

**The Role of Epidemiologic Studies in
Establishing HPV as a
Cause of Cancer**

April 2, 2008

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Outline

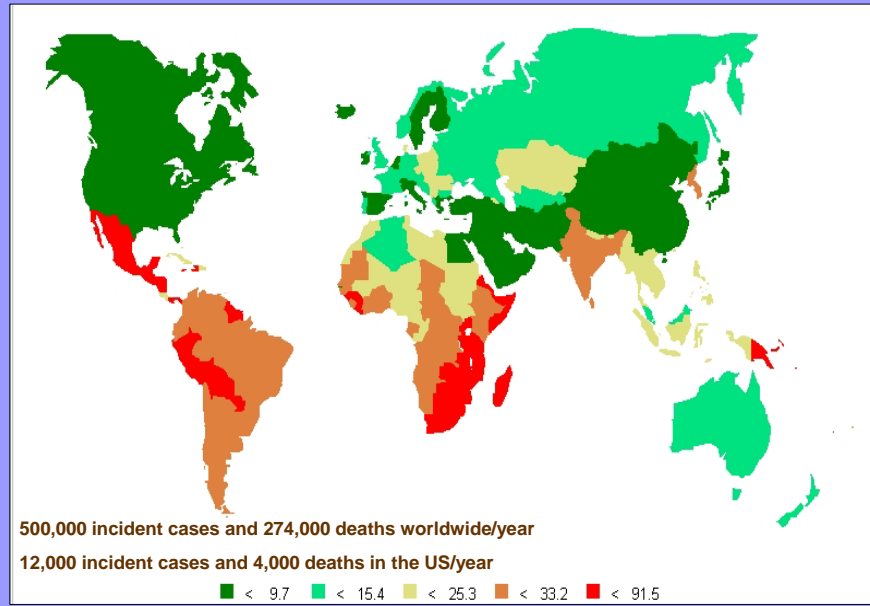
HPV in Cervical Cancer

- Basics: Incidence, screening, and the virus
- Causal criteria
- Cofactors with HPV in cervical cancer

HPV in Other Cancers

Role of HPV in Skin Cancers

Incidence of Cervical Cancer



Cytology, Histology, and Common Names for Cervical Precursor Lesions

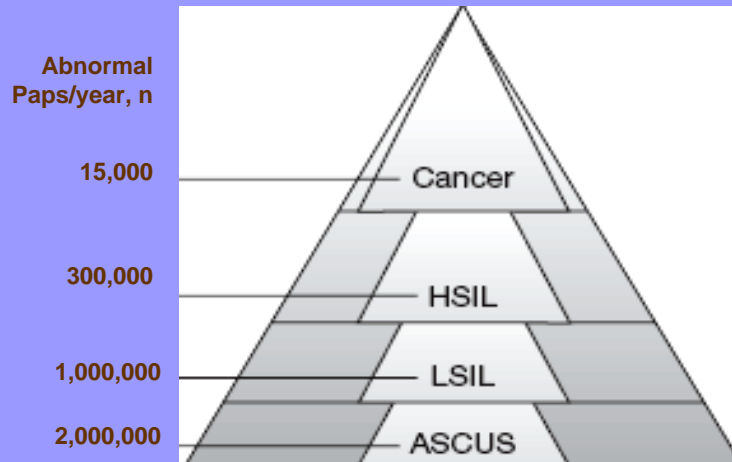
		LSIL	HSIL	
	ASCUS	CIN 1	CIN 2	CIN 3
Normal		Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia Carcinoma <i>in situ</i>

SIL = Low Grade Squamous Intraepithelial Lesion
 CIN = Cervical Intraepithelial Neoplasia
 ASCUS = Atypical Cells of Undetermined Significance

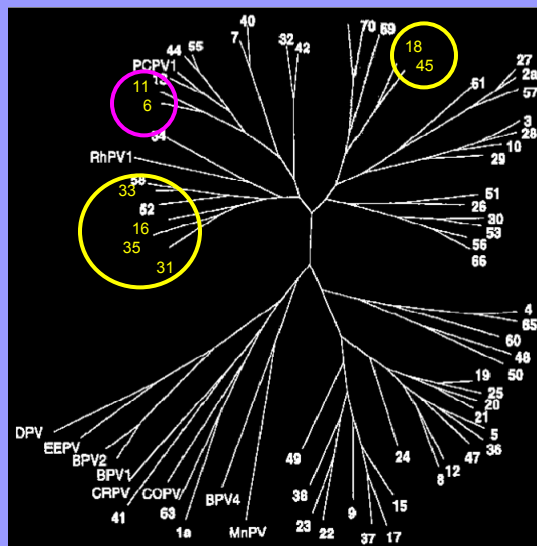
From Figure 6.13, DeMay RM. The Art and Science of Cytopathology. 1999

Annual Prevalence of Cervical Abnormalities in the US

About 55 million Paps/year in US, 3.5 million require follow up



Phylogenetic Relationships of HPV Types based on Sequencing of the L1 Genes



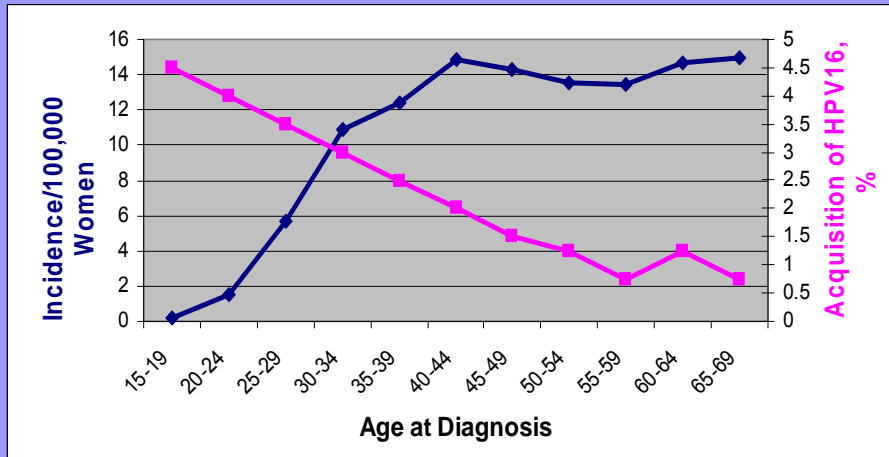
Genus Alpha HPV and Mucosal Lesions:

- About 15 of 40 mucosal types are oncogenic
- **HPV 16 and HPV 18** account for about 70% of cervical cancer
- **HPV 6 and HPV 11** account for about 90% of genital warts

Genus Beta HPV

Cladogram based on the L1 open reading frame (from Li, 2006)

Age-Specific Incidence Rates of Cervical Cancer and Acquisition of HPV16



SEER, 2000-2004 and Guanacaste Cohort

Outline

HPV in Cervical Cancer

- Basics
- Causal criteria
- Cofactors with HPV in cervical cancer

HPV in Anogenital and Oral Cancer

Role of HPV in Skin Cancers

HPV & Cervical Cancer: Evaluating Causal Criteria

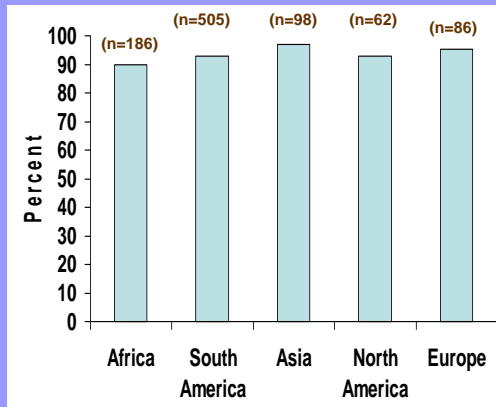
- Strength
- Consistency
- Viral Presence
- Dose Response
- Temporal Sequence
- Mechanistic Coherence

Strength of Association: Risk Estimation and Testing Accuracy Improved Over Time

<u>Location & Year</u>	<u>Cases/Controls</u>	<u>Test</u>	<u>OR (95% CI)</u>
US, 1990	269/400	SB	4 (2.5, 5.3)
Panama, 1989	759/1467	FISH	9 (6, 14)
China, 1991	101/146	PCR	33 (7.7, 141)
Spain/Colombia, 1992	436/387	"	29 (16, 53)
US, 1992	472/453	"	51 (28, 94)
Brazil, 1994	199/225	"	37 (20, 70)
Morocco, 1998	214/203	"	62 (29, 130)
Thailand, 1999	377/261	"	119 (64, 122)
Philippines, 1999	356/381	"	156 (87, 280)
Costa Rica, 2000	34/~10,000	"	710 (110-4500)

FISH = filter in-situ hybridization, SB = southern blot, PCR = polymerase chain reaction

Consistency: Prevalence of HPV in Tumor Tissues *Worldwide Case Series*



- 93% of ~1,000 samples from worldwide study were HPV+ (Bosch, 1995)

- Re-testing negatives with improved assays led to 99.7% HPV+ in these samples (Walboomers, 1999)

Bosch et al. JNCI 87:796-802, 1995.

Viral Presence: Persistence of HPV in Prospective Cohort Study

- 353 women with first abnormal cervical smear followed for progression
- Visits every 3 to 4 months: cytology, colposcopy, HPV test
- Median follow-up of 33 months, biopsy of CIN3 or end of study

<u>HPV Status</u>	<u># Progressing</u>	<u>RR (95% CI)</u>
None (n=81)	0	1.0
Acquire & Clear (n=150)	2	2.9 (0.2, 20)
Persistent (n=122)	33	327.0 (42, 2468)

Nobbenhuis et al. Lancet 354:20-25, 1999.

Dose Response in HPV Epidemiologic Studies: Different Ways to Measure HPV Dose

Some common measures of HPV exposure opportunity correlate with increased risk of disease progression:

- Number of sex partners as a surrogate for escalating chance for critical exposure
- Some HPV types and variants are more aggressive than others
- Increasing viral load with increasing risk of disease, but only in pre-cancers
- Higher levels of antibody correlate with higher risk of disease (even though prophylactic vaccine induces vigorous antibody response, there are higher levels of antibody in cervical cancer cases than controls)

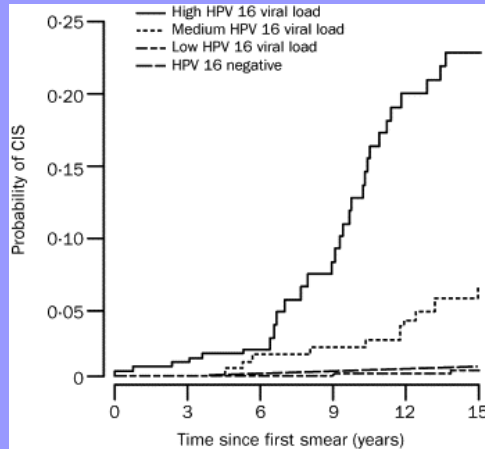
Dose/Response: Increasing Risk of Cervical Cancer by Quartiles of HPV16L1 Antibody

HPV16 quartiles	Control (n=654)		SCC Cervix (n=286)		OR (95% CI)
	N	(%)	N	(%)	
1	164	(25.1)	31	(10.8)	1.0
2	163	(24.9)	41	(14.3)	1.3 (0.8-2.3)
3	164	(25.1)	72	(25.2)	2.3 (1.4-3.8)
4	163	(24.9)	142	(49.7)	4.8 (3.0-7.6)

Unpublished Data

Dose Response: Highest HPV Viral Load Associated with Highest Risk of CIN3

Registry Linkage/Nested Case-Control within a Cohort Study



- In Sweden ~145,000 women w/ abnormal pap smears linked to the cancer registry found ~500 CIN3

- Follow up for up to 26 years

- Probability of CIN3 increased directly with viral load

- 25% of women with high viral load before 25 yo developed CIN3

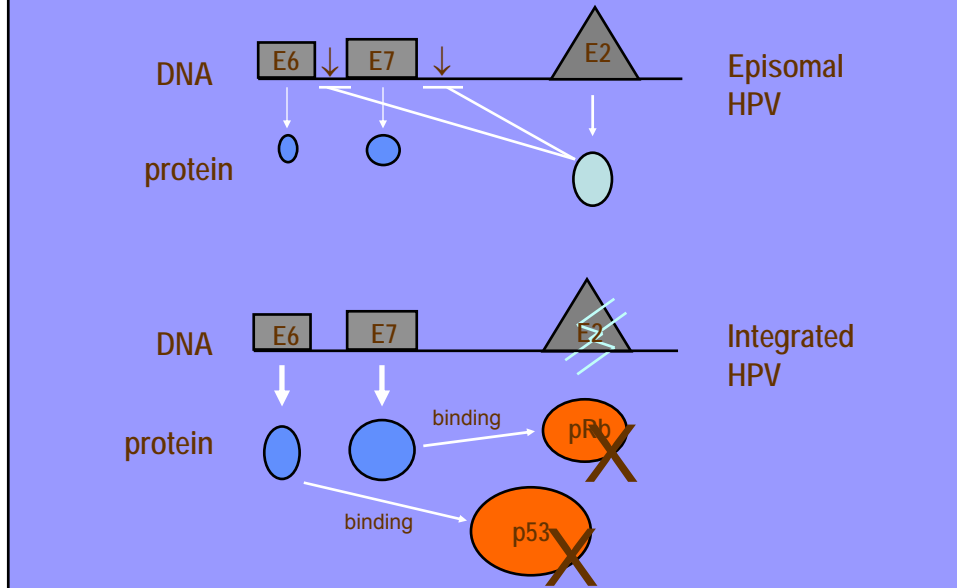
Ylitalo et al. Lancet 355:2194-98, 2000.

Temporal Sequence: Vaccine Efficacy Among 16-26 year-old Females*

Endpoint	Vaccine N	Vaccine Cases	Placebo N	Placebo Cases	Efficacy (95% CI)
HPV 16/18 related CIN2/3 or AIS	8487	0	8460	53	100 (93,100)
HPV 6/11/16/18 related CIN	7858	4	7861	83	95 (87, 99)
HPV 6/11/16/18 related genital warts	7897	1	7899	91	99 (94,100)

*Package insert: Gardasil® , Integrated dataset; results in the per-protocol populations
CIN – cervical intraepithelial neoplasia; AIS – adenocarcinoma *in situ*

Mechanistic Coherence: HPV16 E6 and E7 Proteins Reduce Tumor Suppressor Activity



HPV & Cervical Cancer: Evaluating Causal Criteria

- Strength ✓
- Consistency ✓
- Viral Presence ✓
- Dose Response ✓
- Temporal Sequence ✓
- Mechanistic Coherence ✓

IARC, 1995: "HPV types 16 and 18 are carcinogenic to humans."

Outline

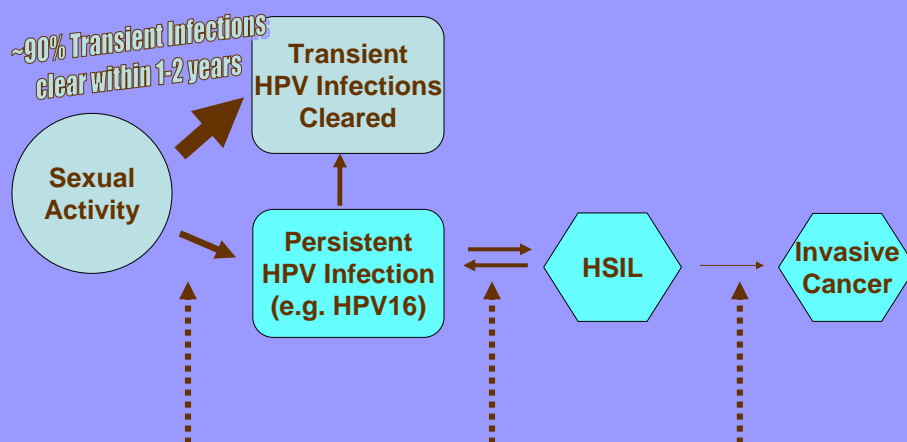
HPV in Cervical Cancer

- Basics
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- Cofactors with HPV in cervical cancer

HPV in Anogenital and Oral Cancers

Putative role of HPV in Skin Cancers

Etiologic Model of HPV in Cervical Cancers



Why do some women progress to invasive cervical cancer?

- **LIFESTYLE:** Screening, Smoking, Parity, Oral Contraceptive Use, other STIs
- **HOST:** Genetic Variation in Immune-Related Genes, DNA Repair Genes
- **VIRUS:** Viral Variants, Viral Load, Integration

How Does Current Smoking Affect Cervical Cancer Risk?

Local immunosuppression in smokers (Barton, 1998) decreased Langerhan's cells in cervical epithelium of current smokers

Direct effect of constituents of cigarette smoke

- eg, Nicotine
 - Cervical mucus of smokers (Prokipczk, 1997)
 - Inhibits apoptosis in tumor cell lines (Wright, 1993)

Epidemiologic evidence

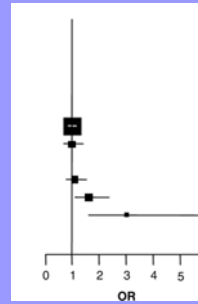
- Increased risk of HPV infection associated with current smoking
- Moderate risk of cervical cancer in most studies, and there is a consistent difference in risk estimates by histologic type

Risk of Cervical Cancer Associated with Current Cigarette Smoking by Histology

Histology	Cases	Controls	OR (95% CI)
Squamous cell (n=730)	39.6	22.1	2.1 (1.6-2.6)
Adenocarcinoma (n=502)	23.0	22.1	0.8 (0.6-1.0)

Current Smoking and HPV Prevalence Trends Among Control Women with 1 Partner

Among Women with 1 Partner				
Smoking	HPV-	HPV+	OR	95% FCI
Never	4753	533	1.00	
Former	414	35	0.97	0.68-1.39
Current, by years smoked				
<5	354	40	1.08	0.78-1.51
5-14	243	32	1.61	1.10-2.34
15+	112	13	3.03	1.60-5.73



Vaccarella, 2008

How do hormones affect risk of cervical cancer?

In vitro evidence

- Hormones enhance HPV E6/E7 expression in cervical cell lines (Mittal, 1993) and
- Cell lines become transformed and tumorigenic in the presence of HPV and progestins (Pater, 1990)

Clinical evidence

- More ectopy with long-term OC use and high parity

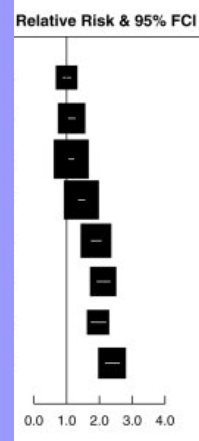
Epidemiologic evidence

- Trends in increasing risk with increasing number of births is consistently found
- Increased risk of cervical cancer associated with oral contraceptive use is found in the majority of studies

Parity and Risk of Cervical Cancer

International Collaboration of 25 Case-Control Studies of Cervical Cancer,
2006 Int J Cancer

Parity	Cases/Controls	OR	95% FCI
0	660/2837	1.00	
1	1051/3474	1.15	1.05-1.27
2	1701/5392	1.14	1.06-1.23
3	1513/4060	1.45	1.35-1.57
4	1260/2862	1.90	1.74-2.07
5	999/1874	2.11	1.91-2.34
6	712/1376	1.96	1.74-2.21
7+	1916/3021	2.39	2.17-2.62



OR adjusted for age, study, number of partners, age first sex.

Oral Contraceptives and Cervical Cancer

IARC, 8 case-control studies, Lancet 2002

Duration of OC Use, Years	Cases/ Controls	OR	95% CI
Never	978/152	1.00	
1	110/28	0.67	0.41-1.08
2-4	156/31	0.80	0.51-1.24
5-9	156/12	2.82	1.46-5.42
10+	172/14	4.03	2.09-7.79

OR adjusted for age, study, number of partners, age first sex

How Does Genetic Variation Affect Susceptibility to Cervical Cancer?

Genetic variation in immune response could act to:

- Attenuate or strengthen immune response to infections
- Allow persistent infections to develop and also allow evolving genetic damage to accumulate
- Aid evasion of tumor surveillance

HLA Region Genes: Essential to Immune Function

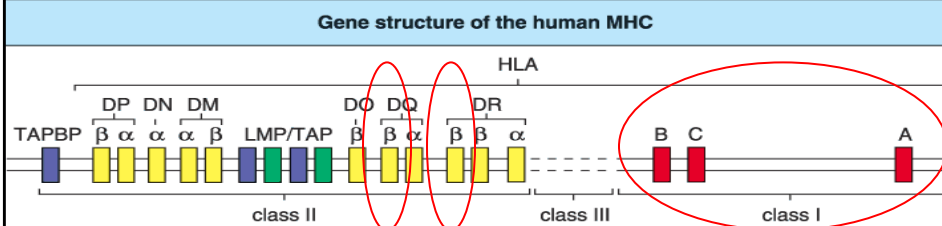


Fig 5.10 © 2001 Garland Science

HPV peptides are presented by HLA molecules

- Class I molecules on nucleated cells present peptide from cytosol to cytotoxic T cells
- Class II molecules on antigen-presenting cells present peptide from outside the cell that are degraded intracellularly and presented to T helper cells

Risk of Cervical Cancer Associated with B*4402-DRB1*1101-DQB1*0301

	Controls N=504 (%)	Cases N=490 (%)	OR	(95% CI)
B*4402	13.9	22.9	1.9	(1.4-2.7)
DRB1*1101	6.1	12.1	2.1	(1.3-3.2)
DQB1*0301	31.4	40.1	1.5	(1.1-1.9)
B*DRB1*DQB1 4402-1101-0301	0.6	5.7	11.5	(3.5-38.5)

Madeleine, 2008

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- Basics
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Other HPV-Related Cancers

- Anogenital
- Oral

Putative role of HPV in Skin Cancers

Percent HPV DNA Positive in Archival Anogenital Tumors by PCR in Case-Control Studies

<u>Site</u>	<u>HPV+ %</u>	<u>HPV16+ %</u>
Vulva	89.0	71.2
In Situ	91.2	74.6
Invasive	79.0*	55.3
Vagina	90.7*	63.0
Anus	95.2	79.5
Penis	79.8*	59.6

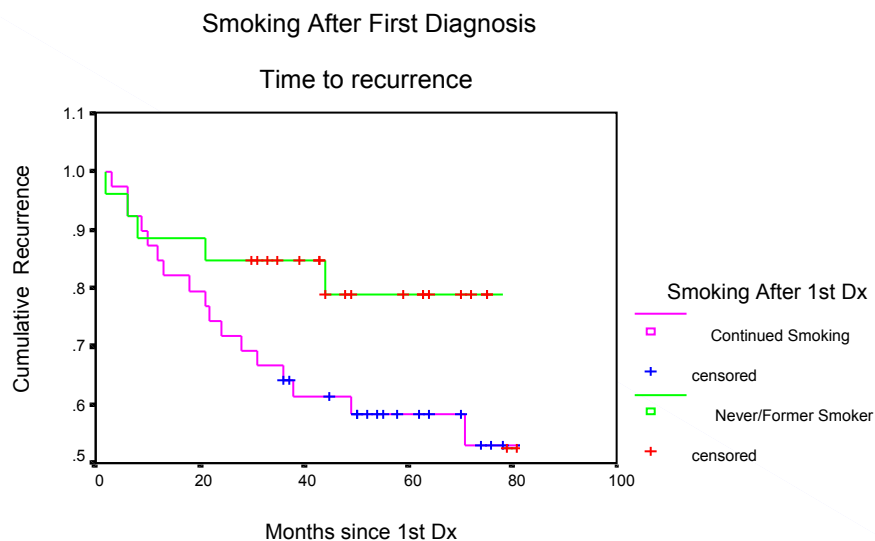
*Highest HPV+ in worldwide studies; others run
40% for vulvar, vaginal, and penile cancers

Daling, 2005; Carter, 2001

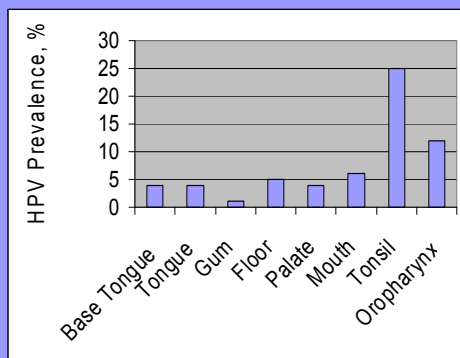
Case-Control Studies in Seattle: Current Smoking and Anogenital Cancers

<u>Site of cancer</u>	<u>Cases</u>	<u>Controls</u>	<u>OR (95% CI)</u>
Vulvar			
HPV+	60.1	25.4	4.7 (3.6-6.1)
HPV-	34.2	25.4	1.7 (1.0-3.0)
Vaginal	42.3	23.2	2.1 (1.4-3.1)
Anal			
Men	56.9	22.2	4.4 (2.1-9.1)
Women	48.2	23.2	3.8 (2.3-6.0)
Penile	35.0	21.8	2.3 (1.4-4.0)

Smoking and Recurrence of VIN3/Vulvar Cancer in Seattle



Higher HPV+ in Oropharyngeal/Tonsillar Cancers Compared to Oral Cavity



- HPV detected in 3.9% of oral cavity and 18.3% of cancers of the tonsil and oropharynx
- HPV16 found among 95% of cases

Herrero, IARC Multicenter Study, 2003

Risk of Oral Cancer Associated with HPV16 Antibody Positivity

	Controls	HPV16+ Cases	HPV- Cases	All Cases
N	446	37	139	259
Seropositive, %	35.0	75.7	52.5	51.4

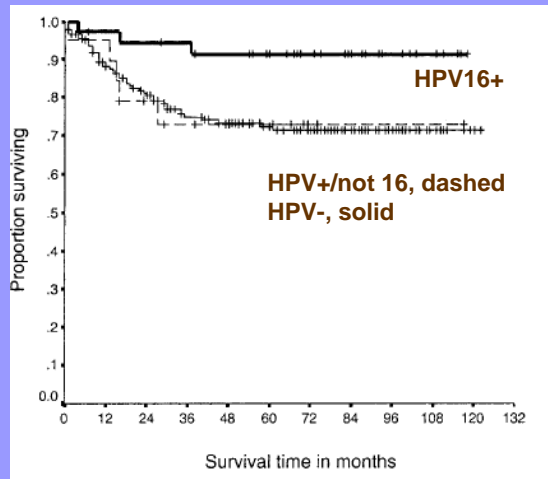
Schwartz JNCI 1988

Characteristics of HPV+ vs HPV- Head & Neck Squamous Cell Cancer

Risk Factors	N=92 HPV+	n=148 HPV-
Sex Partners	X	
Smoking		X
Alcohol		X
Marijuana	X	
Oral Hygiene		X

Gillison JNCI 2008

Survival Differences between HPV Positive and Negative Oral/Oropharynx Cancer



Schwartz, 2001

HPV in Non-Cervix Anogenital Cancers and Oral Cancers: Evaluating Causal Criteria

Criteria	Anogenital	Oral
• Strength	√	?
• Consistency	√-	√
• Viral Presence	?	?
• Dose Response	√	√
• Temporality	√	√
• Coherence	√	?

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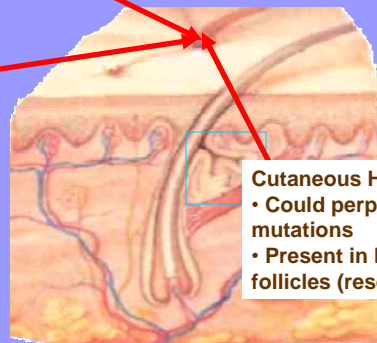
Role of HPV in Skin Cancers

Pathogenesis of Squamous Cell Skin Cancer: Sun Damage, Immunosuppression, and HPV?



Genetic damage
• UVB mutates skin cell DNA, especially p53

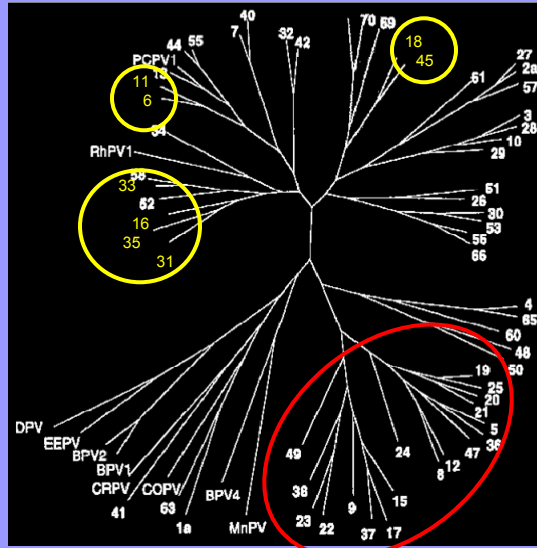
Immune suppression
• Inhibits antigen presenting cells
• Decrease Langerhan's cells
• Increases viral replication



Cutaneous HPV
• Could perpetuate mutations
• Present in hair follicles (reservoir)

Adapted from Euvrard NEJM 2003

Phylogenetic Relationships of HPV Types based on Sequencing of the L1 Genes



Genus Alpha HPV

Genus Beta HPV:

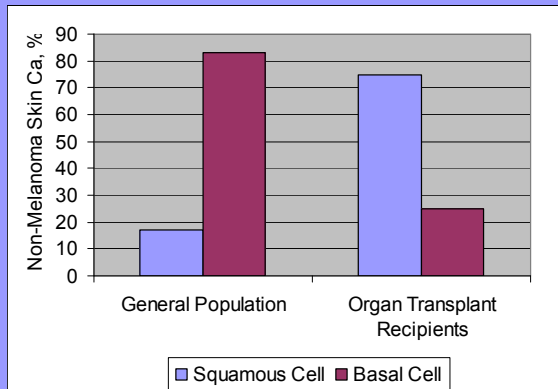
•25 beta HPV types

•Cloned from patients with skin cancers

•Do not integrate or interfere with tumor suppressors

Cladogram based on the L1 open reading frame. (from Li, 2006)

Distribution of Two Skin Cancers: General Population vs Transplant Patients



•Squamous cell cancer is more common and more aggressive in transplant patients than in the general population.

• 82.5% HPV DNA+ in OTR compared to 27% in general population (Harwood, J Med Virol, 2000)

American Academy of Dermatology. <http://www.skincarephysicians.com/skincancer/whatis.html>; Ong J Am Acad Dermatol. 1999.

Risk of Skin Cancers Associated with Alpha and Beta HPV Types

HPV Serology	Basal Cell Skin Ca OR (95% CI)	Squamous Cell Skin Ca OR (95% CI)
Alpha		
Negative	1.0	1.0
Positive	0.7 (0.5-1.0)	1.2 (0.8-1.7)
Beta		
Negative	1.0	1.0
Positive	0.8 (0.6-1.0)	1.5 (1.0-2.1)

Karagas, JNCI, 2006

Beta HPV Prevalence in Superficial Swabs vs Biopsy Samples

Diagnosis	Lesion swab	Lesion biopsy
Actinic keratosis	83% (38 of 46)	11% (five of 46)
Basal Cell	63% (69 of 109)	8% (nine of 109)
Squamous Cell	58% (18 of 31)	19% (six of 31)

Forslund J Invest Dermatol 2004

Skin Cancer after Organ Transplant

The SCOT Study Questions

- Does HPV serology or DNA type from pre-transplant samples predict risk of squamous cell cancer?
- Do measures of HPV change over time after transplant?
- Does level of cell mediated immunity affect HPV infection kinetics?

Antibody Prevalence of Alpha (Mucosa) and Beta (Cutaneous) HPV Types

We tested a newly developed multiplex serology method (Luminex platform) by testing cervical cancer case-control sera to test the hypothesis that beta HPV types are not sexually transmitted

HPV	Controls	Cases	OR (95% CI)
	N (%)	N (%)	
Neither	176 (22.6)	30 (10.3)	1.0
Alpha	86 (11.1)	50 (17.2)	3.1 (1.8-5.2)
Beta	227 (29.2)	45 (15.5)	1.0 (0.6-1.7)
Both	289 (37.1)	135 (56.9)	3.0 (1.9-4.7)

HPV & Skin Cancer: Will Causal Criteria be Met?

- Strength
- Consistency
- Viral Presence
- Dose-response/Escalating Exposure
- Temporal Sequence
- Mechanistic Coherence

Do beta HPV types play a role in squamous cell skin cancer?

HPV Group at the Hutch

Janet Daling
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Greg Wipf
Jolene Weese
Joia Hicks
Peggy Porter
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