Recent Advances in Cervical Cancer Prevention by HPV Vaccination and Screening

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Douglas Lowy, MD, NIH
Sarah Feldman, MD, MPH, Harvard Medical School
An Update on Molecular Biology, Pathogenesis, and Epidemiology of Human Papillomavirus

Yusheng Zhu, PhD, DABCC, FACB
Associate Professor of Pathology
Financial Disclosure Information

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- Fujirebio Diagnostics, Inc.
- Helena Laboratories
- NIH
- AHA
- AACC CPOCT
Outline

- Molecular biology
- Pathogenesis
- Epidemiology
Part I

MOLECULAR BIOLOGY
HPV

- A group of small, non-enveloped, double-stranded DNA viruses
- Belong to the family Papovaviridae
- The main causative agent for cervical carcinomas
- Also associated with oral, esophageal, and other anogenital cancers

Asif et al. 2014
Schematic Representation of HPV

Malik et al. 2014
HPV Genome Structure and Functions of Viral Proteins

E1: Viral replication
E2: Viral replication and transcription regulation
E4: Maturation of virions and facilitation of their release
E5: Tumor progression and augmentation of the oncogenic function of E6 and E7
E6 & E7: Major oncoprotein
L1: Major viral capsid protein
L2: Minor viral capsid

Life Cycle

Malik et al. 2014
Immune Reactions to HPV

- The complete virus cycle takes place above the basal layer without directly triggering cell lysis.
- Limited interaction of viral antigens with the immune cells.
- HPV depletes intraepithelial Langerhans cell (antigen-presenting cells), crucial for T-cell surveillance.
- Lack of local inflammation and poor immune response to HPV

Malik et al. 2014
### Classification of HPVs by Tissue Tropism

<table>
<thead>
<tr>
<th>Location</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous</td>
<td>1, 4, 41, 48, 60, 63, 65, 76, 77, 88, 95</td>
</tr>
<tr>
<td>Mucosal</td>
<td>6, 11, 13, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 42, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 64, 66, 67, 68, 69, 70, 72, 73, 74, 81, 82, 83, 84, 86, 87, 89</td>
</tr>
<tr>
<td>Cutaneous and/or mucosal</td>
<td>2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 61, 62, 78, 91, 94, 101, 103</td>
</tr>
<tr>
<td>Cutaneous associated with Epidermodysplasia Verruciformis</td>
<td>5, 8, 9, 12, 14, 15, 17, 19, 20/46*, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 50, 80, 75, 92, 93, 96, 107</td>
</tr>
</tbody>
</table>
Part II

PATHOGENESIS
Risk Types of HPV

- **Low-risk**: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108
  - Cause low-grade mild dysplasia, genital warts, and respiratory papillomatosis

- **High-risk**: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82
  - Cause dysplasia and cancer

- **Probable high-risk**: 26, 53, and 66

Asif et al. 2014
Comparison of Terminologies Used for Cytologic and Histologic Findings

<table>
<thead>
<tr>
<th>Dysplasia/carcinoma in situ</th>
<th>CIN terminology</th>
<th>Bethesda System for cytologic studies</th>
<th>Modified CIN terminology for histologic studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild dysplasia</td>
<td>CIN 1</td>
<td>LSIL</td>
<td>Low-grade CIN (CIN 1)</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>CIN 2</td>
<td>HSIL</td>
<td>High-grade CIN (CIN 2,3)</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>CIN 3</td>
<td>Invasive cancer</td>
<td>Invasive cancer</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td></td>
<td>Invasive cancer</td>
<td>Invasive cancer</td>
</tr>
</tbody>
</table>

Abbreviations: CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.
**HPV Classification by Tissue Tropism**

<table>
<thead>
<tr>
<th>Group</th>
<th>Prototypes</th>
<th>Site</th>
<th>Acute disease</th>
<th>Chronic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous</td>
<td>HPV 1, HPV 2</td>
<td>Skin</td>
<td>Warts</td>
<td>None</td>
</tr>
<tr>
<td>Cutaneous—high risk</td>
<td>HPV 5, HPV 8</td>
<td>Skin</td>
<td>Flat lesions or warts</td>
<td>SCC</td>
</tr>
<tr>
<td>Mucosal—low risk</td>
<td>HPV 6, HPV 11</td>
<td>Anogenital mucosa</td>
<td>Warts</td>
<td>None</td>
</tr>
<tr>
<td>Mucosal—high risk</td>
<td><strong>HPV 16, HPV 18, HPV 31,</strong></td>
<td>Anogenital mucosa, oral mucosa</td>
<td>Flat lesions</td>
<td>SCC</td>
</tr>
<tr>
<td></td>
<td><strong>HPV 33, HPV 45</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- HPV, human papillomavirus; SCC, squamous cell carcinoma.

Scheurer et al. 2005
The Progression from Precancerous Lesions to Invasive Cancer

Asiaf et al. 2014
The Role of E6 and E7 in Carcinogenesis

- Major oncogenic proteins of HPV
- Both proteins display cell transforming activities in vitro and in vivo
- Immortalize primary human keratinocytes

Ghittoni et al. 2010
## Targets of HPV Oncoproteins

<table>
<thead>
<tr>
<th>E6</th>
<th>Effect</th>
<th>E7</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Antiapoptosis</td>
<td>Rb</td>
<td>Disruption of cell cycle regulation</td>
</tr>
<tr>
<td>Paxillin</td>
<td>Actin cytoskeleton disruption</td>
<td>p107</td>
<td>Disruption of cell cycle regulation</td>
</tr>
<tr>
<td>Bak</td>
<td>Antiapoptosis</td>
<td>p130</td>
<td>Disruption of cell cycle regulation</td>
</tr>
<tr>
<td>IRF-3</td>
<td>Decreased interferon-β transcription</td>
<td>E2F/cyclin A complex</td>
<td>Disruption of cell cycle regulation</td>
</tr>
<tr>
<td>Unknown</td>
<td>Increased telomerase activity</td>
<td>Cyclin E</td>
<td>Disruption of cell cycle regulation;</td>
</tr>
<tr>
<td>PDZ proteins</td>
<td>Increased cellular proliferation</td>
<td>p21</td>
<td>diminished cytostasis by TNF-α</td>
</tr>
<tr>
<td>p300/CBP</td>
<td>Inhibition of transcription</td>
<td>Unknown</td>
<td>Abnormal centrosome duplication</td>
</tr>
<tr>
<td>E6-BP</td>
<td>Unknown</td>
<td>p27</td>
<td>Abrogation of TGF-β growth arrest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p48 and IRF-1</td>
<td>Abrogation of interferon-α signaling</td>
</tr>
</tbody>
</table>
Transforming Properties of E6 Protein

Ghittoni et al. 2010
Transforming Properties of E7 Protein

Ghittoni et al. 2010
Role of E6 and E7 in Escaping Immune Surveillance

- **E6 and E7:**
  - modulate innate immune response by inhibiting a family of toll-like receptors (TLR9), key sensors of evading pathogens.

- **E6:**
  - inhibits interferon regulatory factor-3 (IRF-3), a positive transcriptional regulator of the IFNβ promoter.
  - limits the presentation of viral antigens to the Langerhans cells and promotes viral survival.

- **E7:**
  - binds and prevents IRF-1 from activating the IFNα and β promoters.
  - suppresses cytotoxic response through the downregulation of the transporter associated with antigen protein 1.

Ghittoni et al. 2010
Typical Clinical Course of an HPV in the Uterine Cervix

Kroupis et al. 2011
Part III

EPI DEMIOLOGY
Global Incidence and Prevalence Estimate

- HPV is currently one of the most common sexually transmitted infections in both men and women.
- ~75% of sexually active adults will encounter at least one infection during their lifetime.

Asif et al. 2014
Global Incidence and Prevalence Estimate

- At a given point of time, about **11.4%** of women worldwide with a normal cervical cytology are positive for HPV DNA.
- **30 million** new cases of genital HPV are diagnosed every year worldwide.
- The global prevalence of genital HPV infection is **11.7%**.

Asiaf et al. 2014
### Global Prevalence

<table>
<thead>
<tr>
<th>Region</th>
<th>Adjusted HPV Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caribbean</td>
<td>35.4</td>
</tr>
<tr>
<td>Eastern Africa</td>
<td>33.6</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>21.4</td>
</tr>
<tr>
<td>Western Africa</td>
<td>19.6</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>17.4</td>
</tr>
<tr>
<td>South America</td>
<td>15.3</td>
</tr>
<tr>
<td>South-Eastern Asia</td>
<td>14.0</td>
</tr>
<tr>
<td>Central America</td>
<td>13.0</td>
</tr>
<tr>
<td><strong>World</strong></td>
<td><strong>11.7</strong></td>
</tr>
<tr>
<td>Eastern Asia</td>
<td>10.7</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>10.0</td>
</tr>
<tr>
<td>Northern Africa</td>
<td>9.2</td>
</tr>
<tr>
<td>Western Europe</td>
<td>9.0</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>8.8</td>
</tr>
<tr>
<td>Southern Asia</td>
<td>7.1</td>
</tr>
<tr>
<td>Northern America</td>
<td>4.7</td>
</tr>
<tr>
<td>Western Asia</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Forman et al. 2012*
Incidence and Prevalence Estimate in United States

Estimated number of new sexually transmitted infections
- United States, 2008

Young people (15-24) represent 50% of all new STIs

*HIV incidence not calculated by age in this analysis

Bars are for illustration only; not to scale, due to wide range in numbers of infections

Hepatitis B: 19,000
HIV*: 41,400
Syphilis: 55,400
HSV-2: 776,000
Gonorrhea: 820,000
Trichomoniasis: 1,090,000
Chlamydia: 2,860,000
HPV: 14,100,000

TOTAL: 19,738,800

Incidence and Prevalence Estimate in United States

Estimated number of new and existing (total) sexually transmitted infections
- United States, 2008

<table>
<thead>
<tr>
<th>Disease</th>
<th>Estimated Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>117,000</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>270,000</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>422,000</td>
</tr>
<tr>
<td>HIV</td>
<td>908,000</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1,570,000</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>3,710,000</td>
</tr>
<tr>
<td>HSV-2</td>
<td>24,100,000</td>
</tr>
<tr>
<td>HPV</td>
<td>79,100,000</td>
</tr>
</tbody>
</table>

Total: 110,197,000

Gender totals do not equal overall total, due to rounding
Bars are for illustration only; not to scale, due to wide range in numbers of infections

Medical Care Costs for HPV-Related Conditions in US

- $2.9 billion per year for follow-up of abnormal Pap smear and treatment of cervical neoplasia in the 15-24 years old
- $108.3 million for direct medical costs associated with invasive cervical cancer.
- $123.9 million for the treatment of external anogenital warts

Chesson et al. 2004
Type-specific HPV Prevalence in Women with a Normal Cervical Cytology

Asif et al. 2014
Positivity of HPV types 16, 18, and 45 as a Portion of HPV-positive Samples by Cervical Disease Grade

Guan et al. 2012
The Relationship between Age and HPV Prevalence

- Inverse relationship in many countries
- Uniformly high across all age groups in the poorest areas
- Two-peak pattern in some countries
  - $\leq 25$ years: Higher levels of sex activity? Multiple partners? Lower immunity?
  - $\geq 45$ years: Immunosenescence? Hormonal change? Reactivation of latent infection?

Asif et al. 2014
HPV Infection in Men

- Prevalence among low-risk sexually active men: 1-84%
- Prevalence among high-risk sexually active men: 2-93%
- Prevalence of HPV infection in men remains steady or decline only slightly with age after the peak

Smith et al. 2011
Factors Related to Genital HPV Infection

- Number of recent/lifetime partners
- Age at sexual debut
- Young age
- Socioeconomic status
- Multiparity
- Male circumcision
Factors Related to Genital HPV Infection

- Condom use
- Oral contraceptive use
- Smoking
- Immune suppression
- Viral load
- Genetic polymorphism in HLA system
Summary

- HPV infection is a necessary cause in the development of cervical cancer and other anogenital cancer and cancers in other remote site.
- Viral oncoproteins E6 and E7 are responsible for pathogenesis of cervical cancer.
- HPV 16 and 18 are the most prevalent types of HPV causing cervical cancer.
- HPV and related diseases pose a significant burden on global health care.
- Vaccination, early detection and intervention hold promise for overcoming HPV infection and related diseases.
Thank you
HPV Diagnostics
Principles and Methods for Cervical Cancer Screening

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Financial Disclosures

None

The opinions expressed in this presentation are my own and do not necessarily reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.
Diagnostic methods for HPV (1)

1. Not culturable!

1. Cytology - Low sensitivity, high specificity
   - Used in developed countries since mid-1950s
   - Highly trained cytopathologists
   - High rate of false negatives
   - False positives due to inflammation and sampling variation
   - Abnormal cytology often involves repeat testing, colposcopy, and biopsy.
Diagnostic methods for HPV (2)


Current guidelines for molecular HPV testing:

<table>
<thead>
<tr>
<th>United States</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>To triage women with:</td>
<td>To triage women with:</td>
</tr>
<tr>
<td>1. Atypical squamous cell of undetermined significance (ASC-US)</td>
<td>1. ASC-US</td>
</tr>
<tr>
<td>2. Adjunct to cytology (co-testing) if ≥30 yo.</td>
<td>2. Surveillance after treatment of cervical intraepithelial neoplasia (CIN)</td>
</tr>
<tr>
<td></td>
<td>3. Primary screening without cytology</td>
</tr>
</tbody>
</table>
Specific Genotyping for 16 & 18

- Increasingly important
- Females ≥30 yo HR pos, cytology neg
- Improves specificity
- Progression to CIN2 for 16 & 18:
  - 2x higher compared with other HR
  - >10x higher compared with negative HR

HPV genome

HPV 7900 bp

Linearized

Viral replication

Assembly and release

Capsid proteins

Oncogenic

E6 E7 E1 E2 E4 E5 L2 L1

Arney & Bennett (2010) Lab Med
**FDA approved assays for HPV (1/27/15)**

<table>
<thead>
<tr>
<th>Name of Test</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas HPV test</td>
<td>Roche</td>
</tr>
<tr>
<td>Aptima HPV assay</td>
<td>Hologic (formerly Gen-Probe)</td>
</tr>
<tr>
<td>Aptima HPV 16 18/45 Genotype assay</td>
<td>Hologic (formerly Gen-Probe)</td>
</tr>
<tr>
<td>Cervista HPV HR</td>
<td>Hologic</td>
</tr>
<tr>
<td>Cervista HPV 16/18</td>
<td>Hologic</td>
</tr>
<tr>
<td>Digene Hybrid Capture 2 High-Risk HPV DNA test</td>
<td>Qiagen</td>
</tr>
</tbody>
</table>
## Comparison of the 4 FDA approved HPV assays

<table>
<thead>
<tr>
<th></th>
<th>cobas HPV</th>
<th>Aptima HPV</th>
<th>HC2</th>
<th>Cervista</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manufacturer</strong></td>
<td>Roche</td>
<td>Hologic</td>
<td>Qiagen</td>
<td>Hologic</td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td>✔ (14)</td>
<td>✔ (14)</td>
<td>✔ (13)</td>
<td>✔ (14)</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td>✖</td>
<td>✖</td>
<td>✖</td>
<td>✖</td>
</tr>
<tr>
<td><strong>Genotyping</strong></td>
<td>✔ (16 &amp; 18)</td>
<td>✖ *</td>
<td>✖</td>
<td>✖ *</td>
</tr>
<tr>
<td><strong>Indications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC-US (≥ 21 yo)</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Co-testing (≥30 yo)</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Primary screening</td>
<td>✔</td>
<td>✖</td>
<td>✖</td>
<td>✖</td>
</tr>
</tbody>
</table>

*16 and 18 genotyping available on separate assay
## Roche cobas HPV Overview

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Endocervical brush/spatula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen collection</td>
<td>ThinPrep Pap Test PreservCyt Solution</td>
</tr>
<tr>
<td>Methodology</td>
<td>Real-time PCR (Taqman)</td>
</tr>
<tr>
<td>Assay target</td>
<td>L1 gene (late gene; viral capsid protein)</td>
</tr>
<tr>
<td>Genotypes detected</td>
<td>14 high risk genotypes</td>
</tr>
<tr>
<td></td>
<td>- HPV16</td>
</tr>
<tr>
<td></td>
<td>- HPV18</td>
</tr>
<tr>
<td></td>
<td>- High risk pool</td>
</tr>
<tr>
<td></td>
<td>(31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)</td>
</tr>
<tr>
<td>Indications</td>
<td>1. Triage patients ≥21 yo with ASC-US to determine need for colposcopy</td>
</tr>
<tr>
<td></td>
<td>2. Co-testing with cytology in women ≥30 yo</td>
</tr>
<tr>
<td></td>
<td>3. Primary HPV screening alone ≥25 yo</td>
</tr>
<tr>
<td></td>
<td>If 16 or 18 pos – reflex to colposcopy</td>
</tr>
<tr>
<td></td>
<td>If HR pos, 16/18 neg – reflex to colposcopy</td>
</tr>
</tbody>
</table>
Roche cobas HPV
Principle of the Method

Cobas 4800 System

Cobas x 480 instrument
- Automated sample prep
- Ready to use reagents (magnetic beads)
- 20 min set up for 94 samples

Cobas z 480 analyzer
- Real-time PCR
- 4 fluorophores
  16, 18, HR pool, β-globin
Roche cobas HPV Processing & Result Interpretation

- Up to 96 tests per run
  - 94 samples plus positive and negative control

<table>
<thead>
<tr>
<th>Result</th>
<th>ASC-US cytology ≥21 yo</th>
<th>NILM cytology ≥30 yo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other HR neg HPV16 neg HPV18 neg</td>
<td>Very low likelihood of underlying ≥ CIN2</td>
<td>Lowest likelihood of underlying ≥ CIN2</td>
</tr>
<tr>
<td>Other HR pos HPV16 neg HPV18 neg</td>
<td>Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy</td>
<td>Low likelihood of underlying ≥ CIN2</td>
</tr>
<tr>
<td>HPV16 and/or HPV18 pos</td>
<td>Highest likelihood that underlying ≥ CIN2 will be detected at colposcopy</td>
<td>Increased likelihood of underlying ≥ CIN2</td>
</tr>
</tbody>
</table>
Roche cobas HPV Clinical Performance

- ATHENA study (2008, 3-year longitudinal)
  - 61 participating sites, 5 test centers
  - 46,887 women ≥21 yo
  - Overall HPV prevalence = 12.6%
    - ASC-US group (≥21 yo) = 31.9%
    - NILM group (≥30 yo) = 6.7%
    - Primary screening (≥25 yo) = 10.5%
  - Reference standard = Hybrid Capture 2 (Qiagen)
**HPV Primary** predicts ≥CIN2 and CIN3+ in women ≥25 yo

### ≥CIN2

<table>
<thead>
<tr>
<th></th>
<th>Cytology</th>
<th>Co-testing</th>
<th>Primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>40.6</td>
<td>55.5</td>
<td>69.1</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.3</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>PPV</td>
<td>24.8</td>
<td>19.5</td>
<td>20.2</td>
</tr>
<tr>
<td>NPV</td>
<td>98.7</td>
<td>99</td>
<td>99.3</td>
</tr>
</tbody>
</table>

### ≥CIN3

<table>
<thead>
<tr>
<th></th>
<th>Cytology</th>
<th>Co-testing</th>
<th>Primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>47.8</td>
<td>61.7</td>
<td>76.1</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.1</td>
<td>94.6</td>
<td>93.5</td>
</tr>
<tr>
<td>PPV</td>
<td>17</td>
<td>12.6</td>
<td>12.9</td>
</tr>
<tr>
<td>NPV</td>
<td>99.3</td>
<td>99.5</td>
<td>99.7</td>
</tr>
</tbody>
</table>

Wright TC et al (2015) Gynecologic Oncology
# Aptima HPV Assay Overview

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Endocervical brush/spatula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen collection</td>
<td>ThinPrep Pap Test PreservCyt Solution</td>
</tr>
<tr>
<td>Methodology</td>
<td>Transcription mediated amplification (TMA)</td>
</tr>
<tr>
<td>Assay target</td>
<td>E6/E7 mRNA (oncogenes)</td>
</tr>
<tr>
<td>Genotypes detected</td>
<td>14 high risk genotypes (does not differentiate) 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>Used either with:</td>
</tr>
<tr>
<td></td>
<td>1. Tigris DTS System</td>
</tr>
<tr>
<td></td>
<td>2. Panther System</td>
</tr>
<tr>
<td>Indications</td>
<td>1. Triage patients ≥21 yo with ASC-US to determine need for colposcopy</td>
</tr>
<tr>
<td></td>
<td>2. Co-testing with cytology in women ≥30 yo</td>
</tr>
</tbody>
</table>
Aptima HPV Assay
Principle of the Method

- Single tube reaction
  - Target capture
  - Target amplification by Transcription Mediated Amplification (TMA)
    - Reverse transcriptase to generate DNA
    - RNA polymerase to generate multiple copies of RNA amplicon
  - Amplicon detection by Hybridization Protection Assay (HPA)
    - “Torches” - Single stranded nucleic acid bind to amplicon. Fluorophore separates from quencher
Aptima HPV Assay Instrumentation

Tigris
- Fully automated
- 450 samples in 8 h

Panther
- Random access
- 275 samples in 8 h
- Diverse molecular testing (Trichomonas, CT/NG)
Aptima
Clinical Performance

- CLEAR trial
  - Prospective, multicenter US trial
  - Detection of ≥CIN2
  - Cohort
    - ASC-US ≥21 yo – all women referred to colposcopy
    - NILM ≥30 yo – if positive by molecular, referred to colposcopy. Three year follow up.
  - Reference standard = FDA approved HPV DNA test
## Aptima Clinical Performance

### ≥CIN2 and CIN3+ ASC-US ≥21 yo

### ≥CIN2

<table>
<thead>
<tr>
<th></th>
<th>Aptima Tigris</th>
<th>Aptima Panther</th>
<th>HPV DNA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86.8</td>
<td>84.6</td>
<td>88.8</td>
</tr>
<tr>
<td>Specificity</td>
<td>62.9</td>
<td>62.1</td>
<td>55.8</td>
</tr>
<tr>
<td>PPV</td>
<td>20.1</td>
<td>19.3</td>
<td>18.8</td>
</tr>
<tr>
<td>NPV</td>
<td>97.8</td>
<td>97.4</td>
<td>97.7</td>
</tr>
</tbody>
</table>

### ≥CIN3

<table>
<thead>
<tr>
<th></th>
<th>Aptima Tigris</th>
<th>Aptima Panther</th>
<th>HPV DNA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90.2</td>
<td>90.2</td>
<td>92.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>60.2</td>
<td>59.7</td>
<td>53.3</td>
</tr>
<tr>
<td>PPV</td>
<td>9.4</td>
<td>9.3</td>
<td>8.6</td>
</tr>
<tr>
<td>NPV</td>
<td>99.3</td>
<td>99.3</td>
<td>99.3</td>
</tr>
</tbody>
</table>
# Hybrid Capture 2 Overview

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Endocervical brush/spatula Biopsies</th>
</tr>
</thead>
</table>
| **Specimen collection** | ThinPrep Pap Test PreservCyt Solution  
                          Hybrid Capture Cervical Sampler  
                          Biopsies in Specimen Transport Medium (STM)  
                          TriPath Imaging SurePath Preservative Fluid |
| **Methodology**   | DNA hybridization/Chemiluminescence |
| **Assay target**  | Full genome                         |
| **Genotypes detected** | 13 high risk genotypes  
                          16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 |
| **Indications**   | 1. Triage patients ≥21 yo with ASC-US to determine need for colposcopy  
                          2. Co-testing with cytology in women ≥30 yo |
Hybrid Capture 2

Principle of the Method

1. Denature specimen
2. Hybridize with HPV RNA probe
3. Capture hybrids using monoclonal antibodies on microtiter plates
4. A second monoclonal antibody conjugated to alkaline phosphotase is added
5. Alkaline phosphotase splits a chemiluminescent substrate to produce light

www.papillomavirus.cz/images/hybridcapture.jpg
Hybrid Capture 2
Processing & Result Interpretation

- Performed manually or using the Rapid Capture System
- 352 specimens in 8 h
- Relative Light Units (RLU)

<table>
<thead>
<tr>
<th>PAP result</th>
<th>RLU/CO ratio &lt;1.0 (negative)</th>
<th>RLU/CO ratio ≥1.0 (positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNL</td>
<td>Very low likelihood of underlying ≥ CIN2</td>
<td>Low likelihood of underlying ≥ CIN2. Possible persistent, resolving, or transient infection</td>
</tr>
<tr>
<td>ASC-US</td>
<td>Low likelihood of underlying ≥ CIN2</td>
<td>Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low likelihood of underlying ≥ CIN2</td>
<td>Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy</td>
</tr>
<tr>
<td>HSIL</td>
<td>Uncommon. Repeat testing</td>
<td>High likelihood of underlying ≥ CIN2</td>
</tr>
</tbody>
</table>
## Cervista HPV HR Overview

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Endocervical brush/spatula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen collection</td>
<td>ThinPrep Pap Test PreservCyt Solution</td>
</tr>
<tr>
<td>Methodology</td>
<td>Invader chemistry</td>
</tr>
<tr>
<td>Assay target</td>
<td>E6 and E7</td>
</tr>
<tr>
<td>Genotypes detected</td>
<td>14 high risk genotypes</td>
</tr>
<tr>
<td></td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 over three reaction mixtures</td>
</tr>
<tr>
<td>Indications</td>
<td>1. Triage patients ≥21 yo with ASC-US to determine need for colposcopy</td>
</tr>
<tr>
<td></td>
<td>2. Co-testing with cytology in women ≥30 yo</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>96 well plates</td>
</tr>
<tr>
<td></td>
<td>Fluorescent plate reader</td>
</tr>
</tbody>
</table>
**Cervista HPV HR**

**Principle of the Method**

What is invader chemistry?

- **Two isothermal reactions**
  - **Primary reaction** – target detection using two oligonucleotides (probe and invader)
    - When both probes overlap by a single nucleotide, the “invasive structure” acts as a substrate for Cleavase, which cleaves the 5’ flap along with the single nucleotide
  - **Secondary reaction** – fluorescent signaling (FRET)
    - The 5’ flap along with the single nucleotide binds with hairpin FRET probe, creating another “invasive structure” substrate for Cleavase thus releasing a fluorescent signal
Cervista HPV HR Invader Chemistry

Sequence specific oligonucleotide with 5’ flap

Sequence specific invader probe

2 different flap sequences for HPV (FAM) and human histone 2 gene (internal control; Red)
Cervista HPV HR

Manual or automated
96 samples per 8 h
(medium throughput)
Cervista HPV HR
Result interpretation

- Signal to noise ratio (fold over zero; FOZ) generated for each of the 3 HPV reactions
- Generate a HPV FOZ ratio among the 3 HPV mixtures
- Average signaling of the internal control

<table>
<thead>
<tr>
<th>Result</th>
<th>ASC-US cytology ≥21 yo</th>
<th>NILM cytology ≥30 yo</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG</td>
<td>Low likelihood of underlying ≥CIN2</td>
<td>Low likelihood of underlying ≥CIN2</td>
</tr>
<tr>
<td>POS</td>
<td>Increased likelihood that underlying ≥CIN2 will be detected at colposcopy</td>
<td>Low likelihood of underlying ≥CIN2 (resolving, persistent, or transient infection)</td>
</tr>
</tbody>
</table>
Cervista HPV HR
Clinical Performance

ASC-US population (n=1332; reference standard colposcopy)

<table>
<thead>
<tr>
<th></th>
<th>≥CIN2</th>
<th>≥CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92.8</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>44.2</td>
<td>43</td>
</tr>
<tr>
<td>PPV</td>
<td>8.3</td>
<td>2.9</td>
</tr>
<tr>
<td>NPV</td>
<td>99.1</td>
<td>100</td>
</tr>
</tbody>
</table>

NILM population 3-year follow up (n=1959)

<table>
<thead>
<tr>
<th></th>
<th>≥CIN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>66.7</td>
</tr>
<tr>
<td>Specificity</td>
<td>81.6</td>
</tr>
<tr>
<td>PPV</td>
<td>1.6</td>
</tr>
<tr>
<td>NPV</td>
<td>99.8</td>
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</table>
# Summary of Methodologies

<table>
<thead>
<tr>
<th></th>
<th>cobas</th>
<th>Aptima</th>
<th>HC2</th>
<th>Cervista</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>1 mL</td>
<td>1 mL</td>
<td>4 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Methodology</td>
<td>Taqman</td>
<td>TMA</td>
<td>DNA hybridization</td>
<td>Invader Chemistry</td>
</tr>
<tr>
<td>Target</td>
<td>L1 DNA</td>
<td>E6/E7 mRNA</td>
<td>Full genome</td>
<td>E6/D7 DNA</td>
</tr>
<tr>
<td>Throughput (8h)</td>
<td>375</td>
<td>450 (Tigris)</td>
<td>352</td>
<td>96</td>
</tr>
<tr>
<td>Acceptable specimens</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>ThinPrep Pap</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>HC cervical sampler</td>
<td>✗</td>
<td>✗</td>
<td>✔</td>
<td>✗</td>
</tr>
<tr>
<td>Biopsies in STM</td>
<td>✗</td>
<td>✗</td>
<td>✔</td>
<td>✗</td>
</tr>
<tr>
<td>SurePath Preservative Fluid</td>
<td>✗</td>
<td>✗</td>
<td>✔</td>
<td>✗</td>
</tr>
</tbody>
</table>
Testing timeline

<table>
<thead>
<tr>
<th></th>
<th>cobas HPV</th>
<th>Hybrid Capture 2</th>
<th>Cervista HPV HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N reportables</td>
<td>94</td>
<td>88</td>
<td>48</td>
</tr>
<tr>
<td>Total cycle time (min)</td>
<td>296</td>
<td>405</td>
<td>471</td>
</tr>
<tr>
<td>Hands on time (min)</td>
<td>29</td>
<td>143</td>
<td>126</td>
</tr>
<tr>
<td>Walk away time (min)</td>
<td>267</td>
<td>249</td>
<td>309</td>
</tr>
</tbody>
</table>
HR molecular assays perform similarly. Genotyping is best.

- Compared 3 commercial systems for prediction of ≥CIN2
- Tissue biopsy was used as reference standard
- 350 patients, 81 (23%) showed ≥CIN2 histopathology

<table>
<thead>
<tr>
<th></th>
<th>APTIMA</th>
<th>HC2</th>
<th>cobas HR pool</th>
<th>cobas 16/18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreement</td>
<td>53.4</td>
<td>43.4</td>
<td>45.1</td>
<td>78.6</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>91.4</td>
<td>97.5</td>
<td>91.4</td>
<td>51.9</td>
</tr>
<tr>
<td>Specificity</td>
<td>42</td>
<td>27.1</td>
<td>31.2</td>
<td>86.6</td>
</tr>
<tr>
<td>PPV</td>
<td>32.1</td>
<td>28.7</td>
<td>28.6</td>
<td>53.9</td>
</tr>
<tr>
<td>NPV</td>
<td>94.2</td>
<td>97.3</td>
<td>92.3</td>
<td>85.7</td>
</tr>
</tbody>
</table>
HPV Diagnostics Summary

- Diverse and complex
- Variety of platforms and chemistries
- Generally perform similarly
- No assay will have perfect clinical sensitivity
  - Inherent variability in cervical sampling
- Highly dependent on prevalence
- Test choice dependent on institutional algorithm and physician
The HPV Vaccine: Implications for HPV Disease and Cervical Cancer Screening

Douglas R. Lowy
Laboratory of Cellular Oncology, Center for Cancer Research
National Cancer Institute, National Institutes of Health

AACC Conference, Atlanta
July 30, 2015

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

• The National Institutes of Health (NIH) has patents on papillomavirus L1 virus-like particle (VLP) vaccine technology. I am an inventor of this technology.

• The NIH has licensed the L1 VLP technology to Merck and GlaxoSmithKline, the two companies with commercial versions of the vaccine.

• I will discuss potential off-label use of the FDA-approved vaccines.
Outline of Presentation

• HPV-associated cancers in the US
• The HPV vaccine: development, efficacy, and US uptake
• Recent developments:
  – fewer than 3 doses
  – a second generation HPV vaccine
• Potential impact of HPV vaccination on cervical cancer screening
USA: HPV-associated Non-cervical Cancers Affect Both Genders and are as Common as Cervical Cancer

Annual number of cases

- Pap screening has reduced cervical cancer incidence by ~80%
- No approved screening tests for other HPV-associated cancers
- Incidence of HPV-positive oropharynx cancer 1988-2004 increased >3-fold

Cervical Cancer is Attributable to Multiple HPV Types; HPV16 Predominates

Adapted from Munoz et al, Int J Cancer 111: 278-85, 2004
Developing and Testing The First Generation HPV Vaccines
Laboratory of Cellular Oncology, CCR, NCI

Patricia Day  Nicolas Cuburu
Rhonda Kines  Susana Pang
Cynthia Thompson  Alessandra Handisurya
Hanna Seitz  Carla Cerqueira

John Schiller

Chris Buck, Diana Pastrana - LCO, CCR, NCI Bethesda
Aimee Kreimer, Allan Hildesheim, Mark Schiffman, Mahboobeh Safaeian, Ligia Pinto - DCEG, NCI, Bethesda

Peter Choyke, Marcelino Bernardo - Molecular Imaging, CCR, NCI, Bethesda
Jeffrey Roberts – FDA, Rockville
Rolando Herrero – IARC, Lyon, France
Bryce Chackerian - University of New Mexico
Reinhard Kirnbauer - University of Vienna, Austria
Choosing an appropriate molecular target for a preventive HPV vaccine

• Licensed vaccines against microbial agents are mainly preventive; induction of neutralizing antibodies is critical.

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

• Papillomaviruses encode two proteins that induce neutralizing antibodies, the capsid proteins L1 and L2.
  
  – L1 contains the immunodominant neutralization epitopes. They are conformationally dependent.

• OUR HYPOTHESIS: L1 can self-assemble to make empty particles having a conformation that induces high levels of neutralizing antibodies.
Prophylactic HPV Vaccines Are L1 Virus Like Particles (VLPs)

- L1 Insertion in Baculovirus Expression Vector
- Production in Insect Cells
- Spontaneous assembly of L1 into VLPs
- Induce high titers of virion neutralizing antibodies
- Shown initially for BPV-1, then for HPV16
- Non-infectious, Non-oncogenic

Reinhard Kirnbauer et al. PNAS 1992
The Commercial Vaccines Are Composed of Multiple Types of HPV L1 VLPs

Gardasil (Merck)

HPV16: 70% of Cervix Cancer
HPV18: >90% of Non-cervix Cancer
HPV6: 90% of Genital Warts
HPV11

Cervarix (GlaxoSmithKline)

Three intramuscular injections over 6 months
Summary of phase III HPV vaccine trials

• In uninfected patients, HPV vaccination can confer close to 100% protection against incident persistent infection and disease attributable to the HPV vaccine types
  – HPV vaccination can also protect against non-cervical infection and disease, while screening is only for cervical cancer

• There is limited cross-protection against non-vaccine HPV types; cross-protection is greater with the bivalent vaccine than with the quadrivalent vaccine

• The bivalent vaccine is more immunogenic than the quadrivalent vaccine and induces greater cross-protection

• HPV vaccination does not alter the natural history of prevalent infection, i.e., it is not therapeutic
Monitoring the safety of quadrivalent human papillomavirus vaccine: Findings from the Vaccine Safety Datalink

Julianne Gee\textsuperscript{a,}, Allison Naleway\textsuperscript{b}, Irene Shui\textsuperscript{c}, James Baggs\textsuperscript{a}, Ruihua Yin\textsuperscript{c}, Rong Li\textsuperscript{c}, Martin Kulldorff\textsuperscript{c}, Edwin Lewis\textsuperscript{d}, Bruce Fireman\textsuperscript{d}, Matthew F. Daley\textsuperscript{e}, Nicola P. Klein\textsuperscript{d}, Eric S. Weintraub\textsuperscript{a}

\textsuperscript{a}Immunization Safety Office, Division of Healthcare Quality and Promotion, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333, USA
\textsuperscript{b}Center for Health Research, Kaiser Permanente Northwest, Portland, OR, USA
\textsuperscript{c}Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA
\textsuperscript{d}Vaccine Study Center, Northern California Kaiser Permanente, Oakland, CA, USA
\textsuperscript{e}Institute for Health Research, Kaiser Permanente Colorado, Denver, CO, USA

\begin{itemize}
  \item Prospective post-licensure assessment of 600,558 doses (Gardasil) from 7 managed care organizations
  \item \textbf{No vaccine-related increased risk to prespecified outcomes:} Guillan-Barré syndrome, stroke, venous thromboembolism, appendicitis, seizure, allergic reactions
    \begin{itemize}
      \item Prespecified outcomes were derived from CDC analysis from VAERS (Vaccine Adverse Events Reporting System): Slade et al, JAMA 2009
      \item Similar conclusions in Denmark from 997,585 girls, of whom 296,826 received 696,240 doses (Gardasil): Arnheim-Dahlstrom et al., BMJ, 2013
    \end{itemize}
  \item Rate of anaphylaxis (1 case, 26 y.o.) similar to other vaccines
  \item Rate of fainting similar to that of other adolescent vaccines
\end{itemize}
Initial Population-wide Impact of HPV Vaccination
Goals of HPV Vaccination

• To directly reduce the risk of infection and disease in vaccinees

• To indirectly reduce these risks by reducing the prevalence of the HPV vaccine types in the general population (herd/community immunity)
Age-dependent Decrease in Genital Warts in Australian Women After HPV Vaccine Implementation in 2007

Herd Immunity: Decreased Incidence of Genital Warts in Heterosexual Australian Men Following Female HPV Vaccine Implementation in 2007

Australia: Fall in Prevalence of HPV Vaccine Types after Initiating National Vaccine Program

Figure 1. Differences in human papillomavirus (HPV) genoprevalence between prevaccine and postvaccine populations. *P < .05 for difference in percentages between groups. Abbreviations: CI, confidence interval; excl, excluding; HR-HPV, high-risk HPV.

Tabrizi et al, J Infect Dis 206: 1645-51, 2012
Limited HPV Vaccine Uptake in the United States
**Trends in U.S. Vaccination Rates: Ages 13-17 Yrs**

Abbreviations: Tdap = tetanus, diphtheria, acellular pertussis vaccine; MenACWY = meningococcal conjugate vaccine; HPV-1 = human papillomavirus vaccine, ≥1 dose; HPV-3 = human papillomavirus, ≥3 doses.

* Tdap and MenACWY vaccination recommendations were published in March and October 2006, respectively.
† HPV vaccination recommendations were published in March 2007.
Parents’ Top 5 Reasons for not vaccinating their Children with the HPV Vaccine (CDC, 2013)

<table>
<thead>
<tr>
<th>Reason</th>
<th>Parents of girls</th>
<th></th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of knowledge</td>
<td>15.5</td>
<td>(13.0–18.5)</td>
<td></td>
</tr>
<tr>
<td>Not needed or necessary</td>
<td>14.7</td>
<td>(12.5–17.3)</td>
<td></td>
</tr>
<tr>
<td>Safety concern/Side effects</td>
<td>14.2</td>
<td>(11.8–16.8)</td>
<td></td>
</tr>
<tr>
<td>Not recommended</td>
<td>13.0</td>
<td>(10.8–15.5)</td>
<td></td>
</tr>
<tr>
<td>Not sexually active</td>
<td>11.3</td>
<td>(9.1–13.9)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reason</th>
<th>Parents of boys</th>
<th></th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not recommended</td>
<td>22.8</td>
<td>(20.6–25.0)</td>
<td></td>
</tr>
<tr>
<td>Not needed or necessary</td>
<td>17.9</td>
<td>(15.9–20.1)</td>
<td></td>
</tr>
<tr>
<td>Lack of knowledge</td>
<td>15.5</td>
<td>(13.7–17.6)</td>
<td></td>
</tr>
<tr>
<td>Not sexually active</td>
<td>7.7</td>
<td>(6.4–9.2)</td>
<td></td>
</tr>
<tr>
<td>Safety concern/Side effects</td>
<td>6.9</td>
<td>(5.6–8.5)</td>
<td></td>
</tr>
</tbody>
</table>

Stokley et al, MMWR 63:620-4, July 25, 2014
Mechanism of Action
Several Factors Contribute to the High Efficacy of the Vaccine

- The repetitive structure of the VLP immunogen is intrinsically immunogenic.
- Tissue-associated neutralizing antibodies are exudated at potential sites of infection.
  - Antibody levels at these sites reflect their level in serum, rather than their lower level in the non-disrupted genital tract.
- HPV is highly susceptible to neutralizing antibodies.
Neutralizing L1 Antibodies (in red) Bound to Papillomavirus Particle
VLP Vaccination Induces High Titer Antibodies that Prevent Basement Membrane Binding

Based on Patricia Day et al, Cell Host Microbe 16: 260-70, 2010
Rapid Acquisition of Genital HPV Infection in Young Women With Their First Sexual Partner

UK (15-19 years old; N=242)

US (18-22 years old; N=130)

20% in 4 months

45% in 26 months

UK data adapted from Collins et al, BJOG 109: 96-98, 2002

US data adapted from Winer et al, J Inf Dis 197:279-282, 2008
For Young Adolescents, One or Two Vaccine Doses may be Sufficient
Two vaccine doses: The future is now (except in the US)

• Immune response in girls and boys <15 years old is stronger than in older teenagers

• In young adolescents, 2 doses separated by 6 months produce an immune response similar to those in the responses in the efficacy trials

• European Medicines Agency approval and World Health Organization Strategic Advisory Group of Experts recommendation for 2 doses for HPV vaccines:
  – Bivalent (GSK) girls 9-14
  – Quadrivalent (Merck) girls & boys 9-13
One or two vaccine doses (Cervarix, GSK) can induce 4 years of protection against persistent (6 months) HPV infection with HPV16/18

<table>
<thead>
<tr>
<th>Number of doses</th>
<th>Vaccine arm</th>
<th>Number of women</th>
<th>Number of events</th>
<th>Rate per 100 women</th>
<th>HPV vaccine efficacy % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3 doses</strong></td>
<td>Control</td>
<td>3010</td>
<td>229</td>
<td>7.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>2957</td>
<td>37</td>
<td>1.3%</td>
<td>84 (77-88)</td>
</tr>
<tr>
<td><strong>2 doses</strong></td>
<td>Control</td>
<td>380</td>
<td>24</td>
<td>6.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>422</td>
<td>5</td>
<td>1.2%</td>
<td>81 (63-94)</td>
</tr>
<tr>
<td><strong>1 dose</strong></td>
<td>Control</td>
<td>188</td>
<td>15</td>
<td>8.0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>196</td>
<td>0</td>
<td>0.0%</td>
<td>100 (79-100)</td>
</tr>
</tbody>
</table>

- **Similar protection was seen against 12 month persistent infection**
- **It is unknown whether these results can be extrapolated to Gardasil**

Kreimer et al, JNCI 103: 1444050, 2011
HPV16 GMTs among seronegatives

Stable antibody titers after 1 dose

- There is no precedent for 1 dose of a protein-based sub-unit vaccine to induce stable antibody titers for several years
- May be attributable to two factors
  - VLPs are highly immunogenic
  - ASO4 is a TLR4 agonist
- A possible randomized controlled trial to rigorously test the efficacy of 1 dose
  - Test two commercial vaccines: one with alum, one with AS04
A Second Generation Vaccine: Protecting against a Larger number of HPV Types
A 9-valent VLP vaccine: adding 5 oncogenic HPV types to the quadrivalent vaccine

- A randomized controlled trial that compared the efficacy of the quadrivalent vaccine to the 9-valent vaccine

- **CIN2+ vaccine efficacy against the 5 additional HPV types was 96%**: 1 case in the 9-valent group vs. 27 cases in quadrivalent group
  

- FDA approval, December, 2014

- ACIP recommendation, February, 2015
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
HPV Type Affects the Rate of Development of CIN3 or worse in women with normal cytological findings at baseline: The Danish Cohort Study

A single HPV test predicts 10-fold increased risk of CIN3 for >10 years

*From Kjaer et al, J Natl Cancer Inst 102: 1478-88, 2010*
Doug’s speculative predictions for cervical cancer screening

- In populations with high HPV vaccine uptake, HPV testing will gradually replace cytology as the primary screening modality, and the age of first screening will be increased to older than 21
  - Cytology is relatively insensitive for detecting precancerous lesions associated with HPV types other than HPV16 (Safaeeian et al, Cancer Res 2009)
  - Primary screening by HPV testing is more sensitive than cytology
  - HPV-based screening has greater negative predictive value than cytology-based screening; increase screening intervals?
  - HPV types that rapidly progress to high-grade dysplasia are specifically targeted by the second generation HPV vaccine
Summary and Conclusions

- Basic research identified HPV infection as the main cause of several cancers.

- This knowledge enabled development of effective vaccines to prevent infection and disease caused by HPV.

- The high immunogenicity of the HPV VLP vaccines suggests that long-term protection can be induced with fewer than 3 doses.

- Second generation HPV vaccines with activity against a broader range of HPV types can achieve an even greater reduction in HPV-associated infection and disease.
Update on Screening and Prevention of Cervical Cancer in the US—Understanding the Guidelines

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Brigham & Women’s Hospital
Dana Farber Cancer Institute
Lowell Cancer Center
Harvard Medical School
Financial Disclosures

• None
Objectives

• Review HPV and its association with cervical cancer
• Be familiar with new guidelines
• Understand the data behind the guidelines
• Introduce primary HPV testing
How are we doing with cervical cancer?

<table>
<thead>
<tr>
<th>Cervical Cancer</th>
<th>USA</th>
<th>Worldwide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>12,900</td>
<td>528,000</td>
</tr>
<tr>
<td>Deaths</td>
<td>4,100</td>
<td>266,000</td>
</tr>
</tbody>
</table>

Between 60-70% of cervical cancer worldwide is due to HPV16/18.

The 5 year survival of this preventable disease is 67.9%.

We have to do better... it is preventable.
Successful Cervical Cancer Prevention

Requires a programmatic approach including:

• Primary prevention:
  – vaccination

• Secondary prevention:
  – screening
  – active management of abnormalities to prevent progression
Prevalence of HPV Infection among US females

Dunne EF et al Jama 2007 Feb 28; 297(8) 876-8

14-59 years old
Self collected vaginal swabs
HPV prevalence overall 24.5%
- 14-19: 25%
- 20-24: 45%
- 25-29: 28%
- 30-39: 25%
- 50-59: 20%
- HPV 16: 1.5%; HPV 18: 1.5% overall
Natural History of CIN/dysplasia

Linked to high risk/oncogenic HPV
CIN 1: 60% regress/1 year, 90% /3yrs (Moscicki 2004)
CIN 2: 40% regress/1 year (Fuchs 2007, Moscicki 2010)
CIN 3:
- Untreated CIN 3: 30% risk of invasive CA /30 yrs
- Treated CIN 3: 1% risk of invasive cervical CA

Higher levels of dysplasia - more likely to progress to cancer

*Most HPV infections are transient and do not confer significant risk of cancer or dysplasia*
Duration of HPV infections in young women

- Women aged 16-23
- Studied incidence and duration of HPV 6, 11, 16 and 18 infection
- Mean duration of 6/11 - 8 months
- Mean duration of 16/18 - 14.5 months

HPV 16/18 persists 2x longer than HPV 6/11 on average

Insigna RP, bCancer Epidemiol Biomarkers Prev 2007 Apr; 16 (4): 709-15
Long Term Risk of CIN3+ after HPV infection: role of persistence

- 8656 women in Denmark
- Co-testing-underwent pap and HC2 testing
- 2 exams, two years apart
- Then followed in registry for 12 years
- Estimated risk of CIN3+ for women who were HPV 16+ at both exams=47.4% (over 12 years of f/u)
- Risk of CIN3+ after HPV negative= 3%

- Suggests less frequent follow up appropriate for HPV negative women, and aggressive follow-up should be considered for those persistently positive for HPV 16.
Challenges in managing cervical pre-cancers

• Lesions change over time
• Special populations differ with respect to risk of progression/regression (ie. Adolescents, pregnant, immuno-compromised)
• Fertility desires may affect relative risk of treatment versus observation
• Data is complicated and constantly changing
There are many Screening Options
*(and the technology continues to evolve....)*

- **Cytology** (aka Pap Tests)—conventional or thin layer easier for downstream testing as well as cost.
- **HPV testing**-4 FDA approved types, some for use with specific Pap preparations, some which can differentiate HPV types, some not.
- **Co-testing** refers to a screening test that includes both a Pap and an HPV test
- **Reflex testing** ex. HPV testing after an ASCUS Pap– this is used to triage patients to more or less subsequent evaluation
- **Primary HPV screening** *(Cobas FDA approved 4/2014)*
# Canadian Cervical Cancer Screening Trial

**Women ages 30-69**

**Compared conventional Pap and HC2**

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## Table 4. Comparison of Pap Testing and HPV DNA Testing Using Combined Study Groups According to Different Positivity Thresholds and Test Combinations.*

<table>
<thead>
<tr>
<th>Screening Approach</th>
<th>Definition of Positivity</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>No. of Tests Needed for Screening</th>
<th>Referrals for Colposcopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap only</td>
<td>ASCUS or worse</td>
<td>56.4</td>
<td>97.3</td>
<td>8.5</td>
<td>99.8</td>
<td>9,959</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>LSIL or worse</td>
<td>42.2</td>
<td>99.1</td>
<td>17.5</td>
<td>99.7</td>
<td>9,959</td>
<td>1.0</td>
</tr>
<tr>
<td>HPV only</td>
<td>≥1 pg HPV DNA/ml</td>
<td>97.4</td>
<td>94.3</td>
<td>7.0</td>
<td>100.0</td>
<td>9,959</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>≥2 pg HPV DNA/ml</td>
<td>81.1</td>
<td>95.5</td>
<td>9.1</td>
<td>99.9</td>
<td>9,959</td>
<td>4.8</td>
</tr>
<tr>
<td>Pap screening followed by HPV triage</td>
<td>Triage of all results of</td>
<td>53.8</td>
<td>98.7</td>
<td>14.9</td>
<td>99.8</td>
<td>10,145</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>ASCUS; ≥1 pg HPV DNA/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV screening followed by Pap triage</td>
<td>Triage of all with ≥1 pg</td>
<td>53.8</td>
<td>99.1</td>
<td>21.4</td>
<td>99.8</td>
<td>10,563</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>HPV DNA/ml; Pap threshold of ASCUS or worse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap and HPV cotesting</td>
<td>Pap result of ASCUS or worse, or HPV result of</td>
<td>100.0</td>
<td>92.5</td>
<td>5.5</td>
<td>100.0</td>
<td>19,918</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>≥1 pg HPV DNA/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Estimates are corrected for verification bias according to the conservative case definition and are based on pooled data from 9959 women in the two study groups who had available Pap and HPV results. HPV denotes human papillomavirus, ASCUS atypical squamous cells of undetermined significance, and LSIL low-grade squamous intraepithelial lesion.

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2012 Guidelines for Cervical Cancer Screening

3/14/2012 ACS, ASCCP, ASCP (www.ASCCP.org)
USPSTF (www.USPSTF.org)
ACOG Practice Bulletin #131
Reviewed similar data

• Evidence Based

• Logical, simple to understand and clearly written

• Clearly address areas of patient and provider confusion
Cervical Cancer Screening Guidelines 2012
(Healthy Low Risk Women)

<21  No screening pap/cytology
21-29  Pap q 3 years regardless of sexual activity
       (no HPV screening)
30-65  Pap alone q 3 years or Cotesting/Pap with HPV q 5 years
       if both results negative
       (and normal and adequate screening history)
> age 65 or hysterectomy stop screening
   In well screened women
   – Defined as 3 neg Paps within prior 10 years or 2 neg cotests within
     10 years
   – Poorly screened women still need to be screened in this age group.

Any abnormal findings require more aggressive evaluation and
follow up as per the new management guidelines
Women at increased risk need more frequent screening (2012)

- **Immunosuppressed** (e.g., HIV+, organ transplants, chronic steroids or immunosuppressive drugs, auto-immune illnesses, etc)
- Previously treated CIN2/ CIN3/ Adenocarcinoma in situ (AIS) or cancer—more frequent screening should occur for at least 20 years.
- Women with h/o mildly abnormal Paps or abnormal HPV tests should still be followed closely.
- Diagnostic Paps should be performed for **abnormal symptoms**—even if in between recommended screening intervals.
Increased Cervical Cancer Risk Associated with Screening at Longer Intervals

Makes key points with respect to how data is interpreted and understood with respect to guideline development.

• Annual cytology remains the gold standard for cancer prevention
• Cost and benefits need to be considered and may vary with different life situations/populations.
• Q 3 year Pap or q 5 year cotesting are known to increase cancer rates relative to annual cytology.
• Adverse effects of treatment (LOOP) may have been overstated.
2013 Management Guidelines
ASCCP 2013/ACOG 2013
www.ASCCP.org

- Management of Abnormal Pap Smears (cytology)
- Management of Colposcopy Biopsies (histology)
- Follow up after treatment (excision, ablation)
2013 Management Guidelines
Very complicated and difficult to follow
Management Guideline Problems

- 30 pages long
- 12 algorithms
  - 7 for pap smear follow up
  - 5 for colposcopy finding follow up
- Unclear which are evidence based and which are only expert opinion
What’s the principle behind the new management guidelines ("risk based assessment")?

The guidelines divide patients in many ways:

2 basic cytology categories: ASCUS/LSIL vs. ASC-H/HSIL

Special groups: HIV+, DES, Immunosuppressed, pregnancy

Age groupings: when to do paps and use HPV testing
  <21, 21-24, 25-29, >30, >65 or hyst, “young women”

Sometimes it is not clear which group a patient belongs to or what her actual risk is.
Is it safe to return to the new “routine” screening after an abnormality?

- after any abnormal cytology?
- after any abnormal histology?
- after treatment for histologic abnormality?

Recommendations for surveillance post abnormality - based on weakest data, may be misleading
Update on Management Guidelines-

What’s the data?

ASCCP 2013/ACOG 2013
www.ASCCP.org

• Based mostly on Kaiser’s large dataset
• Health system with excellent tracking, insurance, systems to bring patients back for appropriate testing and management
• **Data based on earlier screening practices** with more frequent evaluation and more aggressive management
• true rates of cancer or pre-cancer with the current guidelines cannot be assessed (since patients are not being detected and treated as often)
• **May not be generalizable to all settings**
Variable Risk of Cervical Precancer and Cancer After a Human Papillomavirus-Positive Test
Castle, P. Obstet Gynecol 2011:117:650-6

- Kaiser data
- >30 year old women, tested positive for HPV
- Past positive HPV test OR abnormal Pap - significantly higher risk CIN2+ than newly acquired infection
- unknown prior screening history
  for ASCUS /HPV+ women with unknown screening history:
  -the 4 year cumulative risk of CIN2 was 23% and of CIN3 was 13% 
  -similar to women known to have had known prior abnormal results

THUS KNOWLEDGE OF THE PAST SCREENING AND RESULT HISTORY MATTERS
Follow-up testing after colposcopy: five-year risk of CIN2 after a colposcopic diagnosis of CIN1 or less


• Kaiser women >25 years old
Screening results antecedent to colposcopy affected 5 year risk of CIN2

<table>
<thead>
<tr>
<th>Pap cytology</th>
<th>Colposcopy histology</th>
<th>5 year risk of CIN2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS/LSIL</td>
<td>CIN1 or less</td>
<td>10 %</td>
</tr>
<tr>
<td>ASC-H</td>
<td>CIN1 or less</td>
<td>16 %</td>
</tr>
<tr>
<td>HSIL</td>
<td>CIN1 or less</td>
<td>24 %</td>
</tr>
</tbody>
</table>

• No group had sufficiently low risk to return to “routine” screening
• If prior Pap showed ASC-H or HSIL, there was no group that could be returned to even less frequent co-testing
Five Year risk of recurrence after treatment of CIN2/CIN3 or ACIS
Katki, HA. J Low Genit Tract Dis 2013

- Kaiser >30 year old women
- 5 year risks of recurrence after treatment varied by antecedent screening result and path

<table>
<thead>
<tr>
<th>Pap –Cytology</th>
<th>Colpo biopsy-histology</th>
<th>5 year risk of recurrence post rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS/HPV+ or LSIL</td>
<td>CIN 2</td>
<td>5%</td>
</tr>
<tr>
<td>ASCUS –H or worse</td>
<td>CIN3/ACIS</td>
<td>16%</td>
</tr>
</tbody>
</table>

- No subgroup of women achieved risk sufficiently low to return to the new routine screening
- Recommendation is co-test at 12, 24, 36 months then “routine”
Factors affecting screening and management of cervical precursors

- The strength of data supporting different options and improved outcomes in actual clinical settings
- A patient’s clinical history, preferences and ability to comply with care
- Systems concerns such as the availability and costs of different tests and support to track and manage abnormalities, insurance issues, patient mobility
Future uses of HPV testing

• HPV 16/18 account for 77% cervical cancers and 54% high grade lesions in US
• As successive cohorts are vaccinated, fewer women may get these infections
• Primary screening with HPV and triage to cytology might be the logical next step
What is the data for primary HPV screening?
Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomized controlled trials


• Combined results of 4 studies (Sweden, the Netherlands, England and Italy).

• Primary HPV  60-70% greater protection against invasive cervical cancer than primary cytology after first 2.5 years.

• Negative  HPV at 5 years had better negative predictive value (NPV) than normal cytology at 3 years.

• However, studies involved many different treatment and management algorithms reporting markedly different costs for screening, depending on strategies used.
Other studies suggesting improved detection of dysplasia or > with primary HPV

• United States (Kaiser-observational, large data base, HMO, has also reviewed value of subtyping and stratification by age)
• Greece (HERMES study in routine clinical practice)
• British Columbia (FOCAL-organized screening program in Canada)
• Although these studies add support for Primary HPV Screening, the study parameters may not apply to all US clinical setting.
Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as first line screening test


Methods:
• Cobas HPV test
• Cytology and HPV co-collected
• Options compared included:
  – Reflex hpv
  – Hybrid (cytology under 30 and cotesting above 30)
  – Primary HPV with 16/18 triage or cytology triage (if HPV12+)

Results: (n=42,209, >25 yo, no prior hx of abn paps)
  – Primary HPV detects more CIN2+ but at cost of more colposcopies and requires the simultaneous collection of the cytology specimen

Limitations of the study:
• Only 3 years of follow up data
• Patients managed by specific study algorithms which may not be available in all clinic settings.
Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance.

- Based on limited long term data and expert opinion
- Recommended HPV 16/18 triage with reflex cytology for HPV12+
- Assumes HPV 16/18 and or reflex cytology are universally available
- These downstream tests may not be readily available and there is little clinical efficacy data or cost data
- Since cytology and/or cotesting are known to be successful approaches for cancer prevention, not everyone may be ready to dismantle this approach all at once, but...
Will we miss cancers if we screen with HPV alone?

• HPV negative screening cancers exist
  – HPV or cytology obtained before the cancer may not have detected the cancer
  – but this may be the wrong question...
• The real question is: **Will primary HPV testing prevent cancer?**
  – In the setting of a well conceived screening program
  – With repetitive rounds of screening, diagnosis and treatment

*Caveats:*

• Pelvic exams and diagnostic testing of *symptomatic women (even teens)* needs to be the standard of care
• Some cancers are not preventable by screening
Primary HPV screening should be offered as a part of a comprehensive cervical cancer prevention program.

How do we manage abnormal primary HPV tests?

- Different situations determine which is best
- Need ongoing studies to guide management
Things are changing....
Looking Towards the Future

Ultimately a combination of vaccine in younger women and screening for carcinogenic HPV in older women may revolutionize cervical cancer prevention.

References


- Related websites with guidelines:
  - Asccp.org
  - Uspstf.org
  - Acs.org