Human Papillomaviruses in Cervical Cancer – History and Perspectives

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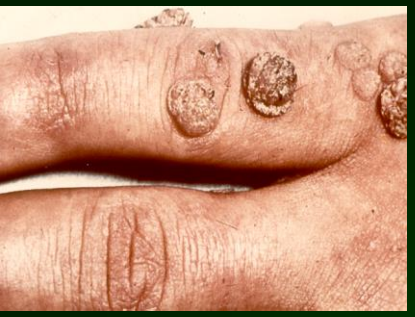
In 1842 Rigoni-Stern in Italy noted that cervical cancer

was linked to sexual contacts

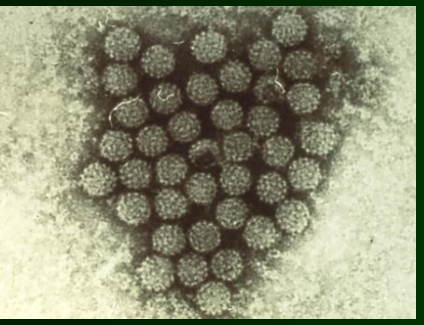
Between 1920 and 1960 a number of studies analyzed a possible role of sexually transmitted agents (syphilis, gonorrhea, trichomoniasis, lamblia infections) in the induction of cancer of the cervix – without conclusive results.

Starting in 1967/68 a first virus was incriminated as the causative agent, Herpes simplex type 2. This was mainly based on seroepidemiological studies. Up to 1982 a larger number of reports appeared confirming this association

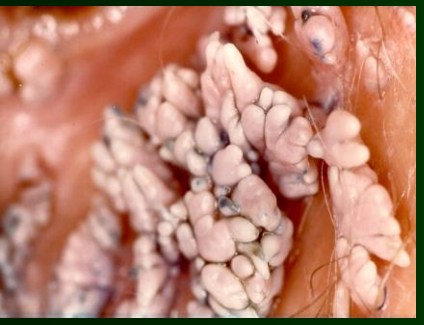
Based on our negative attempts to find Herpes simplex type 2 DNA in cervical cancer cells, I initiated in 1972 a program on a possible link between Papillomavirus infections and cervical cancer



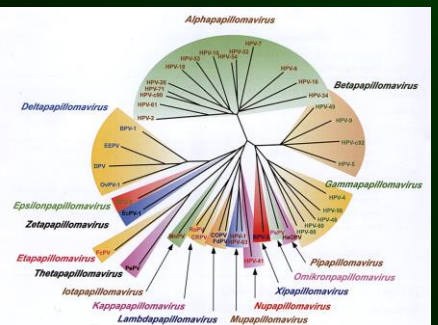
The **infectious nature of warts** was first recorded after autoinoculation of of cell-free extracts of warts in **1907 by Ciuffo** in Italy.



Papillomavirus particles had been detected in human warts by **electron microscopy in 1949 by Strauss** and colleagues in Houston, USA.

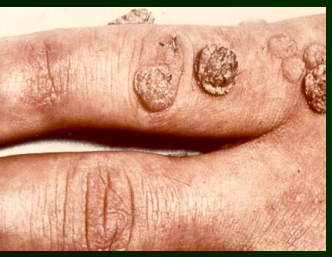


Up to 1975 it has been suspected that the various morphologies of **human papillomas** (e.g. verrucous warts, plantar warts, flat warts, genital warts) **were caused by the same agent**, depending on the type of infected cells.



Between 1975 and 1977 our group as well as the group of Gérard Orth in Paris demonstrated **the plurality of the human Papillomavirus family.**

Between 1974 and 1977 we published the hypothesis as well as some experimental data on a putative role of human papillomaviruses (HPV) in cancer of the cervix



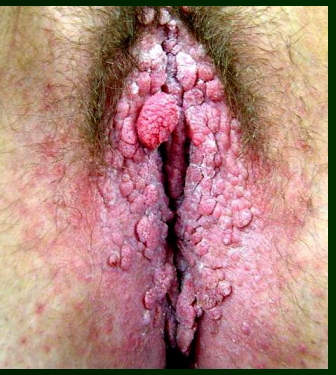
H. zur Hausen et al. Int. J. Cancer 13, 650-656, 1974

H. zur Hausen et al. Bibliotheca Haematologica, 43, 569-571, 1975

H. zur Hausen, Cancer Res. 36, 530, 1976

H. zur Hausen, Current Topics in Microbiol. Immunol. 78, 1-30, 1977

Between 1980 and 1982 we published cloning and characterization of HPV 6 and 11 from genital warts and laryngeal papillomas

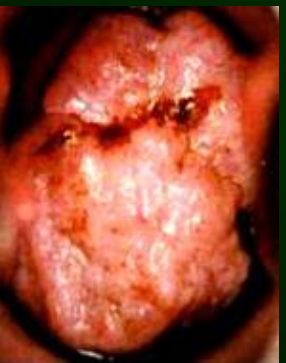


L. Gissmann and H. zur Hausen, Int. J. Cancer 25, 605-609, 1980

E.-M. de Villiers et al. J. Virol. 40, 932-935, 1981

L. Gissmann et al. J. Virol. 44, 393-400, 1982

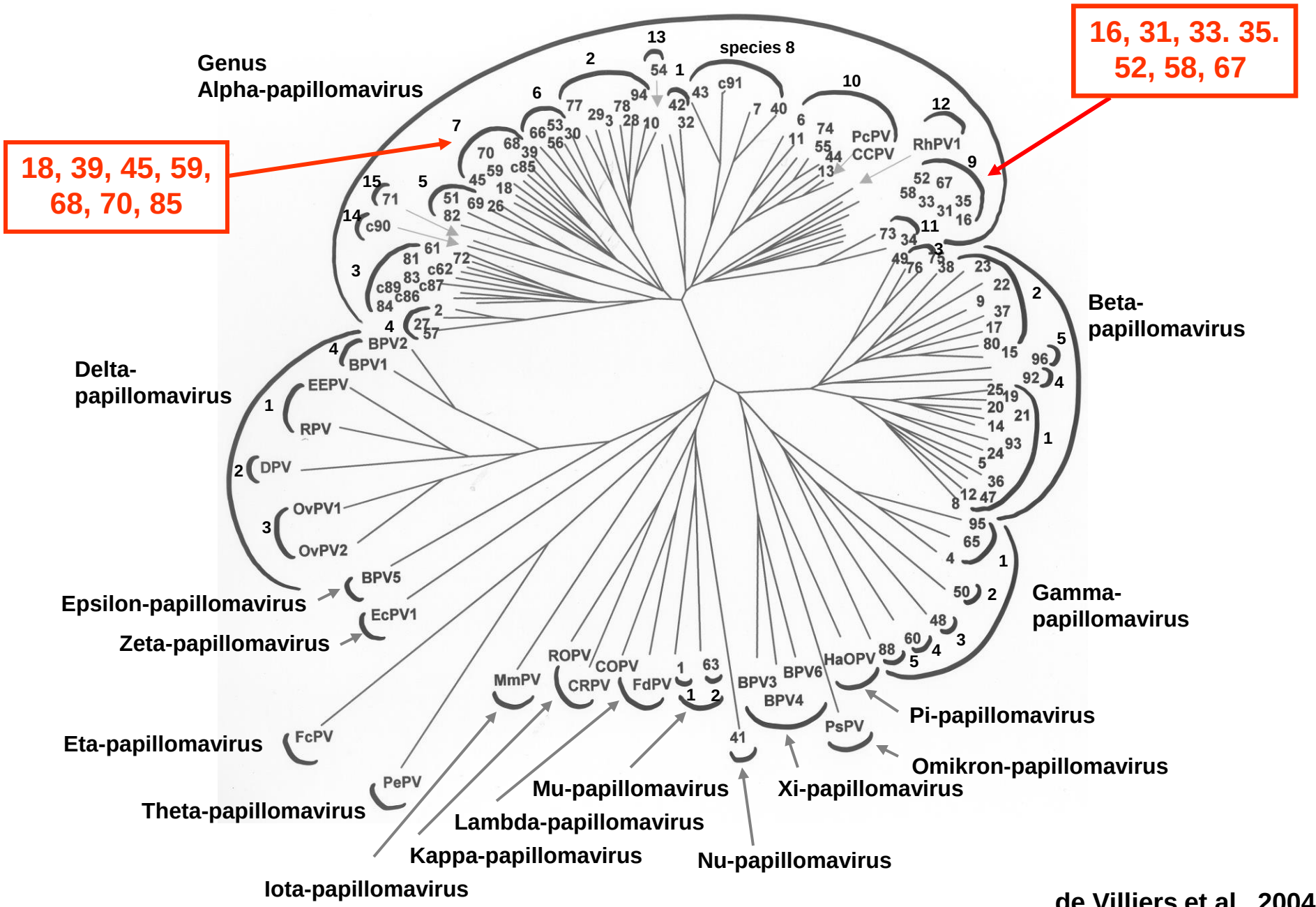
In 1983 and 1984 we cloned and characterized HPV 16 and 18 from cervical cancer biopsies, genital Bowen's disease and cervical carcinoma cell lines



M. Dürst et al. Proc. Nat. Acad. Sci. U.S. 80, 3812-3815, 1983.

H. Ikenberg et al. Int. J. Cancer 32, 563-564, 1983

M. Boshart et al. EMBO J. 3, 1151-1157, 1984.

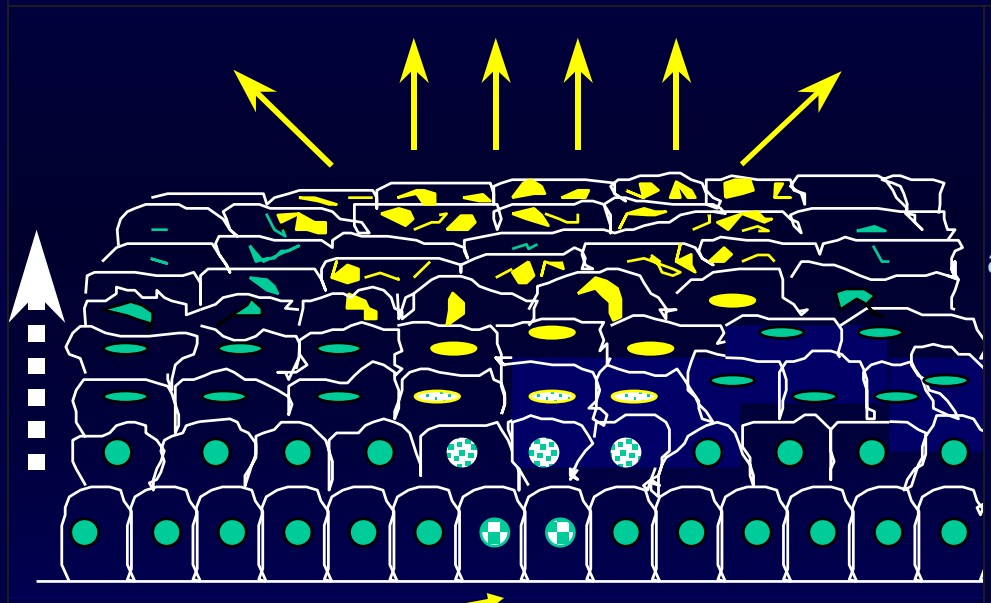

Genus
Alpha-papillomavirus
18, 39, 45, 59,
68, 70, 85
16, 31, 33. 35.
52, 58, 67
species 8
PcPV
CCPV
RhPV1
Beta-
papillomavirus
Delta-
papillomavirus
BPV2
BPV1
EEPV
RPV
DPV
OvPV1
OvPV2
Epsilon-papillomavirus
BPV5
EcPV1
Zeta-papillomavirus
Gamma-
papillomavirus
ROPV
COPV
CRPV
MmPV
FdPV
HaOPV
BPV3
BPV6
BPV4
PsPV
Pi-papillomavirus
Omikron-papillomavirus
Eta-papillomavirus
FcPV
PePV
Theta-papillomavirus
Mu-papillomavirus
Lambda-papillomavirus
Kappa-papillomavirus
Iota-papillomavirus
Xi-papillomavirus
Nu-papillomavirus
de Villiers et al., 2004

Characteristics of Papillomavirus Infections:

- Extreme adaptation to epithelial surface cells;
- Infection of proliferating cells at the basal layer results in more or less silent genome persistence;
- Viral gene codons are „de-optimized“ for human tRNA recognition;
- Promoters are only activated in terminally differentiated cells.

Viral genes are not readily read in proliferating cells

Virus particle release

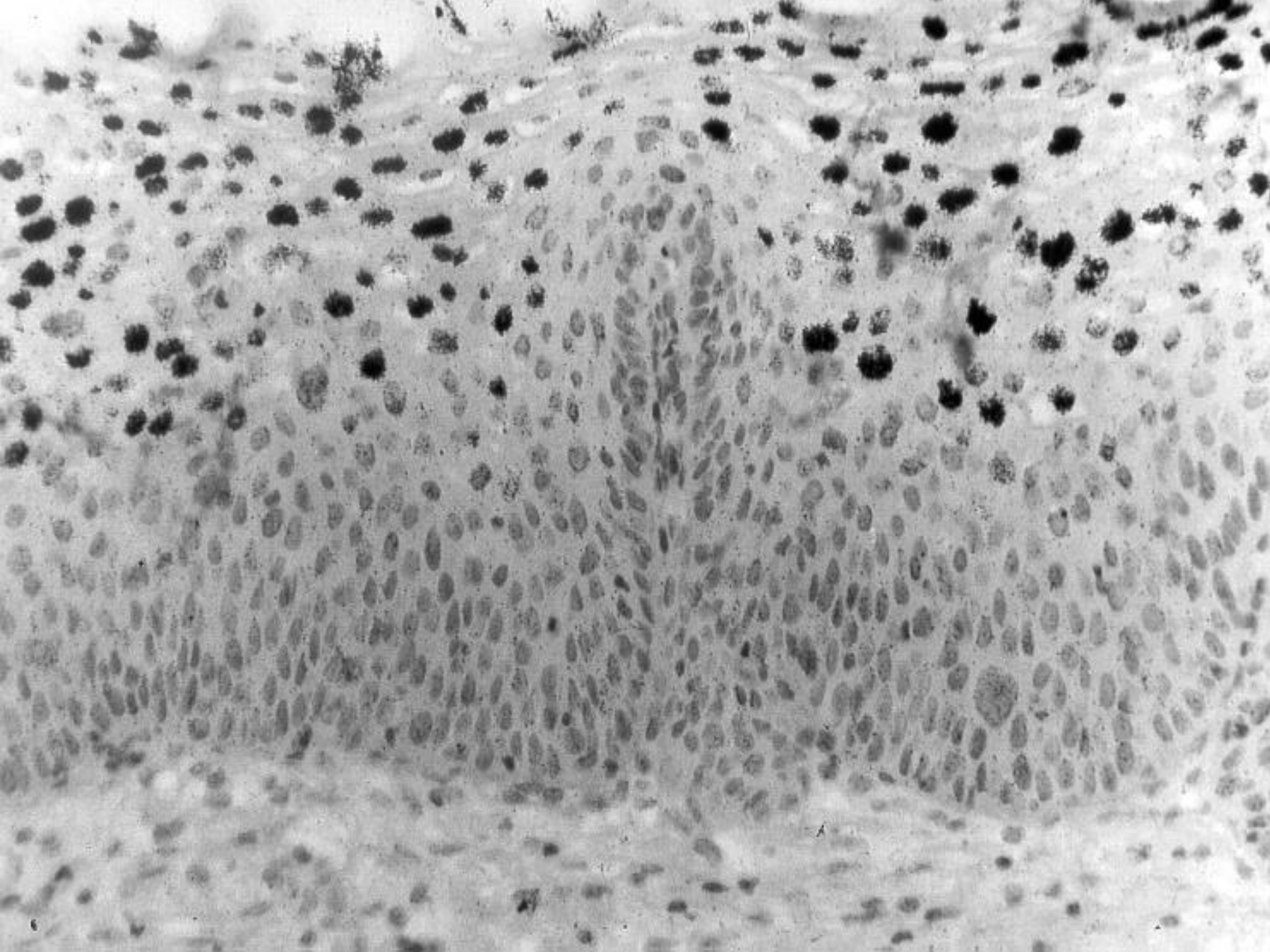


Direction of differentiation

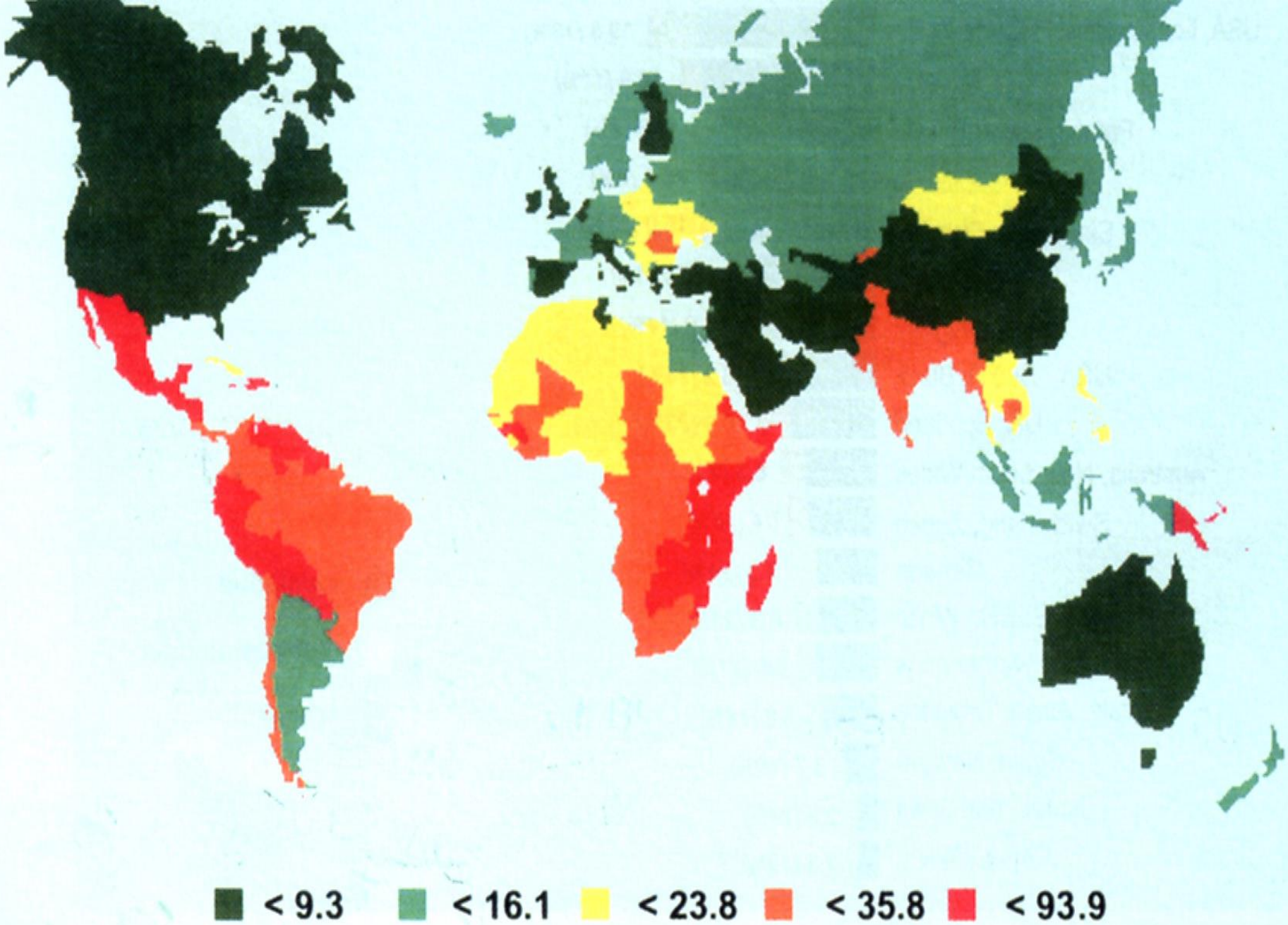
Viral gene activation and viral particle synthesis occurs in the terminally differentiated cell layers

Here most or all viral genes are silenced

Infection requires availability of proliferating cells
(in most instances microlesion)



Type of cancer	Papillomvirus types involved	Percent HPV-positive
Cervical cancer	**16**, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 (26, 68, 73, 82)	>95%
Vulva carcinoma		
basaloid	16, 18	>50%
„warty“	16, 18	>50%
keratinizing	16	<10%
Penile carcinoma		
basaloid	16, 18	>50%
„warty“	16, 18	>50%
keratinizing	16	<10%
Vaginal carcinoma	16, 18	>50%
Anal and perianal cancers	16, 18	>70%
Oral cavity and tonsils	16, 18, 33	~25%
Nail bed	16	~70%


< 9.3
< 16.1
< 23.8
< 35.8
< 93.9
Age-standardized (world) rate (per 100 000)

Global incidence for cervical cancer

per annum ***~ 493.000 new cases globally***

~ 410.000 in resource-constrained countries (83%)

~ 83.000 in resource-rich countries (17%)

230.000-280.000 women die annually of cancer of the cervix

IARC, 2002

HPV-linked anogenital high grade lesions and cancers in women (USA):

~ 330.000 cases of high grade cervical dysplasia are diagnosed
For Germany we estimate between 80.000-120.000 cases annually.

~ 10.000 new cases of cervical cancer per annum (~ 3.000 deaths). In Germany ~ 6.500 new cases and ~ 1.700 deaths.

~ 2.420 new cases of vaginal cancer (820 deaths)

~ 3.740 new cases of vulvar cancer (880 deaths)

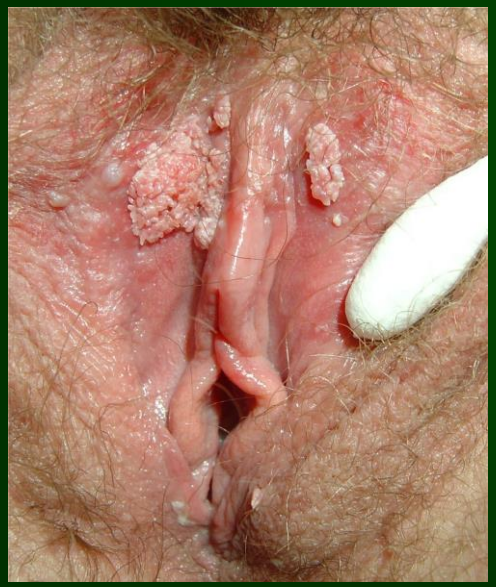
In Men: ~ 5.000 cases of anal cancer (660 deaths)

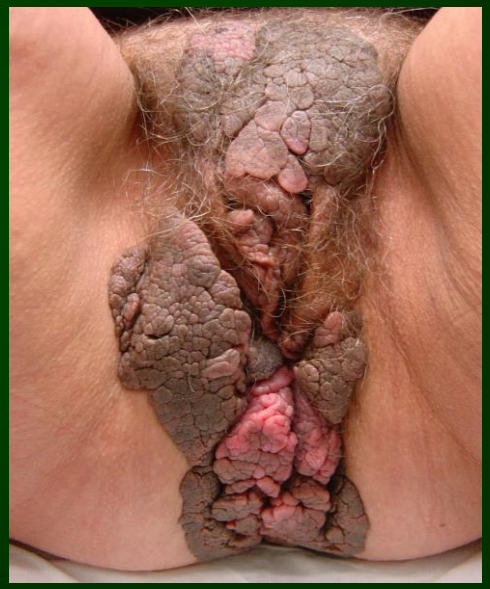
~ 1.400.000 doctor visits for genital warts

Source Merck HPV webside, cited by Lehman Brothers in Major Pharmaceuticals

Low risk HPV are not “no-risk” or “benign” HPV infections

Condylomata acuminata may convert into squamous cell carcinomas





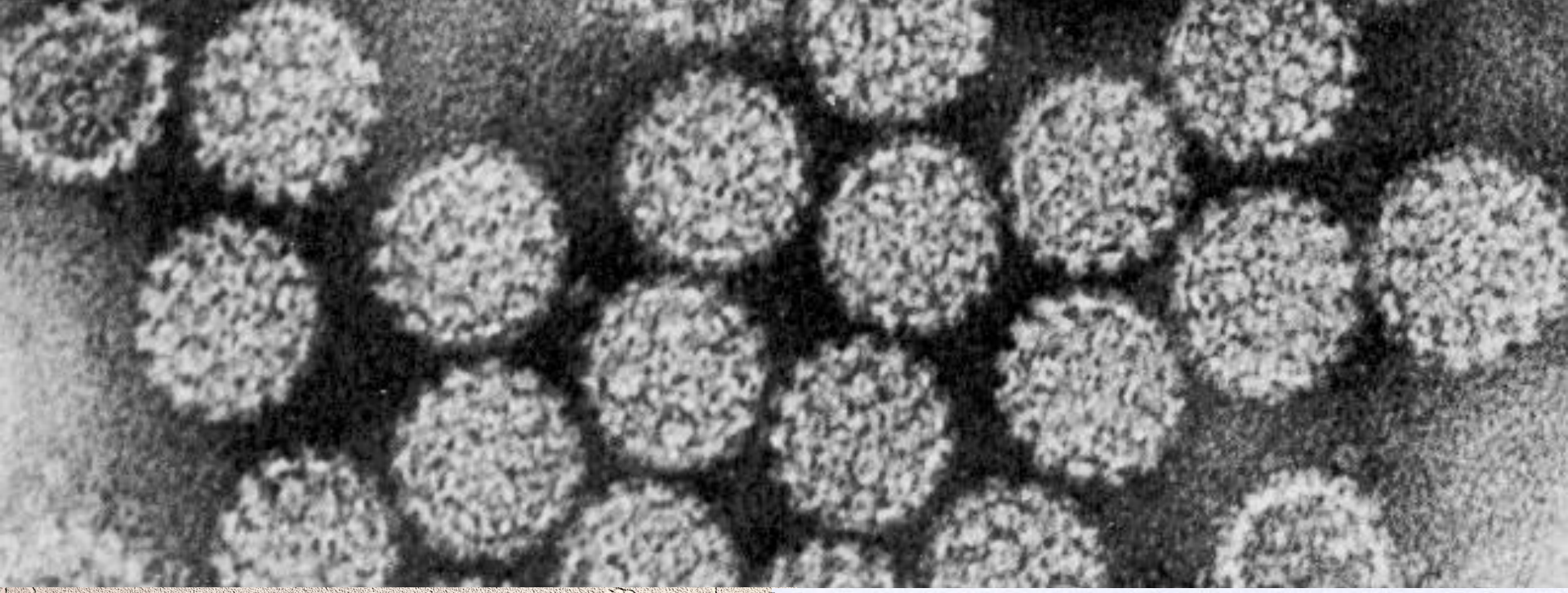
Courtesy of A. Clad, Freiburg

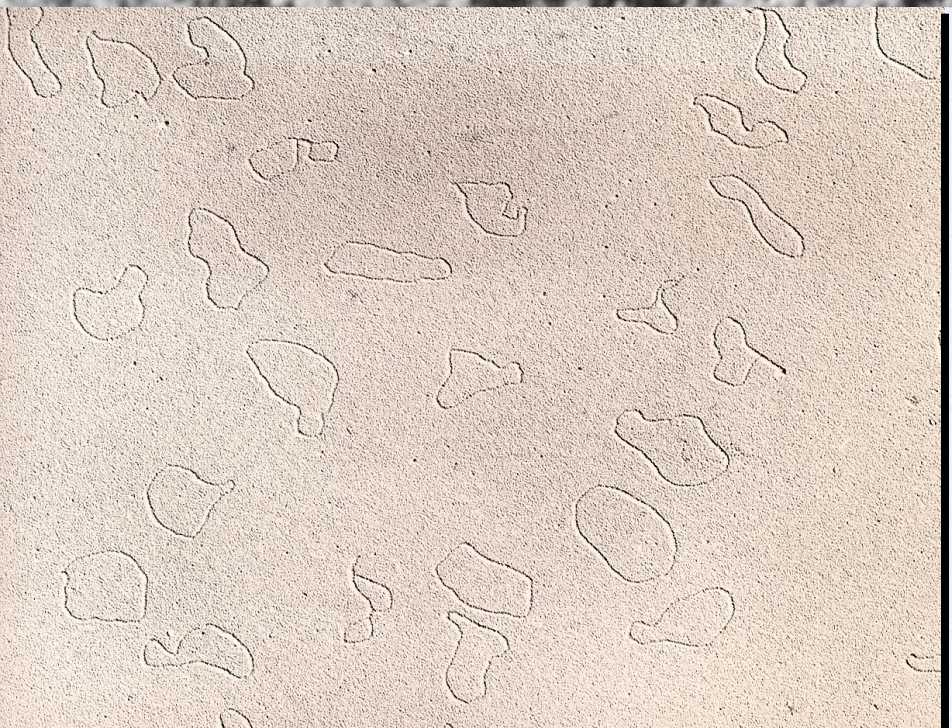
More than one half of all sexually active women and men acquire genital HPV infections at some point in their lives.

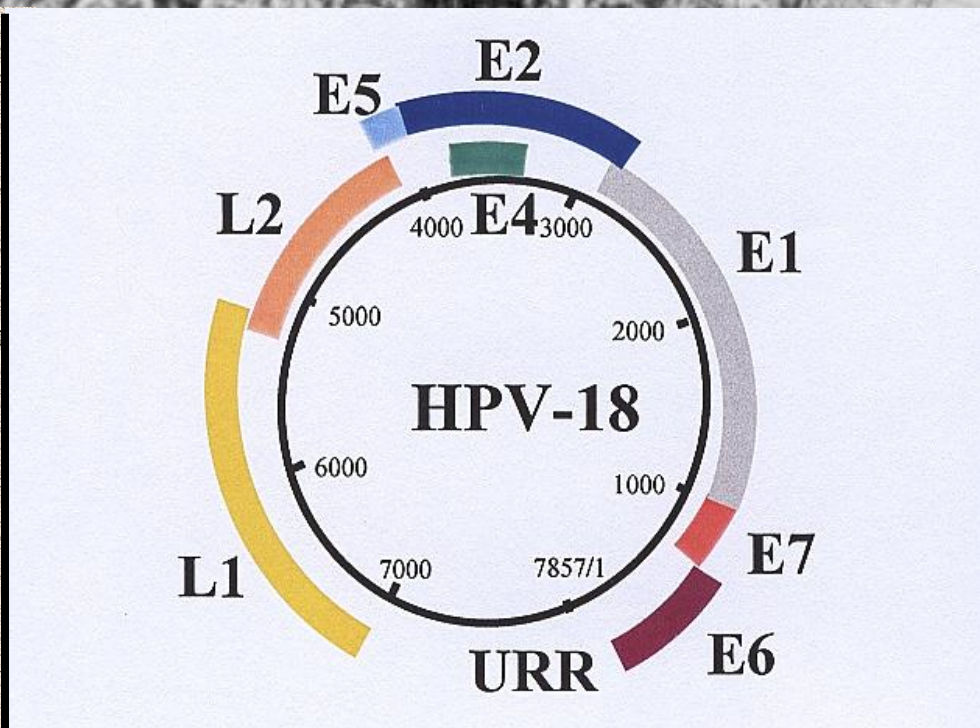
In the USA, about 20.000.000 people are infected with anogenital HPV types. 6.200.000 infections occur every year. 75% of those in young women and men between the ages of 15 and 24 years

HPV infections occur soon after onset of sexual activities with a cumulative incidence of about 40% within 16 months.

Source Lehman Brothers in Major Pharmaceuticals






E2
E5
L2
E4
4000
3000
E1
5000
2000
HPV-18
6000
1000
L1
7000
7857/1
E7
URR
E6

Mean duration of virus persistence is

approximately 8-10 months

~ 30% of infected women remain HPV-positive after one year

~ 9% are still viral DNA-positive after 2 years.

Estimation of the Life Time Risk for Cervical Cancer (in a partially screened, but in general gynaecologically well controlled population)

~1.1% of the infected population (or 0.775% of the total female population).

In a non-screened and gynaecologically poorly controlled population

the life time risk has been estimated to be 4% and higher (depending

on the multiplicity of high risk infections and on hygienic conditions)

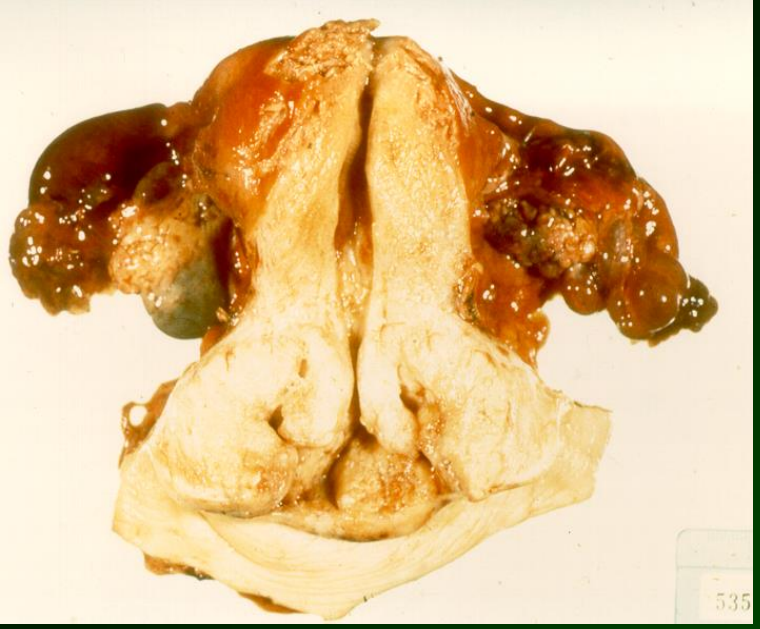
Approximately 20% of routinely screened women develop cervical

lesions during their life time that are considered to require removal.

Why are close to 10% of infected women unable to clear a high risk HPV infection immunologically within a 2 years period?

- Their genetic background may not permit the presentation of viral antigens to the immune system;
- Localization of the infection may not be accessible to an immunological control;
- Viral oncogenes (E6 and E7) do not only stimulate cell proliferation, **both are also mutagenic for host cell DNA.** This may lead to random mutations and to the selection of cell clones with modifications in the HLA class I pathway.
- Chemical and physical carcinogens may also contribute to these modifications.

More than 95% of cervical cancer cells are unable to present HPV antigenic epitopes at their surface



High Risk HPV Are the Primary Cause of Cervical Cancer because:

1. Viral genes (E6/E7) are present and uniformly active in cervical cancer cells.
2. The E6/E7 genes possess growth-promoting and transforming activity.
3. The malignant phenotype of cervical cancer cells depends on the expression of the viral oncogenes.
4. Epidemiological prospective and case / control studies identify high risk HPV as the major risk factor for cervical cancer
5. Vaccines against high risk HPV protect against the essential precursor lesions of cervical cancer

Among many other functions the oncoprotein E6 of high risk HPV interacts with the cellular cell cycle regulatory protein p53.

This interaction results in the degradation of p53 and , among some other effects, to dysregulation of the cell cycle

The oncoprotein E7 also interacts with another cellular growth-regulating protein pRb and mediates its degradation

Both, E6 and E7 genes fulfill dual functions

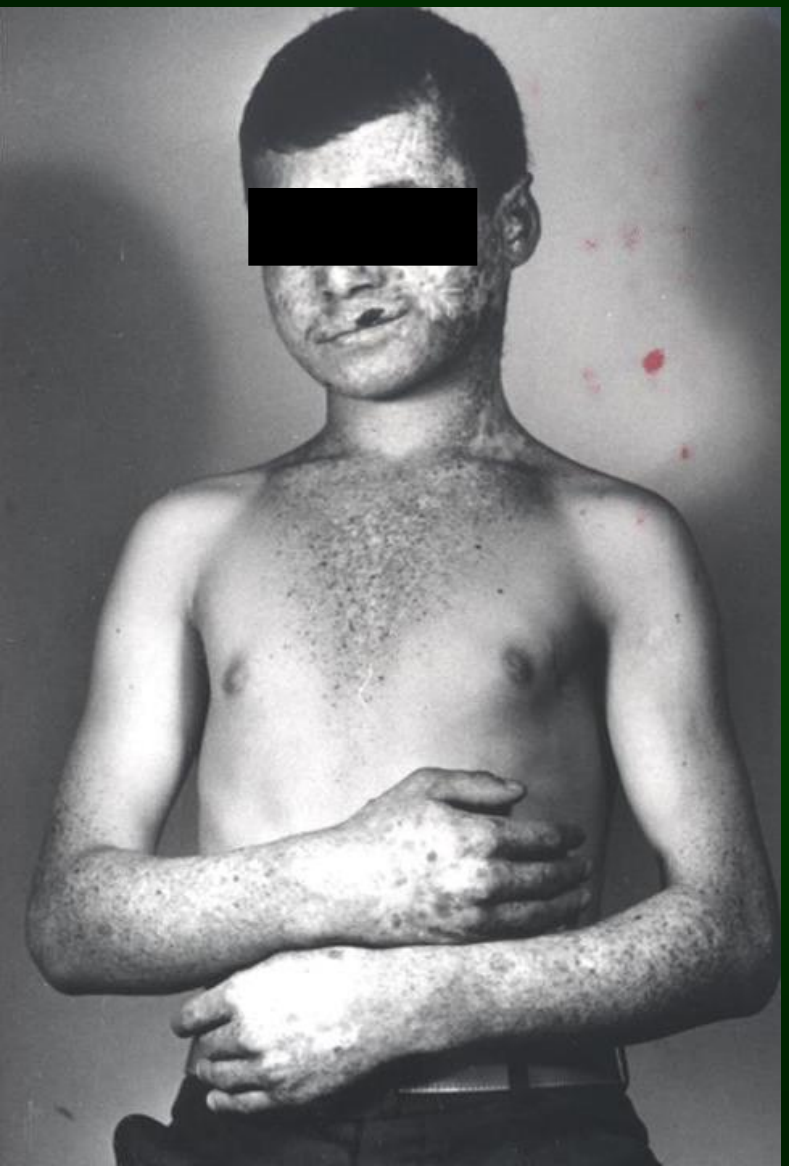
Each of them acts as independent oncogene and induces in addition mutational changes, depending on the degree of their expression.

They synergize with each other, because

E6 abolishes apoptosis, mediated by E7 protein expression
(degradation of p53)

E7 circumvents the $p16^{INK4}$-mediated inhibition of cyclin D / cdk 4/6 complexes
(degradation of pRb results in over-expression of $p16^{INK4}$)

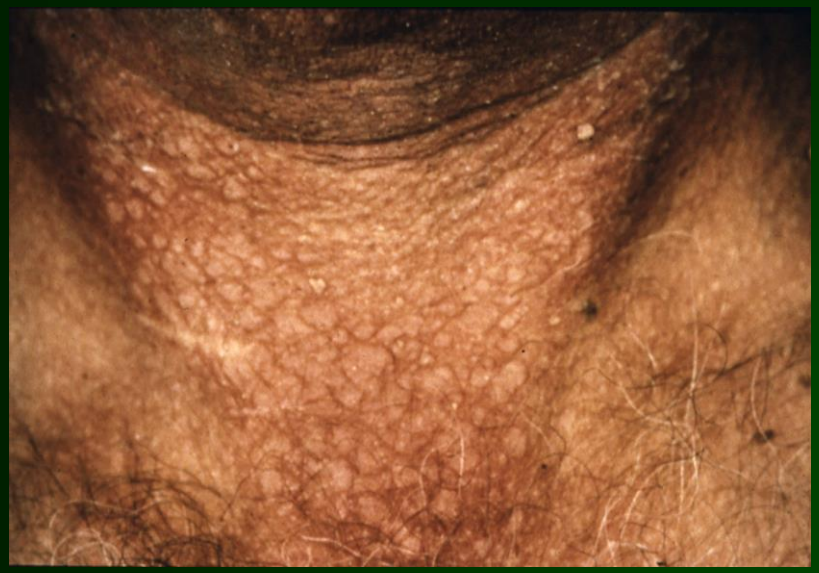
Cutaneous Papillomavirus-Infections

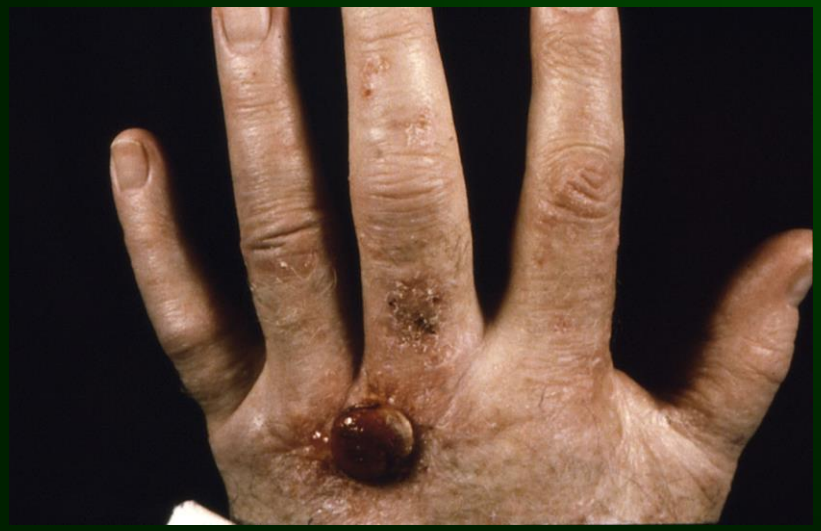


Jablonska, S. and Milewski, B.
Zur Kenntnis der Epidermodysplasia verruciformis Lewandowsky-Lutz
Dermatologica 115: 1-22, 1957.

Jablonska, S., Dabrowski, J., and Jakubowicz, K.
Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis.
Cancer Res. 32: 583-589, 1972.

Cutaneous warts are regularly increased under immunosuppression





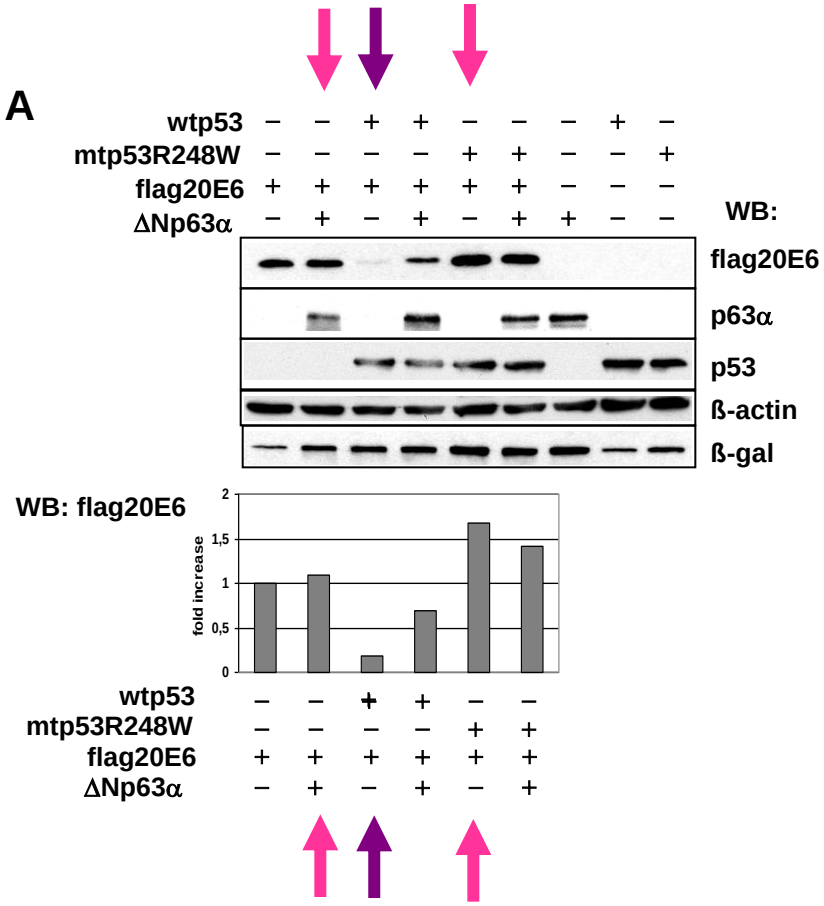
Courtesy of Irene Leigh

Warts are usually clonal and preferentially located at light-exposed sites

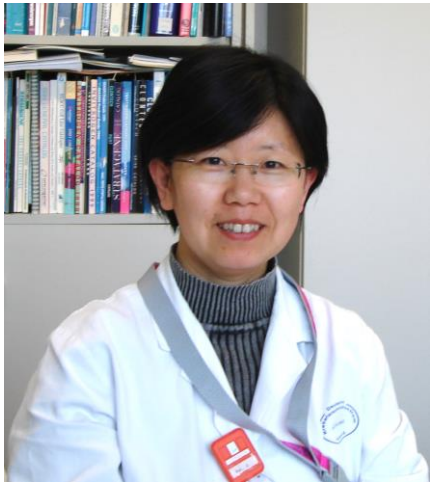
In Epidermodysplasia verruciformis patients as well as under immunosuppression some of these warts at light-exposed sites may convert into squamous cell carcinomas

Is there a role of the UV-part of sunlight in the induction of warts and their malignant conversion?

wtp53 mediates HPV20 E6 degradation:



Ethel-Michele de Villiers

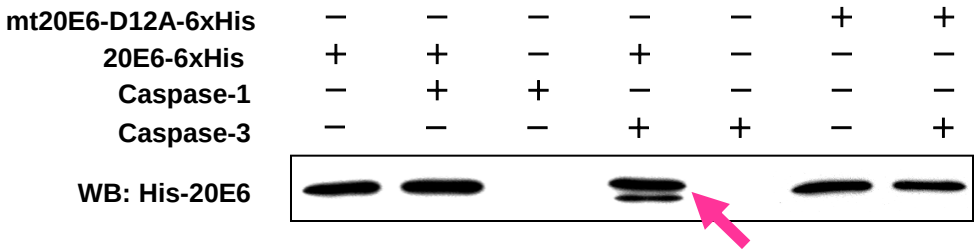


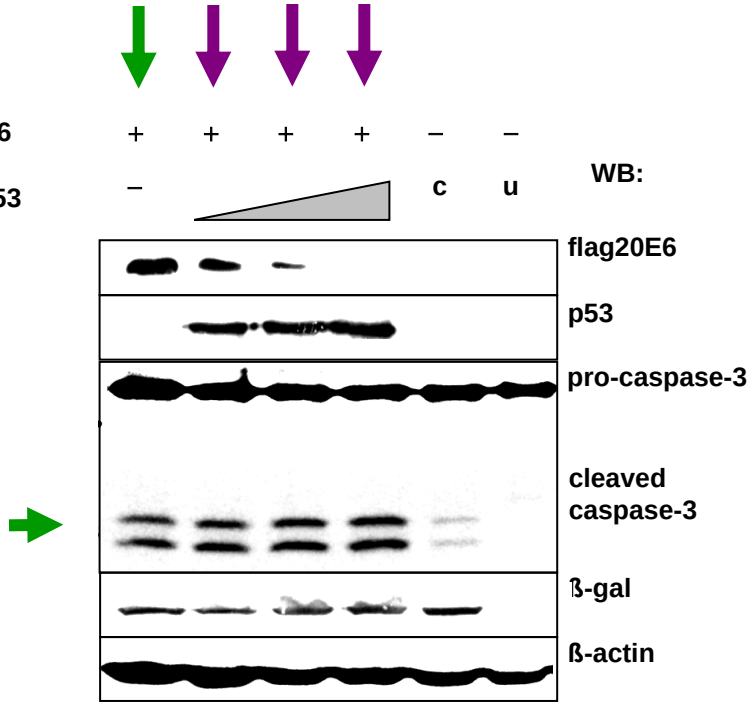
Jian-Wei Fei

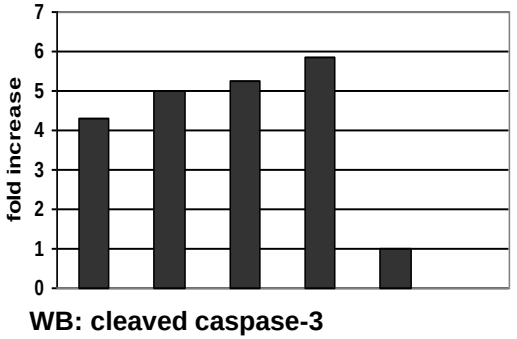
ΔNp63α and mutant p53R248W inhibit this degradation

Caspase-3 induces Wtp53-mediated degradation of HPV20E6

HPV20 E6 activates caspase-3







The data may point to a novel mechanism of host defense against cutaneous HPV infections:

Degradation of E6 by wtp53 of HPV 20 should only permit wart development in cells with functionally modified mutated p53, explaining the preferential occurrence of warts in sun-exposed skin areas. This is remarkably different from high risk HPV infections.

Returning to high risk HPV infections:

Oncogenesis by viruses acting as direct carcinogens

requires genetic or epigenetic modifications

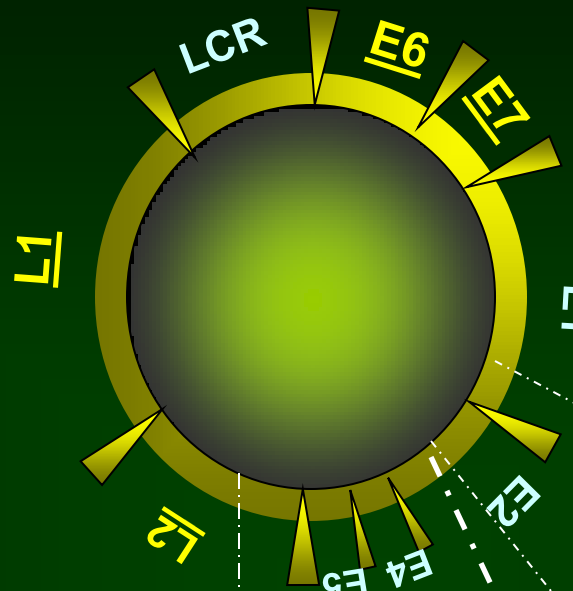
in genes of at least 3 specific host cell signaling

cascades, inactivating the function of the latter.

These cascades normally regulate

- **(I) Immunological surveillance,**
- **(II) the intracellular (functional) control of viral oncoproteins,**
- **(III) the paracrine regulation of viral gene activity.**

HPV genomes in cervical cancer



opening of the viral ring molecule during integratiion

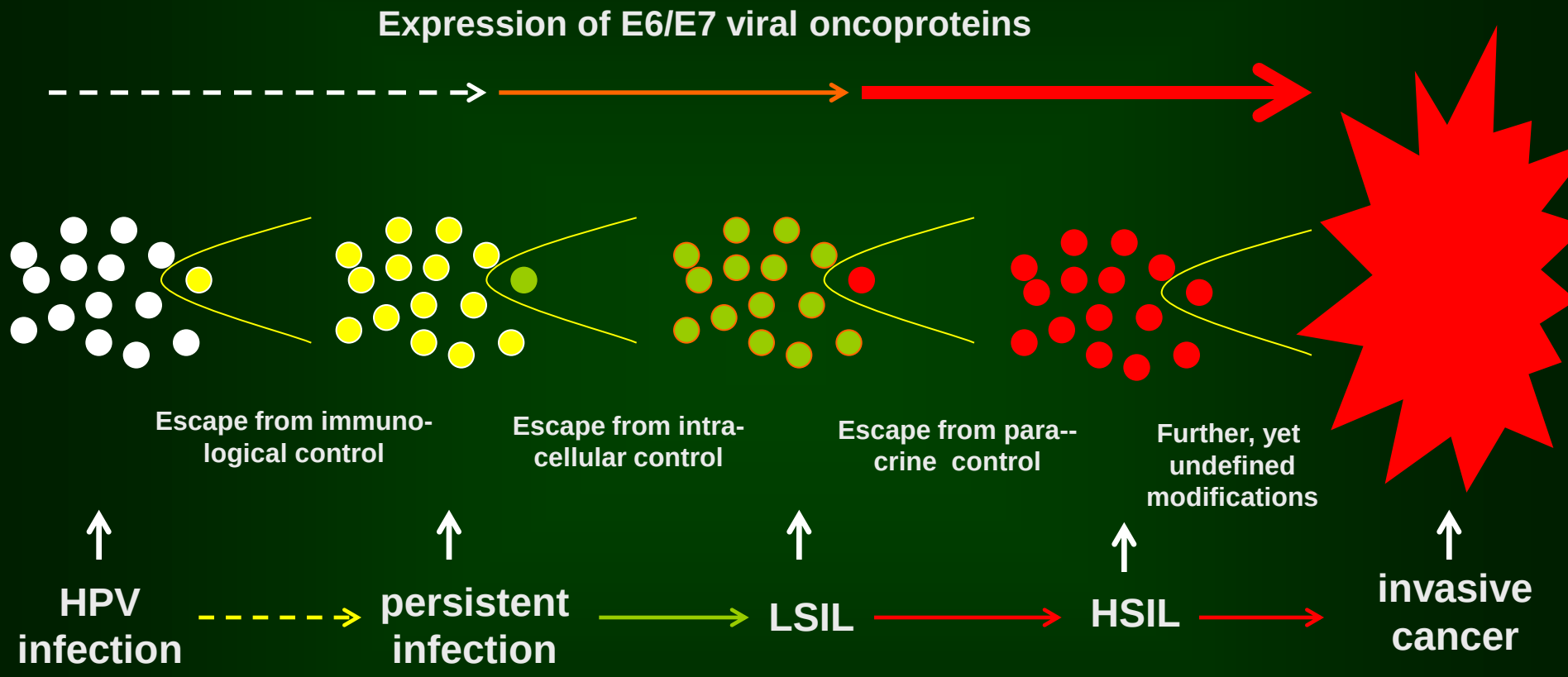
frequently deleted during DNA integration

chimeric transcripts, increased mRNA lifespan

integration

modulation of viral transcription by host cell promoters

Pathogenesis of cancer of the cervix



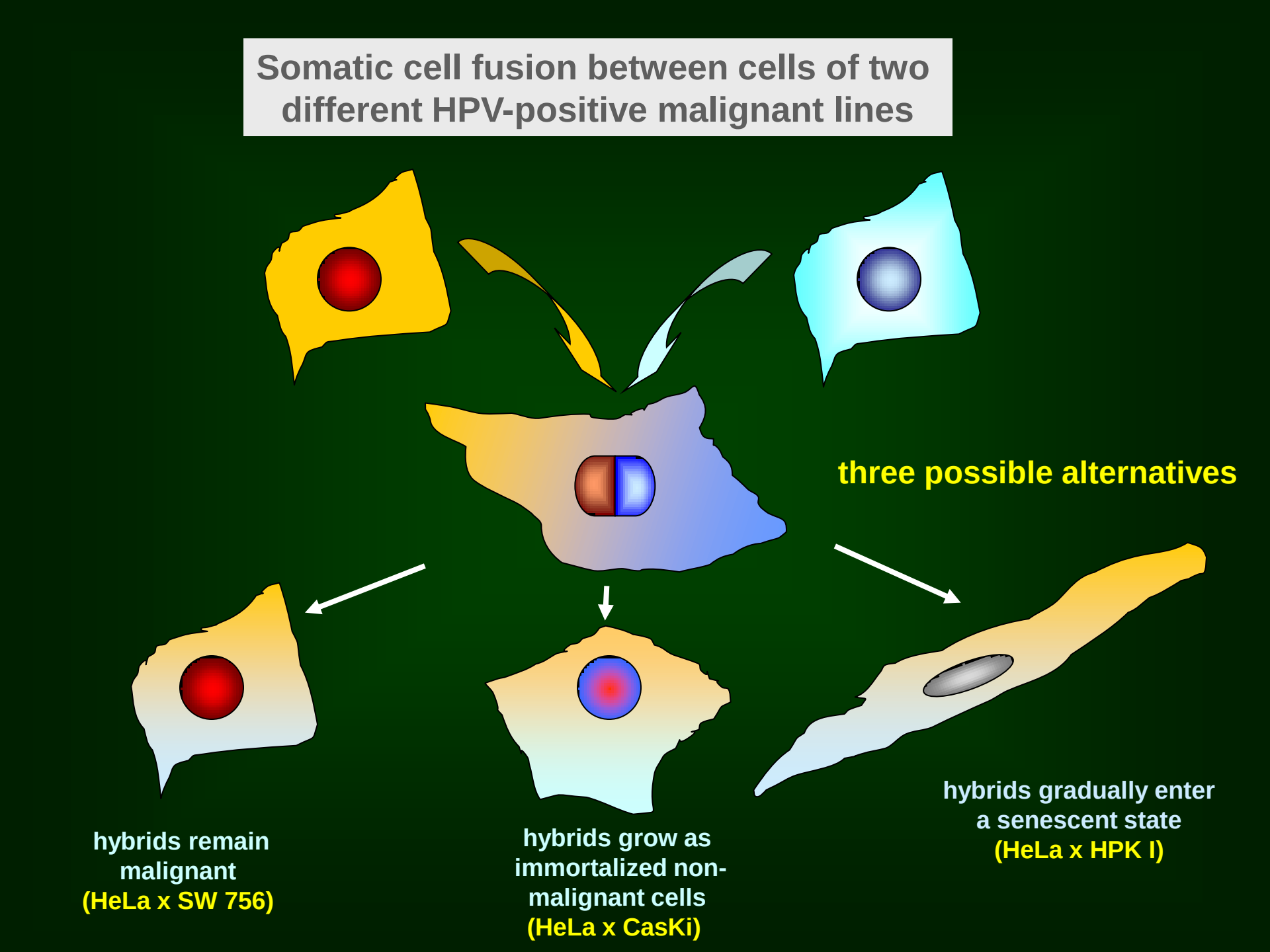
Do additional data support the inactivation of cellular genes as a requirement for malignant transformation by high risk papillomaviruses?

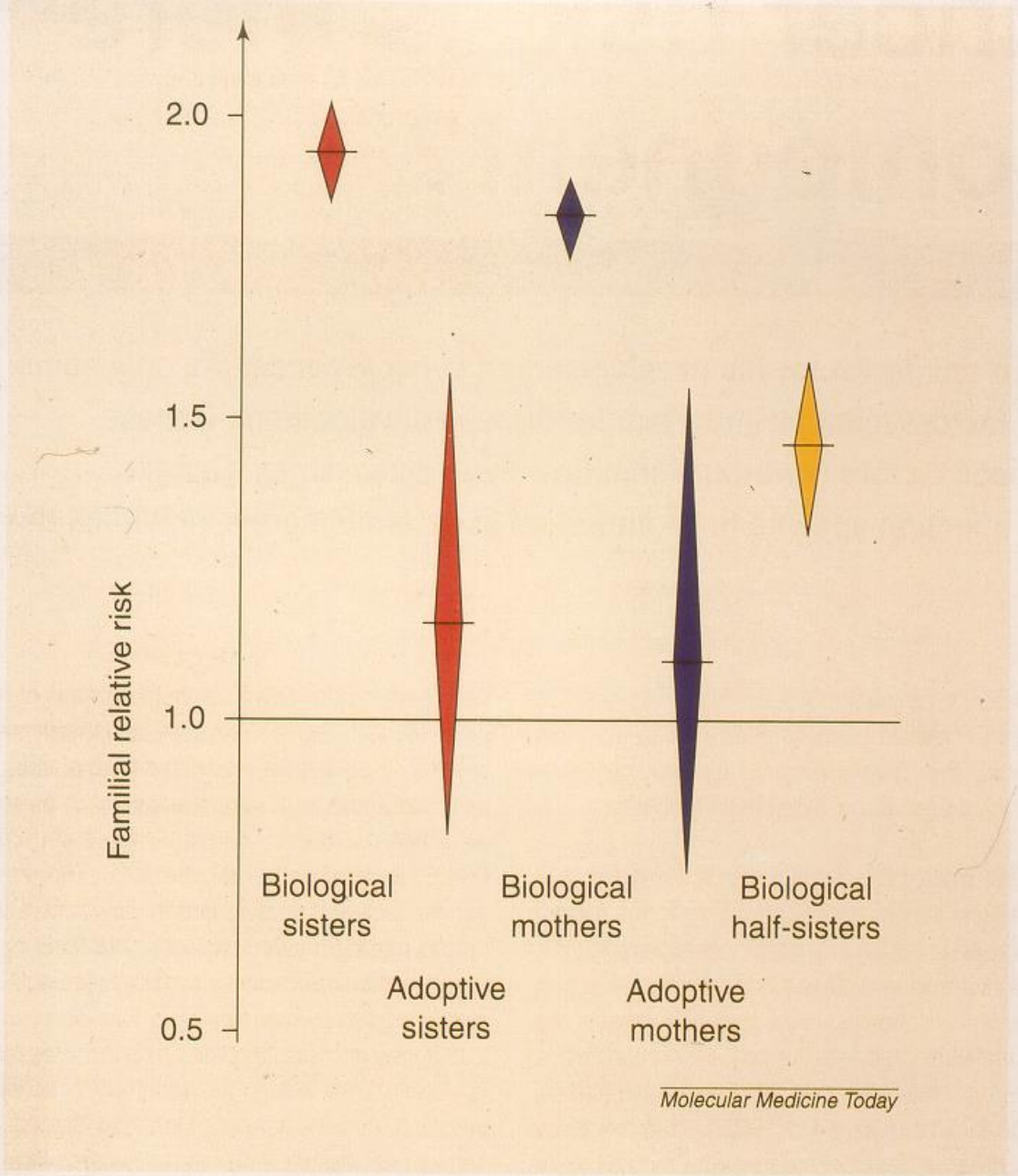
- **somatic cell hybridization (cell fusion)**

  different clones of malignant cells may complement each other to non-maignant growth or senescence in spite of continued E6 and E7 synthesis.

- **Hereditary predisposition to cervical cancer**

Somatic cell fusion between cells of two different HPV-positive malignant lines





Familial relative risk (incidence in relatives of cases divided by incidence in relatives of controls) for cervical tumour diagnosis in Sweden. Black line indicates point estimate and coloured double-triangles indicate the 95% confidence interval. **Pątrik, Magnusson and Gyllensten, Mol. Med. Today 6, 146, 2000**

Since immune mechanisms control

most tumorvirus infections, some

of these infections should

represent suitable targets

for vaccination

Thus, vaccinations against specific types

of cancers are gradually becoming

a reality

A brief history of HPV vaccine development:

Starting in 1984 I approached a number of German pharmaceutical companies with the request to start a collaborative program on HPV vaccination

The Behring Company in Marburg was initially interested, they initiated in 1986/87 a market analysis that eventually indicated that there would be no market for this vaccine.

At the same time period, however, a number of early PCR studies had been published, creating some confusion by reporting in part the same percentage of HPV-positive data in supposedly negative controls.

Salunke, D.M., Caspar, D.L., and Garcea, R.L.
Self-assembly of purified polyomavirus capsid protein VP1. Cell 46: 895-904, 1986.

Burke, K.L., Dunn, G., Ferguson, M., Minor, P.D., and Almond, J.W.
Antigen chimaeras of poliovirus as potential new vaccines. Nature 332: 81-82, 1988

Jenkins, O., Cason, J., Burke, K.L., Lunney, D., Gillen, A., Patel, D., McCance, D.J., and Almond, J.W.
An antigen chimera of poliovirus induces antibodies against human papillomavirus type 16 J. Virol. 64: 1201-1206, 1990

Zhou, J., Sun, X.Y., Stenzel, D.J., and Frazer, I.H.
Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. Virology 185: 251-257, 1991.

Kirnbauer, R., Booy, F., Cheng, N., Lowy, D.R., and Schiller, J.T.
Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc. Natl. Acad. Sci. USA 89: 12180-12184, 1992.

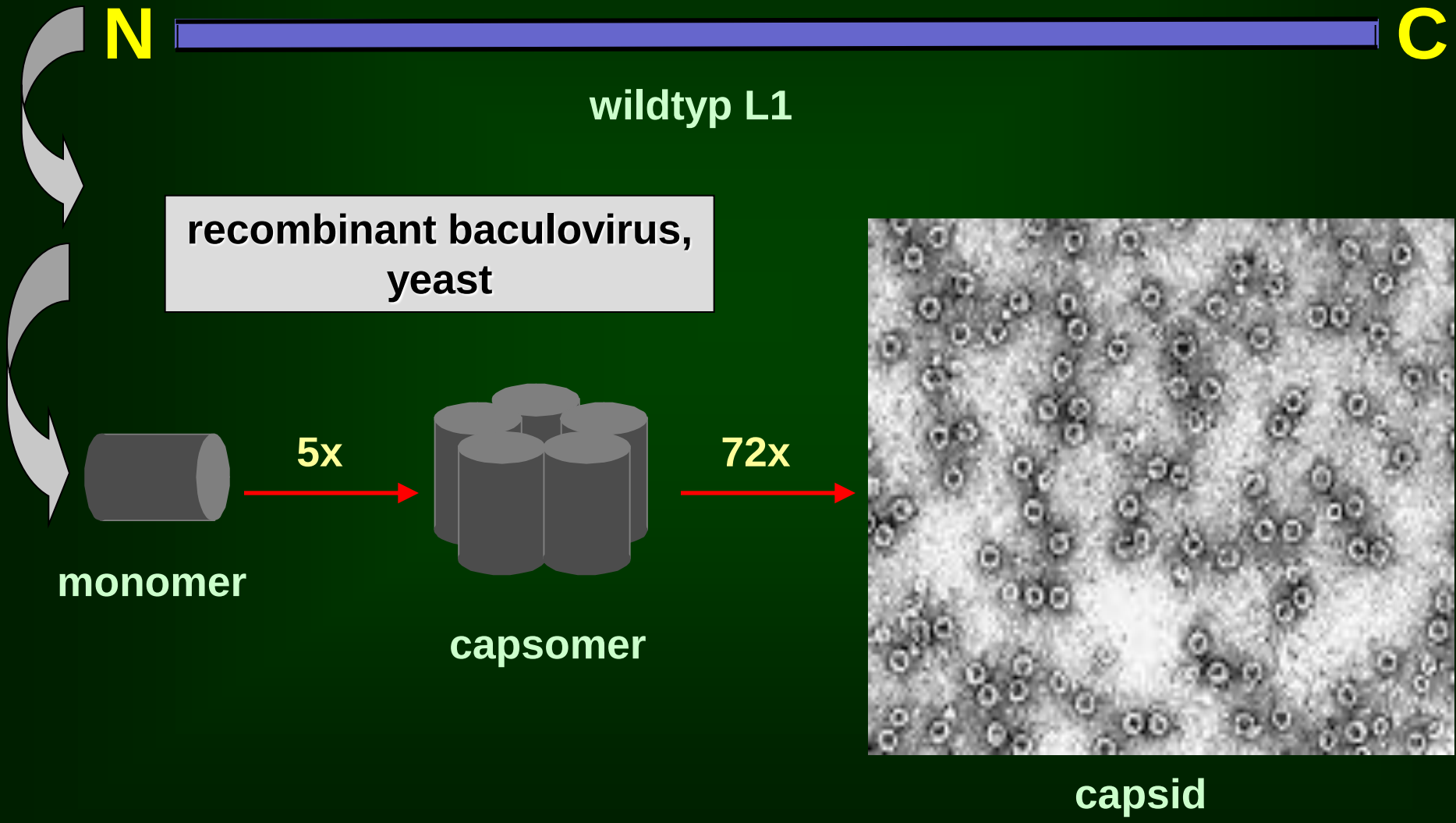
Suzich, J.A., Ghim, S.J., Palmer-Hill, F.J., White, W.I., Tamura, J.K., Bell, J.A., Newsome, J.A., Jenson, A.B., and Schlegel, R.

***Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas**. Proc. Natl. Acad. Sci. USA 92: 11553-11557, 1995.*

Breitburd, F., Kirnbauer, R., Hubbert, N.L., Nonnenmacher, B.,Trin-Dingh-Desmarquet, C., Orth, G., Schiller, J.T., and Lowy, D.R.

Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J. Virol. 69: 3959-3963, 1995.

HPV L1 VLPs



L. Gissmann, 2004

Koutsky et al. **A controlled trial of a human papillomavirus Type 16 vaccine.** New Engl. J. Med. 347: 1645-51, 2002

768 women without signs of HPV infection (age 16-23) were vaccinated with VLP preparations of HPV 16

765 women received placebo vaccinations

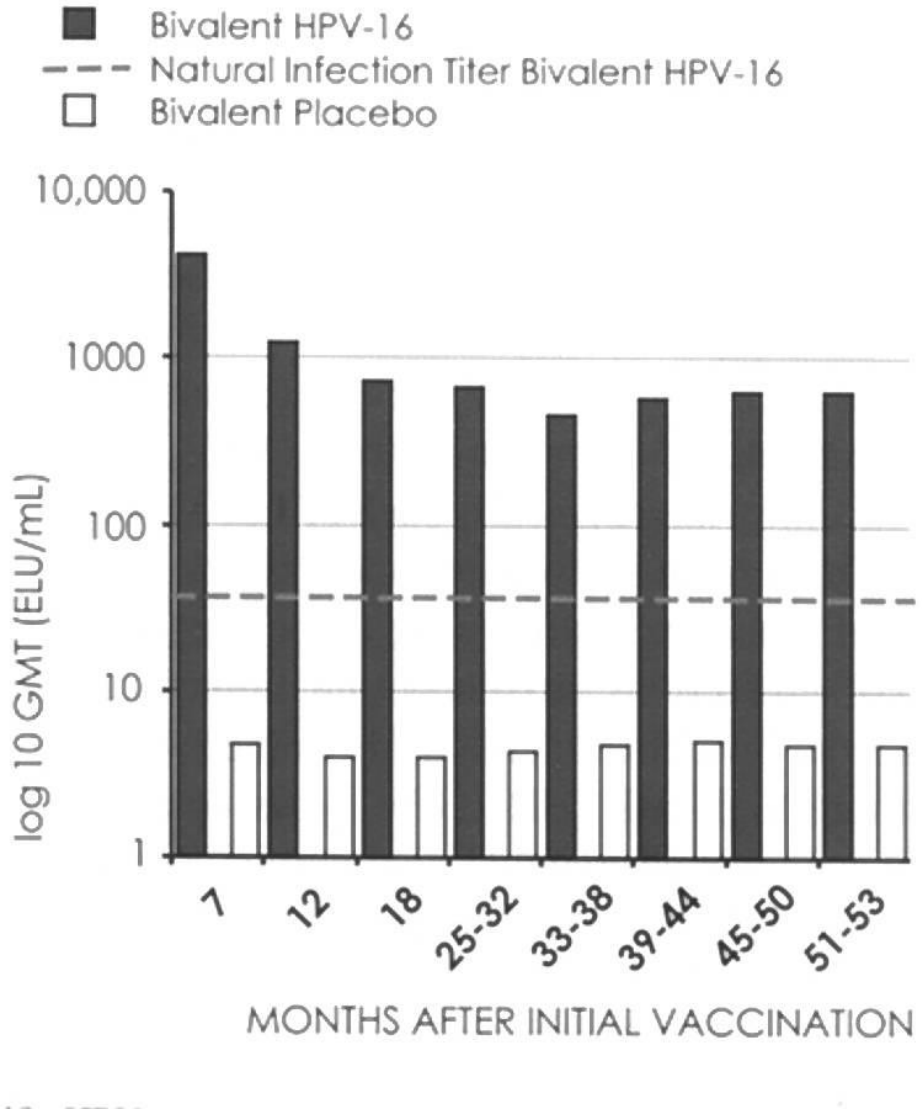
Both groups were followed for a median of 17.4 months.

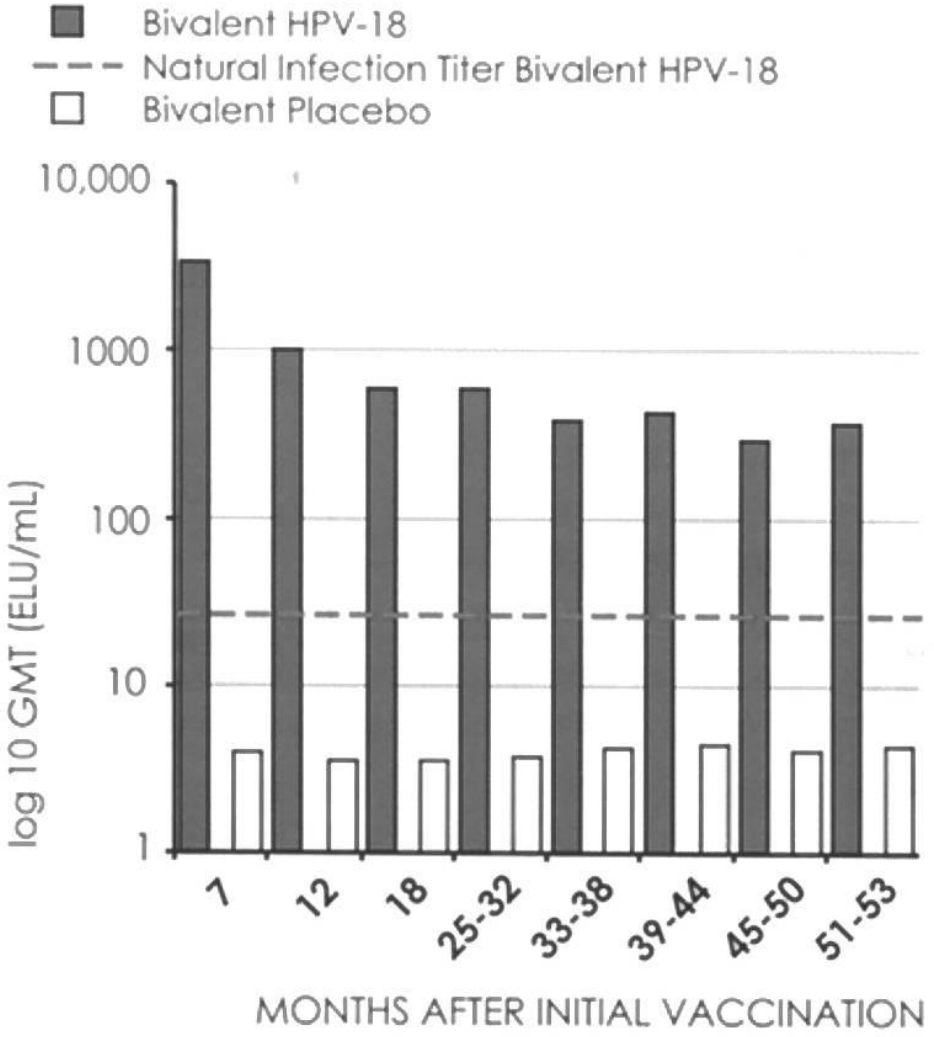
	Transient HPV16 in-fection	Persistent HPV 16 in-fection	CIN 1	CIN II
HPV 16 vaccinated group	6 (0.6 %)	0	0	0
Placebo group	68 (6.3 %)	41	5	4

Two types of vaccines are presently available :

A tetravalent L1 vaccine (Gardasil) for HPV types 16 and 18 (high risk) and 6 and 11 (low risk).

and a bivalent vaccine (Cervarix) against HPV 16 and 18.





From: Koutsky and Harper, 2006

Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial.

- Harper DM,
- Franco EL,
- Wheeler CM,
- Moscicki AB,
- Romanowski B,
- Roteli-Martins CM,
- Jenkins D,
- Schuind A,
- Costa Clemens SA,
- Dubin G;
- HPV Vaccine Study group.

Main conclusions:

Vaccine efficacy was 100% against cervical intraepithelial neoplasia (CIN) lesions associated with vaccine types. Broad protection against cytohistological outcomes beyond that anticipated for HPV 16/18 and protection against incident infection with HPV 45 and HPV 31. The vaccine has a good long-term safety profile. Up to 4.5 years, the HPV-16/18 L1 virus-like particle vaccine is highly immunogenic and safe, and induces a high degree of protection against HPV-16/18 infection and associated cervical lesions. There is also evidence of cross protection.

Lancet. 367: 1247-1255, 2006.

HPV 16 L1 Capsomeres

Δ 1-10

^{175}Cys → Ser

Δ409-431 (Δh4)

N

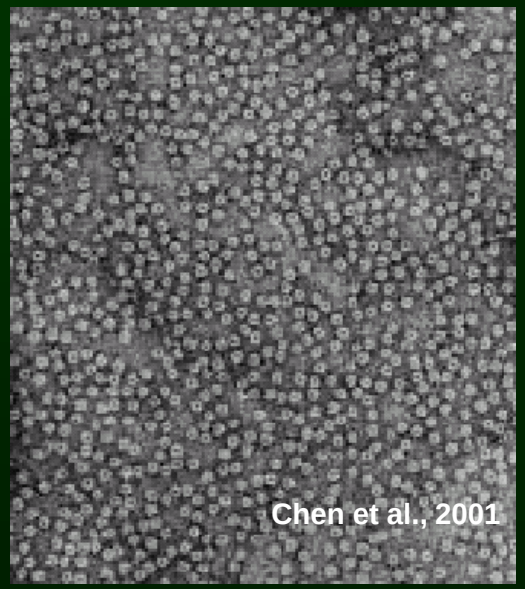
C

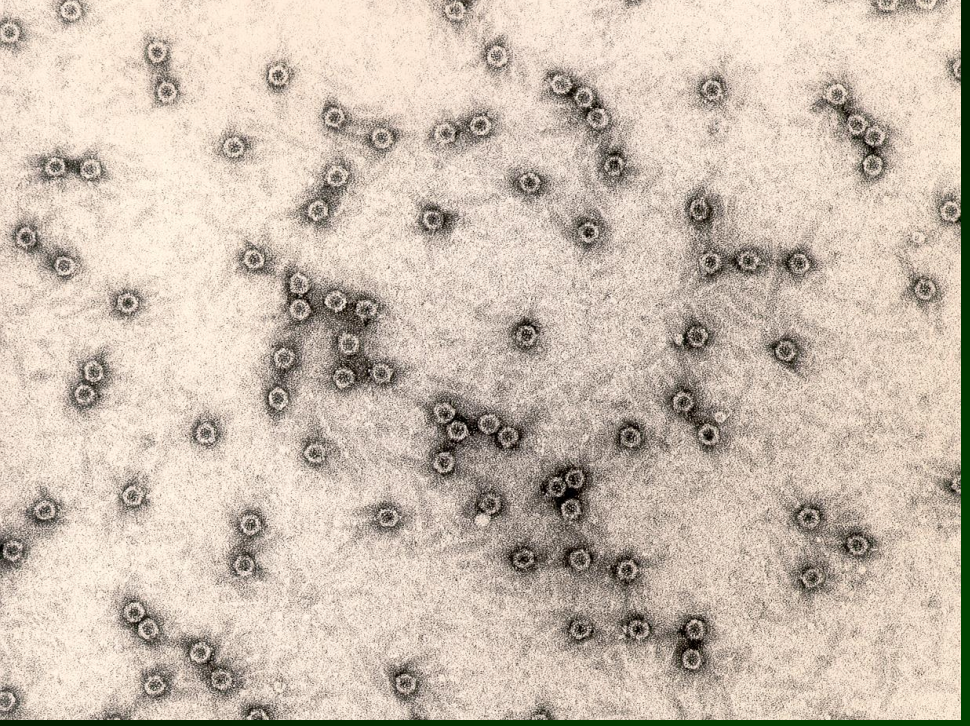
modified L1

E. coli

monomer

5x





Adeno-associated Virus (AAV)-based vector systems

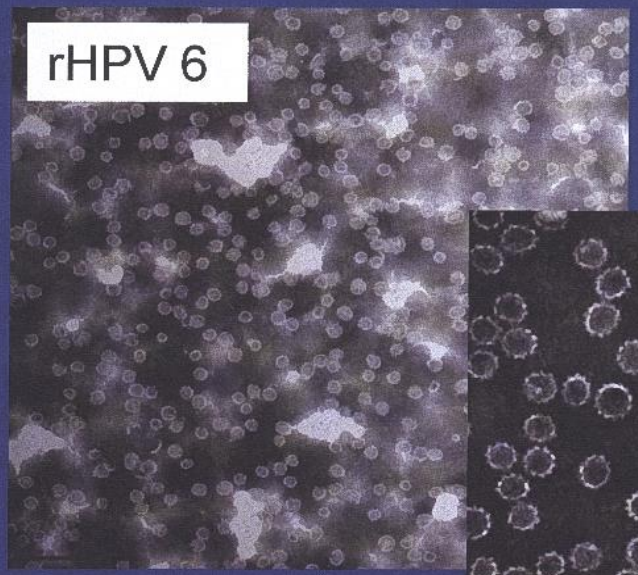
Adeno-associated virus (AAV)-vector systems are heat-resistant (60° C) and may be applied via the mucosal surfaces

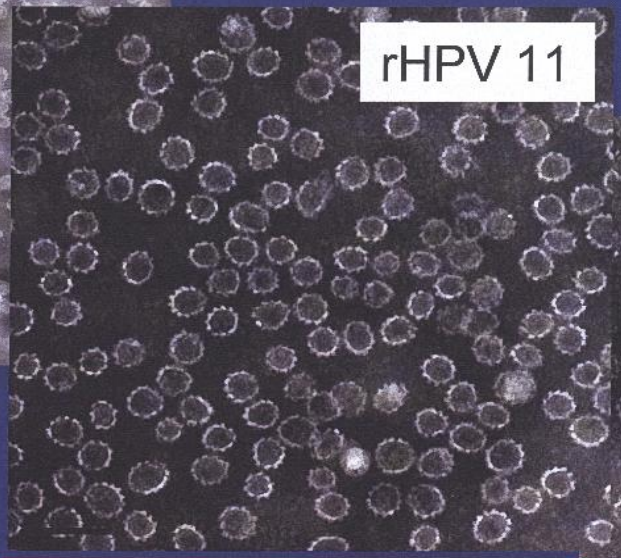
They are specifically promising for intranasal vaccination

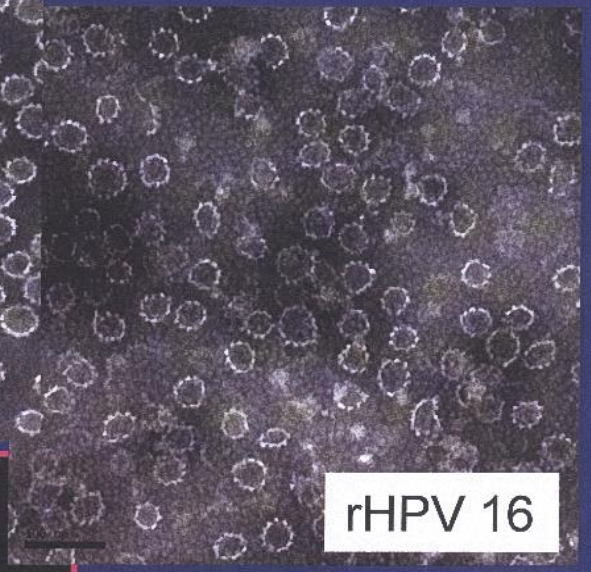
Several additional alternatives exist for future generations of vaccines, e.g.:

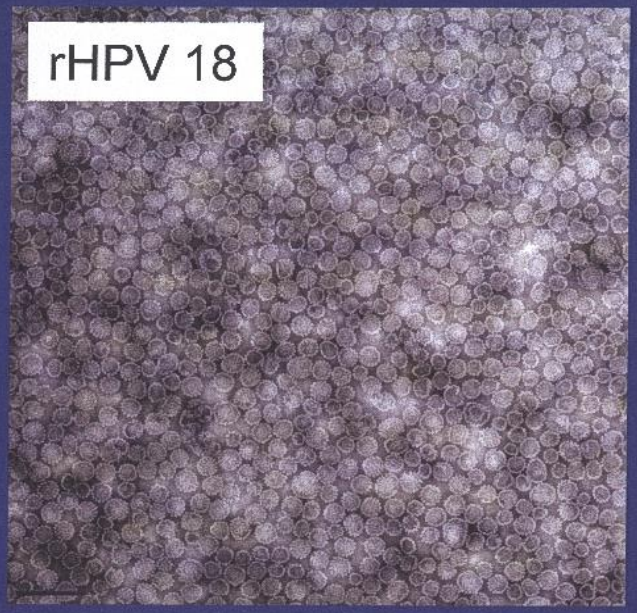
- The **N-terminal part of the L2 protein** induces a broad range of group-specific immunity., resulting in neutralizing antibodies for a large number of HPV types;
- Under certain conditions **virus-like particles can also be produced in bacterial systems**;
- **Viral RNA vector systems** may turn out to be useful for prophylaxctic and therapeutic vaccinations
- Intranasal application of **genetically modified attenuated Salmonella strains expressing HPV proteins**
- **Application of modified naked viral DNA**

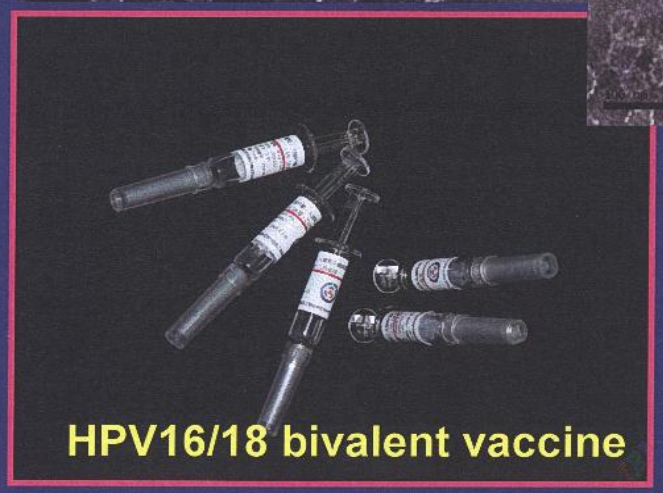
XMU HPV VLP Vaccine Produced in E. coli











James Wai-Kuo Shih
Xiamen University
and Biotech Inc.

Discussed and Open Questions:

- Which age groups should be vaccinated? Only girls between 9 and 17 or 25 years of age?
- Should vaccination only be applied to PCR- and antibody negative women or is it advisable also for a previously not tested female population?
- Should the vaccine also be applied to boys and young adult males?
- Does vaccination result in a prolongation of intervals for cervical cancer screening?
- Does the application of HPV vaccines to young girls result in earlier sexual activity and increased promiscuity?

Perspectives primary prevention of HPV infections:

- will it be possible to eradicate HPV infections?
- development of broad spectrum vaccines
- production of affordable vaccines
- development of organizational plans for countries with high rate of HPV infection
- Improvement of health education

- Identification of more infectious agents involved in human cancers

Diagnosis

- Development of better predictive diagnoses
- Affordable, sensitive and type-specific reliable HPV tests

Therapy

- Targeted chemotherapy
- Inhibition of viral RNA translation

- Immunotherapy against viral persistence and early lesions?
    - against viral antigens?
    - against over-expressed cellular antigens?

“New” Human Pathogenic Viruses 1994-2008

Year	*Virus*	*Symptoms*	*Natural Host*
1994	Sabia virus	Hemorrhagic fever	Rodents
1994	Hum. Herpesvirus 8	Kaposi’s sarcoma	Humans
1994	Hendravirus	Encephalitis	Bats, horses
1997	Influenza H5N1	Avian flue	Birds
1997	TT viruses	?	Humans
1998	Nipah virus	Encephalitis	Bats, pigs
2003	SARS Coronavirus	SARS	Chinese bushcat
2005	Bocavirus (parvovirus)	Acute wheezing	Humans
2005	New coronavirus	Respiratory symptoms	Humans
2007	KI-polyomavirus	?	Humans
2007	WU-polyomavirus	?	Humans
2008	MC-polyomavirus	Merkel-tumor	Humans

Within the same time period at least 30 novel types of human papillonaviruses have been identified

Initial basic studies, identifying high risk viruses and establishing their pathogenicity and carcinogenicity, have been successfully translated into clinical application for cancer prevention by vaccination, risk assessment of HPV infection, and into novel diagnostic tools;

…..but much more remains to be done!