



Il laccio – Giovan Battista De Andreis

HPV Infection and Invasive Cervical Carcinoma: Virus – Host interactions

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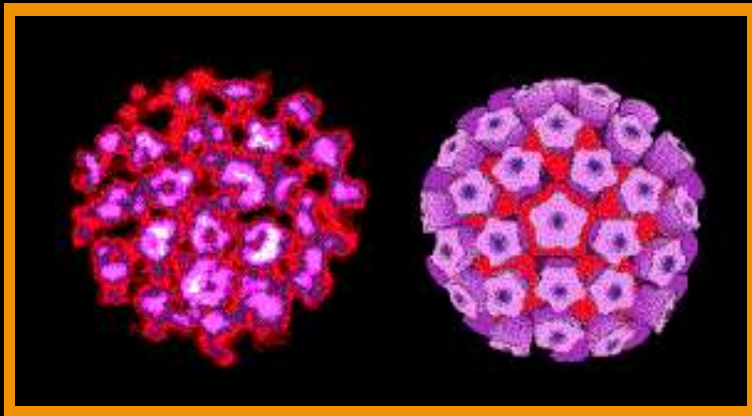
*Training Course in Sexual and
Reproductive Health Research
Geneva 2010*

What HPVs are ?

ds DNA virus with a 8000 pb genome

More than 100 types have been characterised, 1/3 are involved in genital infections

Based on their oncogenic power they are divided in HIGH and LOW risk types

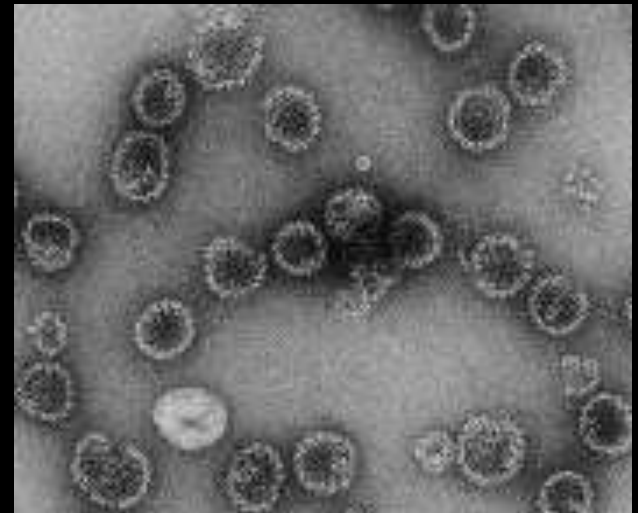
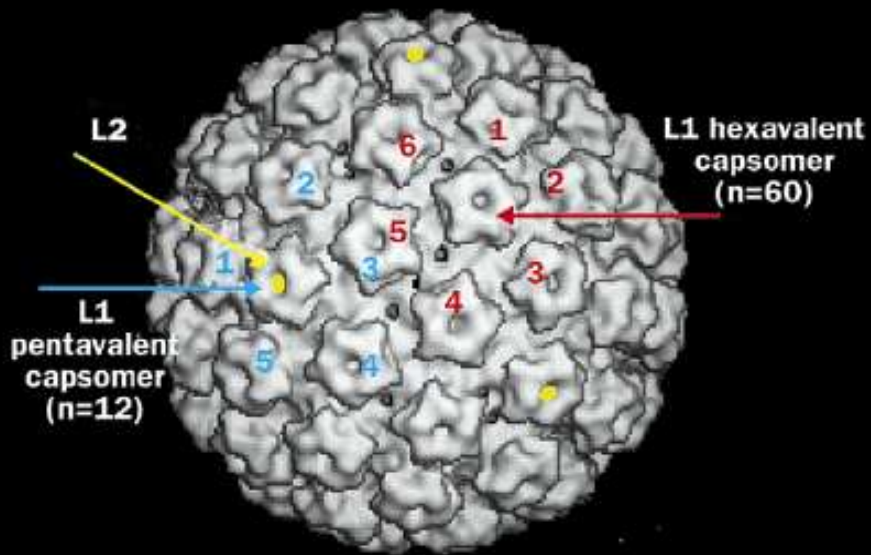
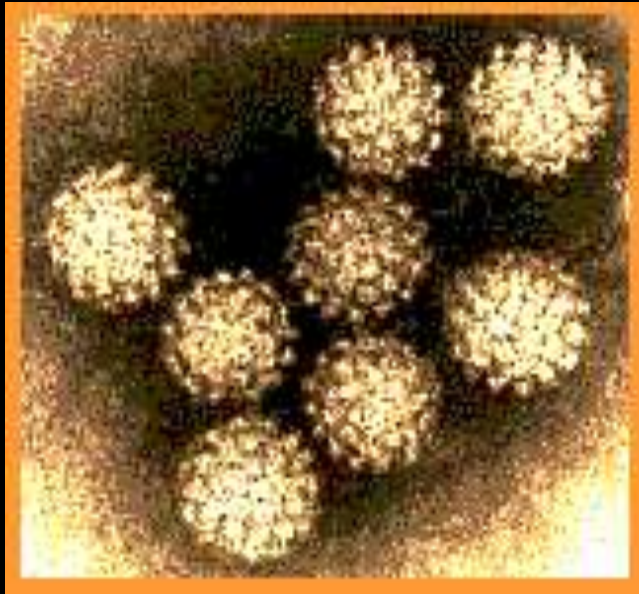


HR-HPV associated tumours:

Squamous Cell Carcinoma

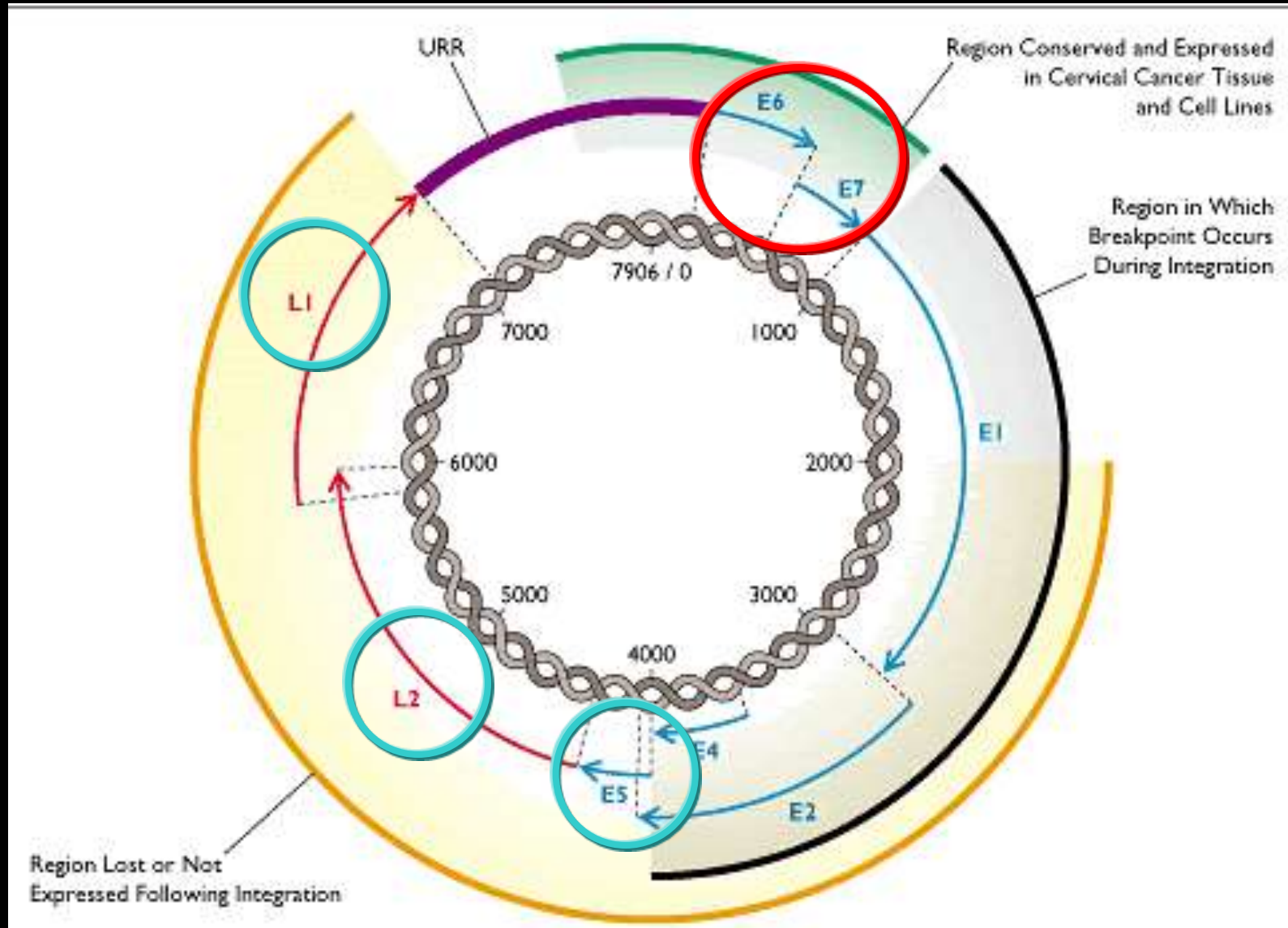
Adenocarcinoma

HPV morphology

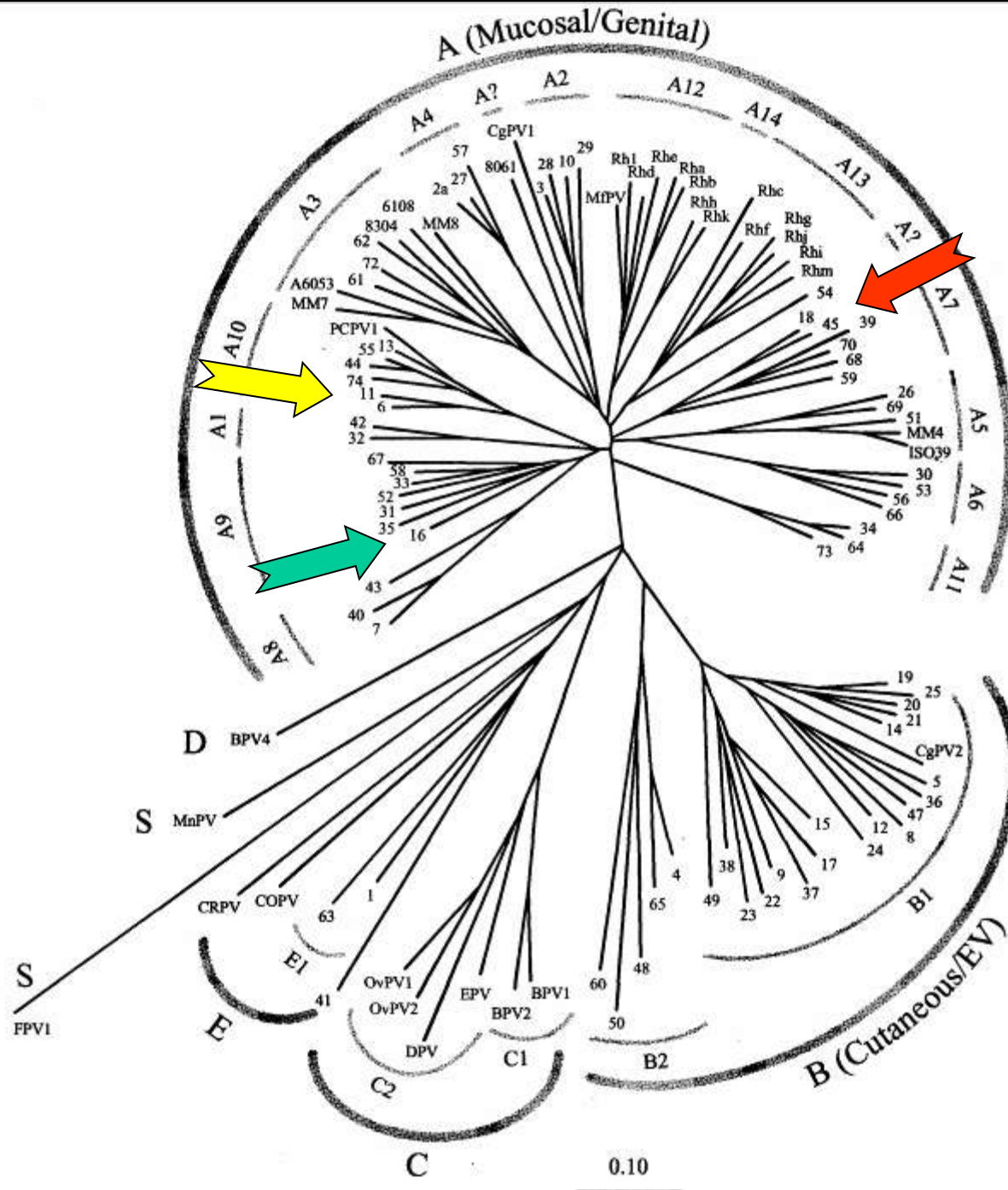


HPV16 L1 VLPs
(J.Schiller, 2000)

HPV genomic structure



HUMAN PAPILLOMAVIRUS PHILOGENETIC TREE



HPV-16 variants and human population migration

(Ho et al Journal of Virology 1993)



BACKGROUND

HR-HPV infection is the necessary cause for the genesis of high grade cervical lesions and invasive carcinoma. HPV type 16 and 18 are Class I human carcinogens

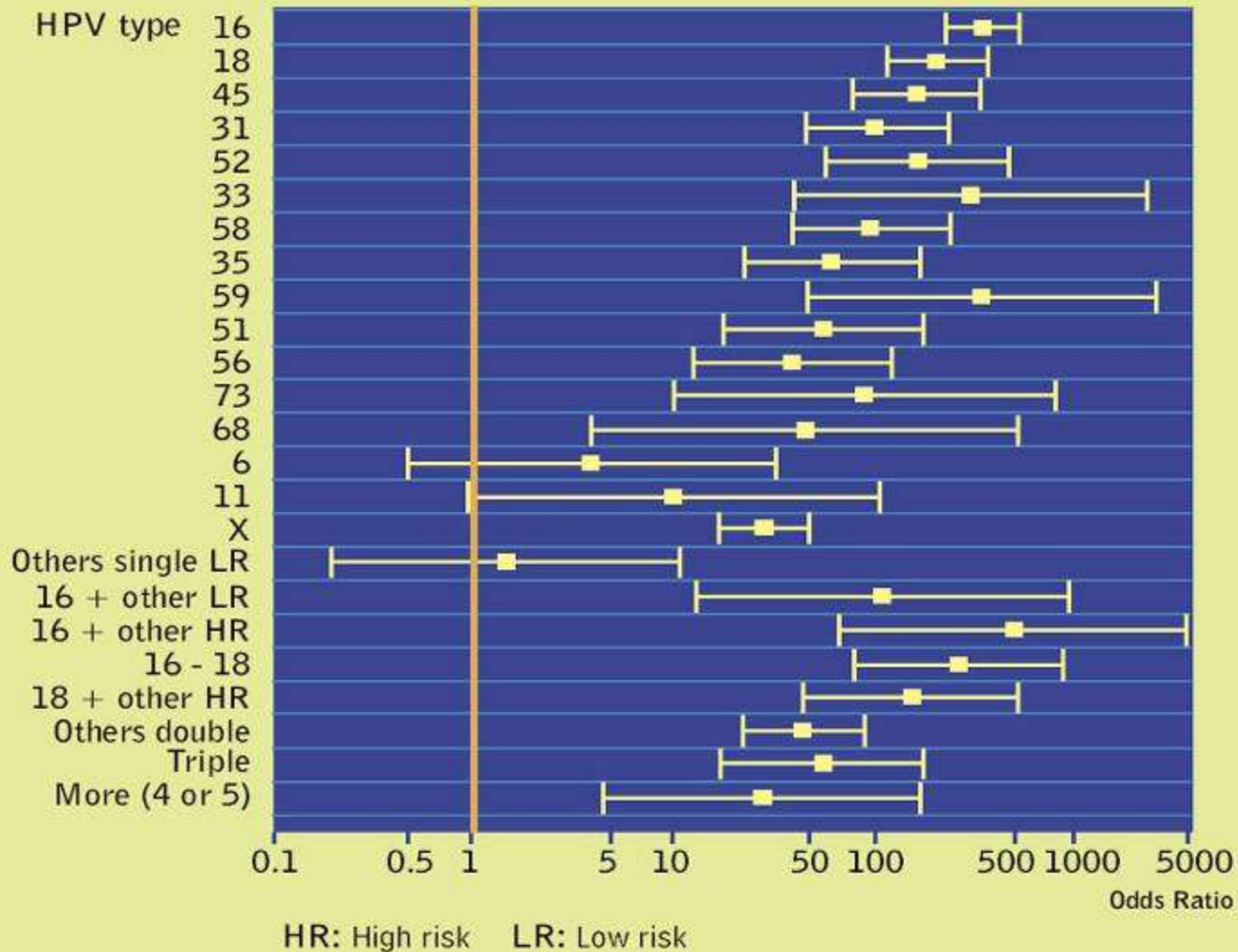
Prospective studies demonstrated that persistent HPV infection precedes the development of lesions and represents an elevated risk for disease

The level of association between HR-HPV infection and tumour development (RR:500) is much greater than cigarette smoking and pulmonary cancer (RR:10)

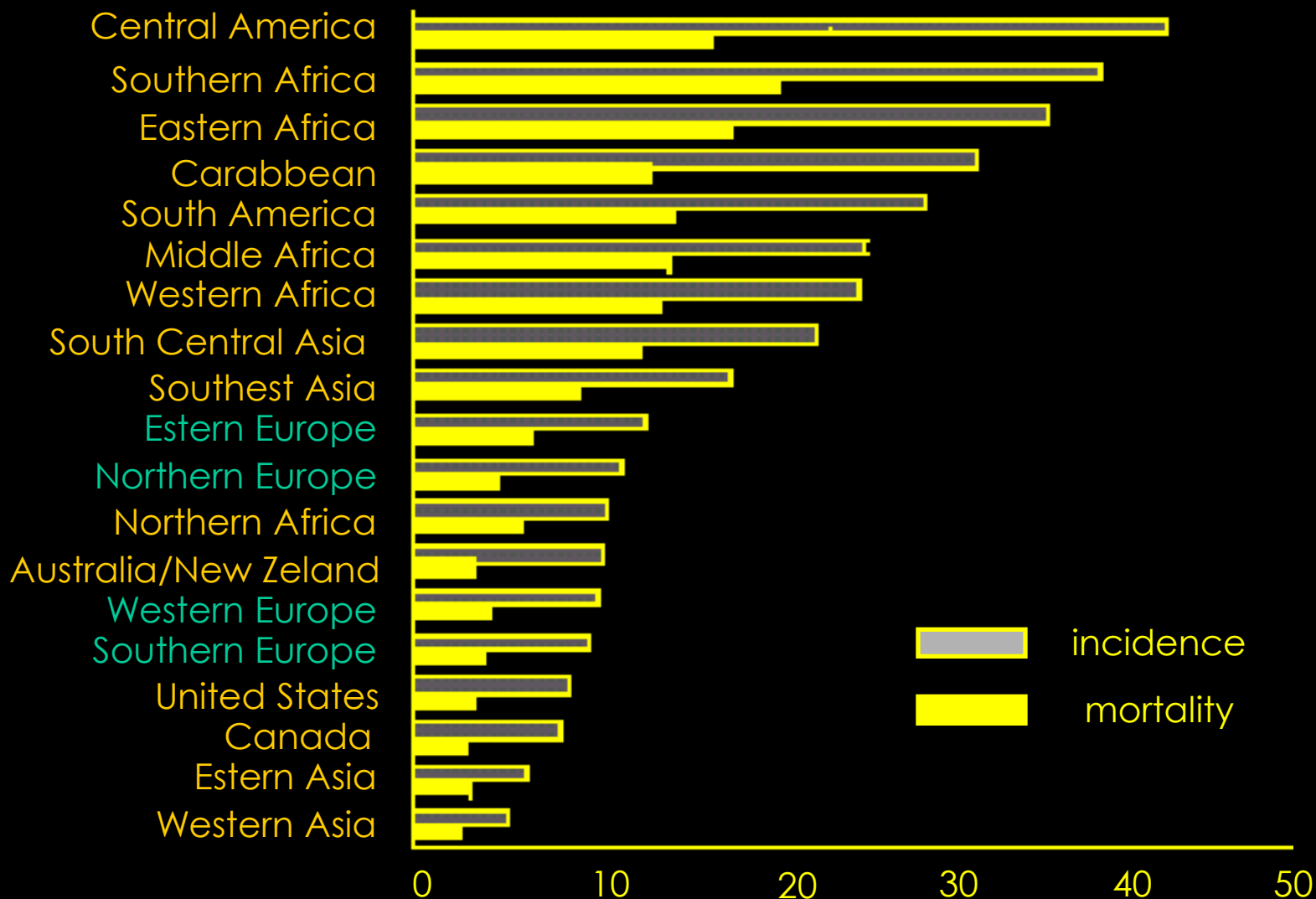
The great majority of infections is transient.
Only persistent ones represent an oncologic risk

HPV TYPE-SPECIFIC RISK ESTIMATES FOR CERVICAL CANCER

Muñoz N et al. Epidemiological classification of Human Papillomavirus types associated with cervical cancer. *New Eng J Med.* 2003, 348(6): 518-527.

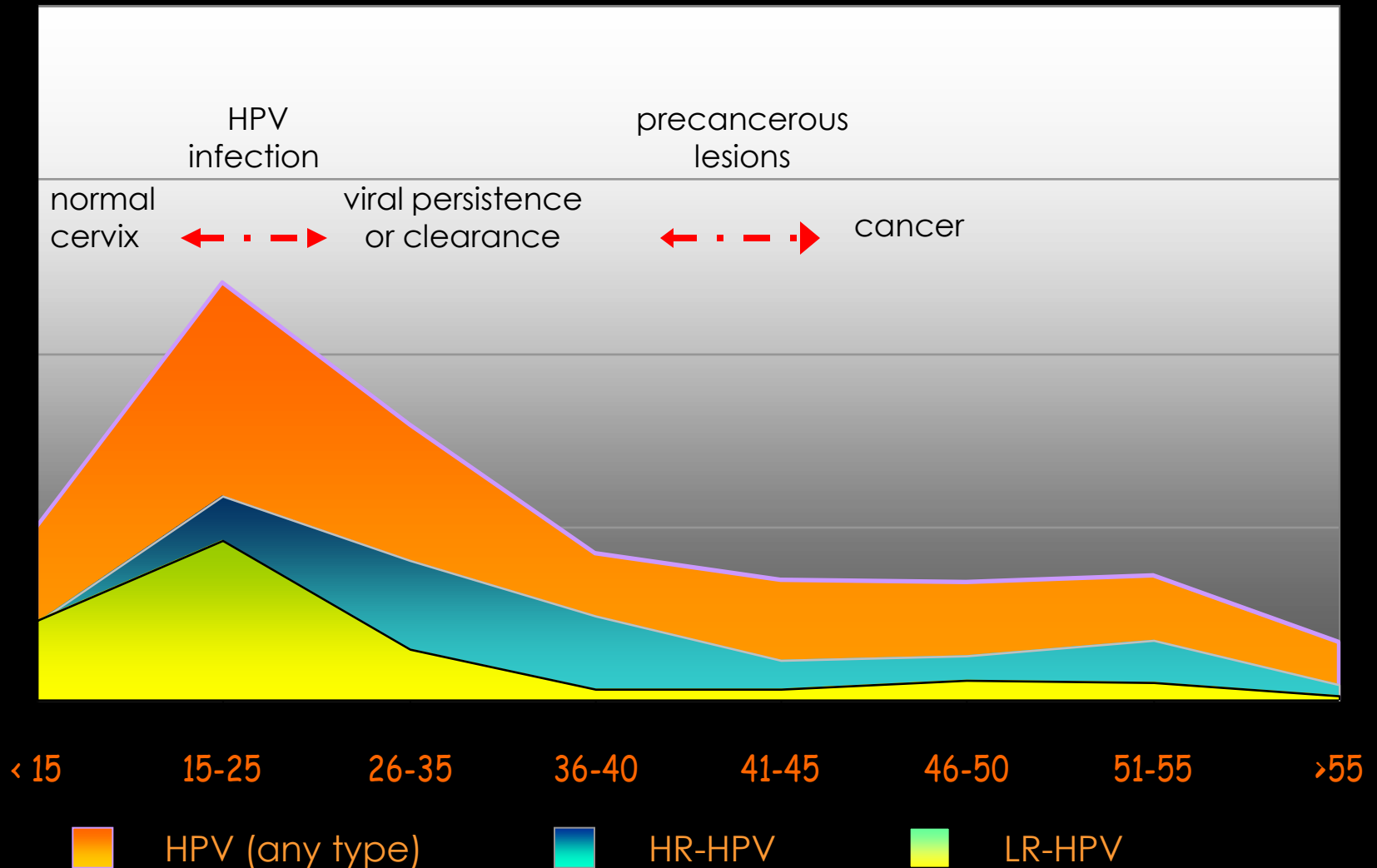


ANNUAL AGE-STANDARDIZED RATE PER 100.000



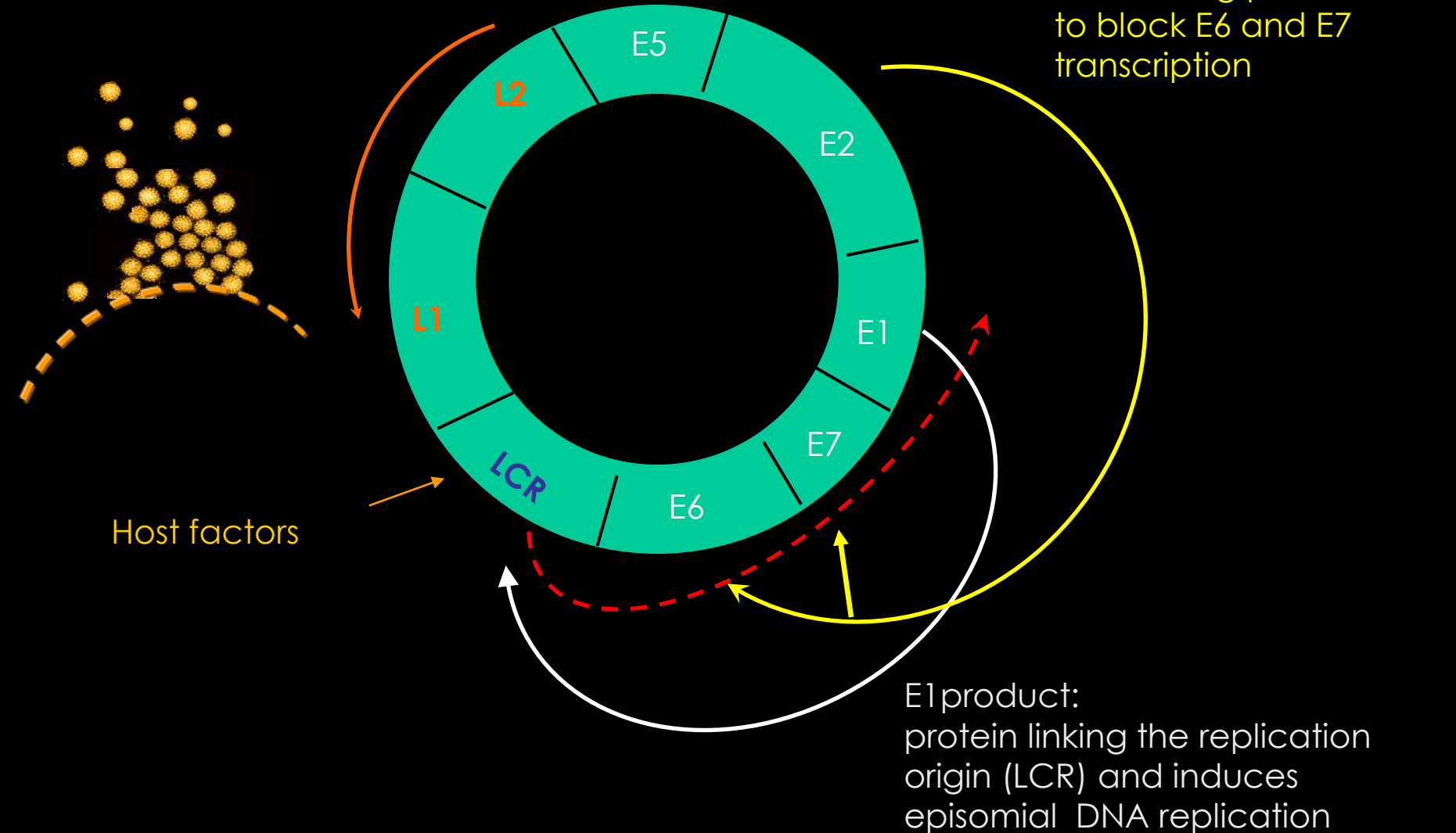
Age-specific prevalence of HPV infection

50%



episomal HPV replication

E2 product:
DNA linking protein able
to block E6 and E7
transcription



virus-cell interaction and oncogenetic effect

- ✓ High risk types have a marked tendency to integrate in the host genome
- ✓ The integration process causes the interruption of E2 gene with the consequence of loss of its control over E6 and E7 expression
- ✓ E6 and E7 products interact with and inhibit the function of cellular anti-oncogenic proteins (p53 e pRB)

E6 and E7

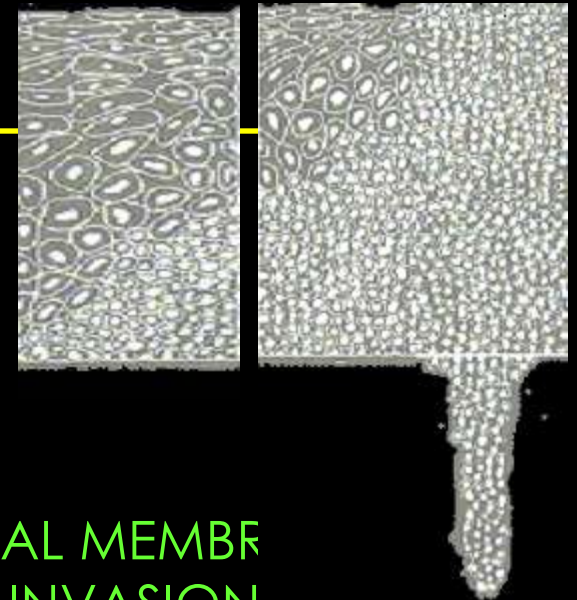
**HAVE A SYNERGISTIC EFFECT
IN THE TRANSFORMATION PROCESS**

BASAL LAYER CELL INFECTION



Suppressor genes
inactivation

Integration



Oncogenesis

BASAL MEMBRANE
INVASION

VIRAL FACTORS

- VIRAL TYPE
 - ✓ INTEGRATION CAPACITY
 - ✓ ONCOGENES EXPRESSION
- VIRAL LOAD
 - ✓ SYNERGY
 - ✓ ANTHAGONISM

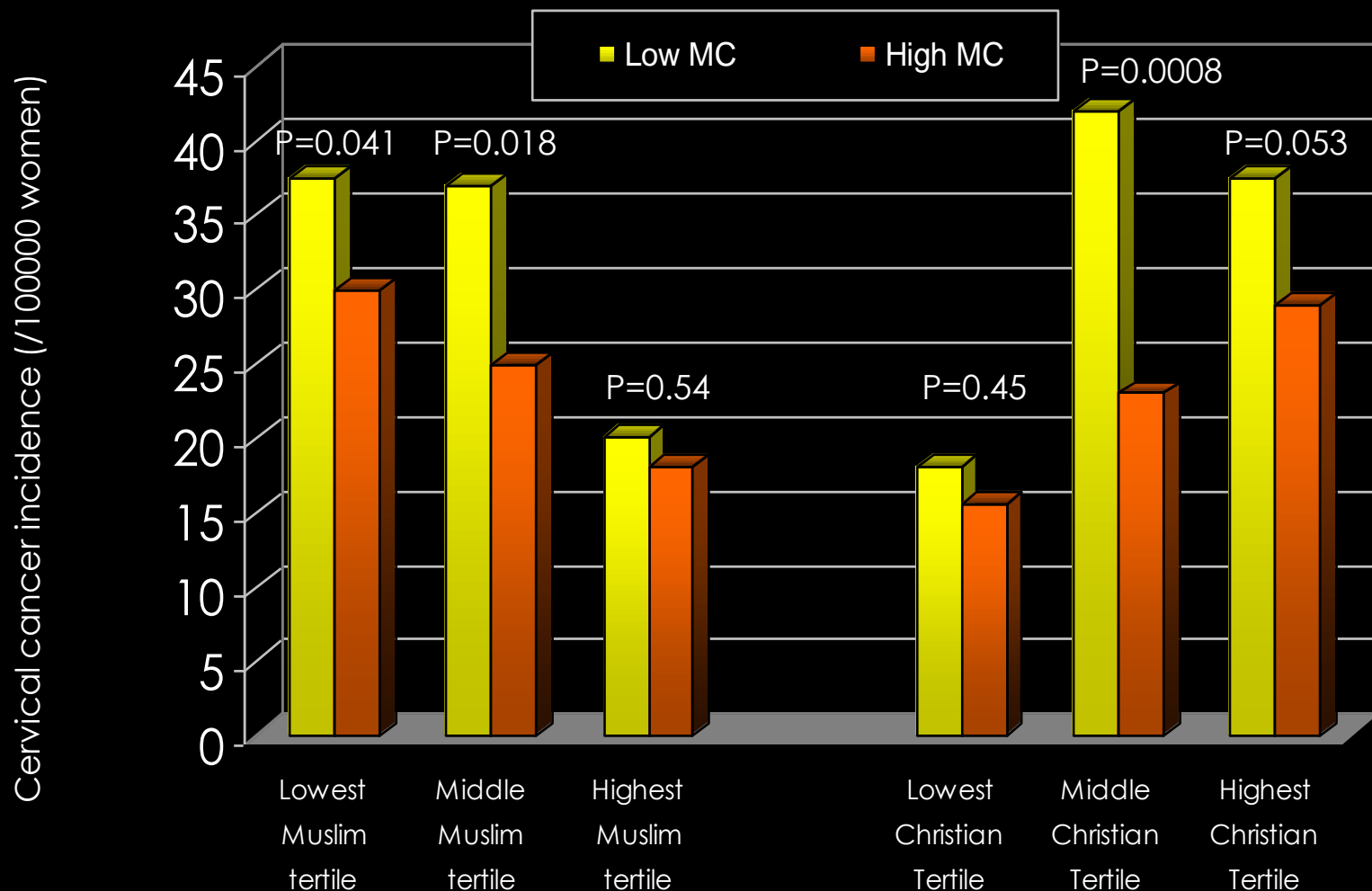
PERSISTENCE

ENVIRONMENTAL FACTORS

- ✓ CIGARETTE SOKING
- ✓ HORMONS
- ✓ SEXUAL BEHAVIOUR
- ✓ PARITY

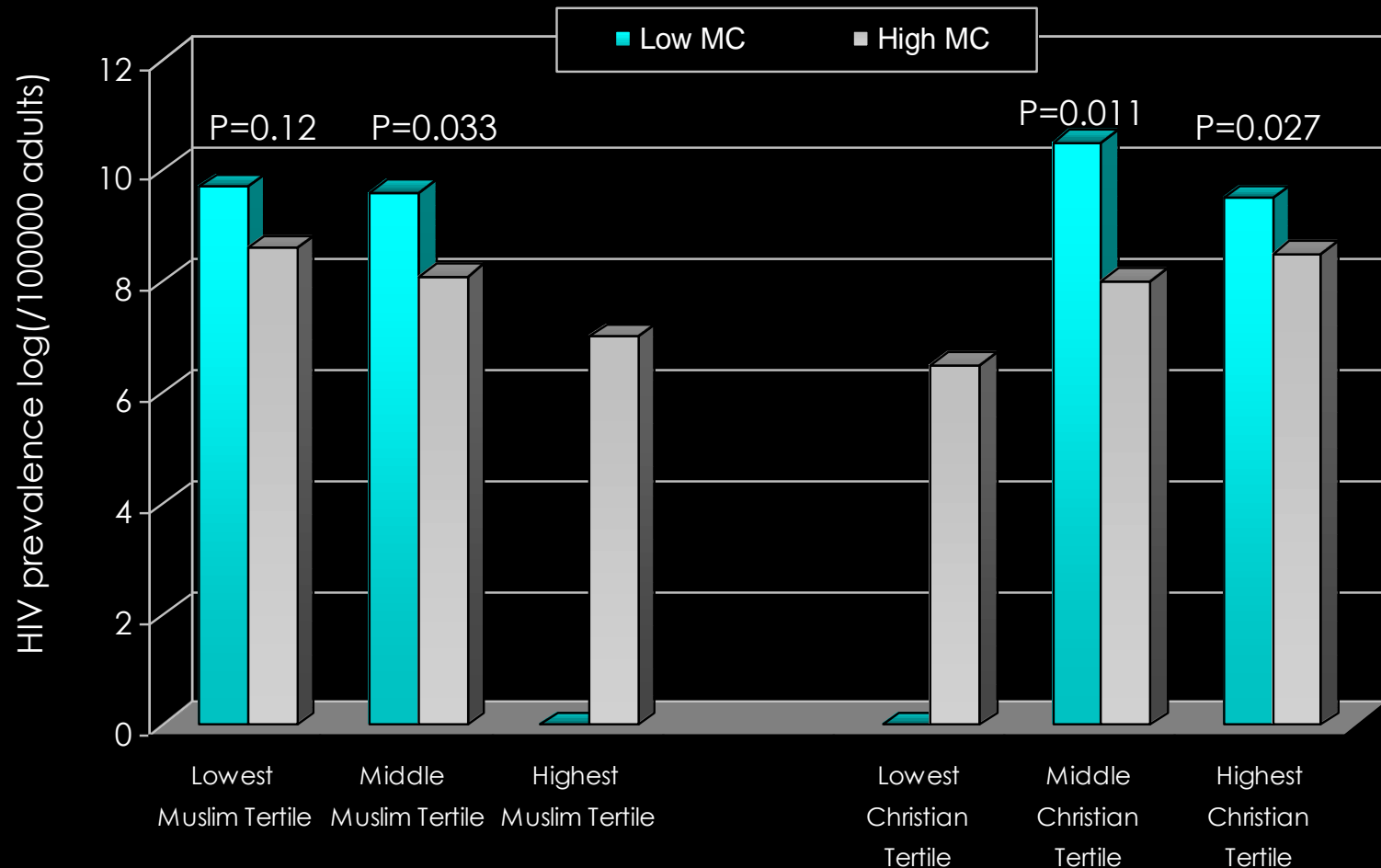
MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES

an ecologic analysis of 121 DC



MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES

an ecologic analysis of 121 DC



HOST FACTORS

- IMMUNE RESPONSE

- ✓ HUMORAL

- ✓ CELL-MEDIATED

- GENETIC FACTORS

- ✓ HLA

- ✓ p53

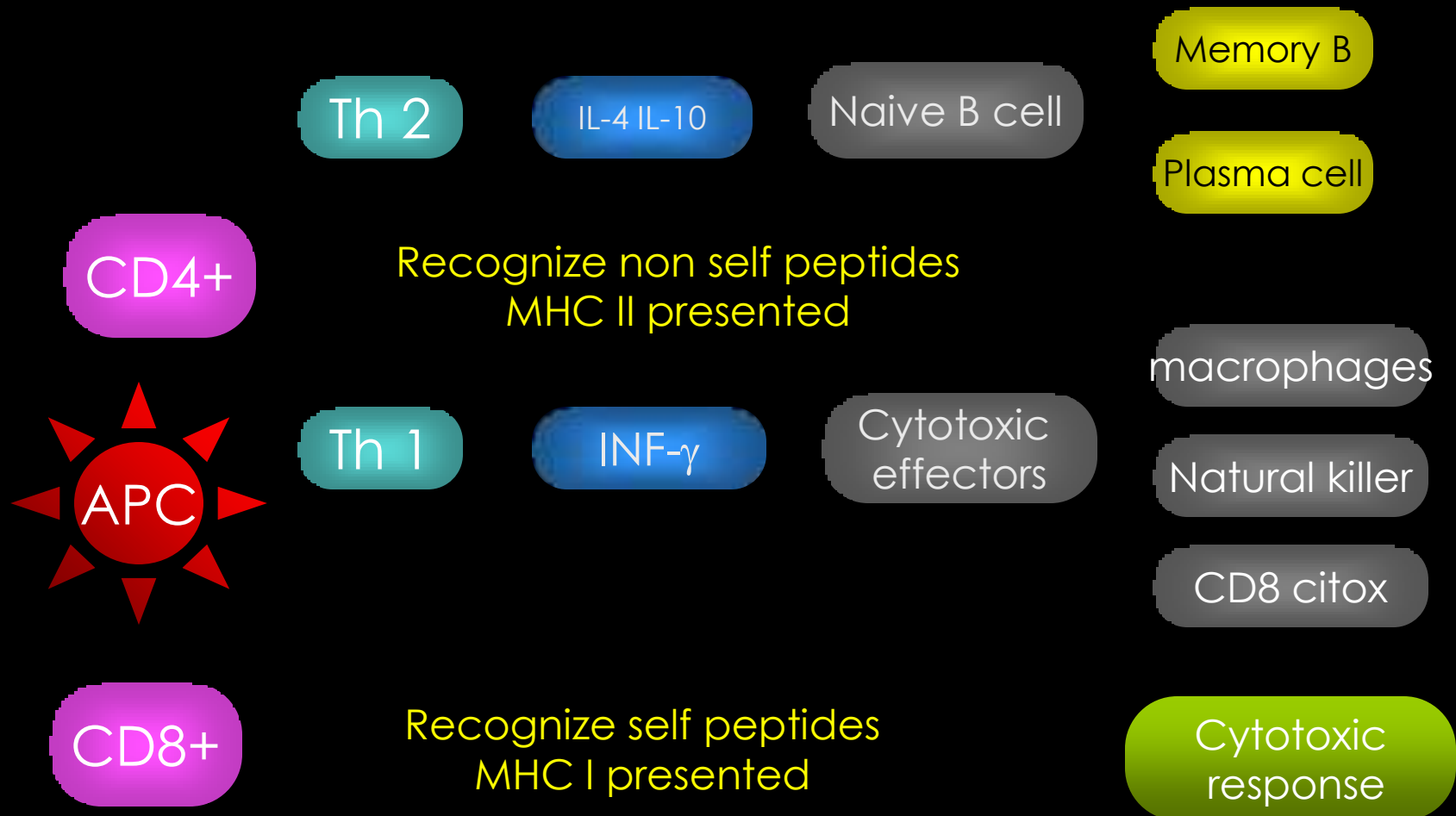
NOT PRODUCTIVE
NO INFLAMMATION

Innate response

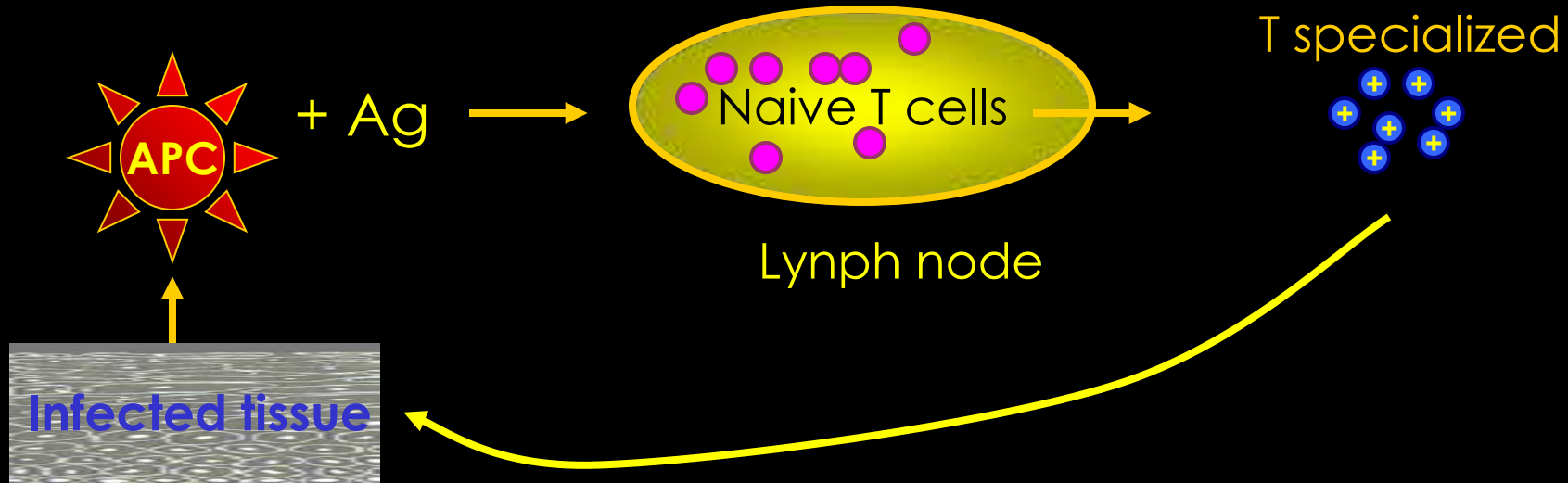
- Function:** to identify highly conserved antigenic structures (pathogen associated molecules-PAM) through superficial molecules (es. Toll-like receptors), system priming toward adaptative response
- Activation mechanisms :** PAM recognition, cell damage and death
- Response:** pro-inflammatory cytokine release
- Consequence:** local effectors recall (APC- dendridic cells), APC migration in the regional lymph-nodes, naive T cells activation, differentiation, migration to the infection site, recognition and disruption of the damaged cell

Adaptative response

T lymphocytes



Why the immune response is scarce and inefficient



1. The keratinocyte is programmed to die of 'natural death'
2. HPV as other DNA viruses interferes with IFN synthesis (natural antivirals, anti-proliferative, anti-angiogenic, immuno-stimulators, pro-inflammatory)
3. LC do not respond to HPV capsid Ag

HPV integration associated events

- ✓ The level of chromosomal instability is related to the level of E7 expression
- ✓ Loss of heterozygosis at FHIT (fragile histidine triad)
- ✓ Disease progression toward invasive cancer correlate with the accumulation of genetic mutation and justify the latency period between precancerous lesion and invasive cancer
- ✓ The greater number of mutations accumulate at the fragile sites 8q24 e 3p14, close to c-myc and FHIT tumour suppressor gene

HPV integration associated events

Loss of heterozygosity (LOH): determines chromosomal instability; is involved in the 'tumour suppressor genes' activation. In a specific chromosomal region the first allelic site is lost, the second is mutated

Microsatellite instability (MSI): diffuse mutations of small repeated DNA sequences (microsatellites). Originate from the inactivation of the DNA mismatch repair gene and from secondary mutations of genes coding for ms as the transforming growth factor receptor II



HIV and HPV

BACKGROUND

Immunesuppression of any origin (genetic, iatrogenic, infectious etc) has been demonstrated to be an additional risk for HPV infection and related pathologies

Cervical HPV infection and CIN are clearly increased in HIV+ women when compared with risk-matched HIV- women

There are few data to suggest a direct role for HIV in the pathogenesis of HPV-associated neoplasia, but HIV-associated attenuation of HPV-specific immune responses may allow for persistence of high-grade CIN and sufficient time for accumulation of genetic changes that are important in progression to cancer

The effect of HAART on HR-HPV infection and CIN has not been established



IDENTIFICATION

STUDIED POPULATION

580 HIV-1 positive women (median 5.1yy)

HR-HPV	26.8%	} 58.2%
HR+LR	31.4%	
LR-HPV	9.4%	
NO HPV	32.4	

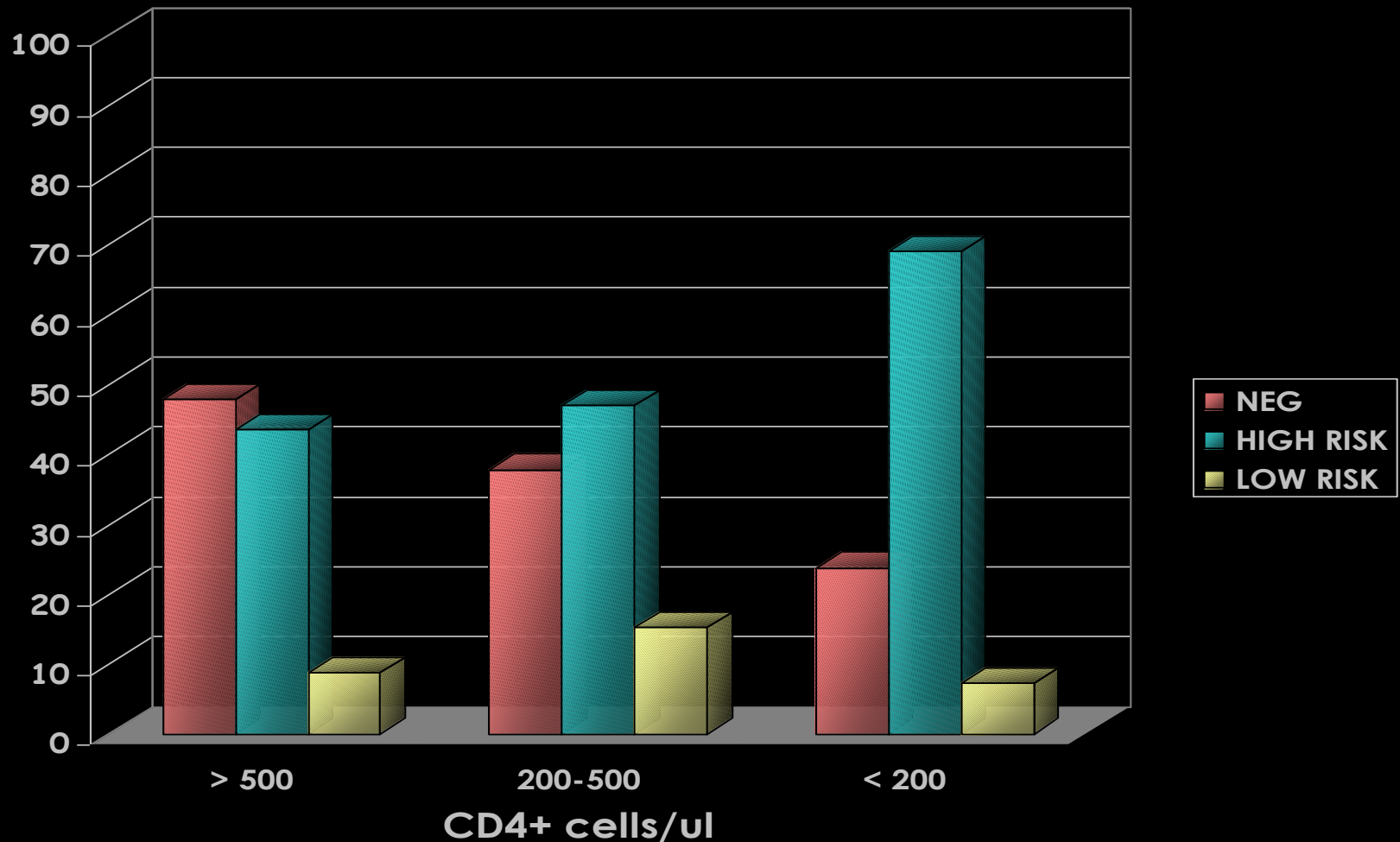
PERSISTENT	39.0%	} MULTIPLE 85.7%
SPORADIC	35.5%	
NEVER HPV	25.5%	

LESION	PAP	HYSTOL
NEG	65.5%	61.0%
LGSIL	23.5%	26.5%
HGSIL	10.2%	12.1%

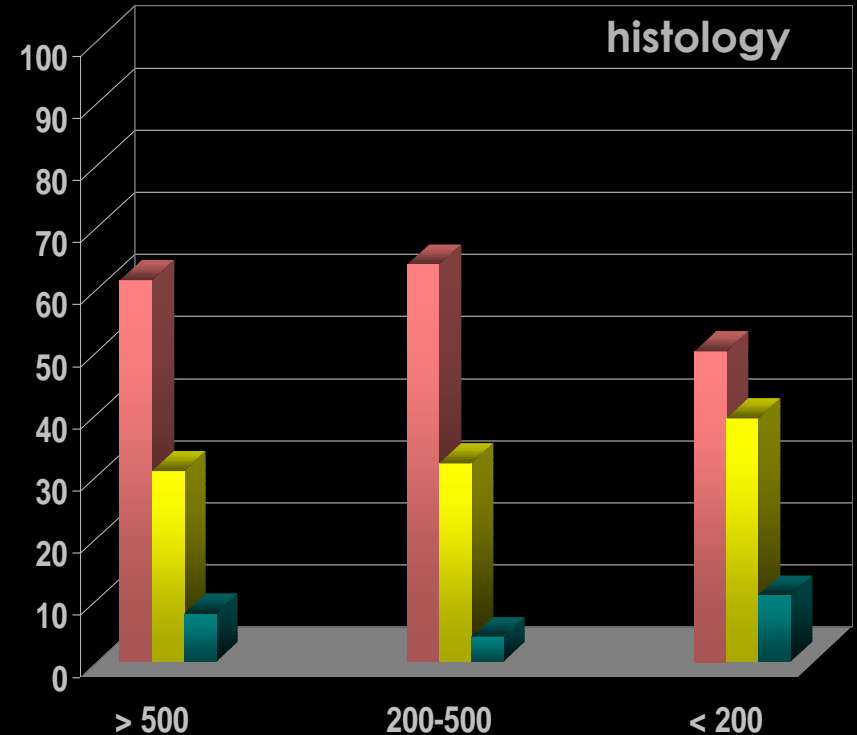
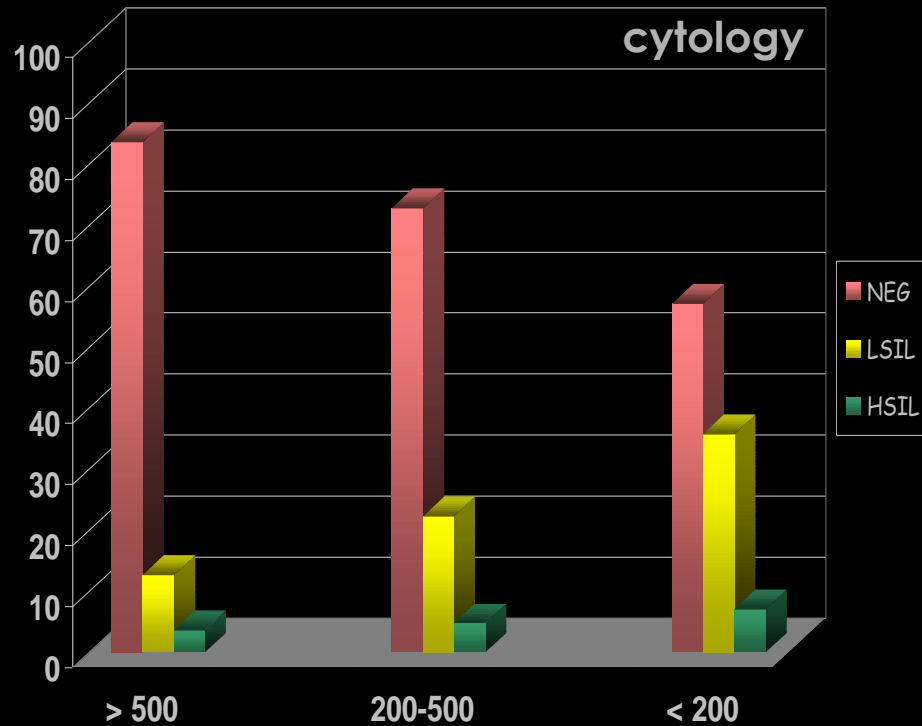
OVERALL PAP-HYSTO AGREEMENT
K=0.379

HGSIL PAP-HYSTO AGREEMENT
K=0.874

HPV TYPES PREVALENCE ACCORDING TO IMMUNE SUPPRESSION



CYTOLOGICAL AND HISTOLOGICAL DIAGNOSIS ACCORDING TO IMMUNE SUPPRESSION

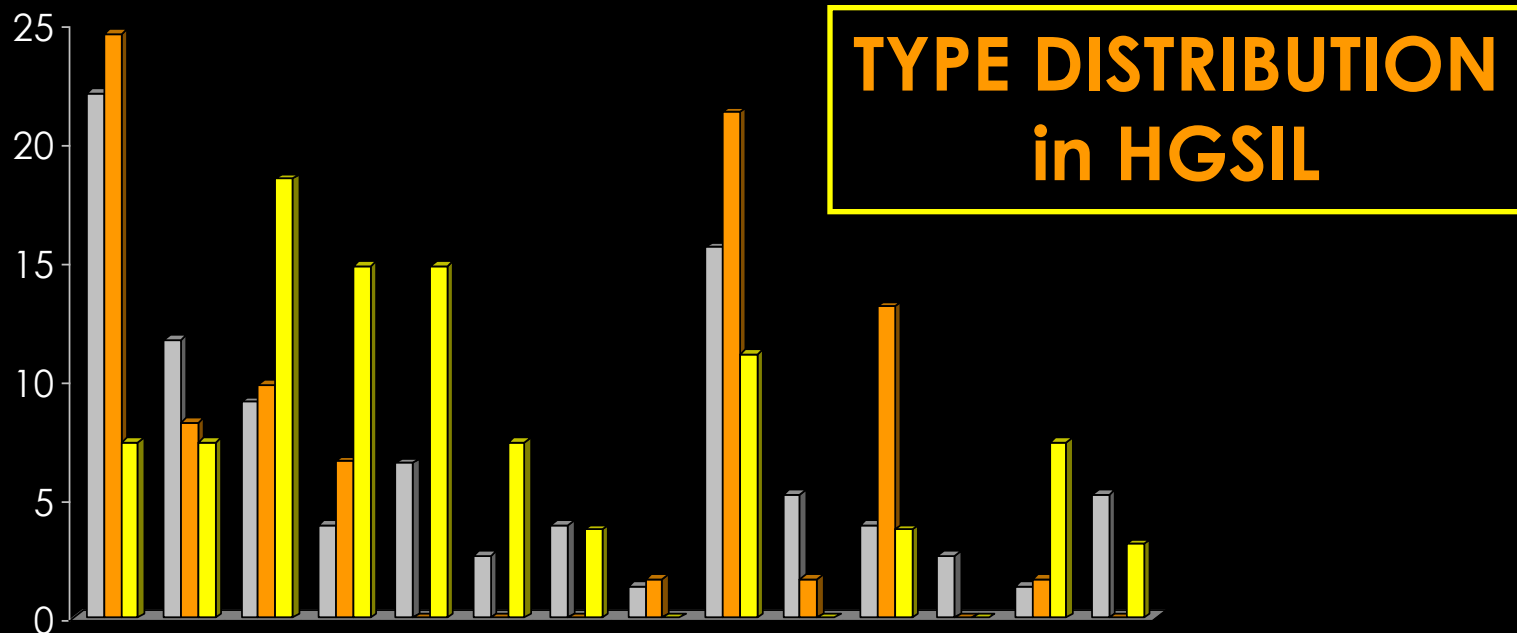


CD4+ cells / μ l

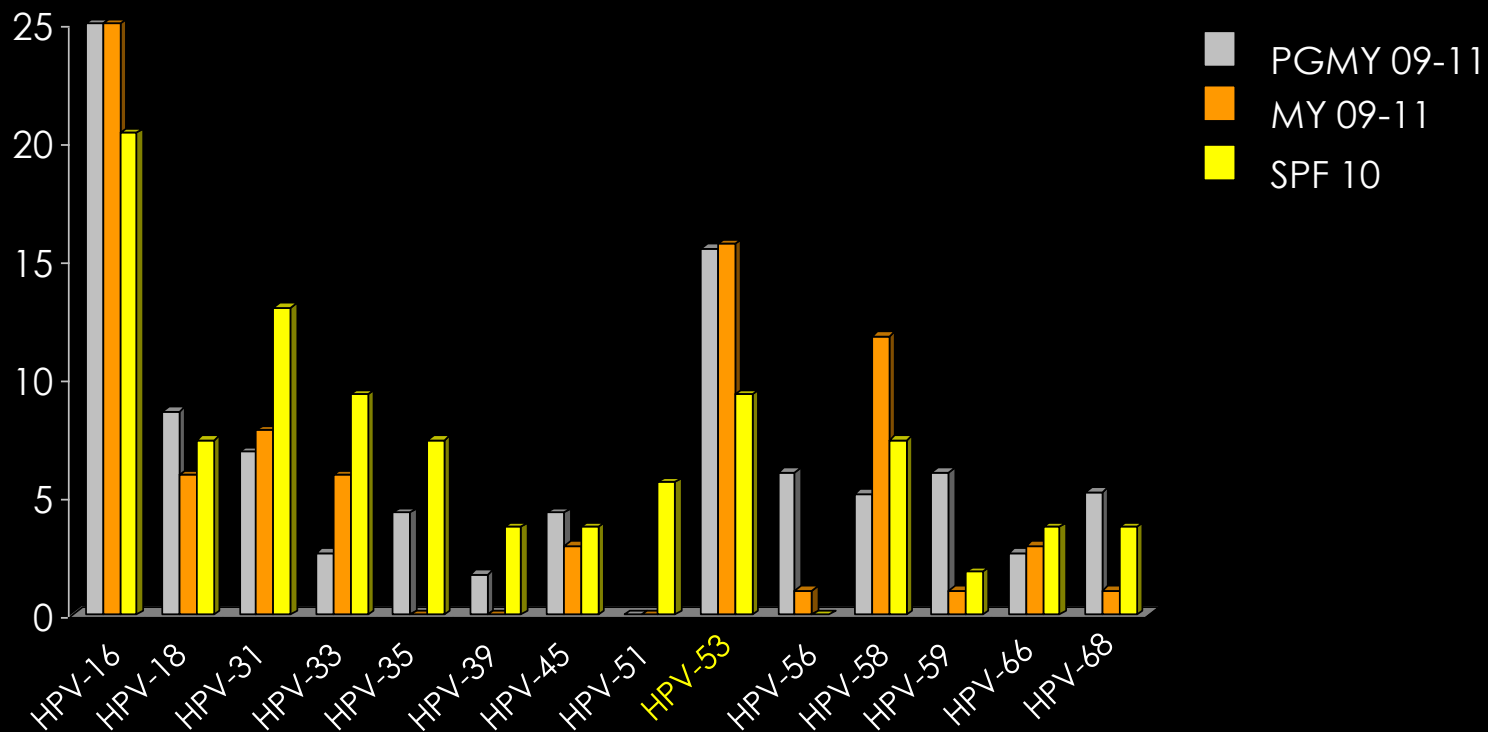


TYPING

PAP



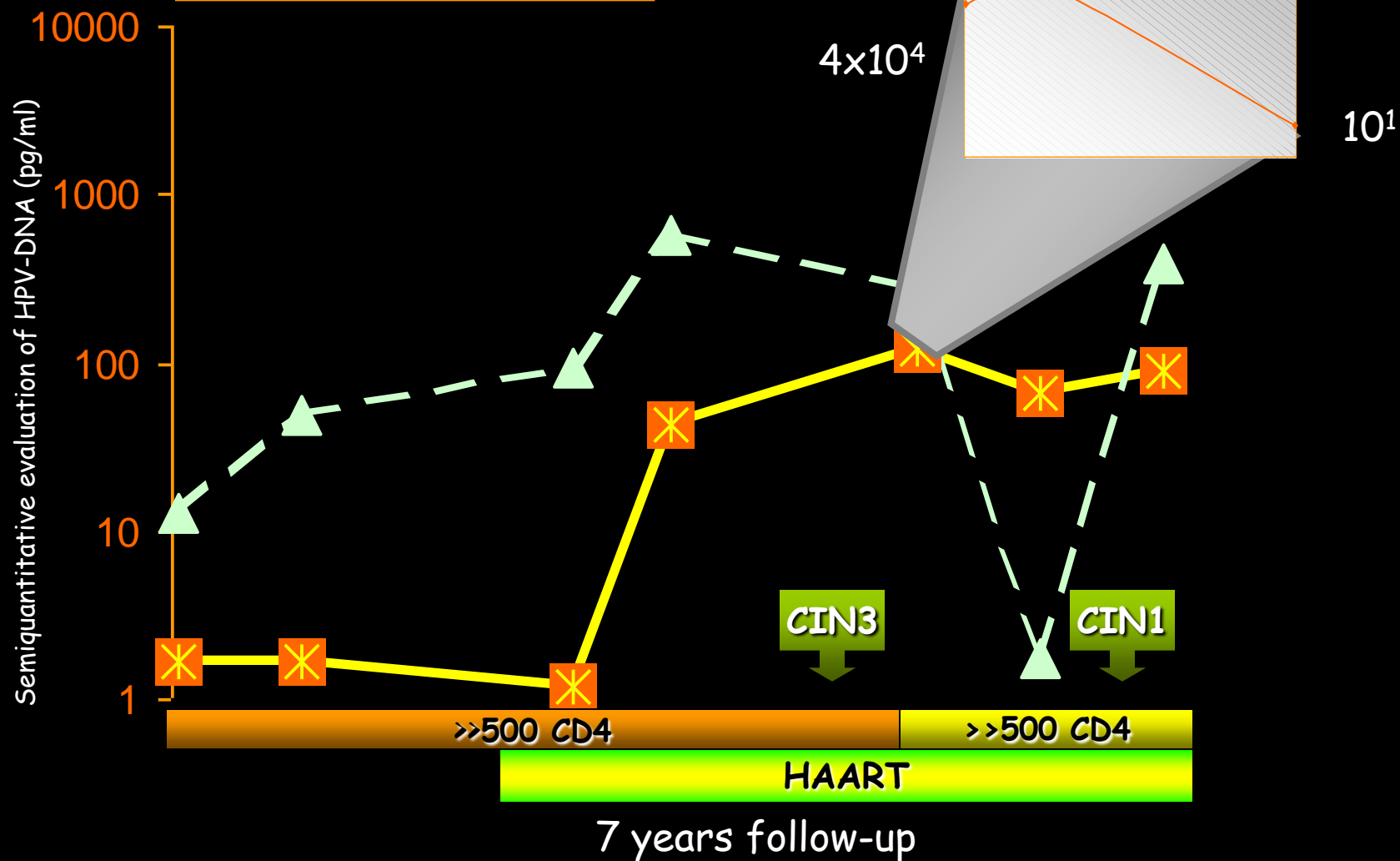
HYSTO



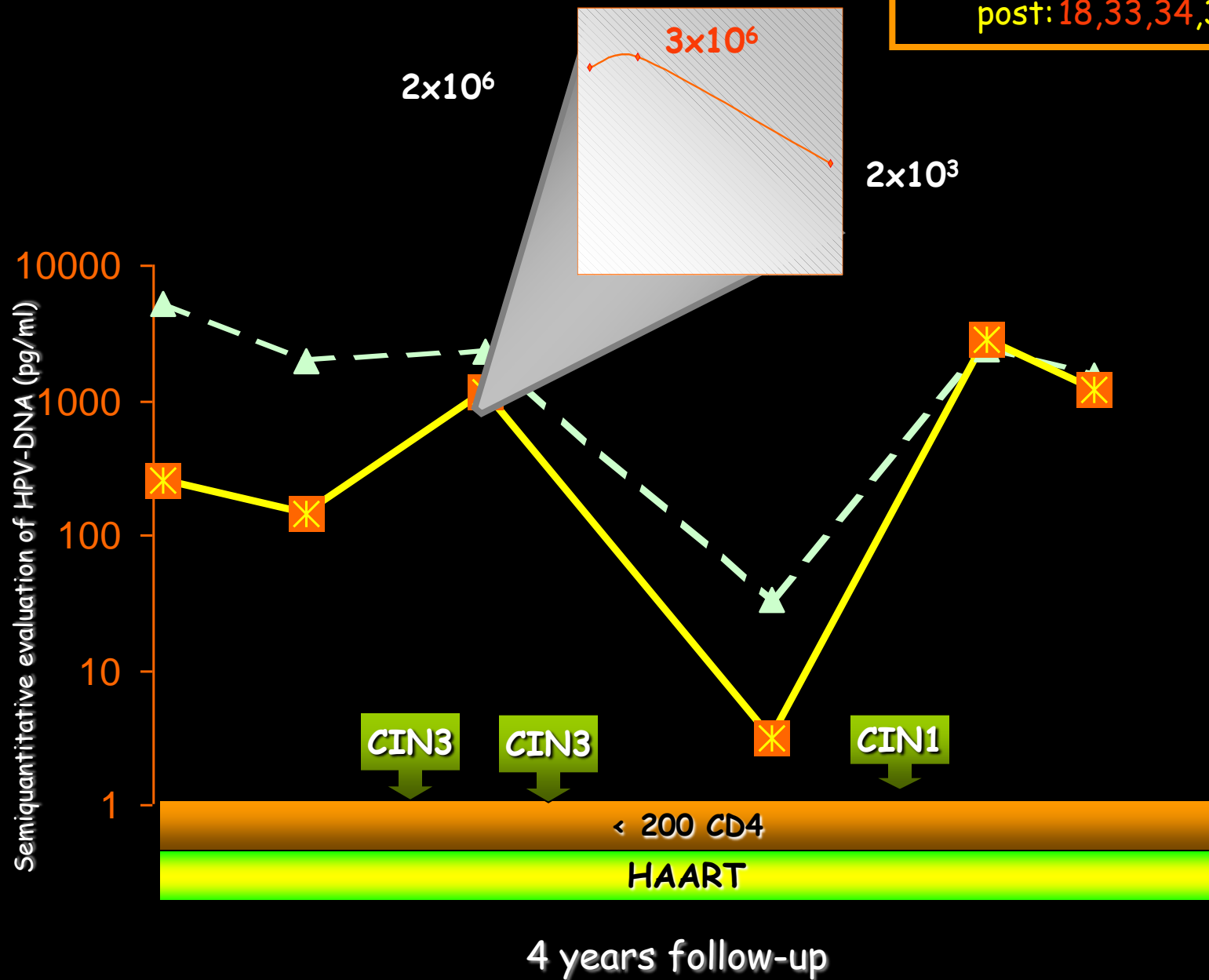
OVERALL AGREEMENT FOR MAJOR ONCOGENIC TYPES

HYSTO/load	ROCHE-GENOMICA K (CI 95%)	ROCHE-LIPA K (CI 95%)	GENOMICA-LIPA K (CI 95%)
Any >150	0.58 (0.46-0.70)	0.75 (0.61-0.88)	0.69 (0.54-0.85)
Any > 400	0.59 (0.48-0.71)	0.78 (0.65-0.91)	0.68 (0.51-0.85)
HSIL >150	0.70 (0.57-0.83)	0.72 (0.56-0.88)	0.71 (0.55-0.88)
HSIL >400	0.70 (0.57-0.83)	0.76 (0.60-0.92)	0.72 (0.54-0.90)

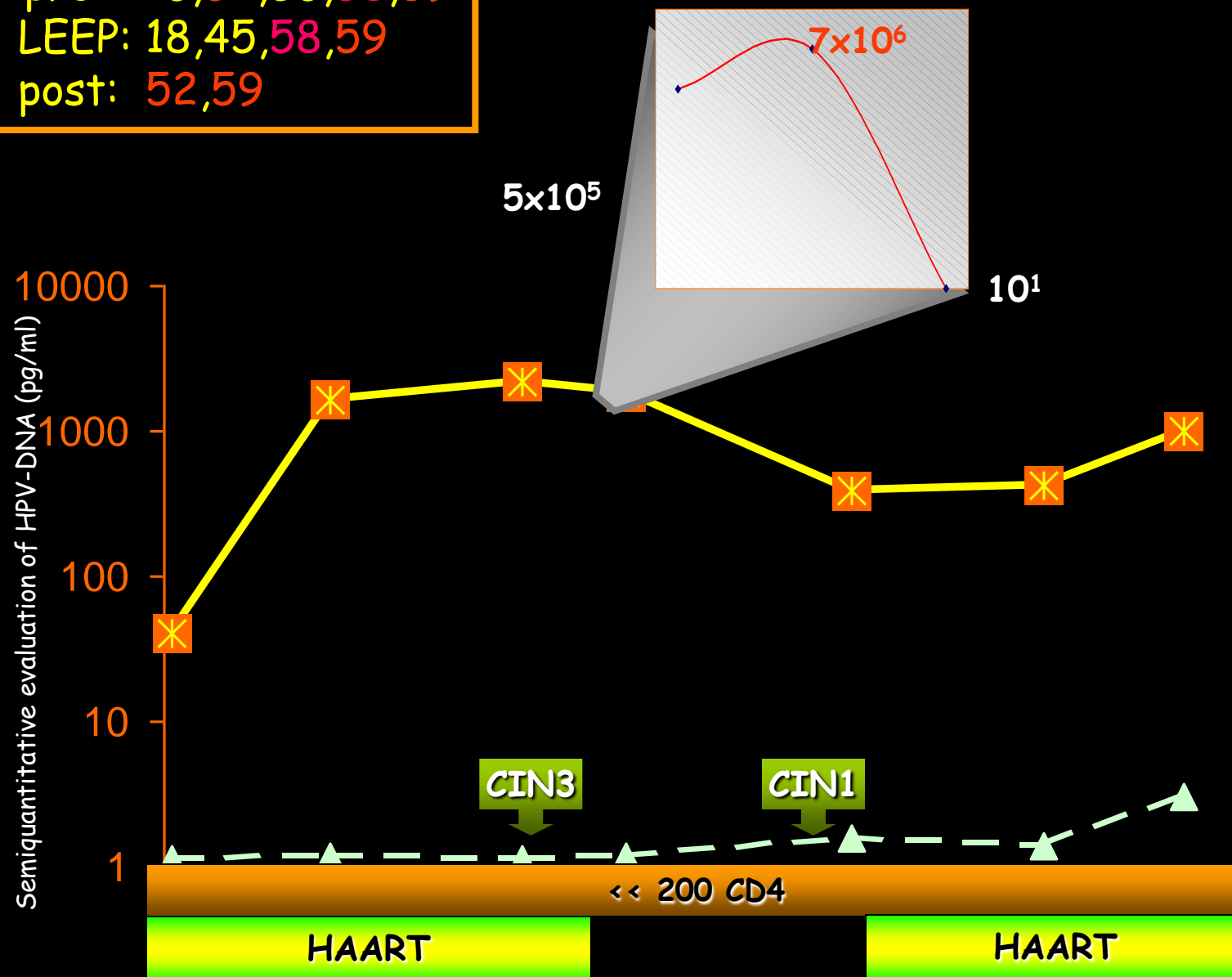
HPV pre:	53
LEEP:	31
post:	31,53



HPV pre: 18,33,34,39,44,45,52,66
LEEP:18,33,34,56
post:18,33,34,39,44,52,66

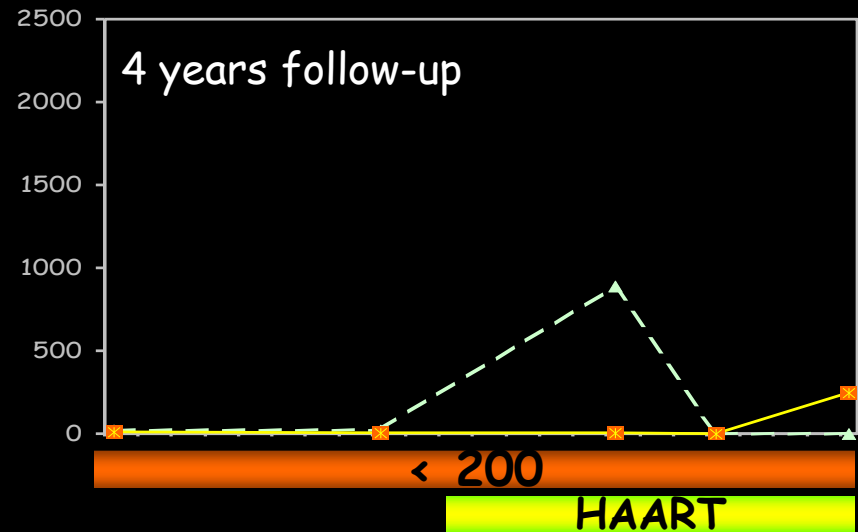
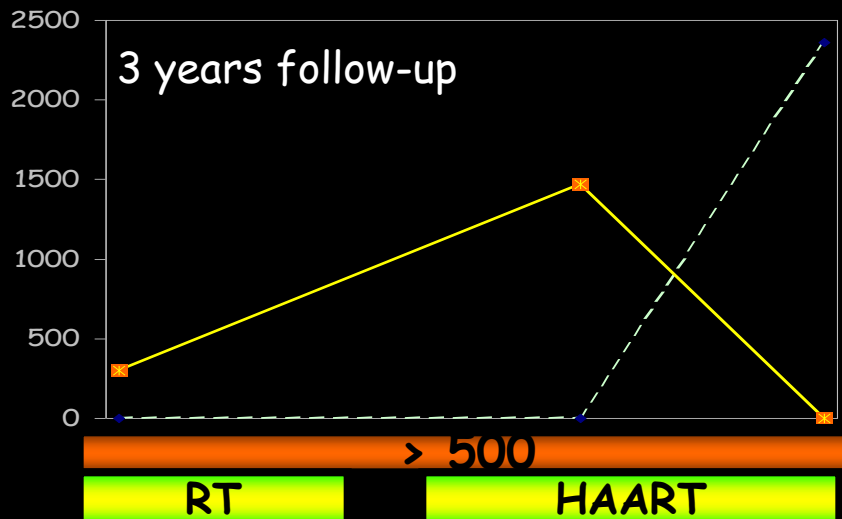


HPV pre: 16,52,56,58,59
LEEP: 18,45,58,59
post: 52,59



5 years follow-up

HR-HPV positive HIV + patients w/o cervical lesions (mean qt:239)



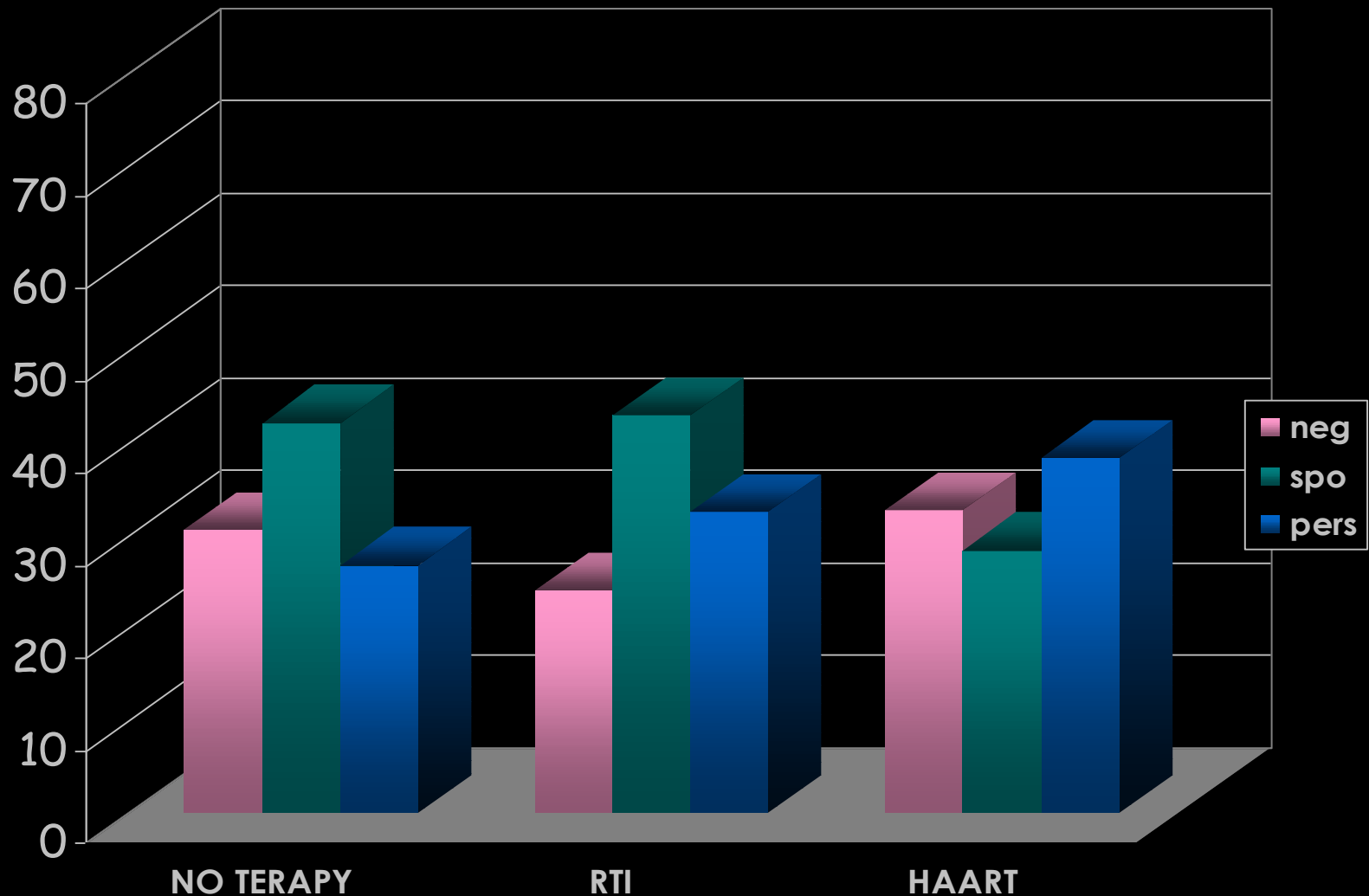
total DNA load and cervical lesions

N°=126	LESION		HR-HPV LOAD (%) HC2 index		
	PAP	HIST.	<150	150-400	>400
22	NEG	NEG	63.6	13.6	22.7
12	LGSIL	CIN1	25	8.3	66.6
32	HGSIL	CIN2+	9.3	3.2	87.5
26	LGSIL	CIN2+	15.4	15.4	69.2
5	NEG	CIN2+	0	0	100
8	NEG	CIN1	62.5	0	37.5
3	HGSIL	NEG	0	0	100
4	HGSIL	CIN1	25	0	75
5	LGSIL	NEG	40	0	60

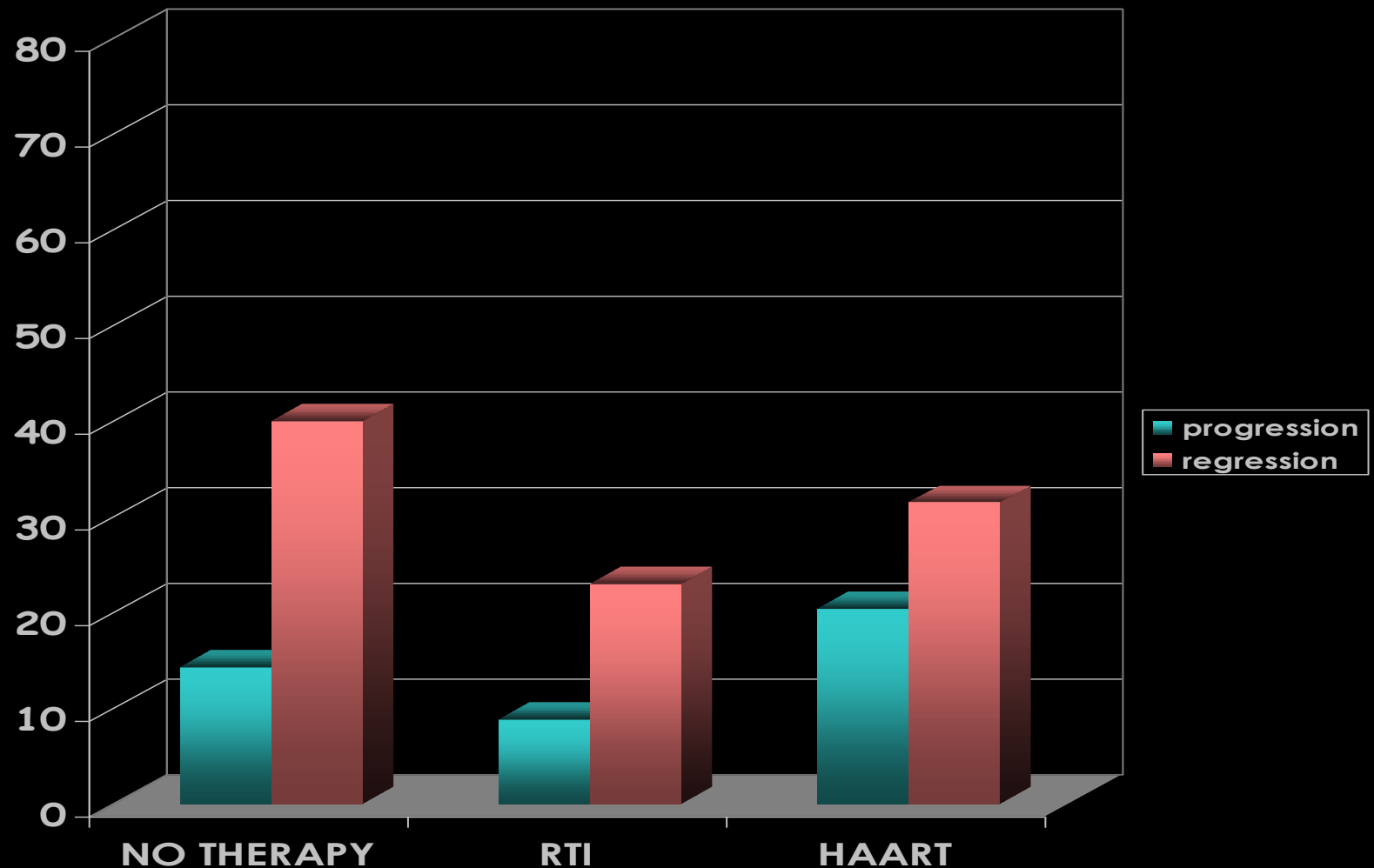


EFFECT OF ARV THERAPY

EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON HPV PERSISTENCE



EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON THE EVOLUTION OF HPV RELATED LESIONS



HPV: prophylactic vaccine

Prophylactic vaccine: *Background*

- "HPV is the necessary cause of cervical carcinoma" (*X.Bosch, 2002*)
- "Identification of an infectious agent as a necessary cause of disease implies that interfering with the infection should prevent development of the disease" (*D.Lowy, 2003*)

Prophylactic vaccine:

Objectives

The major objectives of prophylactic vaccination are:

1. morbidity
2. mortality
3. costs

- 1. removing any risk of disease in women effectively immunised**
- 2. reducing exposure to infection amongst the rest of population**

(G.Garnett, 2000)

Reduction of other genital/non-genital cancer sites

Prophylactic vaccine: *Immunity*

Immune system control HPV infection

1

Epidemiological evidence

HPV infection naturally induces low titre of neutralising antibodies. Nevertheless, prior infection is host protective for the same genotype. (Frazer, 2002)

2

Disease in the immunocompromised

Impaired cellular immune function (HIV or renal transplantation) increase the incidence of HPV lesions (Garzetti G, 1994; Halpert R, 1986; Ozsaran A, 1999)

3

Therapy with immunomodulators

IFN
Imiquimod

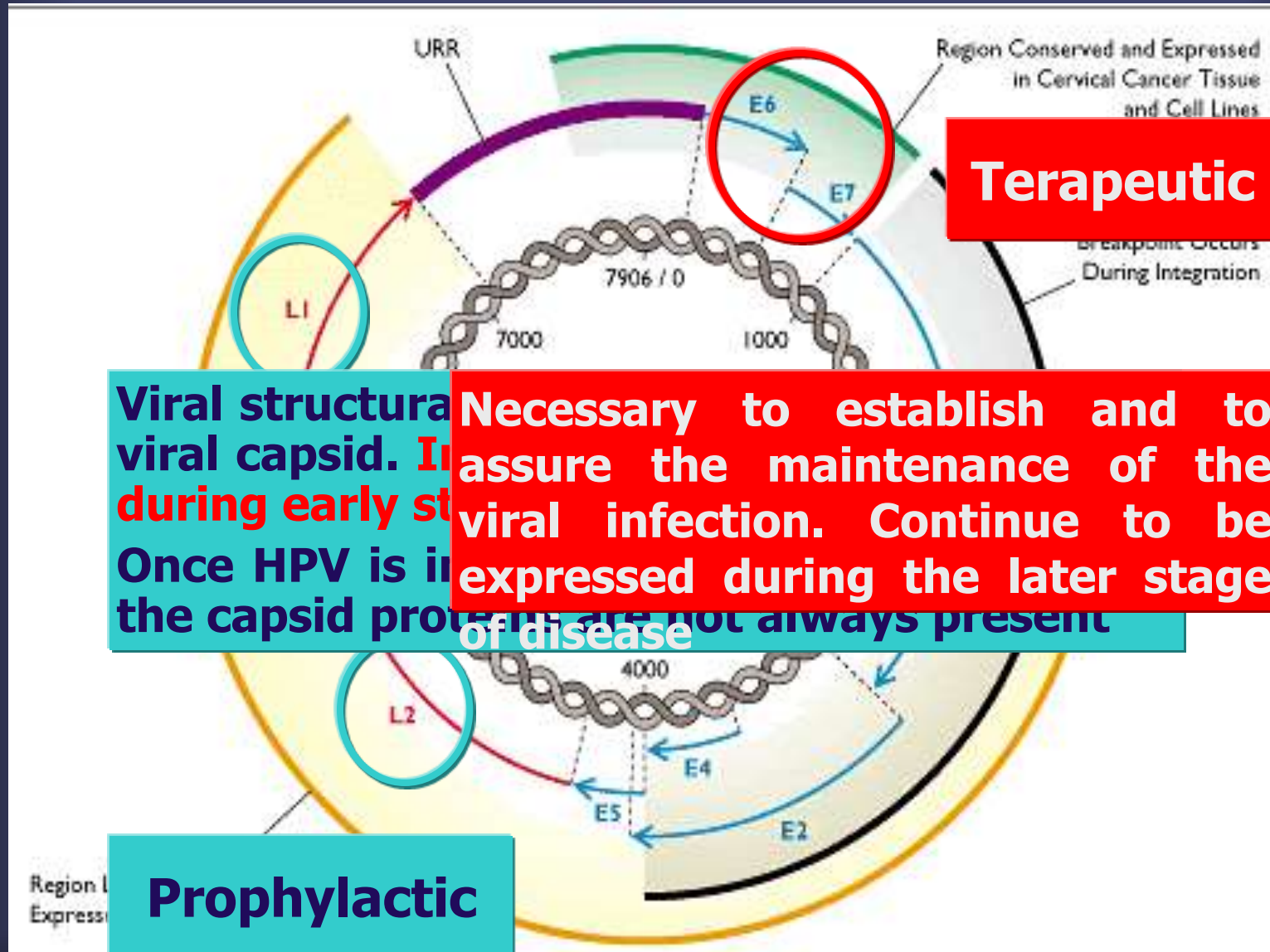
Prophylactic vaccine: *Escape from immunity*



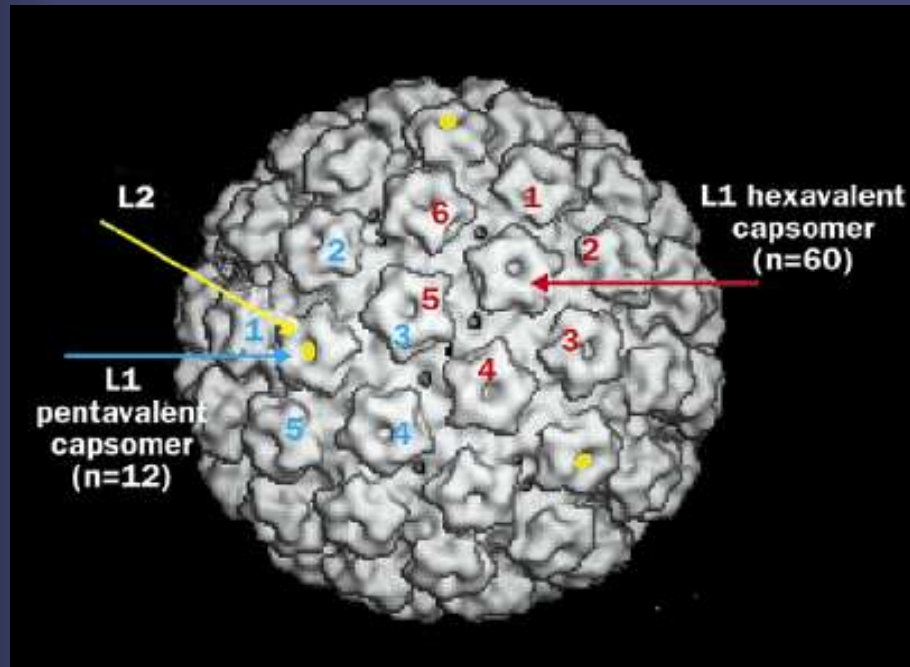
The presence of persisting HPV infection suggests the virus is capable of evading many forms of immune surveillance. (C. Rock, 2000)

- ◆ No viremic phase which stimulate immune response
 - ◆ Viral proteins are produced in very small amounts
 - ◆ HPV gene expression
 - ◆ Release of assembled virus
 - ◆ Keratinocytes are not lysed
 - ◆ No inflammatory response
- } superficial epith. layer where virus antigens are not easily detected
- } there is no a "danger signal" (lack of valid CTLs response)
-
- ◆ Viral early proteins inhibit expression of MHC class I
 - ✓ E7 (HPV16,18) inhibits the induction of IFN
 - ✓ E5 inactivate the antigen processing system

Prophylactic vaccine: *Rationale*



Prophylactic vaccine: *Rationale*



HPV16 L1 VLPs
(J.Schiller, 2000)

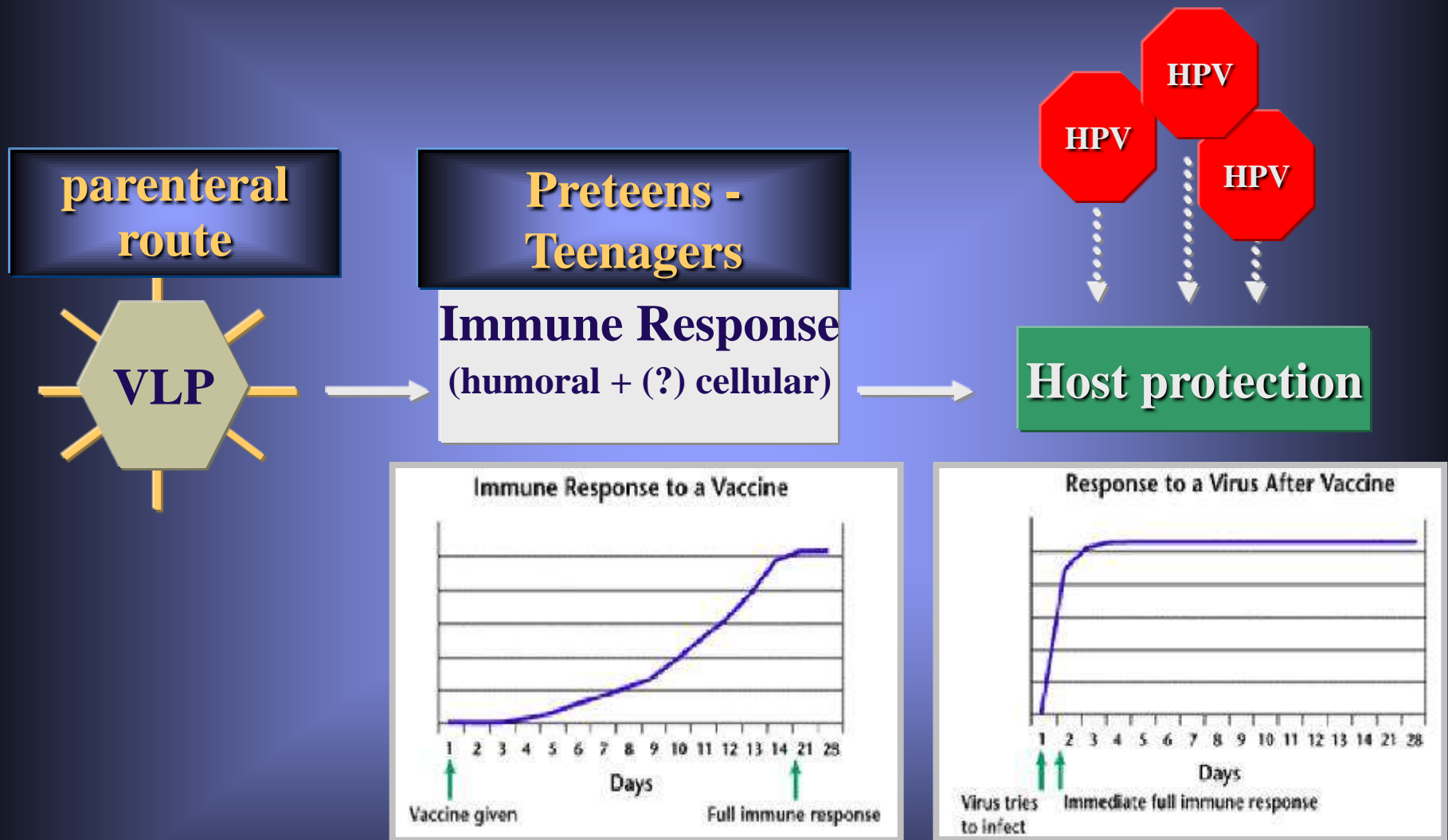
Virus-like particles (VLPs)

**Are neither infectious,
nor potentially oncogenic**

**cross-reaction of
L2 antibodies ?**

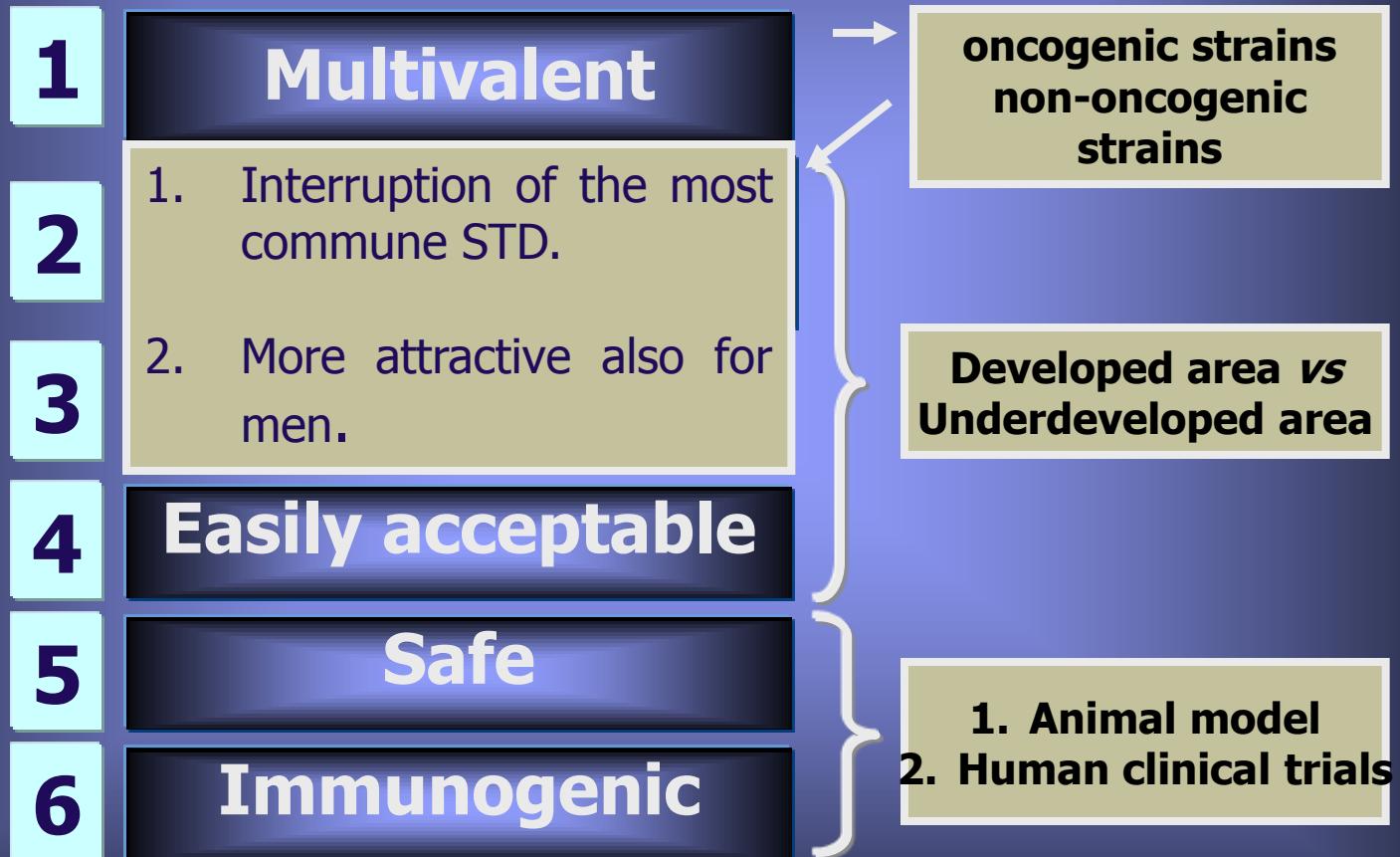
- VLPs resemble the conformation of authentic virions and induce high levels (>10000) of **neutralizing antibodies.**
- The immune-response is type-specific.

Prophylactic vaccine: *Rationale*



Prophylactic vaccine: *Rationale*

Ideal HPV-vaccine



Prophylactic vaccine: *Rationale*

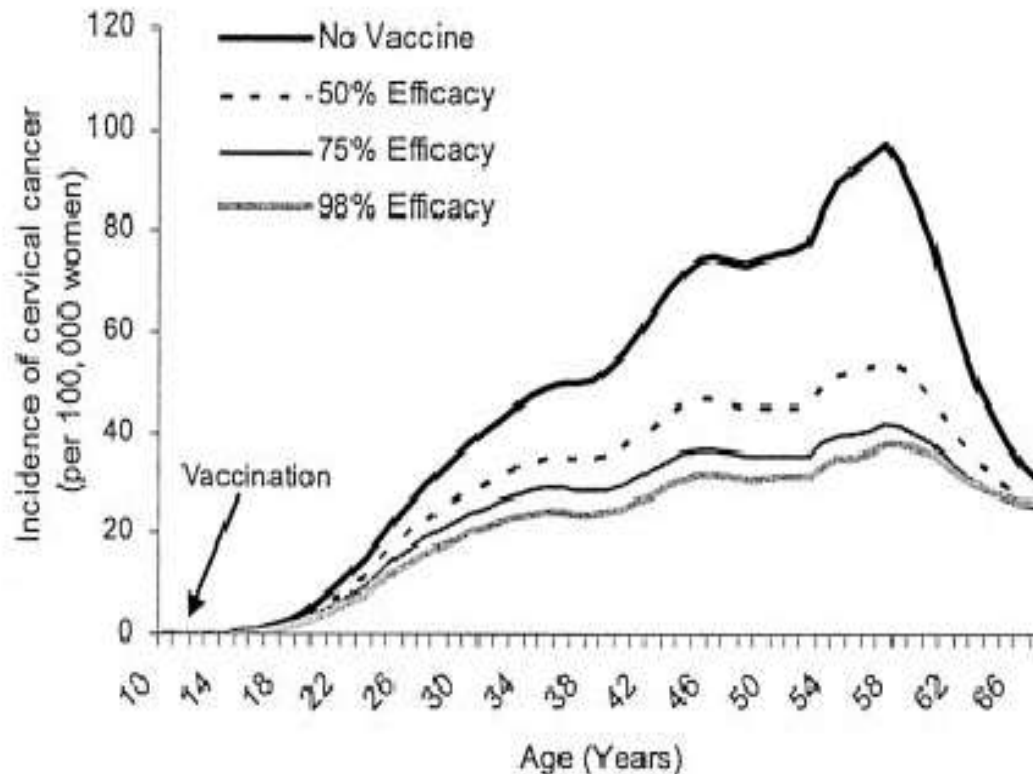
Design of the human clinical trial

Methodology

- Geographic area (*developed vs developing*)
- Population selection (*female vs male and men*)
- (*rural vs urban*)
- **organized mass screening**
- **population based cancer screening**
- **High-risk areas in developing countries (→HBV vaccination)**
- **(J.Pa in Gambia or Taiwan)**
- Biological/immunological (M.Plummer & S.Franceschi, 2002)
- Length and type of follow-up
(ability to follow-up women over a long period)
- Projected population benefit

Prophylactic vaccine: *Rationale*

- ✓ Coverage of the population
- ✓ Theoretical efficacy by HPV-type

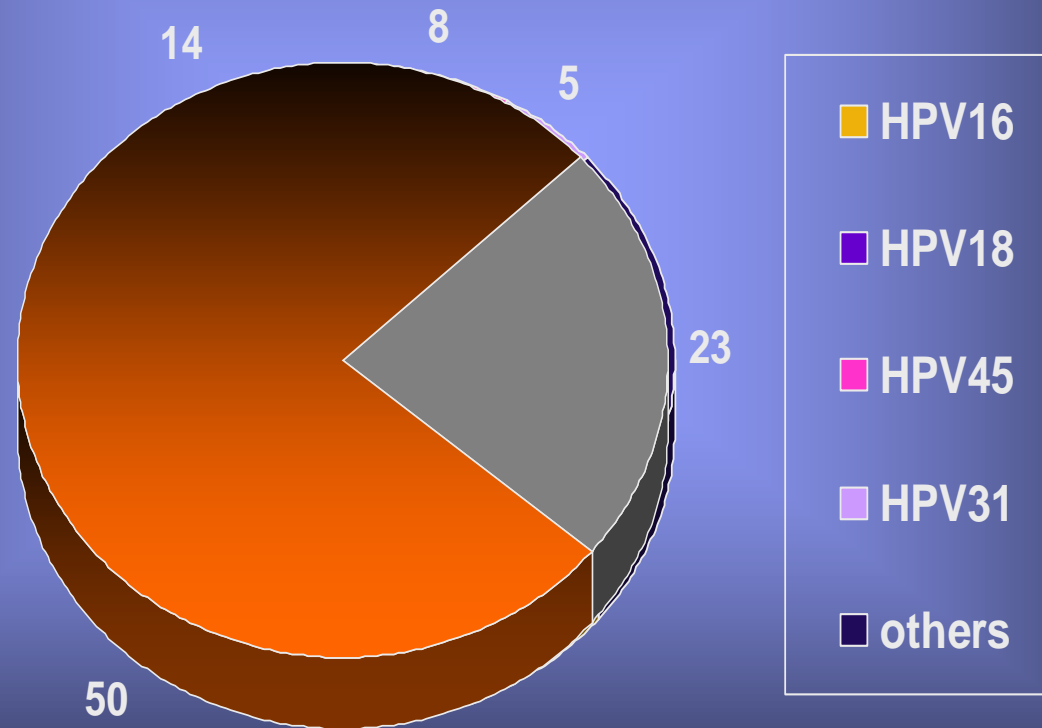


**S. GOLDIE,
2003**

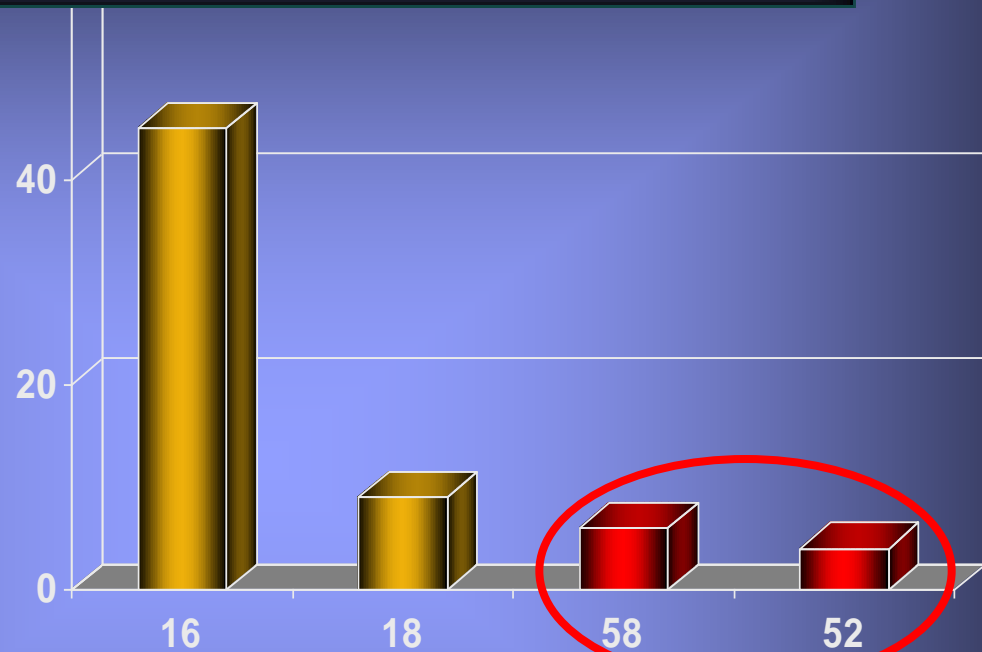
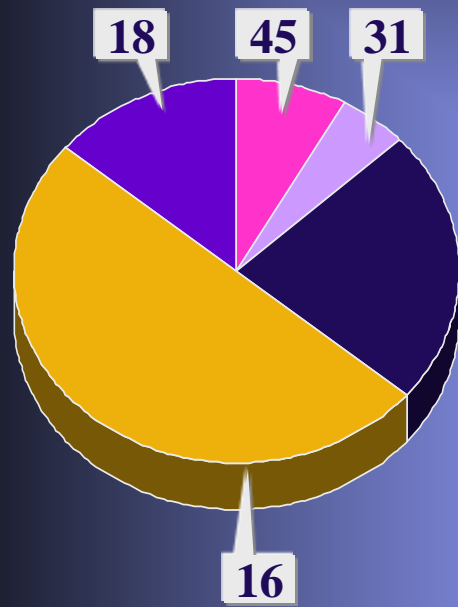
Prophylactic vaccine: *Rationale*

HPV in cervical carcinoma

(X. Bosch, 1995)



Prophylactic vaccine: *open questions*



ASIA
(IARC-FIS-2003)

" The use of papillomavirus vaccines will require better definition of the local prevalence"
(*I.Frazer, 2004*)

Prophylactic vaccine: *open questions*

The rate of detection of new HPV-types appears to have accelerated instead of slowed down (*L. Villa, E. Franco, 2002*)

Five types previously classified as low-risk (HPV 26,53,66,73,82) should now be added to the list of high-risk
(*N. Munoz, 2003*)

Local HPV prevalence

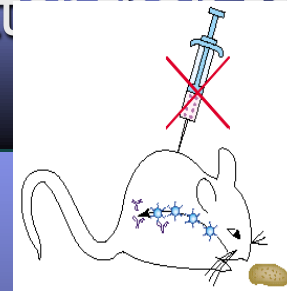
- 1. Life-style*
- 2. Sexual behaviour*

Prophylactic vaccine: *open questions*

Developing countries

- Local production
- Easy and cheap distribution
- Vaccine could be stable, easy storing
- Long-lasting immunity with a single dose
- Oral/mucosal vaccine better than for

EDIBLE VACCINES



Does malnutrition impact on
immune response to HPV-vaccine ?

