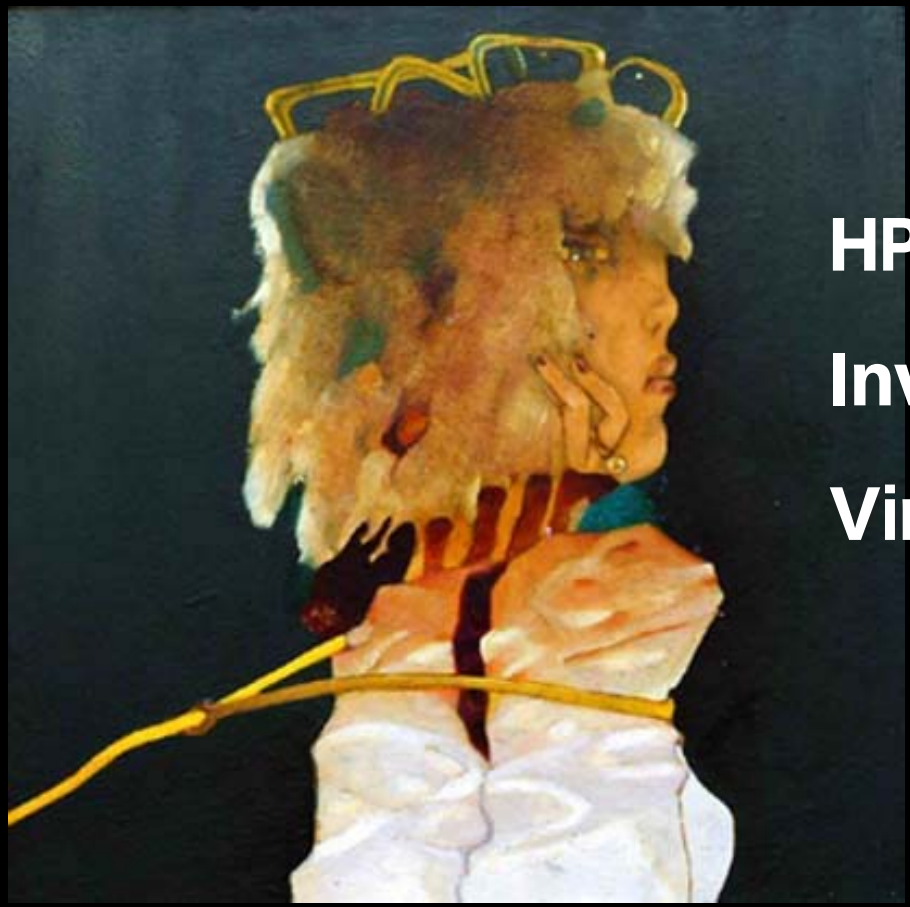
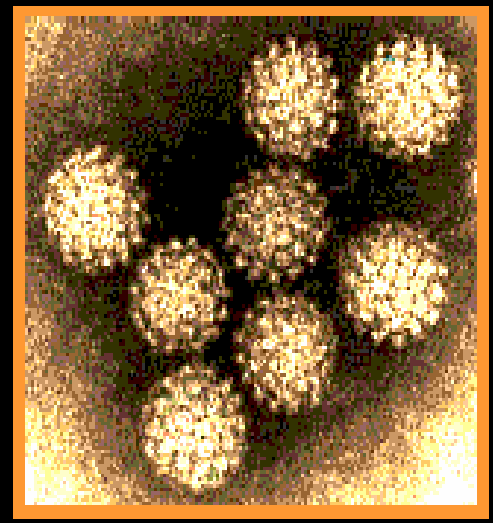


Il laccio – Giovan Battista De Andreis

HPV infection and Invasive Cervical Carcinoma: Virus – Host interactions

Flavia B. Lillo
Laboratory of Virology
IRCCS-Hospital San Raffaele
Milan - Italy

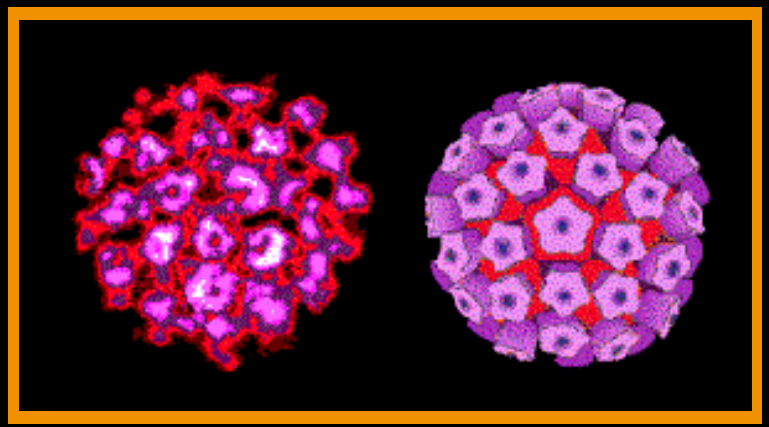
What HPVs are ?



ds DNA virus with a 8000 pb genome

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

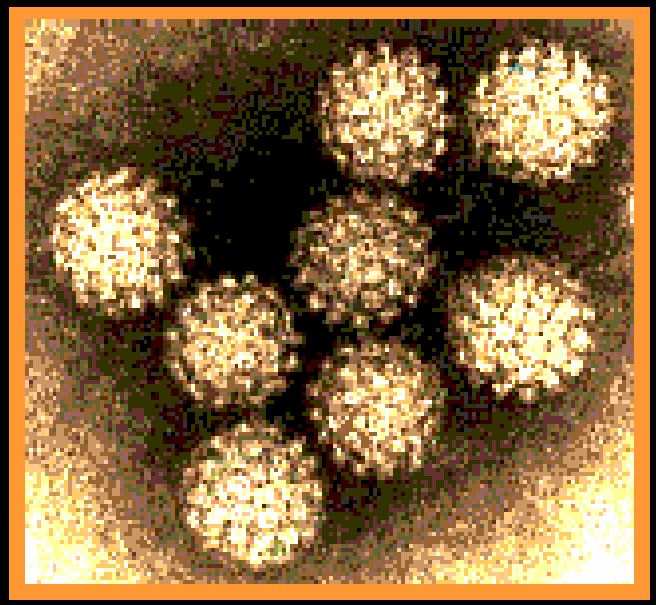
Based on their oncogenic power they are divided in HIGH and LOW risk types



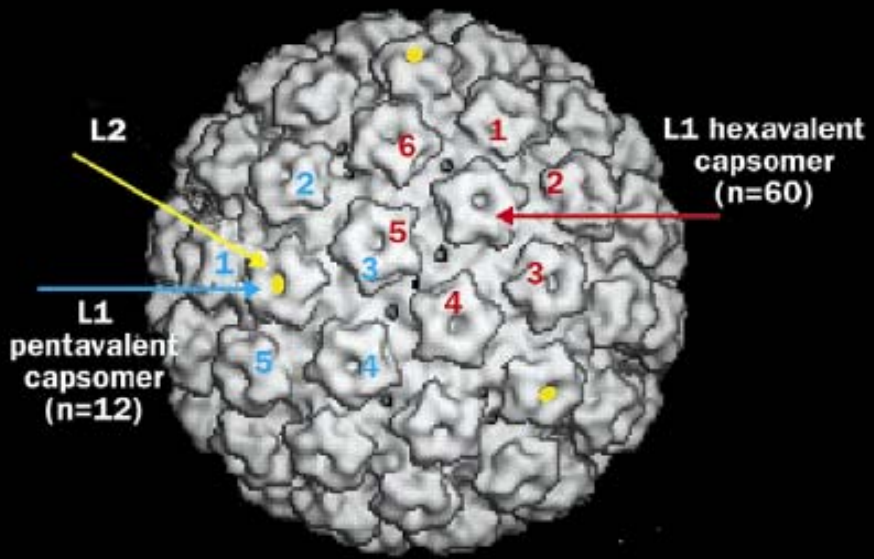
HR-HPV associated tumours:

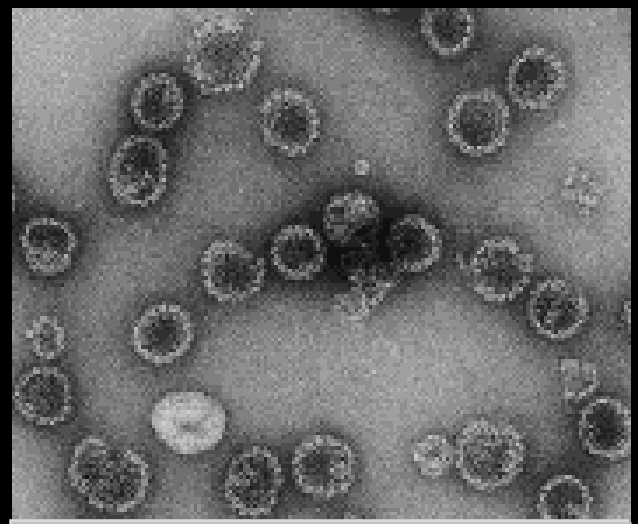
Squamous Cell Carcinoma

Adenocarcinoma



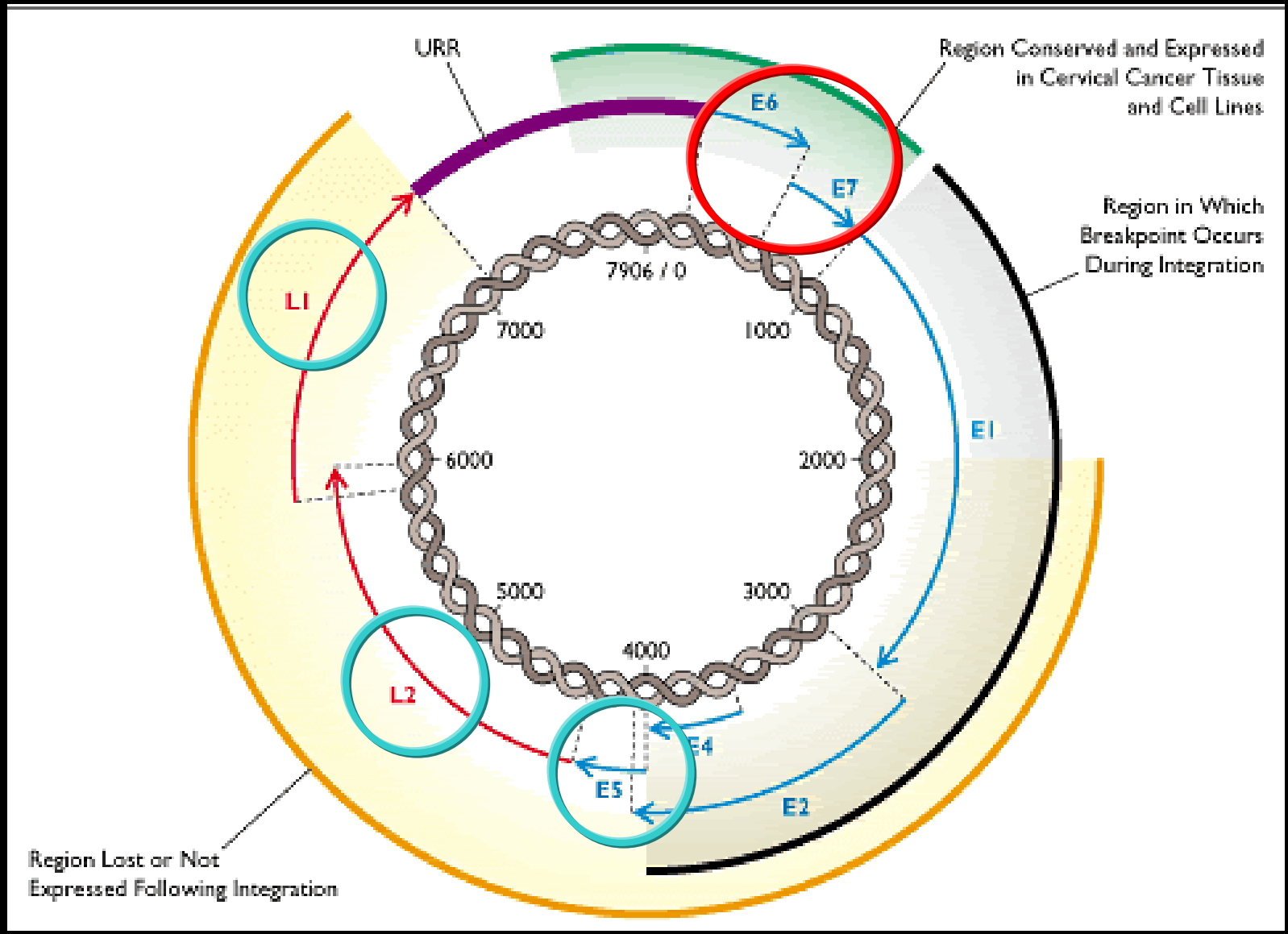
HPV morphology



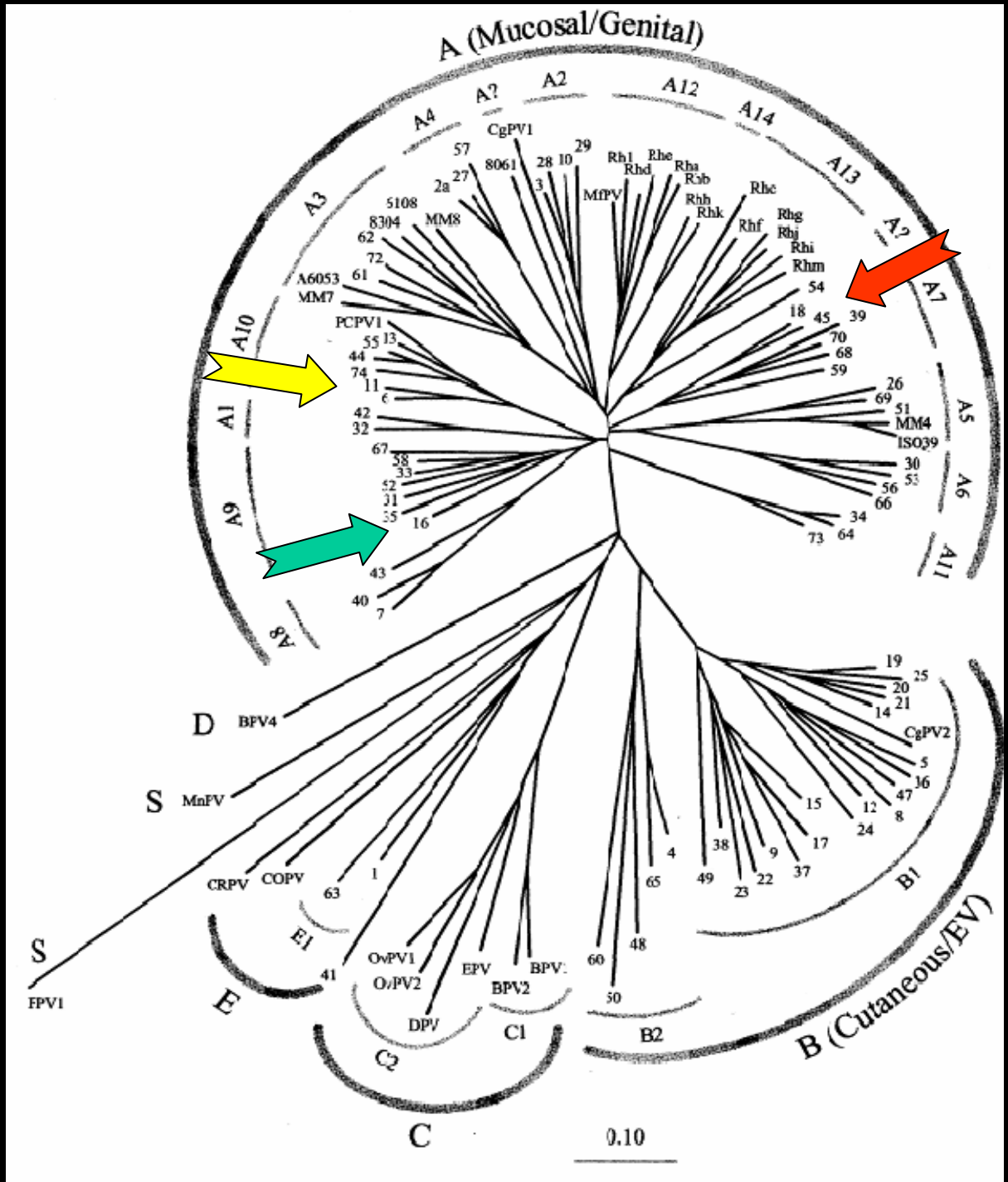


HPV16 L1 VLPs
(*J.Schiller, 2000*)

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

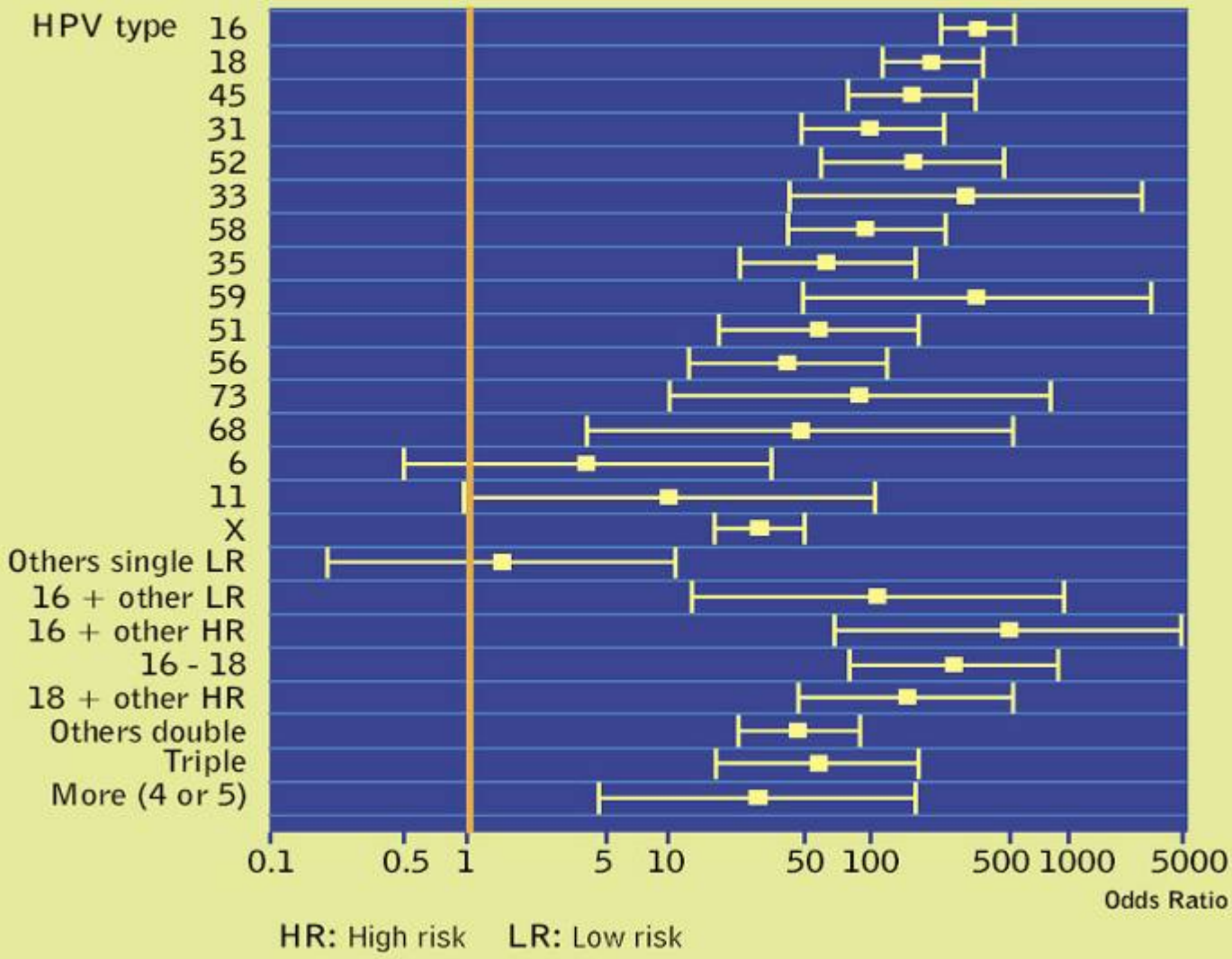
Prospective studies demonstrated that persistent HPV infection precedes the development of lesions and represents an elevated risk for disease

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

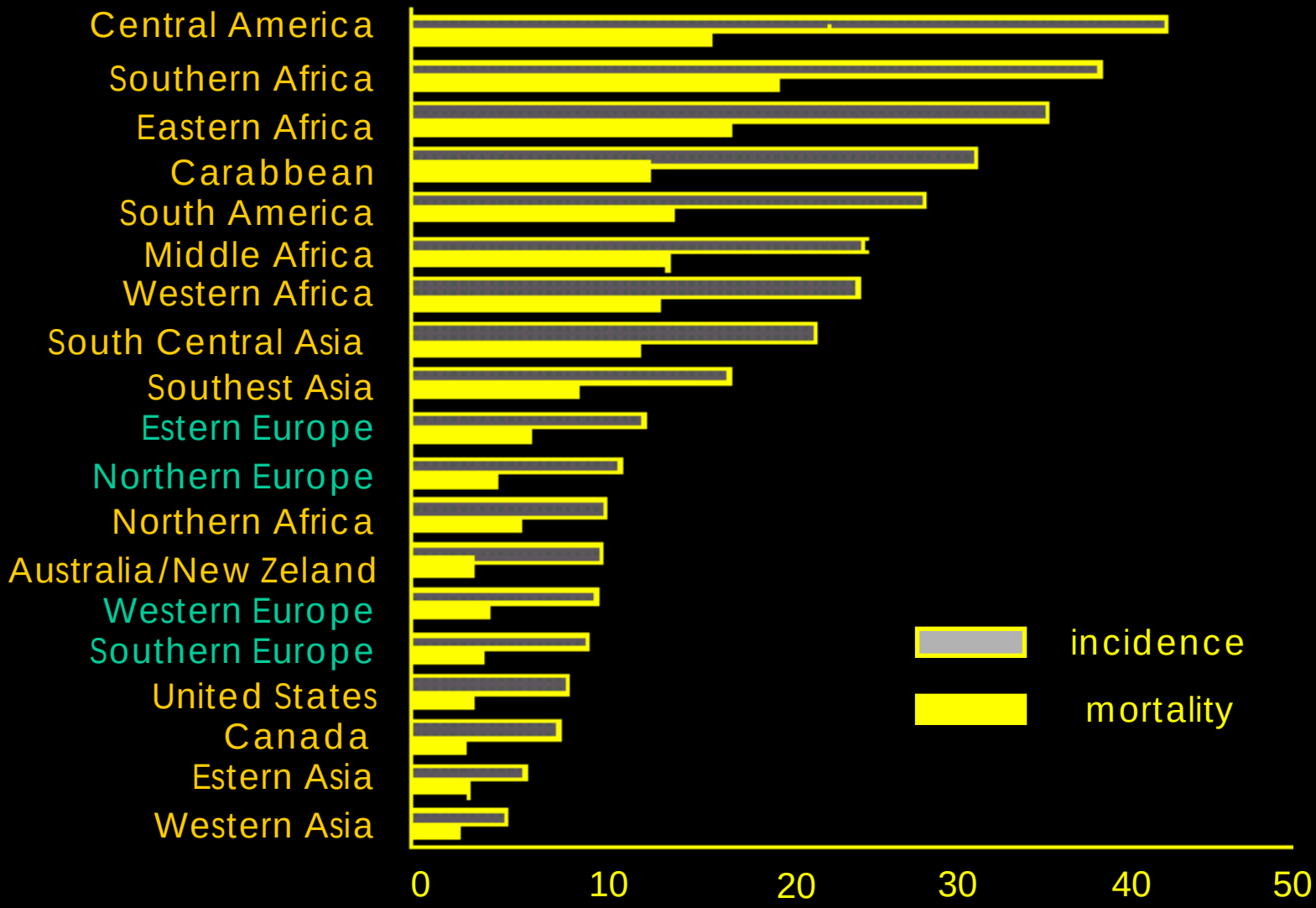
HPV TYPE-SPECIFIC RISK ESTIMATES FOR CERVICAL CANCER

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

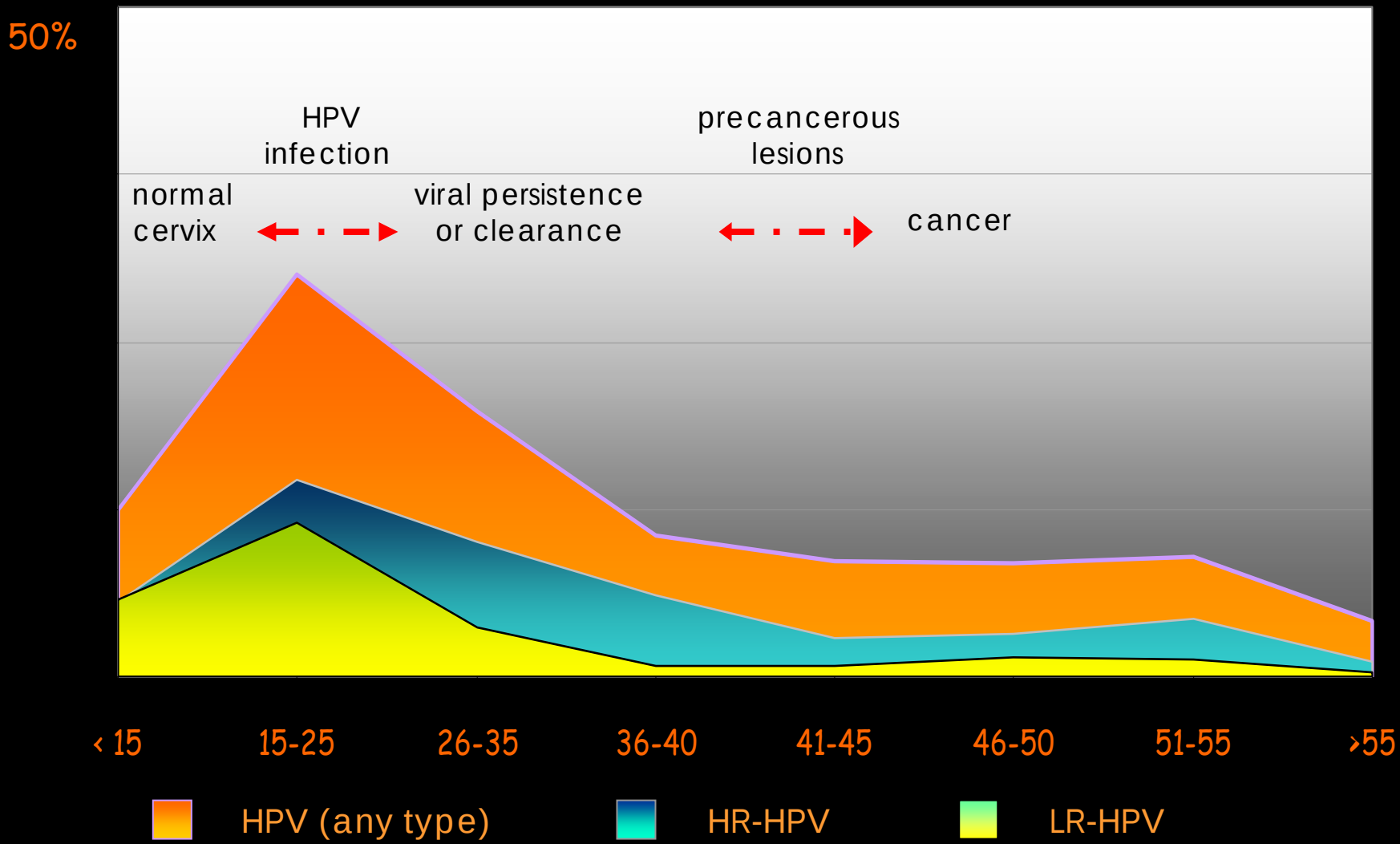


HR: High risk LR: Low risk

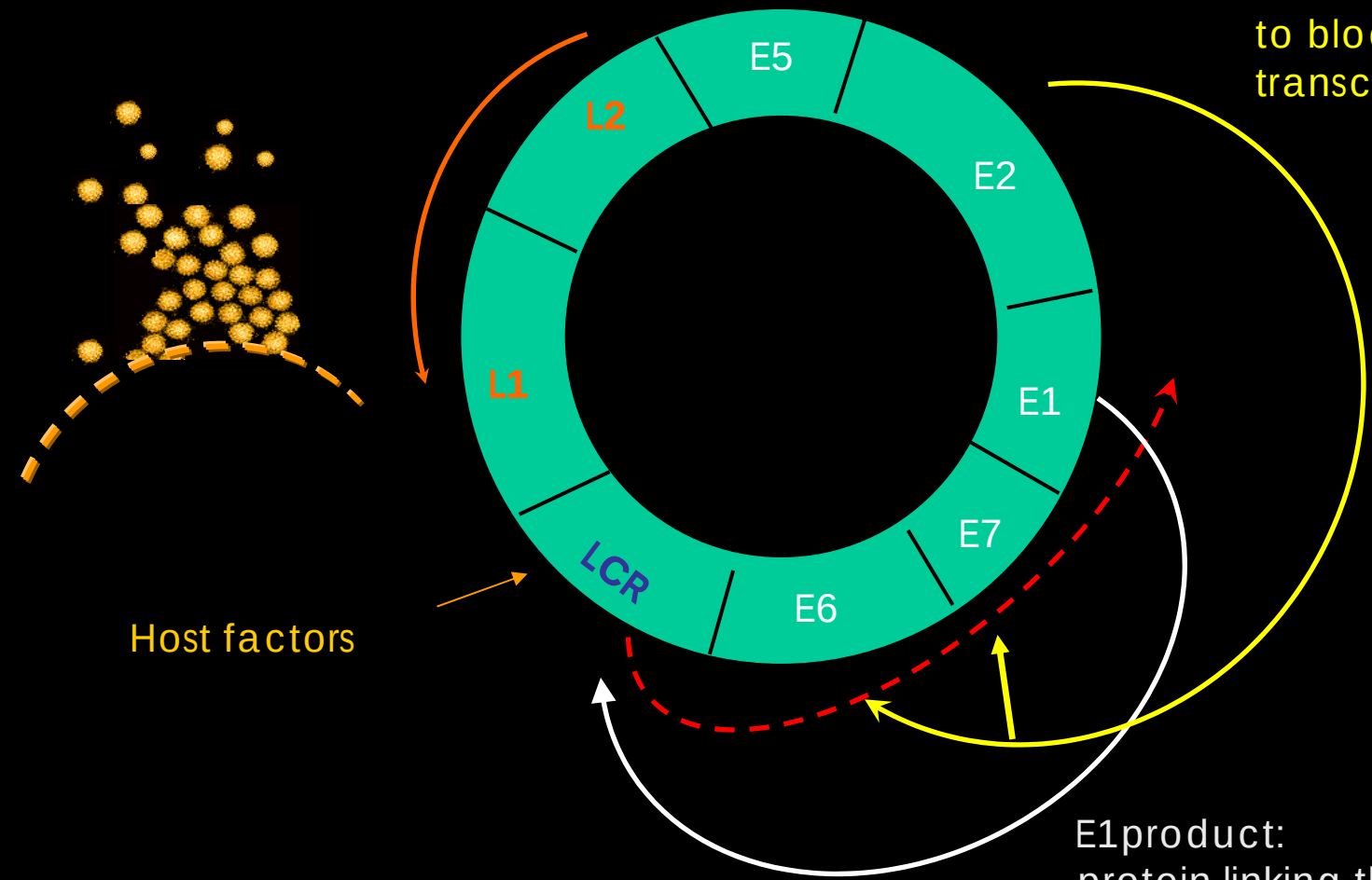
ANNUAL AGE-STANDARDIZED RATE PER 100.000



Age-specific prevalence of HPV infection



episomial HPV replication



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

E1product:
protein linking the replication origin (LCR) and induces episomial DNA replication

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

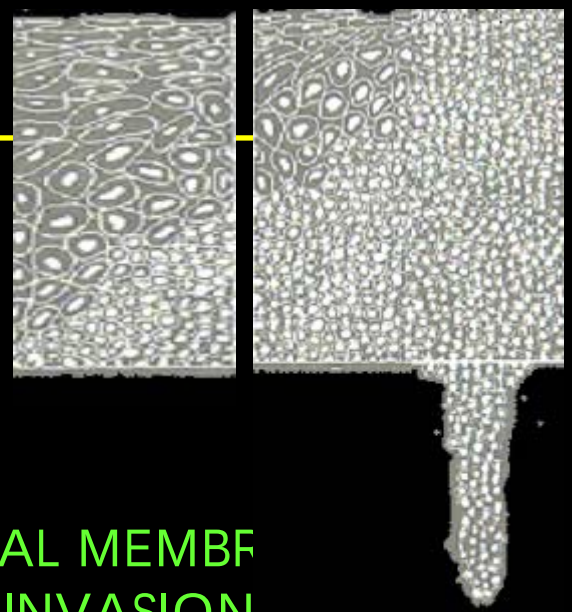
BASAL LAYER CELL INFECTION

uppressor genes

nactivation

Integration

Oncogenesis



BASAL MEMBF
INVASION

VIRAL FACTORS

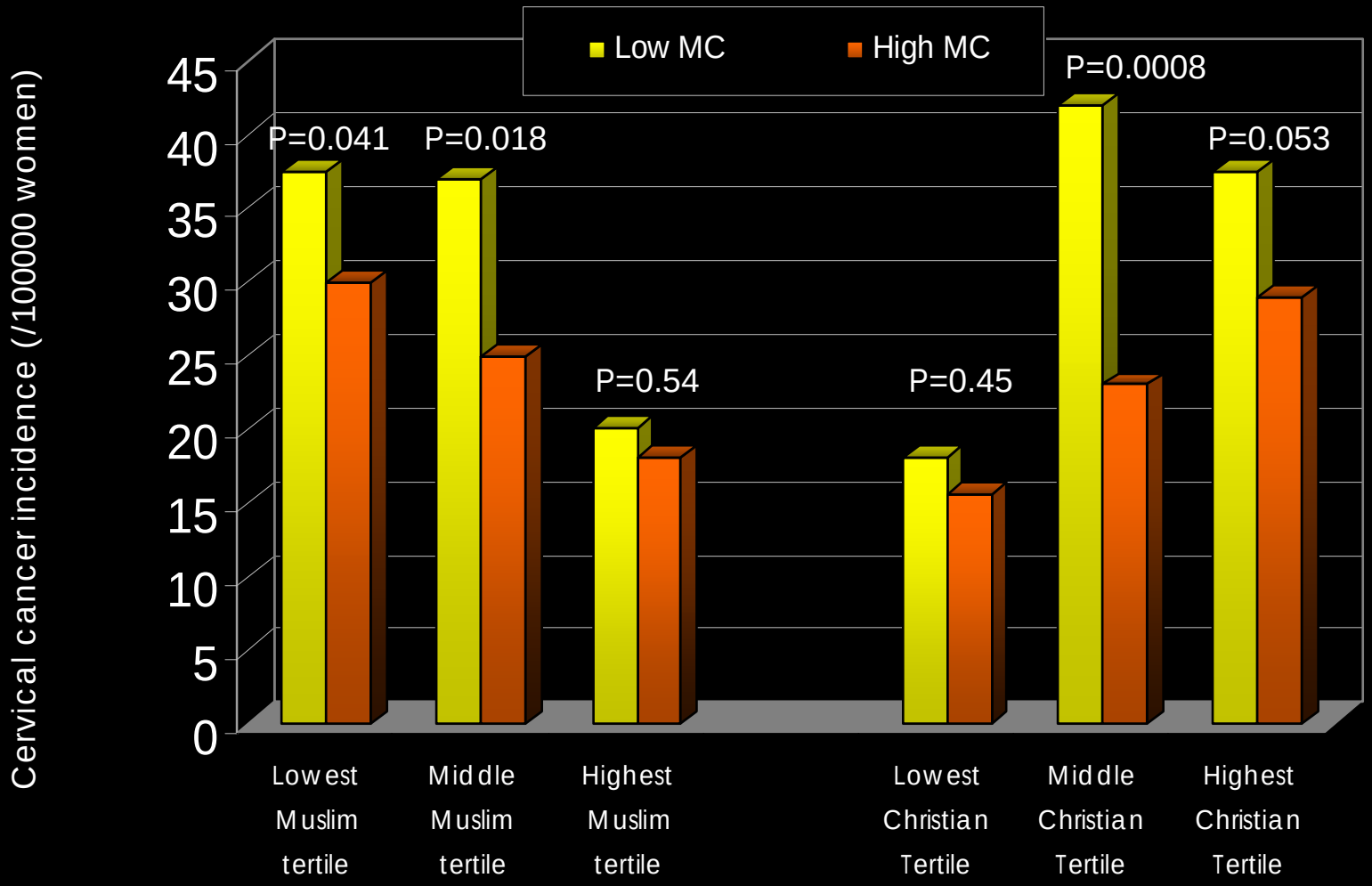
- VIRAL TYPE
  - ✓INTEGRATION CAPACITY
  - ✓ONCOGENES EXPRESSION
- VIRAL LOAD
  - ✓SINERGY
  - ✓ANTHAGONISM

PERSISTENCE

ENVIRONMENTAL FACTORS

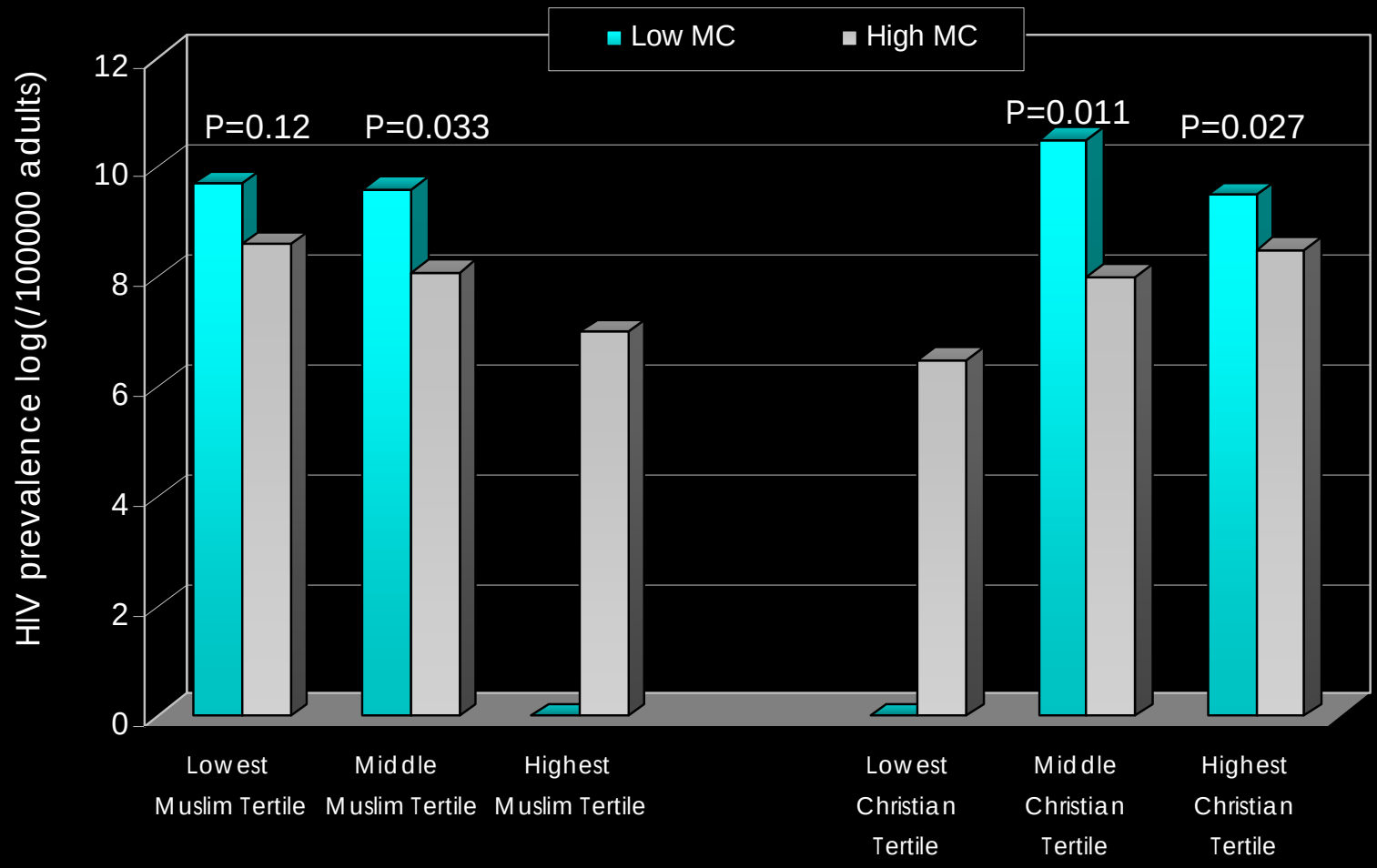
- ✓ CIGARETTE SOKING
- ✓ HORMONS
- ✓ SEXUAL BEHAVIOUR
- ✓ PARITY

MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES
an ecologic analysis od 121 DC



Drain PK et al, BMC inf. Dis nov 2006

MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES
an ecologic analysis od 121 DC



Drain PK et al, BMC inf. Dis nov 2006

HOST FACTORS

- IMMUNE RESPONCE
  - ✓ 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

Th 2

IL-4 IL-10

Naive B cell

Memory B

Plasma cell

CD4+

Recognize non self peptides
MHC II presented

Th 1

INF-γ

Cytotoxic effectors

macrophages

Natural killer

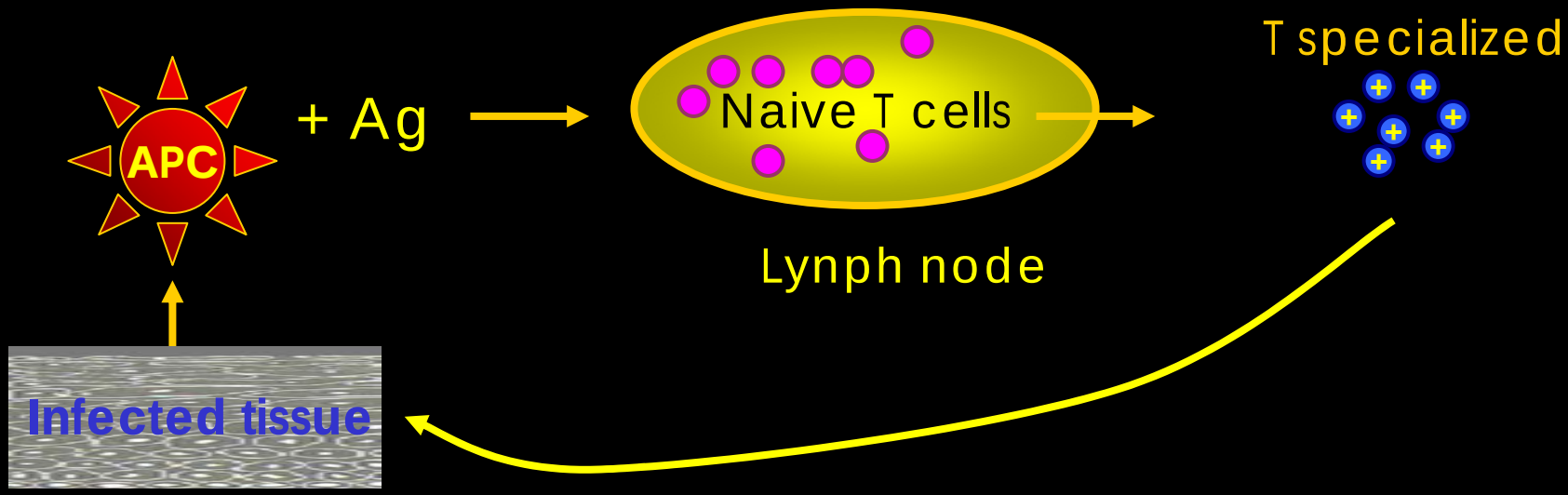
CD8 citox

CD8+

Recognize self peptides
MHC I presented

Cytotoxic response

Why the immune response is scarce and inefficacious



1. The cheratinocyte is programmed to die of 'natural death'
2. HPV as other DNA viruses interferes with IFN synthesis (natural antivirals, anti-proliferativee, anti-angiogenetic, 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%
HR+LR	31.4%
LR-HPV	9.4%
NO HPV	32.4

} 58.2%

PERSISTENT	39.0%
SPORADIC	35.5%
NEVER HPV	25.5%

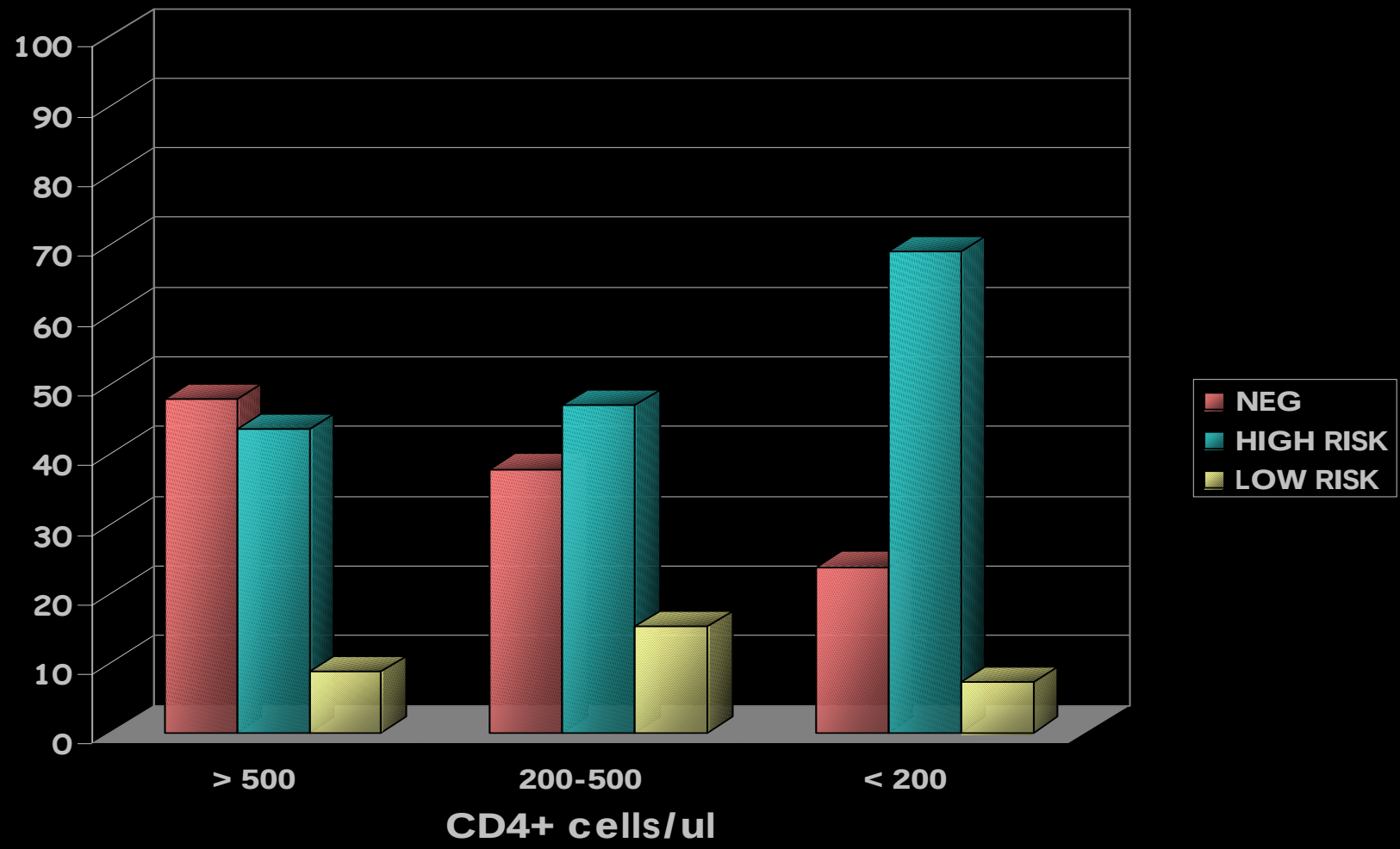
} MULTIPLE 85.7%

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

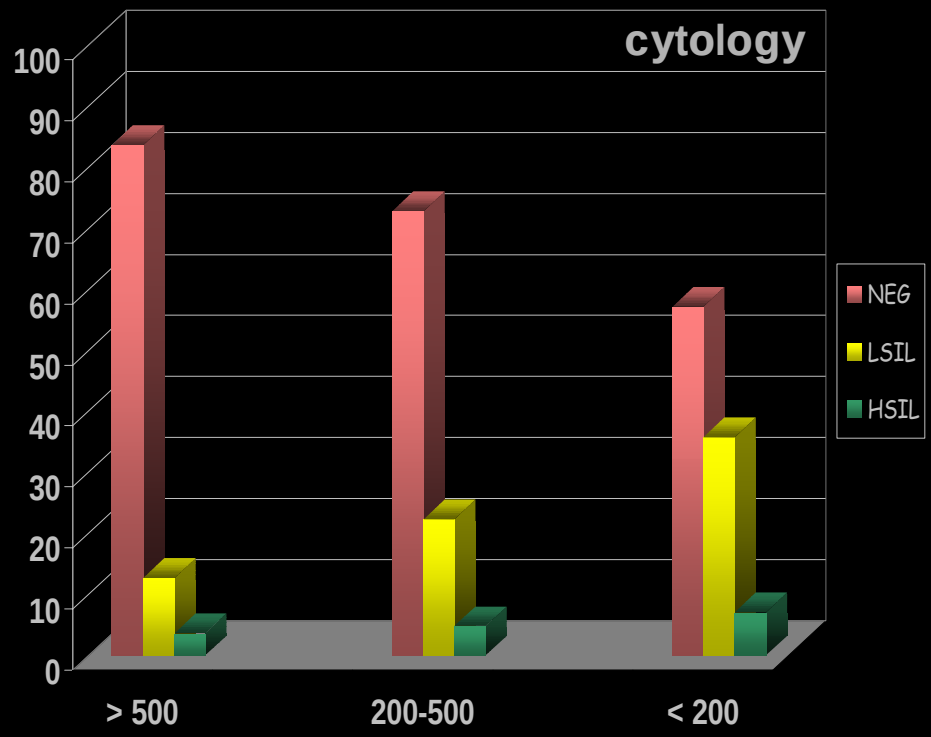
HGSIL PAP-HYSTO AGREEMENT K=0.874

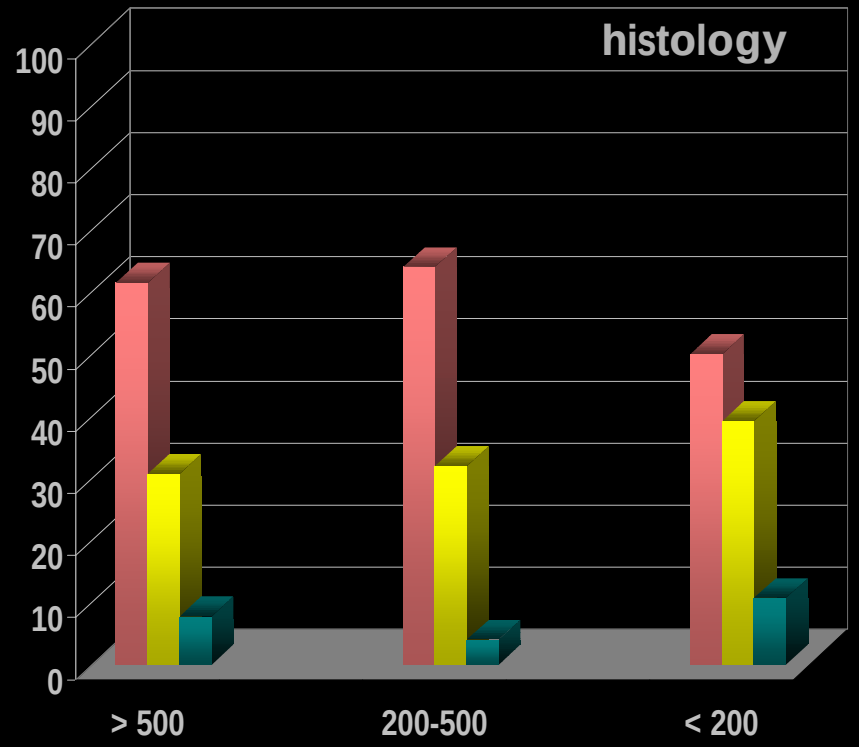
HPV TYPES PREVALENCE ACCORDING TO IMMUNE SUPPRESSION



Lillo et al JID 2001;184:547-51

CYTOLOGICAL AND HISTOLOGICAL DIAGNOSIS ACCORDING TO IMMUNE SUPPRESSION

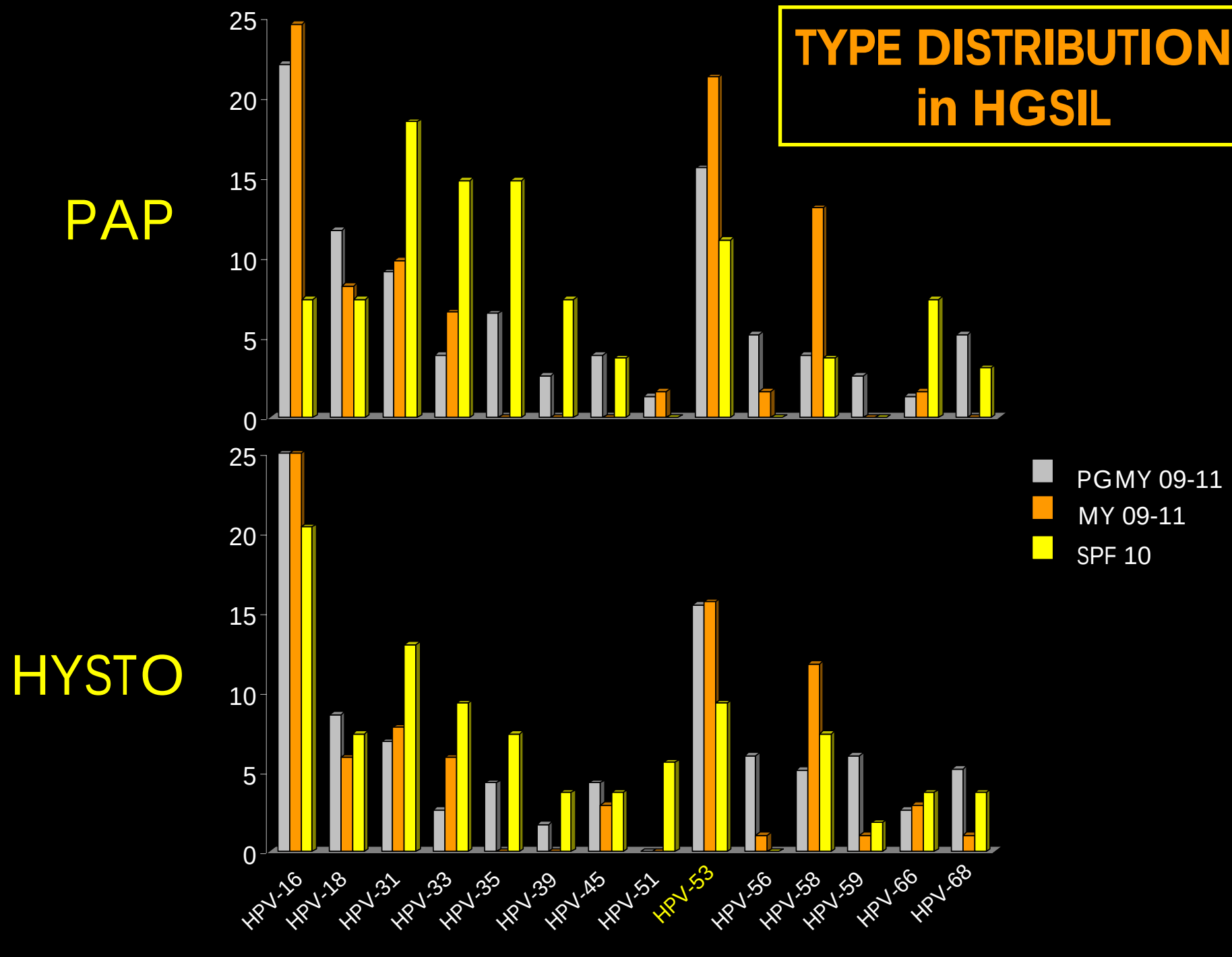




CD4+ cells /μl

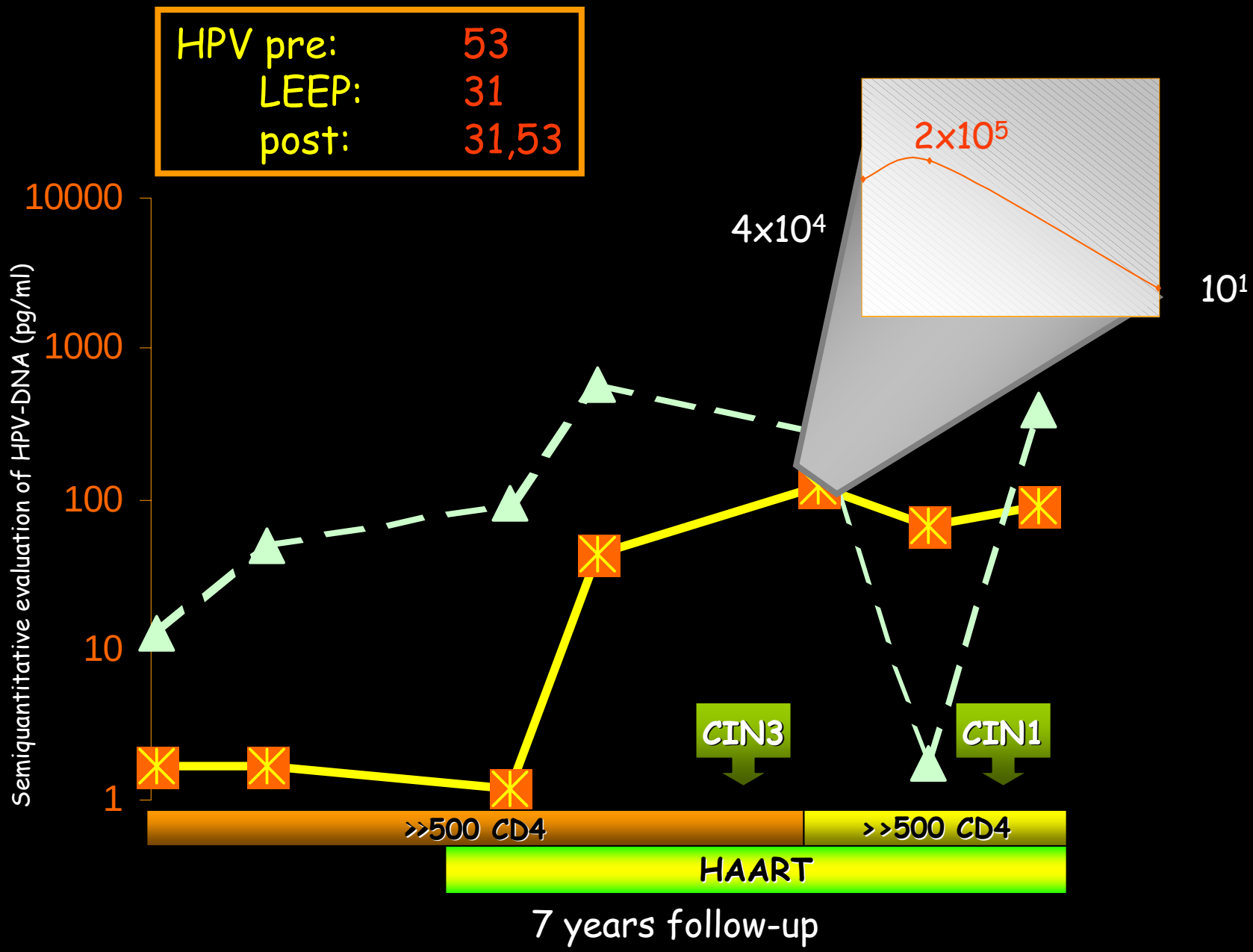
TYPING

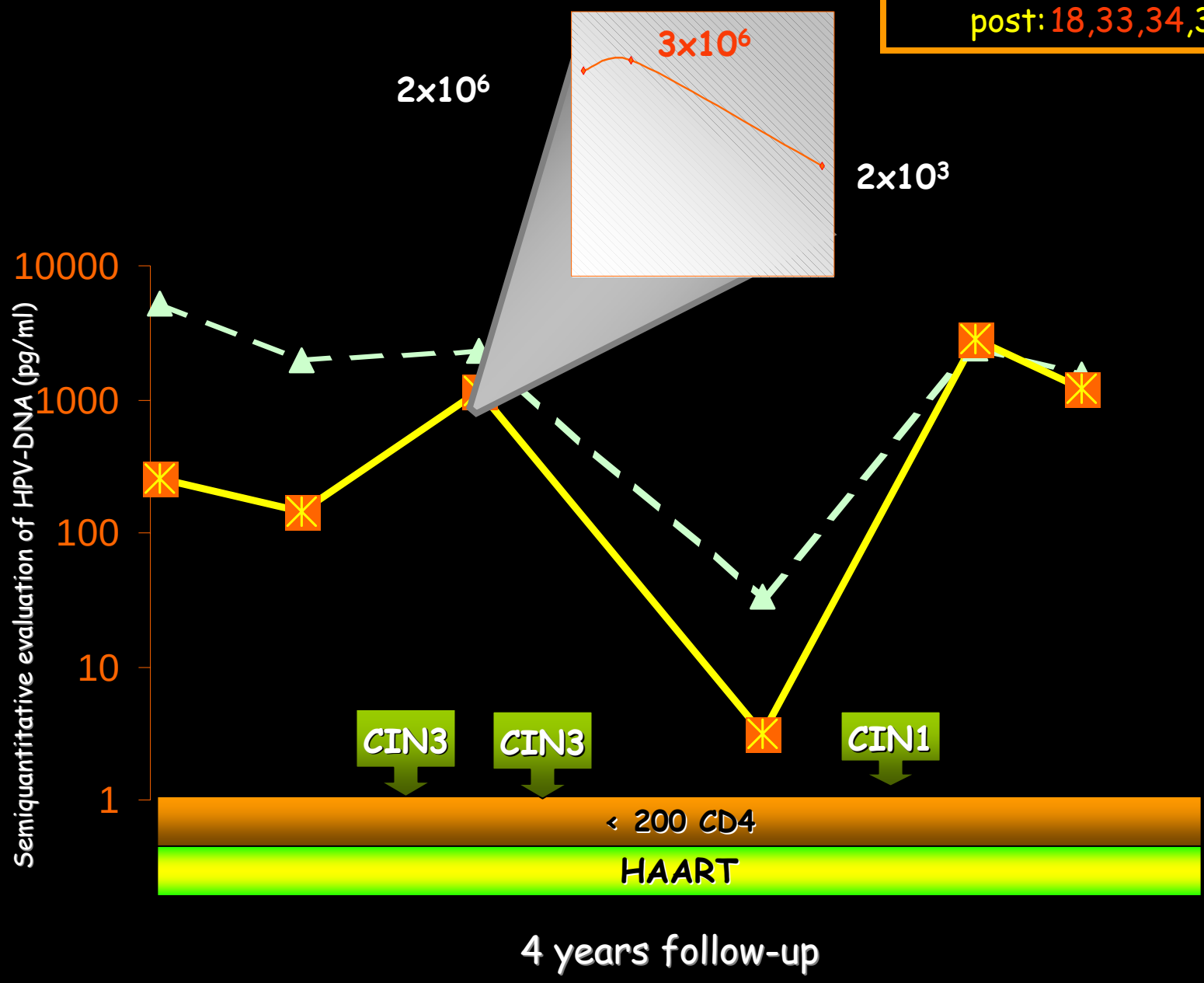
TYPE DISTRIBUTION in HGSIL

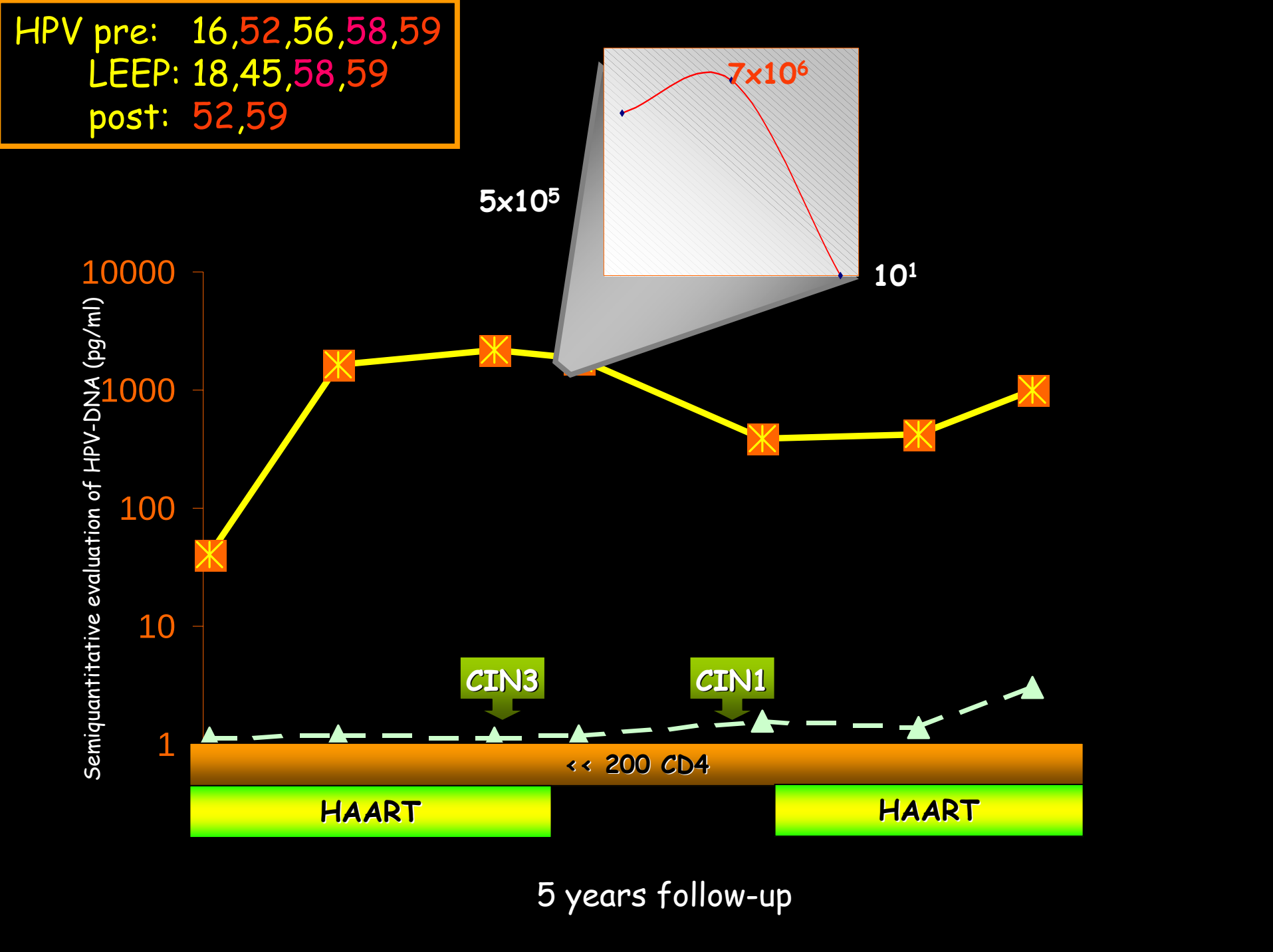


OVERALL AGREEMENT FOR MAJOR ONCOGENIC TYPES

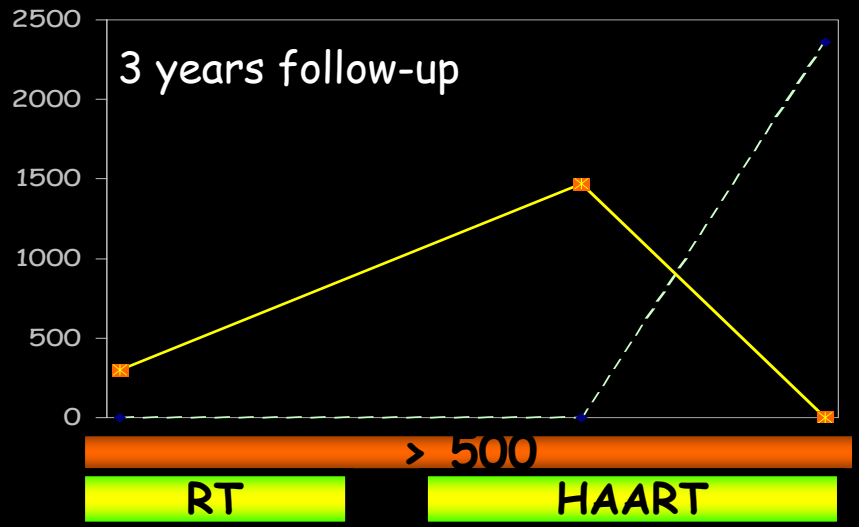
HYSTO / load	ROCHE-GENOMICA K (CI 95%)	ROCHE-LIPA K (CI 95%)	GENOMICA-LIPA K (CI 95%)
Any >150	**0.58** (0.46-0.70)	**0.75** (0.61-0.88)	**0.69** (0.54-0.85)
Any > 400	**0.59** (0.48-0.71)	**0.78** (0.65-0.91)	**0.68** (0.51-0.85)
HSIL >150	**0.70** (0.57-0.83)	**0.72** (0.56-0.88)	**0.71** (0.55-0.88)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

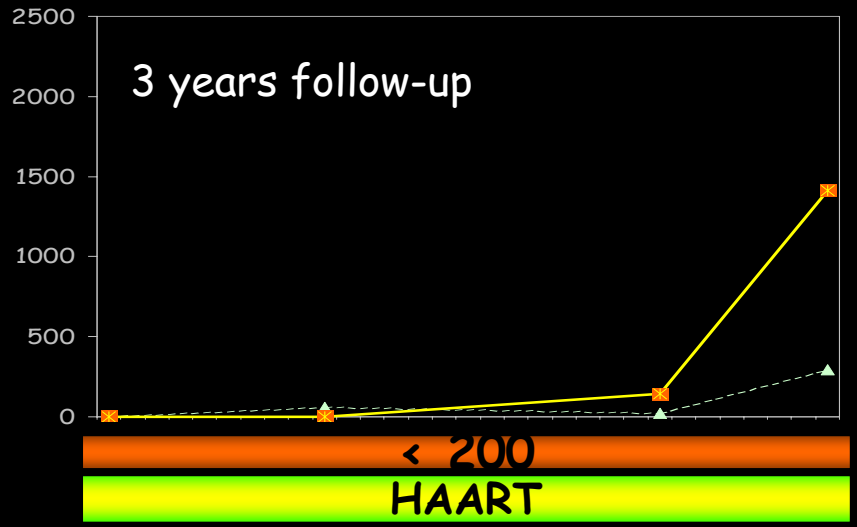

HPV pre: 53
LEEP: 31
post: 31,53
10000
1000
100
10
1
Semiquantitative evaluation of HPV-DNA (pg/ml)
2x10⁵
4x10⁴
10¹
CIN3
CIN1
>>500 CD4
>>500 CD4
HAART
7 years follow-up

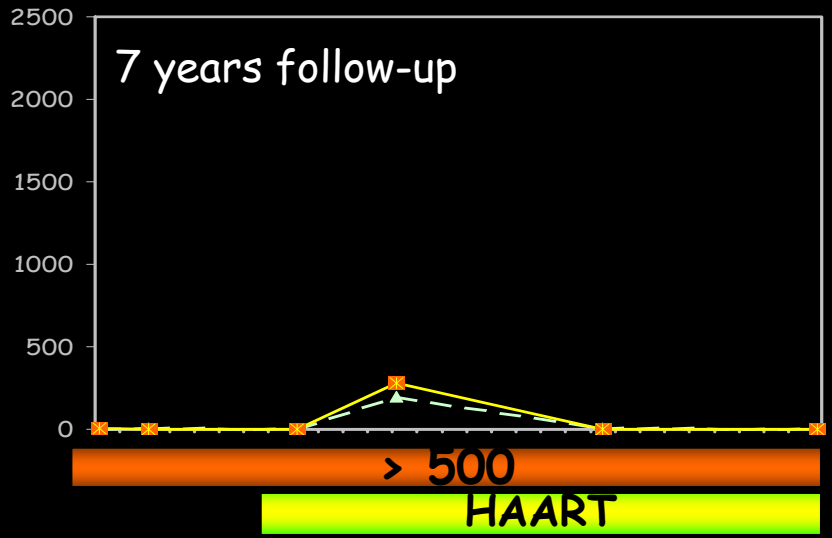

HPV pre: 18,33,34,39,44,45,52,66
LEEP:18,33,34,56
post:18,33,34,39,44,52,66
3x10⁶
2x10⁶
2x10³
10000
1000
100
10
1
Semiquantitative evaluation of HPV-DNA (pg/ml)
CIN3
CIN3
CIN1
< 200 CD4
HAART
4 years follow-up

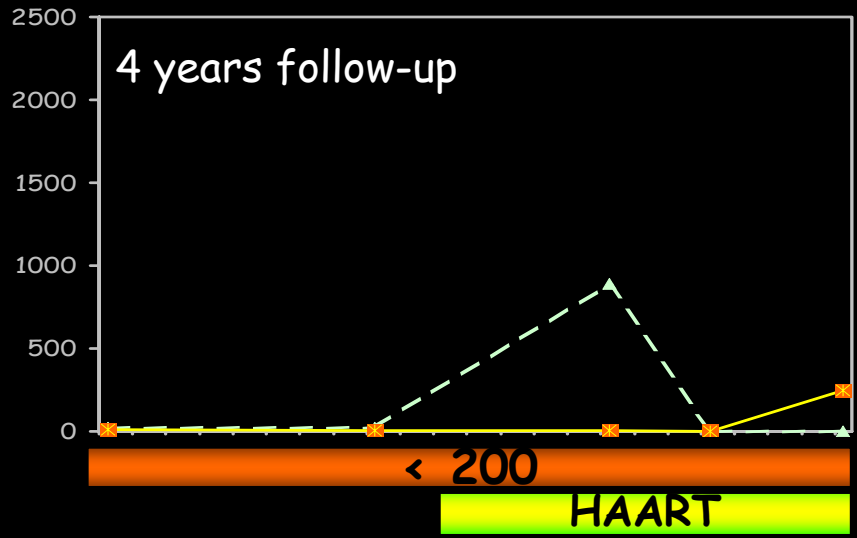

HPV pre: 16,52,56,58,59
LEEP: 18,45,58,59
post: 52,59
7x10⁶
5x10⁵
10¹
10000
1000
100
10
1
Semiquantitative evaluation of HPV-DNA (pg/ml)
CIN3
CIN1
<< 200 CD4
HAART
HAART
5 years follow-up

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







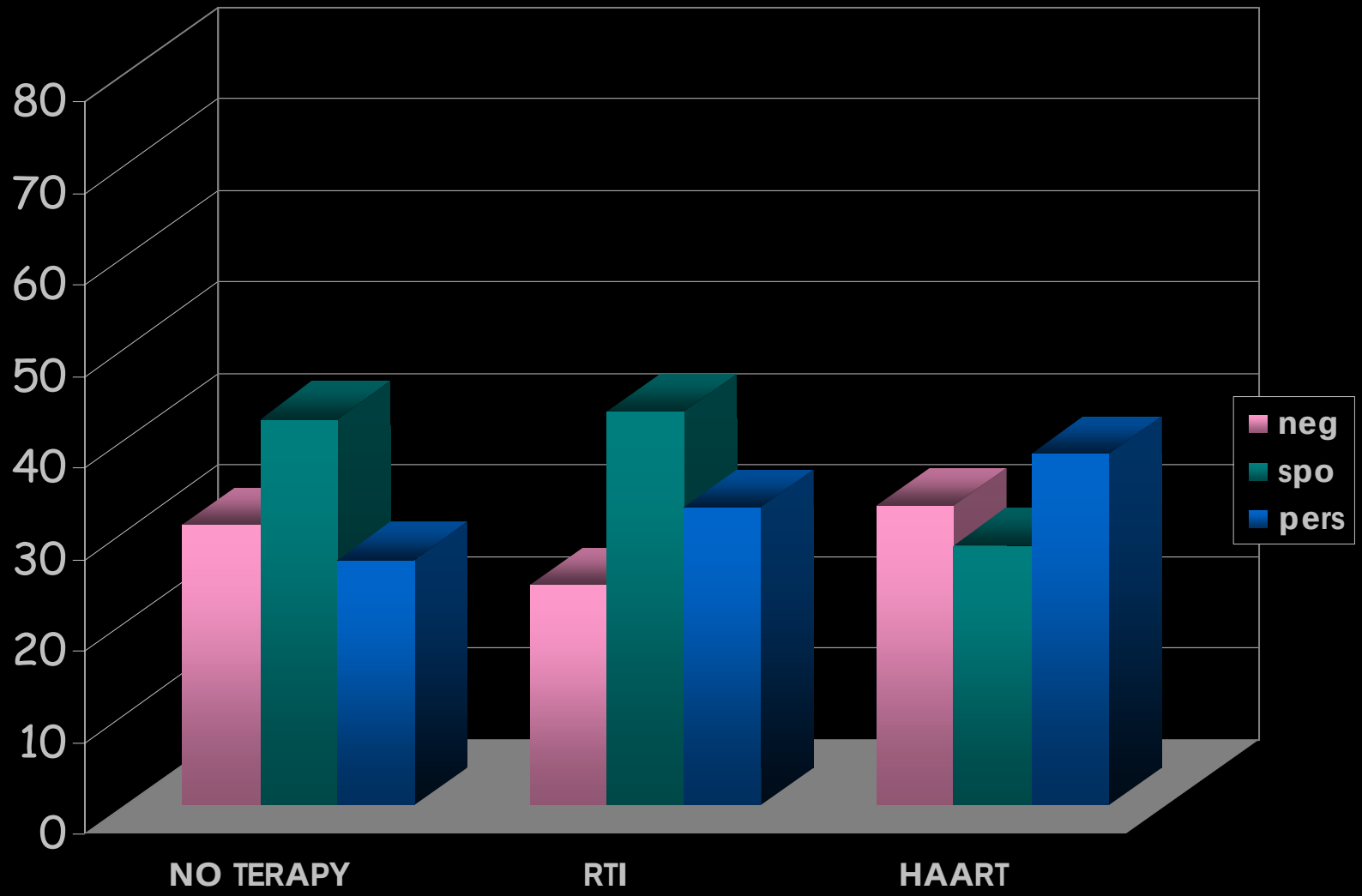


total DNA load and cervical lesions

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

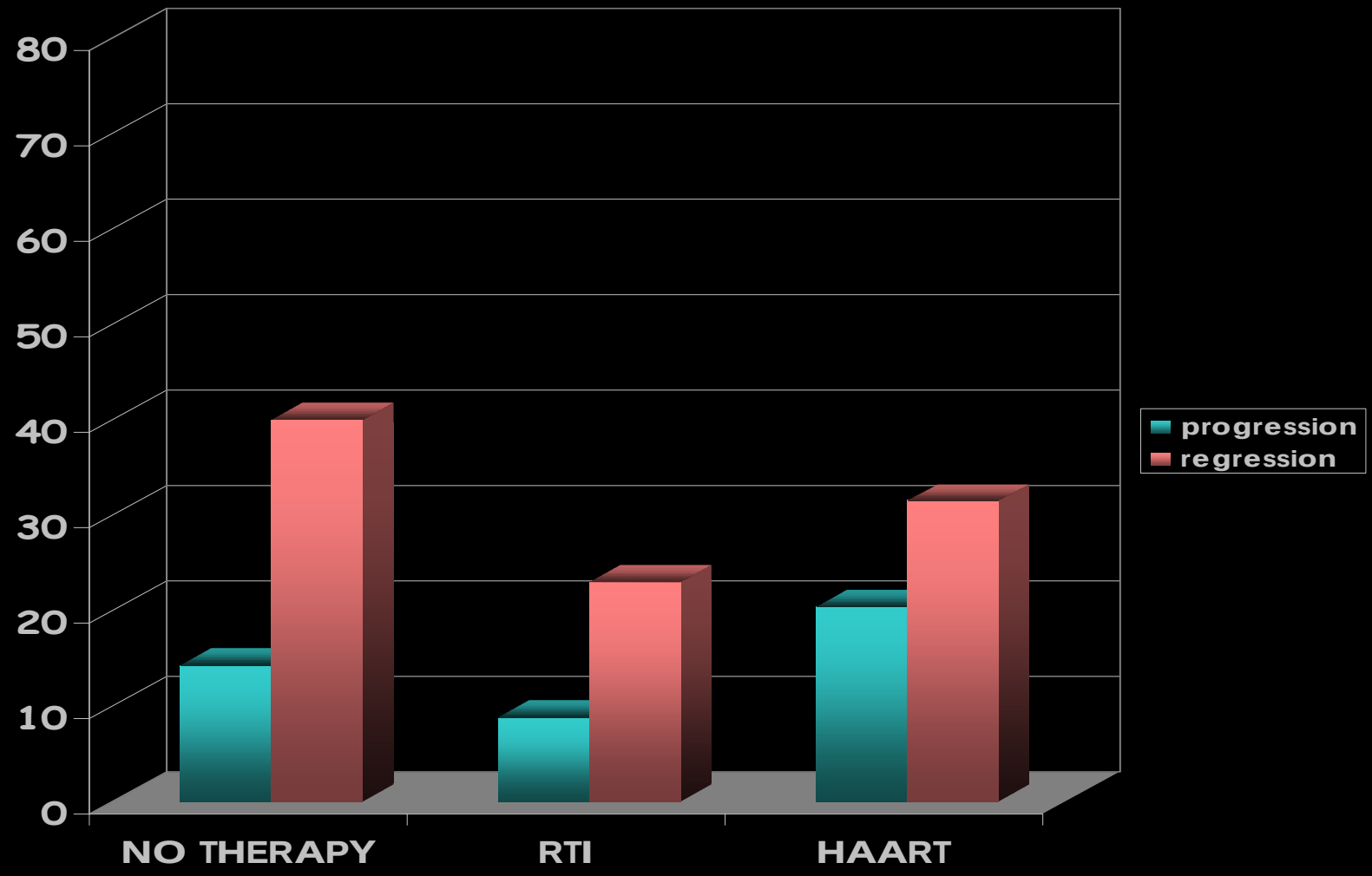
EFFECT OF ARV THERAPY

EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON HPV PERSISTANCE



Lillo et al JID 2001;184:547-51

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



Lillo et al JID 2001;184:547-51

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:

Objectiv

The maj
preventio
1. morbid
2. mortali
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

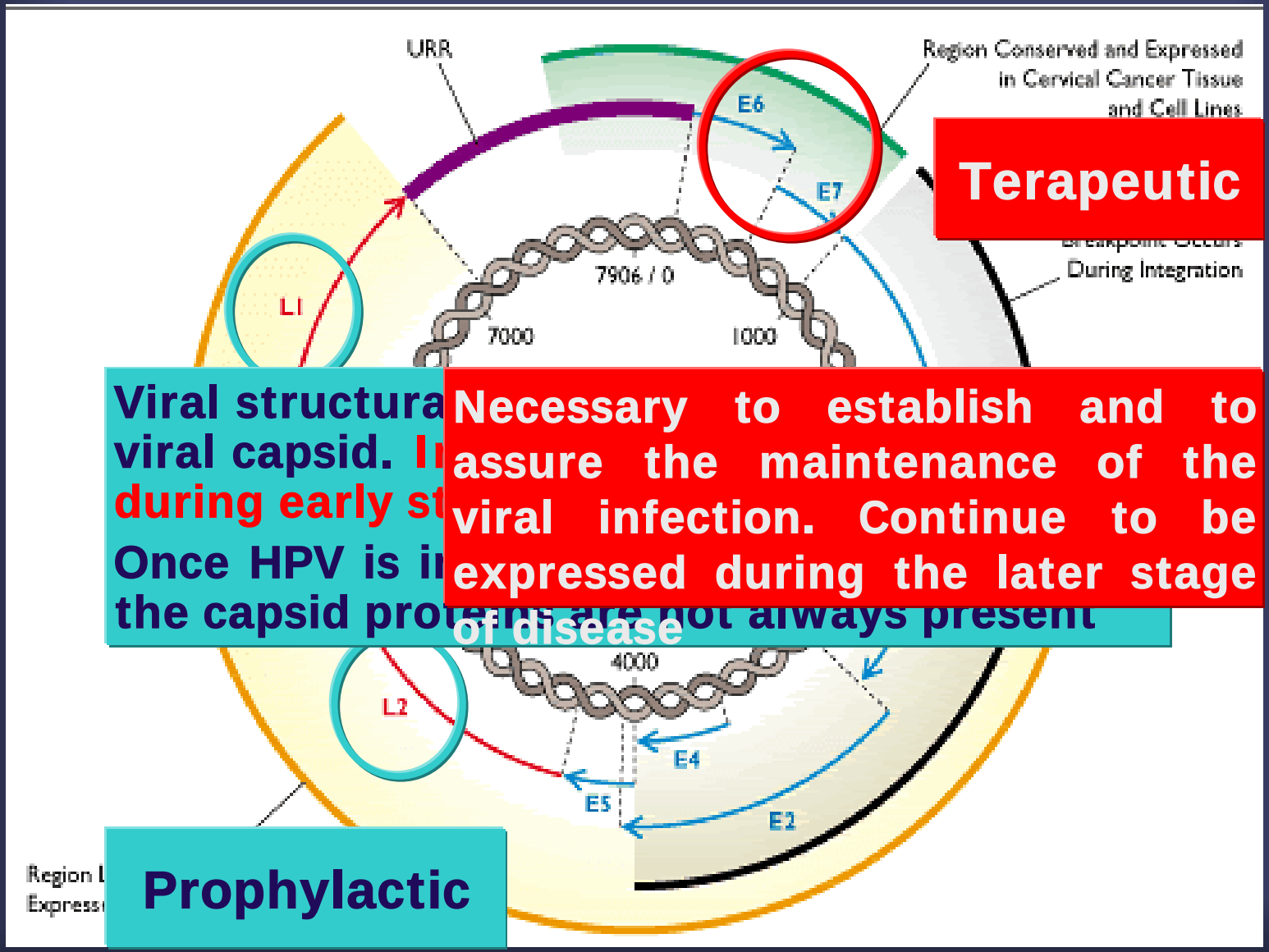
} superficial epith. layer where virus antigens are not easily detected

- Keratinocytes are not lysed
- No inflammatory response

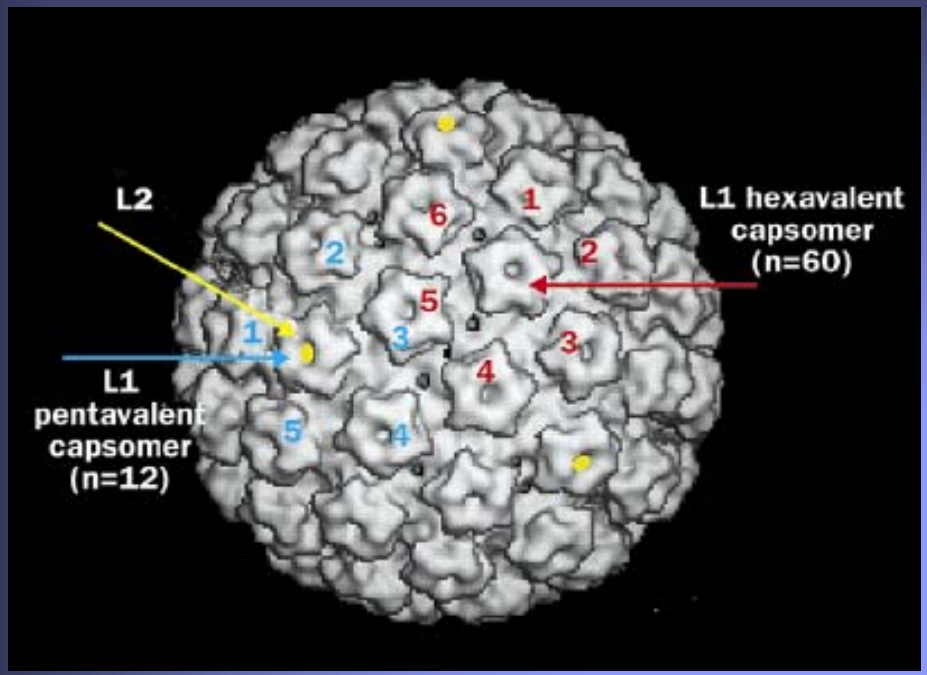
} there is no a “danger signal” (lack of valid CTLs response)

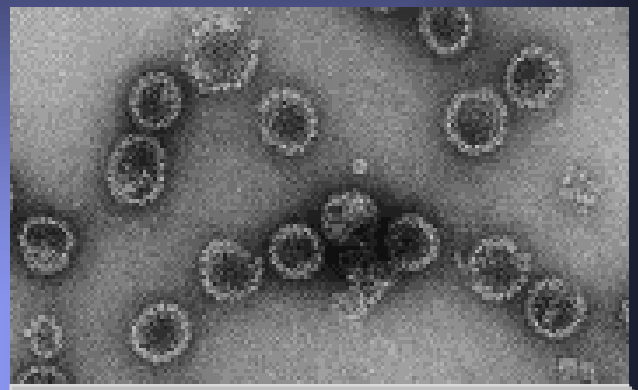
- Viral early proteins inhibit expression of MHC class I
  - ✓ E7 (HPV16,18) inhibits the induction of IFN
  - ✓ E5 inactivate the antigen processing system

Prophylactic vaccine: *Rationale*



Prophylactic vaccine: *Rationale*





HPV16 L1 VLPs
(*J.Schiller, 2000*)

Virus-like particles (VLPs)

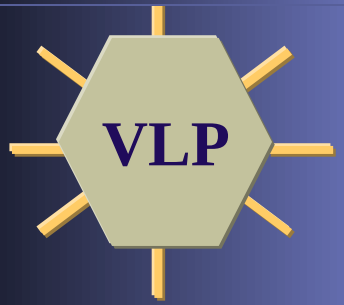
Are neither infectious, no

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.**

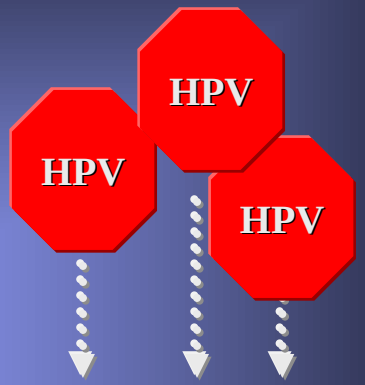
Prophylactic vaccine: *Rationale*

parenteral route

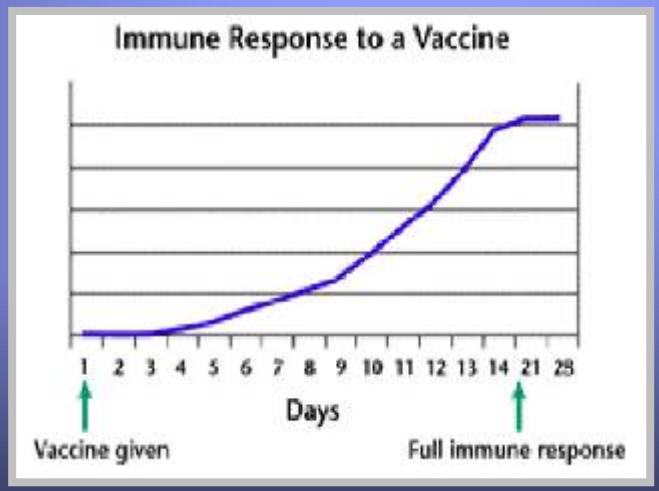


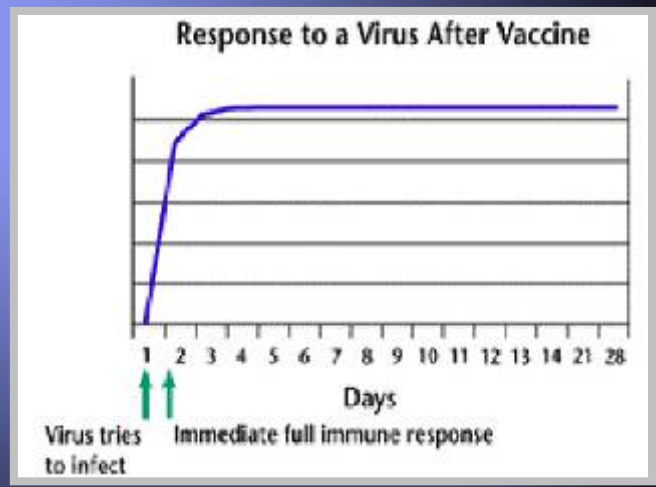
Preteens - Teenagers

Immune Response
(humoral + (?) cellular)



Host protection





Prophylactic vaccine: *Rationale*

Ideal HPV-vaccine

1	**Multivalent**	**oncogenic strains** **non-oncogenic strains**
2	1. Interruption of the most commune STD. 2. More attractive also for men.	**Developed area *vs* Underdeveloped area**
3		
4	**Easily acceptable**	
5	**Safe**	**1. Animal model** **2. Human clinical trials**
6	**Immunogenic**	

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)
- N
- I
- M
- Biological/Immunological
- Lenght and type of follow-up
  (ability to follow-up women over a long period)
- Projected population benefit

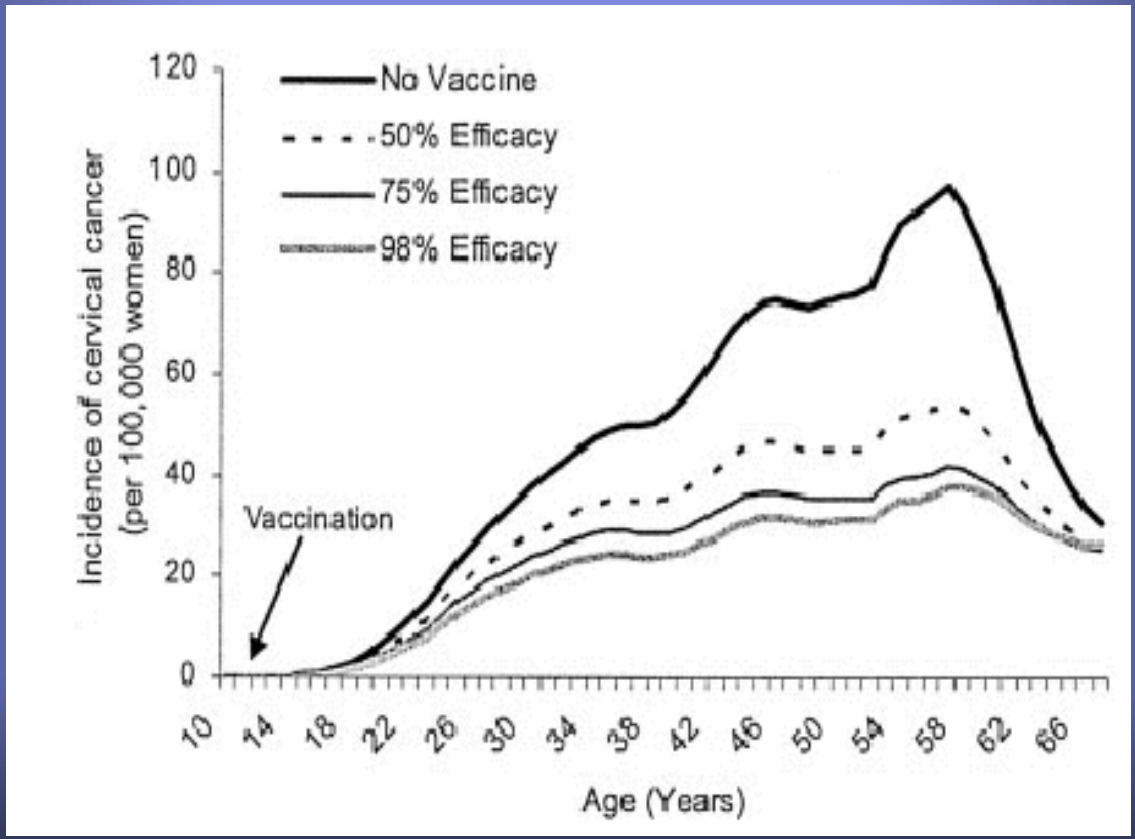
- **organized mass scre**
- **population based car**

(J.Pa

High-risk areas in developing countries (→HBV vaccination in Gambia or Taiwan)
(M.*Plummer & S.Franceschi, 2002)*

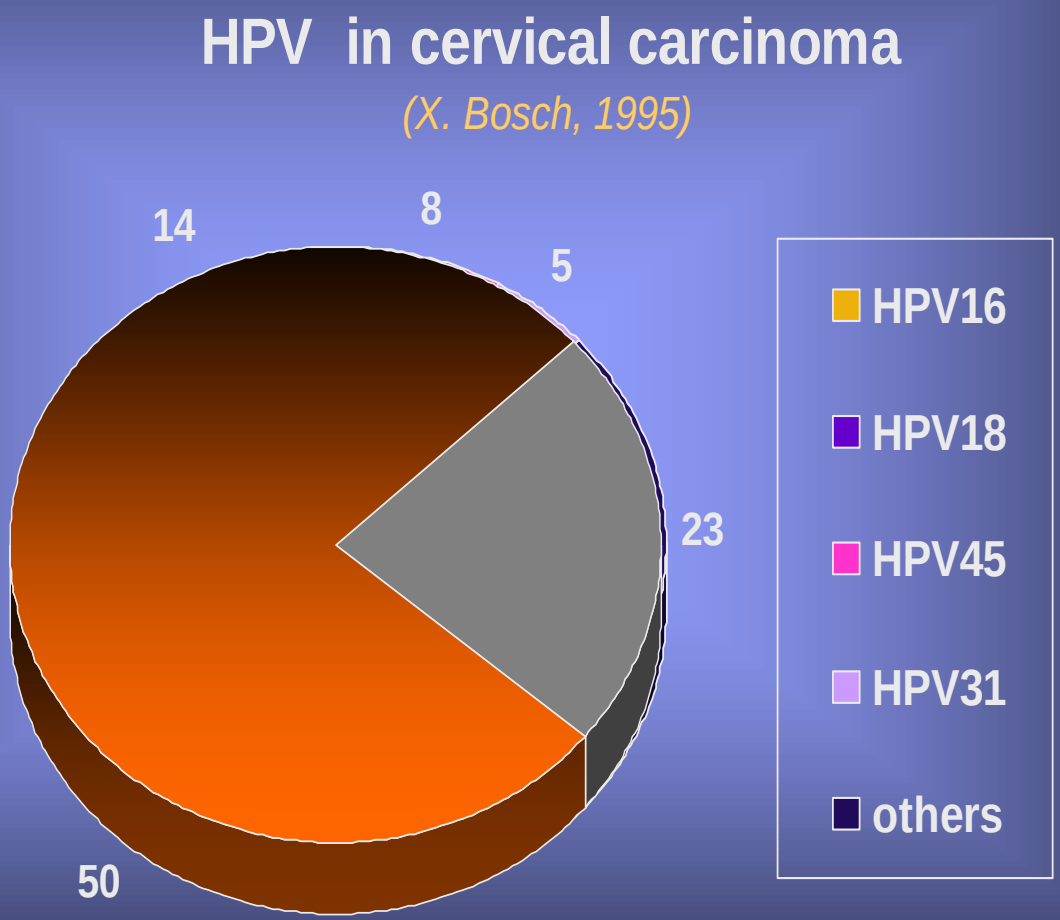
Prophylactic vaccine: *Rationale*

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

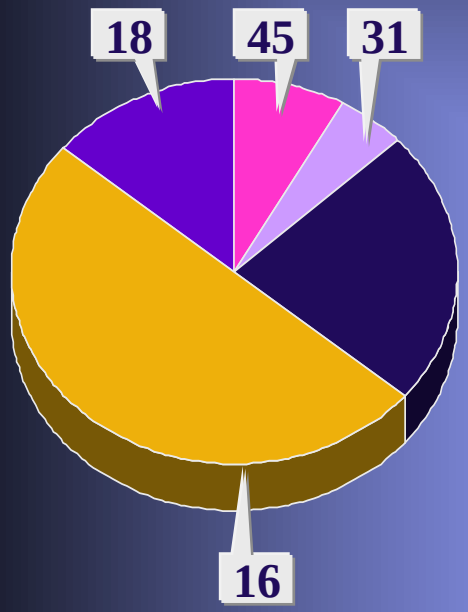


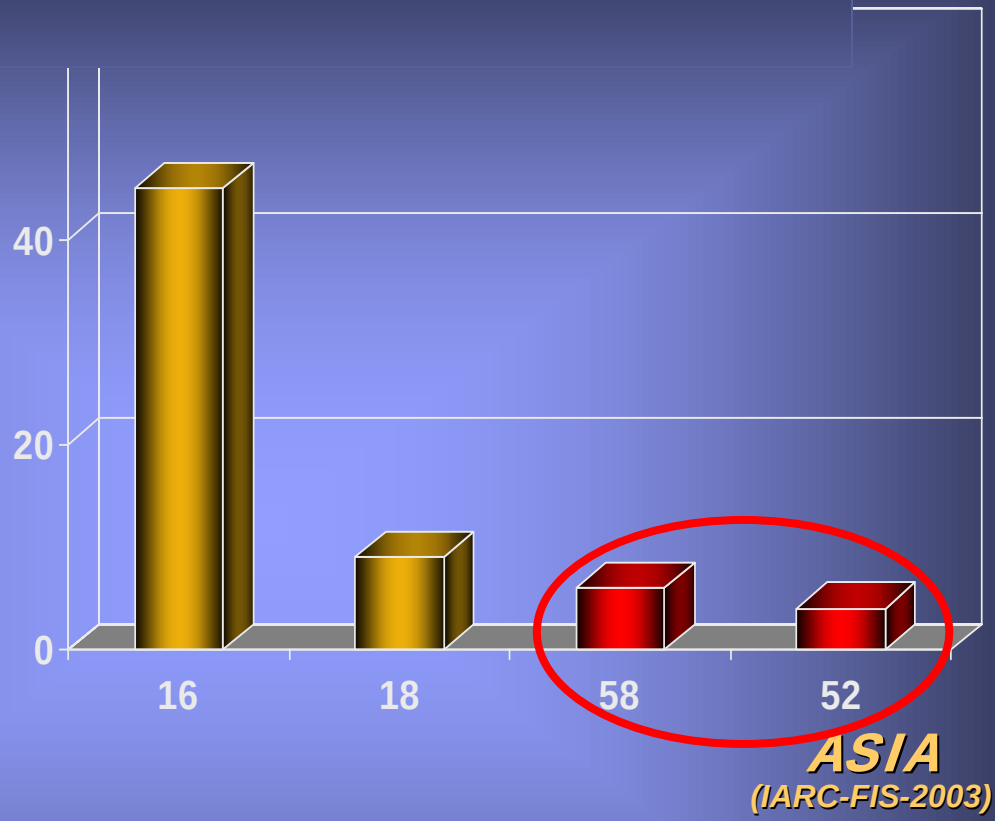
S. GOLDIE, 2003

Prophylactic vaccine: *Rationale*



Prophylactic vaccine: *open questions*





" 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

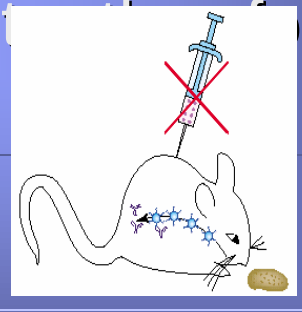
1. *Life-style*
2. *Sexual behaviour*

Prophylactic vaccine: *open questions*

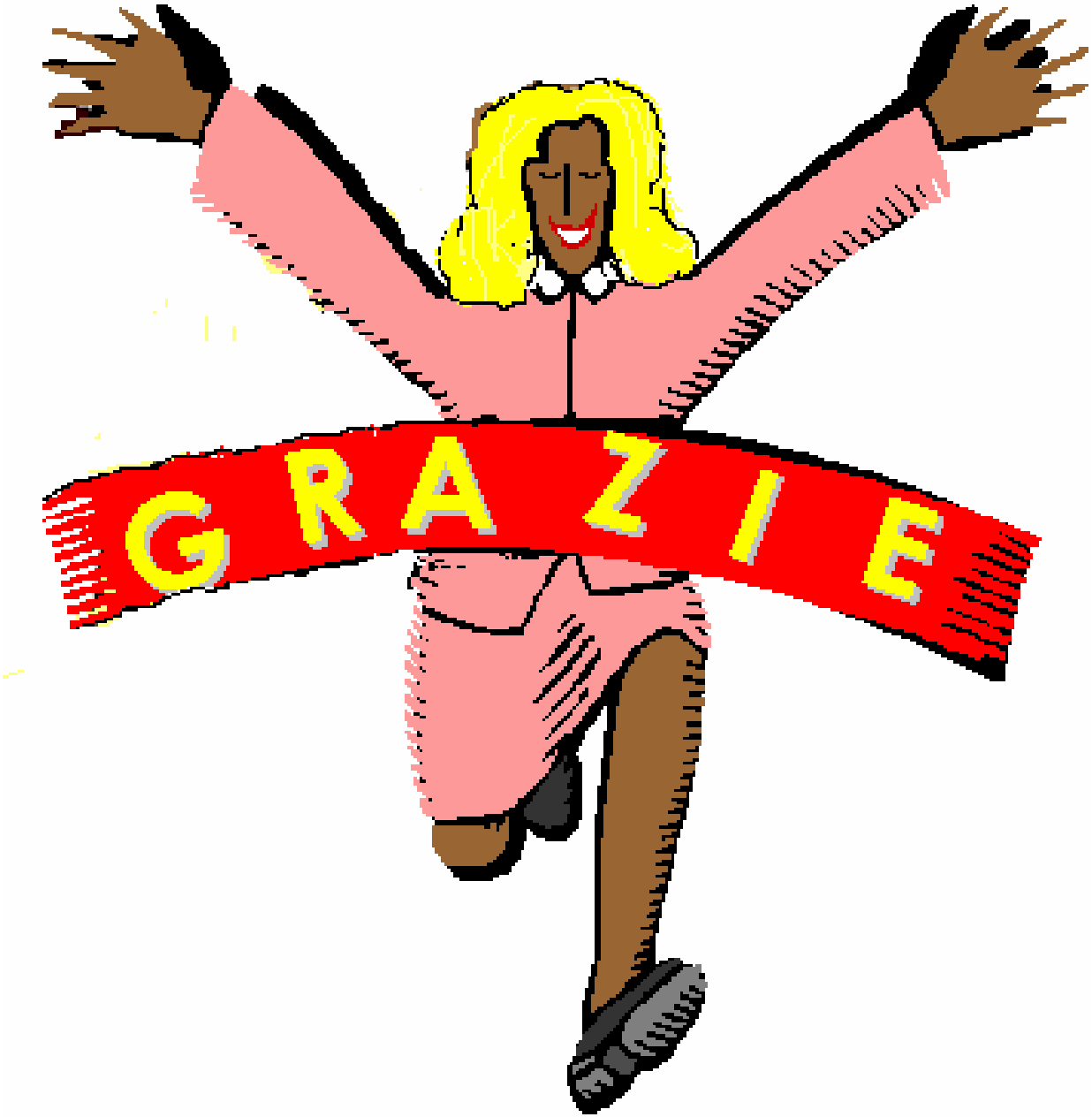
Developing countries

- Local production
- Easy and cheap distribution
- Vaccine could be stable, easy storing
- Long-lasting immunity with a single dose
- Oral/mucosal vaccine bett[illegible]r

EDIBLE VACCINES



Does malnutrition impact on immune response to HPV-vaccine ?


GRAZIE