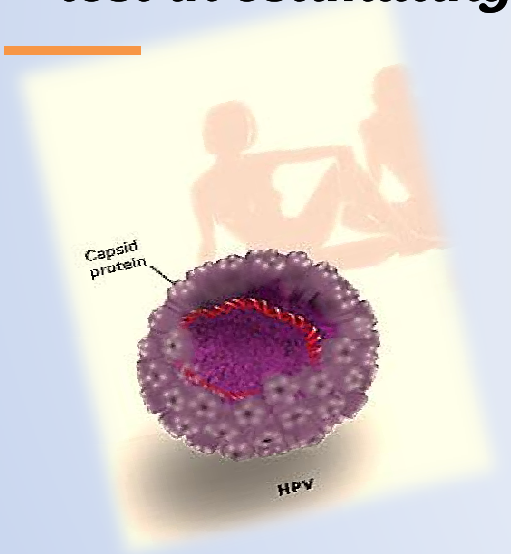
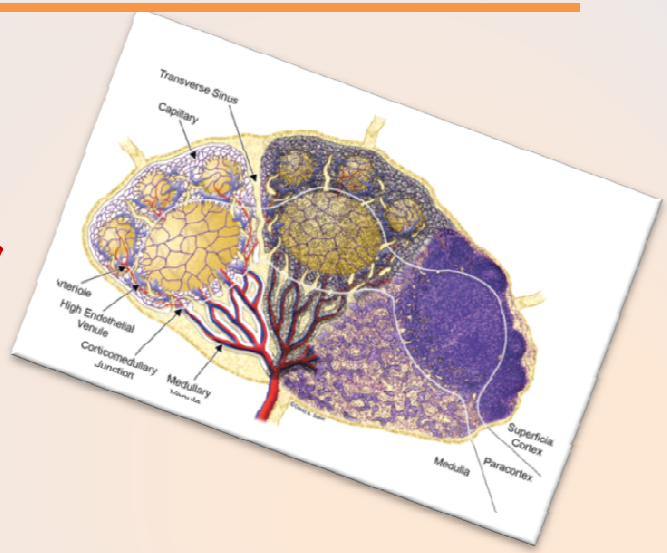
Università degli Studi di Padova
Dipartimento di Scienze Ginecologiche e della Riproduzione Umana
Scuola di Specializzazione in Ginecologia e Ostetricia
Direttore Prof. Giovanni Battista Nardelli

Usefulness, methods and rationale of lymph nodes HPV-DNA test in estimating recurrence risk of early stage cervical cancer.

Systematic Review



- *Dott. Marco Noventa*



HPV molecular biology

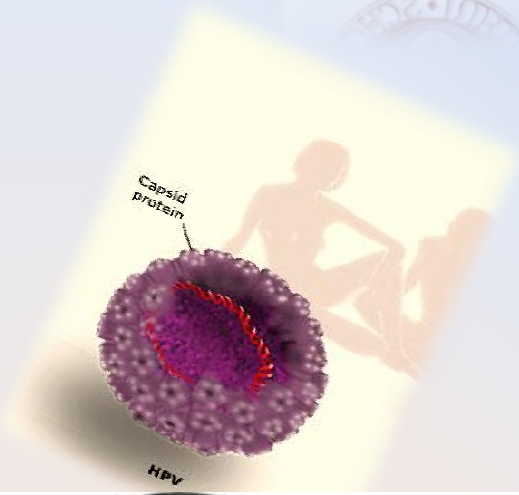
HPV family and genotypes

Contents lists available at SciVerse ScienceDirect
Vaccine
journal homepage: www.elsevier.com/locate/vaccine

Review

The Biology and Life-Cycle of Human Papillomaviruses

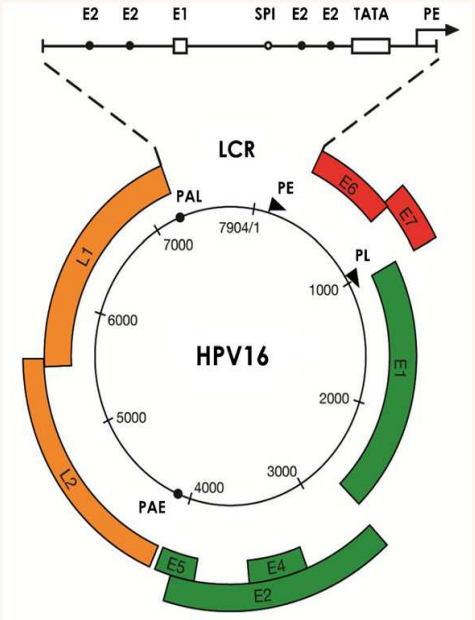
John Doorbar[a,*], Wim Quint[b], Lawrence Banks[c], Ignacio G. Bravo[d], Mark Stoler[e], Tom R. Broker[f], Margaret A. Stanley[g]

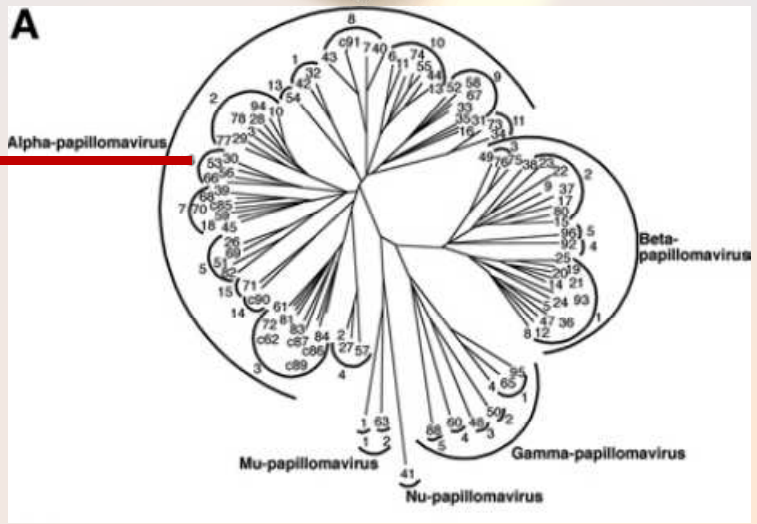


A

Genus + Species	Type	Invasive Cervical Cancer	IARC Category	Squamous Cell Carcinoma	Adeno Carcinoma	Tropism
Alpha 1	HPV32		3			mucosal
	HPV42					
Alpha 2	HPV3					cutaneous
	HPV10					
	HPV28		3			
	HPV29		3			
	HPV77		3			
	HPV94					
	HPV117					
	HPV125					
Alpha 3	HPV61	0.01	3			mucosal
	HPV62		3			
	HPV72		3			
	HPV81		3	0.4		
	HPV83		3	0.4		
	HPV84		3			
	HPV86		3			
	HPV87		3			
	HPV89		3			
	HPV102					
	HPV114					
Alpha 4	HPV2		3			cutaneous
	HPV27		3			
	HPV57		3			
Alpha 5	HPV26	0.37	2B	0.22		mucosal
	HPV51	1.25	1	0.75	0.54	
	HPV69	0.08	2B			
	HPV82	0.07	2B	0.26		
Alpha 6	HPV30	0.37	2B			
	HPV53	0.26	2B	0.04		
	HPV56	0.84	1	1.09		
	HPV66	0.08	2B	0.19		
Alpha 7	HPV18	10.28	1	11.27	37.3	
	HPV39	1.67	1	0.82	0.54	
	HPV45	5.68	1	5.21	5.95	
	HPV59	1.08	1	1.05	2.16	
	HPV68	1.04	2A	0.37		
	HPV70	0.11	2B			
	HPV85		2B			
	HPV97					
Alpha 8	HPV7		3		41.62	cutaneous (mucosal)
	HPV40		3		1.08	
	HPV43				0.54	
	HPV91	0.01	3		1.08	
Alpha 9	HPV16	61.35	1	54.38		mucosal
	HPV31	3.35	1	3.82	0.54	
	HPV33	3.83	1	2.06		
	HPV35	1.94	1	1.27		
	HPV52	2.71	1	2.25		
	HPV58	2.22	1	1.72		
	HPV67	0.31	2B			
Alpha 10	HPV6	0.11	3	0.07		mucosal
	HPV11	0.02	3	0.07		
	HPV13		3			
	HPV44	0.01	3			
	HPV74	0.01	3			
Alpha 11	HPV34	0.07				mucosal
	HPV73	0.52		0.49		
Alpha 12	HPV73					
Alpha 13	HPV54					
Alpha 14	HPV71					mucosal
	HPV90		3			
	HPV106		3			

B





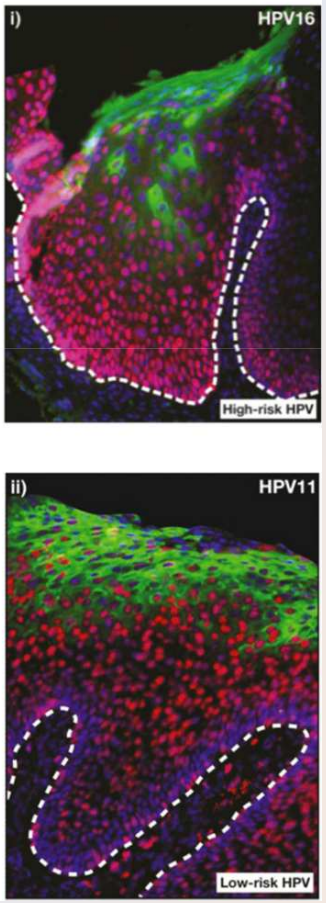
Doorbar J et al. Vaccine. 2012

HPV molecular biology

A

	High-Risk Alpha	Low-Risk Alpha
E6	encodes E6* products	no E6* products
	binding and degradation of… •p53 •specific PDZ-domain proteins (e.g. Dlg, MAGI-1, Scribble)	weaker binding (no degradation) of… •p53 •no binding of PDZ-domain proteins
	interact with the E6AP ubiquitin ligase inhibition of p53 transactivation and acetylation	
	inhibition of apoptosis	unknown
	bypass of growth arrest following DNA damage	normal growth arrest following DNA damage
	inhibition of keratinocyte differentiation	unknown
	inhibition of interferon response	weaker inhibition of interferon response
	activation of signaling pathways… •Akt •Wnt •Notch •mTORC1	unknown
	telomerase activation	no activation
	c-myc activation	no activation
E7	binding and degradation of… •pRb •p107 •p130	weaker binding (no degradation) of… •pRb •p107 •E2F1
	binding (no degradation) of… •E2F1 •Cullin2 •HDAC	binding of… •p130
	binding of regulatory proteins including E2F6, p600, HAT, PP2A induction of cell cycle entry and DNA synthesis role in genome amplification	
	induction of genome instability	no stimulation of instability
	suppression of STAT-1 function	no suppression
	immortalization and transformation functions	no such functions
	activation of signaling pathways… •Akt	unknown

B



Alpha Lr-HPV

- Associated with cutaneus and mucosal genital lesions
- Recurrent respiratory papillomatosis

Alpha Hr-HPV

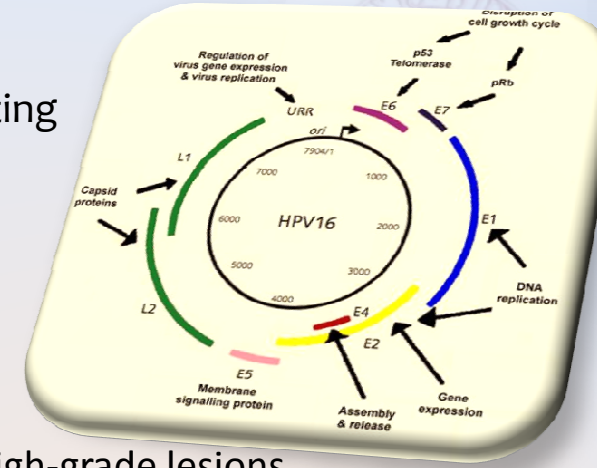
associated with different neoplasia:

- Cervix, Vulva, Vagina, Endocervix
- Head and neck
- Anus
- Penis
- Oropharynx

Bosh FX et al. Vaccine. 2013

HPV molecular biology

Life Cycle of Hr-Risk HPVs in Cervical Epithelium

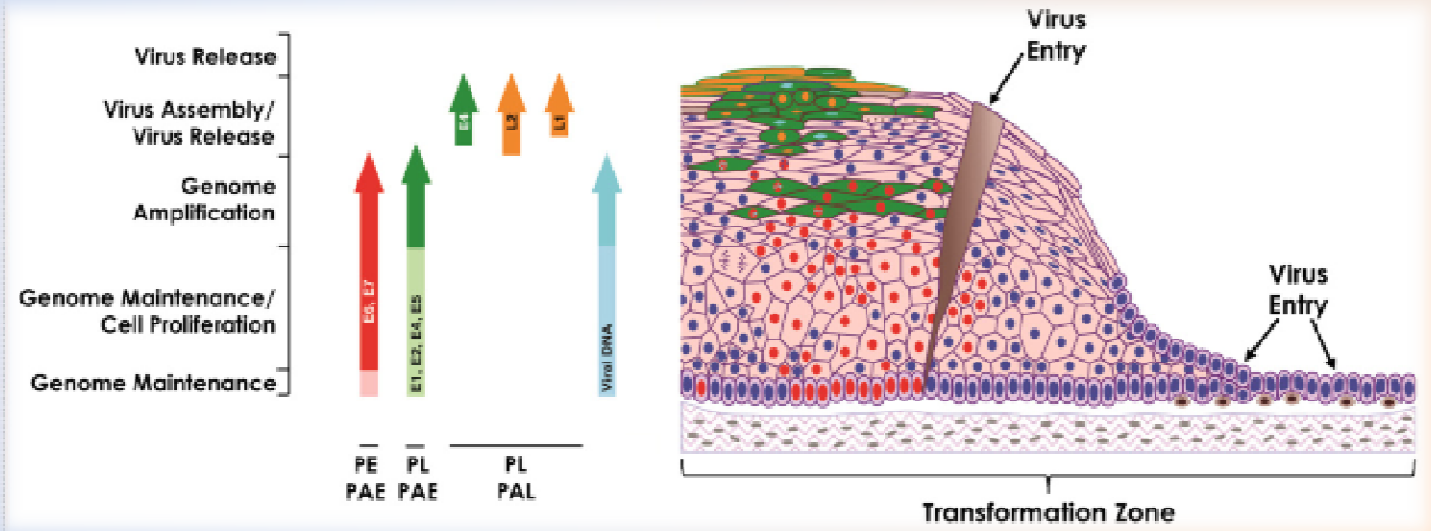


E6/E7 mediate proliferation of the basal and para-basal cells, facilitating lesion growth

Deregulation of **E6/E7** expression is critical in determining neoplastic grade

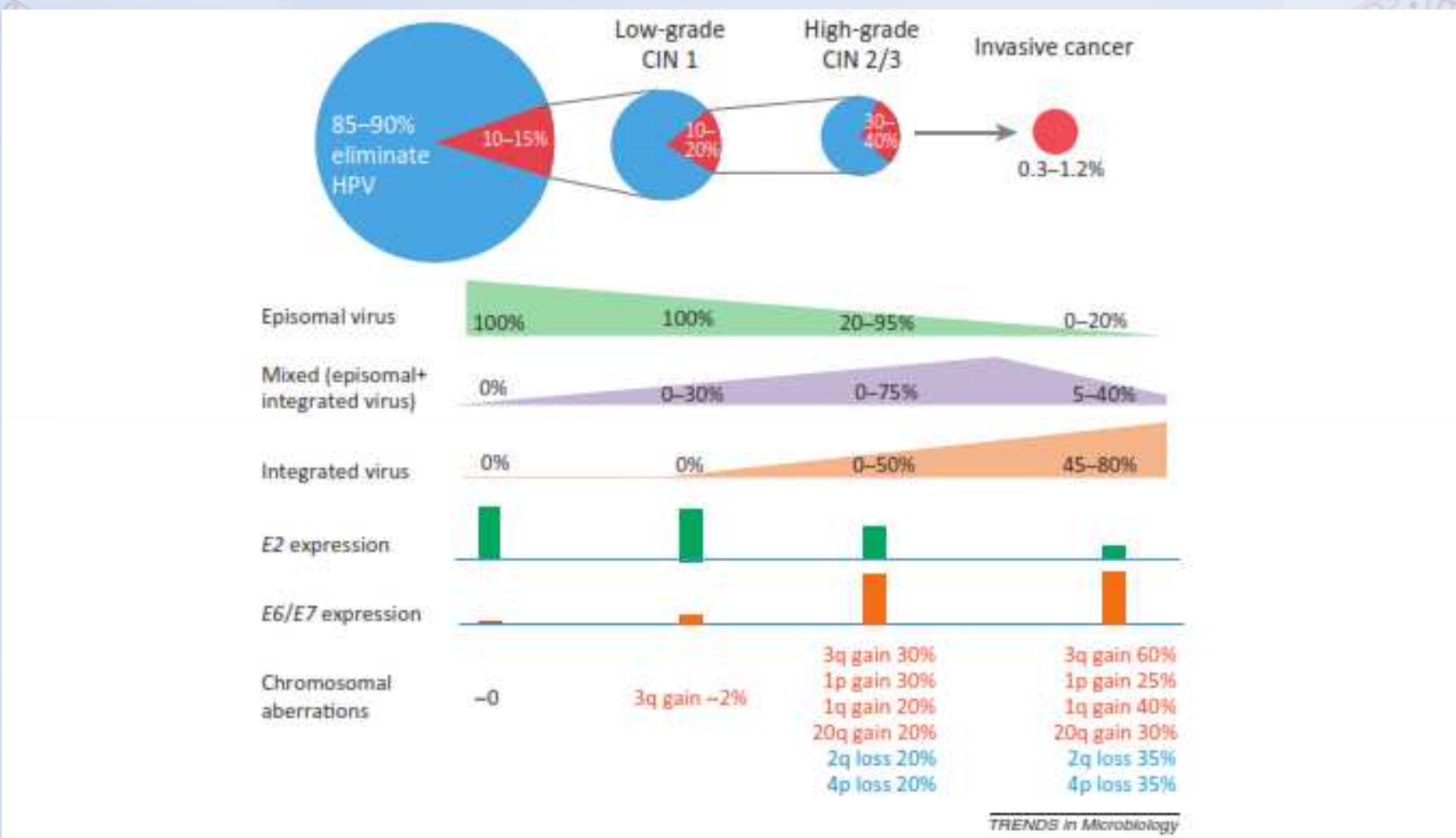
Initial viral replication in the basal cells requires **E1** and **E2** proteins.

Integration of the viral genome into the cell genome occurs in many high-grade lesions, although cancer can arise from cells exclusively containing **episomes**



Doorbar J et al. Vaccine. 2012

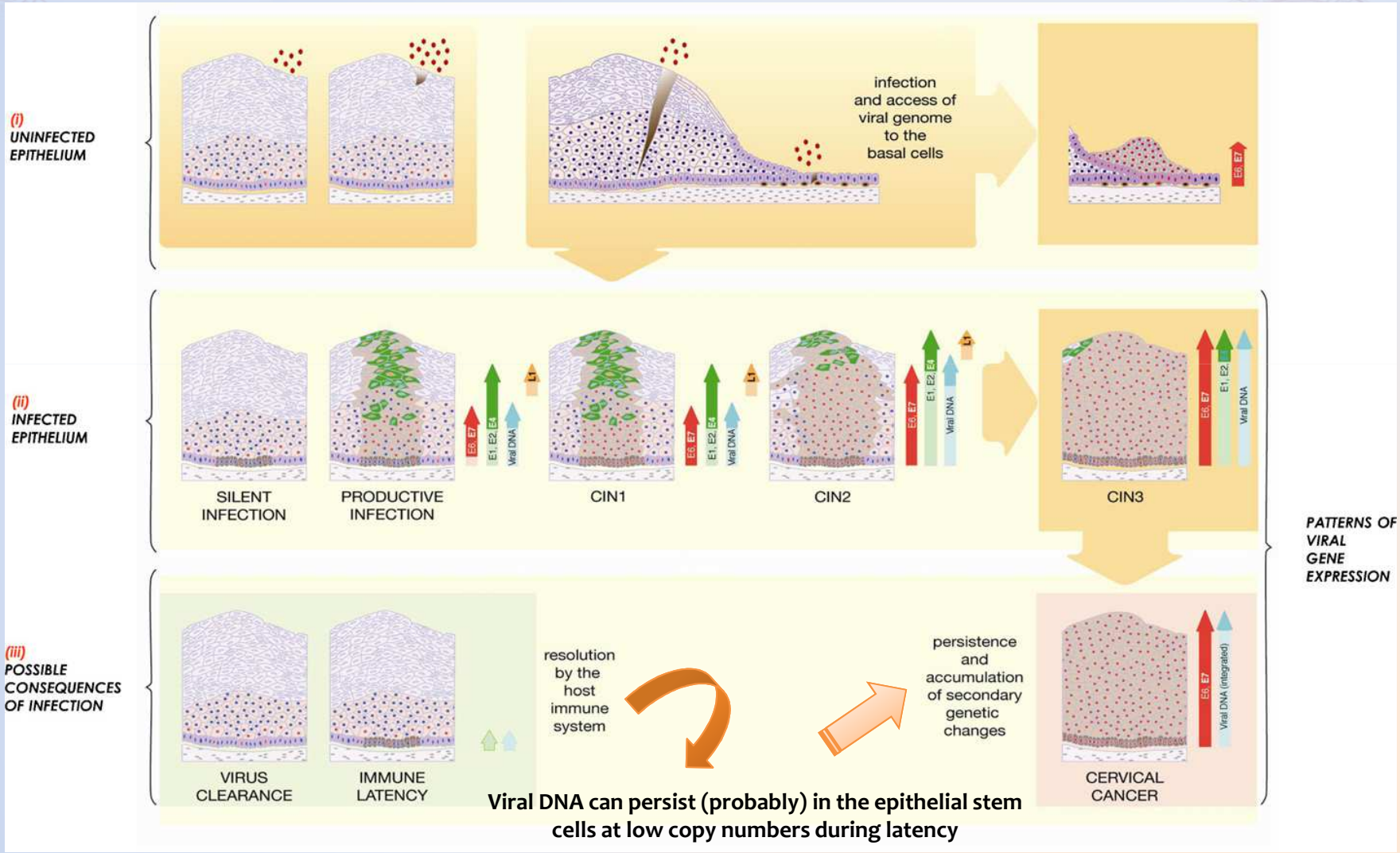
HPV molecular biology



Progression of human papillomavirus (HPV) cervical infection to cancer

Shulzhenko N et al. Trends in Microbiology. 2016

HPV molecular biology

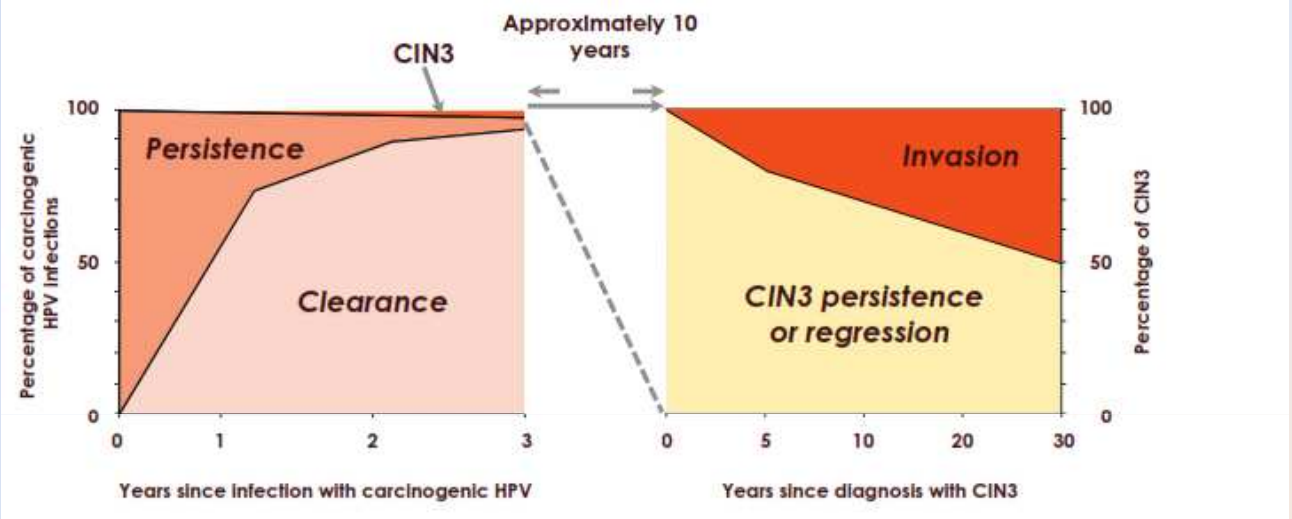


Viral DNA can persist (probably) in the epithelial stem cells at low copy numbers during latency

Bosh FX et al. Vaccine. 2013

HPV molecular biology

Natural history of HPV cervical infection



Infection by HPVs eludes the immune response by down-regulation of multiple pathways, **inhibition of Langerhans cell activation**, and **inadequate recruitment of Dendritic cells**

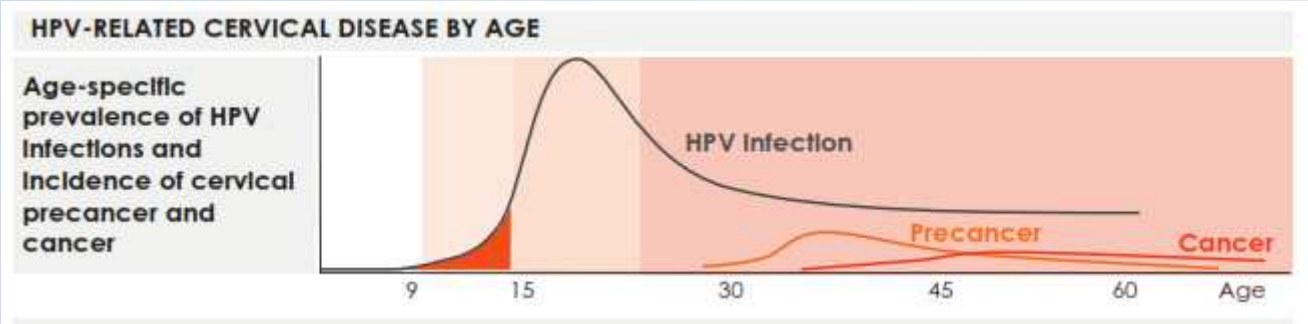
Patients affected by CC have probably a **reduced or non-existent T-cell response** to the antigens of detected HPV type

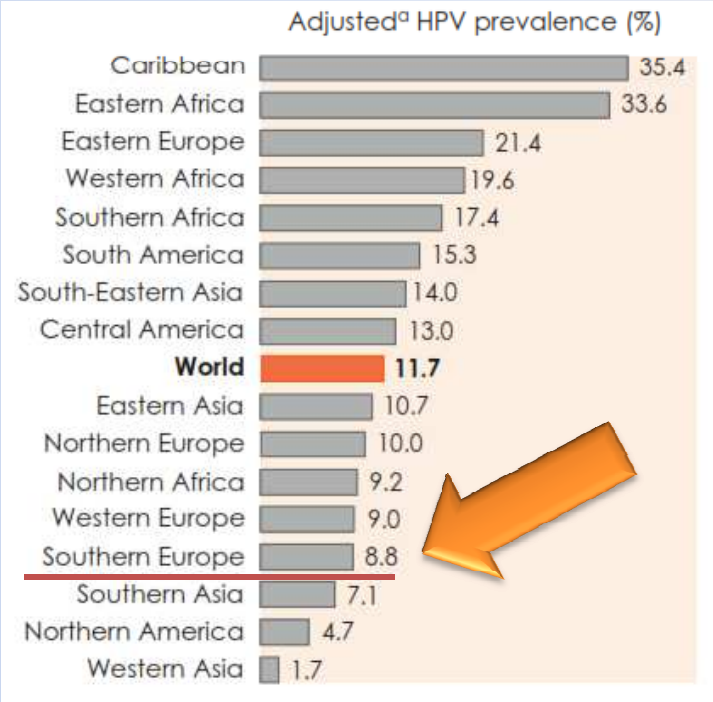
Is confirmed an effective **immune T-cell response** in the cancer progression control

CC are usually infiltrated by **lymphocytes** (both CD8+ and CD4+ T cells) able to recognize the E6 and E7 HPV antigens

Moscicki A-B et al. Vaccine. 2012

HPV women Prevalence and Latency





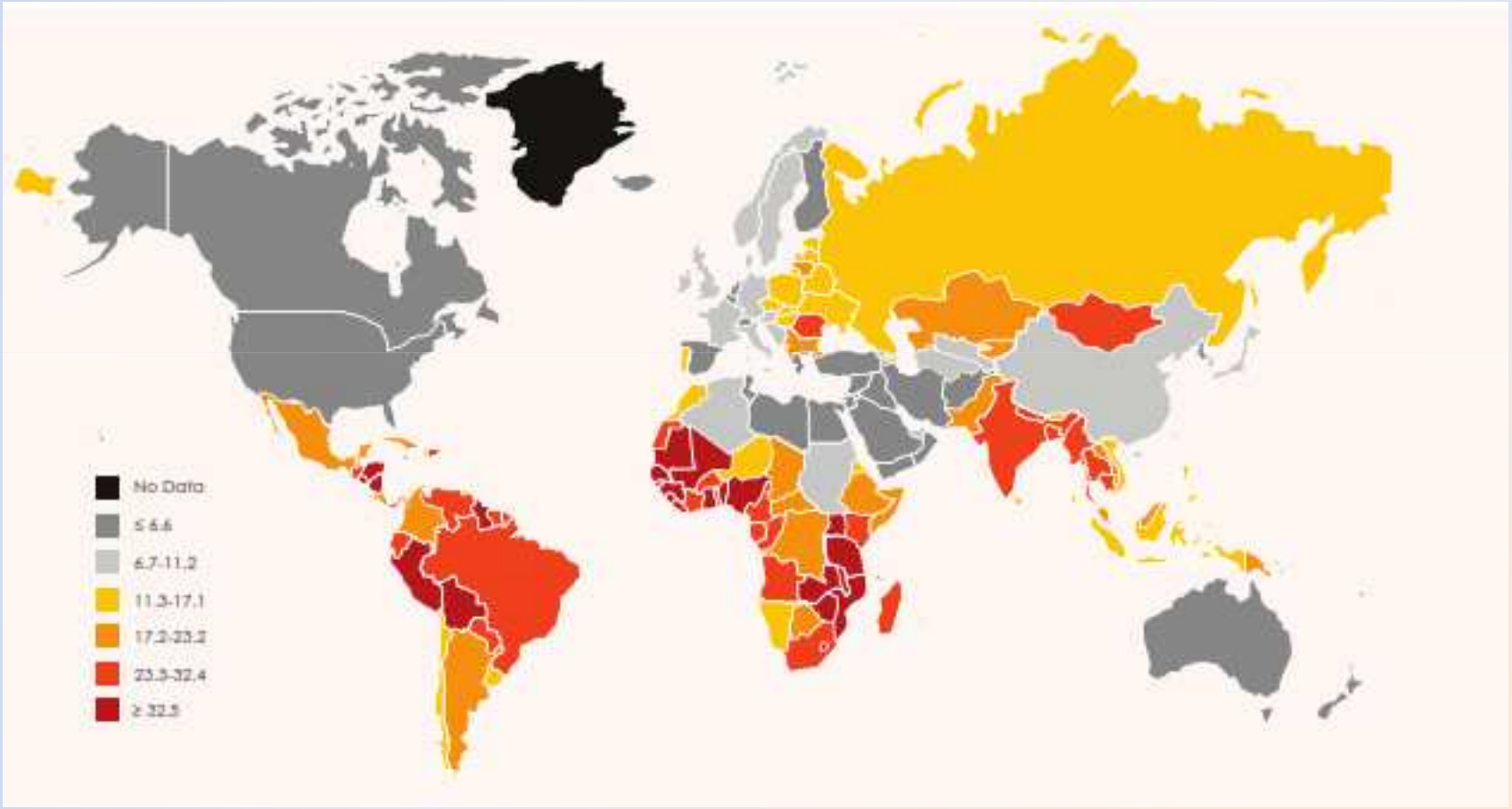
HPV prevalence among women with normal cytology

90% in women with cervical intraepithelial neoplasia (CIN)

Maximum rates of HPV prevalence are observed in women less than 25 years

Forman D et al. Vaccine. 2012

HPV and Cervix neoplasia

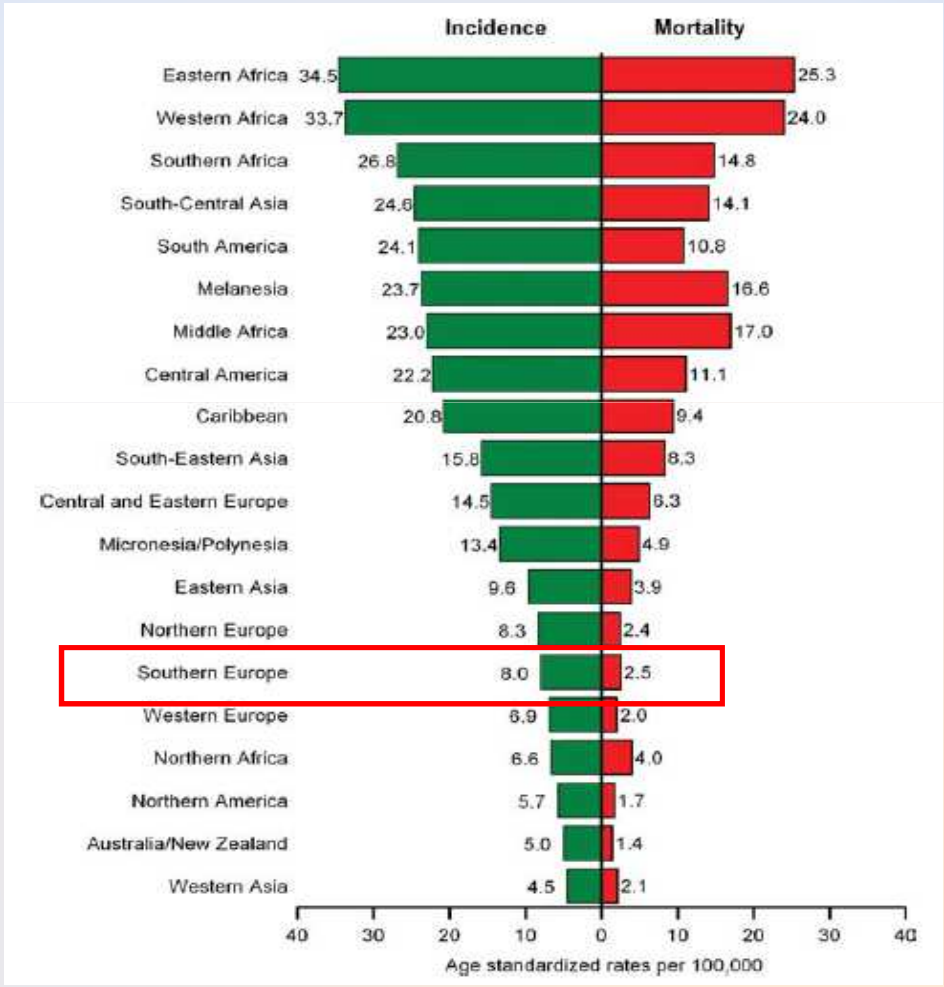


Cervical cancer incidence rate per 100,000 in 2008 (all ages)

Forman D et al. Vaccine. 2012

HPV and Cervix neoplasia

	Estimated New Cases	Estimated Deaths
	Female	Female
Developed Countries	Breast 692,200	Breast 189,500
	Colon & rectum 337,700	Lung & bronchus 188,400
	Lung & bronchus 241,700	Colon & rectum 153,900
	Corpus uteri 142,200	Pancreas 79,100
	Stomach 102,000	Stomach 70,800
	Ovary 100,300	Ovary 64,500
	Non-Hodgkin lymphoma 84,800	Liver 39,900
	Melanoma of the skin 81,600	Leukemia 38,700
	Pancreas 80,900	Non-Hodgkin lymphoma 33,500
	Cervix Uteri 76,500	Corpus uteri 33,200
	All sites but skin 2,584,800	All sites but skin 1,223,200
	Female	Female
Developing Countries	Breast 691,300	Breast 268,900
	Cervix uteri 453,300	Cervix uteri 242,000
	Lung & bronchus 272,000	Lung & bronchus 239,000
	Stomach 247,000	Stomach 202,900
	Colon & rectum 232,400	Liver 177,700
	Liver 186,000	Colon & rectum 134,100
	Corpus uteri 144,900	Esophagus 115,900
	Esophagus 137,900	Ovary 75,700
	Ovary 125,200	Leukemia 75,100
	Leukemia 93,400	Brain, nervous system 50,300
	All sites but skin 3,453,600	All sites but skin 2,122,600



Jemal A et all Global cancer statistics. CA Cancer J Clin. 2011

HPV and Cervix neoplasia

Estimated number of new cancer cases occurring in 2008 attributable to HPV infection by geographic region.

REGION	Total All cancer sites	Total HPV-related cancer sites[a]	Total attributable to HPV	PAF (%)	Cervix uteri	Anus	Penis	Vulva/ Vagina	Oropharynx
AFRICA									
Sub-Saharan Africa	550,000	82,000	78,000	14.2	75,000	1,500	330	940	390
Northern Africa and Western Asia	390,000	12,000	11,000	2.8	9,200	900	<100	620	110
ASIA									
India	950,000	170,000	150,000	15.5	130,000	2,800	3,500	3,400	3,200
Other Central Asia	470,000	48,000	43,000	9.0	39,000	1,800	<100	500	780
China	2,800,000	85,000	80,000	2.8	75,000	1,500	1,200	1,100	440
Japan	620,000	12,000	11,000	1.8	8,900	630	120	360	950
Other Eastern Asia	1,000,000	62,000	55,000	5.4	51,000	1,500	1,000	1,200	710
AMERICA									
Central and Southern America	910,000	84,000	75,000	8.3	68,000	2,300	1,400	2,000	780
Northern America	1,600,000	35,000	26,000	1.6	12,000	3,900	670	2,900	6,200
EUROPE									
Europe	3,200,000	110,000	80,000	2.5	55,000	6,800	2,400	7,400	8,100
OCEANIA									
Australia/New Zealand	130,000	2,100	1,600	1.2	800	280	<100	190	230
Other Oceania	8,800	920	840	9.4	800	<100	<100	<100	<100
Less developed regions	7,100,000	550,000	490,000	6.9	450,000	12,000	7,600	9,800	6,400
More developed regions	5,600,000	150,000	120,000	2.1	77,000	12,000	3,200	11,000	15,000
WORLD	12,700,000	700,000	610,000	4.8	530,000	24,000	11,000	21,000	22,000

[a] HPV-associated cancer sites are: cervix uteri, vulva, vagina, anus, penis and oropharynx including base of tongue and tonsils.
PAF: Population Attributable Fraction.

Results from meta-analysis showing number of women tested for HPV and HPV16, number and percent positive by cervical disease grade.

Grade of cervical disease	Number of women tested	Number of women HPV-positive	Percentage HPV-positive	Percentage HPV16-positive[a]
Normal cytology	266,611	33,154	12	20
ASCUS	12,983	6,810	52	23
LSIL	17,805	13,480	76	25
HSIL	7,743	6,616	85	48
CIN1	11,043	8,108	73	28
CIN2	4,754	4,068	86	40
CIN3	11,618	10,753	93	58
ICC	40,679	36,374	89	63

Forman D et al. Vaccine. 2012

HPV and Cervix neoplasia

Standard treatment

Radical hysterectomy (RH)
Bilateral adnexectomy
Systematic Pelvic lymph nodes (LNs) removal

Radical trachelectomy: The first step of fertility preservation in young women with cervical cancer (Review)

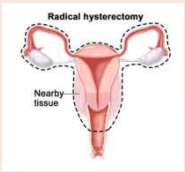
SALVATORE GIZZO[1], EMANUELE ANCONA[1], CARLO SACCARDI[1], TITO SILVIO PATRELLI[2], ROBERTO BERRETTA[2], OMAR ANIS[1], MARCO NOVENTA[1], ANNA BERTOCCO[1], SIMONE FAGHERAZZI[1], MICHELA LONGONE[1], LUCIA VENDEMIATI[1], DONATO D'ANTONA[1] and GIOVANNI BATTISTA NARDELLI[1]

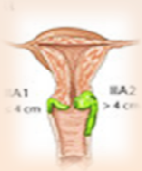
Stage IA1 → **Depends on reproductive age**

- ✓ **Abdominal total Hysterectomy**
- ✓ **Large conization**
- ✓ **Radical Trachelectomy**

Fertility Preservation in Young Women with Cervical Cancer: An Oncologic Dilemma or a New Conception of Fertility Sparing Surgery?

Salvatore Gizzo,[1] Emanuele Ancona,[1] Tito Silvio Patrelli,[2] Carlo Saccardi,[1] Omar Anis,[1] D'Antona Donato,[1] and Giovanni Battista Nardelli[1]





Stage IA2 – IIA2 → **RH**
Bilateral adnexectomy
Systematic Pelvic LNs removal → **Radiotherapy**

Wiebe E et al. Int J Gynaecol Obstet. 2012

HPV and Cervix neoplasia

Risk Factors for recurrences

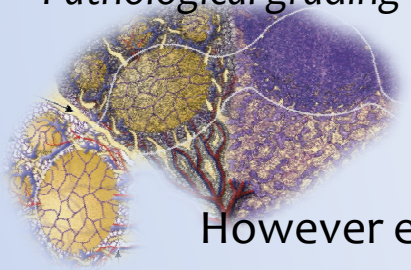
- ***Metastatic involvement of LNs***
- *Parametrial involvement*
- *Positive surgical margins*
- *Primary tumor size more than 4 cm*
- *Lymphovascular space involvement*
- *Pathological grading (GI, GII, GIII)*

Unquestioned parameter to discriminate if performing or not adjuvant therapy after surgery

generally found in **0–29.3%** of patients with *early stages* **(*FIGO* IA1–IB1)**

12–61.8% of patients with *locally advanced disease* **(FIGO IB2–IIB)**

Decrease the overall **5-year survival** by **25%-60%**



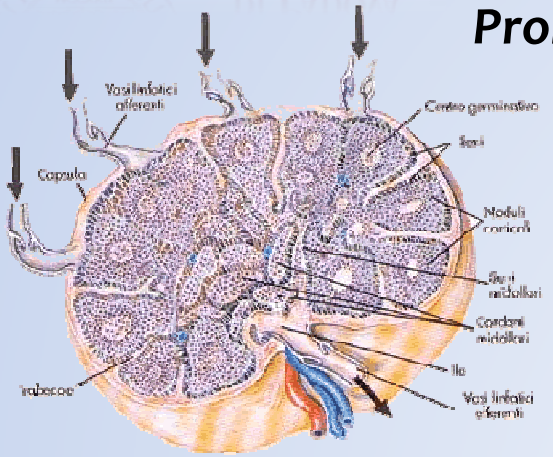
However even in **non-metastatic LNs, recurrence rate reaches 10% to 15%,** affecting the pelvic area in more than 60% of cases

Slama CM et al. Gynecol Oncol. 2013

Wiebe E et al. Int J Gynaecol Obstet. 2012

Ho CM et al. Gynecol Oncol. 2004

HPV and Cervix neoplasia



Probably.....

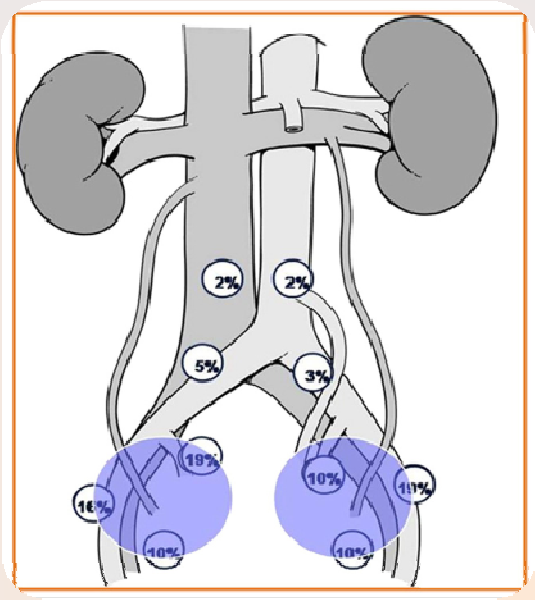
Potential intrinsic ability to neoplastic transformation **of non-atypical cervical cells infected by hrHPV** and **migrated via lymphatic drain**

Macrometastases: tumour deposits >2.0 mm in size

Micrometastases: tumour deposits of 0.2–2.0 mm in size;

ITC: tumour deposits no larger than 0.2 mm

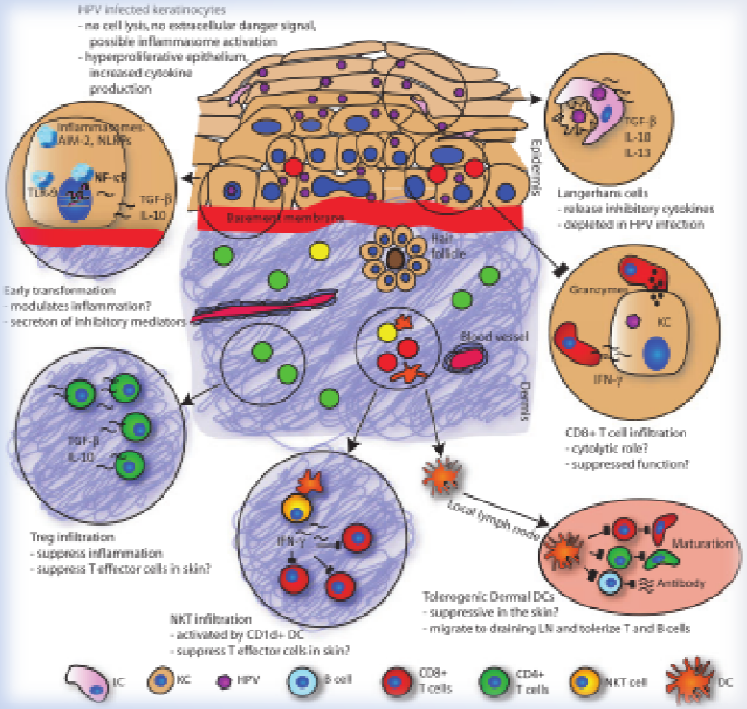
Doubts about their prognostic significance



Slama CM et al. Int J Gynecol Cancer. 2010

HPV and Cervix neoplasia

LNs detection of hrHPV-DNA

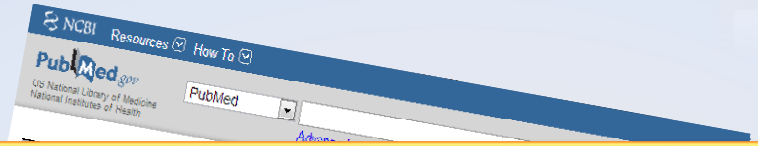


- ✓ **Could be considered a possible marker of LNs recurrence?**
- ✓ **Could estimate the oncological prognosis?**
- ✓ **Could be useful to choose the best surgical treatment, the necessity of adjuvant therapy?**

Aim of our Study

Data Sources

➢ Interval time from 1986 to 2014



➢ Key search terms:

- CC in combination with pelvic and para-aortic LNs metastases and LNs HPV-DNA detection
- Recurrence rate in combination with pelvic and para-aortic LNs metastases and LNs HPV-DNA detection
- Association between LNs metastases positive and LNs HPV-DNA detection
- Association between LNs metastases negative and LNs HPV-DNA detection

➢ Outcomes

- Evaluate the HPV-DNA prevalence in pelvic and para-aortic LNs and the association between HPV-DNA detection and metastatic LNs involvement
- Evaluate the CC recurrence rate in the cohort of patients positive for HPV-DNA detection in regional nodes after systematic LNs (both in metastatic and non-metastatic LNs)
- To understand the prognostic significance of HPV-DNA detection in regional node for the identification of occult metastasis and for the risk stratification of the patients after surgical treatment

Available Methods

Patients with first diagnosis of early stage CC form IA to IIB (early stages)

All patients were surgically treated according to international guidelines available at the time of the study performance

Retrospective studies Formalin-fixed, paraffin-embedded tissue samples taken from CC and regional LNs tissue

Perspective/Observational studies Fresh or frozen tissue samples taken from CC and regional LNs

We considered only the cases in which Authors reported positive HPV-DNA test in primary cervical lesion in order to avoid a sub-cohort of patients in which LNs HPV status was not comparable with the cervical one

Wiebe E et al. Int J Gynaecol Obstet. 2012

Available Methods

HPV-DNA extraction assay (LNs)

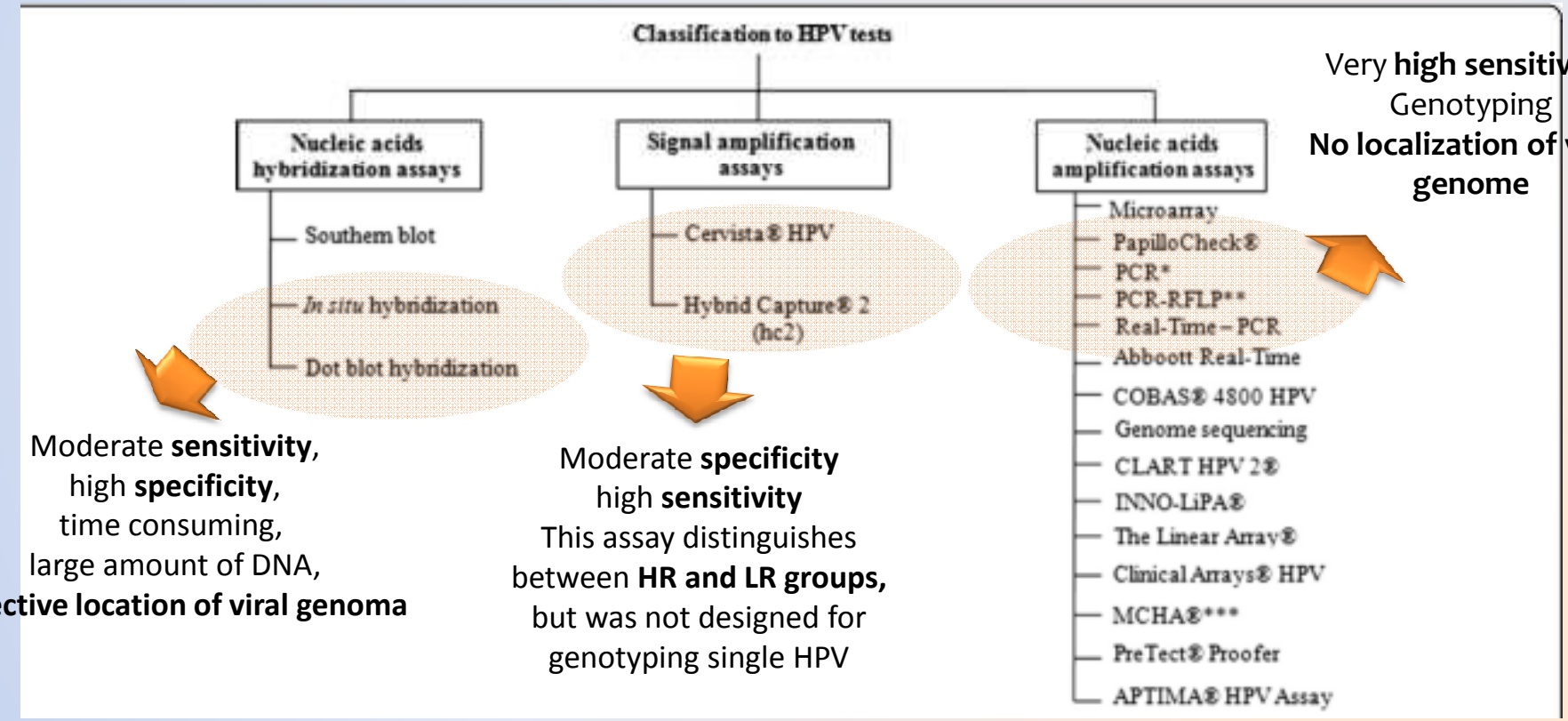
Manuscripts very heterogeneous

Abreu *et al. Virology Journal* 2012, **9**:262
http://www.virologyj.com/content/9/1/262

VIROLOGY JOURNAL

REVIEW Open Access

A review of methods for detect human *Papillomavirus* infection



Moderate **sensitivity**, high **specificity**, time consuming, large amount of DNA, **effective location of viral genoma**

Moderate **specificity** high **sensitivity** This assay distinguishes between **HR and LR groups,** but was not designed for genotyping single HPV

Very **high sensitivity** Genotyping **No localization of viral genome**

Abreu, et al. Virology Journal 2012

Results

General features of the studies

34 studies met all the eligibility criteria for this systematic review

1989 (Fuchs et al.) and 2012 (Zhang et al)

23 retrospective

6 observational

5 perspective

Overall sample size of **1401 patients**

Considering as endpoint the validation of **LNs HPV test as a new prognostic marker 15 studies** were eligible

The remaining eligible manuscripts evaluated only the prevalence of HPV-DNA in metastatic and non-metastatic LNs

Results

General features of the sample

CHARACTERISTICS	TOTAL (1401)
FIGO stage	**(N°)**
IA	78
IB	615
IIA	334
IIB	304
IIIA	1
IIIB	9
Not-reported	60
Histology	**(N°)**
Squamous cell carcinoma	1208
Adenocarcinoma	127
Adeno-squamous	35
Not-reported	31

Pathological grading	**(N°)**
High differentiation (G1)	82
Intermediate differentiation (G2)	61
Low differentiation (G3)	60
Not-reported	1198
Lymphovascular space involvement	**(N°)**
+	463
-	292
Depth of cervical invasion	**(N°)**
+	28
-	24
<10 mm	70
>10 mm	160
Volume of primary lesion	**(N°)**
<20 cm^3	39
>20 cm^3	77
<4 cm	247
>4 cm	149
Corpus uteri invasion	**(N°)**
Not across internal isthmus	114
Across internal isthmus	176
Vaginal invasion	**(N°)**
+	201
-	126
Parametrial invasion	**(N°)**
+	228
-	207

Results

Type of HPV in primary lesion			
Type	**(N°)**	**Type**	**(N°)**
6	2	58	5
11	9	59	1
16	858	68	1
18	164	39	1
31	21	45	4
33	30	52	6
35	5	NS/co-infection	107
Type of HPV in LNs			
Type	**(N°)**	**Type**	**(N°)**
6	3	58	6
11	8	59	1
16	424	39	2
18	102	40	1
31	2	45	5
33	30	52	5
34	1	35	3
NS/co-infection	75		

1) **HPV 16 (70.6%)**
2) **HPV 18 (13.5%)**

> 80%

3) **HPV 33-31 (2.5,1.7%)**

Similar trend

Total Pelvic LNs metastasis involvement	(N°)
M +	488
M -	913

1) **M + 34.8%**
2) **M - 65.2%**

Results

HPV-DNA prevalence in LNs with or without histological metastasis detection

HPV presence in pelvic and/or para-aortic LNs we found that the most representative genotype were **HPV 16 (424 patients)** and **HPV 18 (102 patients)**

Type of HPV in LNs			
Type	**(N°)**	**Type**	**(N°)**
6	3	58	6
11	8	59	1
16	424	39	2
18	102	40	1
31	2	45	5
33	30	52	5
34	1	35	3
NS/co-infection	75		

HPV positive LNs in the whole sample was 51% (725 patients)

Metastasis involvement in HPV+ LNs	(N°)
M+ / HPV+	367
M- / HPV+	358

1) **M+ / HPV + 50.6%**
2) **M- / HPV + 49.4%**

Results

Generally, many Authors showed that **HPV-DNA** was detectable in more than **50% of pelvic LNs independently from the metastatic involvement**

In the recent manuscripts there is a **High correlation between LNs metastatic involvement and HPV-DNA presence**

From **66.6%** (Chan et al) to **100%** (Slama et al)

In patients with **LNs HPV-DNA positivity**, the **rate of non-metastatic nodes resulted very different**

Manuscripts before 2001

From **35.7%** by Hernadì et al. to **90.1%** by Fule et al

lower correlation rate between LNs HPV genome detection and metastasis

very heterogeneous range of HPV genome detection when LNs histology resulted negative for metastasis

Hernádi Z et al. BJOG. 2003
Füle et al. Gynecol Oncol. 2006

Chan et al. J Clin Virol. 2005
Slama et al. Gynecol Cancer. 2009

Results

Correlation of HPV LNs infection and cancer recurrence

Weak point

Only 15 studies, often reporting discordant results affected by the **bias** linked to the **different techniques** used, the **non-homogeneous and comparable cohort of patients investigated, small sample size**

Strenght point

Large part of the manuscripts reported that the **presence of HPV-DNA in LNs increase the risk of recurrences and reduce the overall survival**

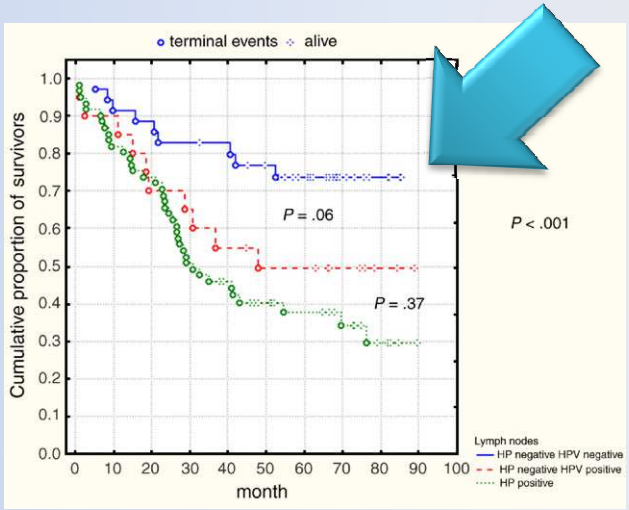
Results

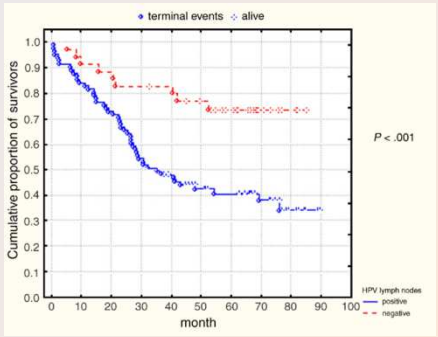
The largest perspective study

Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 104 (2007) 721–726
Gynecologic Oncology
www.elsevier.com/locate/ygyno
ELSEVIER

Predictive value of HPV DNA in lymph nodes in surgically treated cervical carcinoma patients—A prospective study

- 116 patients with early stage cervical cancer
- Survival in groups **LN M+** and **LN HPV+M–** did not differ statistically (p=0.37)
- The survival periods in these two groups **differed when compared with LN HPV-M- patients (p<0.001)**
- The presence of lymph node **HPV DNA is an independent parameters correlating with survival and mortality risk.**



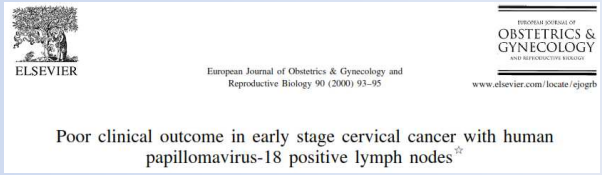


HPV DNA in lymph nodes is an early sign of metastasis

Lukaszuk et al. Gynecol Oncol. 2007

Results

Similar data were reported by **Rolla et al, Nawa et al., Ikenberg et al., Park et al., Kobayashi et al., Sapy et al.** and **Hernadì et al**



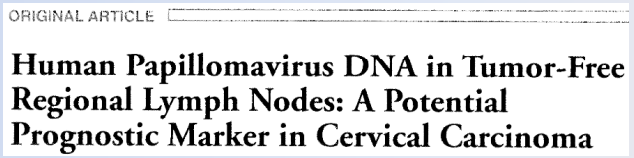
ELSEVIER

European Journal of Obstetrics & Gynecology and Reproductive Biology 90 (2000) 93–95

EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY

www.elsevier.com/locate/ejogrb

Poor clinical outcome in early stage cervical cancer with human papillomavirus-18 positive lymph nodes☆



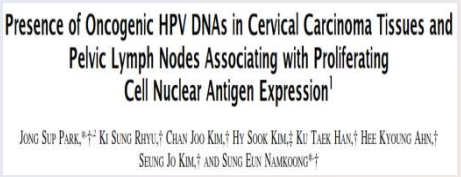
ORIGINAL ARTICLE

Human Papillomavirus DNA in Tumor-Free Regional Lymph Nodes: A Potential Prognostic Marker in Cervical Carcinoma

Eur J Gynaecol Oncol. 2009;30(5):557-61.

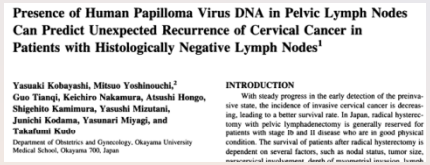
A perspective study on correlation between HPV DNA and lymph nodes in surgically treated cervical carcinoma patients. Preliminary data.

Rolla M[1], Berretta R, Patrelli TS, Merisio C, Gramellini D, Fadda GM, Bacchi Modena A, Nardelli GB.



Presence of Oncogenic HPV DNAs in Cervical Carcinoma Tissues and Pelvic Lymph Nodes Associating with Proliferating Cell Nuclear Antigen Expression[1]

Jong Sup Park,[a,+] Ki Sung Ryu,[†] Chan Joo Kim,[†] Hy Sook Kim,[‡] Ku Taek Han,[†] Hee Kyoung Ahn,[†] Seung Jo Kim,[†] and Sung Eun Namkoong[a,†]



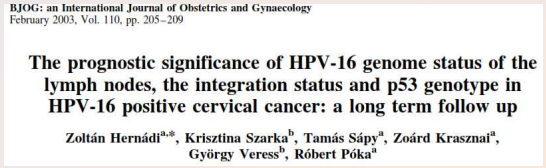
Presence of Human Papilloma Virus DNA in Pelvic Lymph Nodes Can Predict Unexpected Recurrence of Cervical Cancer in Patients with Histologically Negative Lymph Nodes[1]

Yasuaki Kobayashi, Mitsuo Yoshinouchi,[2] Guo Tianqi, Keichiro Nakamura, Atsushi Hongo, Shigehito Kamimura, Yasushi Mizutani, Junichi Kodama, Yasunari Miyagi, and Takafumi Kudo

Department of Obstetrics and Gynecology, Okayama University Medical School, Okayama 700, Japan

INTRODUCTION

With steady progress in the early detection of the preinvasive state, the incidence of invasive cervical cancer is decreasing, leading to a better survival rate. In Japan, radical hysterectomy with pelvic lymphadenectomy is generally reserved for patients with stage Ib and II disease who are in good physical condition. The survival of patients after radical hysterectomy is dependent on several factors, such as nodal status, tumor size,



BJOG: an International Journal of Obstetrics and Gynaecology
February 2003, Vol. 110, pp. 205–209

The prognostic significance of HPV-16 genome status of the lymph nodes, the integration status and p53 genotype in HPV-16 positive cervical cancer: a long term follow up

Zoltán Hernádi[a,*], Krisztina Szarka[b], Tamás Sápy[a], Zoárd Krasznai[a], György Veress[b], Róbert Póka[a]

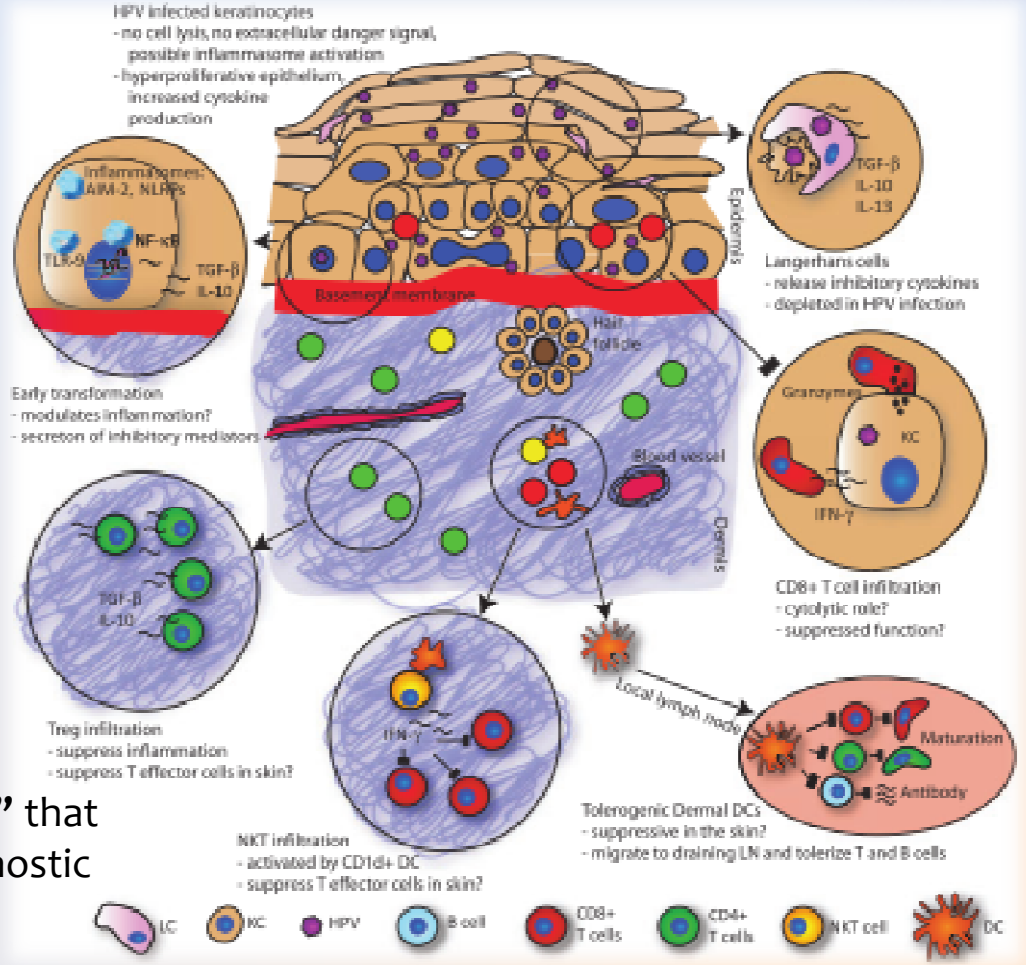
Hording et al. (24 patients), Baay et al. (50 patients), Czegledy et al. (31 patients) Landro et al. (37 patients), Chan et al. (15 cases) and Fule et al. (150 patients) reported *no significant differences in recurrence or overall survival* between patients with positive and negative HPV-DNA LNs status

Conclusions

.....The dilemma persist

The HPV-DNA presence in pelvic LNs is related to the "scavengers" activity of **immune cells**

or it is related to the presence of **micro-metastases or "future metastases"** that cannot be detected through standard diagnostic procedures



Conclusions

The most unresolved dilemma is linked to the very different features reported in cases in which **histology showed negative results** but **HPV test detected viral genome**

Is it logical to consider patients with **positive LNs HPV status** similar to the **negative ones** in *estimating risk of recurrence and overall survival?*

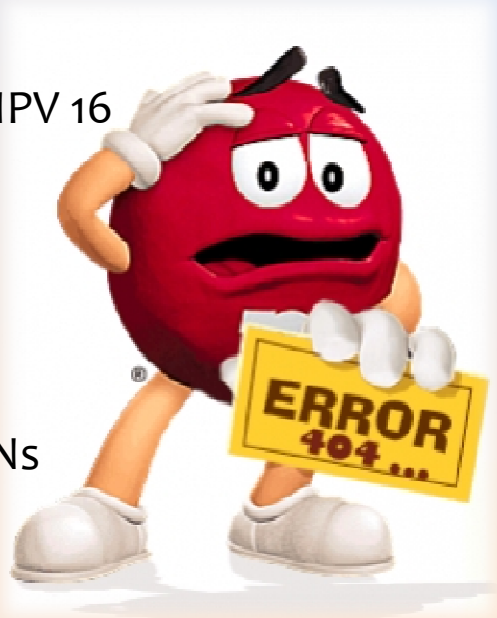
Thanks to few pioneer studies (Lukaszuk et al, Rolla et al) **a correlation between HPV LNs status and worse prognosis has been highlighted**

However **Data are not sufficient to detect a cuse-effect relatioship** between LNs HPV infection (in metastases free LNs) and recurrence risk

Conclusions

Many of these this studies are affected by numerous bias:

- ✓ The use of HPV primers only for HPV 16 Genotype (or only for HPV 16 and 18)
- ✓ The retrospective design of the studies
- ✓ The use of non-fresh LNs material for the HPV detection
- ✓ The non systematic execution of HPV test on all the removed LNs
- ✓ The small sample size
- ✓ The different techniques used



Conclusions

Used techniques to detect HPV-DNA in LNs seem to be not sufficient to solve the question

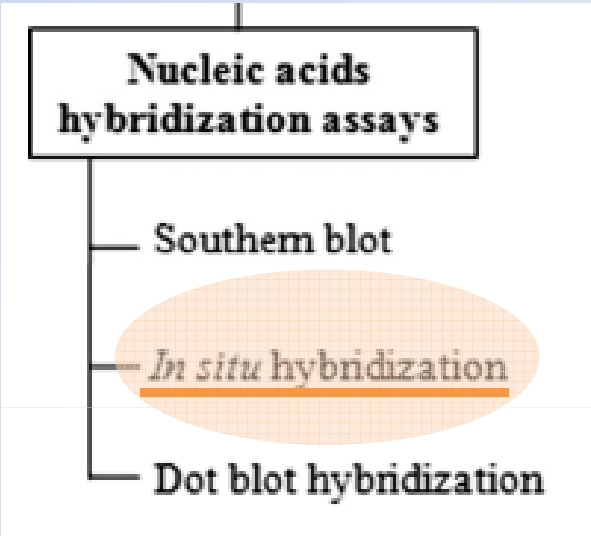
Two different patterns of HPV LNs positivity

(PCR)

Viral genome detection in **squamous cells** (able both to perpetuate the virus replication and to be subjected to oncological transformation) **the HPV-DNA positivity should be considered as an early sign of potential metastases**

viral genome detection **in non-squamous cells** (immune-endothelial) the HPV-DNA positivity should be considered as a **viral spread probably at low risk of LNs metastases recurrence.**

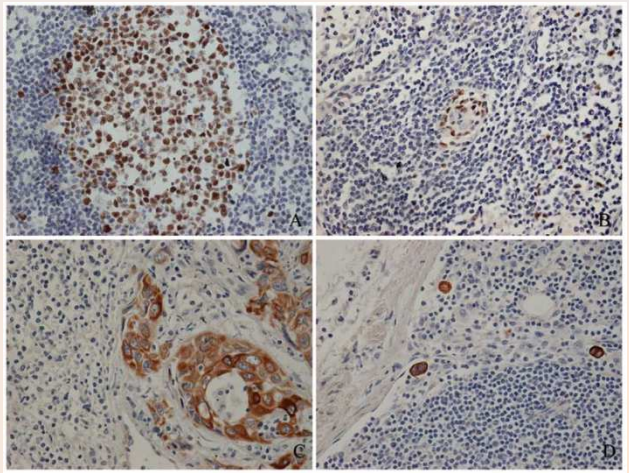
Conclusions



Accurate **detection and localization of HPV-DNA sequences** (different from PCR that is able to detect the presence/absence of HPV-DNA)

Zhang et al ISH is very sensitive for the detection of LNs micro-metastases in early-stage cervicalcancer.

Clinical pathological parameters	N	HPV DNA (+) n	%
Lymph node metastases			
+	13	13	100
-	15	9	60.0



ZHANG et al. ONCOLOGY REPORTS. 2012

Conclusions

It's mandatory to define, through perspective long-term studies (with an adequate standardization of methods and sample size):

- the most **appropriate LNs HPV-DNA detection techniques (PCR + ISH)**
- the **real role of LNs viral genome detection in prognosis estimation**

.....Moreover

To estabilsh the **real role of HPV detection in LNs** could **potentially improve** the **SLNs (sentinel nodes) technique** in case of early stage CC

Could HPV-DNA Test Solve the Dilemma About Sentinel Node Frozen Section Accuracy in Early Stage Cervical Cancer? Hypothesis and Rationale

Marco Noventa, Emanuele Ancona, Carlo Saccardi, Pietro Litta, Donato D'Antona, Giovanni Battista Nardelli, and Salvatore Gizzo

Setting the cohort of **high-risk** and **low-risk** patients which can benefit from different **surgical–oncological strategies**:

- ✓ **Fertility sparing surgery**
- ✓ **Systematic lymphadenectomy**

UNIVERSITÀ DEGLI STUDI DI PADOVA

Thanks for your attention

Title Page

Usefulness, methods and rationale of lymph nodes HPV-DNA investigation in estimating risk of early stage cervical cancer recurrence. A systematic literature review.

Marco Noventa M.D; Enrique A... M.D; Prof. Erich Cosmi M.D; Carlo Saccardi M.D. PhD; Prof. Pietro Litta M.D; Prof. Donato D'Antona M.D; Prof. Giovanni Battista Nardelli M.D; Salvatore Gizzo M.D.

Department of Woman and Child Health – University of Padua, Padua, Italy