

HPV E CANCRO DEL BASSO TRATTO GENITALE

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HPV-UNIT

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BACKGROUND

The progression of HPV-induced lesions toward cancer reflects a classical **selection scenario** in which certain events lead to the clonal outgrowth of single cells in a heterogeneous cell population.

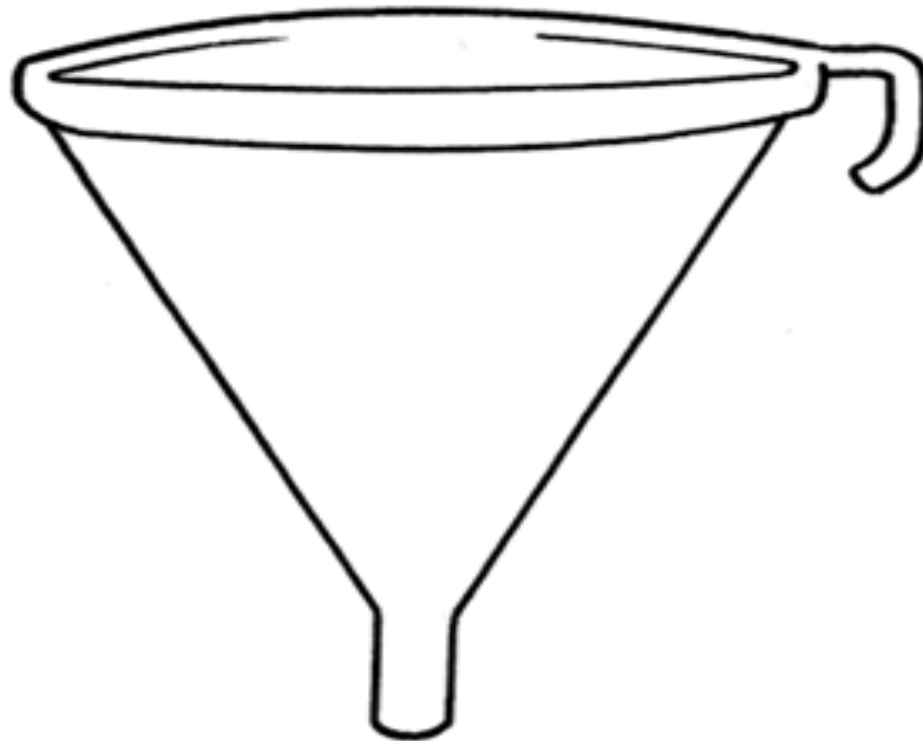
(N.Wentzensen, 2004)

- Selective pressure to confer a growth or survival advantage on the affected cells
- *Causalita', casualita' e dogmi da ripensare*

BACKGROUND

80% popolaz femminile → 23.000.000

10% persistenza HPV+ → 2.300.000

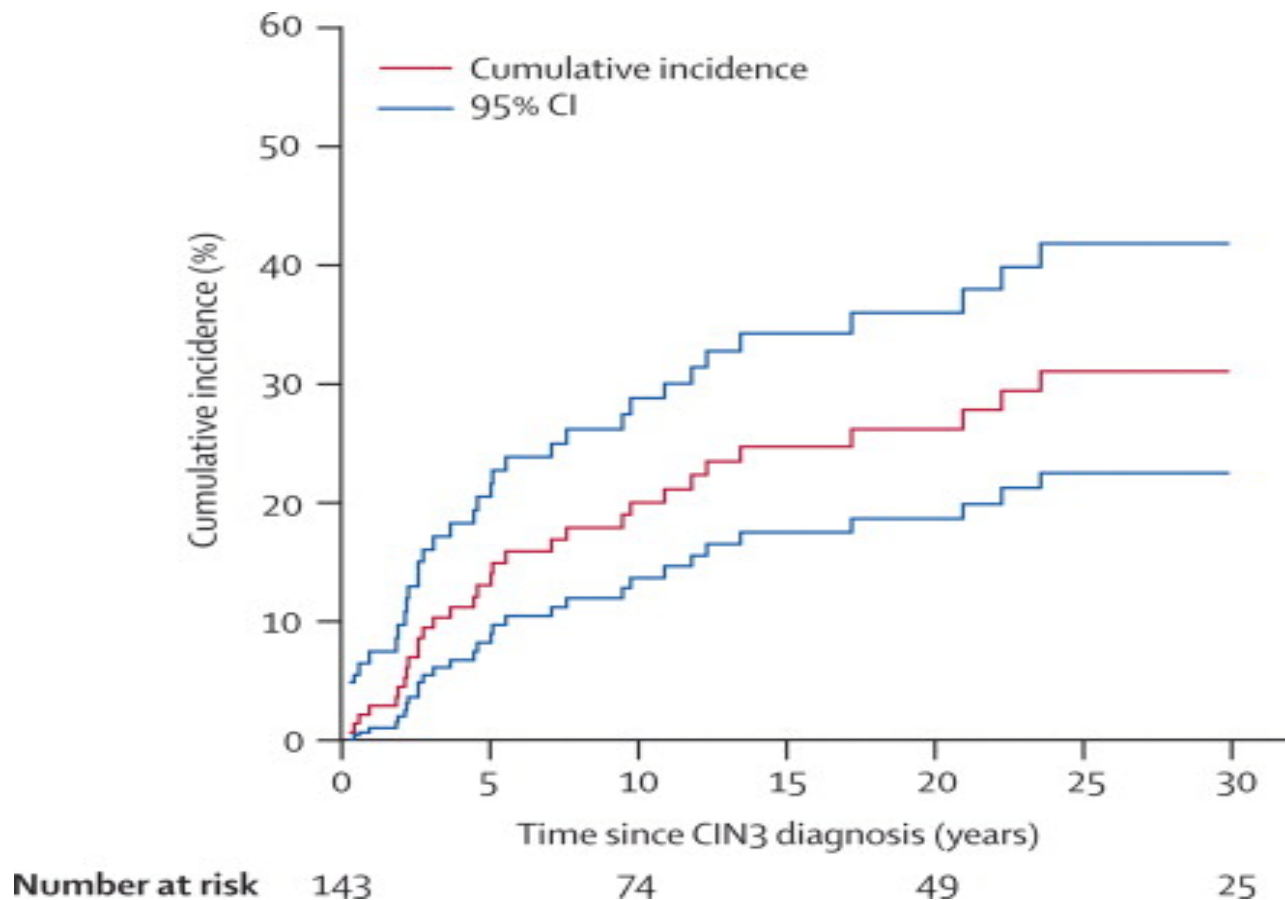


HG-SIL → 20.000

Cancro portio → <3.000

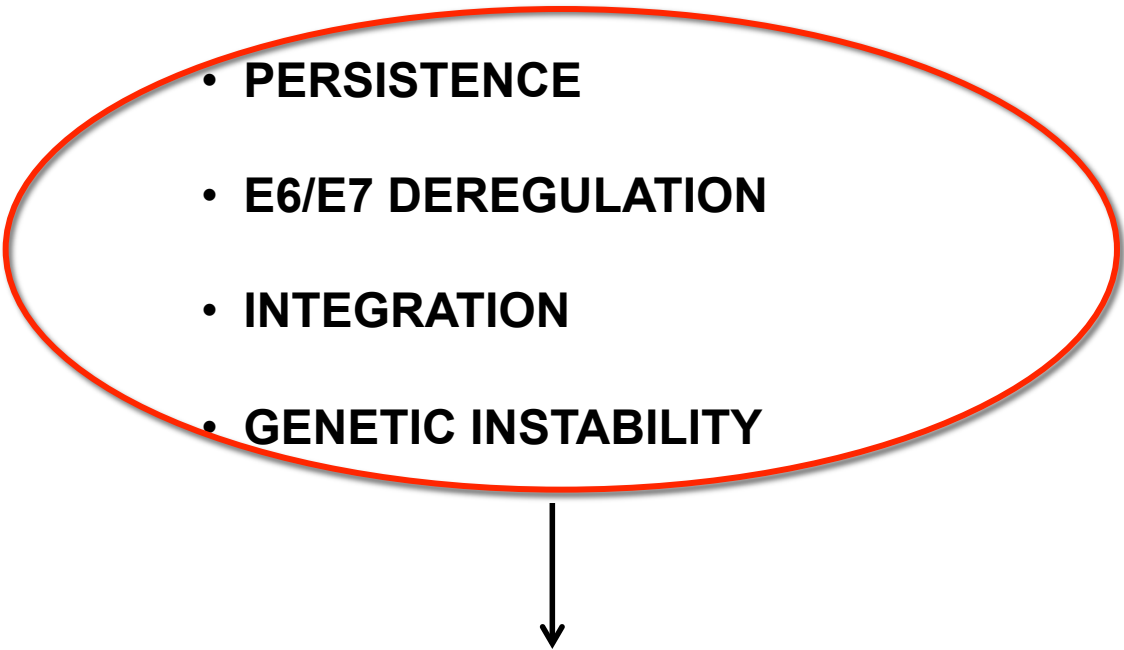
BACKGROUND

The likelihood of progression of CIN3 to invasion has been estimated at 1% per year, and overall 31% within 30 years.
(McCredie et al. 2008)



PERSISTENCE

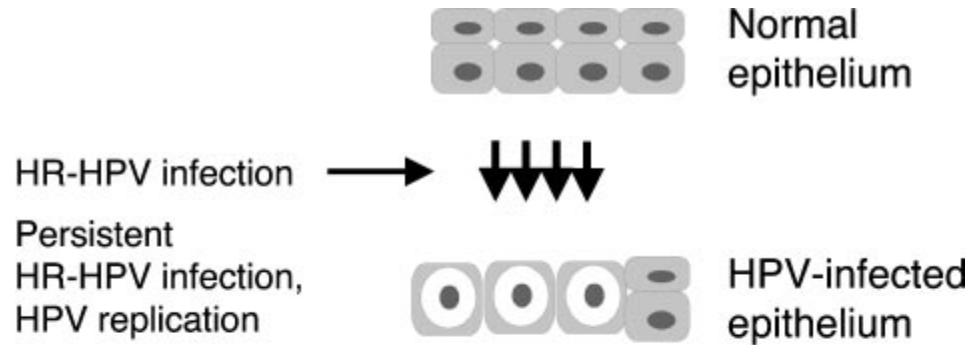
MULTISTEP CARCINOGENESIS

- 
- **PERSISTENCE**
 - **E6/E7 DEREGULATION**
 - **INTEGRATION**
 - **GENETIC INSTABILITY**

Single layer of cuboidal epithelial cells, which represent cancer progenitors in the SC Junction
(M.Herfs, 2012)

PERSISTENCE

MULTISTEP CARCINOGENESIS



The persistence higher the probability within HR-genotypes infection (*in presence of cofactors*) to have a cancer cell clone → **chance occurrence**

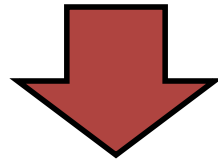
Low-risk genotypes showed varying degrees of persistence, but did not progress to CIN3/ cancer → *carcinogenicity is not strictly a function of persistence.*

(M.Schiffman 2005)

(N.Wentzensen, 2004)

PERSISTENCE

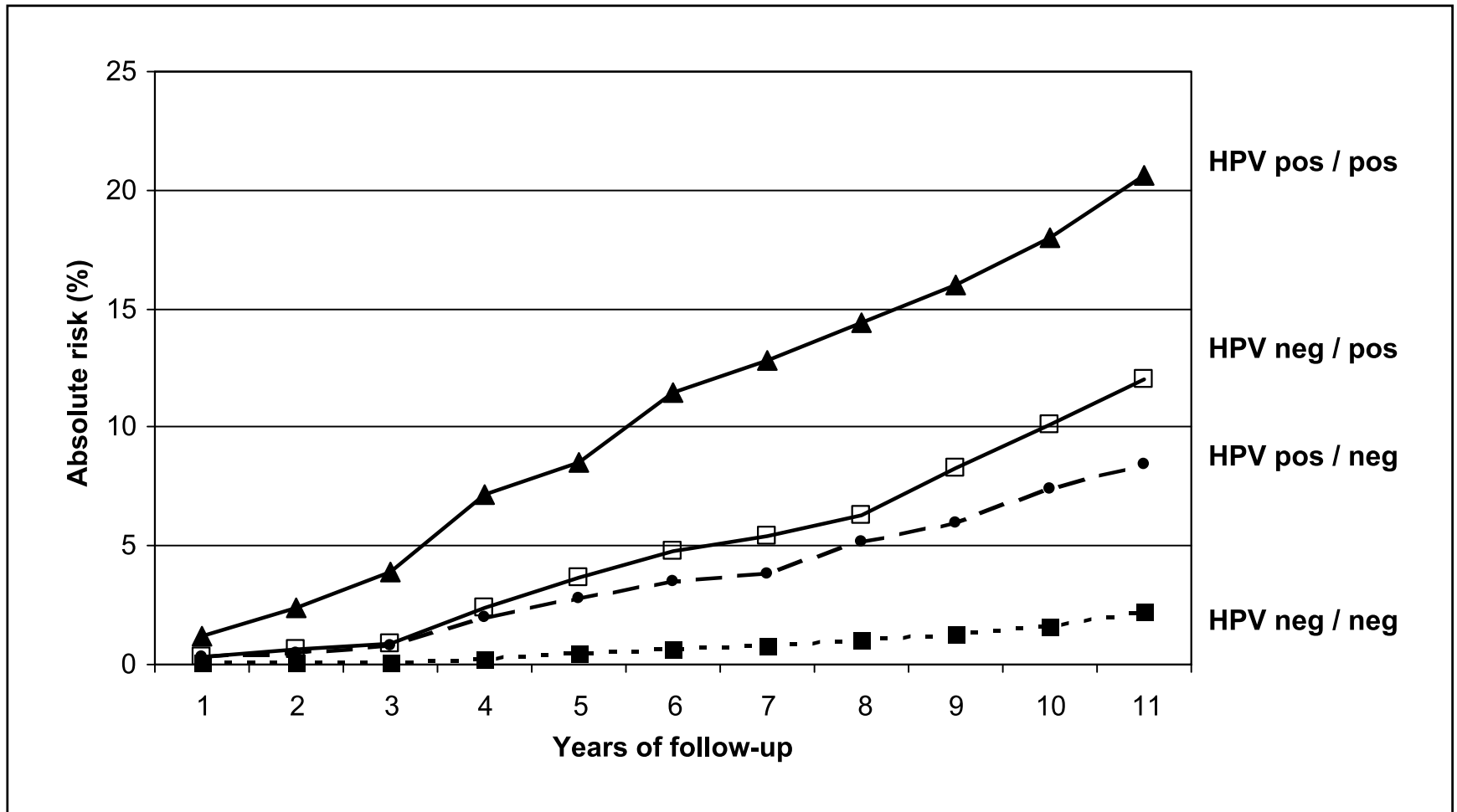
1. Although the vast majority of SILs will regress, it is currently not possible to distinguish between rare lesions destined to progress.
2. HPV persistence may represent a **critical distinction** between infections with risk of progression to CIN3 or cancer and those that are benign or transient (M.Frimer, 2015)



There is a clinical need for additional biomarkers, particularly to select high-risk women among **HPV+/ cytology-**

PERSISTENCE

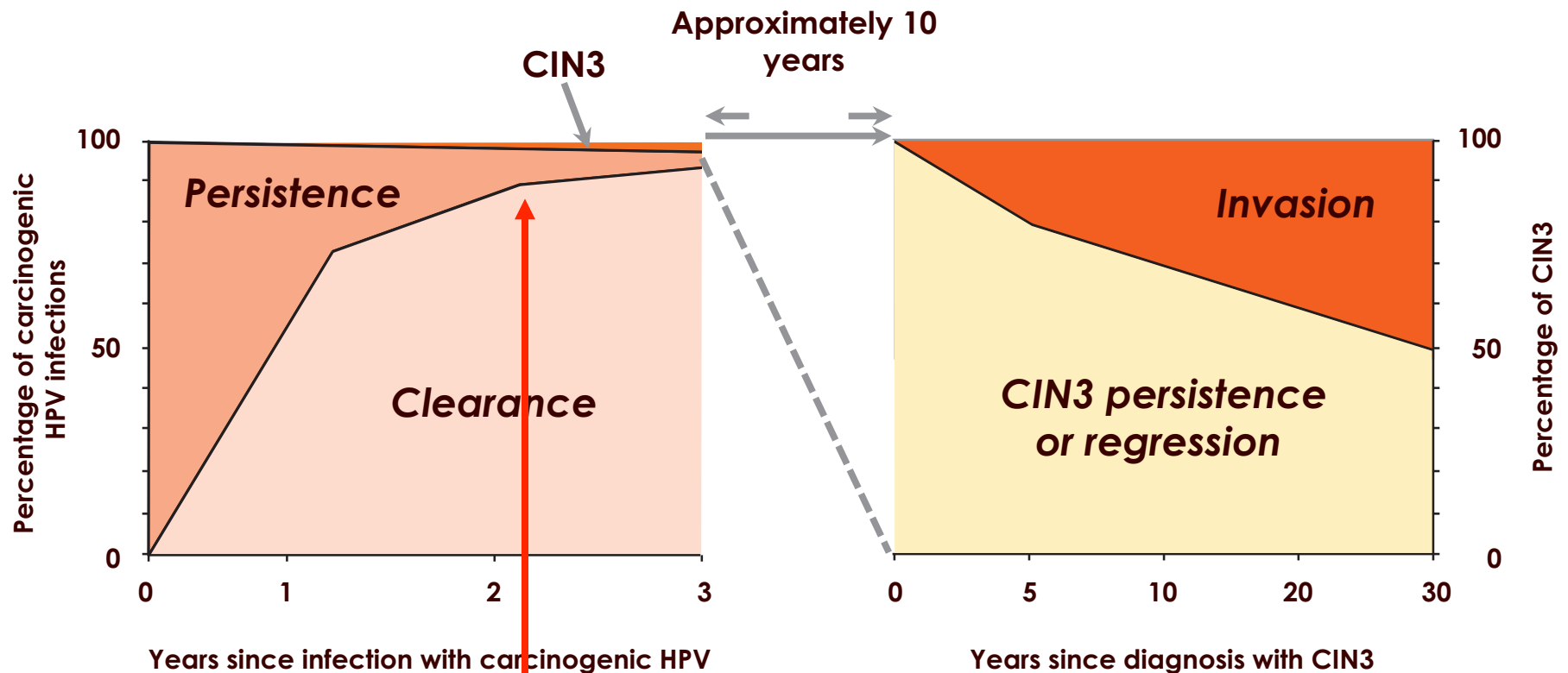
Subsequent CIN3+ in younger women with normal baseline cytology



Susanne Kjaer 2006

PERSISTENCE

Clearance and persistence of carcinogenic HPV and CIN progression (Moscicki A-B et al 2012; AC Rodriguez, 2008)

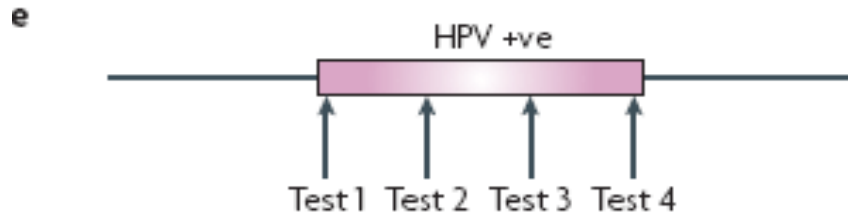


As the rate of clearance slows, the chance of development of HSIL gradually increases, representing the growth of a **clonal high-grade lesion**.

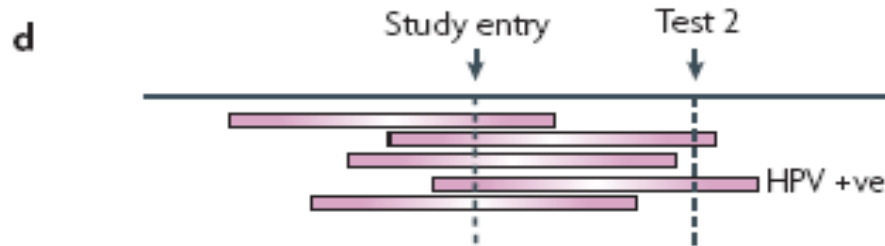
PERSISTENCE

There are numerous conceptual problems with *persistence*

(C. Woodman, 2007)



The shorter the interval, the more likely an infection will be wrongly defined as **persistent**



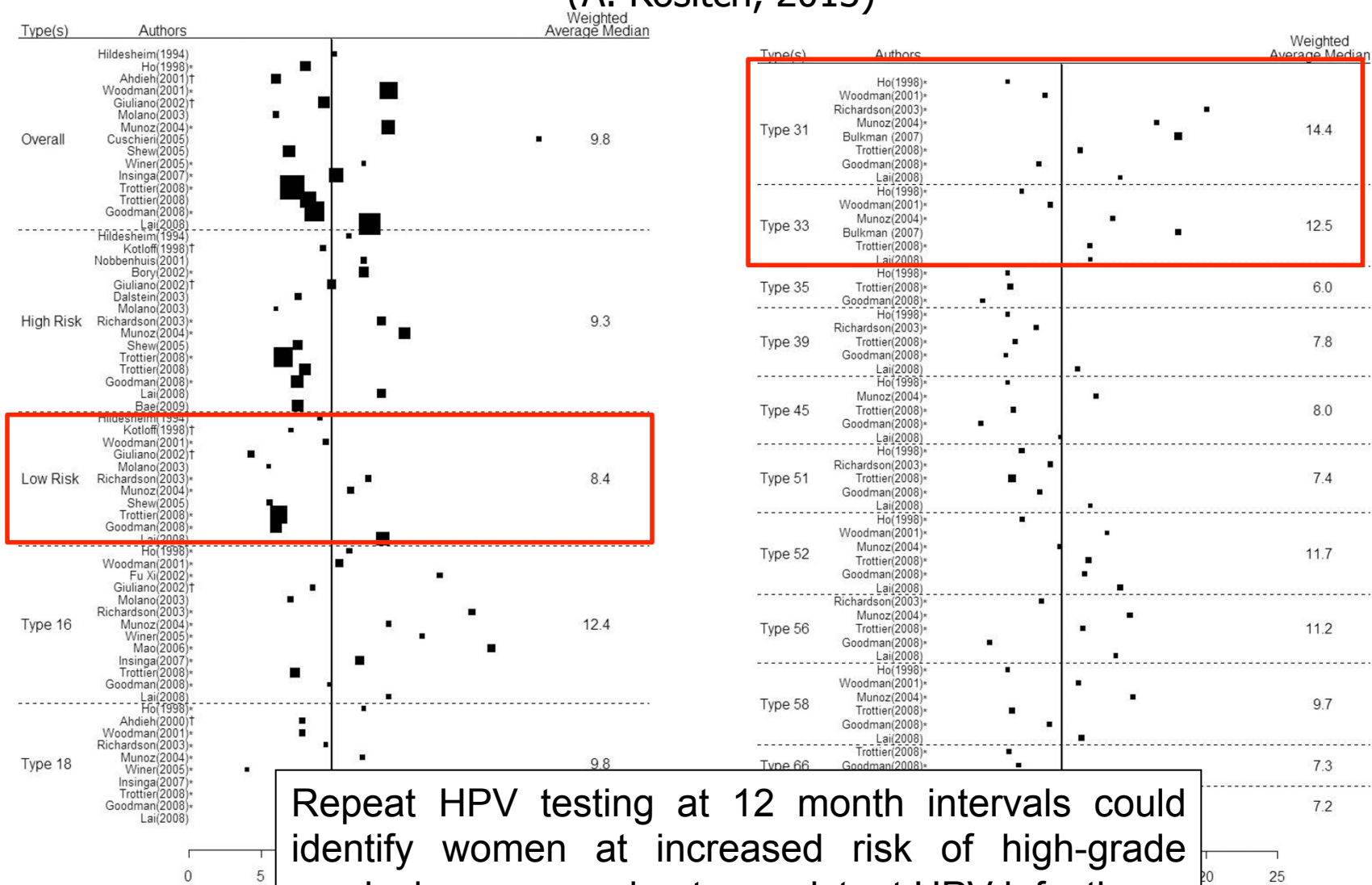
HPV infection of identical duration, but different onset. Two of these infections are wrongly considered **persistent**, and three transient.



True persistent HPV infections

PERSISTENCE

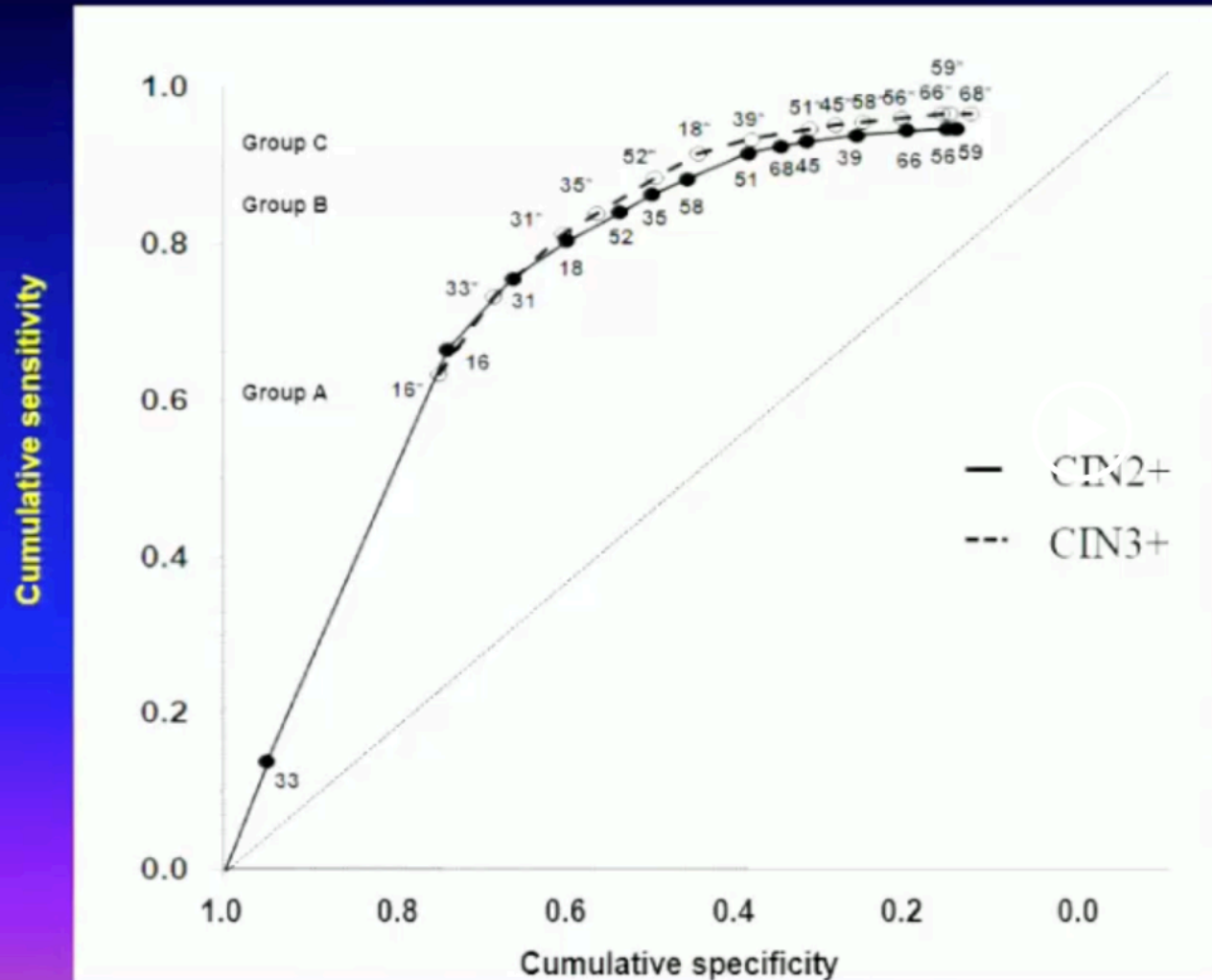
Meta-analysis on persistent HPV (A. Rositch, 2013)



Repeat HPV testing at 12 month intervals could identify women at increased risk of high-grade cervical precancer due to persistent HPV infections.

PERSISTENCE

ROC curve of cumulative sensitivity and specificity Genotypes are ordered according to PPV



PERSISTENCE

HPV GROUPS ACCORDING TO PPV

(J.Cuzick, 2015)

GROUP A (*very high-risk*)

HPV 16, HPV 33

GROUP B (*high-risk*)

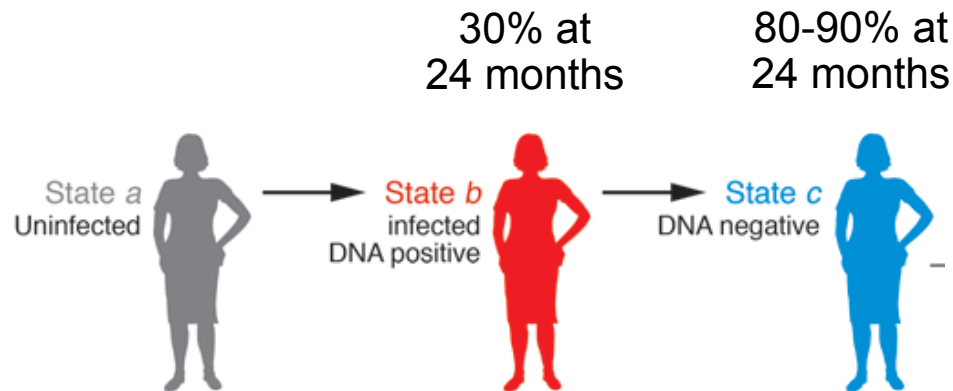
HPV 31, 18, 52, 35, 58

GROUP C (*intermediate risk*)

HPV 51, 68, 45, 39, 66, 56, 59

PERSISTENCE

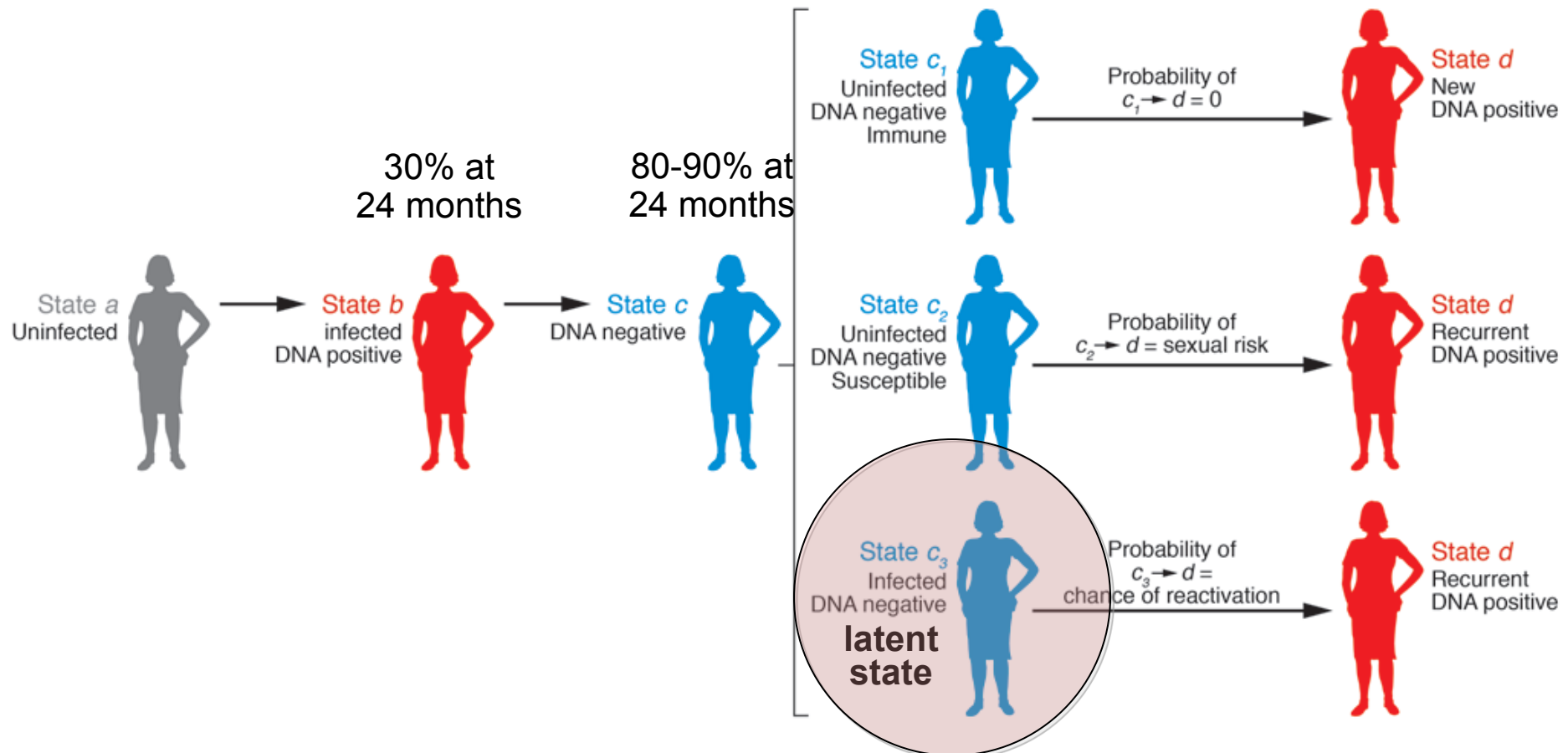
Most HPV infections as detected by molecular assays become **undetectable** after several months (80% by Evander M, 1995; Ho 1998) → *It is not known to which extent the lack of detectability represents viral clearance or **persistence as latent**.*



(Patti E. Gravitt, 2011)

PERSISTENCE

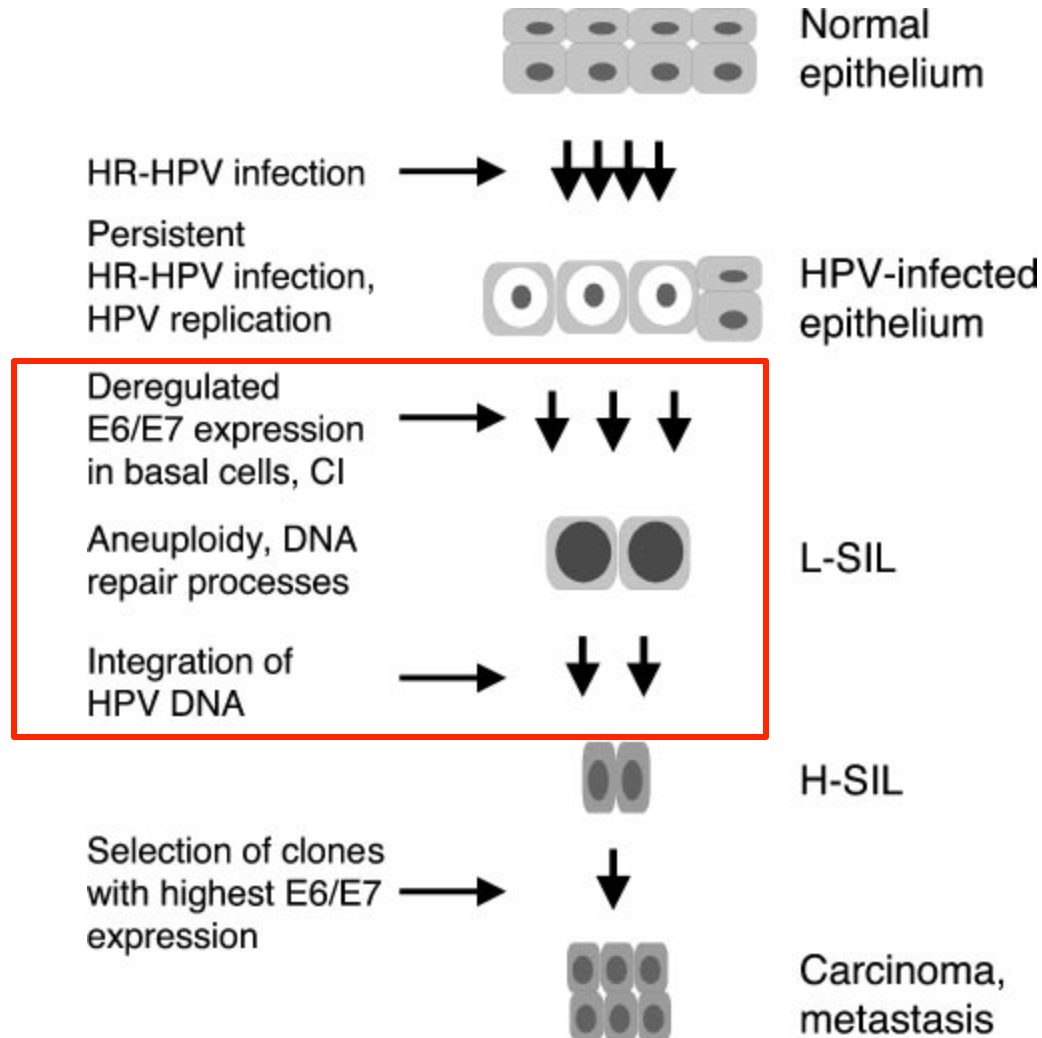
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INTEGRATION

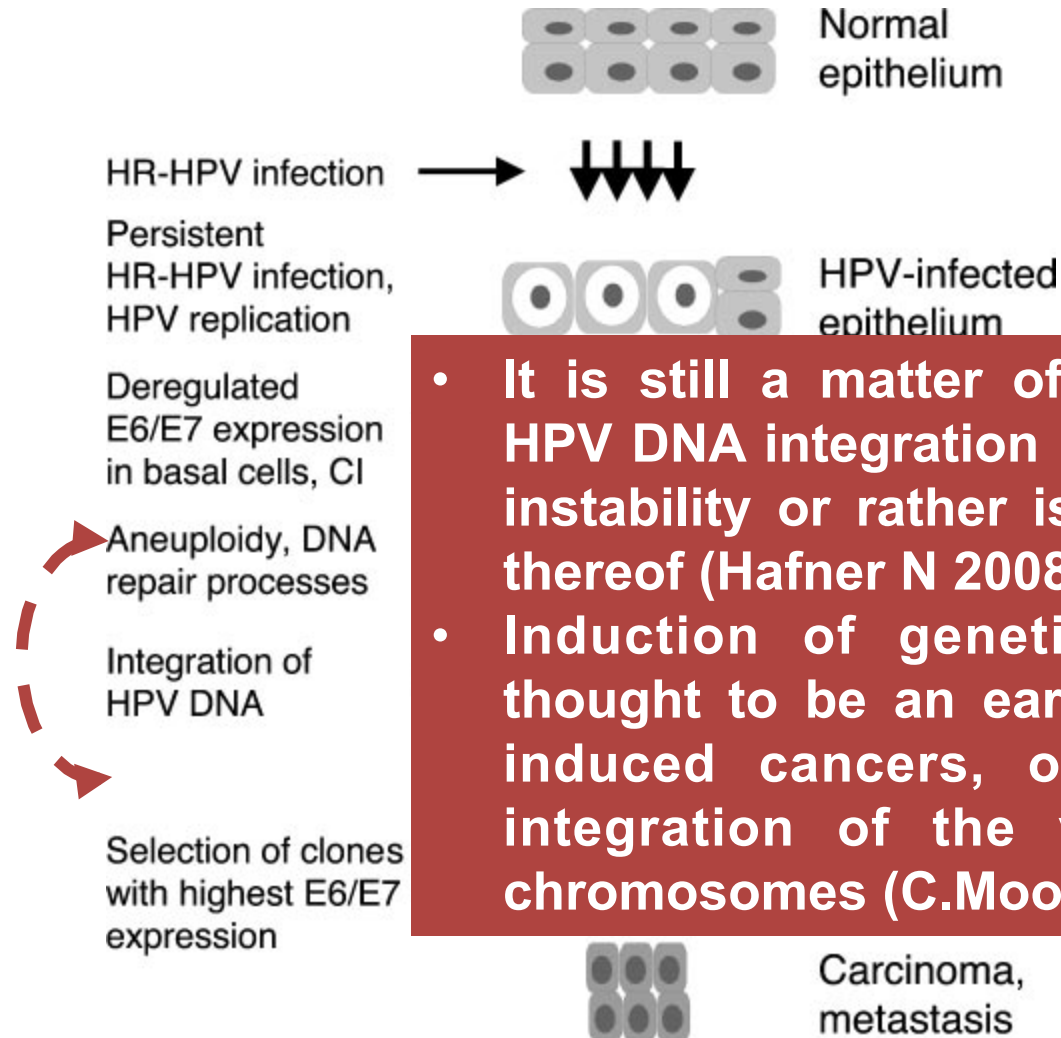
MULTISTEP CARCINOGENESIS



(N.Wentzensen, 2004)

INTEGRATION

MULTISTEP CARCINOGENESIS



- It is still a matter of debate whether HPV DNA integration precedes genetic instability or rather is a consequence thereof (Hafner N 2008)
- Induction of genetic instability is thought to be an early event in HPV-induced cancers, occurring before integration of the virus into host chromosomes (C.Moody, 2010)

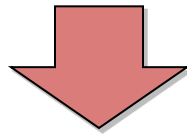
(N.Wentzensen, 2004)

INTEGRATION

Agents that induce DNA damage :

- smoking,
- long-term use of oral contraceptives,
- high parity
- co-infection with other STD → inflammation may facilitate the progression to CIN2+ → C.trachomatis

(V.Williams, 2011)



Any event that increases the frequency of double-strand breaks (DSBs) in host and viral DNA may facilitate the mechanism of integration → **chance occurrence**

INTEGRATION

MULTISTEP CARCINOGENESIS

1.Integration in SCJ cells of HR-HPV genomes into the host genome: decrease of viral load and break at E2 which down-regulate E6/E7 (E5 enhances proliferation and may contribute to cancer progression).

The integration sites are widely distributed all over the human genome (*no obliged hotspots*)

→ **random integration may:**

- (in)activate tumor suppressor genes, or
- (in)activate proto-oncogenes, or
- close to host CFSs (common fragile sites) which offer relative accessibility to HR-HPV with subsequent selective growth advantage.

INTEGRATION

MULTISTEP CARCINOGENESIS

1.integration in SCJ cells of HR-HPV genomes into the host genome: decrease of viral load and break at E2 which down-regulate E6/E7 (E5 enhances proliferation and may contribute to cancer progression).

2.overexpression of viral oncogenes E6/E7 cooperate to:

- *inactivate the tumor suppressors p53 (E6) and RBp (E7)*
- *altered cell cycle control → inhibits cell differentiation, deregulation of proliferation, no apoptosis (immortalization);*
- *accumulation of genetic errors (centrosomal abnormalities, aberrant mitotic spindle pole formation) and thus **chromosomal instability**.*

3. growth and expansion of HPV-dependent **cancer cell clone**

INTEGRATION

QUANTITATIVE DEREGULATION

1. **Highly variable levels of viral oncogene expression** in CIN and CxCa, which were independent of histological grading and the physical state of the viral genome.
2. **How many of the integrated viral genome copies are transcribed into mRNAs ?** → *it was shown that only one locus of the 300 integrated HPV genomes is transcribed, all other copies are transcriptionally silent; Van Tine et al., 2001)* → **chance occurrence**

*The existence of transcriptionally inactive HR-HPV integrants in SIL indicates that **important subsequent events** are necessary for deregulated transcription (M. Pett 2007)*

SPATIAL DEREGULATION

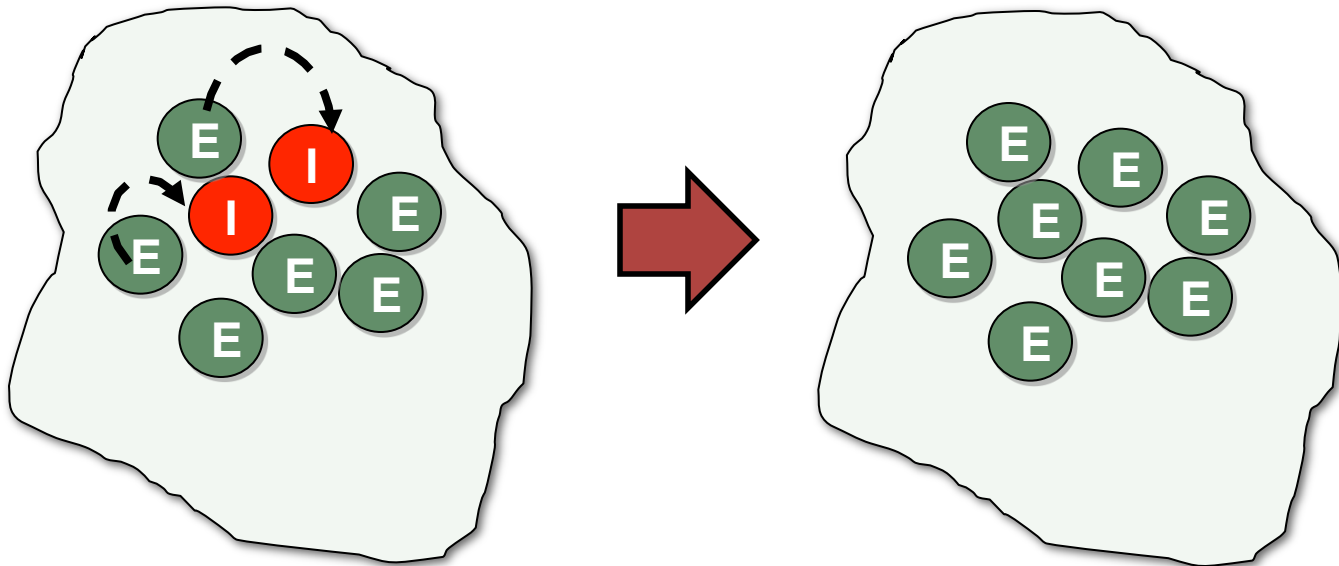
E6/E7 expression in HR-HPV-LSIL is localized to the upper spinous and granular layers, with little/no detectable expression in proliferative basal layers → *deregulated oncogene expression from HR-HPV integrants require **loss of basal repression** of viral gene transcription and insensitivity to inhibitory cytokines.*

HPV16 integration in basal cervical cells does **not necessarily** lead to increased levels of virus oncogenes, or to a competitive growth advantage, when compared with the initiating episome-containing cells.

(C. Scarpini, 2014)

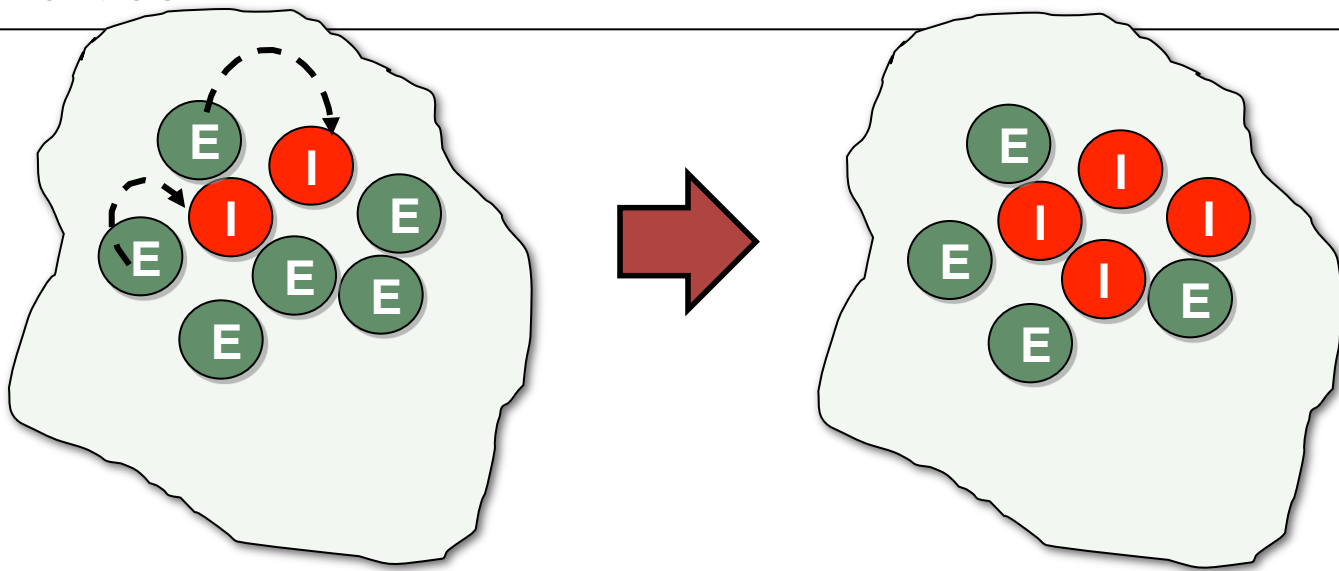
INTEGRATION

1. Within a cell containing a **mixture of episomal and integrated** HR-HPV, E2 expressed from episomes could inhibit expression of the co-existent integrant.
2. Reintroduction of an intact E2 gene in cervical cancer cell lines (HeLa) resulted in growth inhibition due to inhibition of the expression of the E6 and E7 genes (Wells S, 2000)



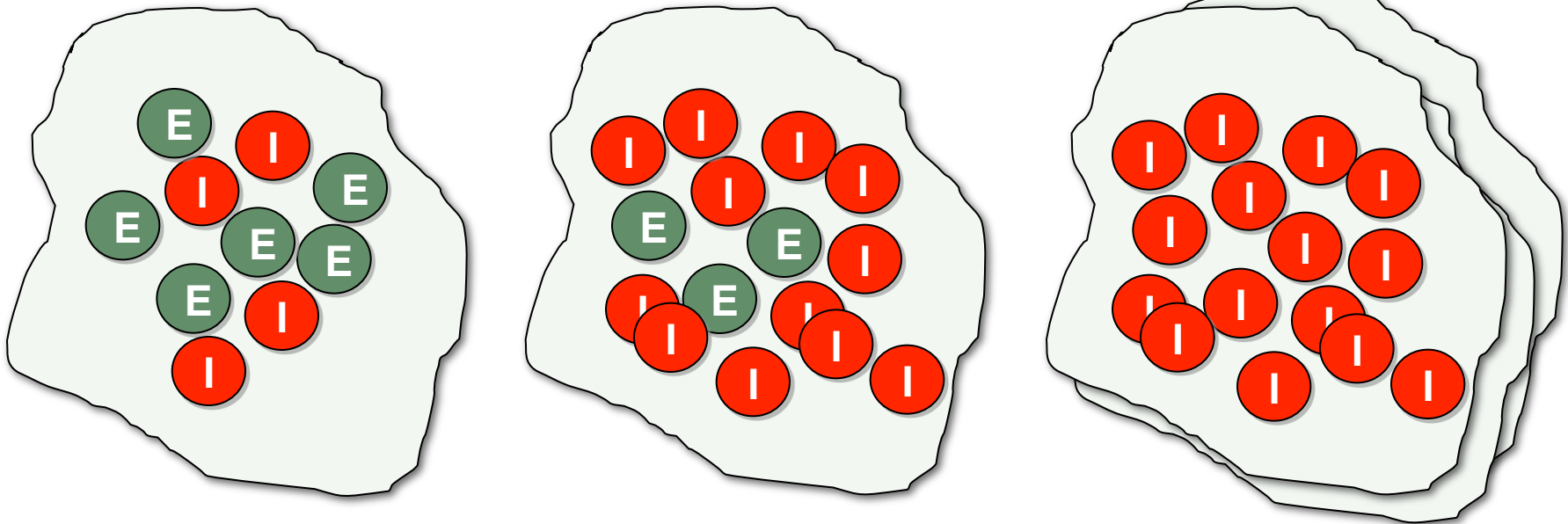
INTEGRATION

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3. *Otherwise (as chance occurrence)...E2 expression from episomes can also initiate DNA replication from integrated viral origins, resulting in their amplification and induction of chromosomal abnormalities.*



INTEGRATION

1. Overcoming the E2 inhibition would represent an important event required for the selection of integrated HR-HPV.
2. Integrant-derived transcripts are more stable than those derived from episomal viral DNA
3. Moreover, integration may confer a selective growth advantage.

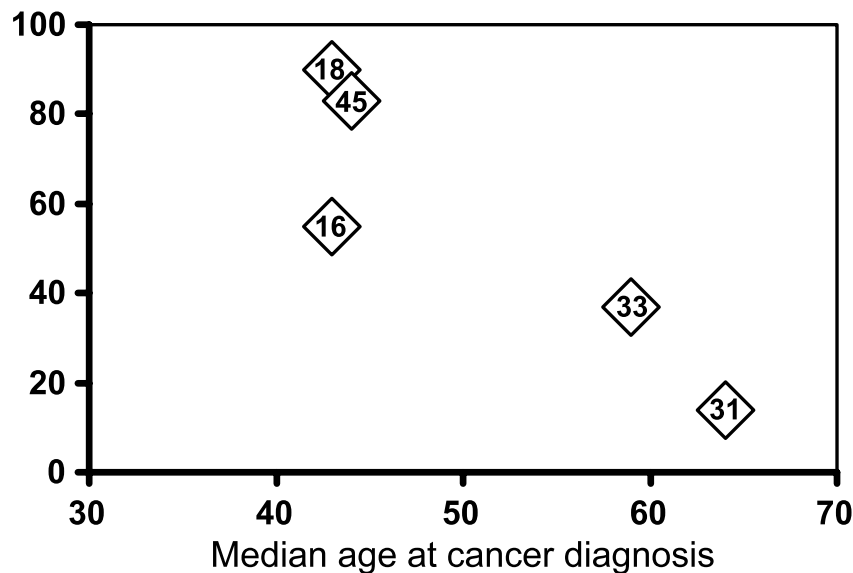


INTEGRATION

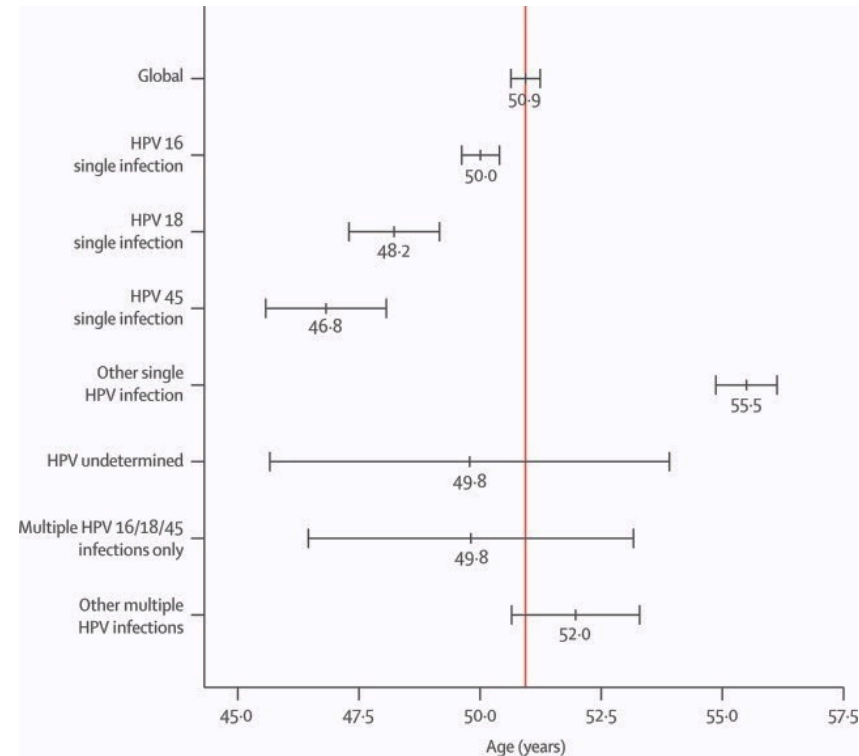
Non-HPV16 and 18 HR-HPV types would confer **less chromosomal instability** than HR-HPV types 16 and 18.

This may be reflected by **less frequent integration** of the respective HR-HPV genomes in advanced cervical lesions and a **longer time interval required for progression** of preneoplastic lesions to invasive cervical cancers. (S. Vinokurova 2008)

Frequency of
integration
%



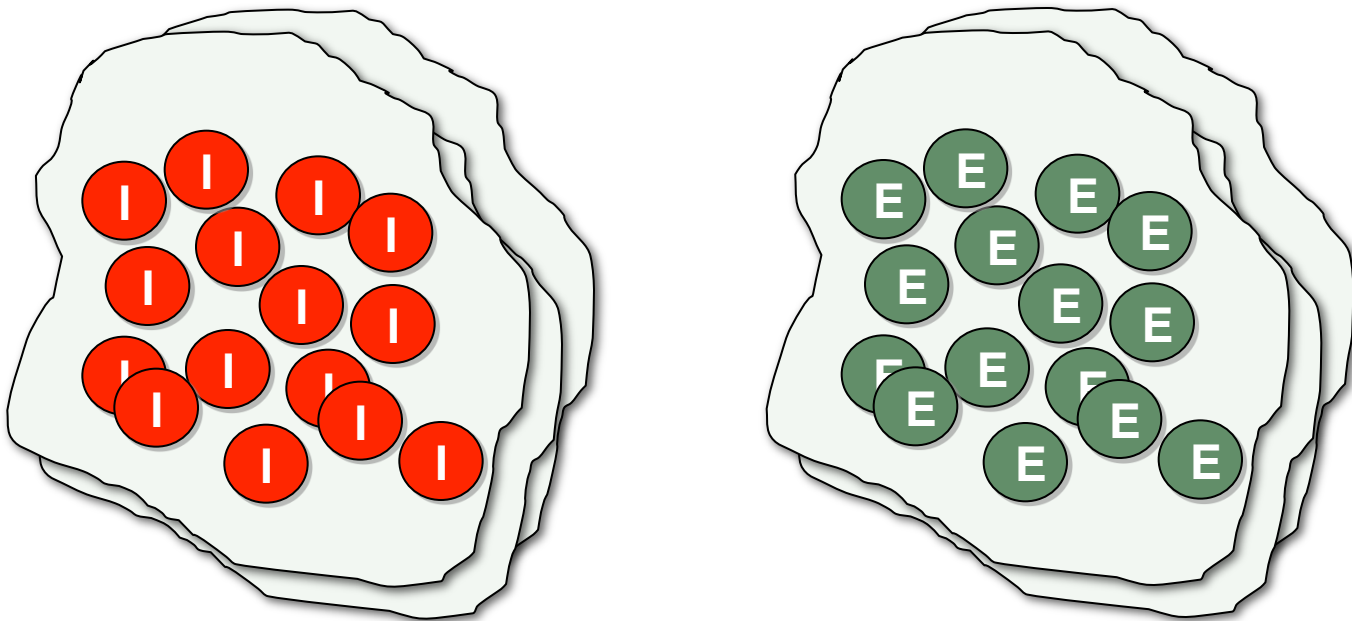
(S. Vinokurova 2008)



HPV genotype attribution by age
in cervical cancer (de Sanjosè S, 2010)

INTEGRATION

1. However, the absence of HPV16 DNA integration in some carcinomas (20%) implies that integration is not always required for malignant progression (Vinokurova S, 2008)
2. **Episome-associated carcinogenesis is poor understood** (E.Gray 2010)
→ *Mutation or methylation of sequences within the LCR may alter E2 functions mimicking the consequence of E2 gene disruption as seen in integration* (A. Chaiwongkot, 2013)



GRAZIE