HPV E CANCRO DEL BASSO TRATTO GENITALE

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CONFLITTO DI INTERESSI

Ho partecipato a eventi congressuali e studi clinici come consulente scientifico per Qiagen, SPMSD, GSK, Roche.

In merito alla presentazione non ho alcun conflitto d’interessi, e le opinioni da me espresse sono totalmente personali.

BACKGROUND

HPV classificato nel Rapporto dell'*American Association for Cancer Research* (AACR) come il **secondo agente patogeno responsabile di cancro nel mondo.**

AACR CANCER PROGRESS REPORT 2014.
http://cancerprogressreport.org/2014/Documents/AACR_CPR_2014.pdf

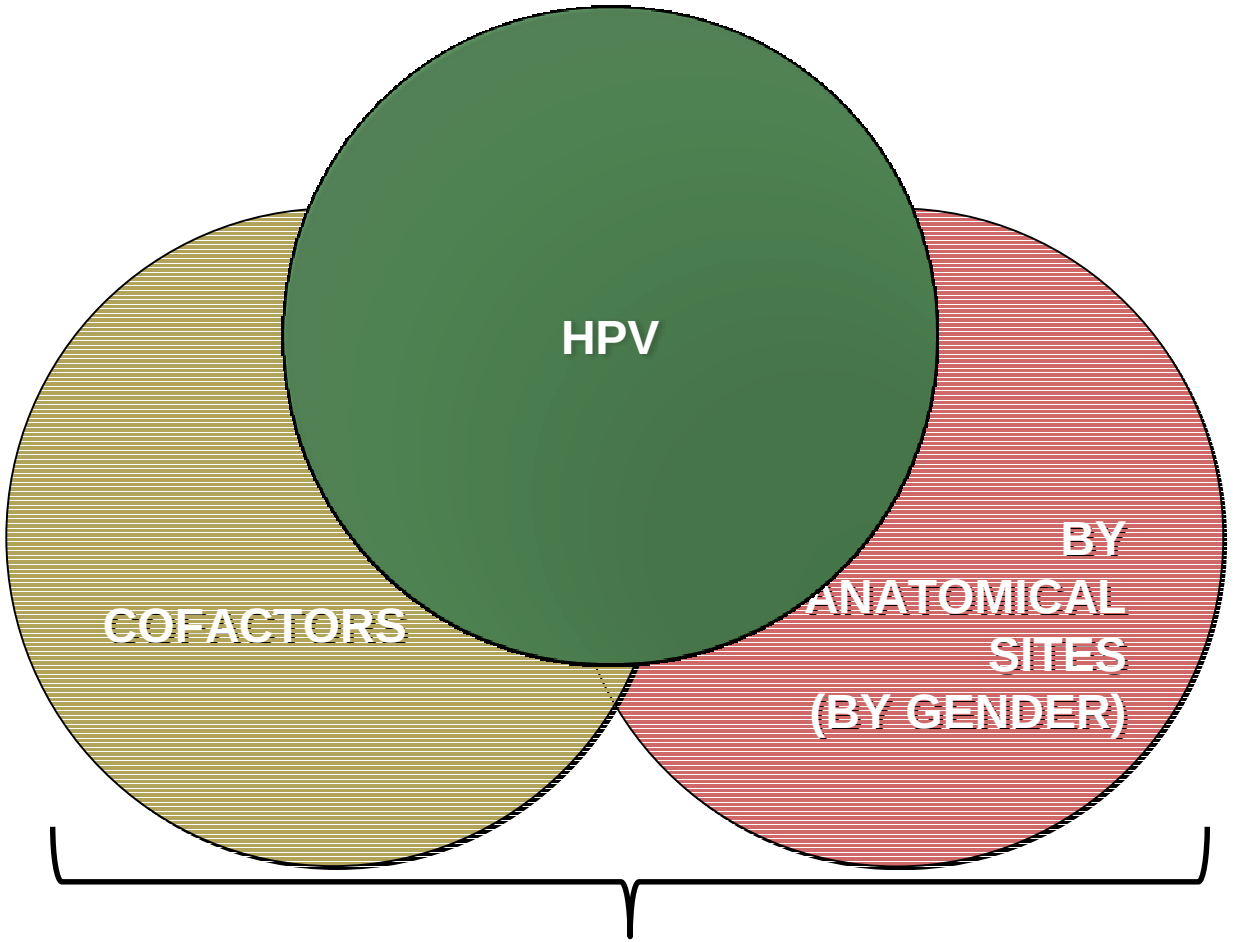
BACKGROUND

	Anno/mondo	Dovuti a HPV	Quali HPV	
Cancro del collo dell'utero	530.000	100%	16,18,45,33, 31,52,58…	HR
Cancro della vagina	7.000	65-90%	16, 18	
Cancro della vulva	14.000	40%	16, 18	HR
Les. pre-tumorali cervice (CIN 2,3)	70 milioni	100%	16,18,45,33, 31,52,58…	
Les. pre-tumorali vulva-vagina	??	40-90%	16, 18	
Cancro dell'ano (F) (M)	13.000 11.000	90-95%	16, 18	HR
Cancro dell'orofaringe (F) (M)	4.000 17.000	25%	16, 18	
Cancro del pene	11.000	40%	16, 18	
Condilomi genitali (F) (M)	4-15 milioni 7-17 milioni	>90%	6, 11	LR
Papillomatosi laringea ric.	4/100.000	>90%	6, 11	HR
Infezioni da HPV (F+M)	300 milioni	100%	16,18,45,33	LR

BACKGROUND

	incidence (100.000)	% HPV	% HPV 16-18	% other genotypes
cervix	10	100	70	30
vagina	0,3-0,7	65-90	88	<20
vulva	0,5-1,5	>40	91	<10
anus	1-2	85	93	<10
penis	<1	47	74	25

FACTORS RELATED TO CLINICAL VARIABILITY OF HPV-DISEASES

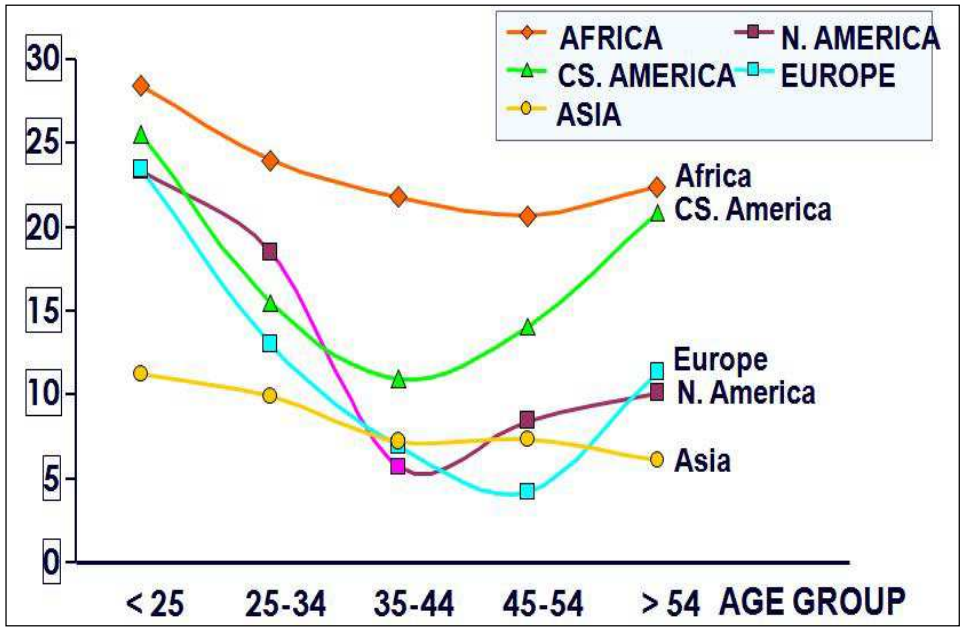


They modulate the risk in the critical transition steps of cervical carcinogenesis:
disease progression

VARIABILITY BY GENDER

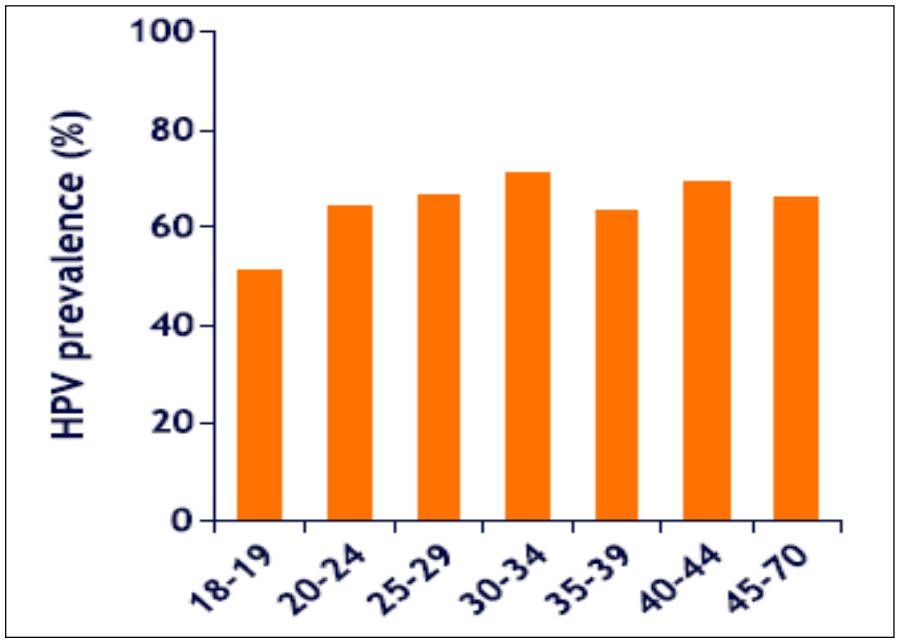
GENITAL HPV PREVALENCE

females



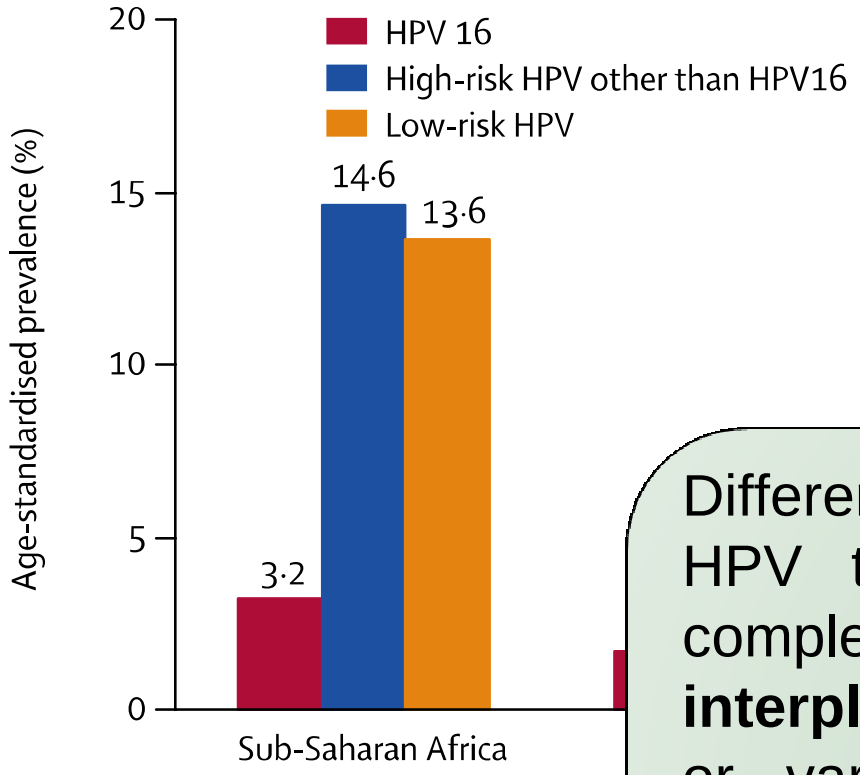
(N Munoz, 2006)

males



(AR Giuliano, 2008)

VARIABILITY BY GEOGRAPHY



Differences in the relative prevalence of HPV types might be related to the complex **geographical and biological interplay** between different HPV types or variants and host immunogenetic factors:

•impairment in cellular immunity, through chronic cervical inflammation, parasitic infection, malnutrition, HIV…

(G Clifford, 2005)

VARIABILITY BY GENOTYPES

BIOLOGICAL VARIABILITY

Group A (very high-risk)
16 – 33

- most persistent
- highest PPV for CIN3 and cancer
- more aggressive management

Group B (high-risk)
31 – 18 – 52 – 35 – 58

- less persistent than 16
- lower PPV for CIN2+ and cancer
- less aggressive management

Group C (intermediate-risk)
51 – 68 – 45 – 39 – 66 – 56 - 59

- low PPV and stronger association with LG-CIN
- less aggressive management

Low-risk (single 6/11)
12 over 8977 cancer cases
(S. de Sanjose, 2010)

- ???

J. Cuzick 2013

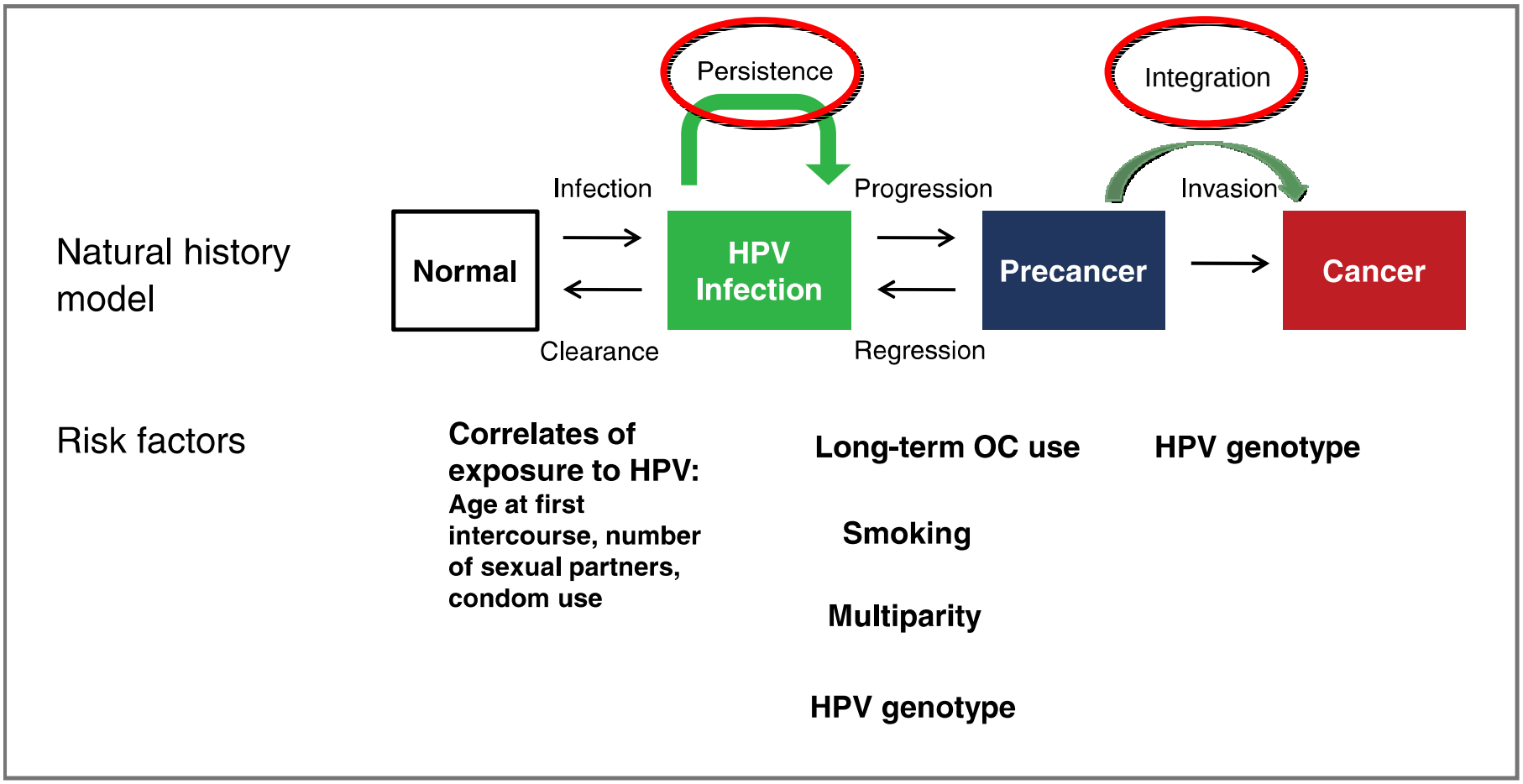
VARIABILITY BY GENOTYPES

Classifica	Cervice	Vulva	Vagina	Pene	Ano	Orofaringe
1	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16
2	HPV 18	HPV 18	HPV 40	HPV 18	HPV 18	HPV 33
3	HPV 33	HPV 33	HPV 6/11	HPV 6/11	HPV 33	HPV 35
4	HPV 45	HPV 6/11	HPV 31	HPV 22	HPV 31	HPV 18
5	HPV 31	HPV 45	HPV 33	HPV 74	HPV 6/11	HPV 26
6	HPV 58	HPV 52	HPV 18	HPV 31	HPV 45	HPV 45
7	HPV 52	HPV 51	HPV 58	HPV 45		HPV 52

[1] de Sanjosé S, et al. Vaccine. 2012;30(Suppl 4):D1-83

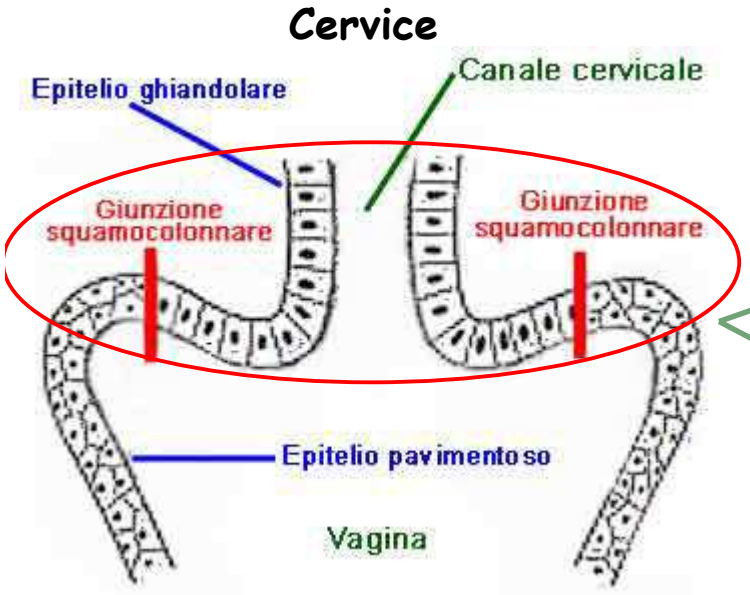
CERVICAL CANCER NATURAL HISTORY MODEL

1-multistep process; 2- chance occurrence; 3- variability of viral integration



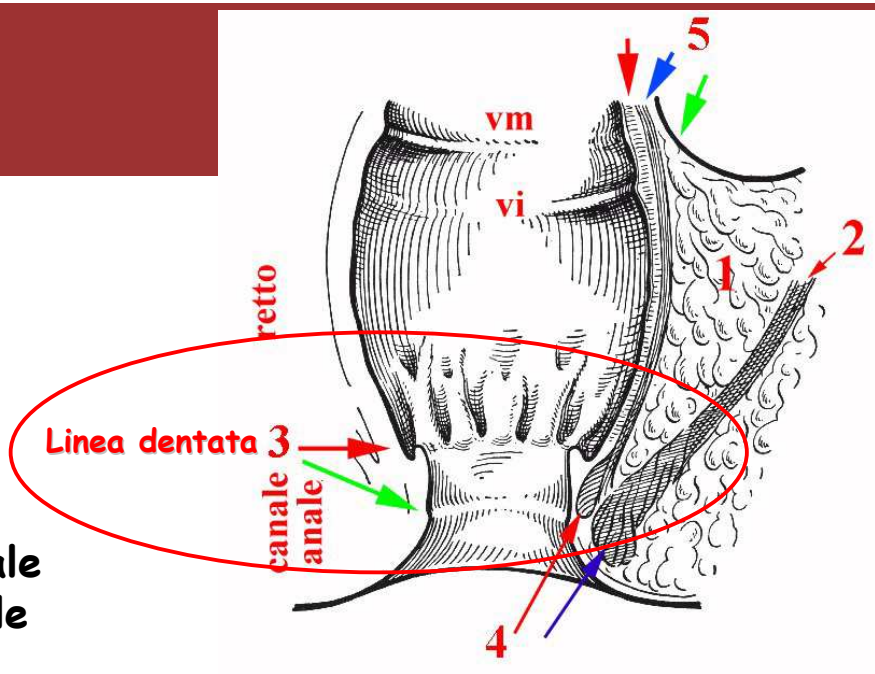
Mark Schiffman and Nicolas Wentzensen, 2013

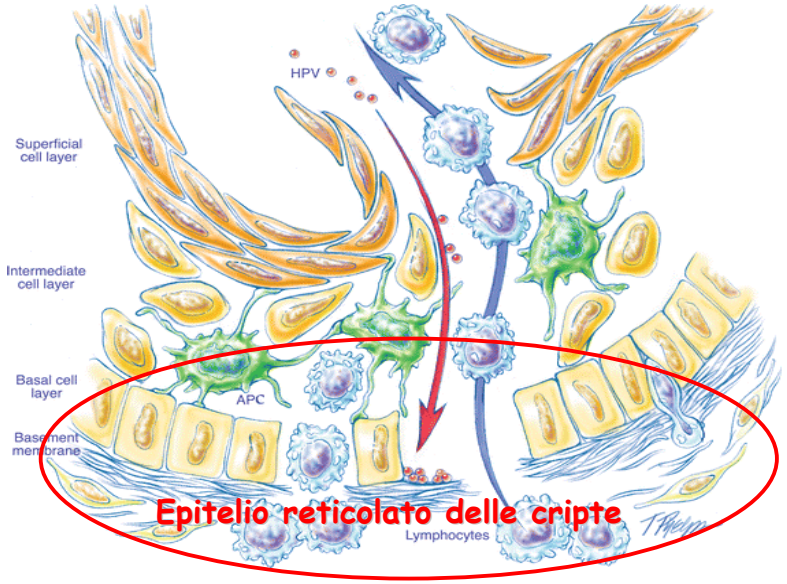
BIOLOGICAL SUSCEPTIBILITY



Canale anale

Tonsilla palatina





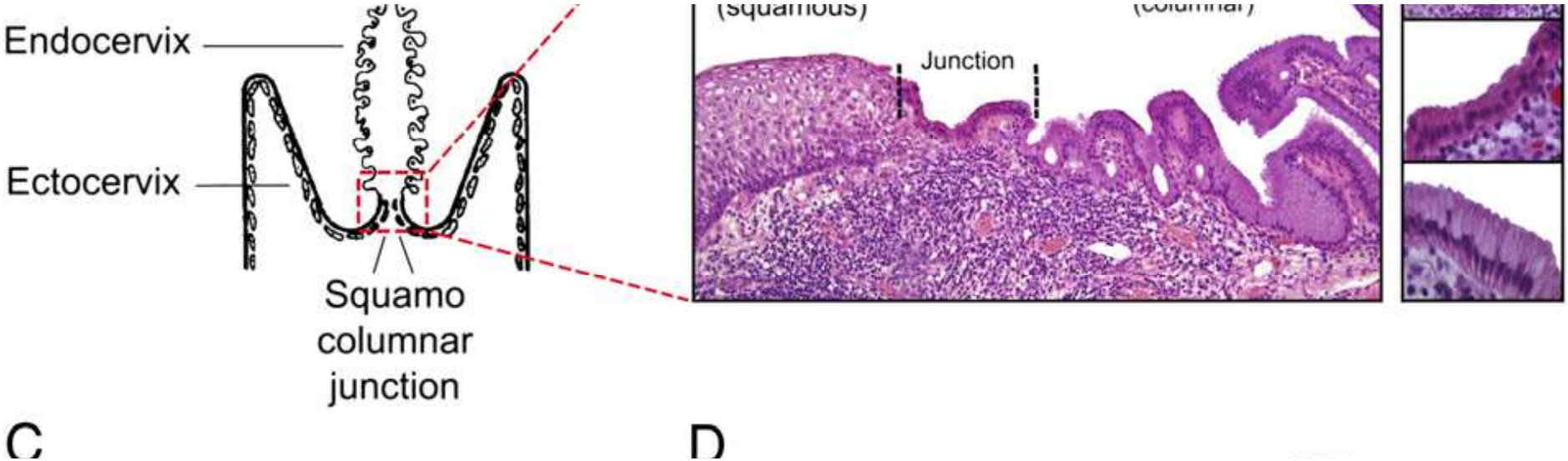
(cortesia A.Del Mistro)

A NEW MODEL OF CERVICAL CARCINOGENESIS ?

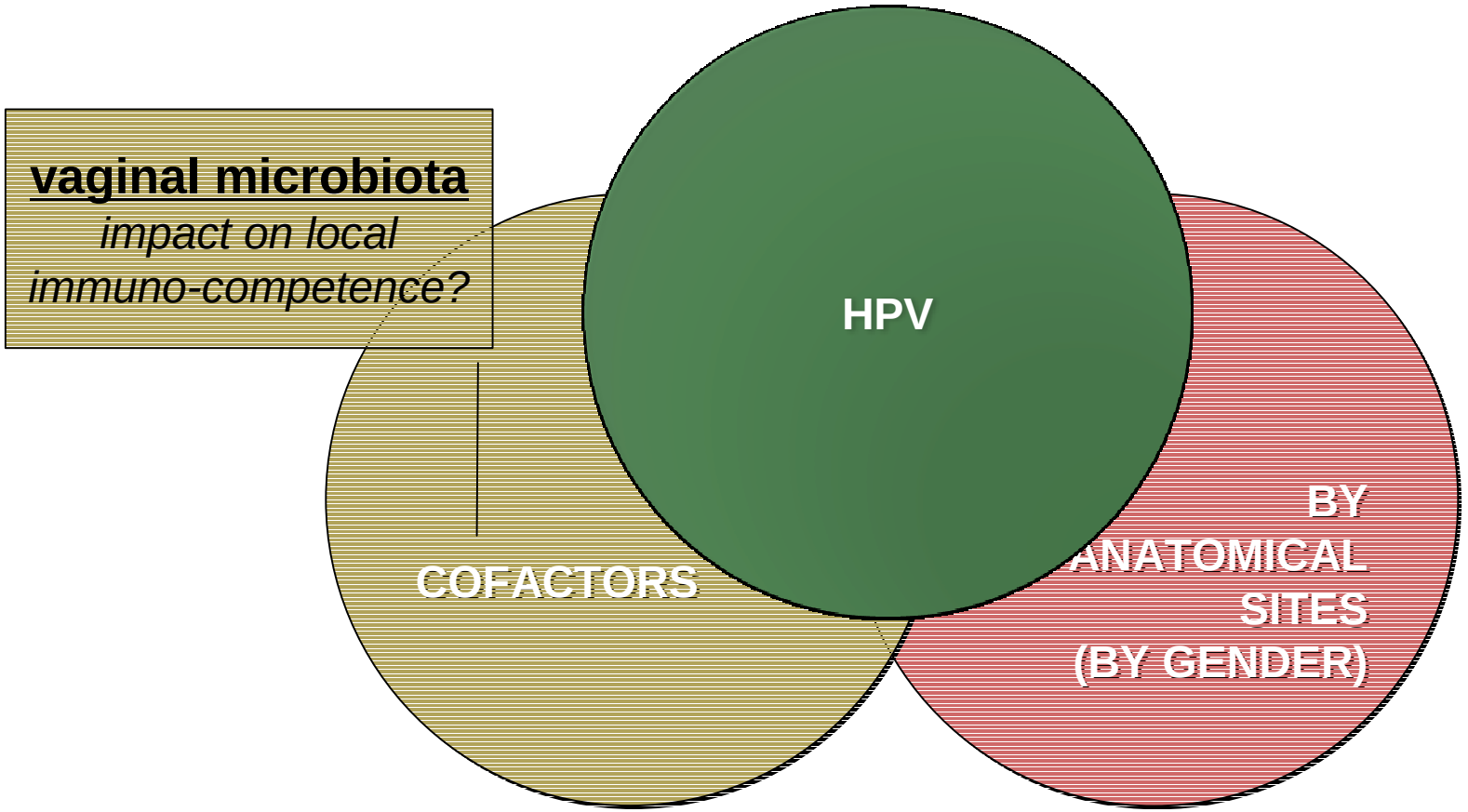
SCJ population

(cancer progenitor cells)

Carcinogenic HPV-related CINs and cervical cancers are linked to a small, discrete cell population that localizes to the SC junction of the cervix, expresses a unique gene expression signature, and is not regenerated after excision. (M.Herfs, 2012)



FACTORS RELATED
TO CLINICAL VULNERABILITY TO HPV-PERSISTENCE



High-risk HPV infection is necessary **but not sufficient** for the development of cervical preinvasive and invasive

VULNERABILITY

1.tobacco smoking,
2.oral contraceptive use,
3.parity,
4.biologic susceptibility of the immature cervical epithelium inherent in adolescents:

- SCJ cell, *cancer progenitor cells,* fetal origin, single layer of cuboidal epithelial cells.
- vaginal micro-environment (local microbiome)

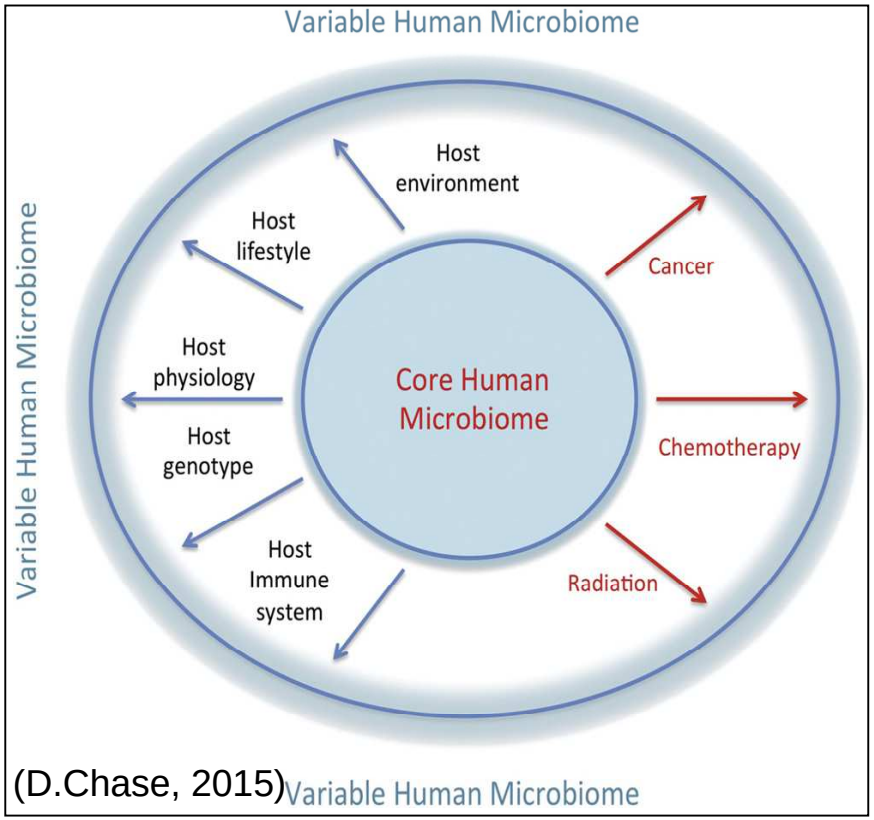
Does the **microbiome** contribute to the vulnerability to HPV infections or persistence? or influences genes such as p53, pRb…?

MICROBIOMA

"the ecological community (microbial ecosystem) of commensal, symbiotic and pathogenic microorganisms that literally share our body space"

Microbial communities populate all mucosal surfaces, the composition of each community varies from site to site within the body depending upon a myriad of host-derived factors.

Biological functions:
- nutrient absorption,
- establishing/regulating the immune system,
- protecting against pathogenic insults.



(D.Chase, 2015)

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Table I. Microbiota changes that have been observed in human cancer cases

Cancer type	Sampling site	Microbiome changes in cases compared with controls
Oral squamous cell carcinoma	Saliva	Incr: *Capnocytophaga gingivalis*, *C. ochracea*, *Eubacterium sabureum*, *Leptotrichia buccalis*, *Streptococcus mitis*
Barrett's esophagus and esophageal cancer	Saliva, biopsied tissue	Incr: *Campylobacter consisus*, *C. rectus*, *Treponema denticola*, *S. anginosus*, *S. mitis*; Decr: *Helicobacter pylori*
Pancreatic cancer	Saliva	Incr: $n = 31$ including *S. mitis* and *Neisseria elongata*; Decr: $n = 25$
Gall bladder cancer	Bile culture	Incr: *Salmonella typhi*, *S. paratyphi*; bile usually free from bacteria but infected in cases
Colorectal cancer	Feces, biopsied tissue	Incr: *S. bovis*, Streptococcus spp., *Escherichia coli*, *Fusobacterium nucleatum*, Clostridium, Bacteroides; Decr: Lactobacillus, butyrate-producing bacteria (including Roseburia and Fecalibacterium), Microbacterium, Anoxybacillus, *Akkermansia muciniphilia* (a mucin-degrading species)

Scott J.Bultman, 2013

MICROBIOMA

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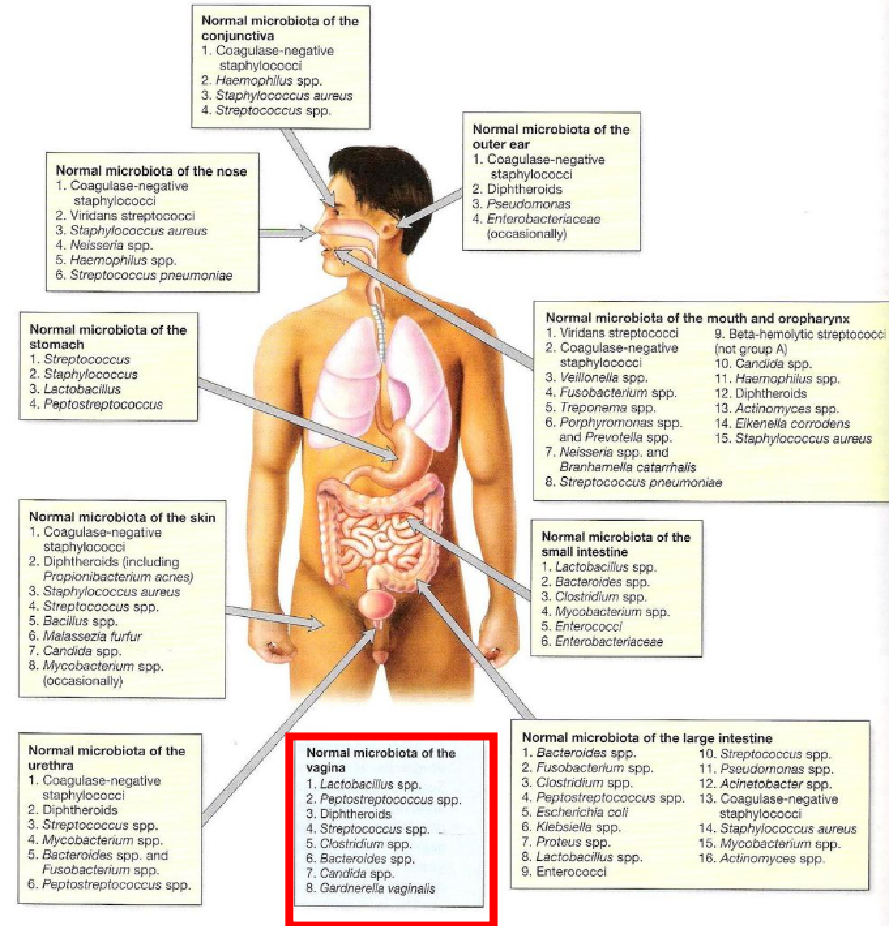


The human body harbors ≥10^{14} microbial cells

VAGINAL MICROBIOMA (VM)

✓Variability through the women's menstrual cycle and reproductive age (also by smoke and oral contraception), usually populated by **Lactobacillus** spp.:

→regarded to ensure a low pH (first-line of defense against pathogenic agents)

→providing peptides, metabolites inhibiting bacterial growth and colonization of amine-producing bacterial species;

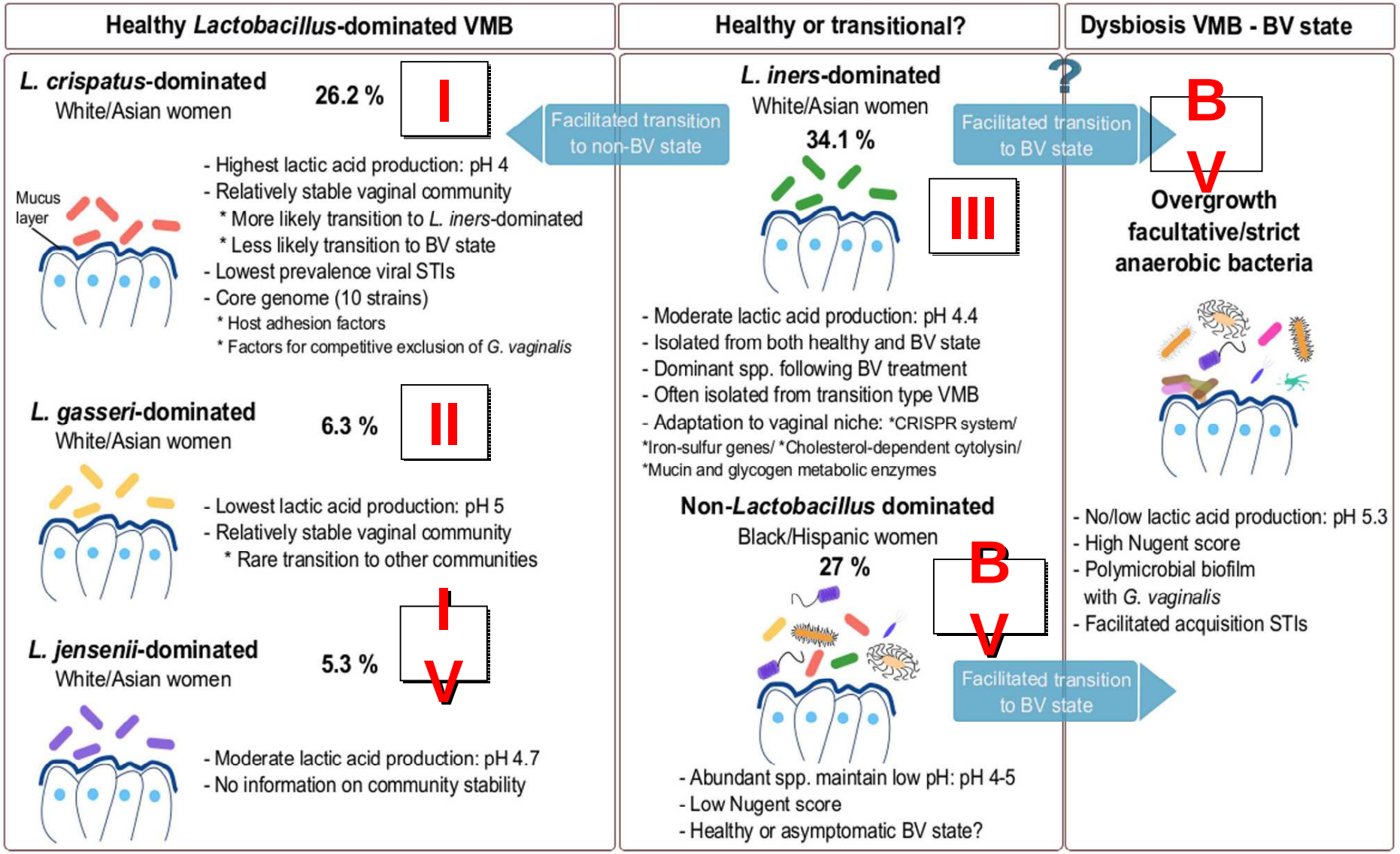
→adherence of vaginal lactobacilli to host cells has been shown to prevent colonization by pathogenic microorganisms.

→promote health in the vaginal ecosystem via immunomodulation mechanisms

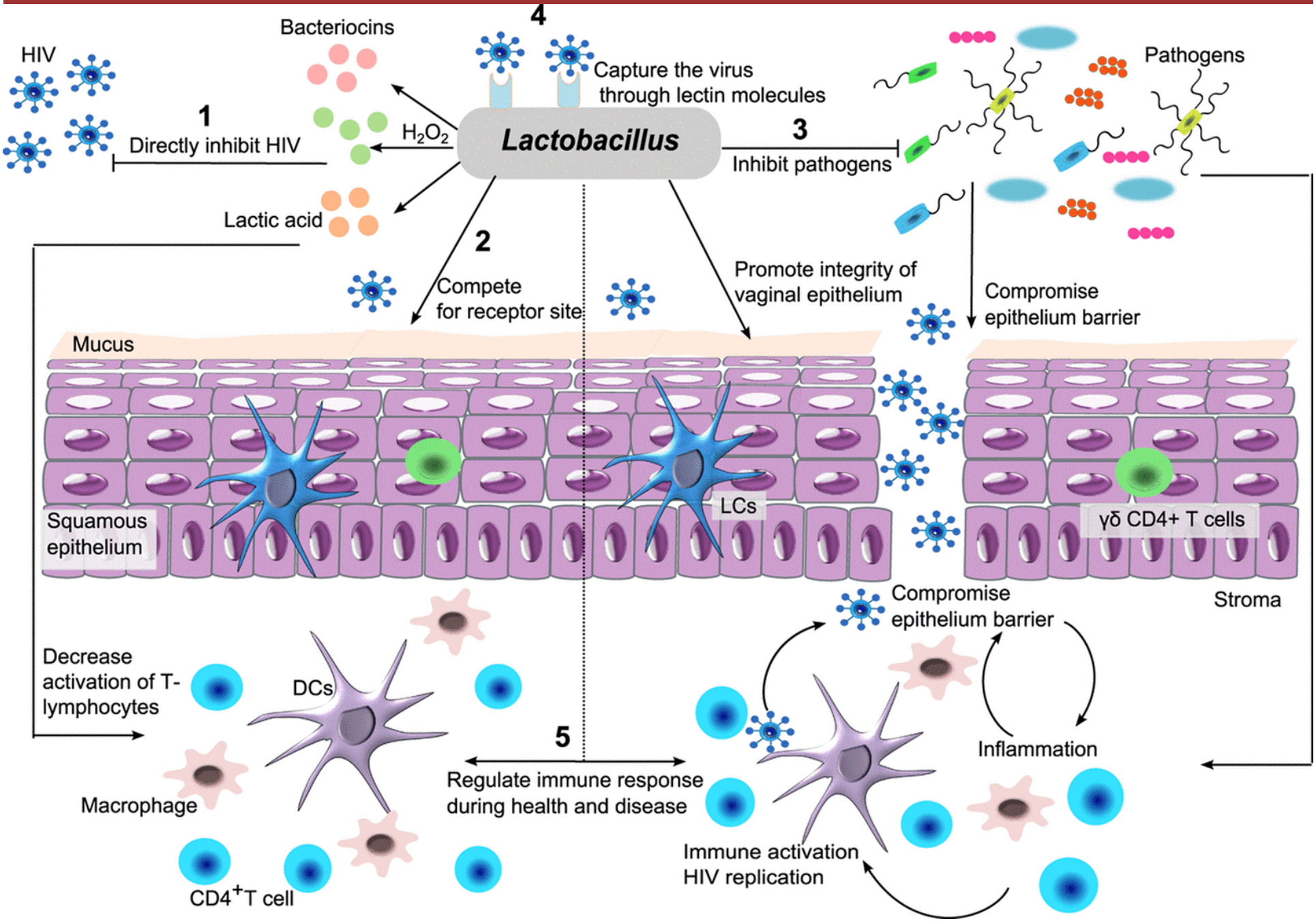
✓**Lactobacillus-dominated microbiota appears to be a good biomarker for a healthy vaginal ecosystem**

CTS (community state types)

- I → dominated by L. crispatus
- II → " L. gasseri
- III → " L. iners
- IV → BV *(associated anaerobic bacteria)*
- V → dominated by L. jensenii



(Petrova M, 2015)


HIV
Bacteriocins
4
Capture the virus
through lectin molecules
Pathogens
1
Directly inhibit HIV
H_2O_2
Lactobacillus
3
Inhibit pathogens
Lactic acid
2
Compete
for receptor site
Promote integrity of
vaginal epithelium
Compromise
epithelium barrier
Mucus
Squamous
epithelium
LCs
γδ CD4+ T cells
Stroma
Compromise
epithelium barrier
Decrease
activation of T-
lymphocytes
DCs
5
Regulate immune response
during health and disease
Inflammation
Macrophage
Immune activation
HIV replication
CD4+T cell

BACTERIAL VAGINOSIS (BV)

the most common vaginal disorder (depletion of Lactobacillus spp. with presence of anaerobic species such as Gardnerella, Megasphera, Sneathia..) may play a role in cervical carcinogenesis

BV increase susceptibility to many STDs: Neisseria gonorrhoeae, Chlamydia trachomatis, HSV-1 and 2, and HIV (Wiesenfeld HC, 2003; Atashili J,2008; Allsworth JE,2008)

↓

- **activates the proinflammatory transcription factor:** nuclear factor (NF)-kB, tumor necrosis factor alpha, interleukin (IL)- 6 and IL-8, macrophage inflammatory protein 3 alpha and regulated on activation, normal T cell expressed and secreted (Anahtar MN 2015)
- **biofilm production** → virulence mechanism that enhances bacterial attachment to epithelial surfaces → increase recurrent rate

WHY BV IS AT RISK FOR HPV?

Possible **biological mechanisms** *(Petrova M, 2013)* include:

1. activation of immune response and inflammation *(modification of the immunological environment of the vaginal niche; higher genotoxic damage through oxidative metabolites)*
2. disruption of the vaginal epithelium
3. decreased Lactobacillus spp. causing **elevated pH**
   - *may arrest squamous metaplasia in the post-pubertal cervix and prolong the period in which the transformation zone is vulnerable to agents promoting dysplasia such as HPV (Hudson MM 1997)*
   - *a vaginal pH above 5 resulted in a 10–20% increased HPV risk and is associated with LSIL diagnosis (Costa Rica trial; Clarke, M 2012)*

Vaginal pH Level	N	Percent with PCR Positive HPV	Odds of Testing HPV Positive[a] (95% CI)	*p*-value (trend)
All Women[b]				
4.0	2,139	19.1	0.7 (0.7-0.8)	
4.5	17,201	23.8	ref[c]	
5.0	7,246	25.6	1.2 (1.1-1.3)	
5.5	1,976	25.2	1.2 (1.1-1.4)	< 0.001

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Vaginal pH Level	N	Percent with LSIL	Odds of LSIL Cytology[a] (95% CI)	*p*-value
All Women[b]				
4.0-4.5	19,272	2.4	ref	< 0.01
5.0-5.5	9,059	2.5	1.3 (1.1-1.6)	

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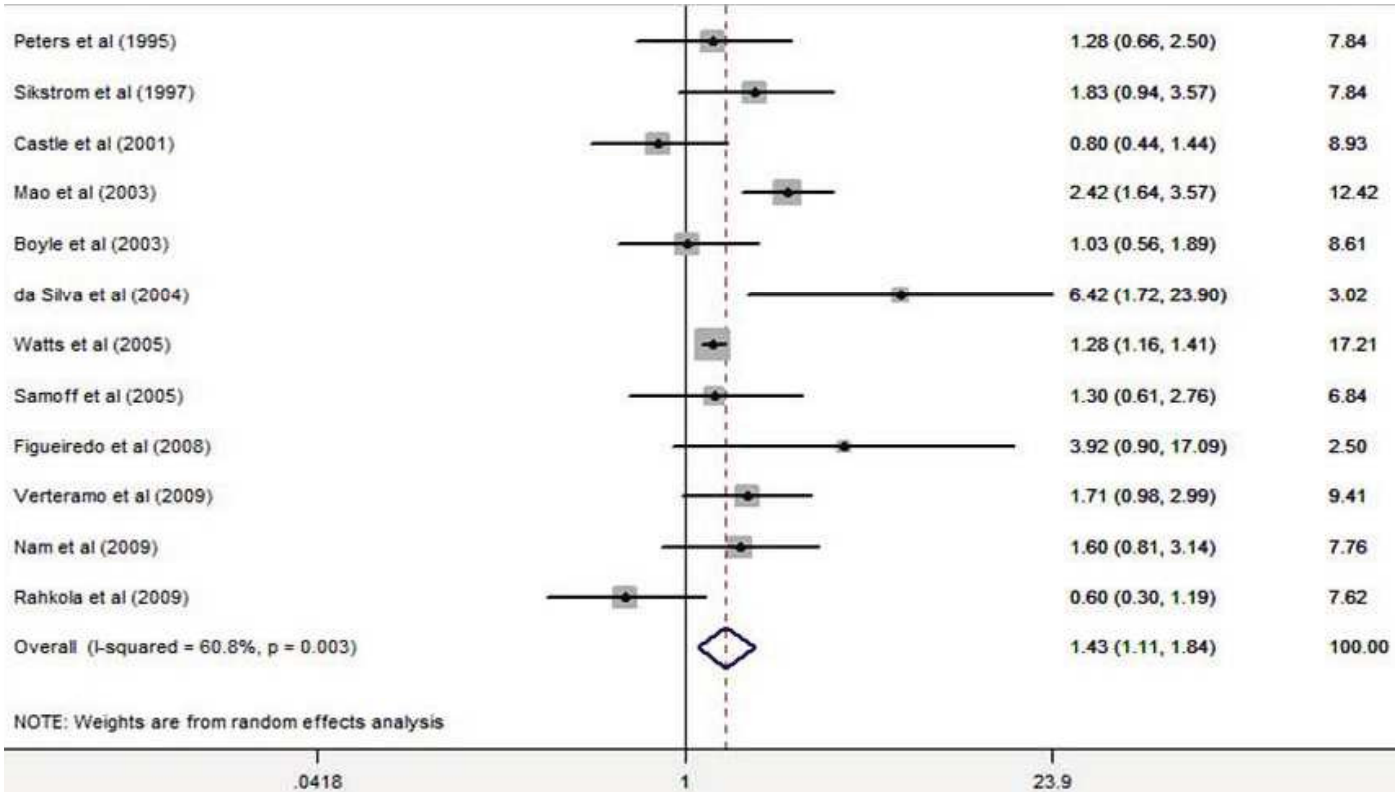
    - *a vaginal pH above 5 resulted in a 10–20% increased HPV risk and is associated with LSIL diagnosis (Costa Rica trial; Clarke, M 2012)*
4. **lowered H_2O_2 concentrations** *(protective effect against vaginal colonization by pathogenic species)*
5. **anaerobes release volatile amines** and form in combination with nitrites nitrosamines *(carcinogenic compounds capable of forming mutagenic events)*

WHY BV IS AT RISK FOR HPV?



The presence of BV was associated with higher rates of HPV infection (12 studies; OR: 1.43), ***suggesting that a diverse, Lactobacillus-depleted microbiome may contribute to HPV persistence*** (Gilet E, 2012)

WHY BV IS AT HIGH RISK FOR HPV?
REDUCTION OF L. CRISPATUS

L. crispatus-dominant microbioma

✓May be a **community protective** against the development of precancerous and cancerous lesions. (Mitra A, 2015 and 2016)

WHY BV IS AT HIGH RISK FOR HPV?
REDUCTION OF L. CRISPATUS

L. crispatus-dominant microbioma

✓May be a **community protective** against the development of precancerous and cancerous lesions.

✓CST IV (lower crispatus and anaerobic agents) was increased:

- 2 fold in women with LSIL,
- 3 fold in women with HSIL,
- 4 fold in women with invasive cervical disease

(Mitra A, 2015 and 2016)

✓Synergistic effect of a microbial pattern defined '**risky microbial pattern**' composed by:

- paucity of L. crispatus
- increased A. vaginae, G. vaginalis, and L. iners

were associated by an almost **6-fold increase** in the risk of cervical LSIL/HSIL disease (HY Ho, 2015)

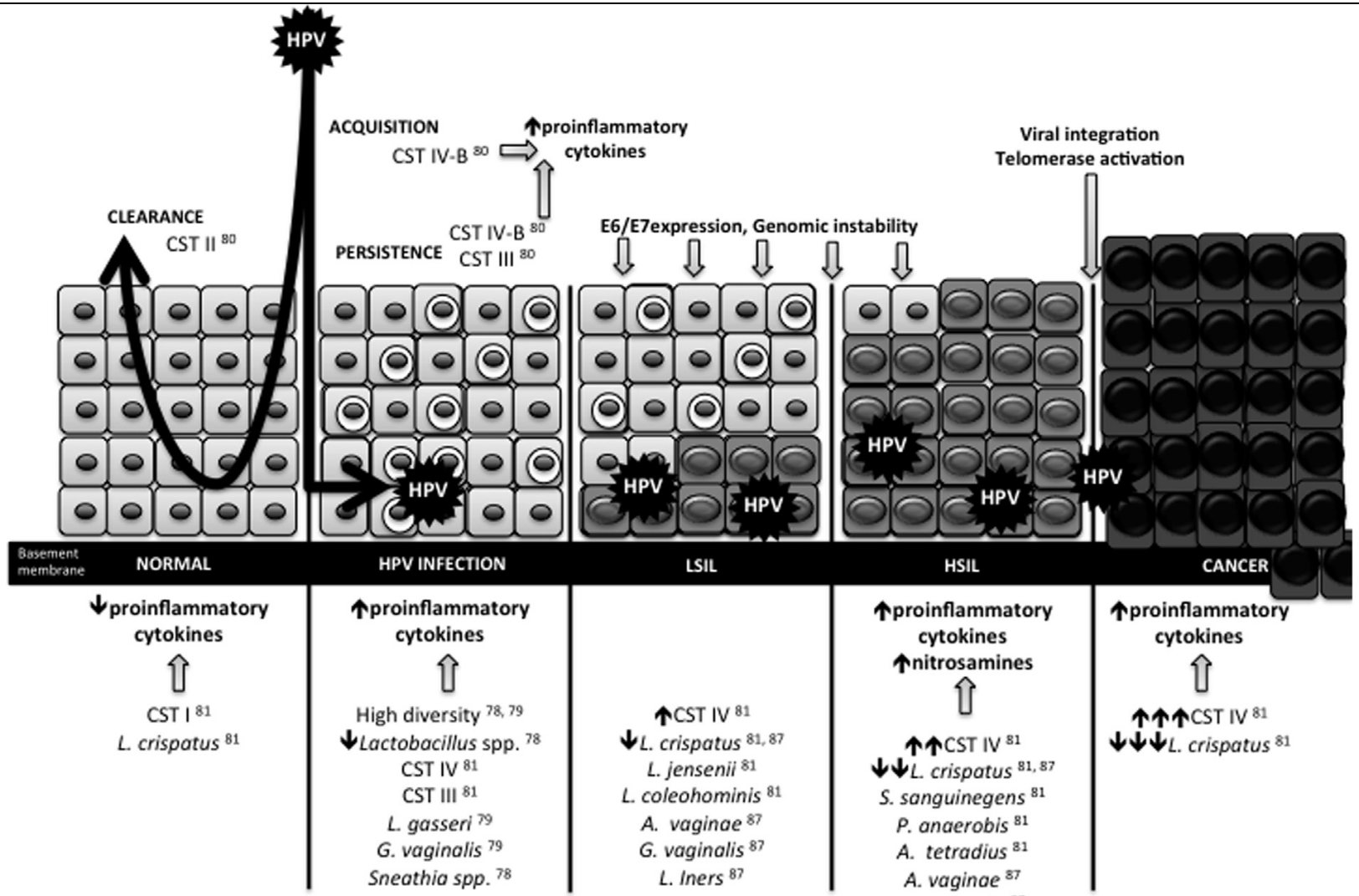
WHY BV IS AT HIGH RISK FOR HPV?
INCREASED OF L. INERS

In general, **microbiota dominated by Lactobacillus iners** may represent microenvironments with **increased risk of HPV acquisition/persistence** (Brotman 2014)

An association between microbiome diversity and CIN severity (CIN2+ vs CIN1) provided suggestive evidence that a cervical microbiome characterized by a predominance of Lactobacillus iners is associated with **high risk (OR 3.4) of CIN 2**+ in women infected with hr-HPVs (Piyathilake C, 2016)

Microbial community types		
A (*L. crispatus*-dominant)	10/21	1 (ref.)
B (*L. iners*-dominant)	24/23	
Model 1		5.72 (1.29–25.4), 0.02
Model 2		5.74 (1.43–23.1), 0.01
Model 3		6.39 (1.52–26.7), 0.01

BACTERIAL VAGINOSIS (BV)



(Kyrgiou M, 2016)

CONCLUSION

- **It is currently unclear if a CST IV microbiome is a causal factor in HPV persistence/ CIN progression or a consequence**

- **Modified vaginal microbiota with increased L. iners and reduced L. crispatus may be considered as an "high-risk" vaginal profile for HPV infection. (***it may be that* ***only*** *certain strains of L. iners predispose to HPV acquisition and persistence, or conversely* ***only*** *certain L. crispatus strains are protective).*

- **Probiotic users** (54 HPV-positive and low-grade SIL-positive women) were twice as likely to have cervical lesion clearance, although no change in HPV detection was observed (Verhoeven et al., 2013).

Clinical trials investigating the impact of probiotics on preventing or alleviating symptoms following gynecologic cancer treatment

References	No. of patients (probiotics/control)	Type of study	Bacterial strain investigated/formulation/frequency of treatment	Clinical setting	% of patients with treatment-induced diarrhea	Severity of treatment-induced diarrhea	Anti-diarrheal medication use following probiotic treatment	Stool consistency
Chitapanarux et al. [64]	63 (32/31)	R, DB, PC	*L. acidophilus* plus *Bifidobacterium bifidum*/capsule/twice a day before meals (morning and evening)	Women undergoing radiation with concurrent cisplatin for cervical cancer	–	–	Use decreased in placebo group ($p = 0.03$)	Improved in probiotic group ($p < 0.001$)
Delia et al. [63]	482 (243/239)	DB, PC	VSL#3 (combination of *L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *L. salivarius* subsp. *thermophilus*)/sachet/three times a day	Patients undergoing adjuvant radiation after surgery for sigmoid, rectal, or cervical cancer	51.8% of placebo group vs. 31.6% of probiotic group ($p < 0.001$)	Less severe in probiotic group ($p < 0.001$)	Mean time to medication use was longer in probiotic group ($p < 0.001$)	–
Giralt et al. [66]	85 (44/41)	R, DB, PC	*L. casei* DN-114 001/probiotic drink/three times a day	Women undergoing radiation for cervical carcinoma or endometrial adenocarcinoma	–	–	–	Improved in probiotic group ($p = 0.04$)
Demers et al. [65]	229 (140/89) Standard dose probiotic: n = 81 High dose probiotic: n = 59	R, DB, PC	*L. acidophilus* LAC-361 plus *Bifidobacterium longum* BB-536/capsule/standard dose of 1.3 billion CFU twice a day OR high dose of 10 billion CFU three times a day	Patients undergoing radiation, with or without chemotherapy, for gynecologic, rectal, or prostate cancer	–	Less severe in group receiving standard dose of probiotic ($p = 0.04$)	–	–

D. Chase et al. / Gynecologic Oncology 138 (2015)

GRAZIE…

Table II. Single microbes that can drive human cancer

Microbe	Cancer type(s)
Helicobacter pylori	Gastric adenocarcinoma, gastric lymphoma, esophageal adenocarcinoma
Human papillomavirus (HPV)	Anogenital carcinomas, oropharyngeal carcinoma
Epstein-Barr virus (EBV)	Lymphomas, nasopharyngeal carcinoma
Human immunodeficiency virus (HIV)	Lymphomas, Kaposi's sarcoma
Hepatitis B virus	Hepatocellular carcinoma
Hepatitis C virus	Hepatocellular carcinoma, lymphomas
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Human herpesvirus 8 (HHV-8)	Kaposi's sarcoma

Scott J.Bultman, 2013

BV and HPV

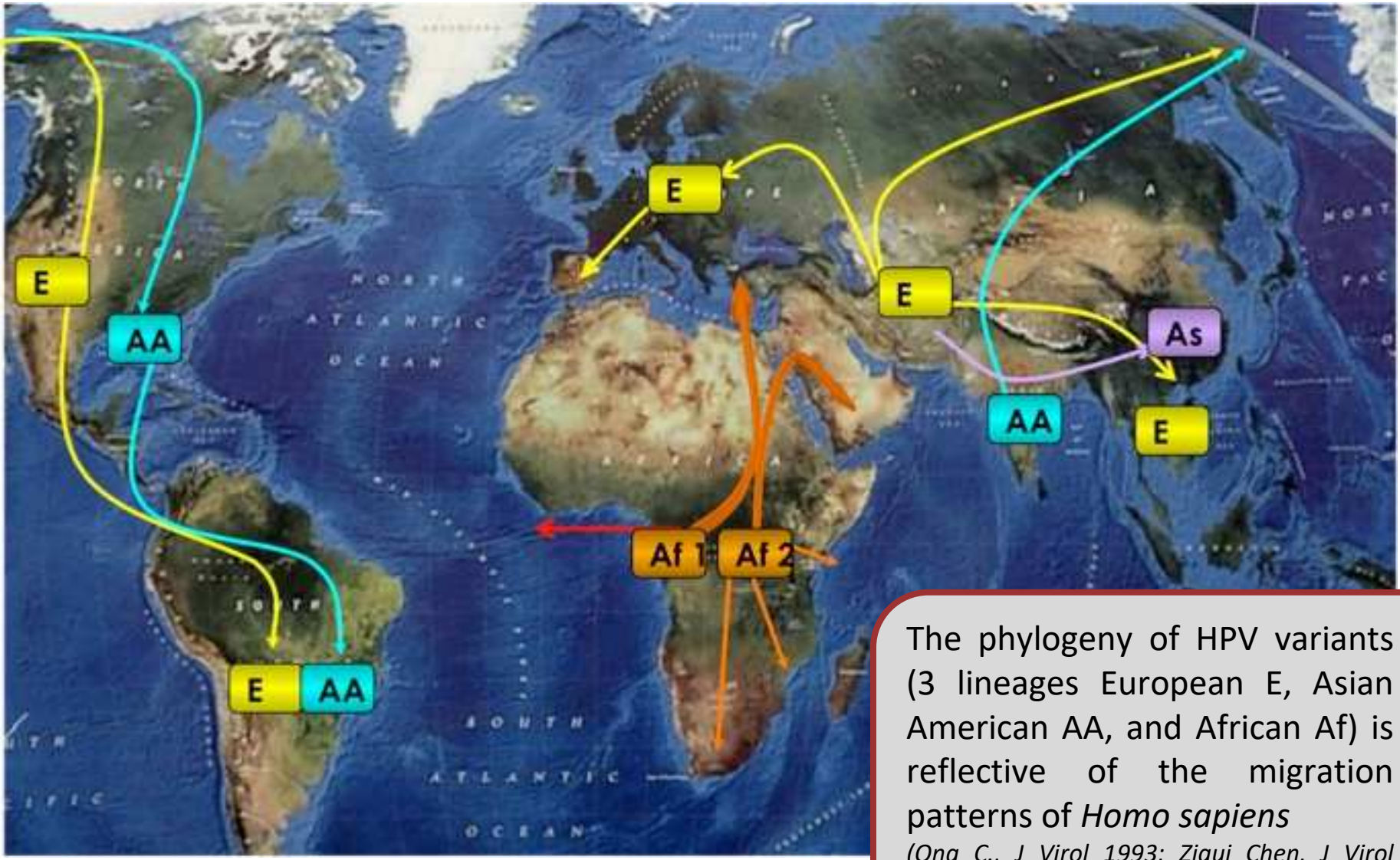
BV was associated with increased odds for prevalent (OR = 1.14) and incident (OR = 1.24) HPV infection and with delayed clearance of infection (HR = 0.84).

(C King 2011)

First, it may be possible to develop rapid bedside tests,

Second, it is possible to manipulate the VM using probiotics

HPV: VARIABILITY



(courtesy Flavia Lillo)

The phylogeny of HPV variants (3 lineages European E, Asian American AA, and African Af) is reflective of the migration patterns of *Homo sapiens*

(Ong C., J Virol 1993; Zigui Chen, J Virol 2008)

HPV: VARIABILITY

From the evolutionary biological standpoint, HPVs are very **successful infectious agents**: they induce chronic infections without systemic sequelae and periodically shed large amounts of infectious virus for transmission to naive individuals.

To achieve this lifestyle,
HPV must avoid the host defense systems

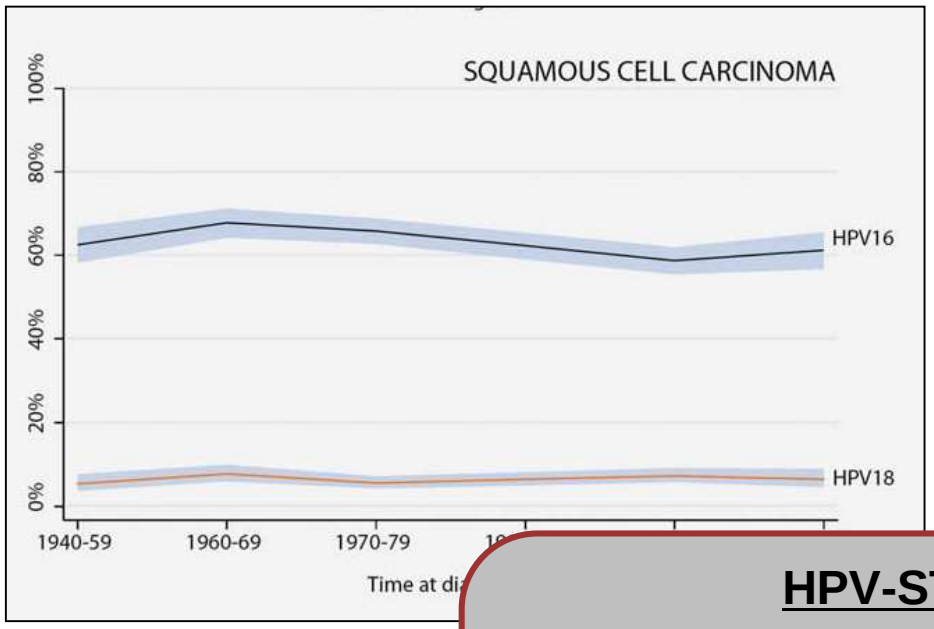
Given time, HPVs and their host reach a **state of equilibrium** where the host is not greatly disadvantaged in its reproductive capacity by virus infection, and the virus is not too limited in reproductive capacity by the host immune response.

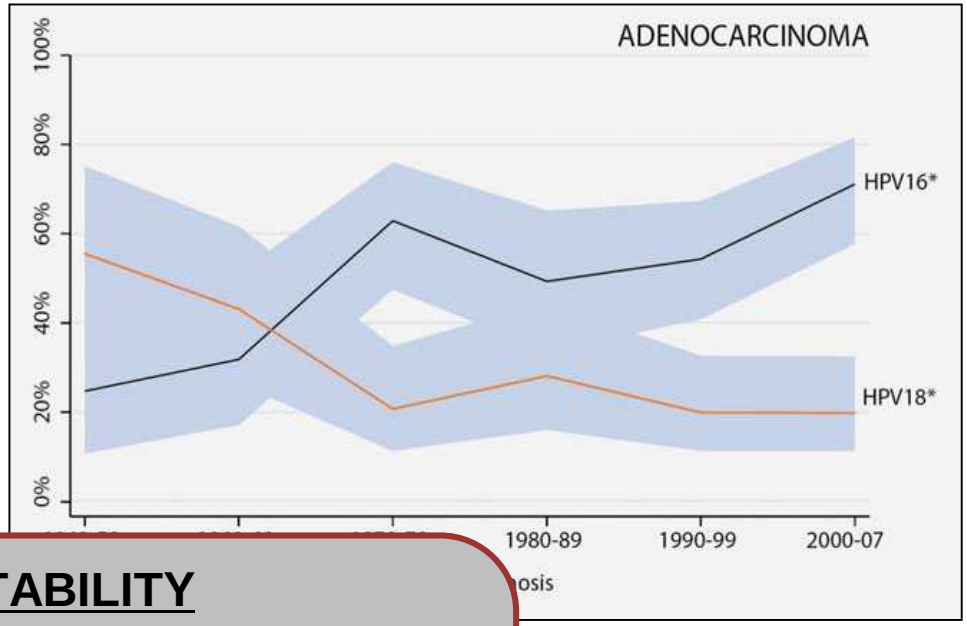
(Ian Frazer, 2008)

HPV: VARIABILITY

Time trends of human papillomavirus types in invasive cervical cancer, from 1940 to 2007

(5,737 ICC cases recruited from 11 countries)





HPV-STABILITY

PRO: genetically stable DNA viruses with low mutation rates *(*Rector A 2007*)*

CONS: 1-changes in the HPV background prevalence after vaccine and screening programmes (Alemany 2013);

2-coinfection with HIV (Clifford G 2006).