Literature review on HPV 6, 11, 16 and 18: Disease and Vaccine Characteristics

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I. Acronyms & Glossary

ACIP	The Advisory Committee on Immunization Practices
ADC	Adenocarcinoma
AGC	Atypical glandular cells
AIS	Adenocarcinoma *in situ*
ASC-H	Atypical squamous cells, cannot exclude high grade
ASCUS	Atypical squamous cells of undetermined significance
ASO4	Adjuvant containing 500 μg aluminum hydroxide and 50 μg 3-deacylated monophosphoryl lipid A
Baculoviruses	Viruses that attack insects and other arthropods
Capsomeres	Protein units that make up the viral capsid or outer shell
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia; this is subclassified into three categories (CIN 1, CIN 2, and CIN 3)
CIS	Carcinoma in situ
cLIA	competitive Luminex based Immunoassay
cRIA	competitive Radio Immuno Assay
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
Epitope	a molecular region on the surface of an antigen capable of eliciting an immune response and of combining with the specific antibody produced by such a response (also known as determinant or antigenic determinant)
FUTURE	Females United to Unilaterally Reduce Endo/Ectocervical Disease
GMT	Geometric mean titres
GSK	GlaxoSmithKline
HR	High-risk (carcinogenic) HPV viruses: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
HSIL	High grade squamous intraepithelial lesion (CIN 2,3)
HPV	Human papillomavirus
IARC	International Agency for Research on Cancer
Icosahedral	A structure with twenty sides
IL	Interleukin
IM	Intramuscular
INF	Interferon
ITT	Intention-to-treat
LR	Low-risk (non-carcinogenic) HPV viruses: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
LSIL	Low-grade squamous intraepithelial lesion (CIN 1)
MITT	Modified intention-to-treat
MPL	Monophosphoryl lipid A
NA	Not available
NACI	The National Advisory Committee on Immunization
NOCD	New onset chronic disease
OCP	Oral contraceptive pills
OR	Odds ratio
PATRICIA	PApilloma TRIal against Cancer in young Adults
Pentamer	A molecule composed of five monomeric units

Prophylactic vaccine	These vaccines are designed to protect an individual against an initial infection; thus the vaccines are given pre-exposure
Pseudovirion	A synthetic virus that is similar in structure and characteristics to a natural virus except that it lacks the capacity to replicate
PHAC	The Public Health Agency of Canada
RCT	Randomized, placebo-controlled trial
RRP	Recurrent respiratory papillomas
Saccharomyces cerevisiae	Baker's or brewer's yeast, also known as the 'top' fermentor
SCC	Squamous cell carcinoma
Therapeutic vaccine	These vaccines are designed to treat an existing infection thus are given post exposure
trichnoplusia ni	Commonly called the cabbage looper, a pest of plants such as cabbage, broccoli, cauliflower, Chinese cabbage
VaIN	Vaginal intraepithelial neoplasia
VIN	Vulvar intraepithelial neoplasia
VLP	Virus like particles

II. Introduction

In 2006, the first of two vaccines for human papillomavirus (HPV) prevention, Gardasil™, was approved for use in Canada. Gardasil™, produced by Merck Frosst Canada Ltd, is a quadrivalent vaccine product against HPV 6, 11, 16 and 18. The National Advisory Committee on Immunization (NACI) statement on the recommendations for the use of HPV vaccine for the prevention of cervical cancer was released in January 2007[1]. A second HPV candidate vaccine, Cervarix™, produced by GlaxoSmithKline (GSK) Inc in Belgium is currently undergoing the regulatory review process by Health Canada. This is a bivalent vaccine against HPV 16 and 18. A need for a systematic literature review on the characteristics of the HPV vaccines was identified. This review was commissioned by the Public Health Agency of Canada (PHAC).

The purpose of this document is to apply the Erickson, De Wals and Farand framework[2] on immunization programs to review the following:

- the disease burden attributed to HPV 6, 11, 16, and 18; and to identify non-HPV risk factors for cervical cancer; and
- the vaccine characteristics of Gardasil™ and Cervarix™.

III. Methods

This report is based on literature retrieved from peer-reviewed publications and Gardasil™ product monograph. In addition, data published in conference abstracts have been used to supplement trial results. Sections 1.4 and 2 of the Erickson, De Wals and Farand framework were used to frame the components of this document[2]. This report will be divided into two sections, the burden of disease caused by HPV 6, 11, 16 and 18 and a review of the HPV vaccines, Gardasil™ and Cervarix™.

Literature search

Section 1: Burden of disease caused by HPV 6, 11, 16 and 18

Information on disease attributed to HPV infection and non-HPV risk factors for cervical cancer was collated from the following sources: the supplement of the journal Vaccine, entitled 'HPV Vaccines and Screening in the Prevention of Cervical Cancer', a literature

search by the Public Health Agency of Canada, the NACI statement on Human Papillomavirus Vaccine, and the reference lists of articles from these sources[1, 3].

Section 2: HPV vaccines, Gardasil™ and Cervarix™
Trial data for Gardasil™, Cervarix™ and components included in the current vaccines are presented. Examples of the latter include information from the Merck monovalent HPV 16 trial as it contributes to data on duration of seropositivity and efficacy of the HPV 16 VLP component of the vaccine authorized for sale. Data on ASO4, a new adjuvant included in the vaccine Cervarix™, are also included.

Literature was identified using a variety of search strategies: systematic search of electronic medical databases (Medline and EMBASE), search of reference lists in relevant articles, pharmaceutical company data[4] and direct contact with a few authors and the two pharmaceutical companies to obtain the documents in press or data presented at peer-reviewed conferences.

Employment of search terms 'Gardasil' and 'Cervarix' in Medline for the search period 1966 to date of search (Nov 29, 2006) yielded only four articles. The same search terms in EMBASE for the period 1974 to present (Nov 29, 2006) yielded 83 articles. The search was narrowed to articles in English which yielded 63 articles. All 67 references were reviewed; articles were excluded if abstracts and/or title indicated program implementation issues or if the authors were anonymous. A total of 23 articles were retained for full text review. This search strategy yielded review articles, commentaries and a primary research article on the thermal stability of Gardasil™. However no randomized control trials were identified.

The systematic literature review identified a single topic supplement of the journal Vaccine, entitled 'HPV Vaccines and Screening in the Prevention of Cervical Cancer'[3]. Additional papers from this supplement (not detected by the systematic literature review) were incorporated in this document. The reference lists of chapters 11-13 and 28 from the same supplement[3] yielded twelve articles detailing the phase II-III trials of the two vaccines. Numerous other articles were identified from reference lists of these and secondary articles that provided insights into immunology following natural infection and vaccination, and were incorporated in the relevant sections of the literature review. Four primary research articles were published following the systematic Medline/EMBASE searches and reviewed for this paper[5-8].

The Gardasil™ product monograph was available for review. The product monograph for Cervarix™ was requested however, this document was unavailable as the vaccine is currently undergoing the regulatory review process by Health Canada. GSK provided two publications on their proprietary adjuvant, and three conference abstracts on 5.5 year phase II trial data and unpublished immunogenicity bridging data for the young and the older populations.

IV. Results

IV. 1. Burden of disease caused by HPV 6, 11, 16 and 18

1. Disease agent
The family of human papilloma viruses consists of over 100 different double-stranded DNA viruses. Of these, approximately 40 can infect the genital tract. The genetic material of the virus is enclosed in an icosahedral protein shell. The outer viral shell contains capsomers of L1, the major capsid protein and L2, a minor internal capsid protein. Type-specific viral

epitopes are located on the external loops of the L1 protein. While there are some shared epitopes among the HPV types, the immunodominant neutralizing antibodies are to type-specific epitopes[9] .

2. Risk of disease from HPV infection

HPV is one of the most common sexually transmitted infections, with highest prevalence in persons under the age of 25 years. Risk factors for HPV infection include behavioural factors that increase probability of exposure to HPV (eg, number of sexual partners, early age of first intercourse, never being married, never being pregnant), endogenous factors (immunosupression secondary to HIV infection or post-transplant, etc), and factors that relate to the cervical microenvironment (sexually transmitted infections)[1]. Receptive anal intercourse is documented to be a risk factor among men who have sex with men in at least one cohort study, with the risk proportionally related to the number of partners. Risk of external genital warts associated with the number of receptive anal intercourse partners (adjusted for age, condom use, tobacco smoking, HIV, and other covariates) compared with individuals not sexually active was 1.1 for 1 partner, 1.5 for 2-5 partners and 5.1 for $\geq$ 6 partners[10].

There is strong epidemiologic evidence to indicate that infection with certain high risk (HR) HPV genotypes are a necessary, but not sufficient, cause of cervical cancer. It can take anywhere from 1 to 10 years post persistent infection for the development of precancerous lesions, and then another ten years for the development of invasive cervical cancer. Thus, there is a long lag period from infection to invasive disease[11].

Case control studies conducted in eleven countries under the auspices of the International Agency for Research on Cancer (IARC) demonstrated consistent and high odds ratios for the risk of cervical cancer associated with persistent HPV infection[12]. The overall odds ratios (OR) for risk of squamous cell carcinoma (SCC) of the cervix was 90.0 (95% CI, 71.3-113.5) and for adenocarcinoma (ADC) of the cervix was 81.3 (95% CI, 42.0-157.1)[12]. HPV 16, 18 were associated with 70% of SCC and 86% of ADC[12]. The odds of cervical cancer with HPV 16 infection is 281.9 (95% CI, 196.3-404.8) and with HPV 18 is 222.5 (95% CI, 130.8-378.4)[12]. Six HPV genotypes account for an additional 20% of cervical cancers worldwide, and these are types 31, 33, 35, 45, 52 and 58[13].

Table 1 demonstrates that HPV infection is associated with many anogenital cancers and non-anogenital cancers. Cancers of the head and neck include tonsillar and conjunctival cancers for which data have not been presented[14].

Table 1: HPV positivity (all genotypes, 16, and 18) for selected cancers and precancerous lesions worldwide[12, 13, 15]

Section 1: Anogenital cancers			
Cancer	HPV positive (%)	HPV 16 positive/all HPV positive (%)	HPV18 positive/all HPV positive (%)
Cervical cancer	94-99	54.6	15.8
HSIL		41-67	
LSIL		16-32	
ASCUS		6-27	
Vaginal cancers	64-91		
VAIN-3	82-100		
Anal cancers	88-94	92	

Vulvar and Penile cancer - basaloid and warty tumors - keratinizing SCC	60-90 < 10		
Section 2: Head and neck squamous cell carcinomas (HNSCC)			
	HPV positive (%)	HPV16 /all HPV positive (%)	HPV 18/all HPV positive (%)
HNSCC	25.9		
Oropharyngeal SCC	35.6 (range 11-100)	86.7	2.8
Oral SCC	23.5 (range 4-80)	68.2	34.1
Laryngeal SCC	24.0 (range 0-100)	69.2	17

The main limitation in studies presented in table 1 is that case ascertainment of HPV infection in the cancer specimens has not been standardized. HPV genotype testing among studies is variable and has usually only been assessed for a few HPV types and in fixed tissue.

HPV infection is felt to be a necessary cause of cervical cancer however it may not be a sufficient cause. Cofactors identified to increase the risk of cervical cancer are presented in table 2.

Table 2: Other risk factors associated with cervical cancer[12]

Agent	**Magnitude of Risk (95% CI)**
Tobacco smoking[12]	RR, 1.60 (1.48-1.73)
High parity[12]	RR, 1.10 for each pregnancy (1.08-1.12)
Decreasing age at first full term pregnancy[12]	RR, 1.07 (1.06-1.09 per year decrease)
Long term oral contraceptive use[12, 16, 17]	RR, 1.6 (1.4-1.7) for 5-9 years of use; 2.2 (1.9-2.4) for 10 or more years
HSV-2 infection[12]	OR, 2.2 (1.4-3.4) for SCC; 3.4 (1.5-7.7) for ADC
Chlamydia trachomatis infection[12]	OR, 1.8 (1.2-2.7)

Risk of cervical cancer is higher in current smokers (RR 1.6; 95%CI 1.48-1.73) than past smokers (RR 1.12; 95%CI 1.01-1.25). In current smokers the risk increases with the number of cigarettes smoked per day.

Hormonal factors have been identified as cofactors. These include the number of full-term pregnancies and long term use of combined estrogen-progesterone oral contraceptives (OCPs). OCPs are recognized to protect women from endometrial and ovarian cancers, however, they have been associated with an increased risk of cervical cancer, liver cancer and breast cancer. It is important to note that the IARC working group on oral contraceptives recognized that the 'net public health outcome [from OCP use] could be beneficial but difficult to quantify[16].

Sexually transmitted infections, such as *Chlamydia trachomatis*, herpes simplex virus and HIV are also identified to increase the risk of precancerous and cancerous cervical lesions. The local mechanisms of action responsible for the increased risk of HPV associated pathology are felt to be an inflammatory response and the generation of free radicals in HSV infection, and a decrease in cell mediated immunity for HIV infection[12]. Other cofactors may include nutritional elements such as antioxidants. There is evidence from observational studies in

support of an inverse relationship between serum levels of β-carotene, lycopene and tocopherol and CIN and invasive cervical cancer[18].

3. Disease associated with low-risk HPV 6, 11 viruses

HPV 6 and 11 account for over 90% of genital warts. Rare complications of HPV 6 and 11 infection include anogenital carcinoma (2.5-5%), Buschke-Lowenstein tumours (verrucous tumors of the vulva and cervix), and recurrent respiratory papillomatosis (RRP)[19].

RRP is a rare condition characterized by airway papillomas in children and adults. The morbidity associated with interventions to maintain an open airway, including chronic inflammation and injury to the vocal cords, can be quite substantial. The juvenile form of the disease is felt to occur from infection acquired *in utero* or during vaginal delivery. The likelihood of transmission of RRP is 200-400 times higher in women with genital warts at the time of pregnancy or delivery compared to women without apparent HPV infection. Adult onset disease may be due to reactivation of a latent infection acquired *in utero* or during vaginal delivery or more recent acquisition of HPV 6, or 11 infection. RRP can rarely transform to a malignant disease[19].

IV. 2. HPV vaccines, Gardasil™ and Cervarix™

1. Nature and characteristics of immunizing agent

The current HPV vaccines consist of L1 proteins of HPV manufactured with the use of recombinant technology. The L1 proteins self assemble into empty non infectious virus-like particles (VLPs).

Gardasil™ is a quadrivalent HPV 6, 11, 16, 18 L1 VLP vaccine developed by Merck and Co. This vaccine targets two high-risk oncogenic HPVs that are associated with approximately 70% of cervical cancers and two low-risk HPVs that are associated with over 90% of genital warts.

Cervarix™ is a bivalent HPV 16, 18 L1 VLP candidate vaccine developed by MedImmune in partnership with GlaxoSmithKline Biologicals. This vaccine targets the two high-risk HPV genotypes.

Both vaccines are prophylactic vaccines and produce a virus neutralization antibody response; thus they are indicated for prevention of infection from respective HPV genotypes and their associated diseases. They are not therapeutic vaccines and are not effective in disease modification once the HPV infection has been acquired[20].

2. Characteristics of the commercial products and administration schedule

Gardasil™

The L1 proteins in Gardasil™ are expressed using a single recombinant yeast producer cell, *Saccharomyces cerevisiae* CANADE 3C-5 (Strain 1895). Gardasil™ contains purified L1 VLPs of HPV 6, 11, 16, 18 at 20/40/40/20 μg per dose formulation along with 225 μg of aluminum (amorphous aluminum hydroxyphosphate sulfate) adjuvant. Inactive ingredients include 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 μg of polysorbate 80, 35 μg of sodium borate, and water. The vaccine product does not contain any preservative or antibiotics. Gardasil™ is approved for use in females 9-26 years of age. The vaccine is supplied in single dose vials or prefilled single use syringes containing 0.5 mL of the vaccine product. It is recommended to be stored at +2 to+ 8 ºC[4].

Thermal stability of Gardasil™ has been evaluated and the vaccine is found to be extremely stable at temperatures up to 25 ºC for a period of 130 months or longer. The half-life of Gardasil™ at 37 ºC is predicted to be 18 months and at 42 ºC approximately three months. If the vaccine is exposed to a rapid rise in temperature to 50 ºC or higher, it undergoes a quick denaturation process resulting in degradation of the vaccine protein[21].

Gardasil™ is recommended to be administered using a three dose schedule at 0, 2 and 6 months. The standard dose of 0.5 mL should be administered by intramuscular (IM) injection in the deltoid region of the upper arm or the anterolateral upper thigh area. The minimum interval between dose one and two is 1 month and between dose two and three is 3 months. Manufacturer recommendations are to provide all three doses within a one year period. Gardasil™ can be administered at the same time as the hepatitis B vaccine[4].

Gardasil™ is not recommended for pregnant women, but may be administered to lactating women.

Cervarix™

The L1 proteins in Cervarix™ are expressed using a novel baculovirus expression system using a *Trichnoplusia ni* insect producer cell line. Vaccine contains purified VLPs of HPV 16 and 18 at 20/20 μg per dose. Cervarix™ also contains a novel proprietary adjuvant, ASO4 comprised of 500 μg of aluminum hydroxide and 50 μg of 3-deacylated monophosphoryl lipid A (MPL). MPL is a deactivated derivate of lipopolysaccharide, a component of bacterial cell walls[9, 22, 23]. ASO4 has been used in other new vaccines manufactured by GSK (eg, hepatitis B and candidate HSV vaccine) that are undergoing the regulatory review processes. It has also undergone extensive safety testing for organ damage, as well as reproductive and genotoxicity testing in animal models[24, 25]. The use of the novel technology (expression system and adjuvant) has created additional characterization and process development work to satisfy the regulatory requirements for this product. The baculovirus expression system is felt to enhance antigen yield, while the new adjuvant produces a balanced immune response by stimulating cell mediated immunity, in addition to the classical humoral immunity[25, 26].

Cervarix™ is not yet approved for use in Canada. It has been administered in the RCTs as a 0.5m L IM dose, using a 0, 1 and 6 month schedule[22].

3. Vaccine manufacturers, production capacity and supply to Canada

Gardasil™ is manufactured by Merck and Company, Inc in West Point, Pennsylvania. The product is packaged in either the United States or Belgium. Vaccine lots intended for Canada need to be lot tested by Health Canada prior to appropriate packaging and distribution to the Canadian market.

Cervarix™ is manufactured by GSK Biologicals in Rixensart, Belgium. The current production technology employed by GSK is amenable to scale up for large vaccine orders. The company does require a six month lead time in order to boost production and secure supply for large scale public health immunization programs.

4. Nature and characteristics of immune response

4.1 General overview

Immune response following natural infection

HPV is a relative immune evader. HPV infection fails to induce an inflammatory response as the virus takes advantage of the pre-programmed cell death of the keratinocytes. Thus,

HPV replication and release is not seen as a danger signal by immune system. HPV also down regulates the interferon (INF) -α-inducible gene expression. INF-α is a bridge between innate and adaptive immunity and thus the viral mediated downregulation interferes with the prompt stimulation of the adaptive immune system[26].

Infection is ultimately followed by a largely Th-1 based cell mediated immune response that activates within 2-3 months of infection. This response is detected at distinct and narrow time points within the HPV infection cycle characterized by viral DNA amplification and period of lesion regression. Low level neutralizing antibodies are detected in the serum coincident with lesion regression. Most HPV infections resolve spontaneously within 5-6 months for low-risk HPV types and 8-14 months for high-risk HPV types. A persistent infection is established if the immune response fails to clear or control the infection[26].

The role of a competent cell mediated immune system in preventing and controlling HPV infections is underscored by HPV incidence and disease in individuals with HIV. Such individuals are at an increased risk of HPV infection, longer persistence of HPV infection, and greater predilection to disease progression for both low-risk genotypes (genital warts) and high-risk genotypes (recurrences of CIN and progression to HSIL and cervical cancer).

The percent of individuals seroconverting and the time period to seroconversion vary by genotype and may be determined by initial high viral load or the nature of infection (transient or persistent infection). In two cohort studies of young college women, 40-60% of women seroconverted following HPV 16 infection and the median time for antibody response varied from 8-12 months. Duration of antibody response also varied and may be determined by initial antigenic stimulus, viral load and ongoing antigenic stimulus (repeat infections by the same or similar genotypes)[27, 28].

In at least one cohort study, presence of naturally induced high-level antibody titre to HPV 16 offered some protection from re-infection by the same and similar genotypes (HPV 31, 33, 35, 52 or 58; RR=0.33 (95% CI, 0.14-0.79, p=0.012)) though the duration of this protection remains unclear[27]. There is abundant animal data to demonstrate that a naturally induced low titre of HPV antibodies confer lifetime protection from viral challenge. Experiments with passive transfer of serum from a naturally infected animal also yielded protection from infection in serum recipients[26, 29]. Despite this evidence, the neutralizing effect of naturally induced antibodies remains incompletely characterized and is likely not as simple as may be implied. HPV infection induces a range of antibodies against several antigens. Some of these antibodies are preferentially found in patients with cervical cancer and are thus a marker for oncogenic antigens (eg, antigens located on E2 proteins) rather than a marker for immunity[30, 31].

Immune response following vaccination

In experimental studies, HPV VLPs alone have been shown to induce innate immunity[26]. ASO4, the adjuvant in Cervarix™, is an additional stimulant of the innate system. This leads to stimulation of antigen presenting cells and secretion of cytokines, Tumor necrosis factor α and interleukin (IL) 2, thus resulting in a stronger adaptive immune response to the vaccine antigens[29].

Humoral immunity

Antibodies are the primary mode of protection from HPV infection. HPV VLPs are highly immunogenic even in the absence of adjuvants[32]. The immunodominant neutralizing antibodies are directed against type-specific viral epitopes located on the external loops of the L1 protein with the exception of a common neutralizing epitope for HPV18 and 45,

HPV 31 and 33 and HPV 6 and 11[9]. In the clinical trials, both vaccines induce a robust humoral response with antibody levels that are sustained at or above those induced by natural infection. There is only one study comparing the addition of AS04 to HPV VLP antigens (baculovirus expression vector) with classic alum adjuvant[29]. At seven months, the ASO4 arm produced a superior neutralizing antibody response (as measured by the pseudoneutralization assay) with higher levels of memory B cells than HPV vaccine with alum. The higher neutralizing antibody titres to HPV 16 and HPV 18 in the ASO4 arm were maintained to 48 months and 24 months respectively.

Cell mediated immunity

Proliferation of peripheral blood mononuclear cells and cytokine production (INF-γ and IL-5) have been demonstrated in *in vitro* assays involving volunteers vaccinated with HPV 11 vaccine (baculovirus expression vector, alum adjuvant). T cell response was not restricted to the vaccine antigen (HPV 11) but was seen also in response to VLPs of other HPV types (specifically HPV 6 and 16). This suggests conservation of T cell epitopes across distinct genotypes[33]. While this trial examined the MedImmune product (later GSK) with an alum adjuvant, the results would likely be similar for a Merck vaccine. GSK indicates that AS04, the adjuvant in Cervarix™, may produce a more balanced immune response, with stimulation of both humoral and cell mediated immunity. The latter response is favoured towards Th1 stimulation[29].

4.2 Immunoassays to measure anti-HPV antibodies

International standards for harmonizing HPV serology and DNA assays have not yet been developed[34]. Both manufacturers have developed their own assays to measure antibody titres, thus, valid inter-study comparison of antibody levels or intra-study comparisons of antibody response to various HPV VLPs is not possible. In addition, antibody levels correlating with immune protection to HPV are currently undefined. The manufacturers have defined a serostatus cutoff level for their assays that reliably distinguishes between a panel of positive blood samples (i.e., from HPV PCR positive individuals) and negative samples (i.e., from HPV PCR negative individuals and individuals at low-risk of HPV infection). The cutoff level has been set to maximize specificity[35].

The enzyme-linked immunosorbent assay (ELISA), competitive luminex based immunoassay (cLIA), and competitive radioimmunoassay (cRIA) have been used in the HPV vaccine trials. None of these assays measure neutralizing antibodies. A new pseudoneutralization assay is used to detect the presence of neutralizing antibodies and has been used to validate the GSK's ELISA binding assay[36]; the pseudoneutralization assay has not been used in any clinical trials to date. The ELISA assays measure both neutralizing and non neutralizing antibodies produced to the HPV VLPs.

The cRIA and the cLIA, used by Merck, are competitive assays that provide an indirect measure of serum antibodies that bind to neutralizing epitopes on the HPV VLPs. The Gardasil™ trials switched from using cRIA to cLIA assays as the cRIA kits were being discontinued. The cRIA technology also employs the use of radioactive iodine (^{125}I) labeled antibodies, and is a more labor intensive process than the cLIA assays[37].

4.3 Induction of immunity to HPV VLPs in the female genital tract

HPV vaccination results in induction of antibodies in the cervico-vaginal lavage fluid[38, 39].

Serum and cervico-vaginal immune response data are presented from two dose ranging placebo-controlled trials of monovalent HPV 11 and monovalent HPV16 (recombinant yeast product, no adjuvant) vaccines[38]. Participants in the HPV 11 study received 10, 20, 50,

or 100μg of antigen, while participants in the HPV 16 study received 10, 40 or 80μg of antigen at 0, 2 and 6 months. This study demonstrated a modest antibody response in the cervico-vaginal lavage fluid of vaccinees. Twenty-four to 52% of women developed measurable anti HPV-11 antibody titres, while a lower proportion (5-10%) of women in the HPV 16 arm had measurable antibodies[38].

In the second study, participants (n=18) received monovalent HPV 16 (baculovirus expression vector, no adjuvant) using two different doses and schedules (2μg, 2μg, 50μg and 50μg given at weeks 0, 4, 12 and 15 respectively or 50μg and 50μg given at weeks 0 and 4 respectively). All participants developed detectable levels of anti HPV 16 IgG antibody in the cervical secretions. The IgG levels were affected by the hormonal cycles, and were highest in the proliferative phase and lowest at ovulation. Use of oral contraceptives resulted in a stable level of cervical IgG throughout the cycle. IgA antibodies were also found in the cervical secretions but at a lower level than IgG[39]. It is unknown whether the low antibody levels at ovulation are associated with an increase in risk of HPV infection.

While the VLPs used in the previous studies were produced by two different mechanisms, variations in other factors such as vaccination dose, schedule, site of antibody sampling, and the antibody assay (cRIA versus ELISA) used likely contributed to the differences seen in the induction of antibodies in the genital tract.

4.4 Description of HPV Vaccine Phase II and III Trials

There are a few publications based upon several large trials of the two HPV vaccines to date[7, 8, 22, 37, 40-43]. Table 3 compares key components of these trials.

Table 3: Comparison of trial protocols for the Merck Frosst Canada Ltd and the GlaxoSmithKline Inc products[7, 43, 44]

	Bivalent vaccine (GSK)	**Quadrivalent vaccine (Merck)**
	Cervarix™	**Gardasil™**
Target population, phase II	15-25 y.o. healthy females from Brazil, Canada and USA; ≤6 sex partners, and no evidence of prior HR HPV infection	16-23 y.o. healthy females from Brazil, Europe and USA; ≤4 sex partners, and no evidence of prior HPV 6, 11, 16 or 18 infection
Target population, phase III		FUTURE I: 16-24 y.o. healthy females at 62 study sites in 16 countries; ≤ 4 sex partners; no history of genital warts or abnormal pap smears FUTURE II: 15-26 y.o. healthy females at 90 study sites in 13 countries; ≤ 4 sex partners and no history of abnormal pap smears
Inclusion criteria for per protocol analysis, phase II	Prescreening to exclude women with abnormal cytology, HPV 16 or HPV 18 serum antibodies or HPV-DNA positive by PCR for 14 HR HPV types ≤90 days before study entry; received all three vaccine doses as per	No prescreening visit. Excluded from type specific analysis if PCR positive for HPV 6, 11, 16 or 18 at day 1 or month 7, or positive for antibodies for these four genotypes at day 1; received all three vaccine doses as per

	protocol	protocol
Inclusion criteria for per protocol analysis, phase III		As above, women with abnormal day 1 pap smear retained in analysis
Inclusion criteria for intention-to-treat analysis (ITT), phase II	Women who received at least one dose of vaccine, were negative for HR HPV DNA at day 1, and who had any data available for outcome measurement	Referred to as modified (M)ITT-1; women who had at least one dose of vaccine, and were naïve (seronegative and DNA negative) to the relevant HPV type at day 1
ITT, phase III		FUTURE I and II: Unrestricted susceptible population, females who were seronegative and pcr negative at day 1, included even if protocol violations present or had abnormal pap smear at day 1 (similar to MITT above) FUTURE I and II: ITT, General study population: females who were randomized to the two study arms at day one regardless of baseline infection or disease; included even if protocol violations present or abnormal pap smears at day 1
Sample size	HPV001: Vaccine 560/Placebo 533 HPV007: 398/389	Phase II, Vaccine 277/ Placebo 275 Phase III, FUTURE I, Vaccine 2723/ Placebo 2732 Phase III, FUTURE II, Vaccine 5305/ Placebo 5260
Vaccine	20 μg of HPV 16 L1 VLP, 20 μg of HPV 18 L1 VLP, ASO4 adjuvant containing 500 μg of AlOH and 50 μg of 3-deacylated MPL	20 μg of HPV-6 L1 VLP, 40 μg of HPV-11 L1 VLP, 40 μg of HPV-16 L1 VLP, and 20 μg of HPV-18 L1 VLP with 225 μg of aluminum hydroxyphosphate sulfate (alum) adjuvant
Vaccine technology	Baculovirus expression system / *Trichnoplusia ni* cell line	Recombinant yeast producer cell, *Saccharomyces cerevisiae*
Placebo	500 μg of aluminum hydroxide	225 or 450 μg of aluminum hydroxyphosphate sulfate (alum)
Immune assay	ELISA	cRIA and cLIA

Outcomes	Incident infection: PCR-based evidence of new cervical infection with HPV16, 18 or both. Persistent infection: two positive HPV-DNA PCR assays for the same viral genotype over consecutive visits; 6 month definition required the absence of negative samples in between two positive samples over a minimum of 5 months; 12 month definition required the detection of positive specimens over a minimum of 10 months	Per protocol analysis: persistent infection detected in individuals who were HPV 16, 18 DNA negative at 0 and 7 months and defined as: a) PCR-based HPV 6, 11, 16 or 18 positive samples from cervical, vaginal, and/or external genitalia collected at consecutive visits at least 4 months apart; or b) a biopsy showing HPV-related lesion that is positive for HPV 6, 11, 16 or 18 DNA; or c) positive for HPV 6, 11, 16 or 18 DNA at last visit before lost to follow-up
	Cytology outcomes (ASCUS, ASC-H, AGC, LSIL, HSIL) and histology outcomes (CIN 1-3) associated with HPV 16 or 18	Histologically confirmed HPV 6, 11, 16 or 18 related CIN
Maximum follow up	Phase II: 5.5 years	Phase II: 5 years Phase III: 3 years for both studies

There are slight but important variations in trial protocols of the two vaccine products for the following parameters, including study population, inclusion criteria, baseline screening, and outcome measurement. One important distinction exists in the ITT analysis of phase II and phase III trials of Gardasil™. In phase II trials, the ITT analysis includes subjects who were negative for the four vaccine genotypes at baseline and had incurred protocol violations. This population in phase III trials is referred to as 'unrestricted susceptible population'. The ITT analysis in phase III trials refers to all subjects randomized to the two arms regardless of their baseline positivity for vaccine genotypes and the analysis is sometimes called ITT general study population.

Table 4: Vaccine trials and source publications

Merck: Gardasil™				
Protocol Number	Vaccine	Subjects (N)	Trial Location	Reference
005	40 μg HPV 16 VLP	2391	United States	45-47
007	Low, medium and high dose quadrivalent vaccine	Phase 1: 1158	United States, Brazil and Europe	37
	20/40/40/20 μg of HPV 6, 11, 16 and 18 L1 VLPs with alum	Phase II : 552	Brazil and Europe	40, 41

013, FUTURE I	20/40/40/20 μg of HPV 6, 11, 16 and 18 L1 VLPs with Alum	Phase III: 5455	16 countries	8
015, FUTURE II	20/40/40/20 μg of HPV 6, 11, 16 and 18 L1 VLPs with Alum	Phase III: 12167	13 countries	7, 48
GSK: Cervarix™				
HPV001	20/20 μg of HPV 16 and 18 L1 VLPs with ASO4	Phase II: 1113	Canada, Brazil and USA	22
HPV007	20/20 μg of HPV 16 and 18 L1 VLPs with ASO4	Phase II: 776	Canada, and Brazil	42, 49
HPV008	PATRICIA: large phase III trial; no publications			None available
HPV009	Large phase III trial funded by the National Cancer Institute, taking place in Costa Rica			None available

4.5 Immunogenicity of HPV vaccines in phase II and III studies

Data are presented on the intensity of immune response immediately following vaccination (time[t]=7 months) and over the duration of follow up. As antibody assays for both products are different, antibody thresholds defining seroconversion in response to vaccination are provided. Due to the absence of a marker for seroprotection (immune correlate of protection), vaccine induced response is referenced to the natural response induced by infection.

The convention for documenting time periods in a vaccine trial usually start at t=0 which represents the time of administration of dose 1.

4.5.1 Protocol 005: Trial of HPV 16 L1 VLP (Merck technology)

A multicentre US-based randomized control trial (RCT) recruited 2,391 women for a study of monovalent HPV 16 L1 VLP vaccine. Participants in the vaccine arm received 40 μg of the HPV 16 L1 VLP while the subjects in the placebo arm received 225 μg of aluminum adjuvant. Data from the interim analysis conducted at 18 months of follow up, and a final analysis at 48 months are presented in tables 5-7; 99.7% of women who received the full course (i.e., three doses of vaccine) seroconverted, and 94% were still seropositive at 48 months (table 5). Seropositivity is defined as antibody titres >20mMU/mL (cRIA). Titres were highest at 7 months, decreased by 18 months and remained stable from 30 to 48 months. Vaccination provided a dramatic immune response with anti-HPV 16 geometric mean titres (GMTs) in vaccinees at 48 months present at a much higher level (22 times) than GMTs in women with evidence of natural infection[45-47].

4.5.2 Trials of Gardasil™

4.5.2a: Protocol 007

The objective of this multicentre RCT was to assess one of three dose vaccine formulations for use in phase III studies. The protocol consisted of dose-ranging assessment of safety, immunogenicity and efficacy. The three formulations tested were low dose vaccine (i.e., 20 μg type 6 L1 VLP, 40 μg type 11 L1 VLP, 40 μg type 16 L1 VLP, and 20 μg type 18 L1 VLP [20/40/40/20] with 225 μg alum adjuvant), intermediate dose vaccine at 40/40/40/40 with 225 μg alum adjuvant and high dose vaccine at 80/80/40/80 with 395 μg alum adjuvant. A total of 1158 women were enrolled in the trial and randomized to the three study arms or

two placebo arms (two different doses of the alum adjuvant). All three vaccine formulations were equivalent in their immunogenicity and similar in their efficacy. At 36 months of follow up, rates of persistent infection due to any of HPV 6, 11, 16, or 18 were 0.7, 1.3 and 0.5 per 100 woman-years for the low, medium and high dose formulations respectively[37].

The US portion of the trial was discontinued at 36 months. Other centres in Europe and Brazil continued the low dose vaccine formulation (now licensed as Gardasil™) trial. Five year follow up data are available for 516 women (256, low dose vaccine group; 260, placebo).

All subjects in the vaccine arm seroconverted at t=7 months with GMT's that were approximately 11 fold (HPV 6), 7 fold (HPV 11), 105 fold (HPV 16) and 19 fold (HPV 18) higher than placebo recipients with evidence of prior natural infection.

Antibody levels reached a peak at 7 months and dropped to a plateau at 18 months. The antibody titres at 18 months were sustained at approximately the same levels at 5 years of follow up. At 3 years and 5 years of follow up, the GMTs for HPV 6, 11, and 18 were statistically similar to those acquired from natural infection while the GMTs for HPV 16 were 18 times and 25 times higher respectively than those from natural infection. Greater than 94% of women were seropositive for HPV 6, 11 and 16 at 36 months of follow up while 76% were seropositive for HPV 18. The lower limits of antibody quantitation by the cLIA assay were 4.1, 3.0, 10.2 and 2.9 mMU/mL with seropositivity defined as titres $\geq$ 20, 16, 20 and 24 mMU/mL for HPV 6, 11, 16 and 18 respectively. The seropositivity rates at 60 months follow up were not indicated[37, 41].

4.5.2b. Protocols 013 (FUTURE I) and 015 (FUTURE II)

FUTURE I is a phase III RCT enrolling 5455 women at 62 study sites in 16 countries; the trial is designed to examine vaccine efficacy against two composite disease endpoints: external anogenital and vaginal lesions and cervical lesions (CIN 1+). FUTURE II is another large phase III RCT enrolling 12,167 women at 90 study sites in 13 countries; this trial is designed to examine vaccine efficacy against advanced cervical lesions (CIN 2+). The current publications pertain to three year follow up data from these trials[7, 8].

Over 99% of subjects seroconverted post dose 3 to all HPV genotypes. Seropositivity rates at 24 months are presented. Per-protocol vaccinees retained over 96% seropositivity to HPV 6, 11, and 16 in both trials but seropositivity to HPV 18 declined to 68% and 74% in FUTURE II and FUTURE I respectively.

4.5.2c. Combined protocols

The immunogenicity data from the combined protocols are unpublished but available from the manufacturer and have been cited in the NACI statement. The sample size has not been provided. At 60 months of follow up, the GMTs for HPV 6 and 18 are similar to that from natural infection; however, the GMTs for HPV 11 and HPV 16 are both higher than that induced by natural infection[1].

4.5.3 Trials of Cervarix™

4.5.3a: Protocol HPV001 and HPV007

The GSK HPV Vaccine Phase II trials are randomized, placebo controlled multi-centre trials that took place at 28 sites in Canada, Brazil and the US. The first trial, HPV 001, enrolled 1100 women who were followed for 27 months[22]. The second trial, HPV007, recruited a subset of participants from the first trial (776 women) for extended follow up with a mean duration of 5.5 years[42, 49]. Seroconversion was defined as ELISA titres $\geq$ 8 units/mL for HPV 16 and $\geq$ 7 units/mL for HPV 18.

Immunologic response to the bivalent vaccine appears to be strong with 100% of women seroconverting to HPV 16 L1 VLP and 99.7% of women seroconverting to HPV 18 L1 VLP at month 7; 100% of women had seroconverted by 18 months. Over 98% of women remained seropositive at all measured time points for the 5.5 years of follow up. Antibody titres were highest at 7 months with vaccinees attaining GMTs that were 107 fold (HPV 16) and 82 fold (HPV 18) higher than those obtained from natural infection. At 18 months, the GMTs were 10 fold (HPV 16) and 16 fold (HPV 18) higher than that of natural infection while at 51-53 months, they were still 17 fold (HPV 16) and 14 fold (HPV 18) higher[42].

4.5.3b. HPV008 and HPV009

These are two large phase three trials of the bivalent vaccine. HPV008 or PATRICIA (PApilloma TRIal against Cancer In young Adults) is a multicentre, multicountry trial that has enrolled 18,000 women to assess outcomes of CIN 2+. HPV009 is a trial coordinated by the National Cancer Institute that has enrolled over 9,000 women in Costa Rica. Immunogenicity and efficacy data have not been released from either trial.

Table 5: GMTs and Seropositivity (SP) from Monovalent HPV 16 VLP Vaccine, Gardasil™ and Cervarix™

1	**HPV 16 L1 VLP**[45-47] Recombinant *Saccharomyces cerevisiae* 40 μg HPV 16 – alum. cRIA. Population: Females 16-23 years									
Antigen	SP level	**7 months**			**18 months**			**48 months**		
		N	GMT	%SP	N	GMT	%SP	N	GMT	%SP[1]
HPV 16	>20mMU/mL[2]	684	1518.8	99.3	649	201.8	98.3	481	131.5	94.0
Baseline HPV 16 +		105	15.9		97	16.2		72	20.0	
2	**Gardasil™ Protocol 007**[37, 41] Recombinant *Saccharomyces cerevisiae* 20/40/40/20 – alum. cLIA. Population: Females 16-23 years									
Antigen	SP level	**7 months***			**36 months***			**60 months**[¶]		
		N	GMT	%SP	N	GMT	%SP	N	GMT	%SP
HPV 6	≥20mMU/mL	208	582	100	184	93	94	77	66.5	NA
Baseline HPV 6 +		17	55		16	68		9	30.5	
HPV 11	≥16mMU/mL	208	697	100	184	94	96	83	67.6	NA
Baseline HPV 11+		4	94		4	96		2	150.4	
HPV16	≥20mMU/mL	194	3892	100	177	509	100	78	395.4	NA
Baseline HPV 16 +		15	37		15	29		8	16.0	
HPV 18	≥24mMU/mL	219	801	100	196	60	76	82	43.7	NA
Baseline HPV 18 +		12	42		10	29		7	32.7	
3	**Gardasil™ Protocol 013**[8] FUTURE I, Population: Females 16-24 years									
Antigen	SP level	**7 months**			**24 months**					
		N	GMT	%SP	N	GMT	%SP			
HPV 6	≥20mMU/mL		552	≥99.5		118	96			
Baseline HPV 6 +						< 12				
HPV 11	≥16mMU/mL		781	≥99.5		150	98			
Baseline HPV 11+						< 12				
HPV 16	≥20mMU/mL		2234	≥99.5		477	99			
Baseline HPV 16 +						< 12				
HPV 18	≥24mMU/mL		469	≥99.5		56	74			
Baseline HPV 18 +						< 12				

4	**Gardasil™ Protocol 015**[7] (FUTURE II Study Group) FUTURE II, Population: Females 15-26 years									
Antigen	SP level	**7 months**			**24 months**					
		N	GMT	%SP	N	GMT	%SP			
HPV 6	≥20mMU/mL			>99	986		96			
HPV 11	≥16mMU/mL			>99	987		97			
HPV 16	≥20mMU/mL			>99	953		99			
HPV 18	≥24mMU/mL			>99	1059		68			
5	**Cervarix™** [22, 42] Baculovirus expression system 20/20 – ASO4. ELISA. Population: Females 15-25 years									
Antigen	SP level	**7 months**			**18 months**			**51-53 months**[4]		
		N	GMT	%SP	N	GMT	%SP	N	GMT	%SP
HPV 16	≥ 8 EU/mL[3]	351	5334.5	100	348	801.4	100	40	NA	100
Baseline HPV 16 +			50			NA			36.3	
HPV 18	≥ 7 EU/mL	351	3364.7	99.7	348	480.5	100	40	NA	100
Baseline HPV 18 +			41			NA			26.5	

[1] %SP= Percentage seropositivity
[2] mMU/mL= milli Merck Unit per mL
[3] EU/mL= ELISA Units per mL
[4] At 5.5 years, seropositivity parameters only (≥ 98% seropositive to both HPV 16 and HPV 18) are available in the abstract cited[49] .

5. Immunogenicity in other populations

Immunogenicity is measured in populations which lack efficacy data in order to compare their immune response with populations for whom immunogenicity and efficacy data are available. These studies are called bridging studies as they attempt to infer efficacy from one group to another based on similarities in immune response[1].

Gardasil™, 9-15 year olds

Immunogenicity bridging data are available for adolescent males and females 9-15 years of age[5, 6]. In the first adolescent non inferiority trial[5], over 1500 subjects were enrolled in three equal arms: males (10-15 years of age), females (10-15 years of age), and older vaccinated female controls (16-23 years of age). Over 99% of all participants (100% adolescent females, 99.7% adolescent males, and 99.1% of older females) seroconverted at month 7. The adolescent males and females mounted a strong immune response to all four HPV VLPs of the quadrivalent vaccine. The GMTs for adolescent females for HPV 6, 11, 16, and 18 were 1.7, 1.7, 1.8 and 2.0 times higher respectively than the respective GMTs in the control group. The GMTs for adolescent males for HPV 6, 11, 16, and 18 were 1.8, 1.9, 2.2 and 2.7 times higher respectively than the respective GMTs for the control[5].

Table 6: GMTs (mMU/mL**) in females, males and older females at 7 months following vaccination with Gardasil™ [5]**

	Females (10-15 years)		Males (10-15 years)		Females (16-23 years)	
	N	GMT	N	GMT	N	GMT
Anti HPV 6	423	959	428	1042	320	575
Anti HPV 11	423	1220	428	1318	320	706
Anti HPV 16	424	4697	427	5638	306	2548
Anti HPV 18	426	916	429	1212	340	453

The same three-dose adolescent noninferiority trial[5] also noted that the antibody titres at t=3 months (i.e., one month post dose 2) in adolescent boys and girls were similar to antibody titres post dose 3 in older females for three of the four HPV genotypes. GMTs (in mMU/mL) post dose 2 to HPV 6, 11, 16, and 18 in adolescent girls were 636, 776, 2834, and 369 respectively while the GMTs in adolescent boys were 678, 796, 3026, and 414 respectively.

The second adolescent RCT study[6] recruited over 1700 subjects 9-15 years of age to assess the immune response of three doses of Gardasil™ compared to placebo. While the overall GMTs for the 9-15 year olds were similar to that in Table 6, the antibody response was much higher for the 9-12 year olds (1.4, 1.5, 1.5 and 1.6 fold higher for the four genotypes) as compared to the 13-15 year old adolescents. The durability of this response was measured to 18 months of follow-up. Over 97% of individuals remained seropositive to HPV 6, 11, and 16 but only 91.5% of girls and 92.5% of boys were seropositive to HPV 18 at month 18.

Cervarix™, 10-14 year olds and 26-55 year olds

Immunogenicity bridging data for Cervarix™ in younger (10-14 years) and older (26-55 years) age groups are available [50-51]. Table 7 summarizes these results. Study subjects, all of whom were female, were vaccinated using a 0, 1 and 6 month schedule. At month 7, all the participants were seropositive. The intensity of the antibody response inversely correlated with age and was highest in the youngest age group. The 10-14 year olds had GMTs that were on average two times that of the 15-25 year olds for both HPV-16 and -18. Local adverse events occurred at a similar frequency (80-85%) among subjects aged 10-45 years, and a lower frequency (69%) was observed in the oldest age group, 46-55 years.

Table 7: GMTs (EU/mL**) at 7 months following vaccination with Cervarix™ in younger and older female age groups**

	10-14 years[50]	15-25 years[50]	26-45 years[51]	46-55 years[51]
N	158	458	226	211
Anti HPV 16	17273	7293	4029.2	2566.8
Anti HPV 18	6864	3319	1837.3	1313.0

6. Short and long-term vaccine efficacy against persistent infection and disease

The World Health Organization consensus workshop on endpoints for HPV vaccine efficacy and effectiveness trials recommends the following endpoints: persistent infection in cervico-vaginal specimens lasting 6-12 months, CIN 2 or CIN 3 histology (including adenocarcinoma *in situ*) or worse accompanied by HPV 16, 18 detection, and invasive cancer with or without HPV 16, 18[52]. Where possible, vaccine efficacy against these endpoints has been provided. The trial publications present vaccine efficacy against several HPV disease outcomes including transient and persistent infections, condyloma, CIN1-3+. Gardasil™ trials employ a more sensitive definition of persistent infection both in terms of duration (4 months rather than the recommended 6-12 months) and site (cervical, vaginal or external genital specimens instead of the recommended cervico-vaginal).

6.1 Trial of HPV 16 L1 VLP, Merck product

The first large RCT of the monovalent HPV 16 vaccine involving approximately 2400 women in the US is presented here as the vaccine efficacy data are available over a four year period of follow up (table 8)[45-47].

Persistent infections, per protocol: At 48 months of follow up, seven cases of persistent infection were identified among the vaccinees (rate, 0.3 per 100 women-years) while 111 cases were identified in the placebo group (rate, 4.9 per 100 women-years) resulting in a vaccine efficacy of 94% (95% CI, 88-98).

Disease outcomes, per protocol: At 48 months, no cases of CIN 2 and CIN 3 were observed in vaccinees while seven cases of CIN 2 (rate, 0.3) and six cases of CIN 3 (rate, 0.3) were detected in placebo group. Vaccine efficacy against CIN 2 was 100% (95% CI, 33-100), and against CIN 3 was 100% (95% CI, 18-100).

Disease outcomes, (M)ITT-1: Includes all subjects who were negative for HPV 16 at baseline and who received one or more doses of the monovalent vaccine. No cases of CIN 2 and CIN 3 were observed in vaccinees while nine cases of CIN 2 (rate, 0.3) and eight cases of CIN 3 (rate, 0.3) were observed in placebo. Vaccine efficacy against CIN 2 was 100% (95% CI, 50-100) and against CIN 3 was 100% (95% CI, 42-100).

Disease outcomes, (M)ITT-2: Includes all subjects randomized to receive the vaccine including those who tested positive for HPV 16 infection at enrolment. Four cases of CIN 2 (rate, 0.1) and one case of CIN 3 (rate, 0.0) were observed among the vaccines while 13 cases of CIN 2 (rate, 0.4) and 11 cases of CIN 3 (rate, 0.3) were detected in the placebo group. The vaccine efficacy against CIN 2 was 69% (95% CI, <0-93) and against CIN 3 was 91% (95% CI, 36-100)[45-47].

6.2 Trials of Gardasil™

6.2 a. Protocol 007

There are two publications outlining results of the efficacy trial that followed 550 women for 36 months and a subset (45% of subjects) for 5 years[40-41].

Persistent infections, per protocol: At five years of follow up, two cases of persistent infection occurred among the vaccinees (rate, 0.3 per 100 women-years), one each from HPV 16 and HPV 18. In the same period, 45 cases of persistent infection occurred among the placebo group (rate, 6.0 per 100 women-years). This yields a vaccine efficacy of 95.6% (95% CI, 83.3-99.5)[41].

Persistent infections, ITT: four cases of persistent HPV infections occurred, yielding a rate of 0.4 per 100 women-years for vaccinees. This compares with 58 cases of persistent infections in the control group yielding a corresponding rate of 6.6 per 100 women-years. Vaccine efficacy against persistent infection was 93.5% (95% CI, 82.5-98.3)[41].

Disease outcomes, per protocol: Over the five years of follow up, no cases of CIN or condyloma were identified among the vaccinees while three cases of CIN 1-3 (rate, 0.4) and three cases of condyloma (rate, 0.4) were identified in the placebo group yielding a total disease rate of 0.8/100 women-years at risk and a vaccine efficacy against all disease of 100% (95% CI, 12.4-100)[41]. Efficacy against the subset of CIN 2/3/AIS outcomes in the per protocol group is not identified in the peer-reviewed publication, but is reported in the product monograph as 100.0% (95% CI, -3734.9-100.0)[4].

Disease outcomes, ITT: No cases of CIN or condyloma were identified in the vaccinees while ten cases of disease were identified in the placebo group. Of these, seven were CIN 1-3 outcomes (rate, 0.8) and three were cases of condyloma (rate, 0.4). This yields a vaccine efficacy of 100% against all disease outcomes (95% CI, 55.3-100)[41].

6.2 b. Protocol 013

Disease outcomes at three years of follow up are available on 5455 women enrolled in the trial. There were two primary outcomes for this trial: first, vaccine genotype specific external anogenital and vaginal lesions (anogenital warts, VIN, VaIN, vulvar or vaginal cancer), and second, vaccine genotype specific cervical lesions (CIN 1-3, AIS, cervical cancer)

Disease outcomes, per protocol:

External anogenital and vaginal lesions: No cases of disease were identified among the vaccinees while 60 cases (rate, 1.1 per 100 person years) were identified among the placebo subjects yielding a vaccine efficacy of 100% (95% CI, 94-100).

Cervical lesions: No cases of disease were identified among the vaccinees while 65 cases (33 CIN 1, 13 CIN 2, 13 CIN 3, 6 AIS) were identified among placebo subjects yielding a disease rate of 1.2 in placebo arm and a vaccine efficacy of 100% (95% CI, 94-100).

Disease outcomes, unrestricted susceptible population:

External anogenital and vaginal lesions: Four cases of disease (rate, 0.1 per 100 person years) were identified among the vaccinees and 81 cases (rate, 1.1) were identified among the placebo arm yielding a vaccine efficacy of 95% (95% CI, 87-99).

Cervical lesions: Two cases of CIN 1 were identified among the vaccinees (rate, <0.1) and 89 cases (46 CIN 1, 17 CIN 2, 20 CIN 3, 6 AIS) were identified among the placebo arm (rate, 1.2) yielding a vaccine efficacy of 98% (95% CI, 92-100). On a retrospective review, it was identified that one of the 'vaccinees' with CIN 1 had received three doses of placebo by mistake.

6.2 c. Protocol 015

Two year follow up results[48] and three year follow up results[7] are available for 12,167 women. Primary composite endpoint of this trial is cervical disease (CIN 2+, AIS, cervical cancer) due to vaccine specific genotypes.

Disease outcomes, per protocol: In the three years of follow up, one case of CIN 3 was identified among the vaccinees (rate, <0.1 per 100 person years) and 58 lesions (28 CIN 2, 29 CIN 3, one AIS) in 42 individuals were identified among the placebo arm (rate, 0.3) yielding a vaccine efficacy of 98% (95% CI, 86-100). The one case of CIN 3 among the vaccinees was positive for HPV 52 at baseline and five histologic specimens collected for diagnosis were also positive for HPV 52. HPV 16 was also detected in one histologic specimen only.

Disease outcomes, unrestricted susceptible population:

Three cases of CIN 2+ (one CIN 2, and two CIN 3) were identified among the vaccinees (rate, <0.1) and 87 lesions (40 CIN 2, 43 CIN 3, four AIS in 62 individuals) were identified among the placebo arm (rate, 0.4) yielding a vaccine efficacy of 95% (95% CI, 95-99).

6.2 d. Combined protocols

The following information has been retrieved from the Gardasil™ product monograph[4]. This data does not include the phase III data published since the product monograph was released.

Disease outcomes, per protocol: Data are presented on 16,947 women who followed identical study entry criteria among the various trials. Median duration of follow up is not provided. No cases of CIN 2/3 or AIS were identified in the vaccine population (n=8487) while 53 cases were identified in the placebo group (n=8460) yielding a vaccine efficacy of 100% (95% CI, 92.9-100). One case of genital warts was identified in 7897 vaccinees and 91

cases were identified in 7899 placebo recipients, yielding a vaccine efficacy against condylomatous disease of 98.9% (95% CI, 93.7-100.0).

6.3 Trials of Cervarix™

6.3 a. Protocols HPV001 and HPV007[22, 53]

This RCT took place in multiple sites across Canada, Brazil and the US. The initial efficacy study enrolled 1100 women who were followed for 27 months; a subset of participants residing in Canada and Brazil (n = 776) were enrolled in an extended follow up of over 5.5 years.

Persistent infection: In the first phase, two locations of infections were sampled and analyzed: cervical (gynecological exam), and cervico-vaginal (self sampling by participants). Cervico-vaginal sampling results in a more sensitive detection of transient HPV infections in both vaccine and placebo groups. Cervical sampling only was conducted in the extended follow-up group.

Persistent cervico-vaginal infection, per protocol: At 27 months of follow up, no cases of persistent infection were detected among the vaccinees and 16 cases (rate, 2.6 per 100 women-years) were detected among the control yielding a vaccine efficacy of 100% (95% CI, 76.8-100.0).

Persistent cervico-vaginal infection, ITT: At 27 months of follow up, four cases of persistent infection were detected among the vaccinees (rate, 0.5), while 31 cases were detected among controls (rate, 4.0) yielding a vaccine efficacy of 87.5% (95% CI, 64.6-95.6).

Persistent cervical infection, per protocol: At 51-53 months of follow up, one case (0.1/100 women-years) of persistent HPV 16 infection (lasting 6 months) and no cases of HPV 18 were detected among the vaccinees while 23 cases (2.5/100 women-years) of persistent HPV 16 or 18 infections (6 month definition) were detected in the control group, yielding a vaccine efficacy of 96.0% (95% CI, 75.2-99.9). Analysis of persistent infections lasting a minimum of 12 months yielded a vaccine efficacy of 100% for per protocol (95% CI, 52.2-100) group; no cases were detected in the vaccinees and nine cases (rate, 1.0) were detected among the controls. At 5.5 years of follow up, vaccine efficacy against persistent infection using a 6 month definition was 100% (95% CI, 81-100) and using a 12 month definition was also 100% (95% CI, 54-100).

Persistent cervical infection, ITT: Two cases of persistent infections (6 month definition) were detected among the vaccinees (rate, 0.1) while 34 cases were detected among the controls (rate, 2.7), yielding a vaccine efficacy of 94.4% (95% CI, 78.2-99.4). One case of persistent infection lasting 12 months was detected in the vaccinees (rate, 0.1) while 16 infections were documented in controls (rate, 1.2), yielding a vaccine efficacy of 94.0% (95% CI, 61.1-99.9).

Disease outcomes, per protocol: data not provided.

Disease outcomes, ITT: At 4.5 years of follow up, 46 cases HPV 16, 18 associated histological abnormalities were documented. Of these, only two cases of histological abnormalities were documented in the vaccinees and both of these were LSIL. The remaining 44 abnormalities were experienced by the women in the placebo group; of these, eight had CIN1+ and five had CIN 2+ abnormalities. The efficacy of the bivalent vaccine against CIN 2+ at 4.5 years of follow-up was 100% (95% CI, -7.7-100). Though detailed raw data at 5.5 years are not available, vaccine efficacy against ASCUS or worse cytology is

100% (95% CI, 85-100) and against CIN 2+ outcomes is also 100% (95% CI, 33-100). No cases of CIN 2+ were detected among vaccinees while seven cases were detected in the placebo arm.

Table 8: Vaccine efficacy in percent (95% confidence interval) against infection or disease from HPV viruses covered by the vaccine

1. HPV 16 L1 VLP[45-47] Females 16-23 years				
Outcome*	18 months		48 months	
	Per protocol	ITT	Per protocol	ITT
Persistent HPV 16 infection of ≥ 4 months duration	100 (90-100)	100 (90-100)	94 (88-98)	
HPV 16 related CIN 2		100 (CI, NA)	100 (33-100)	100 (50-100)
HPV 16 related CIN 3		100 (CI, NA)	100 (18-100)	100 (42-100)
2. Gardasil™ Protocol 007[40-41] Females 16-23 years				
Outcome*	36 months		60 months	
	Per protocol	ITT	Per protocol	ITT
Persistent Infection ≥ 4 months duration in cervical, vaginal or external genital samples	89 (70-97)	88 (72-96)	95.6 (83.3-99.5)	93.5 (82.5-98.3)
CIN 1-3	100 (16-100)	100 (32-100)	100 (<0.0-100)	100 (30.8-100)
Condyloma	NA	NA	100 (<0.0-100)	100 (<0.0-100)
HPV 6 endpoints¶	100 (68-100)		100 (75.7-100)	100 (81.9-100)
HPV 11 endpoints	NA		100 (<0.0-100)	100 (<0.0-100)
HPV 16 endpoints	86 (54-97)		96.6 (79.2-99.9)	91.6 (73.3-98.4)
HPV 18 endpoints	89 (21-100)		90.6 (35.6-99.8)	91.6 (43.3-99.8)
3. Gardasil™ Protocol 015[8] Females 16-24 years				
Outcome*	36 months			
	Per protocol	Unrestricted susceptible		
External anogenital and vaginal lesions	100 (94-100)	95 (87-99)		
Cervical lesions	100 (94-400)	98 (92-100)		
4. Gardasil™ Protocol 015[7, 48] Females 15-26 years				
Outcome*	36 months			
	Per protocol	Unrestricted susceptible		
CIN 2+	98 (86-100)	95 (85-99)		
HPV 16 CIN 2+	97 (84-100)	94 (82-99)		
HPV 18 CIN 2+	100 (61-100)	100 (74-100)		
5. Gardasil™ [4]				
Outcome*	Median duration, not provided			
	Per protocol			
CIN 2+	100 (92.9-100.0)			
Condyloma	98.9 (93.7-100.0)			

6. Cervarix™ [22, 42] Females 15-25 years				
Outcome*	18 months		51-53 months	
	Per protocol	ITT	Per protocol	ITT
Persistent (cervical) infection ≥ 6 months	100 (47.0-100)	95.1 (63.5-99.3)	96.0 (75.2-99.9)	94.4 (78.2-99.4)
Persistent (cervical) infection ≥ 12 months			100.0 (52.2-100.0)	94.0 (61.1-99.9)
Persistent (cervical + cervico-vaginal) infection ≥ 6 months	100 (76.8-100)	87.5 (64.6-95.6)		
CIN 2+				100 (-7.7-100)
Any high-risk HPV type, CIN 2+				67.1 (-31.9-94.3)
Independent of HPV DNA status, CIN 2+				73.3 (-1.0-95.2)

* Unless otherwise indicated, all outcomes pertain to HPV genotypes covered in the vaccine.
¶ Endpoints by genotype refer to persistent infection and disease outcomes.

7. Effect of the vaccine on the transmission of the type-specific and related HPV genotypes

Gardasil™ induces cross-reactive antibodies against HPV 31, 45, 52 and 58 VLPs at levels that are 1.5 to 2 log lower than antibodies induced to vaccine specific antigens[5]. Data on efficacy of Gardasil™ against these HPV genotypes nor against all HPV genotypes in a susceptible population have not been published from Gardasil™ trials.

The Cervarix™ vaccine trials examined the vaccine efficacy against transient infections with 14 high-risk type HPVs and 11 low oncogenic risk HPV genotypes. The vaccine was noted to offer significant protection from HPV 45 infection (vaccine efficacy of 94.2% (95% CI, 63.3-99.9); 17 cases in control and one in vaccinees), modest protection from HPV 31 (vaccine efficacy of 54.5%, 95% CI, 11.5-77.7) and no protection from other HPV genotypes. Efficacy against CIN 2+ outcome from any HR HPV type at 5.5 years was 68% (95% CI, 7-91)[49] .

8. Short and long-term population effectiveness (i.e. impact on reduction of burden of disease, including herd immunity)

Large phase III studies[7, 8] (and population-based studies[54, 55] are currently underway to address this issue. Three year follow-up data are available for Gardasil™ [7, 8]. The following results present data on all subjects randomized to receive three doses of the vaccine regardless of baseline HPV positivity and protocol violations incurred during the trial. Thus, this data provide the current best estimates for vaccine effectiveness in females 15-26 years of age who have four or less life-time sexual partners.

Protocol 013
Disease outcomes, intention to treat general study population: Vaccine efficacy against all vaccine genotype related external anogenital and vaginal lesions was 73% (95% CI, 58-83) and efficacy against same lesions from any HPV type was 34% (95% CI, 15-49).
Vaccine efficacy against cervical lesions due to vaccine HPV genotypes was 55% (95% CI, 40-66) and against cervical lesions from all HPV types was 20% (8-31).

Protocol 015

Disease outcomes, intention to treat general study population: Vaccine efficacy against CIN 2+ due to vaccine genotype was 44% (95% CI, 26-58) and efficacy against CIN 2+ from any HPV was 17% (95% CI, 1-31).

Table 9 presents a summary of the vaccine efficacy outcomes from the Gardasil™ and Cervarix™ trials to date.

Table 9: Vaccine efficacy of Cervarix™ and Gardasil™ in HPV naïve and general populations

		Susceptible population♣	General trial population♠
CIN 2+ from vaccine HPV genotypes	Cervarix™	100% [49]	
	Gardasil™	98% [7]	44-55% [7, 8]
CIN 2+ from any HPV genotype	Cervarix™	68% [48]	
	Gardasil™		17-20% [7, 8]

♣ Subjects who are HPV 16 and HPV 18 seronegative and PCR negative at day 1 and received one or more doses of the vaccine.

♠ Includes all subjects randomized to vaccine and placebo arms, including those who are baseline positive to HPV 16 and HPV 18.

9. Vaccine safety and adverse events

Adverse event profile for Gardasil™: Injection site reactions were 6-8% higher in the vaccine group compared to the placebo group. The proportion of adverse events were higher following dose one than dose two or three[6]. Systemic adverse events such as headache or fatigue were reported by a similar proportion of subjects in the vaccine and placebo arm. Serious adverse events occurring within 15 days of vaccination were reported in 102 of 21,464 total subjects in the Gardasil™ trials. The most frequent were headache (0.03% vaccine versus 0.02% placebo), gastroenteritis (0.03% vaccine versus 0.01% placebo), appendicitis (0.02% vaccine versus 0.01% placebo), and pelvic inflammatory disease (0.02% vaccine versus 0.01% placebo). In addition, one case of bronchospasm and two cases of asthma were reported in the vaccine group. Timing of these events in relationship to vaccination and severity are not provided. An incidental condition potentially indicative of systemic autoimmune disorder following enrollment in the trials was reported by 0.076% of vaccinees (n=9) and 0.031% (n=3) of controls. These conditions for vaccinees included one case of juvenile arthritis, two cases of rheumatoid arthritis, and six cases of other arthritides (general or reactive). One case of lupus and two cases of general arthritis were detected in the placebo arm.

Table 10: Common adverse events reported in the vaccine trials and product monograph

N	Fever %	Injection Site reaction %	Pain at site %	Systemic event %	Headache %	Fatigue %
1. HPV 16 L1 VLP[45]: Recombinant *Saccharomyces cerevisiae* 40 μg HPV 16 – alum						
1194 (Vaccine)			86	42		
1198 (Placebo)			82	44		
2. Gardasil™ [37]: 20/40/40/20 – alum; Placebo, group 1 received 225 μg of alum and group 2 received 450 μg of alum						
275 (Vaccine)	11	86	85	69	40	6
135 (Placebo 1)	10	75	73	71	36	7
140 (Placebo 2)	11	80	79	68	39	6
3. Gardasil™ [5] : 20/40/40/20 – alum; no placebo arm.						
501 (females 10-15 years)	13	81	79	31		
500 (males 10-15 years)	14	74	71	27		
497 (females 16-23 years)	7	88	86	32		
4. Gardasil™ [5] : Data by sex; Placebo - data for both aluminum and non aluminum containing placebo has been combined.						
5088 (Vaccine, F)	13.0		83.9		28.2	
3790 (Placebo, F)	11.2				28.4	
1072 (Vaccine, M)	12.1		69.3		17.9	
274 (Placebo, M)	7.4				15.6	
5. Cervarix™ [22] : Baculovirus expression system 20/20 AS04; placebo, 500μg Aluminum hydroxide						
531 (Vaccine)	17	94*	93*	86	62	58
538 (Placebo)	14	88	87	86	61	54

*Indicates adverse events that were statistically different from the control group.

There were 17 deaths among 21,464 participants (ten in vaccine group and seven in placebo). None of these were judged to be vaccine related[4]

Table 11: Causes of death among participants in the Gardasil™ trials (n=21,464)

	Gardasil™	Placebo
Motor vehicle collision	4	3
Overdose/suicide	1	2
Pulmonary embolus/DVT	1	1
Sepsis	2	0
Pancreatic cancer	1	0
Arrhythmia	1	0
Asphyxia	0	1
Total	10	7

Adverse event profile for Cervarix™: Adverse event profile of Cervarix™ is only available from phase II trials[22, 42]. The first trial publication reports a 6% higher frequency of injection site reactions in the vaccine group, and no difference in the frequency of systemic reactions between the vaccine and alum placebo groups[22]. A total of 41 serious adverse events were reported, of which 54% occurred in the vaccine group. None of these were attributed to the vaccine. Details on the serious adverse events were not provided in the publication. The second publication reports a lower self reported frequency of adverse events in the vaccine group (14% versus 22% in the placebo group)[42]. Frequency of new onset chronic disease was 3% in vaccinees and 5% in placebo arm. The frequency of reported serious events was 5.6% and 5.1% among vaccinees and placebo; none of these were judged to be attributed to the vaccine.

Vaccine experience in pregnancy: Gardasil™ is not approved for use in pregnant women. If women become pregnant during the course of the vaccination series, it is recommended that the vaccine series be postponed until after delivery. Data are available from the clinical trials of Gardasil™ in which women became pregnant before the completion of their series. Pregnancy was reported in 1901 women (944 in vaccine group and 957 in the control group). Overall, the rate of pregnancy related complications between the two groups was similar (4.2% and 4.3%). The number of children with congenital anomalies born to subjects in both groups was similar (13 in vaccinated group, and 12 in control group). However, in the subgroup of births that occurred within 30 days of vaccination, there were five congenital anomalies noted in the Gardasil™ group, compared to zero in the control group[4]. These congenital anomalies were reviewed by an external panel of teratologists in a blinded fashion[56]. The panel unanimously agreed that the congenital anomalies were highly unlikely to be associated with Gardasil™ administration.

Data on vaccine experience in pregnancy are not yet available for Cervarix™.

V. Discussion

Infection with HPV 16 and 18 is associated with a significant burden of cancers of the anogenital and head and neck regions. Approximately 70% of cervical cancer resulting from HPV 16, and 18 would be preventable by the currently approved vaccine (Gardasil™) and by a second vaccine (Cervarix™) that is undergoing the regulatory approval process. Gardasil™ also offers protection against approximately 90% of genital warts.

A comparison of the immunologic performance of these vaccines is limited given the absence of a standard assay to measure anti-HPV antibodies and the absence of a marker for seroprotection to the HP viruses. Merck Frosst Canada and GlaxoSmithKline Inc have developed and validated their own assays to assess the performance of their vaccine products. In the absence of a valid seroprotection correlate, in each trial, post-vaccination antibody titres are compared with those from natural infection. However inter-study comparisons of vaccine induced antibodies between the two manufacturers' products are not valid. GSK is starting a RCT comparing the two vaccine products that will allow comparison of outcomes (immune response and potentially, efficacy) from these two products.

Both vaccines are highly immunogenic and result in an antibody response at seven months that is much higher than that seen with natural infection. The antibody titres decrease up to 18 months and then plateau. Five year follow up data are available for Gardasil™. Antibody titres to HPV 16 and 11 are maintained at levels above those due to natural

infection, while titres to HPV 6 and 18 are maintained at levels approximately the same as natural infection. At 24 months of follow-up, over 96% of individuals are seropositive for three antigens, and 68% seropositive for the fourth antigen (HPV 18). Five and a half years of follow up data are available for Cervarix™. Antibody titres from Cervarix™ follow the same profile as described for Gardasil™, with two differences. The plateau level post 18 months is maintained at a level many fold higher than that from natural infection. At 5.5 years of follow up, over 98% of individuals remain seropositive for both oncogenic HPV antigens.

Disease outcome data are available for the same time period though phase III data are available for Gardasil™ while only phase II data are available for Cervarix™. In the per protocol population, both vaccines are highly efficacious (95%) in preventing persistent infections from HPV genotypes in the vaccine and 98% (Gardasil™) and 100% (Cervarix™) efficacious in preventing precancerous lesions (CIN 2+). Gardasil™ is also 99% efficacious in preventing genital warts. In the Cervarix™ trial, significant cross protection has been documented against both infection from HPV 45 (vaccine efficacy of 94%), and, to a lesser extent against HPV-31 (vaccine efficacy of 54%). This effect has not yet been demonstrated with Gardasil™. HPV 45 and 31 are estimated to cause 7.2% to 9.6% of cervical cancers[13].

Data on effectiveness of Gardasil™ in women ages 15-26 years are available. In the absence of prescreening and exclusion of women who are vaccine genotype positive at baseline, vaccine efficacy against cervical disease due to vaccine genotypes (44-55%) or all HPV genotypes (17-20%) is very low. This confirms several points: Gardasil™ should be used as a prophylactic vaccine and therefore should be offered to women before they are at risk of HPV acquisition, and vaccinated women need to continue to participate in pap smear screening programs.

Both vaccines have a good safety profile; however less data are available from Cervarix™ trials. The frequency of local reactions with both vaccines is high, but not that much higher (6-8%) than an alum placebo.

Despite the fast evolving state of knowledge of HPV vaccines and HPV immunology, there are still a number of outstanding information gaps. These have been documented in the proceedings of the HPV Vaccine Research meeting held in 2005 (http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/06vol32/32s1/index.html). We wished to highlight some of the important information gaps in this document and they are as follows:

- Presence of serum antibody to HPV viruses in animal models confers protection from HPV infection at a mucosal site. In addition, HPV antibodies have also been documented in cervical and vaginal secretions. However, given that the HPV infection is epithelial in nature, it is unclear whether serum antibody will confer protection at non-mucosal sites such as the anus, penis and vulva.
- The level of serum antibody correlating with seroprotection needs to be defined. While one cohort study has demonstrated that the low level immune response from natural infection is adequate to prevent challenge from reinfection by the same HPV genotypes[27], other studies have demonstrated the production of several different antibodies in response to infection or disease, including cancer. Thus, the type and level of antibody needed to confer protection needs to be clarified.
- To prevent infection at the epithelium or cervical mucosa, a minimum level of serum antibody will need to be maintained over time. The level required to maintain this protection is currently unclear. Will the initial vaccine series provide this protection for the duration of the exposure period? Will protection be maintained by exposure to wild virus, or will vaccine boosters be required?

- Additional trial data that have not been published but would be useful to have in the public realm include the following: The range of (antibody titres) response produced post vaccination, percent of low responders and predictors of low response; data on vaccine failures; and outcomes in women who have received less than three doses of HPV vaccine.

In conclusion, both Gardasil™ and Cervarix™ are efficacious and safe vaccines that provide protection from high-risk HPV genotypes 16 and 18 that account for approximately 70% of cervical cancer cases in the world. Phase III Gardasil™ trials have demonstrated vaccine efficacy against high grade vaginal and vulvar lesions. Gardasil™ also offers protection against two genotypes of HPV that are responsible for approximately 90% of genital warts. However, the limited Cervarix™ data published to date have demonstrated two differences from Gardasil™: a more robust immune response at 5.5 years of follow up, and cross protection to two additional oncogenic genotypes responsible for another 7-10% of cervical cancers. Given that the two vaccines contain identical virus-like particle antigens against oncogenic HPVs, long-term follow-up will help discern the significance of these differences.

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VII. References

1. Dobson S, Deeks S, Money D, group atNw. Statement on human papillomavirus vaccine. *Can Commun Dis Rep.* 2007;33(ACS-2):1-31.

2. Erickson LJ, De Wals P, Farand L. An analytical framework for immunization programs in Canada. *Vaccine.* Mar 31 2005;23(19):2470-2476.

3. Bosch F.X., ed. Et al. HPV vaccines and screening in the prevention of cervical cancer. *Vaccine.* August 2006. Volume 24, Supplement 3.

4. Ltd. MFC. Gardasil™ Product Monograph. Vol 102682; 2006.

5. Block SL, Nolan T, Sattler C, et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics.* Nov 2006;118(5):2135-2145.

6. Reisinger KS, Block SL, Lazcano-Ponce E, et al. Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. *Pediatr Infect Dis J.* Mar 2007;26(3):201-209.

7. Future II Study Group, Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* . 2007; 356(19): 1915-1927.

8. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med.* May 10 2007;356(19):1928-1943.

9. Stanley M, Lowy DR, Frazer I. Chapter 12: Prophylactic HPV vaccines: Underlying mechanisms. *Vaccine.* Aug 21 2006;24 Suppl 3:S106-113.

10. Wiley DJ, Harper DM, Elashoff D, et al. How condom use, number of receptive anal intercourse partners and history of external genital warts predict risk for external anal warts. *Int J STD AIDS.* Mar 2005;16(3):203-211.

11. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine.* Aug 21 2006;24 Suppl 3:S42-51.

12. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine.* Aug 21 2006;24S3:S1-S10.

13. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine.* Aug 21 2006;24 Suppl 3:S26-34.

14. Hammarsted L, Lindquist D, Dahlstrand H, Romanitan M, Onelov (Dahlgren) L, Joneberg Jea. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer.* 2006;119:2620-2623.

15. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine.* Aug 21 2006;24 Suppl 3:S11-25.

16. Cogliano V, Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F. Carcinogenicity of combined oestrogen-progestagen contraceptives and menopausal treatment. *Lancet Oncol.* Aug 2005;6(8):552-553.

17. Smith JS, Green J, Berrington de Gonzalez A, et al. Cervical cancer and use of hormonal contraceptives: a systematic review. *Lancet.* Apr 5 2003;361(9364):1159-1167.

18. Castle PE, Giuliano AR. Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients--assessing their roles as human papillomavirus cofactors. *J Natl Cancer Inst Monogr.* 2003(31):29-34.

19. Lacey CJ, Lowndes CM, Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine.* Aug 21 2006;24 Suppl 3:S35-41.

20. Reinis M. Technology evaluation: HPV vaccine (quadrivalent), Aventis Pasteur MSD/CSL. *Curr Opin Mol Ther.* Apr 2004;6(2):206-211.
21. Shank-Retzlaff ML, Zhao Q, Anderson C, et al. Evaluation of the thermal stability of Gardasil. *Hum Vaccin.* Jul-Aug 2006;2(4):147-154.
22. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet.* Nov 13-19 2004;364(9447):1757-1765.
23. Inglis S, Shaw A, Koenig S. Chapter 11: HPV vaccines: Commercial Research & Development. *Vaccine.* Aug 21 2006;24 Suppl 3:S99-S105.
24. Baldrick P, Richardson D, Elliott G, Wheeler AW. Safety evaluation of monophosphoryl lipid A (MPL): an immunostimulatory adjuvant. *Regul Toxicol Pharmacol.* Jun 2002;35(3):398-413.
25. Garcon N, Mechelen MV, Wettendorff M. *Development and evaluation of AS04, a novel and improved adjuvant system containing MPL and aluminum salt.* London, UK: Elsevier Academic Press; 2006.
26. Stanley M. Immune responses to human papillomavirus. *Vaccine.* Mar 30 2006;24 Suppl 1:S16-22.
27. Ho GY, Studentsov Y, Hall CB, et al. Risk factors for subsequent cervico-vaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. *J Infect Dis.* Sep 15 2002;186(6):737-742.
28. Carter JJ, Koutsky LA, Hughes JP, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis.* Jun 2000;181(6):1911-1919.
29. Giannini SL, Hanon E, Moris P, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine.* Aug 14 2006;24(33-34):5937-5949.
30. Nonnenmacher B, Hubbert NL, Kirnbauer R, et al. Serologic response to human papillomavirus type 16 (HPV-16) virus-like particles in HPV-16 DNA-positive invasive cervical cancer and cervical intraepithelial neoplasia grade III patients and controls from Colombia and Spain. *J Infect Dis.* Jul 1995;172(1):19-24.
31. Wikstrom A, van Doornum GJ, Quint WG, Schiller JT, Dillner J. Identification of human papillomavirus seroconversions. *J Gen Virol.* Mar 1995;76 (Pt 3):529-539.
32. Sanchez S, Troncoso G, Ferreiros CM, Criado MT. Evaluation of cross-reactive antigens as determinants of cross-bactericidal activity in pathogenic and commensal Neisseria. *Vaccine.* May 14 2001;19(25-26):3390-3398.
33. Evans TG, Bonnez W, Rose RC, et al. A Phase 1 study of a recombinant viruslike particle vaccine against human papillomavirus type 11 in healthy adult volunteers. *J Infect Dis.* May 15 2001;183(10):1485-1493.
34. Pagliusi SR, Dillner J, Pawlita M, Quint WG, Wheeler CM, Ferguson M. Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays-an update. *Vaccine.* Aug 21 2006;24 Suppl 3:S193-200.
35. Dias D, Van doren J, Schlottmann S, al. E. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. *Clinical and Diagnostic Laboratory Immunology.* 2005;12:959-969.
36. Dessey F, Giannini SL, Bougelet C, Wettendorff M. Assessing the immune response induced by the HPV-16/18 L1 VLP ASO4 vaccine: a correlation of testing methodologies. 2007.

37. Villa LL, Ault KA, Giuliano AR, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine*. Jul 7 2006;24(27-28):5571-5583.

38. Fife KH, Wheeler CM, Koutsky LA, et al. Dose-ranging studies of the safety and immunogenicity of human papillomavirus Type 11 and Type 16 virus-like particle candidate vaccines in young healthy women. *Vaccine.* Jul 29 2004;22(21-22):2943-2952.

39. Nardelli-Haefliger D, Wirthner D, Schiller JT, et al. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J Natl Cancer Inst.* Aug 6 2003;95(15):1128-1137.

40. Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol.* May 2005;6(5):271-278.

41. Villa LL, Costa RL, Petta CA, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer.* Dec 4 2006;95(11):1459-1466.

42. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet.* Apr 15 2006;367(9518):1247-1255.

43. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. *Vaccine.* Aug 21 2006;24 Suppl 3:S114-121.

44. Brown DR, Fife KH, Wheeler CM, et al. Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine. *Vaccine.* Jul 29 2004;22(21-22):2936-2942.

45. Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med.* Nov 21 2002;347(21):1645-1651.

46. Mao C, Koutsky LA, Ault KA, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol.* Jan 2006;107(1):18-27.

47. MRL Clinical Study Report MS. Study of pilot manufacturing lot of HPV 16 virus-like particle (VLP) vaccine in the prevention of HPV 16 infection in 16- to 23-year old females. Vol 005; 2005.

48. Skjeldestad FEFISC. Prophylactic quadrivalent human papillomavirus (HPV) (Types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine (Gardasil^{TM}) reduces cervical intraepithelial neoplasia (CIN) 2/3 risk. Paper presented at: Infectious Disease Society of America 43rd Annual Meeting, 2005; San Francisco, California.

49. Gall SA, Teixeira J, Wheeler CM. Substantial impact on precancerous lesions and HPV infections through 5.5 years in women vaccinated with the HPV-16/18 L1 VLP ASO4 candidate vaccine. Presented at 2007 AACR Annual Meeting, Los Angeles. Abstract number 4900. 2007.

50. Dubin GftHVASI, GlaxoSmithKline Biologicals, Rixensart, Belgium. Enhanced immunogenicity of a candidate human papillomavirus (HPV) 16/18 L1 virus-like particle vaccine (VLP) with novel AS04 adjuvant in pre-teens/adolescents. Abstract no:4042. ICAAC 2005: GSK; 2005.

51. Schwarz TF, Dubin GO. An AS04-containing human papillomavirus (HPV) 16/18 vaccine for prevention of cervical cancer is immunogenic and well-

tolerated in women 15-55 years old. HPV vacine for study investigators for adult women, GlaxoSmithKline Biologicals. Paper presented at: 2006 ASCO Annual Meeting Proceedings Part 1, 2006.

52. Pagliusi SR, Aguado MT. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine.* 2004;23:569-578.

53. Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* May 28 2004;53(RR-6):1-40.

54. Lehtinen M, Apter D, Dubin G, et al. Enrolment of 22,000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. *Int J STD AIDS.* Aug 2006;17(8):517-521.

55. Lehtinen M, Idanpaan-Heikkila I, Lunnas T, et al. Population-based enrolment of adolescents in a long-term follow-up trial of human papillomavirus vaccine efficacy. *Int J STD AIDS.* Apr 2006;17(4):237-246.

56. Advisory Committee on Immunization Practices. June 29-30, 2006. Record of the Proceedings. 'Human Papillomavirus Vaccine' pp5-6. http://www.cdc.gov/nip/acip/minutes/acip-min-jun06.pdf. Accessed April 12, 2007.