Oncogenic Viruses-Human Papillomaviruses

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Introduction

- There is a balance in cell growth, differentiation and cell turnover
- Malignancies arise as a result in defect gene(s) that regulate cell growth and/or differentiation
- Genetic defects may be caused by chemical/physical or biological agents (e.g. viruses)
  - Oncogenic viruses: responsible for 15% of cancers
  - Etiology is either direct (oncogenes) or indirect
- Involve common intracellular signaling pathways
  - Molecular targets for therapeutic development

Terminologies

- **Oncology**: study of tumors (cancers)
  - Greek word ὄγκος (ónkos), meaning "tumor", "volume" or "mass"
- ***Benign tumor***: growth produced by abnormally dividing cells which remain localized and does not invade adjacent tissue
- ***Malignant tumor***: is a cancerous growth that is locally invasive (invades adjacent tissue) and may also be ***metastatic*** i.e. is able to spread via blood and lymphatic circulation to other parts of the body.
- Malignant tumors are also called cancers
  - **Carcinomas**: tumors of epithelial cells
  - **Sarcomas**: tumors of mesenchymal cells
  - **Lymphomas**: tumors arising from leukocytes (solids) or **Leukemia** (if circulating cells are involved)
- **Oncogenesis/Tumorigenesis/Carcinogenesis**: the process of development of tumors
- **Cell transformation**: change in normal cell growth and differentiation to be come a cancer cell (indefinite cell division)

Viral etiologies of cancers

- Retroviruses
  - Adult T cell leukemia (HTLV-1)
- Hepatitis B virus
  - Hepatocellular carcinoma (liver cancer)
- Hepatitis C virus
  - Hepatocellular carcinoma (liver cancer)
- Herpesviruses
  - Epstein-Barr virus
    - Burkitt's lymphoma, B cell lymphomas, Nosalpharyngeal carcinoma
  - Human Kaposi's Sarcoma-associated Herpesvirus (HHV-8)
    - Kaposi's sarcoma
- Papillomaviruses
  - HPV 5,8 (squamous cell carcinoma)
  - HPV 16, 18 (genital carcinomas)
  - HPV types (6,11,16, 18) are associated with 70% of **cervical cancers** and 90% of genital warts

Oncogenes

- Genetic elements associated with cancer induction
  - Include cellular genes with no viral homologues or viral genes of oncogenic DNA viruses with no cellular homologues
    - Retroviruses have *v-onc genes* (corresponding normal genes are *c-onc genes or protooncogenes*)
- Tumor suppressor genes: regulate normal cells (negative regulation of growth)
  - Half of cancers are caused by alterations in tumor suppressor genes
- Proteins coded by oncogenes
  - Growth factors
  - Growth factor receptors
  - Intracellular signal transducers
  - Nuclear transcription factors

Oncogenesis by retroviruses (HTLV-1)

- Cause adult T cell leukemia

- *Tax* is involved in cell transformation
  - Codes for a transcription activator protein
  - Promotes transcription not only from LTR but from regulator sequences of cellular genes
  - Activated the IL-2 receptor gene (creates an autocrine loop promoting lymphocyte proliferation)
  - Also activates other cellular oncogens (*fos* and *PDGF*)

Oncogenesis by DNA viruses

- Polymaviruses (Papillomaviruses) and adenoviruses
- Cause productive and non-productive infection
  - DNA integration into genomic DNA (whole or partial)
  - Integrated DNA behalves like an oncogene
    - Alter patterns of gene expression and regulation of cell growth/differentiation
    - Note: integration does not always result in transformation and integration is not often necessary to cause transformation e.g. Herpesviral (Herpesviruses and EBV) DNA exists as episomal DNA (circular non-integrated plasmids)

Oncogenesis by DNA viruses

Virus	Oncogene	Function	Location
Adenoviruses	E1A	Regulates transcription	Nucleus
	E1B	?	Nucleus, membranes
Papillomaviruses	E5	Cell signaling	Nucleus, membrane
	E6	Transcription/replication	Nucleus, cytoplasm
	E7	Transcription/replication	Nucleus, cytoplasm

Oncogenesis by Hepadnaviruses

- Associated with hepatocellular carcinomas
- Have no known oncogenes
- HCV an RNA virus with no DNA intermediate has no evidence of integration
  - Virus may cause oncogenesis indirectly
  - Chronic infection of HCV and oncogenesis is aggravated by alcoholism, environmental carcinogens, mutagenic oxidants etc

Oncogenesis by Herpesviruses

- Cause malignant B cell lymphoma and Burkitt’s lymphoma
- EBV viral DNA is found in most cases of Burkitt’s lymphoma as episomes
- Burkitt's lymphoma cells have mutation is tumor suppressor gene *p53*
  - EBV infection arrests B cell differentiation and promotes division and chromosomal damage
  - Environmental co-factor e.g. *Plasmodium falciparum* infection impairs T cell function to combat immortal EBV-transformed B cells
  - Chromosomal translocation leads to activation of c-myc oncogene resulting in Burkitt’s lymphoma

Oncogenesis by Herpesviruses

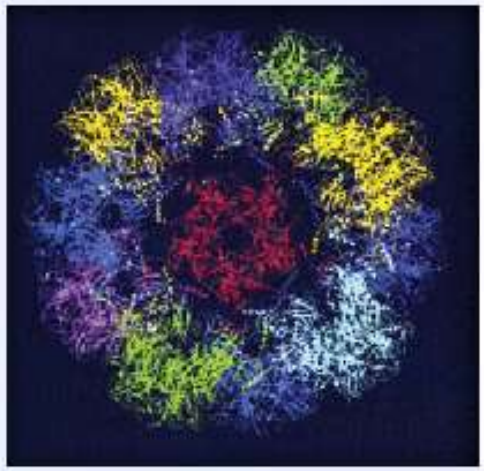
- B cell lymphomas in immunocompromized patients may follow primary EBV infection or reactivation
- Eg in AIDS patients or children with primary immunodeficiencies

Human cancer viruses

Virus family	virus	cancer
Papillomaviridae	Human papillomavirus	Genital/anal tumors, squamous cell carcinoma, oropharyngeal carcinoma, cervical cancer
Herpesviridae	Epstein-Barr virus (EBV)	Nasopharyngeal carcinoma, Burkitt lymphoma, Hodgkin's lymphoma, B cell lymphoma
	Human herpes virus 8	Kaposi sarcoma
Hepadnaviridae	Hepatitis B virus	Hepatocellular carcinoma
Flaviriridae	Hepatitis C virus	Hepatocellular carcinoma
Retroviridae	HTLV HIV (not direct)	Adult T cell leukemia AIDS related malignancies

Human Papillomaviruses

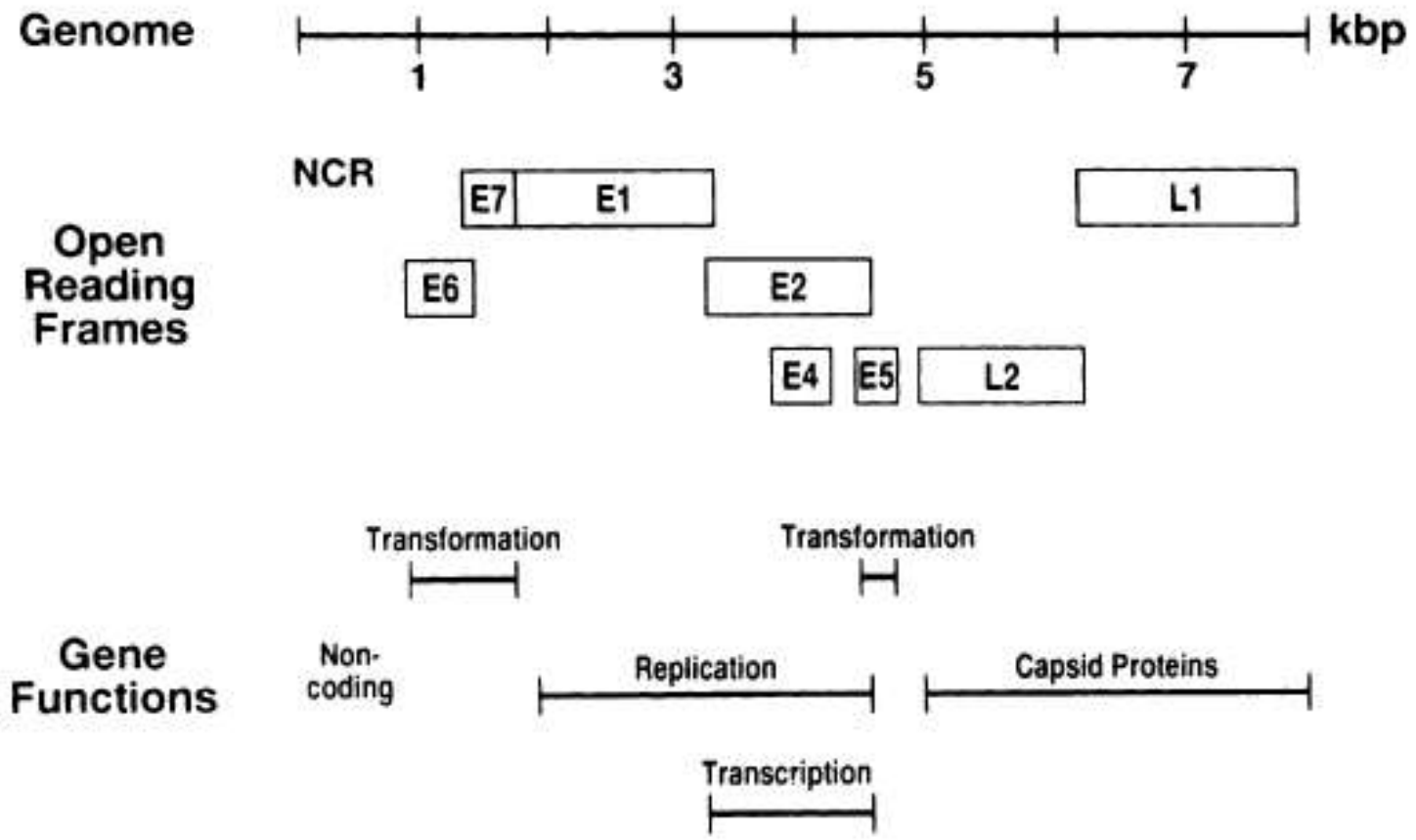
- Very large virus family divided into 16 genera
  - Highly tropic for epithelial cells of skin and mucous membranes
- Large viruses (55nm in diameter)
- Virion
  - Icosahedral 55nm in diameter
- Composition
  - DNA (10%), Protein (90%)



Virus-like Particles (VLPs) Assembled from the L1 Protein of Human Papillomavirus 16

- Genome
  - Double stranded DNA, circular, 8kb; replication in the nucleus
- Envelope: none

Genome


Genome
kbp
1
3
5
7
NCR
E7
E1
L1
Open Reading Frames
E6
E2
E4
E5
L2
Transformation
Transformation
Gene Functions
Non-coding
Replication
Capsid Proteins
Transcription

Genome

- Nine overlapping genes and a single control region (incorporating promoter regions and regulatory regions).
- The genes are distributed into early (E1–E7) and late regions (L1 and L2).
- Early genes encode proteins that are involved with regulation of viral DNA replication and transcription.
- Early genes also are involved with oncogenic transformation in high-risk HPV types.
- The L1 and L2 genes encode the major and minor capsid proteins, respectively.
- The papillomavirus E1 protein demonstrates ATPase and helicase activity: it functions both to promote viral replication and to inhibit viral

Epidemiology

- Cutaneous warts:
  - common in children,
  - contact transmission,
  - self-limiting and spontaneously regress.

Epidemiology

- Condyloma acuminata: (Genital warts)
  - transmitted sexually,
  - one of the most common viral sexually transmitted diseases
  - High-risk HPV types 16 and 18 are found in approximately 40% to 60% and 10% to 20% of all cervical carcinomas, respectively
  - Cervical cancer is the seventh most common cancer overall and third most common in women worldwide
  - HPV DNA is detectable by polymerase chain reaction in more than 99.7% of cervical cancers, making HPV infection the most important risk factor

Clinical Manifestations

- All HPV types target the squamous epithelial cells.
- Infection begins with viral entry through traumatized epithelium and then may follow one of three paths:
  - latent infection, in which there is no gross or microscopic evidence of disease;
  - subclinical infection, in which colposcopy or microscopy reveals evidence of infection in the absence of clinical disease;
  - and clinical disease.
- Most genital HPV infections are latent or subclinical.
- Clinical and histopathologic evidence of HPV infection usually develops 1 to 8 months after initial exposure.
- In the absence of transformation, HPV follows the normal life cycle.
- Physical manifestations of infection include epidermal thickening hyperplasia of the stratum spinosum, and some degree of hyperkeratosis.
- Untreated, these lesions may regress spontaneously, persist as benign lesions, or progress to precancerous lesions and eventually, cancer .

Classifications

- Classified according to genetic similarity with more than 100 HPV types have been identified.
- Also grouped according to their tissue tropism (dermatotropic or mucosotropic) or potential for malignant transformation (high risk and low risk).
- cause a wide range of disease processes, depending on HPV type.

Table 1
Manifestations of various HPV types

Manifestation	HPV types
Nongenital	
Plantar warts	1
Common warts	2, 4, 49
Flat warts	3, 10, 28, 49
Keratoacanthomas	37
Cutaneous squamous cell carcinomas	38, 41, 48
Oral lesions	13, 32, 57
Other nongenital lesions	6, 7, 11, 60, 63, 65, 78
Epidermodysplasia verruciformis (EV) (also found in immunocompromised individuals)	5, 8, 9, 12, 14, 15, 17, 19, 20–25, 36, 47, 50
Anogenital	
Condyloma acuminata	6, 11, 16, 18, 70
Anogenital dysplasias and neoplasias	16, 18, 26, 27, 30, 31, 33–35, 40, 42–45, 51–59, 61, 62, 64, 66–69, 71–74
Other anogenital lesions	6, 11, 16, 18, 33, 39
Immunocompromised hosts	72–77 (also types seen in EV)

Nongenital cutaneous manifestations

- Benign cutaneous lesions associated with HPV
- include common warts, plantar warts, and flat warts.
- also has been linked to keratoacanthomas, actinickeratoses, stucco keratoses, and seborrheic keratoses,
- associated with malignant skin tumors including cutaneous squamous cell carcinoma and possibly basal cell carcinoma.
- Associated with benign and malignant mucosal lesions of the oral cavity, respiratory tract, esophagus, and eyes.

Epidermodysplasia verruciformis

- A genetic disease characterized by HPV infection with multiple types not seen in otherwise healthy individuals.
- Susceptibility to these HPV types is believed to be secondary to a defect in cell mediated immunity.
- Manifests in childhood with lesions on the back, chest, and limbs.
- These lesions undergo oncogenesis in 25% to 63% of cases, especially on areas of the skin exposed to sunlight.

Genital manifestations

- The etiologic agent for condyloma acuminata (genital warts).
- usually are found on the penis and around the anus in men and the vulva, vaginal introitus, perineal area, perianal area, and cervix in women.
- associated with premalignant and malignant genital lesions.
- Anogenital HPV lesions may regress spontaneously or may progress to carcinoma.
- Oncogenic progression most often is associated with high-risk types 16 and 18.
- Malignancies strongly associated with HPV include squamous cell carcinomas of the anal canal, vulva, and penis.
- Papillomaviruses are also responsible for cervical intraepithelial neoplasias and their progression to cervical cancer.

Condyloma acuminata: (Genital warts)

Immunocompromised patients

- less able to effectively mount immune responses
- infected with HPV types not seen in immunocompetent
- cause more severe disease and rapid disease progression
- more frequently leads to oncogenic transformation
- treatment is often less efficacious in immunocompromised

Oncogenic Transformation

- Viral infection and genetic instability:
  - HPV-infected cells often show aberrant mitotic figures.
  - The HPV E7 oncoprotein induces centrosome duplication errors .
  - HPV X protein causes the formation of abnormal centrioles, followed by multipolar mitosis and chromosomal imbalances
  - HPV X protein was found to interact with XAP-1/UVDDB, a putative DNA-repair protein that binds damaged DNA during nucleotide excision repair (NER).
  - HPV E6 protein downregulates the enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which participates in DNA repair and prevents mutation in many critical genes

DNA viruses in human cancer: An integrated overview on fundamental mechanisms of viral carcinogenesis

Deilson Elgui de Oliveira *

Oncogenic Transformation

- Cell immortalization by DNA virus:
  - *TERT* expression was evident in high grade CIN and invasive cervical carcinomas
  - Degradation of p53 by HPV E6 protein is a key event for immortalization of keratinocytes in the presence of HPV E7
- DNA virus-induced malignant transformation:
  - E6 proteins cause proteasomal degradation of p53
  - HPV E7 proteins target the retinoblastoma gene (RB) product (pRb)
- Viral subversion of immunologic surveillance:
  - HPV E5 protein impairs intracellular maturation of MHC class II dimers in keratinocytes *in vitro*.
  - HPV downregulates IFN-signaling pathway, including the Stat1.
  - HPV E6 and E7 proteins also modulate the transactivation of interferons by binding interferon regulatory factors 3 and 1 (IRF-3 and IRF-1)

Detection

- Pap Smear: Conventional and Liquid-based
  - 20% false-negative Pap smears occur with conventional Pap
  - 6% increase in sensitivity of detection of L-SIL or higher and a reduction in the number of unsatisfactory samples with Liquid-based technology
- DNA hybrid capture: Digene
- Colposcopy: Invasive

Diagnosis of Human Papillomaviruses

- HPV does not grow in routine tissue culture
  - Antibody tests are rarely used
- HPV infection leads to perinuclear cytoplasmic vacuolation and nuclear enlargement in epithelial cells
  - These changes can be seen in Papanicolaou smears
  - Abnormal cytology due to HPV infection should prompt colposcopy/biopsy
- Immunoassays
  - To detect viral antigen
  - In situ hybridization
  - PCR to detect viral DNA in vaginal swabs/tissue is more sensitive

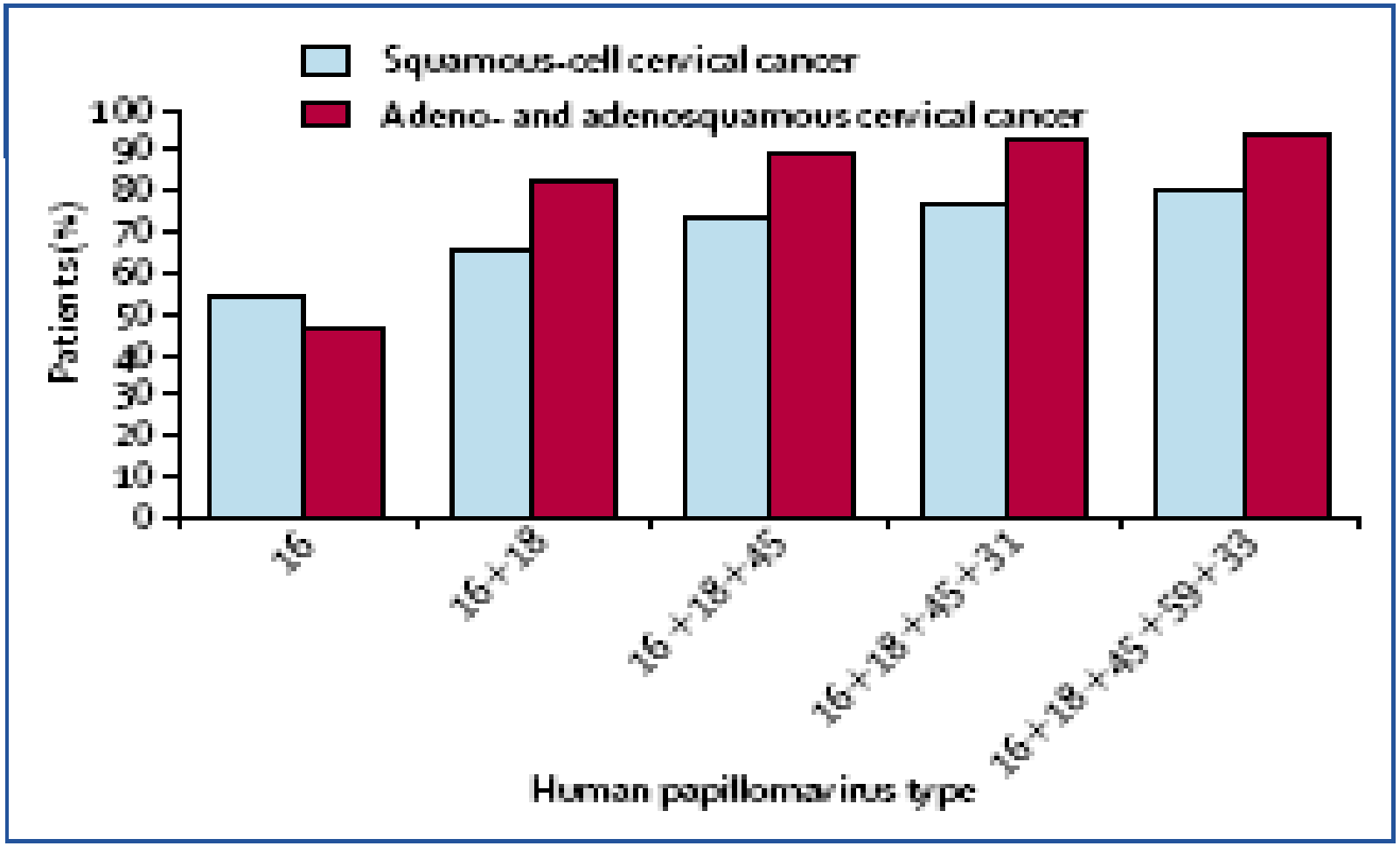
Treatment

- Cytotoxic (Antiviral therapy / Antimetabolite therapy/ Immunomodulation) or surgical/cytodestructive
- Topical cytotoxins
  - Polophyllin
  - Polophyllotoxin
  - 5-fluorouracil
  - Trichloroacetic acid
  - Cidofovir
  - Imiquimod
- Systemic and local interferon therapy
- Electrocautery
  - Recurrences are common following cessation of treatment
    - Survival of virus in the basal layers of the epithelium

Vaccines

- Cost effective way to prevent disease

- HPV vaccines comprise virus like particles generated by recombinant expression of the major capsid protein, L1, in yeast
  - Forms an empty capsid
    - Immunogenetic
      - Produce high titers of antibodies and cell mediated mediators
      - Immunity is type specific



Cumulative prevalence of HPV types in cervical cancer

Quadrivalent HPV Vaccine

- HPV types 6,11,16,18
- Aluminium-adjuvant vaccine
  - Young women negative for HPV
  - Different antibody titers to L1
- Vaccine prevents disease, not infection
- No vaccinee had Cervical intraepithelial neoplasia (CIN)
- In placebo, 7 had CIN and 4 had external genital warts

Table 1: Characteristics of the HPV vaccines Gardasil and Cervarix

Characteristic	Gardasil	Cervarix
Manufacturer	Merck Frosst Canada Ltd.	GlaxoSmithKline Inc.
Type	Prophylactic vaccine consisting of virus-like particles containing L1 capsid proteins	Prophylactic vaccine consisting of virus-like particles containing L1 capsid proteins
Antigens	Quadrivalent vaccine: HPV types 6 (20 µg/dose), 11 (40 µg/dose), 16 (40 µg/dose) and 18 (20 µg/dose)	Bivalent vaccine: HPV types 16 (20 µg/dose) and 18 (20 µg/dose)
Antigen expression system	Yeast	Baculovirus
Adjuvant	Alum: 225 µg aluminum hydroxyphosphate sulfate	AS04: 500 µg aluminum hydroxide and 50 µg 3-deacylated monophosphoryl lipid A
Dose and schedule	0.5 mL intramuscular injection at 0, 2 and 6 months	0.5 mL intramuscular injection at 0, 1 and 6 months
Availability in Canada	Approved for sale	Not yet available

Harald zur Hausen (born 11 March 1936) is a German virologist and professor emeritus. He has done research on cancer of the cervix, where he discovered the role of papilloma viruses, for which he received the Nobel Prize in Physiology or Medicine 2008.

Table 2: Immune response and disease outcomes* for the HPV vaccines Gardasil and Ceravix

Variable	Gardasil	Cervarix
Published trial results	• Phase 2 and 3 trials[21-27]	• Phase 2 and 3 trials[28-31]
Sample size	• 20 583[27]	• 18 644[31]
Maximum duration of follow-up	• 5 years (phase 2)[23] • 3 years (phase 3)[25]	• 5.5 years (phase 2)[30] • 1.25 (phase 3)[31]
Immune response 1 month after completion of vaccine series	• Over 99% seroconversion • Antibody titres 11 times (HPV type 6), 7 times (HPV type 11), 105 times (HPV type 16) and 19 times (HPV type 18) higher than titres following natural HPV infection[22]	• Over 99% seroconversion • Antibody titres 107 times (HPV type 16) and 82 times (HPV type 18) higher than titres following natural infection[28]
Duration of immune response	• 96% seropositive to HPV types 6, 11 and 16 at 24 months • 68% seropositive to HPV type 18 at 24 months[25]	• 100% seropositive to HPV types 16 and 18 at 51-53 months[29]
Persistent infection† from HPV types 16 and 18	• Vaccine efficacy 93.5% (95% CI 83%-98%)[23]	• Vaccine efficacy 80.4% (95% CI 70%-87%)[31]
Cervical intraepithelial neoplasia (grade 2 or higher) related to HPV types 16 and 18	• Vaccine efficacy 98% (95% CI 93%-100%)[27]	• Vaccine efficacy 90.4% (95% CI 53%-99%)[31]
Vaginal and vulvar intraepithelial neoplasia (grade 2 or higher) related to HPV types 16 and 18	• Vaccine efficacy 97% (95% CI 79%-100%)[26]	• No data
Persistent infection† of HPV type 45	• No data	• Vaccine efficacy 59.9% (95% CI 3%-85%)[31]
Persistent infection† of HPV type 31	• No data	• Vaccine efficacy 36.1% (95% CI 0.5%-60%)[31]
Protection from genital warts	• Vaccine efficacy 96% (95% CI 86%-99%)[24]	• No data

Note: CI = confidence interval.
*All disease outcomes are derived from intention-to-treat analyses for populations that were seronegative and polymerase chain reaction negative at enrolment for all HPV genotypes covered by the vaccine and had received at least 1 dose of the vaccine.
†Persistent infection was defined as 4 months in the Gardasil trial and as 6 months in the Cervarix trial.

Box 2: Knowledge gaps

- Will HPV vaccines affect cervical cancer incidence and mortality?
- Is the priming vaccine series sufficient or will a booster dose be required?
- Will exposure to wild-type HPV contribute to natural boosting?
- Will other HPV genotypes fill the niche previously filled by HPV types 16 and 18?
- How will the vaccination program affect current cytology screening programs?
- Will current cytology screening programs need to be adapted to identify vaccine failures?
- Are there rare but serious adverse effects of vaccination that have not yet been detected?

Therapeutic Vaccines

- For high risk HPV types are undergoing clinical trials
  - Immune response: cell mediated factors
  - Focus: HPV E6 and E7 proteins
    - Ubiquitously expressed in cervical cancers
  - BCG + HPV E7 caused tumor regression in mice

Prevention

- Primary: Education about HPV to Vaccines
  - Safe sex practices
- Secondary: Pap smear screening

HPV Vaccine

- The quadrivalent vaccine, Gardasil®, protects against four HPV types (6,11,16, 18), which are responsible for 70% of cervical cancers and 90% of genital warts
- Licensed by FDA on June 8, 2006
- ACIP voted to recommend use of this vaccine in females, ages 9-26 years
- The vaccine is made from non-infectious HPV-like particles (VLP)

HPV Vaccine

- HPV infections are largely shielded from the host immune response because they are restricted to the epithelium

- The best characterized and most type-specific antibodies are those directed against conformational epitopes of the L1 capsid protein assembled as VLPs

HPV Vaccine

- The L1 major capsid protein of HPV is the antigen used for HPV vaccination (*105*).

- Using recombinant DNA technology, the L1 protein is expressed in *Saccharomyces cerevisiae* (yeast), and the proteins self-assemble into conformationally intact, noninfectious VLPs. Each 0.5-mL dose contains 20 *µ*g HPV 6 L1 protein, 40 *µ*g HPV 11 L1 protein, 40 *µ*g HPV 16 L1 protein, and 20 *µ*g HPV 18 L1 protein. VLPs are adsorbed on an aluminum-containing adjuvant.

- Each 0.5-mL dose contains 225 *µ*g amorphous aluminum hydroxyphosphate sulfate. The formulation also includes sodium chloride, L-histidine, polysorbate 80, sodium borate, and water for injection.

- The quadrivalent HPV vaccine contains no thimerosal or antibiotics.

HPV Vaccine

- The vaccine has a 100% efficacy in preventing cervical precancers caused by the targeted HPV types, and nearly 100% efficacy in preventing vulvar and vaginal precancers and genital warts caused by the targeted HPV types

- However, the vaccine has no therapeutic effect on HPV-related disease

HPV Vaccine

- The vaccine should be delivered through a series of three intra-muscular injections over a six-month period. The second and third doses should be given 2 and 6 months after the first dose
- The private sector list price of the vaccine is $119.75 per dose (about $360 for full series)

HPV Vaccine

- A bivalent HPV vaccine is in the final stages of clinical testing in females.
- This vaccine would protect against the two types of HPV (16,18) that cause 70% of cervical cancers

End

Questions