

# HPV- Impfung: Aktuelle Studien und Zukunft

Elmar A. Joura  
Universitätsklinik für Frauenheilkunde Wien

Fortbildungstage, 2. Februar 2010  
Obergurgl

# Outline

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- ▶ **Neue Studienergebnisse 2009**
  - ▶ Bivalent (Cervarix<sup>®</sup>, GSK)
  - ▶ Quadrivalent (Gardasil<sup>®</sup>, Sanofi Pasteur MSD)
- ▶ **Nächste Generation der prophylaktischen HPV Impfung**
- ▶ **Therapeutische Impfung**



# Results CIN2+

## Vaccine efficacy HPV-16/-18 (ATP-E cohort)

HPV-16 or HPV-18 DNA in lesion:

Endpoint	Group	N	n	Vaccine Efficacy (96.1%CI)			
				%	LL	UL	P-value
CIN2+ HPV-16/18	Vaccine	7344	4	<b>92.9</b>	79.9	98.3	<0.0001
	Control	7312	56				
CIN2+ HPV-16	Vaccine	6303	2	<b>95.7</b>	82.9	99.6	<0.0001
	Control	6165	46				
CIN2+ HPV-18	Vaccine	6794	2	<b>86.7</b>	39.7	98.7	0.0013
	Control	6746	15				

HPV-16 or HPV-18 DNA in lesion and in preceding cytology samples (HPV type assignment):

Endpoint	Group	N	n	Vaccine Efficacy (96.1%CI)			
				%	LL	UL	P-value
CIN2+ HPV-16/18	Vaccine	7344	1*	<b>98.1</b>	88.4	100.0	<0.0001
	Control	7312	53				
CIN2+ HPV-16	Vaccine	6303	0	<b>100</b>	91.0	100.0	<0.0001
	Control	6165	45				
CIN2+ HPV-18	Vaccine	6794	1	<b>92.3</b>	45.7	99.9	0.0009
	Control	6746	13				

N = number of evaluable women in each group; n = number of evaluable women reporting at least one event in each group

\* CIN lesion detected at Month 42 with HPV-18 (first detected in the Month 36 cervical sample, and the Month 42 cytological specimen) and HPV-52 DNA (which was present throughout the study).

# Results CIN2+

## *Vaccine efficacy related to individual oncogenic non-vaccine HPV types*

HPV type	group	CIN2+			
		N	n	Efficacy% 96.1% CI	P value
HPV-31	vaccine	8438	11	<b>68.4</b> (34.2; 86.1)	0.0005
	control	8482	35		
HPV-33	vaccine	8559	17	<b>49.8</b> (4.8; 74.6)	0.0239
	control	8582	34		
HPV-45	vaccine	8585	0	<b>100</b> ( 7.0; 100)	0.0312
	control	8586	6		
HPV-52	vaccine	8351	18	-0.4 (-111.9; 52.5)	1.0000
	control	8353	18		
HPV-58	vaccine	8531	10	49.6 (-17.1; 79.9)	0.0985
	control	8575	20		

N = number of evaluable women in each group; n = number of evaluable women reporting at least one event in each group

For single HPV types: women included in the analysis were DNA negative for the corresponding HPV type at Months 0 .

# Bivalente HPV- Impfung Phase II

## Lancet 3.12.2009

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### Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years



The GlaxoSmithKline Vaccine HPV-007 Study Group\*

#### Summary

**Background** Prophylactic human papillomavirus (HPV) vaccines have to provide sustained protection. We assessed efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine up to 6.4 years.

**Methods** Women aged 15–25 years, with normal cervical cytology, who were HPV-16/18 seronegative and oncogenic HPV DNA-negative (14 types) at screening participated in a double-blind, randomised, placebo-controlled initial study (n=1113; 560 vaccine group vs 553 placebo group) and follow-up study (n=776; 393 vs 383). 27 sites in three countries participated in the follow-up study. Cervical samples were tested every 6 months for HPV DNA. Management of abnormal cytologies was prespecified, and HPV-16/18 antibody titres were assessed. The primary objective was to assess long-term vaccine efficacy in the prevention of incident cervical infection with HPV 16 or HPV 18, or both. We report the analyses up to 6.4 years of this follow-up study and combined with the initial study. For the primary endpoint, the efficacy analysis was done in the according-to-protocol (ATP) cohort; the analysis of cervical intraepithelial neoplasia grade 2 and above (CIN2+) was done in the total vaccinated cohort (TVC). The study is registered with ClinicalTrials.gov, number NCT00120848.

**Findings** For the combined analysis of the initial and follow-up studies, the ATP efficacy cohort included 465 women in the vaccine group and 454 in the placebo group; the TVC included 560 women in the vaccine group and 553 in the placebo group. Vaccine efficacy against incident infection with HPV 16/18 was 95.3% (95% CI 87.4–98.7) and against 12-month persistent infection was 100% (81.8–100). Vaccine efficacy against CIN2+ was 100% (51.3–100) for lesions associated with HPV-16/18 and 71.9% (20.6–91.9) for lesions independent of HPV DNA. Antibody concentrations by ELISA remained 12-fold or more higher than after natural infection (both antigens). Safety outcomes were similar between groups: during the follow-up study, 30 (8%) participants reported a serious adverse event in the vaccine group versus 37 (10%) in the placebo group. None was judged related or possibly related to vaccination, and no deaths occurred.

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See Online/Comment  
DOI:10.1016/S0140-6736(09)61789-X

\*Members listed at end of paper

Correspondence to:  
Dr Barbara Romanowski,  
University of Alberta, 1000-8215  
112 Street, Edmonton, AB,  
Canada, T6G 2C8  
broman@docromanowski.com

# Bivalente HPV- Impfung Phase II

## Lancet 3.12.2009

	HPV-16/18-AS04 adjuvanted vaccine		Placebo		Vaccine efficacy (%; 95% CI)
	Total number of women	Women reporting ≥1 event	Total number of women	Women reporting ≥1 event	
<b>Incident infection with HPV 16/18</b>					
2-2 years	366	2	355	23	91.6% (64.5 to 98.0)
4-5 years	414	3	385	51	94.7% (83.5 to 98.9)
5-5 years	401	3	374	66	96.1% (88.1 to 99.2)
6-4 years	401	4	372	70	95.3% (87.4 to 98.7)
<b>≥ASC-US</b>					
2-2 years	560	2	553	27	92.9% (70.0 to 98.3)
4-5 years	505	2	497	44	95.7% (83.5 to 99.5)
5-5 years	505	2	497	51	96.4% (86.3 to 99.6)
6-4 years	505	2	497	54	96.7% (87.3 to 99.6)
<b>CIN1+</b>					
2-2 years	560	0	553	6	100% (37.0 to 100)*
4-5 years	481	0	470	8	100% (42.4 to 100)
5-5 years	481	0	470	11	100% (61.5 to 100)
6-4 years	481	0	470	15	100% (73.4 to 100)
<b>CIN2+</b>					
2-2 years	560	0	553	3	100% (-26.2 to 100)*
4-5 years	481	0	470	5	100% (-7.7 to 100)
5-5 years	481	0	470	7	100% (32.7 to 100)
6-4 years	481	0	470	9	100% (51.3 to 100)

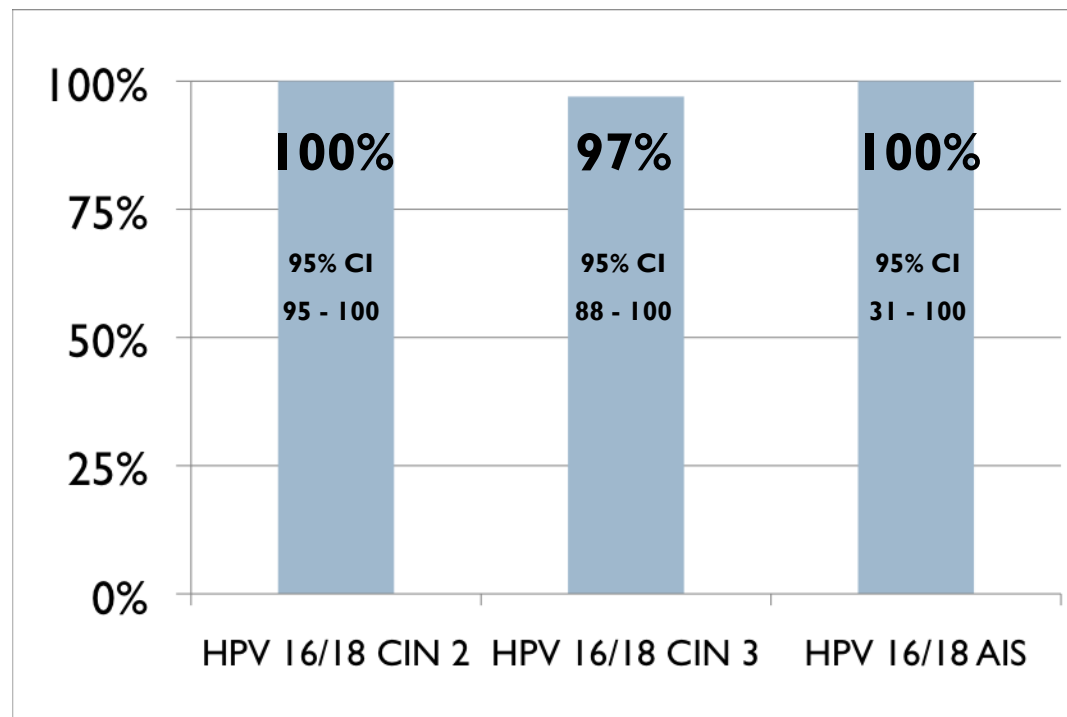
The values shown represent the cumulative number of women reporting an endpoint event associated with HPV 16/18 and related vaccine efficacy up to the maximum follow-up time for the different analyses undertaken: 2-2 years corresponds to the final analysis of the initial study (up to 2-2 years);<sup>21</sup> 4-5 years corresponds to the interim analysis of the combined initial and follow-up studies (up to 4-5 years);<sup>22</sup> 5-5 years corresponds to the interim analysis of the combined initial and follow-up studies (up to 5-5 years), and 6-4 years corresponds to the final analysis of the combined initial and follow-up studies (up to 6-4 years). All infections, cytology results, and lesions are associated with infection with either HPV 16 or HPV 18, or both. Incident infection reported in the according-to-protocol efficacy cohort (women who met all eligibility criteria, complied with study procedures, and had data available for efficacy measures). Atypical squamous cells of undetermined significance (ASC-US) or greater and cervical intraepithelial neoplasia (CIN) reported in the total vaccinated cohort (women who received at least one dose of study vaccine or placebo and for whom endpoint measures were available; this cohort was previously described<sup>13,17</sup> as an intention-to-treat cohort). CIN1+=CIN grade 1 and above. CIN2+=CIN grade 2 and above. \*Calculation of vaccine efficacy against CIN at 2-2 years was a post-hoc evaluation.

**Table 2: Cumulative number of endpoint events associated with HPV 16/18**

# 4- Jahresergebnisse Gardasil CIN2/3 und AIS, Frauen 16 – 26

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**Combined results from 4 phase II/III trials  
~4 year follow up - Per Protocol Population (END OF STUDY)**



**n (GARDASIL®) = 8493; n (placebo) = 8464**

# Prophylactic HPV6/11/16/18 vaccination

## Vulvar disease – 4 year data

up to 4 years follow , phase III studies	HPV-6/11/16/18 vaccine (N = 7,900)*	Placebo (N = 7,902)*	Efficacy against HPV6/11/16/ or 18 lesions	
	Cases	Cases	Efficacy	95% CI
VIN I	0	16	<b>100</b>	(74.1, 100.0)
VaIN I	0	12	<b>100</b>	(64.0, 100.0)
VIN 2/3	0	13	<b>100</b>	(67.2, 100.0)
VaIN 2/3	0	10	<b>100</b>	(55.4, 100.0)



# Wirksamkeit nach HPV- Infektionen

## Seropositive Frauen

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	Vaccine			Placebo				
CIN	n	Cases	Rate	n	Cases	Rate	Efficacy (%)	95% CI
HPV 6/11/16/18	1,243	0	0.0	1,283	7	0.2	100.0	(28.7, 100.0)

	Vaccine			Placebo				
GW+VIN	n	Cases	Rate	n	Cases	Rate	Efficacy (%)	95% CI
HPV 6/11/16/18	1,268	0	0.0	1,301	8	0.2	100%	(39.5, 100.0)

# Primary efficacy Midadult Women

Persistent Infection or clinical endpoint related to HPV 6/11/16/18  
Per-protocol analysis

Age	Gardasil		Placebo		% Reduction	95% CI	P-value
	Cases	pyr	Cases	pyr			
All Subjects	4	2,721	41	2,654	91%	74 – 98	<0.001
24 to 34 Year-Olds	2	1,329	24	1,301	92%	67 - 99	<0.001
35 to 45 Year-Olds	2	1,393	17	1,353	89%	52 - 99	<0.001

# Efficacy Against HPV 6/11/16/18 Related External Genital Lesions (EGL)

*Young Men 16-26 years*

## Per Protocol Efficacy Population

Endpoint	GARDASIL® (n = 1,397)		Placebo (n = 1,408)		% Efficacy	95% CI	p-value
	Cases	Inc. per 100 PY	Cases	Inc. per 100 PY			
<b>EGL any</b>	3	0.1	31	1.1	<b>90.4</b>	69, 98	<0.001

*n = number of subjects randomized who received at least 3 doses, have follow-up after Month 7 and not protocol deviator  
PY = person years; CI = confidence interval.*

*EGLs include external genital warts, penile/perianal/perineal intraepithelial neoplasia (PIN), penile, perianal, or perineal cancer; case counting began after month 7.*

# Clinical efficacy 100% up to 9.5 years HPV16

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PPE	HPV 16 monovalent		PLACEBO		Efficacy (%)	95%CI
	n	cases	n	cases		
HPV-16-related CIN 1 or worse	114	0	118	8	<b>100</b>	47, 100
HPV-16-related CIN 2 or worse	114	0	118	7	<b>100</b>	36, 100

“Proof of Principle” study

# Medien

29. September 2009, 14:24 Uhr

Schriftgröße: A A A Drucken Twittern Bookmarker Versenden Newsletter Gesundheit

## Umstrittener Impfstoff

### Britisches Mädchen stirbt nach HPV-Impfung

In England ist ein 14-jähriges Mädchen nach einer Impfung gegen HP-Viren, die Gebärmutterhalskrebs auslösen können, gestorben. Eine Obduktion sollte klären, ob die Impfung für den Tod verantwortlich war.



In Großbritannien wurden bisher mehr als eine Million junge Frauen gegen HPV geimpft.

© Diether Endlicher/AP

**E**ine 14-jährige Britin ist gestorben, nachdem sie gegen humane Papilloma-Viren (HPV) geimpft wurde. Die Jugendliche hatte an einem nationalen Impfprogramm teilgenommen und kam am Montag kurz nach der Impfung um. Die Gesundheitsbehörde NHS kündigte am Dienstag eine genaue

Untersuchung des Falls an. "Es kann kein Zusammenhang zwischen dem Tod und der Impfung hergestellt werden, solange nicht alle Faktoren bekannt sind", hieß es.

#### MEHR ZUM ARTIKEL

##### Gebärmutterhalskrebs

##### HPV-Impfung in der Kritik

Die Impfstoffe, die Gebärmutterhalskrebs verhindern sollen, schreiben Medizingeschichte: Selten kam ein Medikament so schnell auf den Markt - und war so teuer. Nun regt sich Kritik: Die Wirksamkeit der Impfung sei nicht hinreichend erwiesen, meinen 13 Forscher. Und die Pharmakonzerne halten wichtige Daten zurück. [mehr...](#)

##### Porträt Harald zur Hausen

##### Früher belächelt, heute gefeiert

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# VAERS – Jama 2009

## Postlicensure Safety Surveillance for Quadrivalent Human Papillomavirus Recombinant Vaccine

Barbara A. Slade, MD, MS

Laura Leidel, RN, FNP-C, MPH

Claudia Vellozzi, MD, MPH

Emily Jane Woo, MD, MPH

Wei Hua, MD, PhD

Andrea Sutherland, MD, MSc, MPH

Hector S. Izurieta, MD, MPH

Robert Ball, MD, MPH

Nancy Miller, MD

M. Miles Braun, MD, MPH

Lauri E. Markowitz, MD

John Iskander, MD

ON JUNE 8, 2006, THE FOOD and Drug Administration (FDA) licensed the quadrivalent human papillomavirus recombinant vaccine (qHPV) (Gardasil; Merck & Co, Inc, Whitehouse Station, New Jersey) for females aged 9 to 26 years to prevent infection with genital human papillomavirus (HPV) types 6, 11, 16, and 18.<sup>1</sup> Later that month, the Advisory Committee on Immunization Practices (ACIP) recommended routine vaccination of females aged 11 to 12 years with 3 doses of qHPV and catch-up vaccination for females aged 13 to 26 years. Doses are administered intramuscularly on a schedule of

**Context** In June 2006, the Food and Drug Administration licensed the quadrivalent human papillomavirus (types 6, 11, 16, and 18) recombinant vaccine (qHPV) in the United States for use in females aged 9 to 26 years; the Advisory Committee on Immunization Practices then recommended qHPV for routine vaccination of girls aged 11 to 12 years.

**Objective** To summarize reports to the Vaccine Adverse Event Reporting System (VAERS) following receipt of qHPV.

**Design, Setting, and Participants** Review and describe adverse events following immunization (AEFIs) reported to VAERS, a national, voluntary, passive surveillance system, from June 1, 2006, through December 31, 2008. Additional analyses were performed for some AEFIs in prelicensure trials, those of unusual severity, or those that had received public attention. Statistical data mining, including proportional reporting ratios (PRRs) and empirical Bayesian geometric mean methods, were used to detect disproportionality in reporting.

**Main Outcome Measures** Numbers of reported AEFIs, reporting rates (reports per 100 000 doses of distributed vaccine or per person-years at risk), and comparisons with expected background rates.

**Results** VAERS received 12 424 reports of AEFIs following qHPV distribution, a rate of 53.9 reports per 100 000 doses distributed. A total of 772 reports (6.2% of all reports) described serious AEFIs, including 32 reports of death. The reporting rates per 100 000 qHPV doses distributed were 8.2 for syncope; 7.5 for local site reactions; 6.8 for dizziness; 5.0 for nausea; 4.1 for headache; 3.1 for hypersensitivity reactions; 2.6 for urticaria; 0.2 for venous thromboembolic events, autoimmune disorders, and Guillain-Barré syndrome; 0.1 for anaphylaxis and death; 0.04 for transverse myelitis and pancreatitis; and 0.009 for motor neuron disease. Disproportional reporting of syncope and venous thromboembolic events was noted with data mining methods.

**Conclusions** Most of the AEFI rates were not greater than the background rates compared with other vaccines, but there was disproportional reporting of syncope and venous thromboembolic events. The significance of these findings must be tempered with the limitations (possible underreporting) of a passive reporting system.

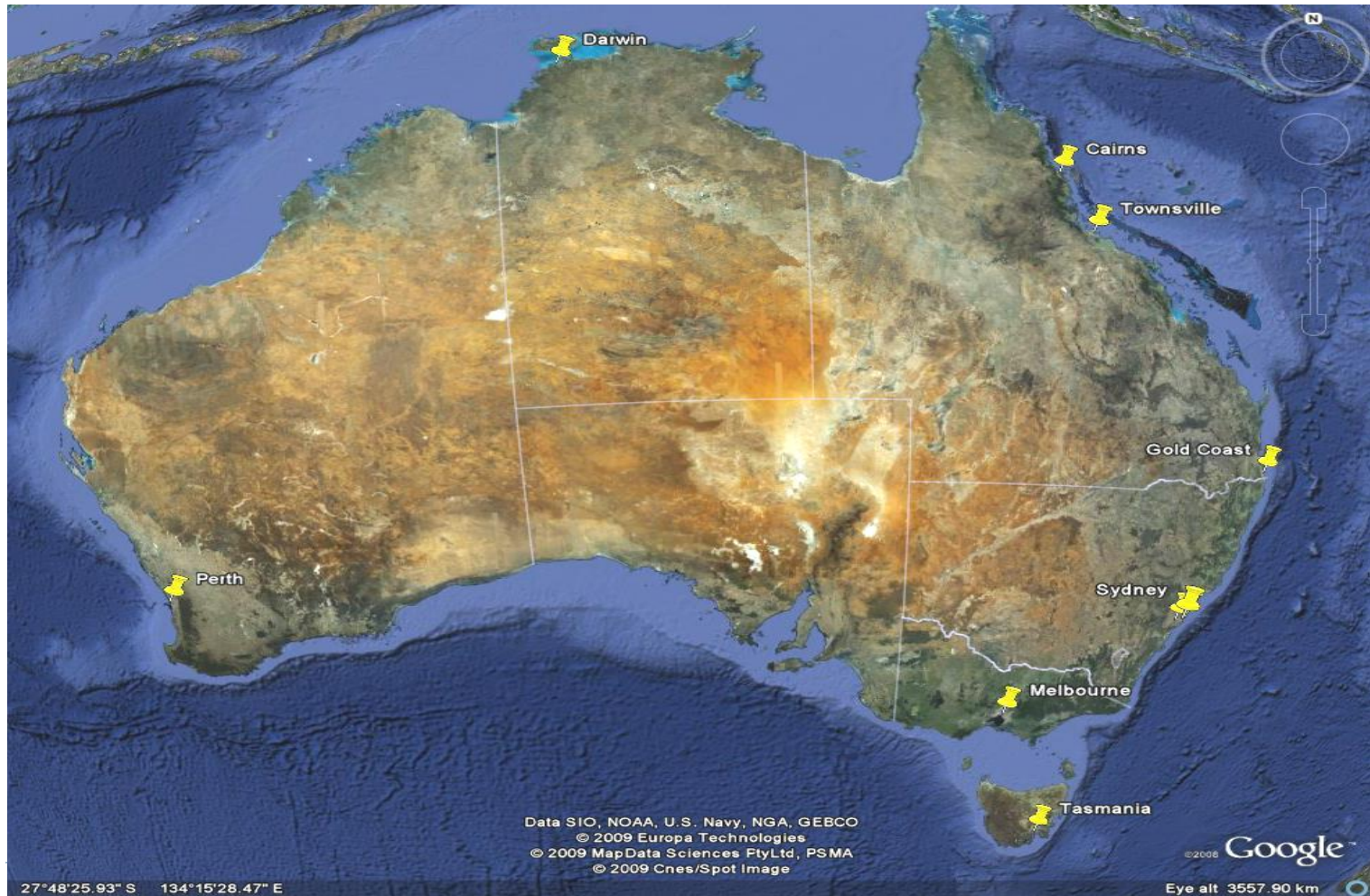
JAMA. 2009;302(7):750-757

www.jama.com

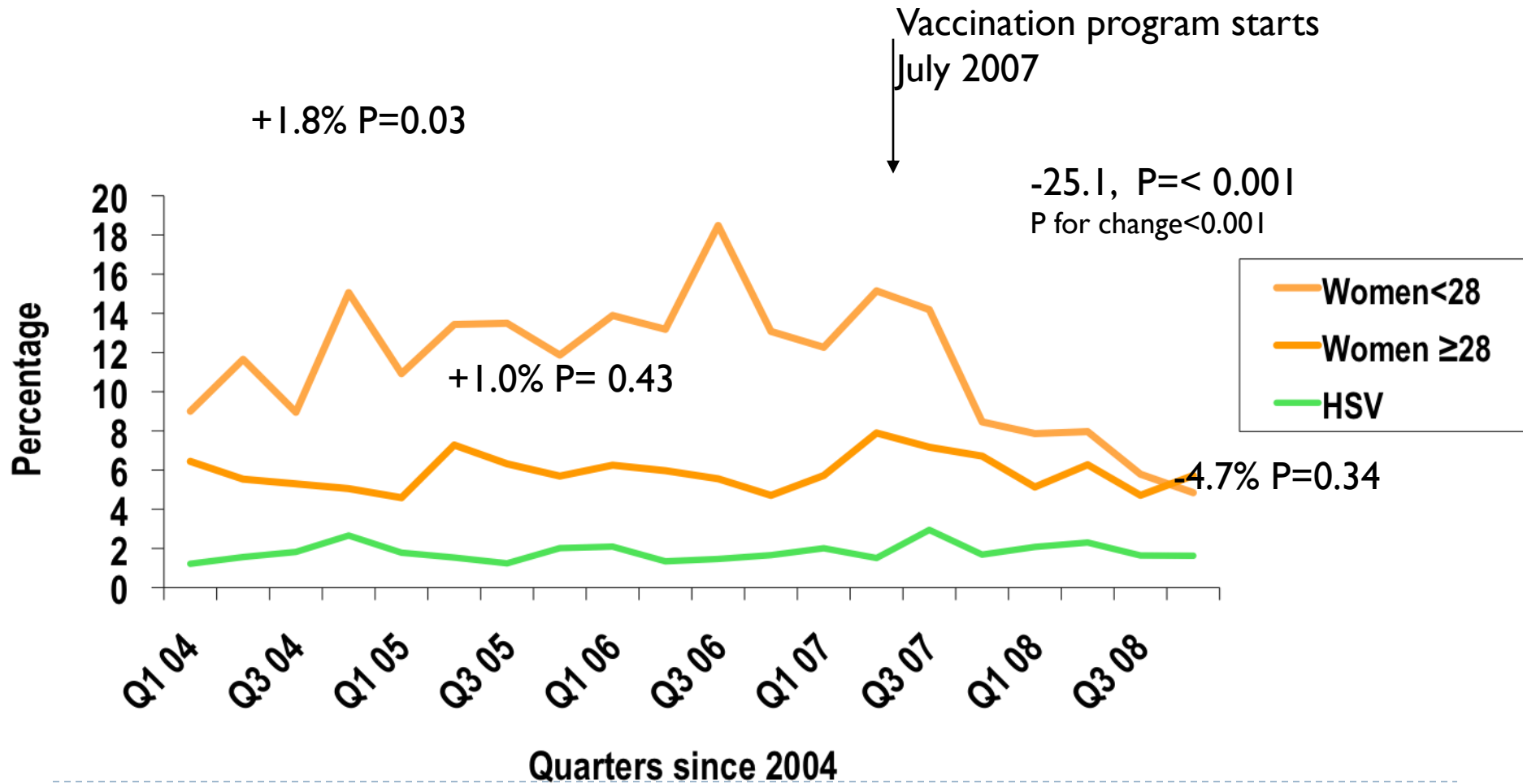
HPV-11 are the most common causes of years who were uninfected prior to vac-

Most of the AEFI rates were not greater than the background rates compared with other vaccines

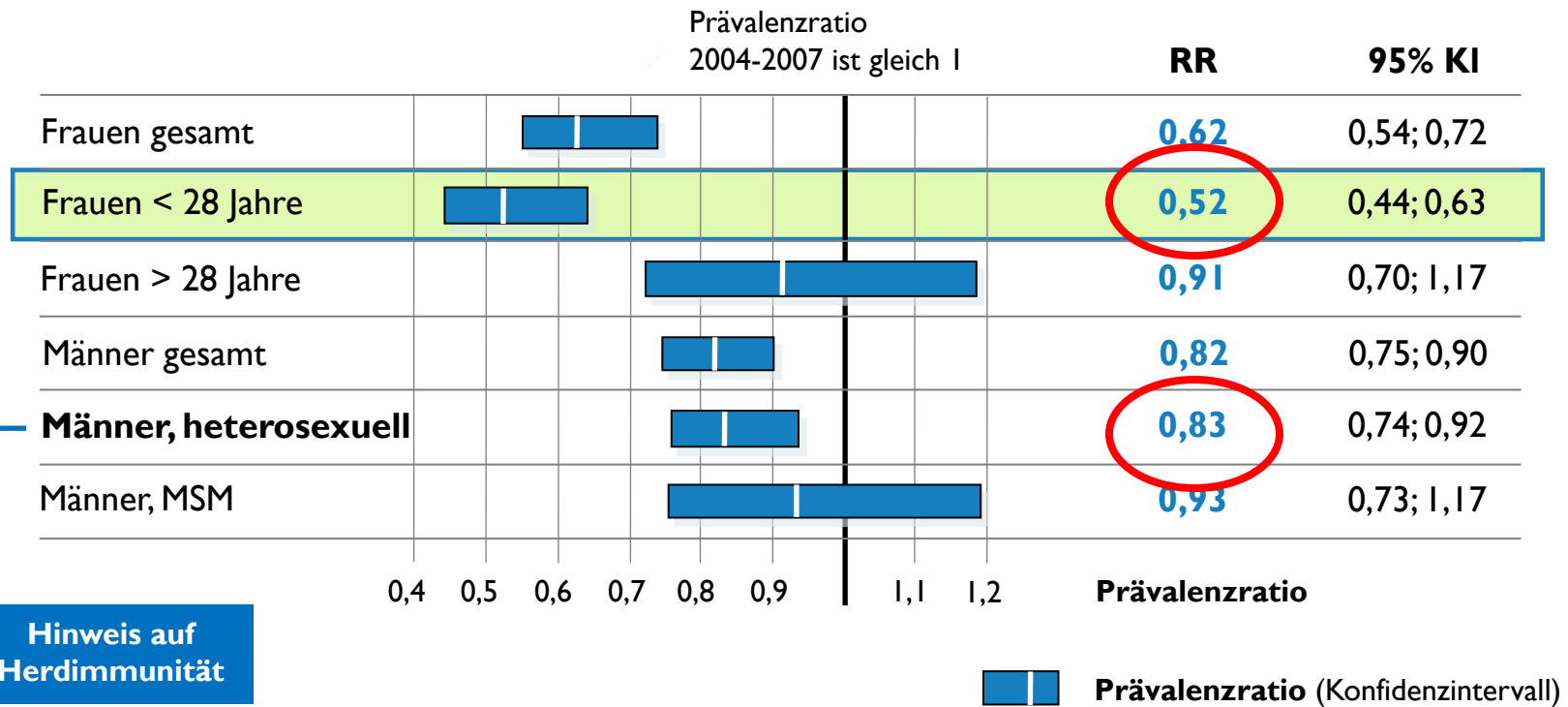
# Sexual Health Clinics - Australia



# Genital warts – Melbourne, AUS



# Genital warts – Melbourne, AUS

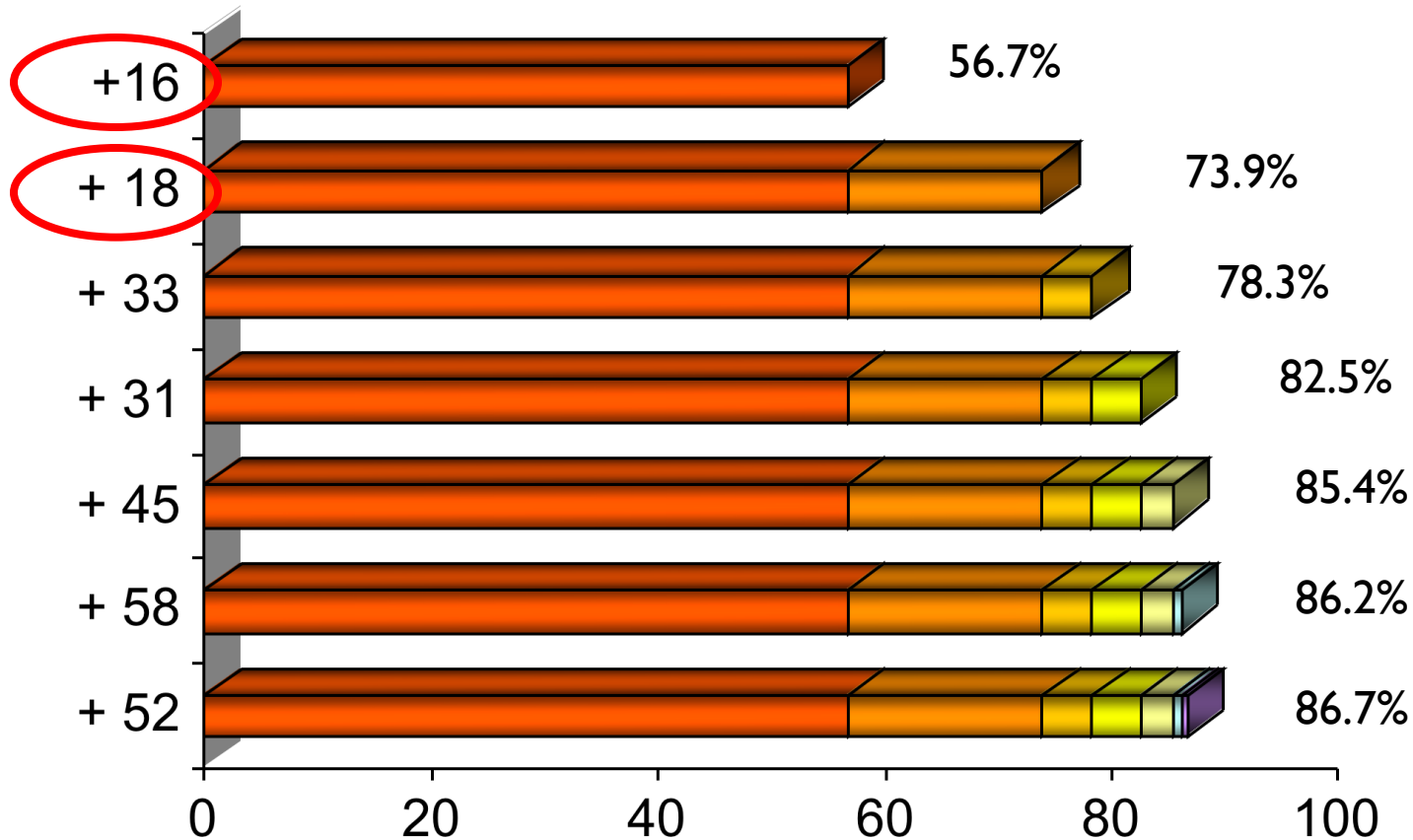


Fairley, C at al, IPC, Malmö, Schweden, Mai 2009; Beobachtungen Melbourne Sexual Health Centre (1/2004 – 12/2008)

\*Australisches Impfprogramm für Mädchen/Frauen (12 -18 J. Schulimpfung & 18 -26 J. durch niedergelassene Ärzte);

# HPV-Typen bei Zervixkarzinom in Europa

HPV-Typ



Ref.: Clifford, G.M. et al.: Br. J. Cancer. 2003; 88: 63-73...

# Prophylaktische HPV-Impfung

## 2. Generation

---

- ▶ Polyvalent
  - ▶ 9 Stämme
- ▶ Ca. 90% der onkogenen Stämme an der Zervix
- ▶ Deutliche Verbesserung bei low grade Läsionen
- ▶ Phase III hat begonnen
- ▶ Studienzentren in Österreich und Deutschland



N Engl J Med 2009;361:1838-47

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*The* NEW ENGLAND JOURNAL *of* MEDICINE

**ORIGINAL ARTICLE**

## **Vaccination against HPV-16 Oncoproteins for Vulvar Intraepithelial Neoplasia**

Gemma G. Kenter, M.D., Ph.D., Marij J.P. Welters, Ph.D.,  
A. Rob P.M. Valentijn, Ph.D., Margriet J.G. Lowik,  
Dorien M.A. Berends-van der Meer, Annelies P.G. Vloon, Farah Essahsah,  
Lorraine M. Fathers, Rienk Offringa, Ph.D., Jan Wouter Drijfhout, Ph.D.,  
Amon R. Wafelman, Ph.D., Jaap Oostendorp, Ph.D., Gert Jan Fleuren, M.D., Ph.D.,  
Sjoerd H. van der Burg, Ph.D., and Cornelis J.M. Melief, M.D., Ph.D.



# N Engl J Med 2009;361:1838-47

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- ▶ single-center, noncontrolled, observational phase 2 study
  - ▶ N=20
- ▶ HPV-16–positive VIN 3
- ▶ vaccinated 3/4 x
- ▶ long peptides HPV-16 oncoproteins E6 / E7
  - ▶ Freund’s adjuvant.
- ▶ End point:
  - ▶ Clinical
  - ▶ HPV-16–specific T-cell responses.



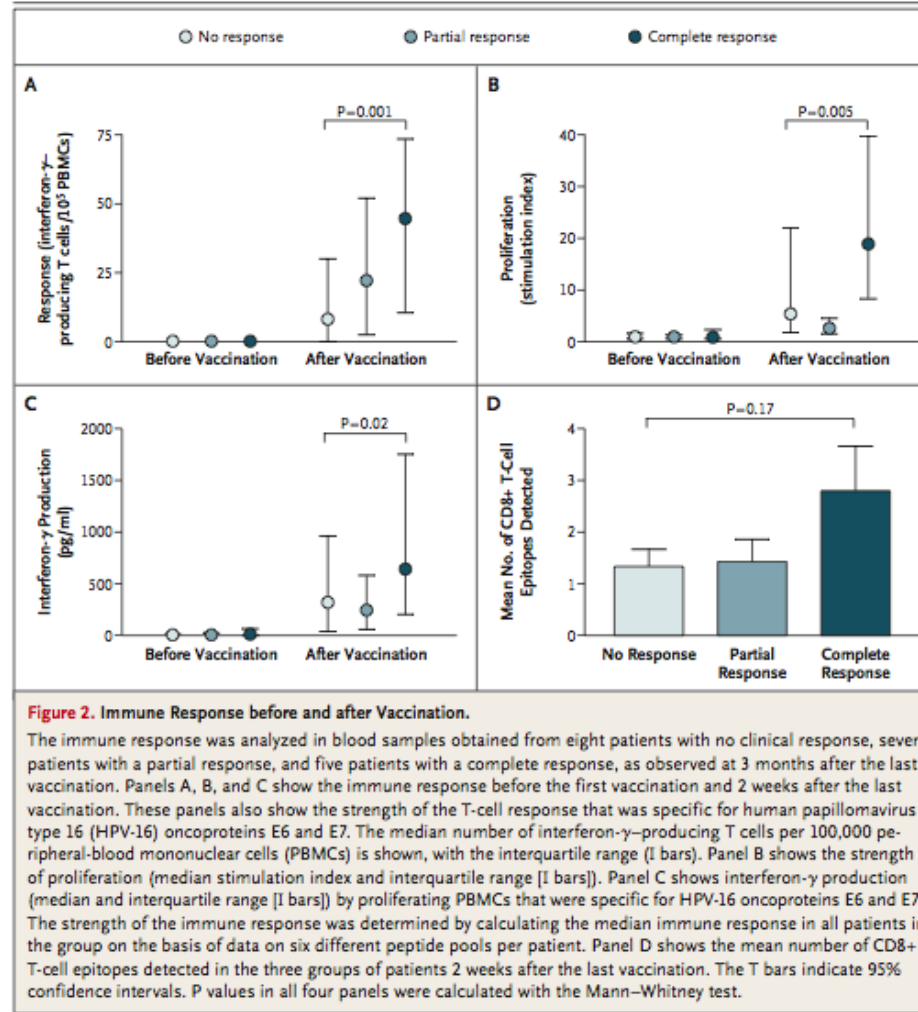
# N Engl J Med 2009;361:1838-47

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- ▶ **3 months follow up:**
  - ▶ 12 of 20 clinical response (60%; 95% CI 36-81)
  - ▶ 5/20 Complete response
  - ▶ 4/20 no HPV 16 detectable
- ▶ **12 months of follow-up:**
  - ▶ 15 /19 clinical responses (79%; 95% CI, 54-94), with a
  - ▶ 9/19 complete response (47%; 95% CI, 24-71)
- ▶ **24 months follow up:**
- ▶ **complete-response maintained**



# N Engl J Med 2009;361:1838-47



# Conclusio - Gegenwart

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- ▶ Hohe klinische Effektivität der Impfungen
- ▶ Kurzfristig: Condylome (quadrivalent)
- ▶ Mittelfristig: CIN, VIN, VaIN
  - ▶ Langfristig: Karzinome
- ▶ Frauen 16-45, Männer 16-26
- ▶ Lange Wirkungsdauer
- ▶ Hohe Sicherheit



# Conclusio - Zukunft

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- ▶ Nächste Generation prophylaktische Impfungen - Phase III
  - ▶ Polyvalent
- ▶ Therapeutische Impfungen Phase II
  - ▶ VIN: Vermeidung von Ops
  - ▶ CIN: Vermeidung von Frühgeburtlichkeit
- ▶ Developing World?

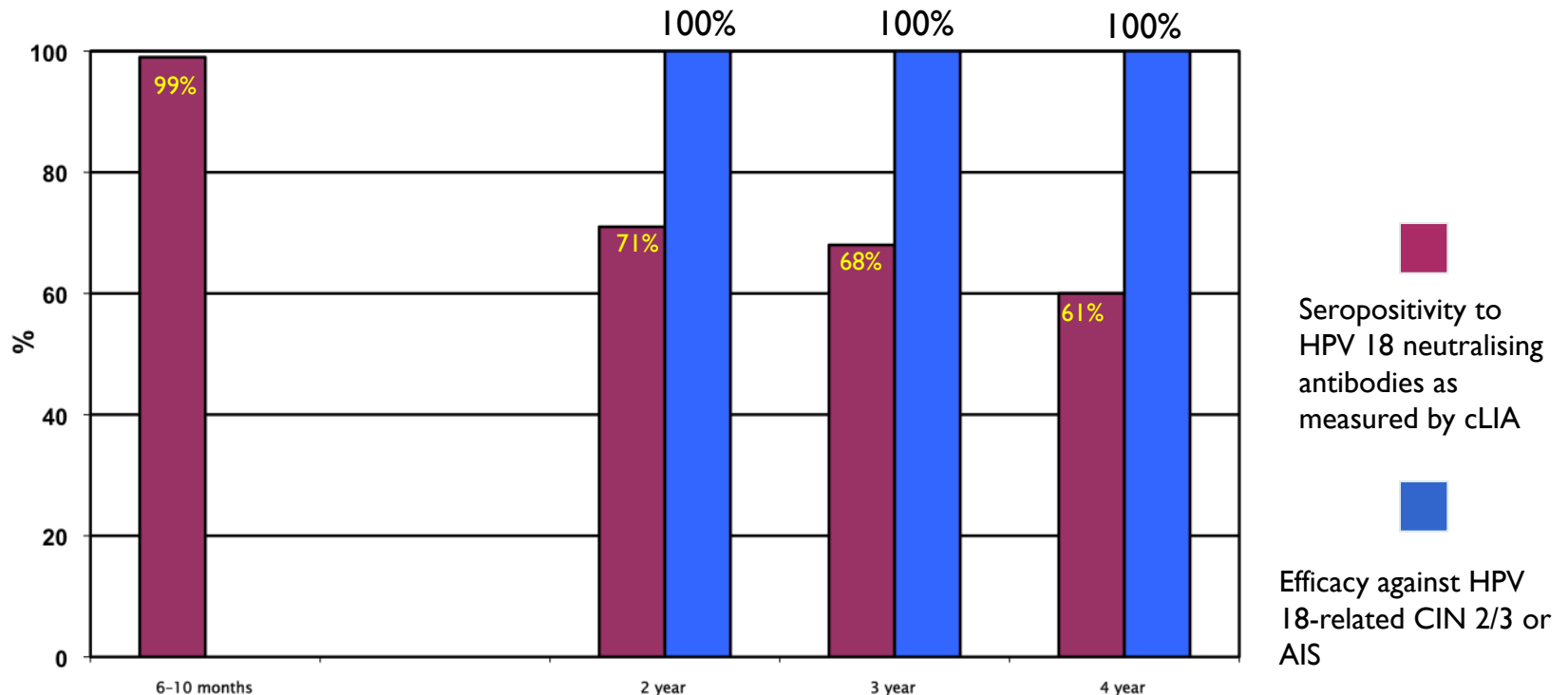


# Humane Papillomaviren

## „low- risk“ - „high-risk“

	häufige Virus- Typen	seltene Virus-Typen	assoziierte Erkrankungen
„nicht onkogene Typen“	6, 11	42, 43, 44, 55	<b>6,11:</b> <b>90% aller Condylome</b> <b>low grade CIN/ VIN</b> <b>Juvenile resp. Papillomatose</b>
„onkogene Typen“	16, 18	<b><u>31, 33, 45,</u></b> 26, 35, 39, 51, 55, <b><u>52, 56, 58, 59,</u></b> 66, 68	<b>16, 18:</b> <b>70% der Zervixkarzinome</b> <b>50% CIN, VIN 2+3, AIS</b> <b>70% aller HPV-Karzinome</b>

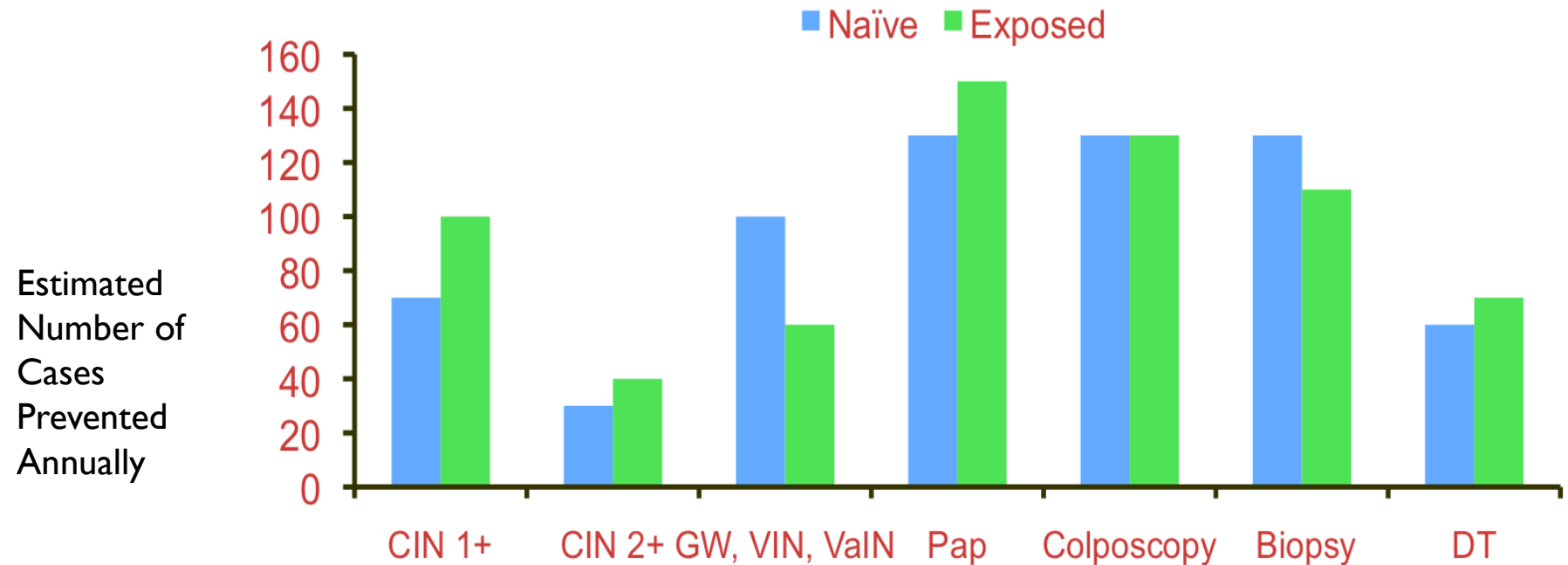
# Seropositivity and Efficacy of GARDASIL<sup>®</sup> against HPV 18 related CIN2/3 or AIS



\*Seropositivity to HPV 18 neutralizing antibodies to a single neutralizing epitope measured by cLIA

No correlation between measured antibody levels and clinical efficacy or duration of protection

# Cases prevented annually per 10,000 women 16–26 years of age vaccinated with GARDASIL FUTURE I/II end-of-study results (mean 3.6 years of follow-up)



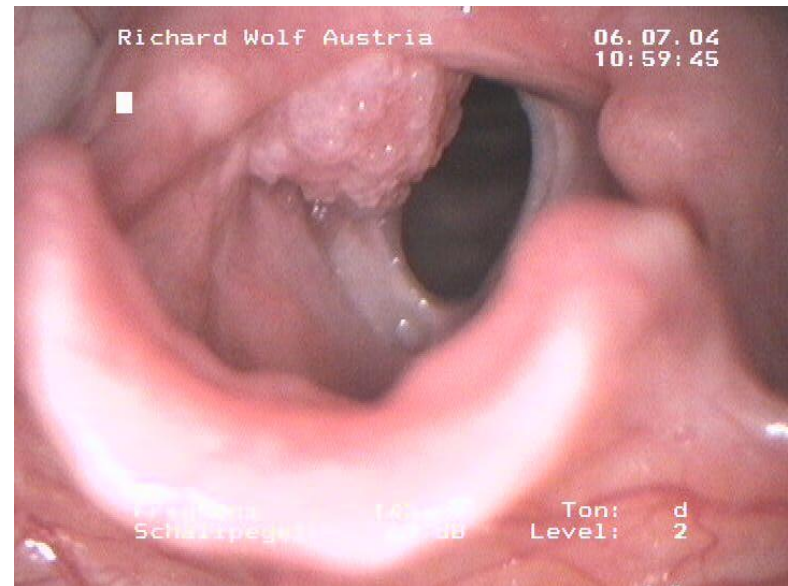
CIN = cervical intraepithelial neoplasia; DT = definitive therapy; GW = genital warts;  
VaIN = vaginal intraepithelial neoplasia; VIN = vulvar intraepithelial neoplasia

Cases prevented = (Incidence in placebo group [per 100 person-years] – Incidence in vaccine group [per 100 person-years]) × 100

# Juvenile Larynxpapillome

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- ▶ 20 jährige Patientin
- ▶ 43 Operationen!
- ▶ Keine Stimme

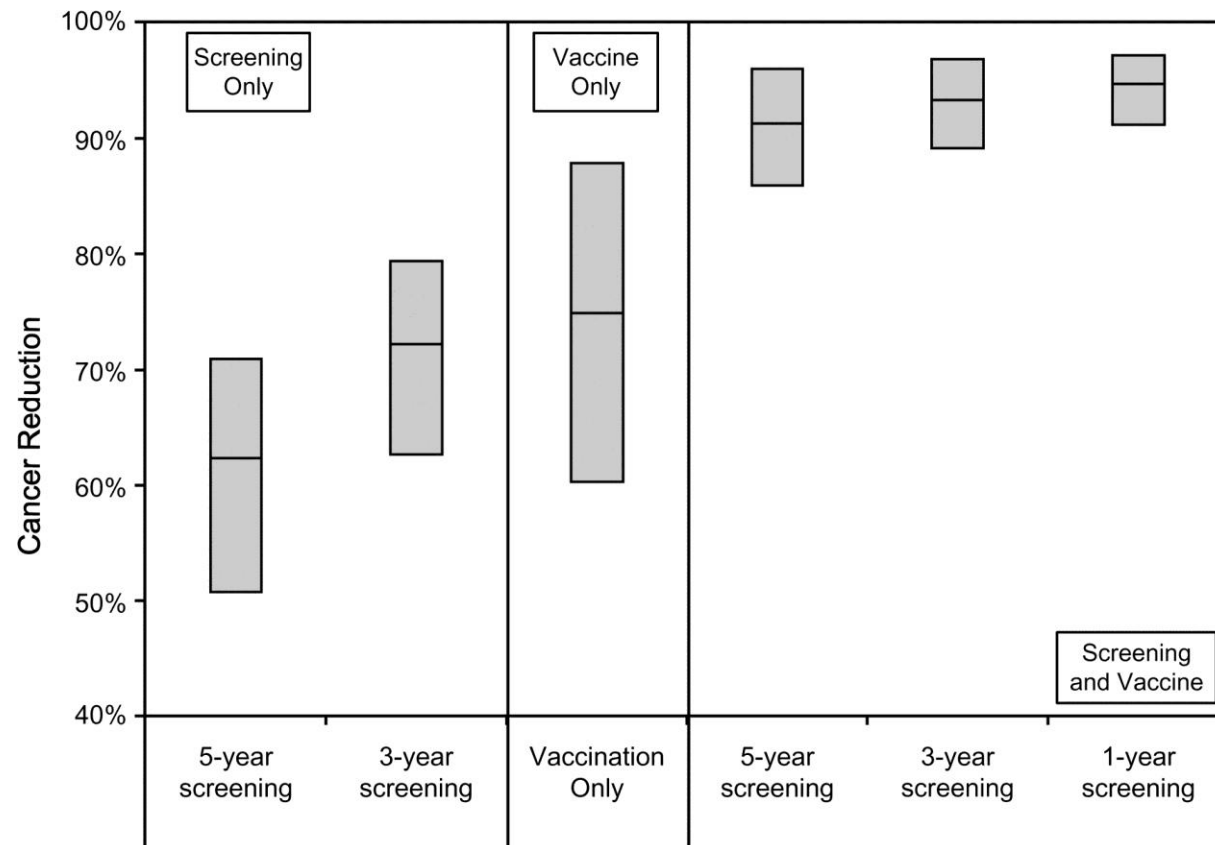


Prof. Biegenzahn HNO AKH Wien

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# Reduktion des Lebensrisikos Screening und Impfen



# Prophylactic Efficacy Against Vulvar condyloma

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Vulvar condyloma	GARDASIL® (N = 9075)			Placebo (N = 9075)			Efficacy	95% CI
	n	Cases	Rate*	n	Cases	Rate*		
<b>HPV 6-related</b>	<b>7,374</b>	<b>1</b>	<b>&lt;0.1</b>	<b>6,867</b>	<b>71</b>	<b>0.7</b>	<b>99</b>	<b>(93, 100)</b>
HPV 11-related	7,374	0	0.0	6,867	16	0.2	<b>100</b>	(77, 100)
HPV 16-related	7,071	0	0.0	6,474	14	0.1	<b>100</b>	(73, 100)
HPV 18-related	7,879	0	0.0	7,355	6	0.1	<b>100</b>	(23, 100)

Per-Protocol Population

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# Prophylactic Efficacy Against VIN 2/3 or VaIN 2/3

*Type Specific Per-Protocol Population(s)*

	GARDASIL® (N = 9075)		Placebo (N = 9,075)		%Efficacy	95% CI
	n	Cases	n	Cases		
HPV 16 or 18- related VIN 2/3	7,771	0	7,742	8	<b>100</b>	(42, 100)
HPV 16 or 18- related VaIN 2/3	7,771	0	7,742	7	<b>100</b>	(31, 100)



# Nobelpreis für Medizin und Biologie 2008

## Harald zur Hausen



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### Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers

(molecular cloning/blot hybridization/perinatal infection/genital cancer)

LUTZ GISSMANN\*, LUTZ WOLNIK†, HANS IKENBERG\*, URSULA KOLDOVSKY‡, HANS GEORG SCHNÜRCH‡, AND HARALD ZUR HAUSEN\*

\*Institut für Virologie, Zentrum für Hygiene, Universität Freiburg, Hermann-Herder-Strasse 11, 7800 Freiburg, Federal Republic of Germany; †Universitäts-Frauenklinik, Filgimstein 3, 3550 Marburg, Federal Republic of Germany; and ‡Universitäts-Frauenklinik, Mohrenstrasse, 4000 Düsseldorf, Federal Republic of Germany

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**ABSTRACT** Human genital tumors as well as recurrent laryngeal papillomas were analyzed for the presence of human papillomavirus (HPV) 6 and HPV 11 sequences. HPV 11 DNA was found in 7 of 14 laryngeal papillomas; in the 7 other tumors no HPV DNA was demonstrated. HPV 11 DNA was also found in all five atypical condylomata of the cervix included in this study. Condylomata acuminata mainly contained HPV 6 DNA. From 63 biopsy specimens, 41 clearly harbored HPV 6 DNA and 13 harbored HPV 11 DNA. In three tumors accurate typing was impossible, and in six additional ones neither HPV 6 nor HPV 11 DNA could be demonstrated. The data support a genital origin of laryngeal papillomavirus infections. In 4 of 24 malignant tumors, HPV 11 DNA or related sequences were demonstrated; 2 of the 4 were biopsy specimens from invasive cancer, and the other 2 originated from carcinomata *in situ*. A possible role of this or related papillomavirus types in the induction of malignant genital tumors remains to be elucidated.

#### MATERIALS AND METHODS

**Extraction of Cellular DNA.** Biopsy materials were examined histologically and stored at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$  until further processing. Extraction of cellular DNA was done as described (6).

**Labeling of HPV DNA.** HPV 6 DNA has been cloned into pBR322 in two fragments representing approximately one-third and two-thirds of the total genome, respectively (5). HPV 11 DNA, which has been identified from a genomic library of laryngeal papilloma constructed in  $\lambda$  L47 (7), was subcloned in pBR322 at the single *Bam*HI site.

Both DNAs were prepared as described (10) and labeled with deoxynucleotide [ $\alpha$ - $^{32}\text{P}$ ]triphosphate by the nick-translation procedure to a specific activity of  $>10^8$  cpm/ $\mu\text{g}$  (6).

**Blot Hybridization.** About 10  $\mu\text{g}$  of papilloma DNA was cleaved with restriction enzyme; the products were separated