HPV Virus-Like Particles Vaccines To Prevent Cervical Cancer

John Schiller, National Cancer Institute, NIH, USA

- HPV and Cancer
- Vaccine composition
- Clinical Trial Results
- Implementation Issues
- 2nd Generation Vaccines
Incidence and Distribution of Cancers Attributable to HPV

- Cervix: 450,000
- Oropharynx: 100,000
- Anus: 20,000
- Oral cavity: 250,000
- Larynx: 100,000
- Vulva: 50,000
- Penis: 10,000

Annual number of cases

HPV cases
Total cases
Cervical Cancer Disproportionately Strikes Disadvantaged Women

More developed countries

Less developed countries

Breast

Cervix

Ovary

Endometrium

Colon/rectum

Lung

Stomach

“Pap” smear

Annual number of cases (thousands)

Adapted from Parkin et al, Eur J Cancer 37:S4, 2001
99% of cervical cancer are HPV DNA positive. 15 types are thought to cause cervical cancer. Worldwide, 70% caused by HPV16 or HPV18.
Natural History of Cervical HPV Infections And Progression to Cancer

* Lifetime risk of genital HPV infection > 50%
Productive Papillomavirus Infections Only Occur in Stratified Squamous Epithelium

Precursor lesions for cervical cancer

Therapeutic vs Prophylactic HPV Vaccines

• Therapeutic vaccine attractive since Cervical CA develops slowly from well defined and routinely identified viral lesions. E6 and E7 expressed.

• Approved vaccines against other viral infections are preventive (based on neutralizing antibodies), not therapeutic (based on cell mediated immune responses).

• We initially concentrated on Prophylactic Vaccines
Live Attenuated Viruses Are Not Suitable For an HPV Prophylactic Vaccine

- Papillomavirus cannot be efficiently grown in cultured cells
- The viral genomes contain oncogenes
- Virion protein-based subunit vaccines are preferable, if they could efficiently induce neutralizing antibodies.
Papillomaviruses Encode Two Structural Proteins

- **L1**: the major structural protein. Each viral particle has 360 copies in 72 pentamers.

- **L2**: the minor structural protein. Up to 72 copies per particle.
Production of HPV Virus-Like Particle Based Vaccines

Insertion in Baculovirus or Yeast Expression Vector

Production in Insect Cells or Yeast

Spontaneous assembly of L1 into VLPs

Purification

VLPs: Non-infectious, Non-oncogenic
VLP Vaccination in Animal PV Models Implicate Antibodies as Immune Effectors

(Cottontail Rabbit PV, Bovine PV 4, Canine Oral PV)

- VLPs induce high titers of type-restricted virion neutralizing antibodies *
- Vaccination with VLPs of animal PVs ** induces type-specific protection from experimental infection with high dose virus
- Protection can be passively transferred ** in serum antibodies
- No regression of established lesions **
- No HPV or sexual transmission model

Human Serum HPV16 VLP ELISA Titers*

NCI/Johns Hopkins Phase II Trial

Geometric Mean Titer

1 Month = 9,400
6 Months = 2,900

* 3 Non-Adjuvanted IM Immunizations of 50 μg VLPs
How Could IM Injection of a VLP Vaccine Prevent Mucosal Infection at the Cervix?

- Transudation of serum IgG
- Exudation of Abs at sites of trauma
- VLP-specific IgG in cervical mucus ~10-fold lower than in the serum after IM vaccination*

* Nardelli et al., JNCI 95: 1128-37, 2003
T Cell Responses To L1 Unlikely to Induce Regression: Virion Protein Expression is Lost During Progression

CIN 1  CIN 2  CIN 3
Virus Producing  Non-productive  Non-productive

Decreasing Epithelial Differentiation

Doorbar, J Clin Virol 32:7-15, 2005
HPV VLP Vaccines in Phase III Trials

GlaxoSmithKline: HPV16
HPV18
ASO4 Adjuvant (MPL + Alum)
Made in insect cells

Merck:
HPV16
HPV18
HPV6
HPV11
Alum Adjuvant
Made in yeast

70% of Cervical Ca
90% of Genital Warts

IM Injections at 0, 1 or 2, and 6 months
### Three Phase III Trials Are in Progress

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>VLP Types</th>
<th>Trial Sites</th>
<th>Enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI:</td>
<td>HPV16, 18</td>
<td>Costa Rica</td>
<td>7,500</td>
</tr>
<tr>
<td>GSK:</td>
<td>HPV16, 18</td>
<td>Multicentric</td>
<td>18,000</td>
</tr>
<tr>
<td>Merck:</td>
<td>HPV16, 18, 6, 11</td>
<td>Multicentric</td>
<td>25,000</td>
</tr>
</tbody>
</table>

Women followed for several years

Virologic Endpoint: Persistent Cervical HPV DNA

Clinical Endpoint: Intermediate and High Graded Cervical Dysplasia (CIN 2/3)
Design, Safety and Immunogenicity

• Design: double blind; placebo controlled

• Young women, 16-26 yrs old, fewer than 4 (or 6) lifetime sex partners

• Primary analyses for types in the vaccine and exclude women HPV DNA or seropositive at entry

• Tolerability:
  Slightly more injection site pain than Alum;
  No effect on drop out rates
  No vaccine-related SAEs

• Immunogenicity:
  >99% Seroconversion;
  GMTs 10-50 fold higher than natural infection
**VLP Vaccines Exhibit Strong Protection For 4 Years**

<table>
<thead>
<tr>
<th>Study</th>
<th>Mao 2006</th>
<th>Harper 2006</th>
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<tr>
<td>VLP Types:</td>
<td>16</td>
<td>16,18</td>
</tr>
<tr>
<td>Adjuvant:</td>
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<td>AS04</td>
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<td>Trial Sites:</td>
<td>US</td>
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<td>Age:</td>
<td>16-23</td>
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<td>N (ATP):</td>
<td>1505</td>
<td>951</td>
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<td>3.5</td>
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<td>Persist. Infection:</td>
<td></td>
<td></td>
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<td>Vaccine/Placebo % Efficacy</td>
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<td>1/23</td>
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<tr>
<td>% Efficacy</td>
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<td>96%</td>
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<td>CIN1+:</td>
<td></td>
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<tr>
<td>Vaccine/Placebo % Efficacy</td>
<td>0/24</td>
<td>0/8</td>
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*ATP analyses for types included in the vaccines

**19 of 111 controls and 7 of 7 vaccinees were DNA positive only at the last visit

US=United States, BR=Brazil, CA=Canada, Persist=Persistent

CIN=Cervical Intraepithelial Neoplasia
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Protection After Plateau of VLP Antibody Titers Suggests Long Term Protection

Merck data, Mao et al. 2006
Merck Phase 3 Trertravalent Vaccine: Interim Analysis (Unpublished)

ATP analysis. HPV6,11,16, 18 Associated Disease Only

<table>
<thead>
<tr>
<th></th>
<th>Vaccine (N = 2717)</th>
<th>Placebo (N = 2725)</th>
<th>Efficacy (%)</th>
<th>CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN or worse</td>
<td>2240</td>
<td>0</td>
<td>2258</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>Genital warts, vulvar/vaginal neoplasia</td>
<td>2261</td>
<td>0</td>
<td>2279</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

ATP = received 3 doses of vaccine; HPV sero(-) at day 1 and HPV DNA(-) from day 1 to month 7; cases counted starting after month 7.

Average Duration of Follow-up: 1.5 Years After the Last Vaccination
Low-risk HPV types

High-risk HPV types

Based on Chan et al. (1995) J Virol
Efficacy Against Incident Infection by Other High Risk HPV Types

GSK HPV16/18 Vaccine; ITT Analysis; ca. 500 per arm

<table>
<thead>
<tr>
<th>HPV Type</th>
<th># Vaccine</th>
<th># Placebo</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>2</td>
<td>25</td>
<td>93 (71-99)</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>12</td>
<td>100 (66-100)</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>17</td>
<td>94 (63-100)</td>
</tr>
<tr>
<td>31</td>
<td>14</td>
<td>30</td>
<td>54 (11-78)</td>
</tr>
<tr>
<td>33</td>
<td>12</td>
<td>13</td>
<td>1 (-117-61)</td>
</tr>
<tr>
<td>52</td>
<td>40</td>
<td>48</td>
<td>19 (-27-48)</td>
</tr>
<tr>
<td>58</td>
<td>14</td>
<td>16</td>
<td>14 (-88-61)</td>
</tr>
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Phylogenetic Tree
Anogenital HPV Types

Low-risk HPV types

High-risk HPV types

Based on Chan et al. (1995) J Virol
Potential Reduction in Cervical Cancer from the Addition of Multiple HPV Types to Vaccine

Cumulative Presence of HPV Types in Cervical Cancer

- 16: 57.6%
- +18: 71.7%
- +45: 77.4%
- +31: 81.3%
- +X: 85.0%
- +33: 87.9%
- +52: 90.1%
- +58: 91.8%
- +35: 93.3%
- +59: 94.6%
- +56: 95.7%

From X. Bosch and N. Munoz: IARC, IBSCC and multicentric studies (N=3045)
# Overall Efficacy in Preventing HPV Cervical Disease

GSK HPV16/18 Vaccine; ATP analysis; 3.5 Yr Follow-Up; ca. 500 per arm

<table>
<thead>
<tr>
<th>HPV Types</th>
<th>Endpoint</th>
<th># Vaccine</th>
<th># Placebo</th>
<th>Efficacy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16/18</td>
<td>LSIL+</td>
<td>2</td>
<td>26</td>
<td>93 (70-99)</td>
</tr>
<tr>
<td>16/18</td>
<td>CIN1+</td>
<td>0</td>
<td>8</td>
<td>100 (42-100)</td>
</tr>
<tr>
<td>16/18</td>
<td>CIN2+</td>
<td>0</td>
<td>5</td>
<td>100 (-8-100)</td>
</tr>
<tr>
<td>Any Type</td>
<td>LSIL+</td>
<td>41</td>
<td>70</td>
<td>45 (17-63)</td>
</tr>
<tr>
<td>Any Type</td>
<td>CIN1+</td>
<td>12</td>
<td>24</td>
<td>51 (-1-80)</td>
</tr>
<tr>
<td>Any Type</td>
<td>CIN2+</td>
<td>3</td>
<td>11</td>
<td>73 (-1-95)</td>
</tr>
</tbody>
</table>

*Harper et al. Lancet 2006*
Potential Impact of the Vaccines in the US

US Cases
- 10,000,000 Subclinical HPV infection
- 1,400,000 Low-grade dysplasia
- 300,000 High-grade dysplasia
- 11,000 Cancer

HPV infection
- Spontaneous regression
- Spontaneous regression
- Pap screen- surgical treatment
- Many years (20+)

*The number of US cases and percentages attributed to each disease category are estimates.*
Outstanding Efficacy Questions

• Duration of Protection, need for boosters

• Extent of cross-protection against CIN by types not in the vaccine. How long will it last?

• Impact on prevalent infection. Might prevention of successive rounds of auto-inoculation diminish the likelihood of persistence and progression?

• Will the vaccine protect men from infection and disease?

• Will the vaccines be safe and effective in HIV+ and other immuno-suppressed patients?

• Will antibody levels be an immune correlate of protection?
Conclusions: HPV VLP Vaccine Efficacy

• VLP vaccines are high effective at protection against a spectrum of cervical HPV endpoints from incident infection to high grade precancer.

• A VLP vaccine is also highly protective against lesions of the external genitalia in women.

• Protection is type-restricted/type-specific, consistent with protection being antibody mediated.

• Duration of protection is unknown, but strong protection at 3.5 years, after antibody levels had reached a plateau, is very encouraging
When Might the Vaccines Be Available to the Public?

- Merck filed with FDA in Dec. ‘05.
- FDA Advisory Panel (VRBPAC) recommended approval for prophylactic vaccination of females 9-26 yr., May ‘06.
- A decision is expected in June ‘06.
- GSK filed in Europe Mar. ‘06, decision expected within one year. FDA filing expected before end of the year.
Who Should Be Vaccinated?

In descending order of importance:

• **10-13 year old girls** - the ultimate target group since they have not yet been exposed to these sexually transmitted viruses

• Sexually active women - since some may have not yet been exposed to these viruses; may reduce auto-inoculation and transmission

• Adolescent boys and men - only if the vaccines are shown to prevent infection, only 10% of HPV cancer in men; may be small impact on herd immunity if coverage of women high
Percentage of Teenagers in USA Who Have Had Vaginal Sex

From Mosher et al., Vital and Health Statistics 362, 2005
Who Should Be Vaccinated?

In descending order of importance:

• 10-13 year old girls - the ultimate target group since they have not yet been exposed to these sexually transmitted viruses

• Sexually active women - since some may have not yet been exposed to these viruses; may reduce auto-inoculation and transmission. *Most of the vaccine will initially go to this group.*

• Adolescent boys and men - only if the vaccines are shown to prevent infection, only 10% of HPV cancer in men; may be small impact on herd immunity if coverage of women high
Who Should Be Vaccinated?

In descending order of importance:

• 10-13 year old girls - the ultimate target group since they have not yet been exposed to these sexually transmitted viruses

• “Older” women - since some may have not yet been exposed to these viruses; may reduce auto-inoculation and transmission

• Adolescent boys and men - only if the vaccines are shown to prevent infection, only 10% of HPV cancer in men; may be small impact on herd immunity if coverage of women high. Protection against genital warts benefit for males.
Outstanding Questions For Implementation in U.S.

• Price of Vaccine

• ACIP* Decision - recommended?, for what age?

• Delivery of 3 IM doses to early adolescents

• Acceptance of an STD vs Cervical Cancer vaccine

• Effects of vaccine on Cervical Ca screening recommendations and compliance

*Advisory Committee on Immunization Practices
We Can’t Give Up Screening

• Pap screen probably decreases cervical cancer rates by at least 80% in the U.S.

• If type specific, the current VLP vaccines could decrease cervical cancer rates by at most ~70% (% caused by 16/18).

• Vaccination without screening would lead to higher rates of cervical cancer in the U.S.

• Need to convince vaccinated women to comply with screening programs.
Will the HPV Vaccine Reach the Women Who Need it Most?

Adapted from Parkin et al, Eur J Cancer 37:S4, 2001
Factors That Could Limit Underserved Women’s Access to the Vaccines

- Vaccine availability: especially during catch up phase
- Cost of Vaccine
- Distribution Costs and Hurdles:
  - Cold chain
  - 3 IM injections
  - Early adolescent vaccination
Potential Ways to Get More Vaccine To More Underserved Women

• Purchase by benefactors: Competition from other new vaccinates? Sustainability of Programs?

• Regional production: reason HBV vaccine is $0.30

• Second Generation Vaccines: strategies that lower cost of production and distribution.
2nd Generation Prophylactic Vaccines Moving Toward Phase I Clinical Trials

• L1 recombinant Ty21 Salmonella:
  Academic Collaborator: Denise Nardelli, U. Vaudois, Lausanne
  Industry Collaborator: Indian Immunologicals, Hydrabad

• L2 polypeptide:
  Academic Collaborator: Richard Roden, Johns Hopkins Univ.
  Industry Collaborator: Shantha Biotechnics, Hydrabad
Mucosal Vaccination with L1-Salmonella Live Vaccine: Murine Model*

* Codon Modified HPV16 L1 expressed inside the bacteria

Ty21a Kan³-L1S

Ty21a is an attenuated and widely used typhoid vaccine strain

Live Bacterial Vector Expressing VLP

• Advantages: Inexpensive production and delivery if oral, perhaps fewer doses

• Potential Disadvantages: trials expensive w/ GM organisms, variability in response?

• Approach hasn’t been tested clinically, but trials with Indian Immunologicals possible within the next year
L2 Induces Cross-Neutralizing Antibodies

Richard Roden (NCI, now JHU)

Papillomavirus Particle

Immunogen: L1/L2 VLPs

Immunogen: L2 protein

Neutralization assay: HPV 6 16 18

Neutralization assay: HPV 6 16 18

Neutralization assay: HPV 6 16 18
**Broad Cross-Neutralization of Mucosal And Cutaneous Types by N-terminal L2 Peptides**

<table>
<thead>
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<th>Anti-Serum</th>
<th>BPV1</th>
<th>HPV16</th>
<th>HPV18</th>
<th>HPV31</th>
<th>HPV6</th>
<th>CRPV</th>
</tr>
</thead>
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<tr>
<td>HPV16 a.a. 1-88:</td>
<td>50</td>
<td>1780</td>
<td>150</td>
<td>90</td>
<td>90</td>
<td>360</td>
</tr>
<tr>
<td>BPV1 a.a. 1-88:</td>
<td>3460</td>
<td>4740</td>
<td>7020</td>
<td>220</td>
<td>340</td>
<td>780</td>
</tr>
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Pastrana et al., Virology 337: 365-71, 2005
(collaboration with Richard Roden)
Psuedovirus Types Cross-Neutralized By HPV16 L2 N-terminus

foot warts

cutaneous, mostly asymptomatic

hand warts

mucosal, cancer-associated

genital warts
Immunization with heterologous N-terminal L2 peptides protects from cutaneous CRPV challenge

Neil Christensen, Penn State, Hershey

CRPV = Cottontail Rabbit Papillomavirus

Papillomas in the control group were larger than papillomas in the immunized groups.
Conclusion: L2 Minor Vaccines

- L2 induces a broadly cross-neutralizing antibody response so possibility for an monovalent vaccine against cutaneous and mucosal types.

- Production in bacteria could make it relatively inexpensive.

- Delivered as part of infant vaccination?

- Disadvantages: Neutralizing titers lower than induced by VLPs.

- Working to increase titers and initiate early phase clinical trials, with JHU and Shanta.
Key Collaborators

National Cancer Institute

Doug Lowy

Past Members of the Lab:
  Reinhard Kirnbauer
  Richard Roden (now at JHU)
  Diana Pastrana

Present Members of the Lab
  Chris Buck  Susana Pang
  Patricia Day  Cindy Thompson

Outside
  Clayton Harro: Johns Hopkins University- Baltimore
  Denise Nardelli: Universitaire Vaudois - Lausanne