an evolving understanding of an ancient and established virus
Disclosures-1

• Merck
  – Speaker Panel for Gardasil
• Hologic
  – Speaker Panel for Cervista and Thin Prep
Disclosures-2

Gynecologic Oncologist

Parent

Vaccine Enthusiast
Objectives

• HPV
• Cervical cancer
• The Cervix
• Epidemiology
• Screening tests
• Breast Cancer
Mortality over the years

- Pneumonia
- TB
- GI infections
- Cardiac disease
- Cerebrovascular dz
- Nephropathies
- Accidents
- Cancer
- Senility

- Cardiac disease
- Cancer
- Pulmonary causes
- Cerebrovascular dz
- Accidents
Concept of Cancer

- Largely dependent upon knowledge of normal
- No concept of prodromal cancer
- Early diagnosis: desired, though treatment options limited
- Prevention: unclear
Screening Test

- Performed on asymptomatic people
- Common disease
- Plausible test
  - Cost, Access, Reliable
- Sufficient “lead time” to intervene
- Intervention that can prevent death or morbidity
SCREENING

- Public Health mechanism
- For the asymptomatic patient
- Cost to patient and society
- Balance benefit versus harm
Diagnostic Test

- A test performed on someone who is symptomatic and needs DIAGNOSIS
- Xray, blood, biopsy
- Pap smear can be diagnostic
  - Vaginal bleeding
- Mammogram can be diagnostic
  - Breast lump
- Colonoscopy can be diagnostic
  - Rectal bleeding
Enter the Pap Smear

- Georgios Papanikolaou (1883-1962)
- 1920s describing the normal vagina
- 1928 presented his findings
- 1943 published his findings
- 1965 ACS recommended screening
- 1999 reflex HPV testing
- 2009 co-testing HPV and cytology
- 2014 HPV primary testing
Major Advances in Cervical Cancer Screening

1941 Pap Smear

1940s

1970s

Research by Harald zur Hausen linking HPV to cervical cancer\(^1\)

1970s

1996

ThinPrep® Pap Test

1990s

1996

Hybrid Capture® 2 HPV Test

1999

SurePath® Pap Test

1999

ThinPrep® Imaging System

2000s

2006

Gardasil® HPV Vaccine

2009

Cervista® HPV HR Test and Cervista® HPV 16/18 Genotyping Test

2006

ThinPrep® Receives Glandular Indication

2009

Cervarix® HPV Vaccine

ThinPrep® Liquid-based Cytology: Mitigates Sampling Error and Improves Preservation

**Conventional Smear**
- Majority of cells discarded
- Nonrepresentative transfer of cells
- Clumping and overlapping of cells
- Obscuring material

**ThinPrep Pap Test**
- Virtually all of sample is collected
- Randomized, representative transfer of cells
- Even distribution of cells
- Minimizes obscuring material

Screening Has Dramatically Reduced Cervical Cancer Incidence

US Cervical Cancer Incidence*

US Cervical Cancer Mortality*

* Insufficient data available for time trend analysis for American Indians/Alaskan Natives.

** Incidence and mortality data not available before 1992.

Limitations of Cytology

• Epidemiology
  – changing face of the disease

• Anatomy
  – screens ectocervix reliably but not endocervix

• Labor Intensive
  – limitations on cyto-technologist work force

• Cost
Advantages of HPV Screening

- HPV testing is more sensitive than cytology
- HPV testing has a high negative predictive value
- For women 30 and older, a negative Pap and HPV test would allow for a patient to extend to a 3-year screening interval

2. J Clin Oncol May 2011 vol. 29 no. 15_suppl 1508
Changing Epidemiology
HPV ONCOGENESIS
HPV in Cancer: History

• 1935 Francis Peyton Rous
  – caused skin cancer in rabbit

• 1972 Stefania Jablonska
  – identified HPV 5 in skin cancer

• 1976 Harald zur Hausen
  – hypothesized HPV cause of cervical cancer

• 1983 HPV 16

• 1984 HPV 18

• 2008 Nobel Prize in Medicine
Peyton Rous and Rous Sarcoma Virus (RSV)

1910  1966
HPV EPIDEMIOLOGY
Could YOU have HPV?
YES... if you have...

- a cervix
- a vagina
- a penis
- tonsils
- a throat
- an anus
- ever had sex
HPV is Ubiquitous

- 85% have come in contact with it
- The majority “clear it”
- More common in smokers
- More transmissible female to male
- Resides in epithelial layer
  - Basal layer required for replication
- Conserved in mammals
  - Across time and geography
“My patient is not at risk”

- Wife of Korean WHO Chief
- 4J school teachers
- Librarians, nurses
- The girl next door
- Anesthesiologists
- Social workers
Human Papillomavirus (HPV) Is a Cause of Cervical Cancer

• Over 100 types identified\(^2\)
• 30–40 anogenital\(^2,3\)

- 15–20 oncogenic\(^2,3\) types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 58\(^4\)
  • HPV 16 (54%) and HPV 18 (21%) account for the majority of cervical cancers worldwide\(^5\)
- Nononcogenic types include:
  6, 11, 40, 42, 43, 44, 54\(^4\)
  • HPV 6 and 11 account for 90% of external genital warts\(^3\)

HPV Facts: Most common STD in the U.S.

Approximately 20 million Americans are currently infected.¹

- Estimated incidence of new cases 6 million per year¹
- 80% sexually active adults in U.S. infected w/ at least one HPV type by age 50¹
- Peak prevalence during adolescence and young adulthood
  - Among sexually active 15-24 year olds:
    - 74% new infections occur in this age group²
    - ~9.2 million currently infected²

¹ Centers for Disease Control & Prevention, Rockville MD: CDC National Prevention Information Network; 2009
“My patient is not at risk

- HPV is ubiquitous virus
- Risk assessment does not work
  - HIV
  - Hep B
  - HPV vaccination
US Cervical Cancer Statistics

• Approximately 12,710 new cases/year\textsuperscript{1}
• Approximately 4,290 deaths/year\textsuperscript{1}
• Approximately 10 million cases of HPV infection without cytologic abnormalities\textsuperscript{2}
• Approximately 1 million cases CIN 1\textsuperscript{2}
• Approximately 300,000–700,000 cases of CIN2/3
• Direct cost of prevention and treatment of cervical cancer is $6 billion annually in the US

33,000 cancer cases annually
HPV in Laryngeal Cancers

![Bar chart showing the distribution of HPV genotypes in males vs females. The chart displays:
- HPV 16
- HPV 18
- HPV 33
- Other carcinogenic (HPV 31, 35, 39, 51, 66)
- Non-carcinogenic (HPV 6, 11, 54, 70, 89)

The x-axis represents the number of genotypes (0 to 10), and the y-axis lists the HPV genotypes. The chart indicates that HPV 16 is more prevalent in females compared to males. Other carcinogenic types are more evenly distributed between genders. Non-carcinogenic types have a similar distribution to males.]

PLoS One 2014 Dec 29, 9(12)
Internationally

- 530,000 cases of cervical cancer
  - 230,000 deaths
- 30,000 cases of anal cancer
  - more female than male
- oropharyngeal cancer
  - new area of tabulation
- >600,000 cancer cases
  - >300,000 deaths
Why Are HPV 16/18 Important?

> 75% of Squamous Cancers in the United States Are Caused by HPV 16/18

Proportion of Cancers Associated with HPV Types

How does HPV do it?

• interferes with the normal work of the cell
  – invades epithelium
  – evades “surveillance”
  – integrates into host genome
• E6
  – interrupts important work of p53
• E7
  – interrupts important work of RB
• Able to create immortal cell lines
  – HeLa cells: HPV 18
HPV enters the epithelium

- trauma of intercourse
  - micro-abrasions
  - coexisting infections
- “dry” intercourse
- anal intercourse
- areas of metaplasia/transition
- oropharyngeal trauma
Normal Cervix with Ectopy
Progression from HPV infection to Cervical Cancer

- **Within 12 months**
  - HPV Clearance
  - HR HPV Infection
  - CIN 1/2
  - CIN 2/3
  - Cervical Cancer

- **1-5 years**
  - Persistent Infection

**Progression to cervical cancer may take up to decades**

**HR** = High risk

**CIN** = Cervical intraepithelial neoplasia

HPV targets the squamous epithelium found beneath the foreskin of the penis and also the cervix. Infection of basal epithelial cells is necessary for HPV replication and it is thought that virus particles gain access to these cells through microabrasion or microwounding that exposes the basement membrane. The L1 capsid protein on the surface of the HPV virion interacts with α6β4 integrins that are upregulated on basal epithelial cells during wound repair. Interaction with α6β4 integrin promotes internalisation of virus. Circumcision may reduce the risk of HPV infection via the removal of target cells present in the squamous epithelium beneath the foreskin. Antibodies to the capsid proteins L1 and L2 of HPV may be important in blocking attachment of virus to receptors on basal epithelial cells during microwound healing.
HPV replication in squamous epithelial cells

HPV takes advantage of the differentiation pathway of keratinocytes that are destined to die naturally (anoikis). Since HPV is not cytolytic and does not cause viraemia, there is no inflammation and subsequent activation of the immune system. Infection of basal epithelial cells establishes a latent infection with low level replication of the viral episome and minimal viral protein expression. Following differentiation of the keratinocyte, early HPV genes are expressed and the viral episome is further amplified to higher copy numbers. Viral late protein expression and virus assembly occurs during terminal differentiation of the keratinocyte and viruses are shed from the outermost layer of epithelial cells.
The role of p53 protein is to respond to DNA damages and functions as a nuclear transcription factor that activates transcription of genes involved in arrest of the cell cycle and induction of DNA repair systems or the induction of apoptosis. Retinoblastoma protein (pRb) functions to inactivate the transcription factor E2F-DP that is required to initiate transcription of genes involved in DNA replication. These two proteins are essential to prevent cells with damaged DNA from dividing and are known as tumor suppressor proteins. In the absence of HPV E6 and E7, p53 and pRb function normally and reduce the risk of malignant cell transformation.
Integration of the HPV genome with a disrupted E2 gene into host chromosomal DNA is a necessary event that can lead to the development of carcinoma. The E2 gene encodes a transcription factor that regulates the transcription of HPV E6 and E7 oncoproteins. In the absence of E2, increased synthesis of E6 and E7 protein occurs. E6 binds to p53 in the cytosol and also recruits the E6AP ubiquitin ligase that ubiquitinates p53 and targets it for proteosomal degradation. Similarly, HPV E7 binds to pRb in the cytosol and recruits the cullin 2 ubiquitin ligase that ubiquitinates pRb and promotes proteosomal degradation. Loss of cellular p53 and pRb tumour suppressor proteins allows a cell with DNA damage to divide and thereby increases the risk of cancer development.
Papillomaviruses are absolutely species specific and tissue specific.

Virus particles assembled
Differentiated cells
E and L viral genes expressed
Dividing cells
Only E genes expressed
Very low levels of protein made

Virus laden cells ready for desquamation and infection of naive individual L1/L2, L1, L2, E4
Viral genomes at 1000’s per cell E6, E7, E1, E2, E5
Viral DNA amplification in non-dividing cells
Virus and cell replicate together E1, E2
Virus infects a primitive basal keratinocyte E1, E2, ?, E5, E6, E7

6–12 weeks
0 weeks

Why Are HPV 16/18 Important?

> 75% of Squamous Cancers in the United States Are Caused by HPV 16/18

Proportion of Cancers Associated with HPV Types
Risk Stratification with HPV Types 16 and 18 in Women ≥ 30 Years of Age with Negative Cytology

In women ≥ 30 years of age, 10-year cumulative incidence of ≥ CIN 3 was 20% and 18% for HPV 16 and 18, respectively.
Papanicolaou to zur Hausen
Cervical Cancer Prevention: Get with the times...

“This dial phone has always worked for me…”

“My patients would never be able to understand a more modern test…”
FUTURE DIRECTIONS
Vaccines

• Gardasil 4 (Merck) FDA approved 2006
  – 6,11,16,18
• Cervarix (GSK) approved 2009
  – 16,18
• Gardasil 9 (Merck) FDA approved 2014
  – 6,11,16,18,31,33,45,52,58
• Vaccines are controversial
Vaccine Efficacy

• 99-100% immunogenicity
• 92-99% efficacy
• Decrease CIN3 17-33%
• Decrease colposcopy by 10%
• Decrease treatment by 25%
• Impeccable safety record
Vaccine Eligibility

• Gardasil 4 and Cervarix
  – Girls 9-26
  – Boys 9-26
  – Safety data exists for the “older woman”

• Gardasil 9
  – Girls 9-26
HPV adjunctive testing

• Primary testing
  – controversial
• Cotesting
  – here to stay
• Enhanced HPV testing
  – Aptima test (Hologic) tests for mRNA
• Testing intervals
  – 3 lifetime screening tests?
Oropharyngeal Cancers

- 20-40% are HPV positive
- HPV 16
- Better prognosis
- Decreased morbidity from scaled back treatment regimens
The Future

• Vaccines prevent cervical cancer
• Therapeutic “vaccines” eliminate any remaining HPV
• Cervical cancer goes the way of small pox
  – ? (and measles?)
Adjunctive Testing (Pap + HR HPV)
Use of HPV Genotyping to Manage HPV HR*-Positive, Cytology-Negative Women 30 Years and Older

- **HR HPV Positive, Cytology Negative**
  - HPV 16/18 Positive
    - Colposcopy
  - HPV 16/18 Negative
    - Repeat BOTH tests at 12 months
    - Cytology Negative, HPV-Positive
      - Routine Screening at 3 Years
    - Cytology Abnormal, Any HPV Result
      - Manage per ASCCP Guidelines

- **Both Negative**
  - Routine Screening at 3 Years

- **Cytology Negative, HPV-Positive**
  - Colposcopy

- **Cytology Abnormal, Any HPV Result**
  - Manage per ASCCP Guidelines

*Test that detects any of the 14 HR (oncogenic) types of HPV.

ASCCP = American Society for Colposcopy and Cervical Pathology

ASCCP. ASCCP Clinical Update. ASCCP: Hagerstown, MD; 2009.
1st Review

- Imager scans every cell and cell cluster on the slide, measuring DNA content

2nd Review

- Cytotechnologist reviews 22 fields containing “objects of interest”
  - Full slide screened if any cells judged abnormal

Clinical benefits over manually reviewed ThinPrep® Pap Test¹:

- Increased sensitivity
- Increased specificity
- Reduced false-negative fraction

Imaging System Focuses Slide Review

ThinPrep® Imaging System Is Statistically More Sensitive Than Manual Screening

- TIS more sensitive than manual screening for:
  - ASC-US
  - Higher-grade lesions with equivalent specificity for ASC-US
  - LSIL

- Glandular malignancies not included in original studies, but data now support

Cells Are Collected in Liquid for Laboratory Processing

**Healthcare Provider Office**
- Sample collected

**Laboratory**
- Representative sample
- Even distribution of cells
- Minimal obscuring material

Dispersion/Collection/Transfer
BREAST CANCER

• Annual exams
  – Clinical exams and mammograms

• Epidemiologic concerns
  – parity, HRT, breastfeeding

• Treatment concerns
  – Tamoxifen, aromatase inhibitors

• Genetic concerns
  – BRCA mutation status
BREAST CANCER EPIDEMIOLOGY

• Being female (100:1)
• Age (stats determined through age 90)
• Exposure to estrogen
  – Obesity
  – Alcohol
  – Breastfeeding
  – Pregnancy history
  – HRT
• Prior radiation
  – lymphoma
“whether it would be permissible to make the ladies old more quickly by removing their ovaries…”

Schinzinger, 1889
SIXTY-EIGHTH ANNUAL MEETING
OF THE
BRITISH MEDICAL ASSOCIATION
Held at IPSWICH, July 31st, August 1st, 2nd, and 3rd, 1900.

PROCEEDINGS OF SECTIONS.

SECTION OF SURGERY.
Howard Marsh, F.R.C.S., President.

ON ÖÖPHORECTOMY IN CANCER OF THE BREAST.
By Stanley Boyd, M.B., F.R.C.S., Surgeon, Charing Cross Hospital.
Distant History

• Dr. George Beatson (Lancet, 1896)
  – Capitalized on experience of Scottish sheep farmers
  – Removed ovaries of a premenopausal patient with advanced breast cancer

• Dr. Stanley Boyd (Br Med Journal, 1900)
  – Reported on 46 patients
  – 37% response rate

Endocrinology Unveiled

- 1923 “estrus stimulating principle”
  - “estrus” from the Latin “frenzy”
- 1962 synthesis of radioactive estradiol
  - Allowed identification of target tissues
- 1966 estrogen receptor isolated
Estrogen Receptor

- Separated tumors for treatment purposes
- Allowed stratification of tumors for prognostic purposes
- **1974:** Estrogen Receptor discussed at NCI
  - ER+ tumors had 60% response rate
  - ER- tumors had 10% response rate
Endogenous Hormonal Manipulation

- Obesity
- Pregnancy
- Breastfeeding
- Terminations
- Hormonal Contraception
Obesity

- Single most modifiable risk factor associated with diagnosis and prognosis
  - Complex association with medical comorbidities, therapy dosing
  - Multiple measures of obesity
- Across age ranges
  - Post and pre menopausal
- Associated with increased aromatase