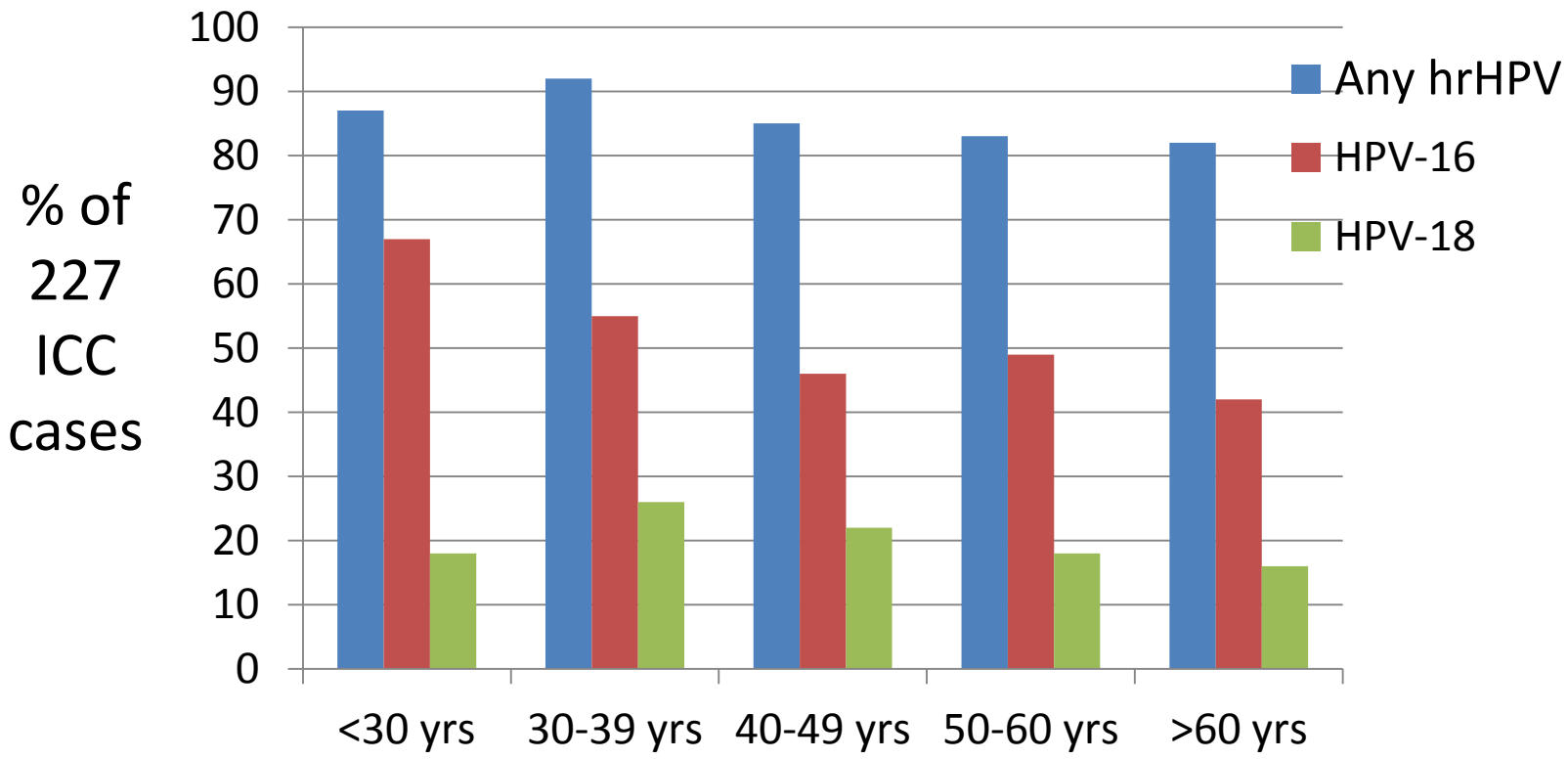
Frequent non-16/18 types were types 31, 45, 52, 59 and 33

Two low-risk HPV types (11,70) = one SCC each

Multiple subtypes

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

Prevalence of hrHPV types by age



- The prevalence of any HPV type was highest in women 30-39 years
- HPV-16 was more frequent in younger women than in older women.

Results: Ethnicity

- 15% of the women (n=34) with invasive cervical carcinoma were of Maori ethnicity
- There was no significant association between ethnicity and either HPV detection rate, histological cancer type or stage of disease at diagnosis
- Subtype prevalence was similar:
  - HPV 16: 58.8% Maori 49.7% non-Maori
  - HPV 18: 11.8% Maori 22.3% non-Maori
  - Minor variations in non-16/18 subtype distribution

International Comparison

HPV infection and subtype distribution rates are comparable with other international studies

Supports the evidence that the biology of HPV infection and its role in cervical pathology is consistent internationally and across different ethnicities

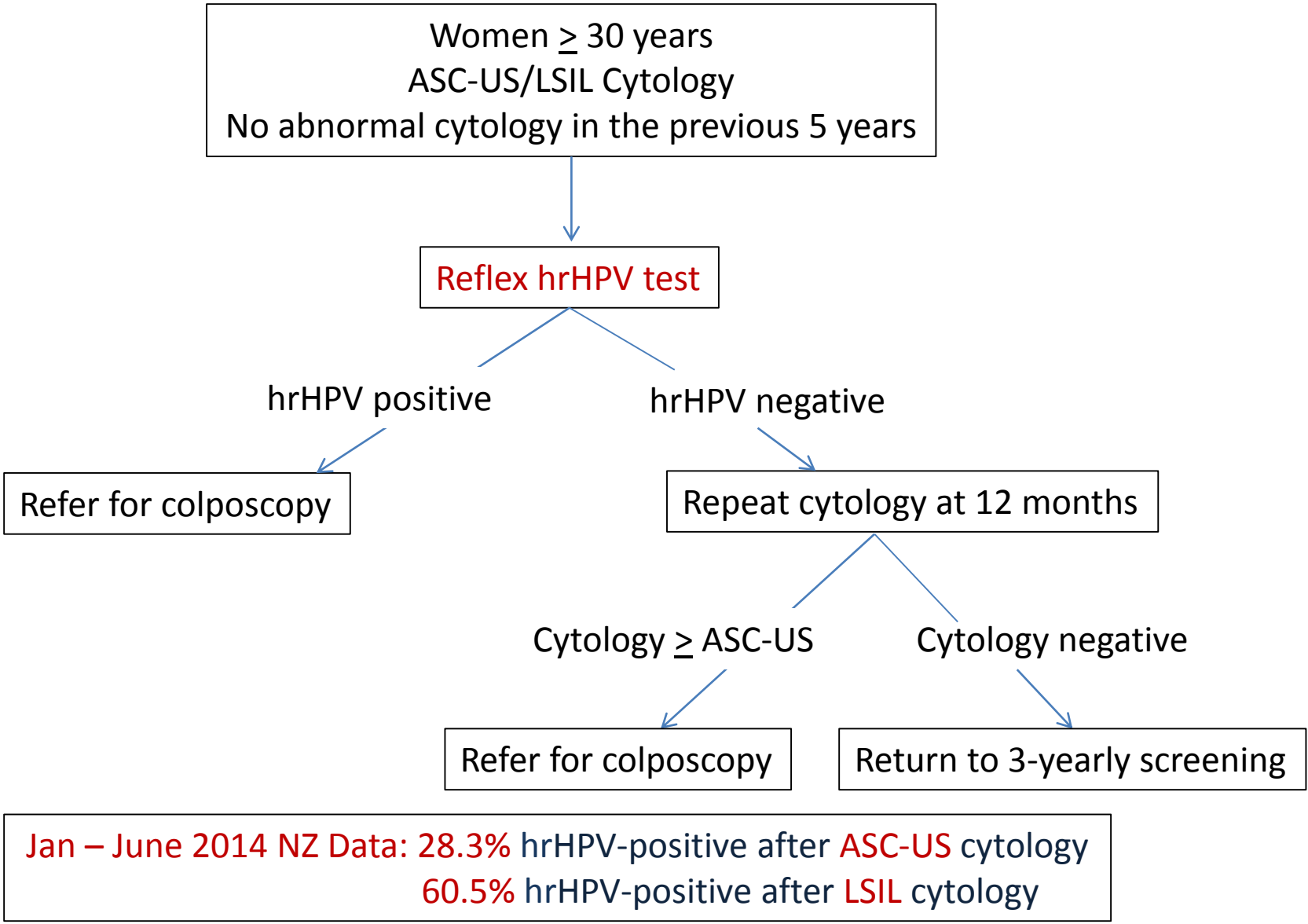
hrHPV testing in New Zealand

Introduced nationally on 1 October 2009

Funded for use in three clinical situations

1. Triage of ASC-US and LSIL in women 30+ years (with no abnormal cytology in the last 5 years)
2. Test of Cure after treatment of HSIL
3. Discordant results at colposcopy

1. ASC-US and LSIL triage

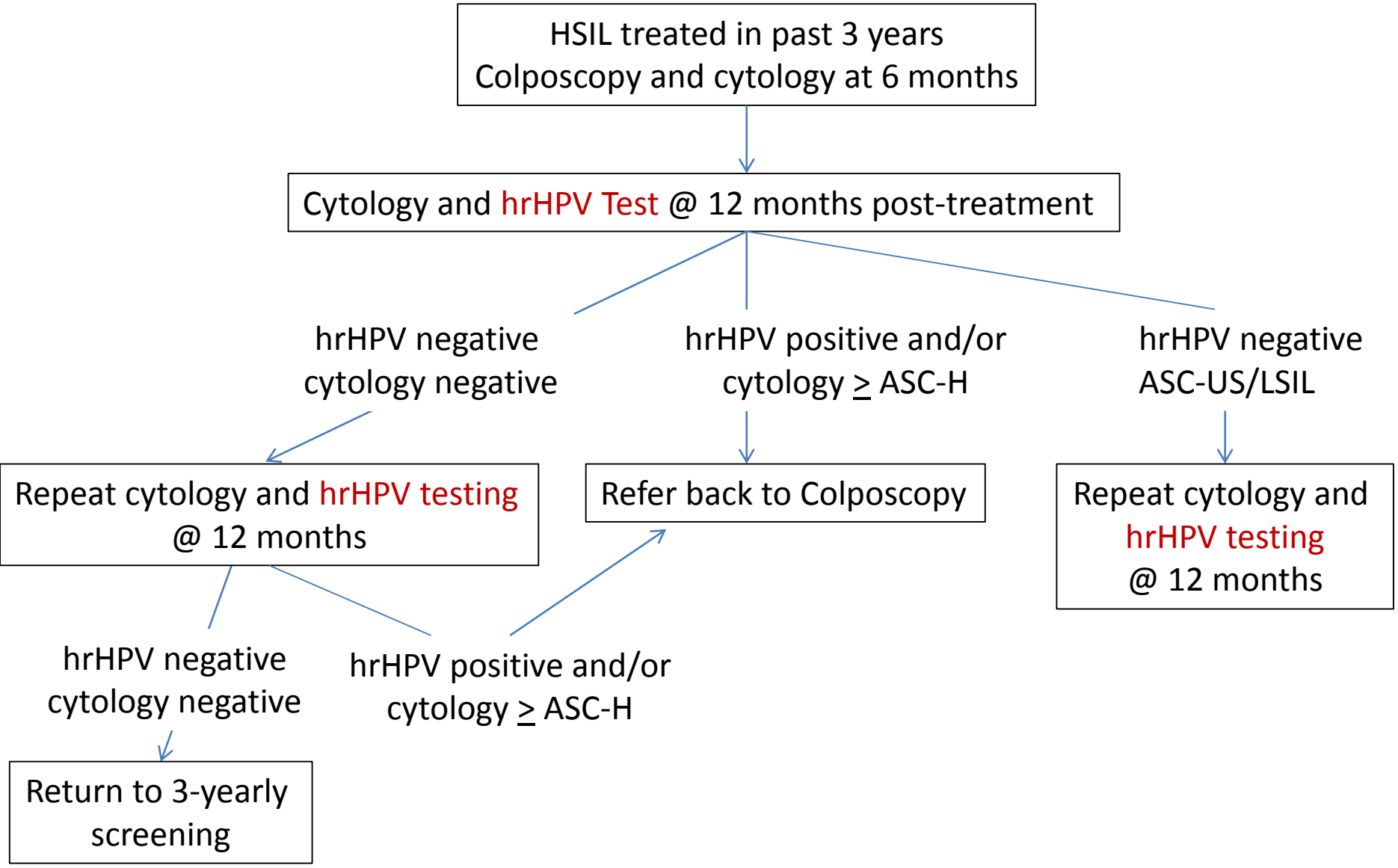


Jan – June 2014 NZ Data: 28.3% hrHPV-positive after ASC-US cytology
60.5% hrHPV-positive after LSIL cytology

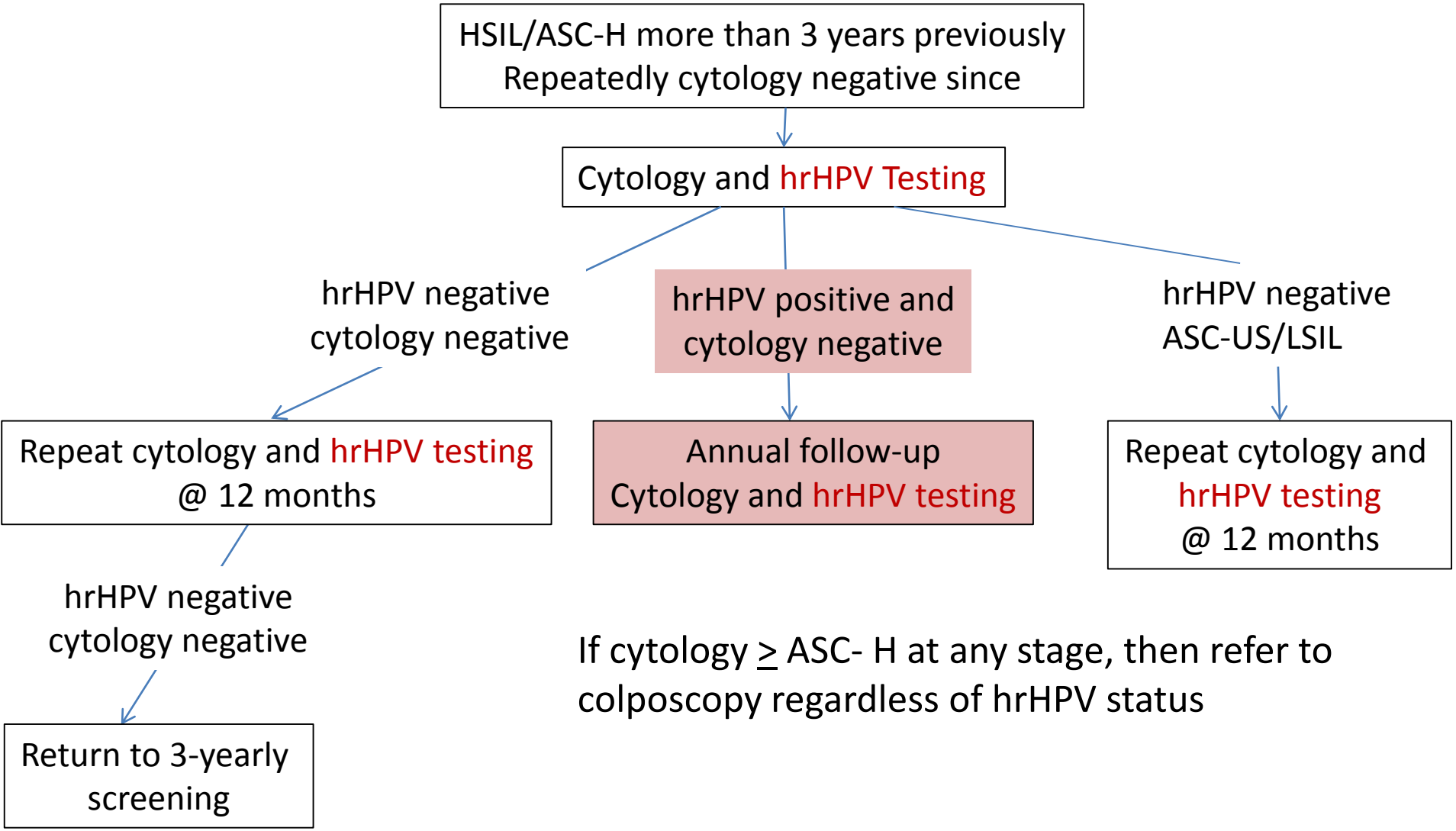
2. Test of cure after treatment of HSIL

- Used to identify women who have been successfully treated for high-grade squamous lesions.
- involves Cytology plus hrHPV testing performed on two occasions 12 months apart
- If all four results are negative, women can return to three yearly screening instead of remaining on annual screening for life.

High-grade lesions treated in the past 3 years



High-grade lesions > 3 years previously, with subsequent repeated negative cytology



If cytology ≥ ASC- H at any stage, then refer to colposcopy regardless of hrHPV status

3. Specialist testing

HrHPV Testing is ordered by colposcopists to assist with case management, particularly when there are discordant results (colposcopy/histology/cytology)

In practice, laboratories perform any HrHPV tests ordered by colposcopists

Glandular lesions

Using hrHPV testing as part of a Test of Cure regimen is not recommended after treatment for high-grade glandular lesions

Reason: The hrHPV positivity rate in high-grade glandular lesions is lower than that of high-grade squamous lesions

Problems in practice

- Some NCSP-Register records do not specify if historical lesions were squamous or glandular, particularly those pre-dating the NCSP-Register (1991)

- Women who have cervical lesions treated outside NZ may not be able to accurately report the nature of their lesions and obtaining records can be very difficult

Using hrHPV testing for primary screening

- More sensitive than cytology for detecting high-grade disease but less specific because the test detects infection, not the presence of a lesion

- ARTISTIC trial data: of 18, 386 women (revealed arm, screening)
  Cytology +ve = 2,061
  Cyto –ve, HPV +ve = 1,675

- At any given time, 8-10% of women in a screening population will be cytology negative and hrHPV+ve but this rate varies considerably with age

  The ARTISTIC Trial: HPV primary screening trial in the UK
  hrHPV +ve rates: 20-24 years = 40%
  25-29 years = 28%
  30-39 years = 15%
  40-49 years = 9%
  50-64 years = 7%

- 80% of sexually active women are estimated to have an HPV infection at some time in their lives: young women particularly, acquire lesions readily and clear them quickly.

A negative HPV test gives greater reassurance than a negative cytology test

Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow up of four European/UK randomised controlled trials comparing HPV screening and cytology-only screening

Italy - NTCC
Netherlands - POBASCAM
Sweden - Swedescreen
England – ARTISTIC

- Detection of invasive cancer was similar for the first 2.5 years but was significantly lower in the HPV screening arm thereafter

- HPV-based screening provided 60-70% greater protection against invasive cancer compared with cytology

Ronco et al Lancet 2014:383:524053

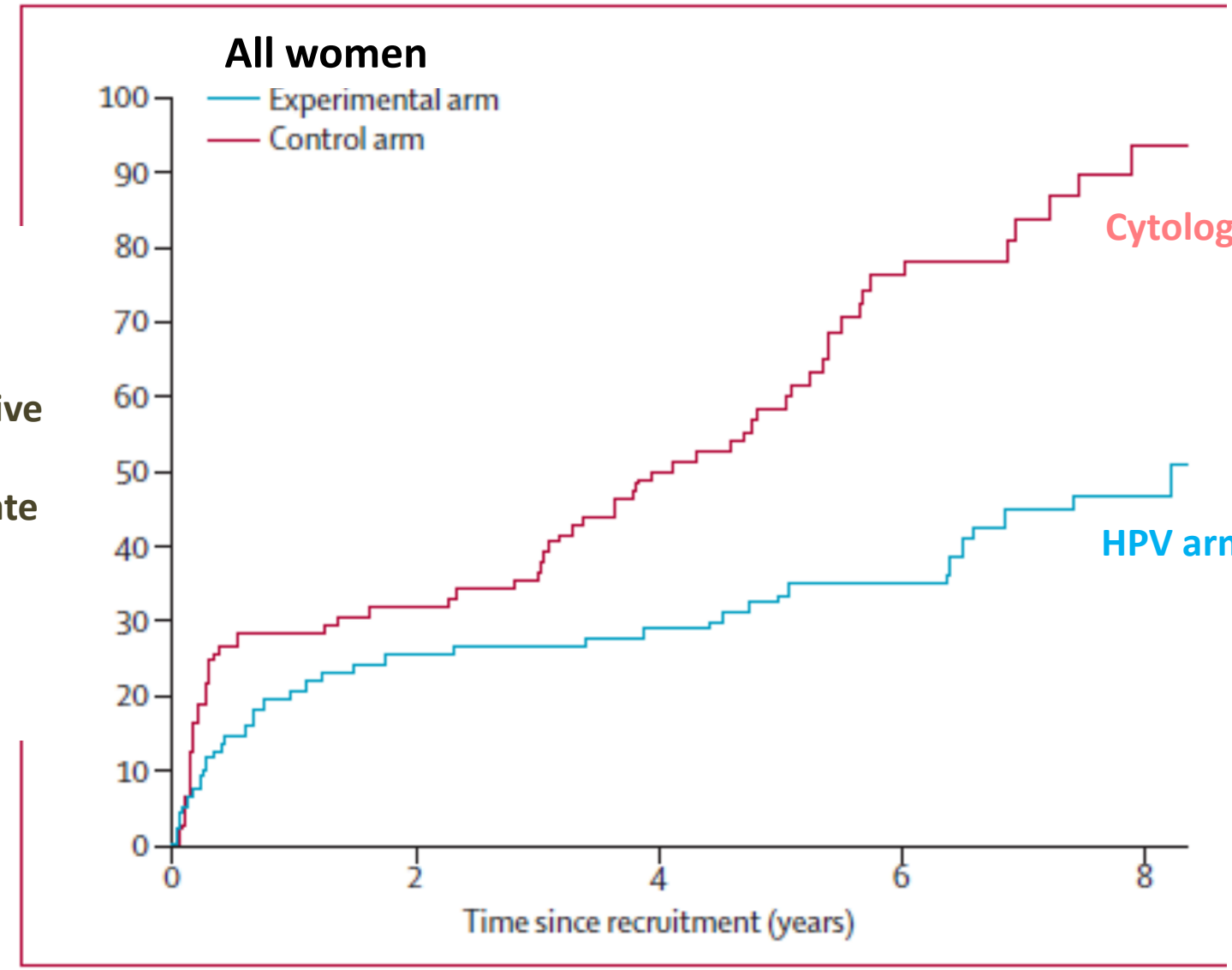
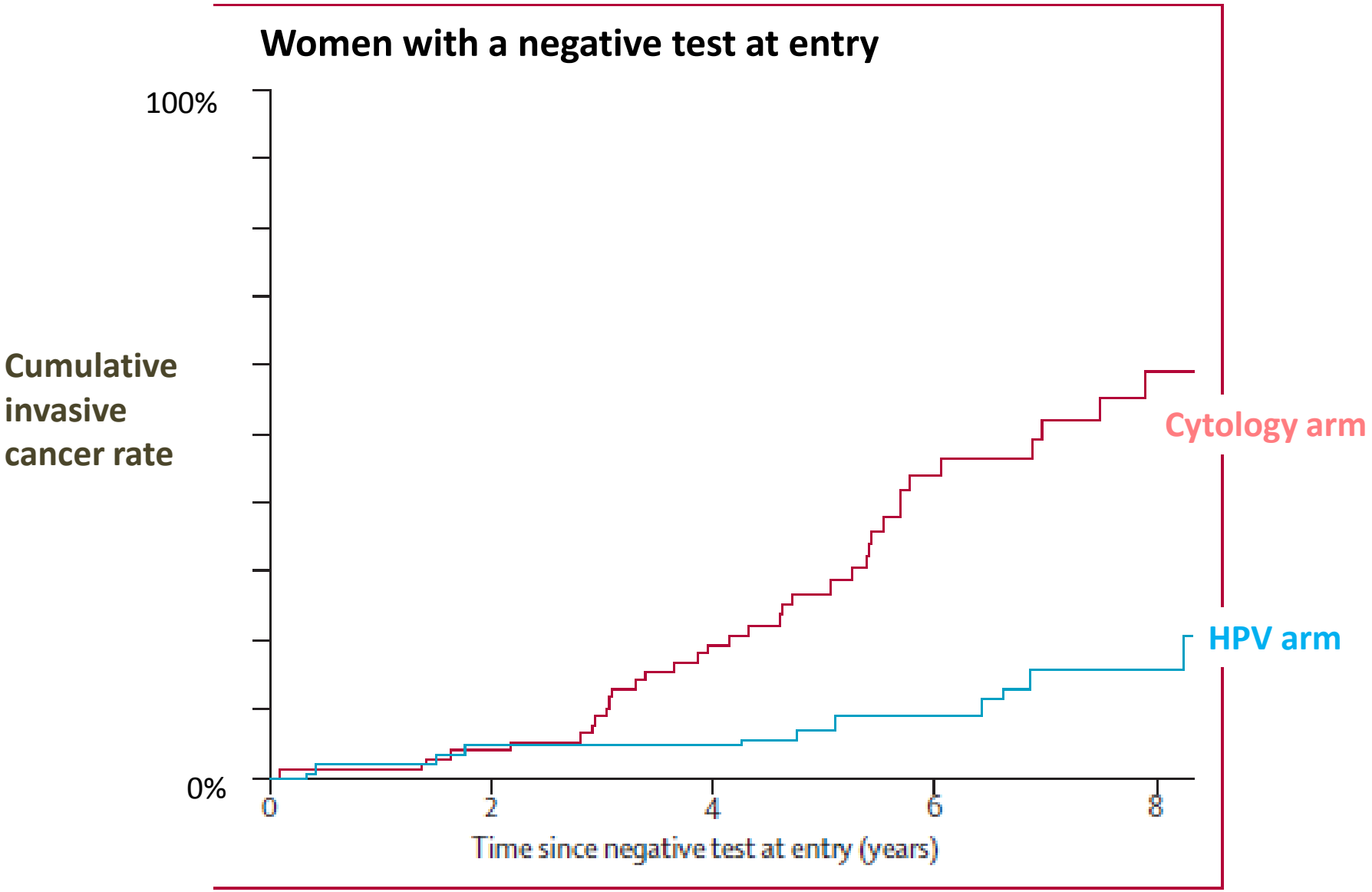


Figure 2: **Cumulative detection of invasive cervical carcinoma**

*Observations are censored 2·5 years after CIN2 or CIN3 detection, if any.

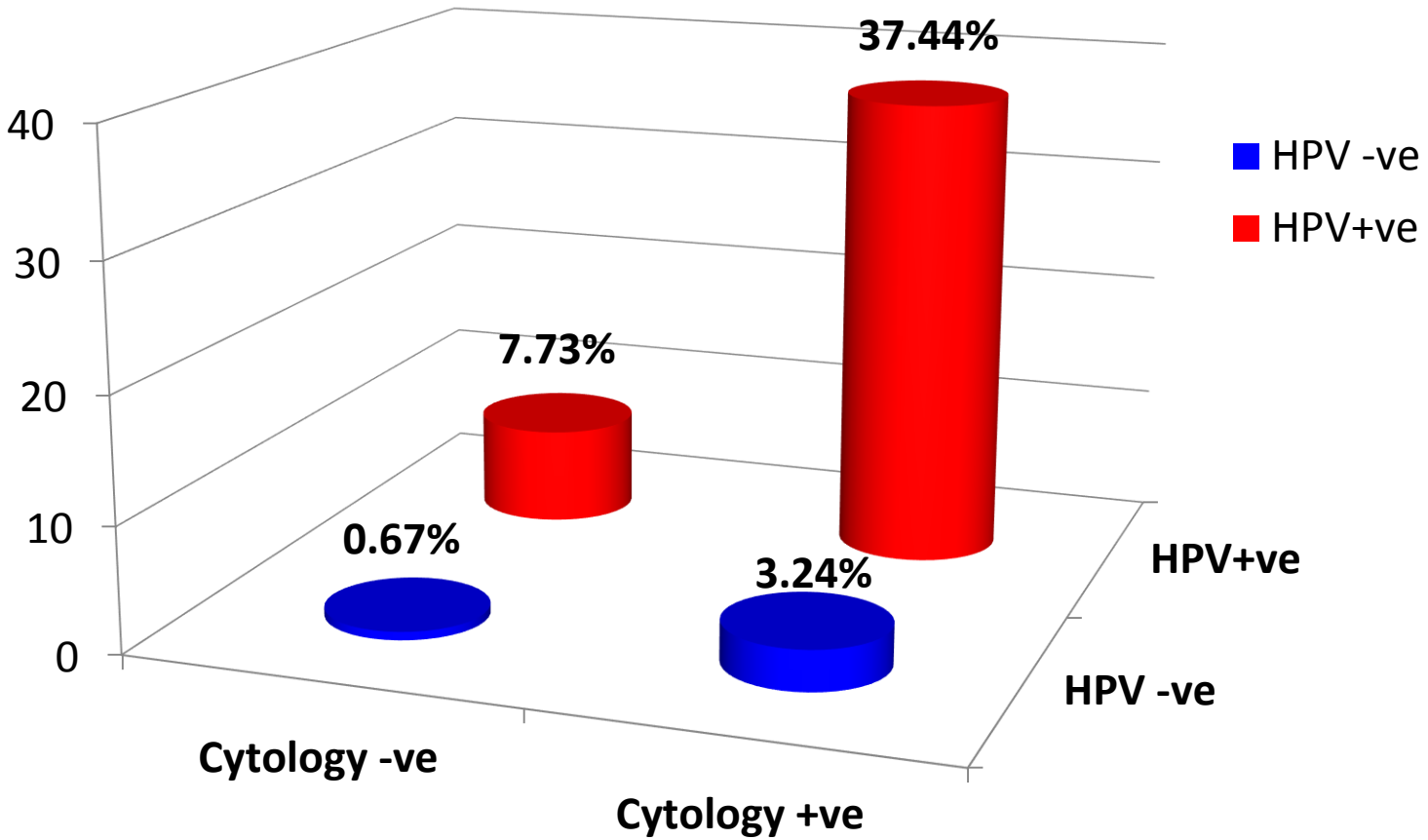
Ronco et al Lancet 2014:383:524053



Ronco et al Lancet 2014:383:524053

The ARTISTIC Trial results: LBC Cytology

Cumulative % CIN 2+ outcome by cytology and HPV status at entry



Cervical cancer risk for 330,000 women undergoing concurrent HPV testing and cervical cytology in routine clinical practice at a large managed care organisation *Lancet Oncology* 2011;12(7):663-72 Katki et al

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- Prospective study analysing the cumulative CIN3+ and invasive cancer rates for 331,818 women aged 30+ who were cotested and followed for 5 years.

Findings for invasive cancer

- 5-year cumulative incidence was extremely low for

  315,061 HPV-ve women: 3.8/100,000

- only slightly higher than for

  306,969 co-test -ve women: 3.2/100,000

- and half that of the rate for

  319,177 cyto –ve women: 7.5/100,000

.....

- HPV+ve cyto –ve women had 29% of the cancers and 63% of the adenocarcinomas

Cervical cancer prevention strategy

Primary prevention:

HPV Immunisation

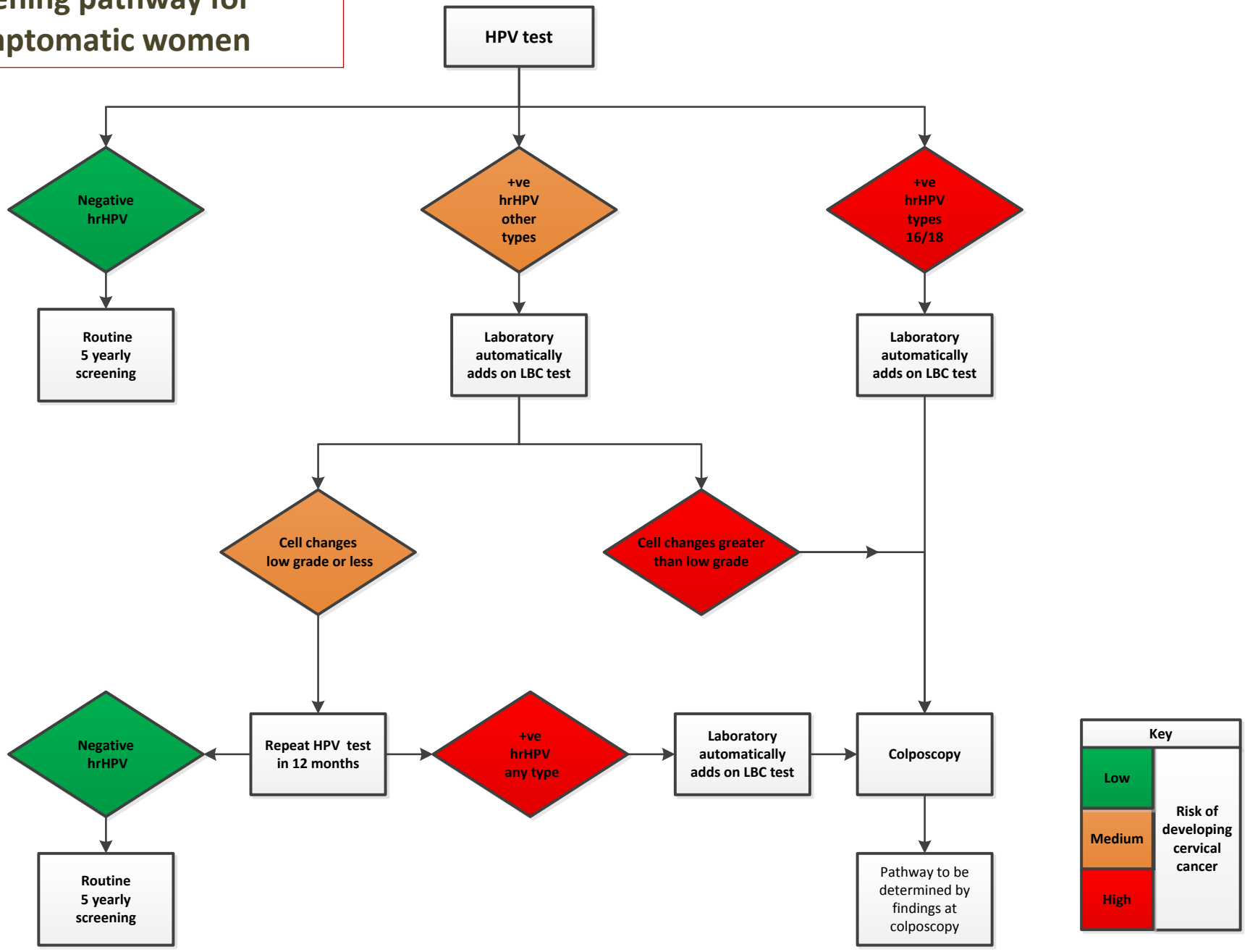
Secondary Prevention:

Screening tests

Currently LBC cytology every 3 years

2018: hrHPV Testing with partial genotyping and cytology triage every 5 years

Screening pathway for asymptomatic women



Potential for self sampling for hrHPV screening

- A sample for cytology needs to be taken from the transformation zone so that abnormal cells are sampled whereas hrHPV testing doesn't require such as specific sample –a high vaginal swab can be used
- Opens up the possibility for self-sampling, particularly as a way to increase screening coverage for women who aren't currently being screened
- If the hrHPV test is positive, the woman will need to have a speculum examination for a clinician-taken sample for cytology
- Need to ensure: a self-sample is as good or nearly as good as a clinician-taken sample, accurate sample identification can be assured, screening coverage will be increased
- NZ research projects about to commence to investigate the acceptability of this option for NZ women
- Self-sampling will NOT be offered initially as an option in 2018

HPV immunisation currently

- Offered free to 12-13 year girls from 2009
- Mainly a school-based programme. Primary care can follow up girls who were not vaccinated in the school based programme
- Currently free for girls and young women up to their 20th birthday.
- protects against hrHPV types 16 and 18 (70% of cervical cancers) and low-risk types 6 and 11 (90% of genital warts)
- HPV immunisation 2-dose coverage: currently 68% for all ethnicities – better for Pacific, Asian and Māori (78%) women
- Immunised or not, women still need to participate in regular cervical screening. The vaccine does not protect against all hrHPV types.

Immunisation against HPV: the future

From 2017 immunisation against HPV will be funded for:

- Girls and boys
- school based programme at 12-13 years of age with free vaccination up to the age of 26 years
- Nonavalent (Gardasil-9) will be used; includes 7 high-risk types plus 6 and 11
- Two doses at least 6-months apart: will assist with coverage

The future is bright for cervical cancer prevention

- HPV primary screening, effective 9-valent vaccination and the possibility of self-sampling to increase coverage will combine to have a major impact to further reduce invasive cervical cancer rates
- Coverage remains as important as ever: women who aren't screened or immunised don't get the benefits of all the research and development that has occurred in the last 20 years
- Disparities in coverage between different ethnic groups in NZ remains a major challenge for the NCSP