HPV Vaccines: Past, Present, and Future

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The views expressed are my own and do not necessarily reflect those of NCI/NIH
Disclosure

The National Institutes of Health (NIH) has patents on papillomavirus L1 VLP and L2 vaccine technologies. John Schiller and I are inventors of these technologies. The NIH has licensed the L1 VLP technology to Merck and GlaxoSmithKline, the two companies with commercial versions of the vaccine. The L2 technology has been licensed to Acambis and PaxVax and is the subject of a CRADA with Shantha Biotech and Johns Hopkins University (Richard Roden).
Outline of presentation

• Background: HPV & cancer
• Current preventive HPV vaccines
• Two potential second generation vaccines
• Mechanisms of protection by vaccination
My perspective (1)

• Overall clinical goal: to reduce the incidence of cervical cancer
• Two medical approaches: HPV vaccination & cervical cancer screening
• Vaccination is mainly for the next generation of women; cancer develops many years after infection
• Screening is the most effective prevention modality for the current generation of women
My perspective (2)

- Over the past 50 years, cervical cancer screening in the US has led to a major reduction (>75%) in cervical cancer.
- This reduction is especially noteworthy, as the incidence of cervical HPV infection has increased substantially during this period.
- To maximize further cost-effective reductions in cervical cancer, it will be important to integrate screening with vaccination.
- A goal of my presentation is to contribute to consideration of this process by providing information about the current vaccines and prospects for improvement.
Worldwide Incidence and Distribution of Cancers Attributable to HPV

- Cervical cancer represents ~10% of all female cancers worldwide
- ~80% of cervical cancer occurs in the developing world

Adapted from Parkin, Int J Cancer 118:3030, 2006
United States: Incidence and Distribution of Cancers Attributable to HPV

- Pap screening has reduced the incidence of cervical cancer at least 75%

Implications of identifying HPV as the main cause of cervical cancer

- Natural history of HPV infection/pathogenesis of cervical cancer; co-factors
- Identification of other HPV-associated cancers
- HPV-based (i.e., etiology-based) cervical cancer screening
  - HPV DNA. HPV RNA?
  - p16-Ink4a? (E7 inactivates pRb, which increases p16 expression)
- HPV-based interventions
  - Preventive vaccine.
  - therapeutic vaccine, antivirals, etc?
Laboratory of Cellular Oncology, CCR, NCI, Bethesda

Patricia Day  
Rhonda Kines  
Cynthia Thompson  
Jeffrey Roberts  
Susana Pang  
Katie Johnson  
John Schiller

Chris Buck, Diana Pastrana - LCO, CCR, NCI Bethesda
Peter Choyke, Marcelino Bernardo - Molecular Imaging, CCR, NCI Bethesda
Mark Schiffman, Allan Hildesheim, Phil Castle, Ligia Pinto - DCEG, NCI Bethesda
Brian Murphy, Heather Greenstone - NIAID, Bethesda
Benes Trus, Frank Booy - NIAMS, Bethesda
Richard Roden, Clayton Harro, Ruth Karron - Johns Hopkins, Baltimore
Neil Christensen - Hershey Medical Center, Hershey
Rolando Herrero, Ana Cecelia Rodriguez, Maria Bratti - SSC, Costa Rica
Reinhard Kirnbauer - University of Vienna, Austria
Denise Nardelli-Haefliger - University of Lausanne, Switzerland
Choosing an appropriate molecular target for a preventive HPV vaccine

• Licensed vaccines are mainly preventive; induction of neutralizing antibodies appears to be necessary (sufficient?).

• HPVs contain viral oncogenes (E5, E6, E7). Implies you need a subunit vaccine lacking the oncogenes.

• Papillomaviruses encode two proteins that contain neutralization epitopes, the capsid proteins L1 and L2.

• **L1 contains the immunodominant neutralization epitopes.** However, they are conformationally dependent. **L1 can self-assemble to make empty particles with a conformation that induces high levels of neutralizing antibodies.**
**L1 self-assembles to form virus-like particles (VLP)**

*That induce high levels of neutralizing antibodies*

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**L1 Pentamers**

![Image of L1 Pentamers]

**Self-assembly**

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**Into VLPs**

![Image of L1 Virus-like Particle (VLP)]

**VLPs can induce neutralizing antibody titers that are many times higher than after natural infection.**

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*Reinhard Kirnbauer et al, PNAS, 1992*
**Systemic VLP Vaccination is Protective in Animal Papillomavirus Models**

- High protection in 3 animal papillomavirus models:
  - prophylactic, not therapeutic
  - *passively transferred with immune IgG (neutralizing antibodies)*
  - type-specific (VLPs from a divergent papillomavirus were not protective)

- Extrapolation to human HPV disease?
  - Yes, despite a poor track record of vaccines against sexually transmitted local infections
Two Distinct Commercial HPV L1 VLP Vaccines

GlaxoSmithKline:  
Cervarix  
- HPV16
- HPV18
  ASO4 Adjuvant (Aluminum + MPL)
  Made in insect cells

Merck:  
Gardasil  
- HPV16
- HPV18
- HPV6
- HPV11
  Aluminum Adjuvant
  Made in yeast

70% of Cervical Ca
90% of Genital Warts

Three intramuscular injections given over 6 months
## Merck vaccine: prophylactic efficacy results against HPV types in the vaccine (2006)

<table>
<thead>
<tr>
<th></th>
<th>Vaccine</th>
<th>Placebo</th>
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<tr>
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<td>Efficacy</td>
<td>C.I.</td>
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<tr>
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<td>8487</td>
<td>0</td>
<td>8460</td>
<td>53</td>
<td>100%</td>
<td>93–100</td>
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<td>CIN 2/3 or AIS</td>
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<tr>
<td>**HPV16 or 18</td>
<td>8641</td>
<td>0</td>
<td>8667</td>
<td>24</td>
<td>100%</td>
<td>83-100</td>
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<tr>
<td>VIN2/3 or ValN2/3</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>*HPV6, 11, 16, 18</td>
<td>7897</td>
<td>1</td>
<td>7899</td>
<td>91</td>
<td>99%</td>
<td>94–100</td>
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<tr>
<td>Genital warts</td>
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</tbody>
</table>

Results in HPV DNA and seronegatives at baseline after three doses (*) or after at least one dose (**), as reported in Gardasil package insert.

Average Duration of Follow-up: 1.5 Years After the Last Vaccination

Note: Protection against infection by other HPV types is limited.
Geometric Mean Titer (mMU/mL)

HPV 16 L1 VLP Vaccinees
(>99.5% seroconversion)

Placebo recipients who were HPV 16 Sero(+)/PCR(-) at Day 1

Protection After Plateau of VLP Antibody Titers Suggests Long Term Protection

Merck data, Mao et al. 2006
The vaccine does not hasten clearance of established infection: L1 is not expressed in basal cells

Conclusion from: Hildesheim et al., Effect of viruslike particle vaccine among young women with pre-existing infection. JAMA 298:743-53, 2007

Figure adapted from: Doorbar, The papillomavirus life cycle. J Clin Virol 32:7-15, 2005
HPV Vaccine Characteristics

• Strengths:
  – Systemic immunization with a non-infectious HPV vaccine induces high efficacy against mucosal and cutaneous infection caused by HPV types in vaccine
  – Can protect against ~70% of cervical cancers and (for Merck vaccine) ~90% of genital warts

• Limitations:
  – Only protects against new infections, not against established infections
  – Protection is type-restricted; current vaccine will not protect against ~30% of serious infections
  – Vaccinated women need to continue regular cervical cancer screening
  – Expensive
Regulatory status of HPV VLP vaccines

- Merck’s Gardasil approved 2006 in USA (females 9-26); EU (females 9-26 + males 9-15); many other countries
- GSK’s Cervarix approved 2007 in EU (females 10-25), other countries; filed with FDA in March 2007
- Main target group for vaccine: 11-12 y.o. girls; prior to becoming sexually active
- Catch-up vaccination for 13-26 y.o. girls/women
- Inclusion in Federal Vaccines For Children program (provides vaccine for girls <19 y.o. from poor families)
Acquisition of Genital HPV infection in young women with their first sexual partner

Adapted from Collins et al, BJOG 109: 96-8, 2002
Influence of Age of Vaccination on Prevention of HPV16-associated Cervical Cancer: Finland

- 70% of females in each age group are immunized in 2008

Some Outstanding Medical Issues

- Will the vaccine continue to have a good safety profile?
- How long will the vaccine remain highly protective? Will a booster be needed?
- Will the vaccine be effective in boys/men?
- Might the eradicated HPV types be replaced by other HPV types?
- Will the vaccine prevent non-genital HPV infection and disease caused by HPV types in the vaccine?
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Some Outstanding Medical Issues

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- How long will the vaccine remain highly protective? Will a booster be needed?
- Will the vaccine be effective in boys/men?
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- Will the vaccine prevent non-genital HPV infection and disease caused by HPV types in the vaccine?
What impact could the vaccine have on overall HPV infection and disease?

• If the vaccine is widely implemented, it should:
  – Reduce overall incidence of CIN2/CIN3 by ~50% and cancer by ~70%
  – Reduce overall incidence of HPV infection by only ~20%
  – Reduce the prevalence of the “vaccine types;” HPV16/18 (HPV6/11 for Merck vaccine)

• If the vaccine is not widely implemented, the population-wide impact of the vaccine will be much less
Cumulative Incidence of Progression to CIN3+ in Women Over 30 by HPV Status at Entry: Portland, OR

Follow-up time (years)

Cumulative incidence rate (%)

HPV16

HPV18

High-risk HPV other than 16/18

Negative for high-risk HPV

From Khan et al, J Natl Cancer Inst 97:1072, 2005
Cumulative Incidence of Progression to CIN3+ in Women Over 30 by HPV Status at Entry: Portland, OR

From Khan et al, J Natl Cancer Inst 97:1072, 2005
Pap smear screening without vaccination would prevent more cervical cancer deaths than HPV16/18 vaccination without Pap screening.

Assumptions: 1) Current Pap screening protects against 80% of cancer deaths. 2) HPV16 & 18 vaccination will be 90% effective.
Possible Goals of Second Generation HPV Vaccines

• To add a therapeutic component to a prophylactic vaccine

• To simplify vaccine production and/or administration

• To broaden coverage against more HPV types
Potential Reduction in Cervical Cancer from the Addition of Multiple HPV Types to L1 VLP Vaccine

Adapted from Munoz et al, Int J Cancer 111: 278-85, 2004
Possible Impact of Increasing the Valency of an L1 VLP Vaccine

• Could prevent a higher proportion of serious HPV infections

• Could help to reduce the cost of cervical cancer screening
  – Fewer positive tests
  – Might permit longer screening intervals

• Paradoxically, the increase in vaccine complexity might delay widespread vaccine implementation in the developing world
  – Vaccine cost might remain high longer
  – Problematic to use lower valency VLP vaccine in developing world?
Papillomaviruses Have Two Structural Proteins: \textbf{L1 and L2}

- 60 nm DIAMETER
- NAKED ICOSAHEDRAL CAPSID
- 72 PENTAMERS OF MAJOR CAPSID PROTEIN, L1
- \(\sim 72\) COPIES OF THE MINOR CAPSID PROTEIN, L2,

L2 Polypeptides as Candidate Preventive Vaccines

• The N-terminus of L2 (the minor capsid protein) induces antibodies that can cross-neutralize a spectrum of HPVs. An L2-based vaccine offers the possibility of a pan-HPV vaccine.

• L2 polypeptides can be produced in bacteria, which should be relatively inexpensive.

• A caveat: L2 is not as immunogenic as L1 VLPs.

• Not yet tested in humans: Working with Richard Roden at Johns Hopkins University and Shantha Biotechnics in India towards an early phase trial.
BPV1 L2 a.a. 1-88 Peptide: Neutralizes a Wide Spectrum of Papillomaviruses

Neutralizing antibodies induced by BPV1 L2 1-88 peptide

<table>
<thead>
<tr>
<th>Neutralization Assay</th>
<th>Antibody Titer for BPV1 L2 peptide</th>
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<tbody>
<tr>
<td>BPV1</td>
<td>3460</td>
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<tr>
<td>HPV5</td>
<td>6400</td>
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<tr>
<td>CRPV</td>
<td>780</td>
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<tr>
<td>HPV16</td>
<td>4740</td>
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<td>HPV18</td>
<td>7020</td>
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<td>HPV31</td>
<td>220</td>
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<tr>
<td>HPV45</td>
<td>6400</td>
</tr>
<tr>
<td>HPV6</td>
<td>340</td>
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</table>

Pastrana et al, Virology, 2005
(Collaboration with Richard Roden, Johns Hopkins)
Heterologous (BPV) L2 Vaccine Prevents HPV16 Pseudovirus Infection in Mouse Cervico-vaginal Challenge Model

Jeff Roberts, unpublished data
**A multi-type L2 fusion peptide induces High titer neutralizing antibodies in rabbits**

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Neutralization Titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV16</td>
<td>HPV16</td>
</tr>
<tr>
<td>409,600</td>
<td>3,200</td>
</tr>
</tbody>
</table>

Rabbits vaccinated 3x in Complete and Incomplete Freund’s Adjuvant

*Jagu, Karanam, Gambhira, Chivukula, Chaganti, Lowy, Schiller, and Roden, unpublished*
How might the HPV vaccine induce sterilizing immunity?

- Must try to explain the high degree of effectiveness against both cervical infection and genital warts
  - Mucosal secretions have immunoglobulins (mainly IgG), but the skin is not bathed in secretions
  - Antibodies in mucosal secretions are therefore less likely to be the main cause of protection, although they may help

- Related to the process of HPV infection

- The key event required for HPV infection is microtrauma/wounding
  - In vaccinees, it would lead to exudation of systemic antibodies at site of trauma
Microtrauma/Wounding: Critical to HPV Infection and Vaccine Protection

Modified from Lowy & Schiller, J Clin Invest 116:1167-73, 2006
How Intramuscular Injection of a VLP Vaccine May Prevent HPV Infection: Epithelial Microtrauma & Exudation of Systemic Antibodies
Formation of high titer (>10^9/ml) infectious papillomavirus pseudoviruses

Pseudovirus infection mimics the initial steps in HPV infection

Codon optimization of L1 & L2 is critical to high titer virus
HPV16 Capsids Bind to the Basement Membrane of Disrupted Stratified Squamous Epithelia in the Female Genital Tract

**Intact columnar epithelium of cervical canal is resistant to infection**

1 hour post-inoculation

1. Intact Epithelium (CMC pretreatment *)
2. Disrupted Epithelium (CMC + N-9 pretreatment **)

72 hours post-inoculation

- Green = PsV particles
- Red = RFP expression, infection

* CMC = carboxymethylcellulose inert vehicle
** N-9 = Nonoxynol-9 detergent

Summary and Conclusions

- The current HPV L1 VLP vaccines can reduce the incidence of benign and malignant genital HPV infections.
  - Their type-restricted protection means that some serious HPV infections will still occur in vaccinated women.

- For the foreseeable future, cervical cancer screening will remain crucial to cervical cancer prevention.

- To achieve maximum reduction in HPV-associated cancers will require:
  - Development of second generation vaccines with activity against a broader range of HPV types.
  - Widespread implementation of vaccination.
  - Integration of screening and vaccination.