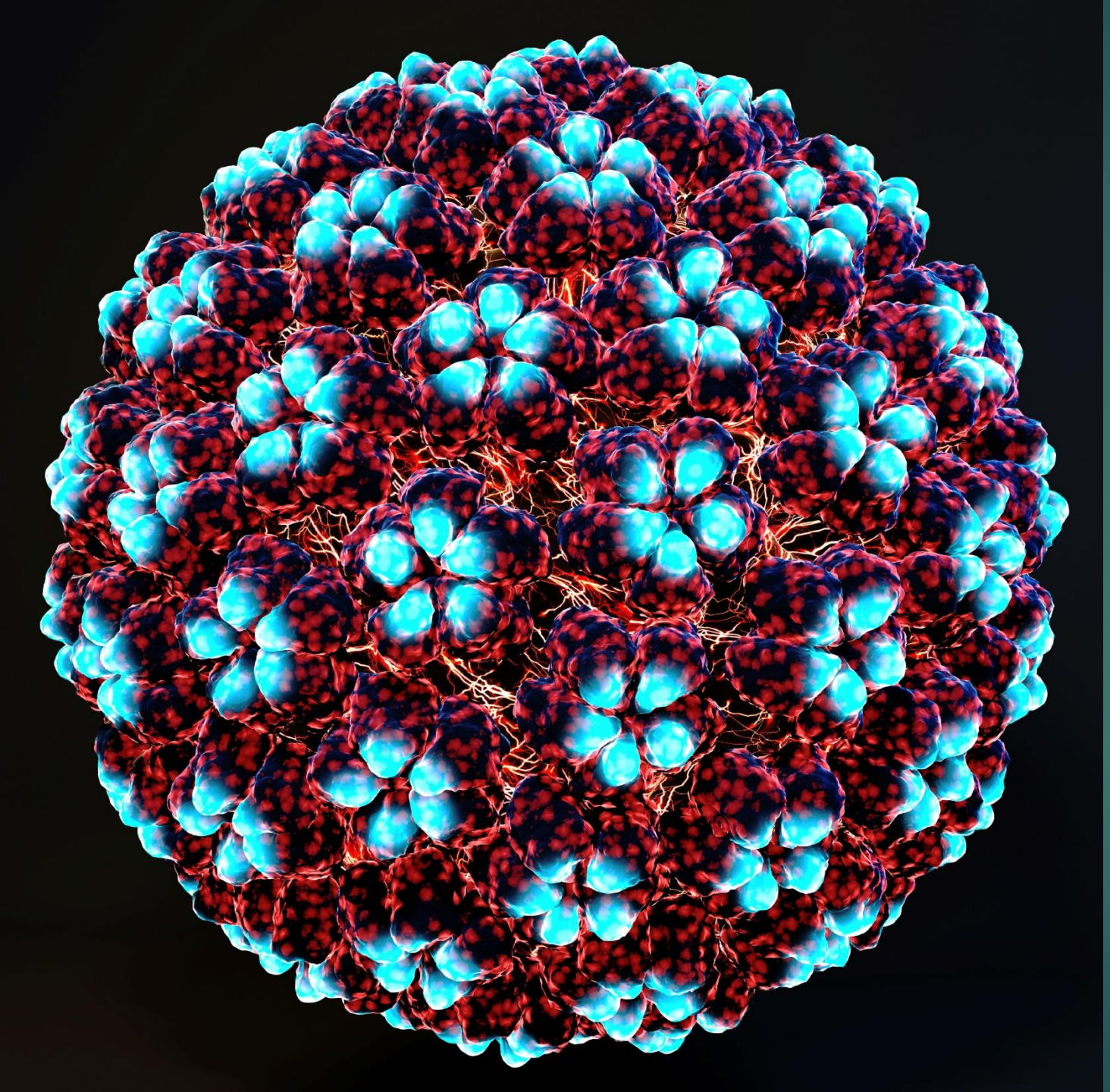

Advances in HPV Detection for Cervical Cancer Screening

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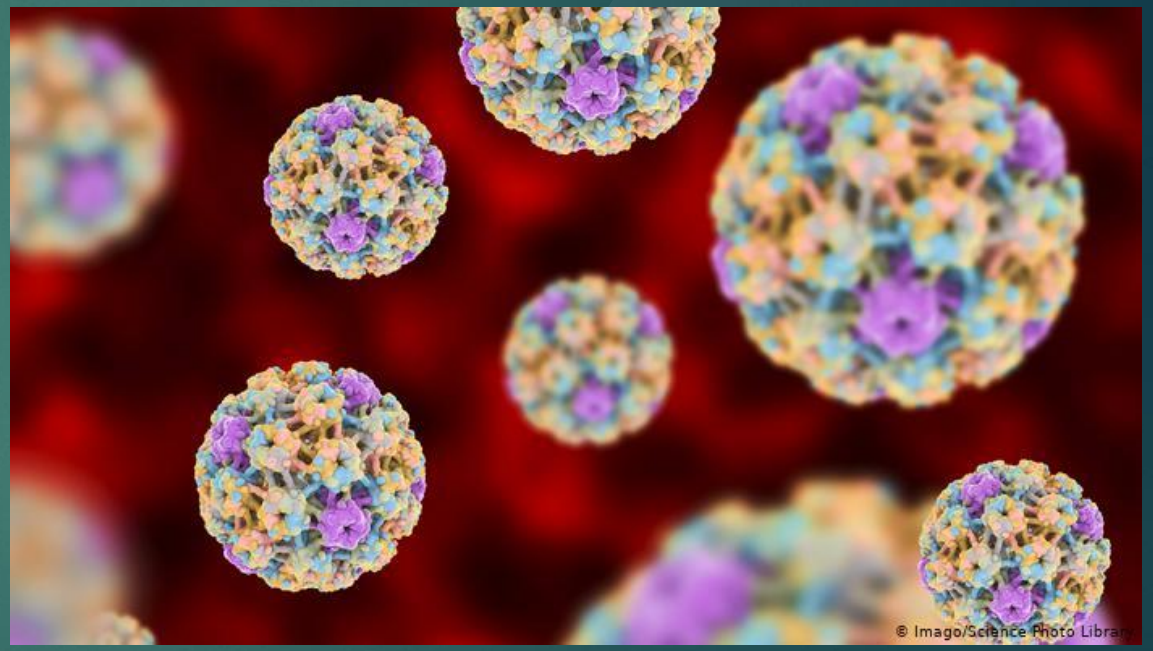
NATIONAL CERVICAL PATHOLOGY TRAINING SERVICE - 2020

Introduction

- There more than 150 types of HPV that live on the body and only a small number (high-risk HPVs) cause problems by transforming cells into cancer cells.
- Skin infection with low-risk HPV types can cause external genital warts (e.g. HPV6 and HPV11) which are rarely associated with pre-cancer or cancer.
- High-risk HPV is recognised as the single major cause of cervical cancer and is found in 99.7% of cervical carcinomas. High-risk HPVs can also cause cancers of the anus, oropharynx, vagina, vulva and penis.
- The risk of developing cervical cancer increases up to 400 fold for women with persistent high-risk HPV infection
- Without HPV infection cervical cancer is rare
- Cancer prevention is based on recognizing and treating precursor lesions before they become invasive cancer
- There are more than 100 commercially available HPV assays
- HPV test technologies play a critical role in cervical screening in countries where HPV testing has become the primary screening test

Introduction

- HPV test technologies are constantly evolving and will continue to evolve for future years
- New factors to consider are:
  - Extended Genotyping
  - Viral Load
  - Viral Integration
  - Methylation Status
  - HPV Variants



Extended Genotyping

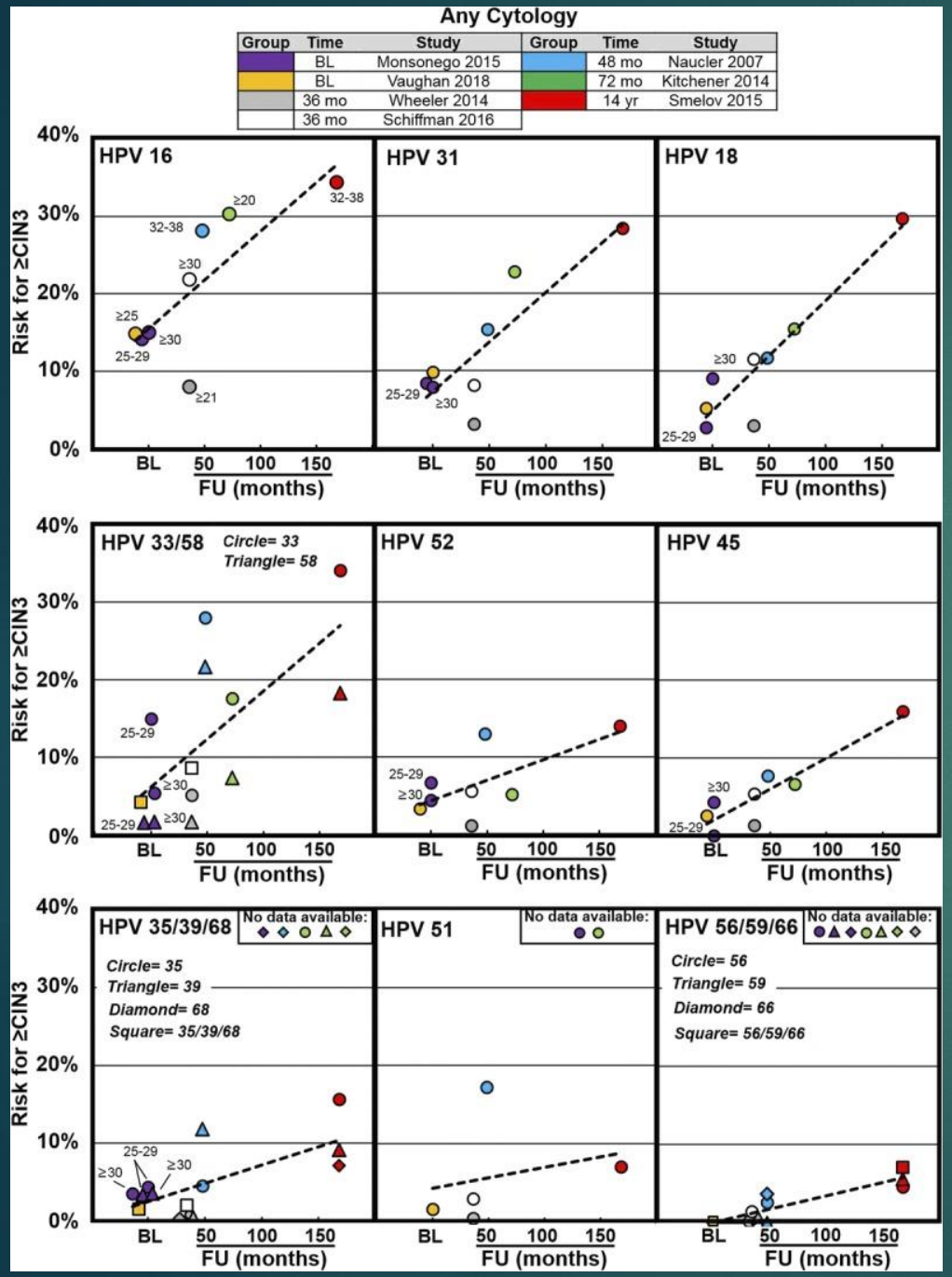
- Focus for HPV genotyping has been mostly on HPV 16 and HPV 18 due to their high prevalence in cervical cancer
- However screening focusses on detecting high-grade precursor lesions CIN 2 and CIN 3 where 'other' HPV types also have a role
- Most current partial genotyping systems separately identify HPV 16 and 18 (and sometimes 45), and group all remaining high-risk HPV types as 'other'
- Emerging evidence has demonstrated that the 'other' HPV genotypes display a range of different risks. Some types present risks for high-grade disease and cancer at least as high if not greater than the risks associated with HPV18.
- Further discrimination of risk based on extended HPV typing is clearly important
- Extended genotyping separately identifies the genotypes of 16, 18 and a variable number of other high-risk types (such as 45, 31, 33) and then groups the remainder of high-risk types into 2-3 risk stratified groups.

Extended Genotyping

- HPV types 16, 18, and 45 are the 3 most common types in cervical cancer. Together, they account for 75% of squamous cell carcinoma and 94% of adenocarcinoma
- In all the studies, HPV 16 was associated with the greatest risk for developing CIN 3 or worse
- Various studies have shown that genotypes 31, 33, 52, and 58 confer risks similar to HPV 18 and 45
- Human papillomavirus 31, 18, 33, and 58 were frequently the genotypes of next highest risk, and HPV 31 and 33 had similar or higher risks than HPV 18, including after 6 to 14 years of follow-up
- Genotypes 35, 51, 56, 59, 66, and 68 were consistently associated with risks lower than the overall risk for pooled HPV positive and lower than the colposcopy threshold risk
- Some authors recommend separate genotyping of HPV 16, 18, 31 & 33 and pooled genotyping of other high-risk types (HPV 35, 45, 51, 52, 58) and intermediate-risk types (HPV 39, 56, 59, 68

HPV type varies with tumour type and age

- The role of different HPV types in the causation of cervical adenocarcinoma is also different from squamous cell carcinoma. HPV-18 and HPV-45 are significantly more common in cases of adenocarcinoma than in cases of squamous cell carcinoma.
- HPV 16 is more common in women under 30 years with cervical cancer compared with older women. For older women the relative contribution from "other" high-risk HPV types is greater compared with younger women.
- A finer level of genotyping deserves further research to find combinations that optimally use sample information to stratify risk of high-grade disease, and to use this information to improve management algorithms (Cuzick & Wheeler 2016)

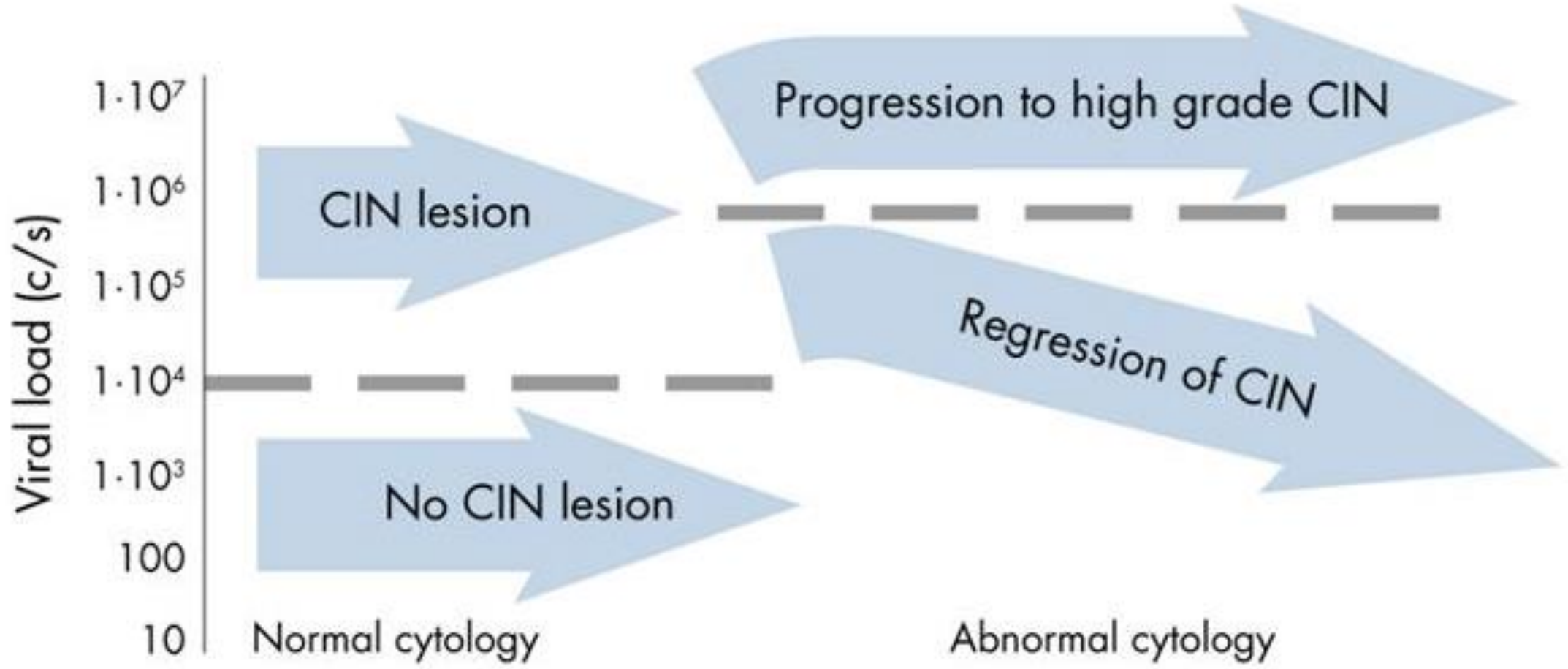


Cervical intraepithelial neoplasia 3 or worse risk values associated with individual HPV genotypes from previously described screening populations—regardless of cytology result. The x-axis represents time to follow-up in months or years (where indicated) and the y-axis represents increasing risk for CIN 3 or worse. Data were extracted from 7 articles that represent baseline results (Monsonego et al, 015; Vaughan et al, 2018) and results at 36 months (Wheeler et al, 2014; Schiffman et al, 2016) 48 months (Naucler et al, 2007), 72 months (Kitchener et al, 2014), and 14 years (Smelov et al, 2015) following baseline in each of the respective studies. Trend lines are superimposed across time points from baseline to 14 years to help visualize the increasing risk associated with long-term HPV infection. Abbreviation: BL, baseline.

Bonde at al 2020

Viral Load

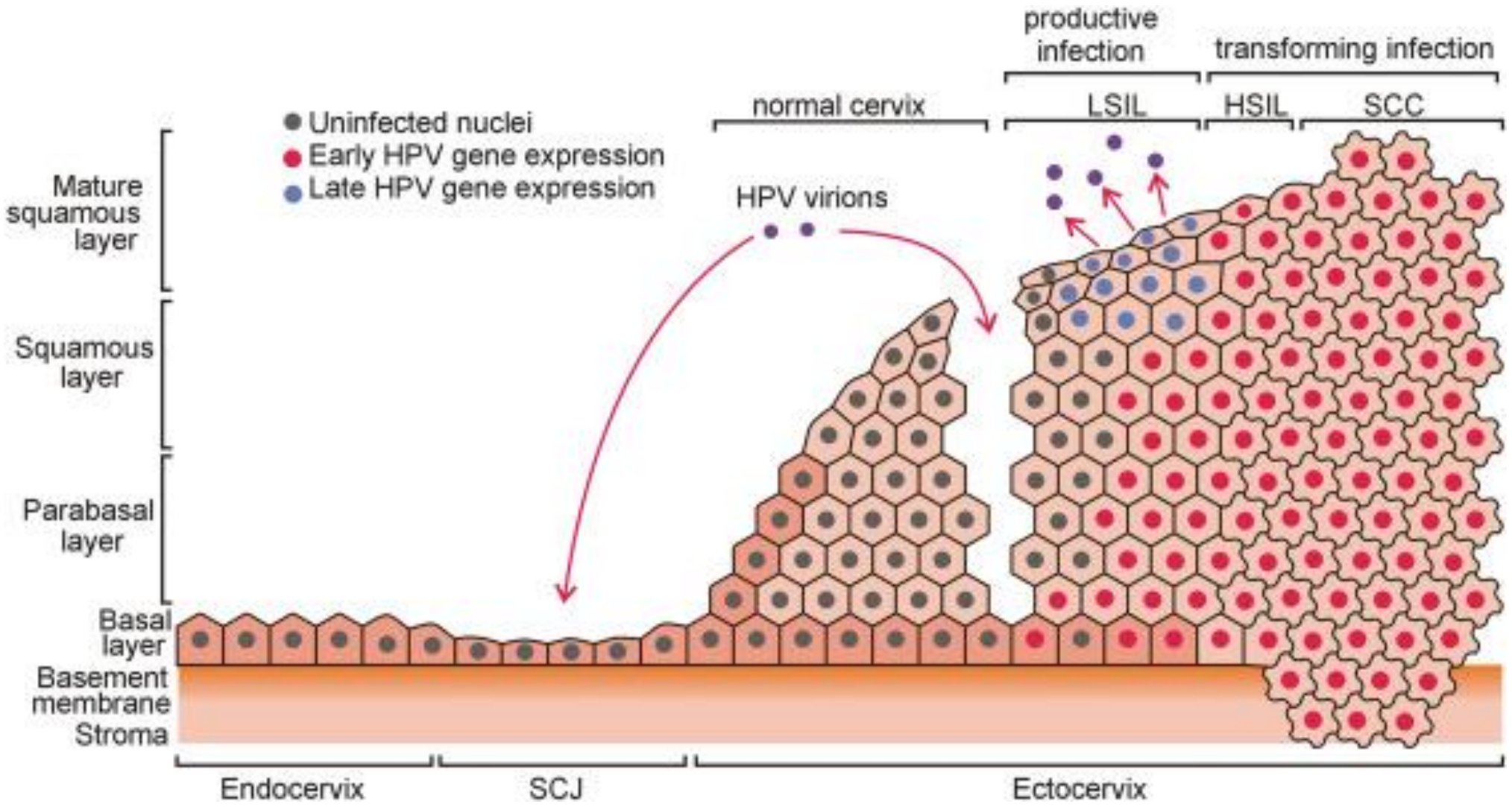
- Determining viral load and integration have been proposed as ways of increasing the specificity of HPV tests
- Persistent infection with hrHPV is the key event in development of cervical cancer and precursor lesions
- A hrHPV DNA test can't discriminate between a persistent and transient infection
- An increase in viral load is associated with an increased risk of CIN 2+
- Reduced amounts of viral DNA can be associated with viral clearance
- Increased viral loads of HPV 16, 31 & 33 were predictive of cumulative high-grade CIN+ diagnosed within an 18 month period in various studies



Hesselink et al 2007

Viral Integration

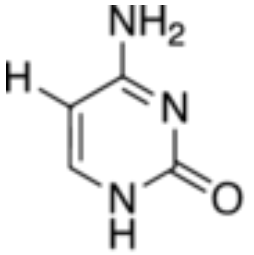
- The integration status of high-risk HPV in premalignant cervical lesions might be a further promising risk marker for progression of cervical cancer
- Viral DNA integration into the host cell genome usually disrupts the E1 and E2 open reading frames, while those of E6 and E7 remain intact
- This results in disruption of expression of E2 protein and subsequent upregulation of the transcription of the oncogenic E6 and E7 proteins
- Continuous production of oncogenic E6 and E7 proteins contribute to the malignant state in infected tissue
- New molecular technologies are currently facilitating these discoveries



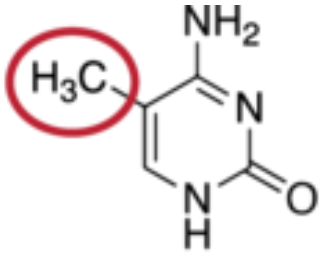
Durzynska et al 2017

What is methylation?

- Epigenetics is where additional information is layered on top of the sequence of letters that makes up DNA
- There are different types of epigenetic markers and each one tells the proteins in the cells to process those parts of the DNA in certain ways
- An example of this is methylation which is a process by which methyl groups are added to DNA which can suppress gene transcription
- Two of DNAs four nucleotides can be methylated - cytosine and adenine



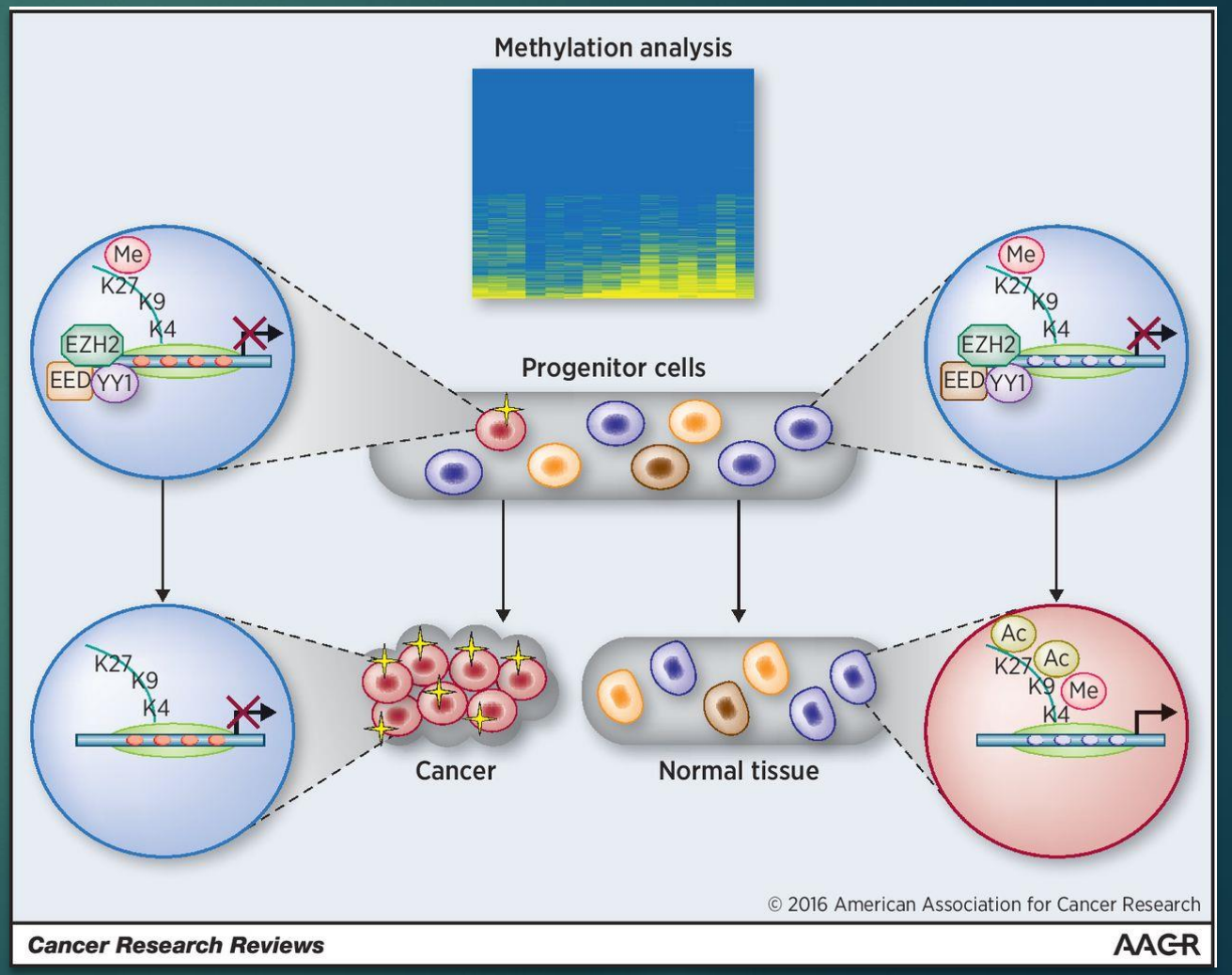
Cytosine



methylated Cytosine

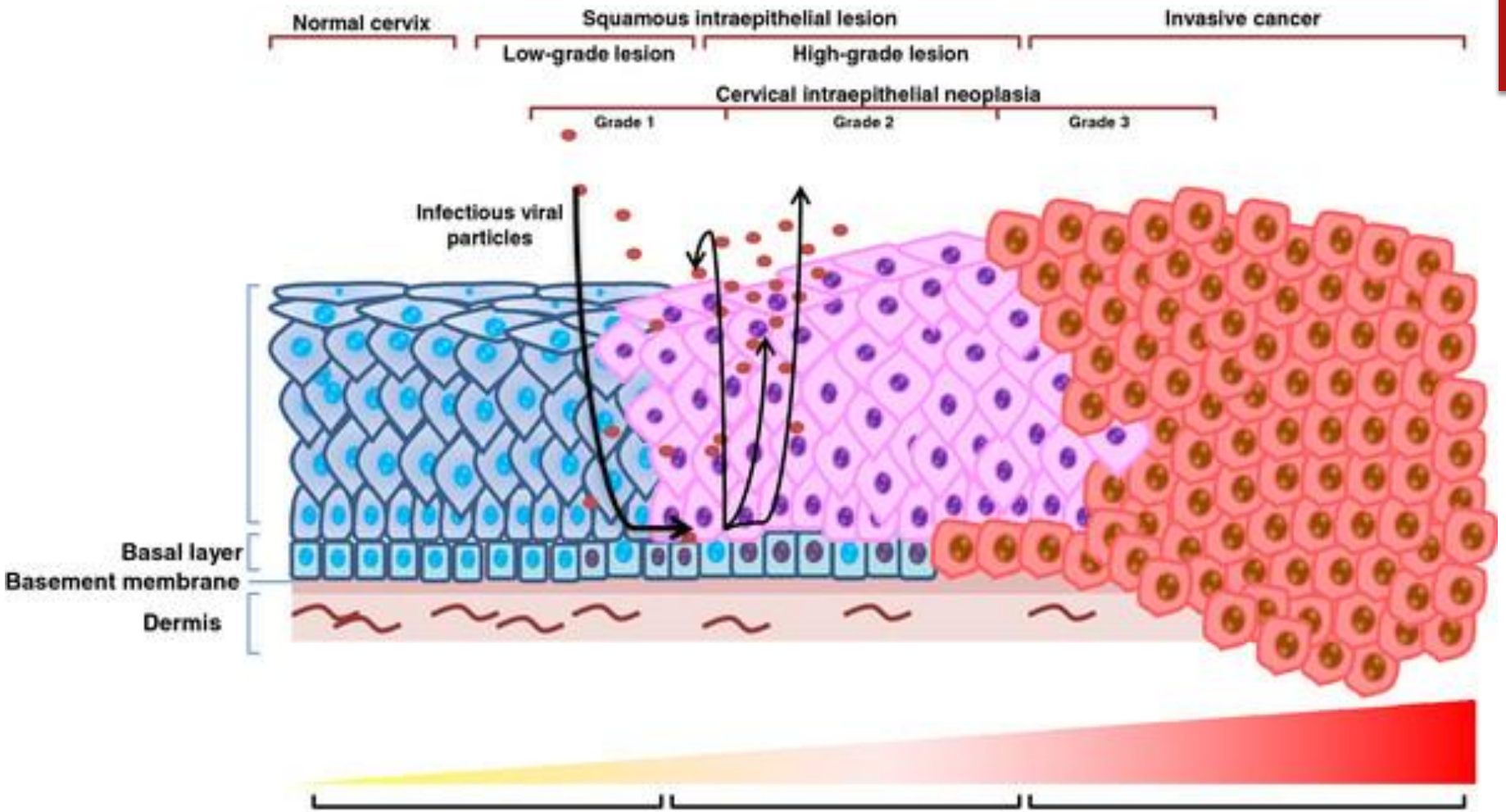
What is DNA methylation?

- Methylation is the main way gene activity is adjusted during life, especially during early development
- There are proteins that specifically seek out and bind to these methylated areas and shut it down so that genes in that region are inactivated in that cell



Methylation Status

- Hypermethylation of HPV and host genes has been reported in cervical cancer
- The degree of methylation of different HPV types may be relative to the severity of cervical lesions
- Genomic DNA methylation has been proposed as an additional marker to increase sensitivity for detecting cervical pre-cancerous lesions
- A study found that the degree of L1 methylation of HPV 16, 18 & 52 but not 58 is associated with the severity of cervical lesions (Hsu et al 2017)
- Several DNA methylation markers involved in carcinogenesis are being developed as tools for predicting progressive lesions. Some markers measure methylation of HPV genes and some measure methylation of host genes.



Methylation Markers

CCNA1			36,6%	93,9%
SFPR2	4,4%	15,6%	16,3%	80,7%
p73	3,2%			38,8%

Woodman et al., 2007

HPV Variants

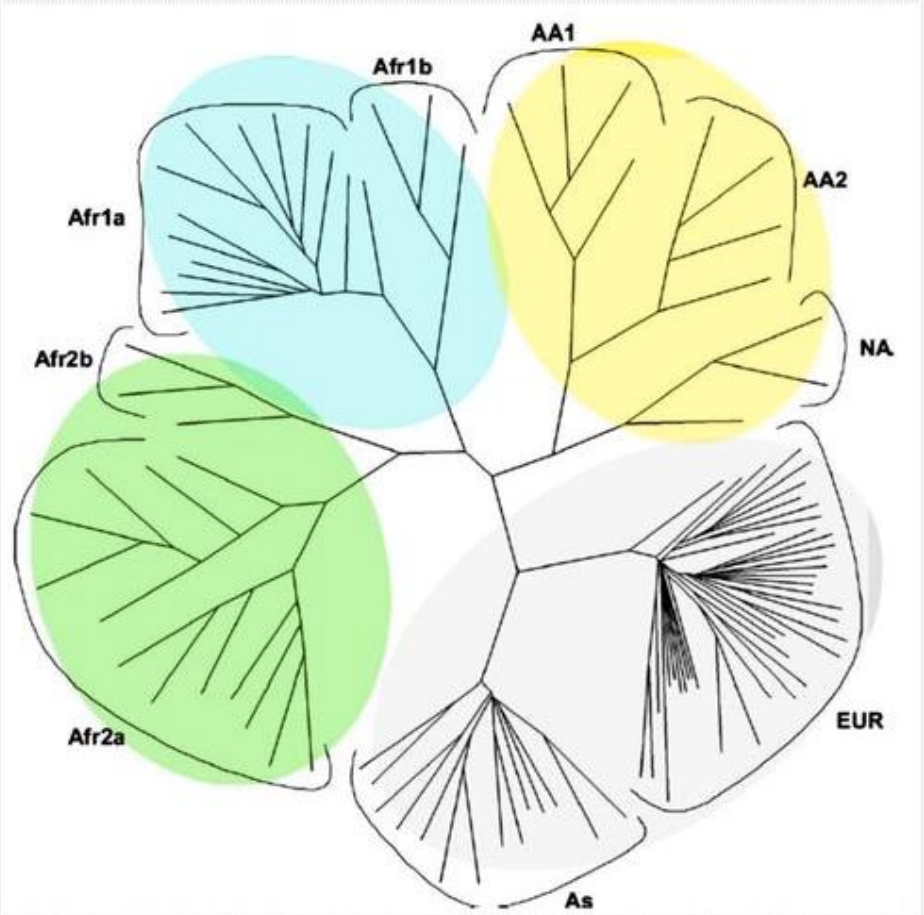
- Different HPV genotypes are involved in the pathways underlying cervical carcinogenesis
- HPV variants co-evolved during human evolution and are often unique to specific ethnic groups
- They can be classified into species and types based on genetic distances between viral genomes
- HPV variants of a type share >90% nucleotide identities
- Variant lineages of a given type differ by approximately 1–10%
- Variant sublineages of a given type differ by approximately 0.5–1.0%
- Despite phylogenetic relatedness, HPV variants can differ in pathogenicity

HPV 16 Variants

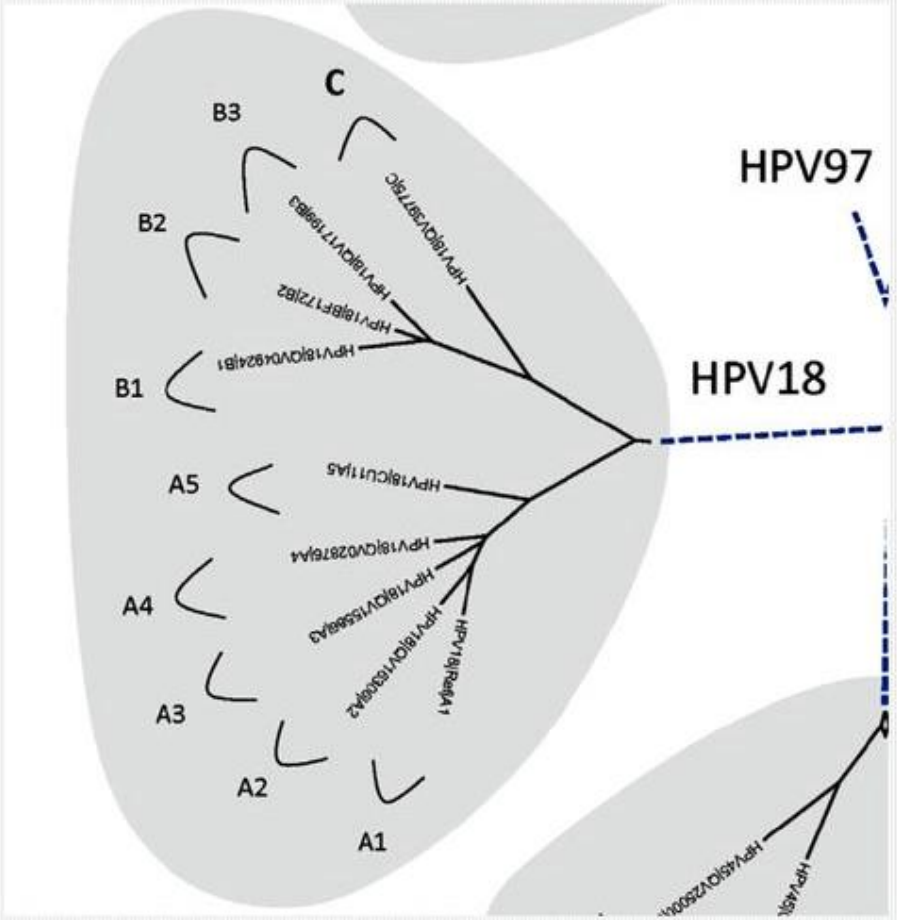
- There are a number of phylogenetic variants of HPV16 originally classified as European, Asian, African, North American and Asian American
- HPV type 16 can be divided into four main variant lineages (A/B/C/D) and nine sub-lineages
  - A – includes A1, A2 & A3 (European) and A4 (Asian)
  - B – B1 (African1a) and B2 (African1b)
  - C – African 2a
  - D – D1 (North American), D2 (Asian American AA2), D3 (Asian American AA3)
- Most studies implicate the non-European lineages as being more pathogenic in comparison to the European lineages
- There is a 2 fold increase for cervical neoplasia in Non-European vs. European variants (Schiffman et al 2010, Villa et al 2000)

HPV 18 Variants

- HPV 18 also has a number of variants (A, B & C) from Europe and Africa
  - A1 & A2 (Asian American); A3, A4 &A5 (European)
  - B - African
  - C – African
- Some variants of HPV18 are more likely to be detected in adenocarcinomas and others in squamous cell carcinomas (Chen et al 2015)
- Other studies have shown there is no compelling evidence that different HPV18 variants are associated with risk of cancer (Arias-Pulido et al 2005)



Cornet et al. /Journal of Virology 86 (2012) 6855–6861



R.D. Burk et al./Virology 445 (2013) 232–243

9 HPV16 variant sub-lineages

3 HPV18 major lineages (A, B C)

HPV 31 Variants

- HPV type 31 has 3 variant lineages (A/B/C) and 7 sub-lineages
  - A1, A2
  - B1 & B2
  - C1, C2 & C3
- Two recent studies provide consistent data that HPV 31 lineage C is more persistent that A and/or B (Schifmann et al 2013)
- Another study showed that HPV31 lineages A/B are more commonly associated with development of CIN3 (Schifmann et al 2012)

HPV 58 Variants

- HPV type 58 has 4 variant lineages (A/B/C/D) and 7 sub-lineages
  - A1, A2 & A3
  - B1 & B2
  - C
  - D1 & D2
- Certain variants of HPV 58 are associated with an increased risk of high grade squamous intraepithelial lesions and cervical cancer
- Comparison of A vs. B/C/D suggested the A lineage was associated with persistence and possibly CIN3+ (Schifmann et al 2010)

Conclusions

- A finer level of genotyping along with other discriminators not used in current HPV testing algorithms such as viral load, methylation status, and HPV variant status deserves further research
- We need to find combinations that optimally use sample information to stratify risk of high-grade disease, and to use this information to improve management algorithms.
- Cervical cancer screening is based on recognising and treating precursor lesions before they become cancer
- Doing this effectively and still avoiding over treatment should be the primary goal of a cervical cancer screening programme