Human Papillomaviruses: Basic Virology to Clinical Management

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Basic HPV Virology

HPV Virus Structure

- Papillomavirus
- 55 nm
- 8000 bp
- Non-enveloped
- Icosahedral nucleocapsid
- No fibers
Papillomavirus DNA Replication

- Viral DNA maintained as stable multicopy plasmid replicating once per cell cycle.
- Viral DNA replicates vegetatively in differentiated cells. No cellular DNA synthesis occurs, and viral DNA is synthesized for progeny virions.

Human Papillomaviruses

- ds DNA viruses
- No envelope
- Replicate in nucleus
- Species specific

- Capsids are made of late proteins: L1 and L2

HPV DNA Detection

- In situ hybridization
- Southern blot hybridization
- Hybrid capture assay
- Polymerase chain reaction (PCR):
  - Consensus and type specific
  - Nested PCR with PGMY09/11 and GP5(+)/6(+) primer sets improves detection of HPV DNA in cervical samples*  

* Fuessel Haws et al. J Viral Methods 122: 87-93; 2004

Clinical Manifestations of HPV Types

- >150 HPV genotypes characterized
  - 30 types infect genital epithelium
  - Distinguished by different DNA sequences at L1 capsid proteins via DNA hybridization
- Transmission enhanced by abrasions (intercourse or other microtrauma)
- Host immune response determines persistence
- Intracellular and epidermal location of HPV may protect virus from the immune system
- Not cultured

HPV: Viral Characterization and Expression

HPV Transmission and Life Cycle

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HPV 6, 11: Anogenital Warts

- HPV 6 and 11 responsible for >90% of anogenital warts
- Peak prevalence
  - Women 20–24 years of age (6.2/1,000 person years)
  - Men 25–29 years of age (5.0/1,000 person years)
- Clinically apparent in ~1% of sexually active US adult population

Clinical manifestation and HPV types

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantar warts</td>
<td>1</td>
</tr>
<tr>
<td>Common warts</td>
<td>2, 4, 29</td>
</tr>
<tr>
<td>Flat warts</td>
<td>3, 10, 28, 59</td>
</tr>
<tr>
<td>Condyloma acuminata</td>
<td>4, 6, 12, 14, 16, 17, 19–22, 30, 41, 50</td>
</tr>
<tr>
<td>Genital warts, laryngeal papillomas, Buschke-Loewenstein</td>
<td>6, 11</td>
</tr>
<tr>
<td>Borrworn papilloma, anogenital carcinoma</td>
<td>18, 31, 33, 35, 39, 41–45, 51–56</td>
</tr>
<tr>
<td>Bacterial warts</td>
<td>7</td>
</tr>
<tr>
<td>Oral focal epithelial hypoplasia (Heck’s)</td>
<td>13, 32</td>
</tr>
<tr>
<td>Keratoacanthoma</td>
<td>37</td>
</tr>
<tr>
<td>Cutaneous SCC…oral SCC</td>
<td>33, 41, 48, 56</td>
</tr>
<tr>
<td>Cutaneous SCC…beta SCC</td>
<td>Data HPV 6, 11, 17, 20, 24, 38</td>
</tr>
</tbody>
</table>

Genital Warts: An Important Healthcare Issue

- 15% (20 million) of the US population infected in 2004
- 1 million estimated new cases per year in the United States
- 1 in 100 estimated number of sexually active adults with clinically visible genital warts
- 1 in 10 estimated number of people who will develop genital warts in their lifetime

Estimated number of new cases per year in the United States:
- 1 million

Estimated number of sexually active adults with clinically visible genital warts:
- 1 in 100

Estimated number of people who will develop genital warts in their lifetime:
- 1 in 10

Secret to Treating Warts:
- Manage patient/parent expectations
- No secret treatment
- Variety of rx options for pts
  - Warn pts that they may get bigger, rather than better
  - Warn patients that warts can spread
  - Each treatment session will be a “lesson” for their immune system to figure out their wart problem, & we don’t know how many sessions it may take

Local Therapy: Common Warts

- Liquid nitrogen/ shave excision
- Topicals under duct tape
- Topical 5-fluorouracil
- Topical cantharidin
- Topical potassium hydroxide
- Topical glycolic acid
- Topical retinoid

Local treatments for cutaneous warts: BMJ 2002;325:461 (>80% studies poor quality)
Local Therapy: Recalcitrant Common Warts

- Topical imiquimod (Aldara)
- Intralesional candida
- Topical Squaric acid
- Oral therapy
- Intralesional bleomycin
- Intralesional 5-FU
- Topical Cidofovir

Gibbs S et al Local treatments for cutaneous warts. BMJ 2002;325:461 (>80% studies poor quality)

Synergy between Imiquimod and Retinoids

- Imiquimod induces interferons
- Retinoids up-regulate interferon response proteins (e.g. STATs)
  - Faluhelyi et al. Anticancer Res 24: 807-809; 2004
- Cell is better able to respond to the interferon induced by imiquimod

Intralesional Candida

- Inject 0.1-0.3 cc total at 1 session
- Inject into the wart(s) itself
- Immune response on other warts, even those not injected, on the patient
- Inject every few weeks if not resolved
- 47% complete response with 31% distant response

Squaric Acid or DCP for Warts:

Home Treatment

- Send home: Apply every other day
  - Weaker concentration of 0.4%
    - (2% stock so dilute 1:5)
- Apply salicylic acid next and tape or bandaid
- Beware of spilling since may develop a rash wherever it touches
- If not improving, may increase concentration to 0.6%

Office Treatment

- Apply in office: if patient not trusted to apply correctly
  - Use the 2% squaric acid or DCP
  - Protect the surrounding skin with barrier such as vaseline petroleum jelly or zinc oxide so it doesn’t spread
- Repeat every 2-3 weeks
Oral HPV Treatment

- H2 blockers orally
  - Cimetidine 400mg orally twice daily
  - Ranitidine 150 mg orally twice daily
- Allicillin in garlic 60-180 mcg orally per day
  - 55-60 mcg per 180 mg capsule (300 mcg/gram)
- Herbal treatment:
  - Echinacea augustifolia, Green tea, Astragalus, Goldenseal Root, Cordyceps sinensis, Shiitake, Monolaurin (lauric acid), Andrographis paniculata, Beta 1,3 glucan, Larch Tree, Elderberry, Maitake, Reishi

Allicillin Extracts

- Ajoene and allicin extracts, with thiosulfinates
- 60-180 mcg orally per day
- Small placebo-controlled trials with topical extracts with no recurrence after 3-4 months

Combination Therapy

- Flat warts: monotherapy or alternating with topical retinoids or topical glycolic acid
- Verruca vulgaris: shave (pare) top of wart, +/- liquid nitrogen, then topical nightly under occlusion (occlusive wrap) for 4-8 weeks or
- Add intralesional Candida
- Add H2 blocker or oral allicillin

Chemotherapies for Condyloma Acuminatum

- Podophyllin;
- Podofilox;
- 5-FU;
- Bichloroacetic acid; trichloroacetic acid:
  - All painful, some carcinogenic; dangerous for normal skin and associated with high recurrence rates

Surgical Therapies for Condyloma Acuminatum

- Cryotherapy (liquid nitrogen)
- Excisional surgery
- Electro-surgery
- Laser surgery
  - All associated with pain, potential for blood loss; potential for scarring; high recurrence rates

Immune Therapies for Condyloma Acuminatum

- Interferon alpha:
  - Effective
  - Must be given by an injection (by physician or nurse)
  - Systemic side effect
- Imiquimod (Aldara/Zyclara)
- Sinecatechins (Veregen) :
  - Effective
  - Self-applied by patient
  - Local inflammation but no systemic side effects
- All with low recurrence rates
**Effects of Interferon**
- Antiviral
- Immune modulation
- Phenotypic reversion
- Down-regulation of oncogenes
- Up-regulation of tumor suppressor genes
- Anti-proliferative

**Imiquimod**
- Acts through innate immune response TLR7 and TLR8
  - In vitro: induces Th1 immunity
  - Production of interferon alpha, tumor necrosis factor alpha, interleukins
  - Infiltrate of effector cells
  - Activation of Langerhans cells
- Immunological memory: antiviral and antitumor
- Not mutagenic
- Not carcinogenic
- Not teratogenic

**Complete Response Rates of Imiquimod in Condyloma Acuminatum Patients**
- Imiquimod 5% cream was used overnight three times per week for up to 16 weeks
- Overall complete responses: 56%
  - Female complete responses: 77%
  - Male complete responses: 42%
- Low relapse rate:
  - After 3 months:
    - Imiquimod: 13%
    - Surgery: 50%
  - After 5 to 7 (additional) years:
    - Imiquimod: 16%
    - Surgery: 57%

**Local Inflammation following Imiquimod Consistent with MOA**
- Erythema: 77%
- Erosions: 23%
- Mild pruritus: 11%
- Scarring: 0%
- Systemic side effects: 0% (i.e. no different from placebo)

**Catechins: Present in Green Tea**
- Major polyphenols/flavonoids in green tea leaves (*Camellia sinensis*)
  - Fermentation reduces catechin content (black and oolong tea)
  - Green tea is dried by heating after picking (to prevent fermentation); catechins are preserved

**Theoretical Properties of Catechins**
- Antioxidant
  - Scavenge reactive oxygen and nitrogen species
  - Chelate redox-sensitive transition metal ions
  - Inhibit pro-oxidant enzymes (eg, nitric oxide synthase, lipooxygenases, cyclooxygenases, and xanthine oxidase)
  - Induce antioxidant enzymes (eg, glutathione-S-transferases and superoxide dimutases)
- Antiviral
  - Inhibit viral growth and reproduction
  - Demonstrated effects against influenza, adenovirus, and herpes simplex
- Immune-stimulatory
  - Possibly related to antioxidant activity
  - May lead to the release of inflammatory mediators
Complete Response Rates of Veregen® Ointment in Condyloma Acuminatum Patients

- Veregen® Ointment, 15% is a patient-applied botanical alternative for treatment of EGW
- Veregen® demonstrated:
  - Statistically significant early onset of action
  - Complete clearance at 6 weeks in a small number of patients
  - High rates of complete clearance (53.6%)\(^1\)
  - Male complete response 47%
- Low rates of recurrence (6.8%)\(^5\)


Veregen® (sinecatechins) 15% Ointment: Complete Clearance of Warts\(^1,2\)

Complete Clearance (%)

<table>
<thead>
<tr>
<th></th>
<th>All Pts</th>
<th>Females</th>
<th>Males</th>
</tr>
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<tbody>
<tr>
<td>Veregen® 15%</td>
<td>53.6%</td>
<td>60.4%</td>
<td>47.3%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>43.8%</td>
<td>28.8%</td>
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</tr>
</tbody>
</table>

\(P<.001\) (n=397) \(P=.010\) (n=192) \(P=.001\) (n=205)

The most common adverse reactions are local skin and application site reactions including (incidence ≥20%) erythema, pruritus, burning, pain/discomfort, erosion/ulceration, edema, induration, and vesicular rash.

Veregen® (sinecatechins): Low Recurrence Rates at 12 Weeks in Patients With Complete Clearance

Among the 35.3% of vehicle patients with complete clearance, 5.8% experienced recurrent warts at 12 weeks post-treatment.

Veregen® (sinecatechins) Phase III Trials: AEs (>3%)

<table>
<thead>
<tr>
<th></th>
<th>Veregen® (N=397)</th>
<th>Vehicle (N=207)</th>
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<tbody>
<tr>
<td>Erythema</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Pruritus</td>
<td>69</td>
<td>45</td>
</tr>
<tr>
<td>Burning</td>
<td>67</td>
<td>31</td>
</tr>
<tr>
<td>Pain/discomfort</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>Erosion/ulceration</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>Edema</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>Erythema</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td>Vesicular rash</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Regional lymphadenitis</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Desquamation</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Discharge</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Other Possible Therapies for Recalcitrant EGW

- Topical cidofovir
- topical ingenol mebutate
- Topical nitric oxide

Oncogenic Potential of Certain HPV

Factors Influencing Progression of HPV Infection to Cancer

- HPV type
- Upregulation of oncogenes
- Downregulation of tumor suppressor genes (i.e., p53 and pRB)
- Cigarette smoking
- Other sexually transmitted diseases
- Immune status
- Diet
- Genetics (i.e., HLA)

HPV E6: p53 Binding and Degradation

- HPV Early (E) proteins E6 and E7 are viral oncogenes
- E6 and E7 bind and degrade tumor suppressor genes p53 and pRB

HPV DNA: episomal vs. integrated

- HPV DNA is episomal in non-oncogenic HPV
- HPV DNA is integrated in oncogenic HPV

Biomarkers for Progression from HPV Infection to SCC in EV

- HPV types 5 and 8
  - 14d
- Susceptibility loci:
  - EVER1 (integral membrane protein of the endoplasmic reticulum) and EVER2
- Tumor suppression
- Cytokines

HPV in Cancer of the Tonsils

- The overall prevalence of HPV DNA in tonsillar cancer tissues was 65.1%


Genetics and HPV-associated Cancers

- Genetics:
- Smoking:
HPV Types 16 and 18

- HPV Types 16/18 are known to 70% of cases:
  - Cervical cancer
  - Adenocarcinoma in situ (AIS)
  - Cervical intraepithelial neoplasia 3 (CIN 3)
  - Vulvar intraepithelial neoplasia 2 and 3 (VIN 2/3)
  - Vaginal intraepithelial neoplasia 2 and 3 (VaIN 2/3)

Major Consequences of HPV in the United States

- 12,000 cases of cervical cancer
- 300,000 cases of HSIL
- 1 million new cases of EGW
- 1.25 million cases of LSIL

Cervical Cancer: Worldwide Prevalence, Incidence, Mortality

- Estimated incidence of invasive cervical cancer by selected region:
  - Europe: 14,545
  - Africa: 21,596
  - South America: 13,265
  - Southcentral Asia: 14,845
  - United States/Canada: 950
  - Australia/New Zealand: 39,648
  - Southeast Asia: 51,266
  - Eastern Asia: 21,596
  - Central America: 151,297

- Mortality: Second leading cause of female cancer-related deaths (288,000 annually)

Anal SCC

- Anal SCC in HIV MSM = cervical cancer before Pap smears
- Rate of anal SCC: 35/100,000 in HIV- MSMs and 70/100,000 in HIV + MSMs
- Abnormal anal cytology in approximately 80% of HIV+ MSMs with CD4 less than 200
- Treatment: surgery, imiquimod, 5-FU, TCA, infrared coagulation, cidofovir

Treatment of HPV Malignancies

- Antisense therapy: clinical trials did not show success for benign lesions
- HspE7 (heat shock proteins) therapeutic vaccine: 40% response rate in high grade cervical dysplasia
- Cidofovir: highly efficacious in patients (including HIV+ persons) who failed other treatments

Experimental Therapies for HPV
HPV Vaccines

HPV-6/11/16/18 VLPs
HPV6/11/16/18/31/33/45/52/58 VLPs = Gardasil 9

- L1 capsid proteins made in yeast (Gardasil) or insect cells using baculovirus system (Cervarix)
- Formulated with alum (Gardasil) or alum & monophosphoryl lipid A (Cervarix)
- Elicits neutralizing antibody to HPV-6, -11, -16 and -18. Th1 dominant CMI
- 3-doses; months 0, 1 or 2, and 6

L1 Surface Loops: Immune Targets

In clinical studies, both the bivalent and quadrivalent HPV vaccines were safe and almost 100% effective in preventing infection with HPV 16 or 18

Gardasil: HPV 6,11,16,18

- Generally safe and well tolerated
- Highly immunogenic
- 11,502 women between the ages of 15-25 years were randomized to vaccine or placebo (13 countries)
- GARDASIL is highly safe and effective in preventing cervical cancer, CIN 2/3, AIS, and other anogenital diseases, including genital warts caused by HPV 6, 11, 16, and 18 in 15- to 25-year-old women naive to the relevant HPV types.
- Widespread vaccination of young women should help reduce cervical cancer as well as other anogenital diseases related to HPV 6, 11, 16, and 18.

Cervarix: HPV 16/18 (bivalent) Vaccine

- L1 capsid proteins of HPV 16 and 18
- AS04 adjuvant system
- Intramuscular injection at 0, 1 and 6 months
- High efficacy preventing HPV 16/18(+) CIN 2+
  - Phase II or III trials
  - >19,000 women aged 15-25 years
  - 6.5 years follow-up (recently shown 6.4 yrs follow up)
  - Greater than with aluminum salt adjuvant
- Cross-protection against HPV 45 or HPV 31
- Injection-site reactions were the most common vaccine-related adverse events

What's New in Prevention

- 9-valent HPV vaccine
- Potentially can prevent >90% of cervical cancers (and other ano-genital/oral HPV-related cancers)
- Three doses (IM): months 0,2 and 6
- Information regarding cross-protection against related HPV types is pending

The Future of HPV Vaccination

- FDA approval (for women) of Gardasil in 2006
- FDA approval of Gardasil in males: 2009
- FDA approval of Cervarix in 2009
- FDA approval of Gardasil 9: 2014
- Duration of protection: unknown
- Use as therapeutic vaccines: under study
- Oct 2016: Recommended age of vaccination: ages 11 or 12
- 3 shots needed at months 0, 2, and 6 after age 15.
- Gardasil-9 will soon be the only HPV vaccine available in the U.S., as Cervarix and Gardasil (quadrivalent HPV vaccine) will no longer be sold in the United States
- Acceptance: cancer vaccine>> >STD vaccine

Use of vaccine as therapy? Litt le information available, but has scientific basis and case reports

- Acceptance in men?
- Use in females (or males) <9 or >26 years?
- Duration of protection? 5 years+
- Role of dermatologists?
  - Educate patients about the link between HPV and ano-genital cancer
  - Remind male patients about recent FDA approval & about pap smears and HPV vaccine for partners
  - Identify pts at high risk or the target age group for HPV vaccine

HPV Vaccine with BCC and SCC

- SCC, and perhaps BCC, development, may be driven in part by HPV in immunocompetent patients
- Reduction of SCCs and BCCs was observed in 2 patients after administration of the quadrivalent HPV vaccine
- Quadrivalent HPV papillomavirus vaccination may represent an efficacious, cost-effective, readily available, and well-tolerated strategy for preventing BCCs and SCCs
- Nichols AJ1, Allen AH2, Shareef S3, Rадиаvас EV, Kirsner RS4, Ioannides T4
Summary

- Over 150 types of HPV, with varied clinical presentations
- Oncogenic potential of HPV types:
  - HPV 5, 8
  - HPV 16
  - HPV 16, 18
- HPV: treated with interferon, imiquimod or sinecatechins
- Oncogenic HPV 16/18/31/33/45/52/58 as well as HPV 6 and 11 can be prevented with VLP vaccines
- Possible prevention of BCC/SCC with HPV vaccine

What's New in Therapy of HPV?

- Verruca Vulgaris and Verruca Plantaris
  - Imiquimod cream: Under occlusion overnight to each wart
  - Glycolic acid cream or lotion
  - Intralvesional candida: Inject 0.1-0.3 cc per session
  - Squaric acid or DCP: sensitize and then apply thin layer to lesions
- Genital HPV
  - Imiquimod 3.75% cream (Zyclara)
  - Sinecatechins Thin layer TID (16 wks)
- HPV Vaccine: Oct 2016
  - Recommended age of vaccination: ages 11 or 12
  - All boys and girls before first sexual contact and HPV exposure
  - 2 shots needed at months 0 and 6-12 months between ages 9 and 14
  - 3 shots needed at months 0, 2, and 6 after age 15+
  - 3 shots needed at months 0, 2, and 6 if immunosuppressed
- Gardasil-9 will soon be the only HPV vaccine available in the U.S