Genital Human Papillomavirus Infections

Impact, Control, and Prevention

Overview of Human Papillomaviruses

- Nonenveloped viruses enclosing a double stranded, circular DNA genome
- Species specific
- Tissue specific: Infect the stratified squamous epithelia and the cervical glandular epithelium
- 107 fully characterized types (L1 gene DNA sequence); many more types exist
- Cause latent, subclinical, and clinical infections
  - Benign (warts, papillomas, condylomas)
  - Potentially malignant (precancers/incipient cancers = intraepithelial neoplasias = dysplasias)
  - Malignant (cancer)

HPV Types and Disease Association

<table>
<thead>
<tr>
<th>Anatomic Distribution of HPVs</th>
</tr>
</thead>
</table>
| Cutaneous HPVs
| Epidermodysplasia verruciformis HPV
| Mucosal/Genital HPVs (low risk)
| Mucosal/Genital HPVs (high risk) |

Human Papillomaviruses (HPV)

- Nonenveloped viruses enclosing a double stranded, circular DNA genome
- Species specific
- Tissue specific: Infect the stratified squamous epithelia and the cervical glandular epithelium
- 107 fully characterized types (L1 gene DNA sequence); many more types exist
- Cause latent, subclinical, and clinical infections
  - Benign (warts, papillomas, condylomas)
  - Potentially malignant (precancers/incipient cancers = intraepithelial neoplasias = dysplasias)
  - Malignant (cancer)
HPV Induces Epidermal Proliferation

Koilocytosis Is a Characteristic Hallmark of HPV Infection

The Transformation Zone Is Most Vulnerable to HPV-related Disease

From Condyloma to Cancer

HPV16 GENOMIC ORGANIZATION
E6 E7
From Condyloma to Cancer

The prevalence of high-risk oncogenic HPVs increases with the severity of the lesion


Clinical Spectrum of Genital Infections

Genital Warts  Laryngeal Papilloma

From Condyloma to Cancer

Clinical Spectrum of Genital Infections

Cervical Condyloma  CIN 2,3  Invasive Cancer

Courtesy of JT Cox, MD.

Prevalence and Incidence of HPV Infection in the US *

- Approximately 20 million people are currently infected with HPV in the United States1
  - 50% are aged 15 to 24 years
- Annual incidence of sexually transmitted HPV infection is ~6.2 million1
  - 74% of new infections occur in 15 to 24 year olds2
- Overall, an estimated 80% of sexually active men and women are exposed to HPV at some point in their lives3

HPV=human papillomavirus.

The Economic Burden of Genital HPV Infections in the United States, 20001

- General Population
  - $3.6 billion
  - $146.4 million
  - $167.4 million
- Persons 15–24 Years of Age
  - $2.7 billion
  - $108.3 million
  - $123.9 million

$2.7 billion  $146.4 million  $167.4 million

Follow-up of Abnormal Paps and Treatment of CIN
Treatment of Invasive Cervical Cancer
Treatment of External Anogenital Warts

**EPIDEMIOLOGY**

**HPV Cervical Infections Descriptive Epidemiology**

**HPV Infections in the United States**

- Genital warts: 25%
- Detected by colposcopy or cytology: 10%
- HPV DNA positive: Colposcopy negative: 6%
- Presence of antibodies (negative HPV test): 4%
- Not currently infected: 1%

- ~75% of population exposed to HPV

**Factors Associated With Higher Risk of HPV Infection**

**Women**
- Age
- Sexual behavior
  - Increased risk associated with a greater number of male sexual partners
  - Risk increases with earlier age of first sexual intercourse
- Sexual behaviors of previous male sexual partners
- Immunological status
  - HPV more likely in immunosuppressed women

**Men**
- Lifetime number of sexual partners
- Number of recent sexual partners
- Uncircumcised
- Same sex encounters

**Age-Specific Prevalence of HPV Infection**

- In the US, there is a constant decline with advancing age
- In other countries (e.g., Costa Rica), there is an unexplained recrudescence past age 50

**Prevalence of HPV Infection Among Women in the US**

- In Portland, OR
- In Guanacaste, Costa Rica

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Portland, OR (%)</th>
<th>Guanacaste, Costa Rica (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20</td>
<td>3.4</td>
<td>10</td>
</tr>
<tr>
<td>21-25</td>
<td>2.6</td>
<td>4.3</td>
</tr>
<tr>
<td>26-30</td>
<td>2.1</td>
<td>4.8</td>
</tr>
<tr>
<td>31-35</td>
<td>2.4</td>
<td>4.1</td>
</tr>
<tr>
<td>36-40</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>41-45</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>46-50</td>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>51-55</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>56-60</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>61-65</td>
<td>1.3</td>
<td>2.7</td>
</tr>
<tr>
<td>66-70</td>
<td>1.2</td>
<td>2.6</td>
</tr>
<tr>
<td>71-75</td>
<td>1.1</td>
<td>2.5</td>
</tr>
<tr>
<td>76+</td>
<td>1.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Sample Source**

<table>
<thead>
<tr>
<th>Source</th>
<th>Add Health</th>
<th>NHANES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

**Relative Sensitivity of Assay**

<table>
<thead>
<tr>
<th>Source</th>
<th>Add Health</th>
<th>NHANES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td></td>
<td>75%</td>
</tr>
</tbody>
</table>

**Prevalence**

<table>
<thead>
<tr>
<th>Overall</th>
<th>Add Health (%)</th>
<th>NHANES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.9</td>
<td>26.8</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Add Health (%)</th>
<th>NHANES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages 18-24</td>
<td>26.9</td>
<td>44.8</td>
</tr>
<tr>
<td>Ages 25-34</td>
<td>27.4</td>
<td>44.8</td>
</tr>
<tr>
<td>Ages 35-44</td>
<td>28.0</td>
<td>44.8</td>
</tr>
<tr>
<td>Ages 45-54</td>
<td>28.5</td>
<td>44.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Type</th>
<th>Add Health (%)</th>
<th>NHANES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>10%</td>
<td>4%</td>
</tr>
</tbody>
</table>

**All High-Risk HPV Are not Equally Carcinogenic**


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**HPV Cervical Diseases Descriptive Epidemiology**

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**Prevalence of HPV Cervical Diseases**

- Low-grade squamous intraepithelial lesion (LSIL) 1.97%
- High-grade squamous intraepithelial lesion (HSIL) 0.51%
- Squamous cell carcinoma 0.026%
- Adenocarcinomas 0.0046%

- Atypical squamous cells (ASC) 2.8%
  - Of unknown significance (ASC-US)
  - Cannot exclude HSIL (ASC-H)


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**Epidemiology of Cervical Cancer**

Cervical Cancer is the second most common cancer in women worldwide

- Lung 5.2 million cases 1.7 million deaths
- Breast 2.0 million cases 600,000 deaths
- Colon/Rectum 1.8 million cases 800,000 deaths
- Stomach 0.9 million cases 700,000 deaths
- Liver 0.8 million cases 700,000 deaths
- Prostate 0.6 million cases
- Cervix uteri 0.6 million cases
- Oesophagus 0.5 million cases
- Bladder 0.5 million cases
- Non-Hodgkin lymphoma 0.4 million cases
- Kidney 0.4 million cases
- Pancreas 0.3 million cases
- Oral cavity 0.3 million cases
- Pancreas 0.3 million cases
- Ovary 0.1 million cases

Epidemiology of Cervical Cancer

Cervical cancer is more common in the developing world.


More developed Less developed

Women

Breast 1,175,000
Cervix uteri 114,342
Stomach 5,476
Colon/rectum 471
Lung 475
Ovary 114,342
Corpus uteri 475
Liver 475
Esophagus 34
Non-Hodgkin lymphoma 34
Leukemia 34
Pancreas 34

(Thousands)

2,176,000 2,562,000

Epidemiology of Cervical Cancer, United States

• Estimated incidence is 9.1/100,0001
  – ~11,150 new cases and ~3,670 deaths annually1
• Cervical cancer is the 14th cause of cancer in incidence and 16th in mortality1
• >50 million Pap smears are performed each year2


Epidemiology of Cervical Cancer, United States

• Characteristics associated with lower rates of screening based on women 18 years of age and older with no history of hysterectomy (N=13,745)3:
  – No contact with primary care physician in the past year
  – Lack of usual source of care
  – Low family income
  – Less than high school education
  – Unmarried
  – No health insurance (~65 years of age only)


HPV AND CANCER

How Much Cervical Cancer Is Caused by HPV?

- HPV is present in virtually all cervical cancers, estimated at 99.7%1, 2


How Much Cervical Cancer Is Caused by HPV?

- HPV meets all of the epidemiologic criteria for causality1
- Cervical cancer may be the first human cancer identified to have a single necessary, if not sufficient, cause.1, 2
- 5.2% (estimate for 2002) of all new human cancers are attributable to HPV3


Genital HPV and Other Cancers

<table>
<thead>
<tr>
<th>Cancer 1</th>
<th>% Associated With Certain HPV Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>100</td>
</tr>
<tr>
<td>Vaginal</td>
<td>40</td>
</tr>
<tr>
<td>Vulvar</td>
<td>40</td>
</tr>
<tr>
<td>Penile</td>
<td>40</td>
</tr>
<tr>
<td>Anal</td>
<td>90</td>
</tr>
<tr>
<td>Oropharyngeal2</td>
<td>70</td>
</tr>
</tbody>
</table>

1. Parkin DM, Bray F. Vaccine 2006;24 Suppl 3:S1-S17-25

Incidence of Various Invasive Genital Cancers Attributable to HPV (per 100,000)

<table>
<thead>
<tr>
<th>Cancer 1</th>
<th>Females</th>
<th>Males</th>
<th>Males or Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>8.3</td>
<td>8.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Vulva2</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Vagina3</td>
<td>0.4</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Anus4</td>
<td>1.2</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Penis4</td>
<td>.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

3. Frisch M and Melbye M. Chap. 43. ibid. pp. 830-840
4. Wideroff L and Schottenfeld D. Chap. 61. ibid. pp. 1166-1172

Head and Neck Cancer (HNSCC)

Tonsillar Cancer HPV Prevalence by Calendar Period, Swedish Cancer Registry

<table>
<thead>
<tr>
<th>Period</th>
<th>HPV Prevalence</th>
<th>Chi Square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970-1979</td>
<td>7 of 30, 23%</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>1980-1989</td>
<td>12 of 42, 28%</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>1990-1999</td>
<td>48 of 84, 57%</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td>2000-2002</td>
<td>32 of 47, 68%</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Two Distinct Head and Neck Cancers**

<table>
<thead>
<tr>
<th>Anatomic site</th>
<th>HPV-positive</th>
<th>HPV-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonsil / Base of Tongue</td>
<td>All sites</td>
<td></td>
</tr>
<tr>
<td>Paranasal sinuses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histology</th>
<th>Basaloid</th>
<th>Keratinized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Younger</td>
<td>Older</td>
</tr>
<tr>
<td>Gender</td>
<td>3:1 men</td>
<td>3:1 men</td>
</tr>
<tr>
<td>HPV Serologic status</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Sexual behavior</td>
<td>Alcohol / tobacco</td>
</tr>
<tr>
<td>Cofactors</td>
<td>Marijuana</td>
<td>Diet, hygiene</td>
</tr>
<tr>
<td>Survival</td>
<td>Improved</td>
<td>Worse</td>
</tr>
<tr>
<td>Incidence</td>
<td>Increasing</td>
<td>Decreasing</td>
</tr>
</tbody>
</table>

from Maura Gillison, M.D.

**HPV Type Distribution in HPV DNA- Positive Oropharynx Cases**

<table>
<thead>
<tr>
<th>HPV Type</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>92</td>
</tr>
<tr>
<td>HPV 18</td>
<td>3</td>
</tr>
<tr>
<td>HPV 33</td>
<td>2</td>
</tr>
<tr>
<td>HPV 35</td>
<td>0.6</td>
</tr>
<tr>
<td>HPV 45</td>
<td>0.3</td>
</tr>
<tr>
<td>HPV 59</td>
<td>0.3</td>
</tr>
</tbody>
</table>


**Prevalence of Genital Warts in the US**

- 5.6% of 18- to 59-year olds surveyed reported ever having genital warts. More women (7.2%) than men (4%) reported a history.1

Weighted percentage of sexually active persons aged 18 to 59 years who reported ever having a diagnosis of genital warts by sex and age group, NHANES, 1999-2004.


**Incidence of Genital Warts in the US**

<table>
<thead>
<tr>
<th>Year</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td>1999</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td>2000</td>
<td>175</td>
<td>150</td>
</tr>
<tr>
<td>2001</td>
<td>200</td>
<td>175</td>
</tr>
</tbody>
</table>


**Age Prevalence of Genital Warts**
- Peak age for genital warts is between 20 and 29 years
- Consistent with a sexually transmitted route of infection

**HPV Types Distribution in Genital Warts**
- 97% of genital warts (n = 65) contain HPV either type 6 or 11
- In non-immunosuppressed patients HPV-6 and -11 are present in 68% and 46% of genital warts respectively. In immunosuppressed patients, the figures are 29% and 71%, respectively

**Recurrent Respiratory Papillomatosis (1)**
- Caused primarily by HPV-11 and HPV-6
- HPV-11 may be more aggressive
- HPV-6 and HPV-11 are not "low-risk" in lung and aerodigestive tract
  - Malignant transformation occurs with either type
- Overall, 3-5% of patients convert to cancer
  - Lung involvement: 85% conversion
  - Rare in larynx
  - If radiation therapy, then about 15%

**Recurrent Respiratory Papillomatosis (2)**
- Two ages of onset - juvenile and adult
- Most juvenile cases before age 5, 15% by age 1
- Younger age of onset - more frequent surgeries
- Adult onset primarily between ages 20-50

**Recurrent Respiratory Papillomatosis (3)**
- US prevalence estimates
  - JORRP 4.3/100,000, AORRP 1.8/100,000
- Risk increases to about 1/200 if mother has active genital condylomas at time of birth
- But 20% of population has HPV DNA in the airway
- Most infections never diagnosed
- Mystery: Why is RRP uncommon in HIV+ MSM patients?
Transmission of HPV

- Sexual contact with an infected partner is necessary for transmission
  - Intromissive intercourse is not strictly necessary
  - Men implicated in epidemiological chain of the infection ("vectors," "carriers")
- Source contact usually has subclinical infection
- Incubation period ranges from 3 weeks to several months
- Fomite transmission may occur (rare)
- Perinatal transmission causes recurrent respiratory papillomatosis in infants and young children (rare)


Many Women Become Infected With HPV Soon After Beginning Intercourse


Risk of Female HPV Acquisition Associated with First Male Sex Partner


New HPV in Men Are At Least Equally Common

240 heterosexually active male university students aged 18-20 years, sampled at 4-month intervals from the glans, shaft, scrotum, urine, and fingernails.

Sexual Behavior among High School Students in the U.S.; YRBS, 1 2003

- Sexual initiation
  - 7.4% before age 13 (4.2%)
  - 32.8% by 9th grade (27.9%)
  - 61.6% by 12th grade (62.3%)
- 4+ lifetime sex partners
  - 10.4% of 9th graders (6.4%)
  - 20.3% of 12th graders (19.2%)

1. MMWR. Youth Risk Behavior Survey 2004; 53: No. SS-2
HPV Is Easily Transmitted

HPV requires access to the basal epithelium

- Dead keratinocyte releasing viral particles
- Dead keratinocyte S. corneum
- Keratin S. granulosum
- Keratohyaline granule
- Koilocyte Vacuole
- Nucleus
- Inclusion body S. spinosum
- Tonofibrils S. basale
- Basal lamina

Epithelium that is naturally thin or immature

SITE OF MICRO OR MACRO TRAUMA

Infection

Natural History of HPV Infections

HPV Is Easily Transmitted

HPV requires access to the basal epithelium

- Dead keratinocyte releasing viral particles
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Epithelium that is naturally thin or immature

SITE OF MICRO OR MACRO TRAUMA

Infection

Natural History of HPV Infections

Most HPV Infections Are Transient

Proportion HPV positive

0 0.25 0.50 0.75 1.00

Months

0 2 4 6 8 10 12 14


CIN Grade Predicts Natural History

<table>
<thead>
<tr>
<th>CIN Grade</th>
<th>Regression (%)</th>
<th>Persistence (%)</th>
<th>Progression to CIN3 (%)</th>
<th>Progression to Cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN1</td>
<td>57</td>
<td>32</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>CIN2</td>
<td>43</td>
<td>35</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>CIN3</td>
<td>32</td>
<td>56</td>
<td>—</td>
<td>&gt;12</td>
</tr>
</tbody>
</table>


Natural History of Cervical Neoplasia

<table>
<thead>
<tr>
<th>Time Period</th>
<th>CIN Grade</th>
<th>Initial HPV Infection</th>
<th>Continuing Infection</th>
<th>CIN 2/3</th>
<th>Invasive Cervical Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 Year</td>
<td>CIN 1</td>
<td>18 yo</td>
<td>28 yo</td>
<td>42 yo</td>
<td>48 yo</td>
</tr>
</tbody>
</table>

Potential Factors for Progression:

- HPV-related: type, variants, viral load
- Parity, oral contraceptive use
- Smoking
- Host factors: immune response, HLA type
- Chlamydia, HSV-2 coinfection
- Diet (vitamin A, C, E, carotenoids, folic acid, etc.)

PREVENTION

HPV Transmission and Condoms

- Meta-analysis of 20 studies suggested condoms may not prevent HPV infection, but may reduce genital warts, CIN 2/3, and cervical cancer.

- In a prospective study of 82 college-aged, initially virgin women:

### Effect of condom use on HPV clearance:

- In a randomized study of 125 couples of women with CIN and their male sexual partners, there was a greater rate of CIN (×1.5) and HPV DNA clearance (×5.7) in the women from the couples assigned to condom use.

- Similarly, there was a faster (×1.9) rate of clearance of HPV-associated flat lesions in the males.

- The clearance effect of condoms in males was seen only in the couples with concordant HPV types (HR 2.63; 95% CI 1.07-6.48).

### ACS* Guidelines for Cervical Cancer Screening (2002)

<table>
<thead>
<tr>
<th>First Screen</th>
<th>Women &lt;30 Years of Age</th>
<th>Women ≥70 Years of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximately 3 years after first sexual intercourse, but no later than 21 years of age</td>
<td>Conventional Pap smear every year with liquid-based Pap smear every 2 years.</td>
<td>Women with 3 consecutive normal Pap smears can be rescreened every 2-3 years.</td>
</tr>
<tr>
<td>Women ≥70 years of age with ≥3 normal Pap smears in a row and no abnormal results in the 10-year period prior to age 70 may stop screening.</td>
<td>Women with total hysterectomy (with removal of the cervix) and no history of cervical cancer or precancer may stop screening.</td>
<td></td>
</tr>
</tbody>
</table>

*ACS = American Cancer Society

**Women who have had in utero exposure to DES and/or who are immunocompromised (including HIV positive) should continue cervical cancer screening for as long as they are in reasonably good health and do not have a life-limiting chronic condition.**

**Women who have a history of cervical cancer should continue screening.**

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### Natural History of Cervical Neoplasia

- HSIL: High-grade squamous intraepithelial lesion; LSIL: Low-grade squamous intraepithelial lesion.

Development of an HPV Vaccine

Requirements for HPV Capsid Formation

Assembly of VLPs

Use of the Baculovirus Expression System To Make Virus-Like Particles

Animal Papillomavirus VLP Vaccines Are Effective

Phase II Study of an HPV16 VLP Vaccine

- Cottontail rabbit papillomavirus (CRPV)
  - L1/L2 VLP: 59% (17/29) protection (0% in controls)
  - L1 VLP: 17% (5/29) protection
  - None of the animals developed cancer (20/30 in controls)
  - Passive transfer of antibodies resulted in protection

- Canine oral papillomavirus (COPV)
  - L1 VLP: 100% (7/7) protection (0% in controls)
  - Passive transfer of antibodies resulted in protection

- Bovine papillomavirus (BPV) type 4
  - L1/L2 VLP or L1 VLP: 87% (13/15) protection (10% in controls)

**Phase II Study of an HPV16 VLP Vaccine**

- **Randomization**
  - 2392 women (1533 evaluable)
  - Ages 16-23 years
  - No prior abnormal Pap smear
  - No more than five male sexual partners
  - Virgins enrolled if seeking contraception

- **Primary endpoint**
  - Persistent HPV16 infection, defined as the detection of HPV16 DNA in samples obtained at 2 or more visits

- **Secondary endpoint**
  - Tolerability of Vaccine

- **Adjuvant/dose**
  - HPV16 VLP Vaccine (N=768)
  - Placebo (N=765)
  - 225 µg aluminum adjuvant/dose

- **Injections**
  - Day 0
  - Month 2
  - Month 6

- Not pregnant
- No prior abnormal Pap smear
- No more than five male sexual partners
- Virgin enrolled if seeking contraception

**Efficacy Analysis of an HPV16 VLP Vaccine: HPV16 Infections Were Completely Prevented**

- Incidence of adverse events was similar in both groups; the most frequent adverse event was pain at the injection site

**Phase II Study of a Bivalent HP16/HP18 VLP Vaccine - CERVARIX**

- **Randomization**
  - 1,113 women
  - Ages 15-25 years
  - No prior abnormal Pap smear
  - No cervical ablation treatment
  - No ongoing treatment for genital warts
  - No more than six male sexual partners
  - Cytologically negative.
  - Seronegative for HPV16, HPV18, and HPV DNA negative for 14 high-risk types

- **Primary endpoint**
  - Prevention of the acquisition of HPV16, HPV18, or both infection

- **Secondary endpoint**
  - Prevention of cytologic or histologic CIN and SCC

- **Follow-up**
  - Median Follow-up of 17.4 months

- **Number of cases**
  - Persistent HPV16 Infection
  - Any CIN
  - CIN 2/3

**HPV 16/18 Vaccine (CERVARIX) Efficacy**

- 100% efficacy against persistent infections, ATP
- 95% efficacy against persistent infections, ITT
- 93% efficacy against cytological abnormalities

**HPV 16/18 Vaccine (CERVARIX) Efficacy**

- According to protocol cohort n=721
- According to cohort n=1113

**Phase II Study of a Bivalent L1 VLP Vaccine (CERVARIX) in Prevention of HPV16/HP18 Infection**

- Every 6 months participants were tested for cervical HPV DNA, cytology, and serum antibody levels; every 3 months, participants were tested for HPV DNA by PCR

- **Primary endpoint**
  - Prevention of the acquisition of HPV16, HPV18, or both infection

- **Secondary endpoint**
  - Prevention of cytologic or histologic CIN and SCC

- **Randomization**
  - Intramuscular Injections
  - Day 0
  - Month 1
  - Month 6

- **Extension Phase**
  - 18 mos. to 27 mos.
**Serious AEs in HPV 16/18 Study**

<table>
<thead>
<tr>
<th>Adverse events reported</th>
<th>Vaccine Group (n=531)</th>
<th>Placebo Group (n=538)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site</td>
<td>499 (94.8%)</td>
<td>472 (87.8%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Pain*</td>
<td>496 (93.4%)</td>
<td>469 (88.2%)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Systemic</td>
<td>455 (86.3%)</td>
<td>462 (85.9%)</td>
<td>0.860</td>
</tr>
<tr>
<td>Headache*</td>
<td>331 (62.3%)</td>
<td>329 (61.2%)</td>
<td>0.760</td>
</tr>
</tbody>
</table>

Serious adverse events

During study†: 22 (4.0%) 19 (3.5%) 0.636

Related to vaccination: 0 0 -

*Most common AE.
†Participants who reported a serious adverse event during the entire study period (months 0–27).
AE=adverse event.

**HPV16 and HPV18 VLP Immunization (CERVARIX) Appears to Offer Cross-Protection Against Other HPVs Incident Infections**

* Data at up to 4.5 years

<table>
<thead>
<tr>
<th>HPV types</th>
<th>Vaccine Efficacy (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-related</td>
<td>31</td>
<td>54.5</td>
</tr>
<tr>
<td>33</td>
<td>8.6</td>
<td>-117.3 to 61.9</td>
</tr>
<tr>
<td>52</td>
<td>18.6</td>
<td>-26.5 to 47.8</td>
</tr>
<tr>
<td>58</td>
<td>14.0</td>
<td>-87.9 to 61.1</td>
</tr>
<tr>
<td>18-related</td>
<td>45</td>
<td>94.2</td>
</tr>
</tbody>
</table>


**Protection Offered by HPV16/HPV18 VLP Immunization**

- HPV16 and HPV18 represent 70.7%.
- HPV31 and HPV45 are an additional 7.6% of oncogenic HPVs.


**Phase III Study (HPV-008) of CERVARIX in Prevention of HPV16/HPV18 Infection**

- Every 6 months participants were tested for cervical HPV DNA, and cytology, and every year for serum antibody levels
- Randomization
- Interim Analysis
- Mean follow-up 18 mos.
- Done on HPV DNA and seronegative subjects
- Primary endpoint
- Prevention of the acquisition of CIN2+ associated with HPV16, HPV18, or both infection
- Dubin G. ACIP Meeting June 2007

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Group</th>
<th>N</th>
<th>n</th>
<th>Vaccine Efficacy (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV</td>
<td>778</td>
<td>2</td>
<td>90.4</td>
<td>53.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7838</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis considering patterns of HPV types in preceding infection

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Group</th>
<th>N</th>
<th>n</th>
<th>Vaccine Efficacy (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV</td>
<td>778</td>
<td>0</td>
<td>100</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7838</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pre-Specified Case Definition based on PCR detection in lesion only

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Group</th>
<th>N</th>
<th>n</th>
<th>Vaccine Efficacy (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>Control</td>
<td>7838</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Review of the Studies of the Merck Quadrivalent (HPV6, HPV11, HPV16, HPV18) VLP Vaccine - GARDASIL

### Clinical Program for GARDASIL®: Selection of Trial End Points

<table>
<thead>
<tr>
<th>Necessary Criteria</th>
<th>End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate precursor for cervical cancer</td>
<td>√</td>
</tr>
<tr>
<td>Detection and removal have been shown to prevent cancer</td>
<td>√</td>
</tr>
</tbody>
</table>

- HPV Infection
- CIN 1
- CIN 2/3

### Clinical Studies for GARDASIL®: Analysis in Per-Protocol Efficacy (PPE) Population

- Primary analysis of efficacy conducted in PPE population:
  - Received all 3 vaccinations within 1 year of enrollment
  - Did not have major deviations from the study protocol
  - Were naïve to the relevant HPV type(s) prior to Dose 1 and through 1 month Postdose 3 (Month 7)
- Efficacy measurements started after Month 7 visit.

### Prophylactic Efficacy (Per-Protocol Population)

<table>
<thead>
<tr>
<th>AERCH Protocol #</th>
<th>Lesion Prevented</th>
<th>Caused by HPV</th>
<th>Number of Subjects</th>
<th>Vaccine Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VIN/VAIN</td>
<td>CIN</td>
<td>1</td>
<td>2/3</td>
</tr>
<tr>
<td>005, 007, 013, 015</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>013</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>007, 013, 015</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>013</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Others</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

### Populations Used to Evaluate GARDASIL®

- **PPE:** per-protocol efficacy; **ITT:** intention-to-treat; **MITT:** modified intention-to-treat; **ASCUS:** atypical squamous cells of undetermined significance; those who had a normal Pap smear at baseline were considered part of a restricted cohort of MITT-3 called R-MITT-3.

### Prophylactic Efficacy (Unrestricted Susceptible Population)

<table>
<thead>
<tr>
<th>AERCH Protocol #</th>
<th>Lesion Prevented</th>
<th>Caused by HPV</th>
<th>Number of Subjects</th>
<th>Vaccine Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VIN/VAIN</td>
<td>CIN</td>
<td>1</td>
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</tr>
<tr>
<td>005, 007, 013, 015</td>
<td>X</td>
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<tr>
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<td>X</td>
<td>X</td>
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<td>013</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>012</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

### Impact of GARDASIL in the General Population

**Page 16**
Populations Used to Evaluate GARDASIL®

- Day 1 (+) to non-vaccine HPV type included.
- Sero (+) and/or PCR (+) to the Relevant Vaccine HPV Type at Day 1 included.
- PCR (+) to the Relevant Vaccine HPV Type During the Vaccination Phase excluded.
- Day 1 Pap >= ASCUS included.
- Protocol Violators/ < 3 Doses excluded.
- Sero (+) and/or PCR (+) to the Relevant Vaccine HPV Type at Day 1 included.

MITT-3 General Population
- MITT-2 Unrestricted
- MITT-2 Susceptible
- PPE: per-protocol efficacy; ITT: intention-to-treat; MITT: modified intention-to-treat; ASCUS: atypical squamous cells of undetermined significance; those who had a normal Pap smear at baseline were considered part of a restricted cohort of MITT-3 called R-MITT-3.

Impact of GARDASIL® Against HPV16/18-Related CIN2/3 and AIS

<table>
<thead>
<tr>
<th>Lesion Prevented</th>
<th>Number of Subjects</th>
<th>Vaccine Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIN/VAIN</td>
<td>570</td>
<td>44% (31-55%)</td>
</tr>
<tr>
<td>CIN</td>
<td>5,627</td>
<td>55% (40-66%)</td>
</tr>
<tr>
<td>VIN/VAIN/CIN</td>
<td>51</td>
<td>71% (37-88%)</td>
</tr>
<tr>
<td>VIN/VAIN/CIN</td>
<td>5,785</td>
<td>63% (40-88%)</td>
</tr>
<tr>
<td>VIN/VAIN/CIN</td>
<td>5,856</td>
<td>76% (61-84%)</td>
</tr>
</tbody>
</table>

Prophylactic Efficacy (Intention-to-Treat Population) (1)

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Lesion Prevented</th>
<th>Number of Subjects</th>
<th>Vaccine Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>570</td>
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<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>VIN/VAIN/CIN</td>
<td>5,856</td>
<td>76% (61-84%)</td>
<td></td>
</tr>
</tbody>
</table>

Prophylactic Efficacy (Intention-to-Treat Population) (2)

Impact of GARDASIL® in the General Population

- GARDASIL is a prophylactic vaccine.
- There was no clear evidence of protection from disease caused by HPV types for which subjects were PCR positive and/or seropositive at baseline.
- Individuals who were already infected with 1 or more vaccine-related HPV types prior to vaccination were protected from clinical disease caused by the remaining vaccine HPV types.

Immunogenicity - Two Challenges

- Because the vaccine has been so effective, the minimum protective anti-HPV-6/11/16/18 antibody levels are unknown.

- Yet, because young children are not sexually active and do not develop genital HPV infections or diseases, one had to rely on the development of an antibody response to assess the vaccine efficacy.

Bridging the Efficacy of GARDASIL® From Young Adult Women to Adolescent Girls

The Antibody Immune Response Is Durable and Can Be Boosted

Adverse Events with GARDASIL

Vaccine-related Experiences

Fever
- GARDASIL 10.3%
- Placebo 8.6%

- Few subjects (0.1%) discontinued due to adverse experiences.

The vaccine-related adverse experiences that were observed among recipients of GARDASIL were at a frequency of at least 1.0% and also at a greater frequency than that observed among placebo recipients.

All-Cause Serious Adverse Experiences*

- One case of bronchospasm and 2 cases of asthma were reported as serious adverse experiences that occurred during Days 1–15 of any vaccination visit.

*Most frequently reported.
New Medical Conditions After Enrollment*

<table>
<thead>
<tr>
<th>Potential Autoimmune Disorder</th>
<th>GARDASIL® (N = 11,813)</th>
<th>Placebo (N = 9,701)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Terms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile arthritis</td>
<td>3 (0.022%)</td>
<td>1 (0.010%)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other Terms</td>
<td>6 (0.051%)</td>
<td>2 (0.021%)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Reactive Arthritis</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

N = Number of subjects enrolled

*Potentially indicative of a systemic immune disorder.

In addition, since the vaccine has been released, there have been 3 cases of Gullain-Barré syndrome, 2 occurring after concomitant administration of the meningococcal and Tdap vaccines.

Summary of Pregnancies in the Phase 3 Program of Gardasil

<table>
<thead>
<tr>
<th></th>
<th>GARDASIL® (N = 10,418)</th>
<th>Placebo (N = 9,120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with pregnancies</td>
<td>1,115</td>
<td>1,151</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>1,344</td>
<td>1,272</td>
</tr>
<tr>
<td>Pregnancies with unknown outcomes / Ongoing pregnancies</td>
<td>258</td>
<td>263</td>
</tr>
<tr>
<td>Pregnancies with known outcomes</td>
<td>996</td>
<td>1,018</td>
</tr>
<tr>
<td>Live births (% pregnancies with known outcomes)</td>
<td>621 (62)</td>
<td>611 (60)</td>
</tr>
<tr>
<td>Fetal loss (% pregnancies with known outcomes)</td>
<td>375 (38)</td>
<td>407 (40)</td>
</tr>
</tbody>
</table>

N = number of subjects who received 1, 2, or 3 doses of only the clinical material in the given column. The GARDASIL group included more 9- to 15-year-olds than the placebo group.

Data from Vaccine Adverse Event Reporting System (VAERS)

- As of April 30, 2008, 12 million doses of Gardasil distributed and 7,802 reports received by VAERS1

- Demographics:2
  - 12-18 year-olds comprise 39% of reports
  - 96% reports were in females

1. Data from CDC/Office of the Chief Science Officer
   Data analyzed up to May 8, 2007

VAERS GARDASIL

- 7 most frequently reported symptoms after quadrivalent HPV vaccine
  - Dizziness 13% (n=224)
  - Syncope 10% (n=176)
  - Injection site pain 19% (n=170)
  - Nausea 9% (n=160)
  - Pain 7% (n=122)
  - Rash 7% (n=122)

* Each report may be coded with more than one symptom

Data from John Iskander, M.D., M.P.H., ACIP, June 28, 2007
Data analyzed up to May 8, 2007

VAERS GARDASIL

- Guillain-Barré - 31 reports
  - 10 confirmed
    - 5 reported vaccination with Menactra (meningococcal vaccine) and Gardasil at the same time
    - 1 did not meet the case definition
    - 1 had symptoms before vaccination
    - 4 were unconfirmed
  - 21 unconfirmed
  - 1 unconfirmed and additional follow-up

- Deaths - 15 reports
  - 10 reports with sufficient information. NO causal association could be shown for any of these cases
  - Before licensure, 10 Gardasil recipients and 7 placebo recipients died. None of these deaths was considered vaccine related

Data from CDC/Office of the Chief Science Officer
Data analyzed up to April 30, 2008, current as of June 16, 2008

GARDASIL® New Results
Impact of GARDASIL® Against HPV6/11/16/18-Related CIN
Protocols 007, 013, and 015 – MITT-2 Analysis, 44 mos mean follow-up

Seronegative and HPV DNA Negative
Efficacy: 95% (92, 97)
GARDASIL Cases | Placebo Cases
16 | 309

Seronegative and HPV DNA Positive
Efficacy: 22% (-6, 42)
GARDASIL Cases | Placebo Cases
83 | 101

Seropositive and HPV DNA Negative
Efficacy: 100% (29, 100)
GARDASIL Cases | Placebo Cases
0 | 7

Seropositive and HPV DNA Positive
Efficacy: 5% (-28, 26)
GARDASIL Cases | Placebo Cases
105 | 103

Gardasil recipients, n= 9,075; placebo recipients, n= 9,075.

Sex Partners in Past 12 Months by Marital Status, NSFG, 1 2002 2

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>No. Sex Partners in Past 12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Divorced/Separated</td>
<td>22.3 56.6 21.1</td>
</tr>
<tr>
<td>Married</td>
<td>3.4 95.2 1.4</td>
</tr>
<tr>
<td>Never Married</td>
<td>30.4 55.8 13.8</td>
</tr>
</tbody>
</table>

1 NSFG: National Survey of Family Growth
2 Leichliter. Unpublished analyses, 2008. cited in

Safety, Efficacy, and Immunogenicity Study of GARDASIL in Women Aged 24-45

<table>
<thead>
<tr>
<th>Population</th>
<th>Vaccine N</th>
<th>Placebo N</th>
<th>PYR Reduction</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td>4 2,721</td>
<td>41 2,654</td>
<td>91%</td>
<td>74, 98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-34 yo</td>
<td>2 1,329</td>
<td>24 1,301</td>
<td>92%</td>
<td>67, 99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>35-45 yo</td>
<td>2 1,393</td>
<td>17 1,353</td>
<td>89%</td>
<td>52, 99</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Luna J. et al. 24th International Papillomavirus Conference and Clinical Workshop. Beijing, China, November 3-9, 2007. Available at

Vaccines Availability

- **Merck, Gardasil (HPV types 6, 11, 16, 18)**
  - Approved by FDA on June 8, 2006
  - Available on the U.S. market on June 19, 2006
  - Approved by the Advisory Committee on Immunization Practices (ACIP) on June 29, 2006
  - Approved in the European Economic Community on September 22, 2006
  - Currently approved in 103 countries (June 25, 2008)
  - June 25, 2007, FDA issued a “complete response letter” for use of Gardasil in women ages 27 through 45 years and use against non-vaccine types

- **GSK, Cervarix (HPV types 16, 18)**
  - No submission to FDA yet, but expected towards the end of 2006
  - Filing in the U.S., February 28, 2006
  - Currently approved in 65 countries (June 23, 2008)
  - December 17, 2007, FDA issued a “complete response letter” to GSK

GARDASIL® USAGE
Who Should Be Receiving the Vaccine?

- Official Approval (ACIP)¹
  - Recommendation for 11-12 year-old girls (9-26 years)²
  - Catch-up vaccination for 13-26 year-old girls²

2. Vaccine for Children Program recommendations apply to the 9-18 year-olds.

Indications and Usage for GARDASIL®

- GARDASIL is a vaccine indicated in girls and women 9 to 26 years of age for the prevention of the following diseases caused by HPV types 6, 11, 16, and 18:
  - Cervix
    - Cancer
    - Adenocarcinoma in situ (AIS)
  - CIN grades 1, 2, and 3
  - Vagina
    - VIN grades 2 and 3
  - Vulva
    - VAIN grades 2 and 3
  - Genital warts (condylomata acuminata)

Contraindications for GARDASIL®

- Hypersensitivity to the active substances or to any of the excipients of the vaccine (Saccharomyces cerevisiae)

- Individuals who develop symptoms indicative of hypersensitivity after receiving a dose of GARDASIL should not receive further doses.

Precautions: General

- Not intended to be used for treatment of active HPV infection; genital warts; cervical cancer; CIN, VIN, or VAIN

- Has not been shown to protect against diseases due to nonvaccine HPV types (current official approval)

Precautions: Information for the Patient, Parent, or Guardian

- Vaccination does not substitute for routine cervical cancer screening
  - Women should continue to undergo cervical cancer screening per standard of care

- GARDASIL® is not recommended for use in pregnant women. (Pregnancy Category B)
  - If immunization has been unknowingly initiated during pregnancy, complete the series AFTER the pregnancy

- It is not known whether vaccine antigens or antibodies induced by the vaccine are excreted in human milk

- Completion of the immunization series is important unless contraindicated

Precautions: Studies With Other Vaccines

- Coadministration* of GARDASIL® with recombinant hepatitis B vaccine¹
  - Safety and immunogenicity evaluated in randomized study of 1,871 women aged 16 to 24 years
  - Immune response to both vaccines was non-inferior whether administered at same visit or at different visit
  - Frequency of systemic or injection-site adverse events similar to that with GARDASIL or hepatitis B vaccine administered alone

- Coadministration of GARDASIL with Tdap and meningococcal vaccines is being studied

¹ Same visit, injections at separate sites.
Recommended Immunization Schedule for Persons Aged 7-18 Years - US, 2007

Precautions: Drug Interactions in PPE Population

- Use with Hormonal Contraceptives
  - Use of hormonal contraceptives or lack of use of hormonal contraceptives among study participants did not alter vaccine efficacy

- Use with Systemic Immunosuppressive Medications
  - Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune responses to vaccines

PPE = Per-protocol efficacy

Who Should Be Receiving the Vaccine?

- ACIP and ACOG recommend vaccination regardless of previous HPV infection or abnormal Pap test results (no need for HPV prescreening), a positive Hybrid Capture II high-risk test, or genital warts.
  - Continue Pap testing after vaccination.1,2

- GARDASIL benefits sexually active women.
  - Although GARDASIL will not protect from disease caused by HPV types that these patients have already contracted, GARDASIL will still protect against the types patients have not encountered.

How Much Does It Cost?

- $120/shot (GARDASIL)
- $360 for the complete immunization series
- The vaccine is free for the beneficiaries of the Vaccine for Children Fund

The End