



Review

Prophylactic HPV vaccines: New interventions for cancer control

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ABSTRACT

Human Papillomavirus (HPV) infection causes cervical cancer, a significant portion of anal, vulvar, vaginal, and oropharyngeal cancers, genital warts, and recurrent respiratory papillomatosis (RRP). HPV 16 and 18 cause 70–90% of HPV-related cancers whereas HPV 6 and 11 cause 90% of RRP and genital wart cases. Together these four types cause 30–50% of all cervical intraepithelial neoplasia such as those detected by Papinicalou screening. In June 2006, a quadrivalent HPV (6, 11, 16, 18) vaccine was licensed in the United States, and subsequently in the European Union (September 2006), both following expedited review. We describe the primary objectives of the quadrivalent HPV vaccine clinical trial program including studies in females aged 9–45 and males aged 9–26. Planned long-term efficacy and safety evaluations, as well as programs to evaluate vaccine impact on oropharyngeal cancer are also described.

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1. Diseases associated with HPV infection

There are several infectious agents that have been associated with malignancies. *Helicobacter pylori* (*H. pylori*) was defined as a carcinogen by World Health Organization (WHO) in 1994. In gastric cancer *H. pylori* is responsible for several pathologies, with up to 15% of them evolving into duodenal ulcer, gastric ulcer, gastric B-cell lymphoma, or gastric adenocarcinoma [1]. AIDS-related non-Hodgkin lymphomas are associated with Epstein-Barr virus, ranging from approximately 30–100% of cases, depending on the histologic subtype of the lymphoma [2]. Epstein-Barr virus is also linked to Burkitts lymphoma [1]. Infections with hepatitis B and C are present in more than 80% of hepatocellular carcinomas (liver cancers) worldwide [3].

1.1. Cervical cancer

Nearly 100% of cervical cancer can be attributed to Human Papillomavirus (HPV), in contrast with smoking, which accounts for 80% of lung cancer [4,5]. HPV is a double-stranded, non-enveloped DNA virus belonging to the Papillomavirus genus of the family Papillomaviridae [6]. Nearly 120 HPV types have been identified and 70–80% have been sequenced and classified [7]. Approximately 30–40 HPV types cause anogenital infections and approximately 15–20 are highly oncogenic [8].

HPV DNA has been identified in 99.7% of cervical cancer biopsies [1,4]. In all regions where extensive data are available, HPV 16 and 18 are found in the majority of cervical cancer cases [9]. Cervical cancer is the second most common cancer among women worldwide. Cervical cancer has a disproportionate impact on women in developing countries who account for about 85% of both the yearly cases of cervical cancer (estimated at 493,000 cases worldwide [2002]) and the yearly deaths from cervical cancer (estimated at 273,500 deaths worldwide [2002]) [10].

1.2. Vulvar and vaginal cancer

HPV also causes a portion of vulvar (20–50%) and vaginal (60–65%) cancers [11]. Unlike cancer of the cervix and breast, there are no screening programs for vaginal and vulvar malignancies and there are no preventative measures. An increasing number of invasive vulvar cancers are being diagnosed among young women globally. Using the SEER database, the change in incidence of vulvar cancer over a 28-year period (1973–2000) was evaluated. Vulvar carcinoma in situ (VIN 3) increased 411% from 1973 to 2000. Invasive vulvar cancer increased 20% during the same period. The increasing incidence of VIN 3 in the United States correlates with reported increases in HPV infection [12]. In a review of 405 cases of VIN 2/3 between 1962 and 2003 in New Zealand, more than one third of the women with VIN had been previously treated for preinvasive or invasive disease of the cervix or vagina [13]. In this country, an increase of squamous cell carcinoma (SCC) of the vulva in women younger than 50 years of age has been observed [14]. Most of these were associated with a background of HPV-related warty or basaloid VIN. HPV 16 appears to be the dominant HPV type

associated with high-grade VIN [15] whereas 6 and 11 have been associated with low-grade VIN. An analysis of pooled results from several studies showed that approximately 65% of the 127 VIN 1 specimens tested were positive for Types 6 and 11 only [16].

The recommended treatment of VIN is surgical excision, including vulvectomy or wide local excision. Laser ablative techniques have had variable outcomes and can be associated with painful healing [17]. Patients with widespread disease can be difficult to manage, may require more radical surgery, and often experience considerable morbidity. Disease recurrence in these patients is also common [17]. While some cases of VIN spontaneously regress (particularly among women under 30 years of age) [18], untreated VIN 3 does have significant invasive potential that has not been well recognized to date [13].

The true incidence of vaginal intraepithelial neoplasia (VaIN) is unknown but is far lower than for CIN [19]. The incidence of VaIN is expected to rise due to increased disease awareness and ascertainment of cases through wider application of cytologic screening and colposcopy [20]. The main predisposing factor for VaIN is exposure to HPV, explaining why VaIN is often found in conjunction with CIN [19]. The majority of VaIN cases have been described in patients who have had previous hysterectomy or a history of cervical or vulvar neoplasia [21]. In vaginal smears of 616 women with a prior hysterectomy, vaginal HPV was found 2.4 times more frequently in women with a history of CIN or cervical cancer than in all other women [22]. Of 110 HPV-positive patients, 5 had VaIN, whereas none of the 506 HPV-negative women had VaIN. All 5 VaIN cases were positive for HPV 16 [22]. HPV Types 16 and 18 were more often identified in patients with a history of cervical carcinoma, whereas HPV Types 6 and 11 were more often seen in patients with a history of benign uterine disease [22]. VaIN is often asymptomatic and difficult to diagnose. Diagnosis is made via colposcopically directed biopsy. Response of VaIN is unpredictable—in some cases it regresses, in others it persists, while in some it progresses (probably less than 10%) to invasive cancer [23,24].

1.3. Anal cancer

Anal cancer accounts for about 1–2% of gastrointestinal cancers. Compared with cervical disease, there is less known regarding the natural history of high-grade anal squamous intraepithelial lesions and the effect of treatment for pre-cancerous lesions [25]. Therefore, anal cancer screening is not routinely recommended. About 4000 new cases of anal cancer are diagnosed each year in the United States [26]. Age-standardized world incidence rates show that anal cancer is predominantly SCC, and in most populations, SCC is twice as common in females as in males [11]. An estimated 85% of anal cancers worldwide are attributable to HPV infection, with HPV 16 accounting for the vast majority of the HPV-positive cancers [27]. One study found that approximately 93% of anal cancers among women were positive for any HPV DNA and 89% were positive for high-risk HPV DNA. The predominant types found in invasive anal cancer included HPV 16 (77%) and HPV 18 (6%) [28].

1.4. Genital warts

Two low-risk types, 6 and 11, cause >90% of anogenital warts [25,29]. The overall incidence of external genital warts is high among both males and females with an estimated lifetime risk of approximately 10% [30,31]. An estimated 1 million new cases of genital warts occur every year in the United States [32] and the incidence of genital warts is increasing [33]. Based on a review of insurance claims, the rate of new genital warts cases increased from 117.8 per 100,000 persons in 1998 to 205.0 in 2001 [33]. The highest rates of genital warts are observed in individuals 20–29 years of age. In the United Kingdom, diagnoses of genital warts increased by 2% between 2002 and 2003 [34]. The highest rates are concentrated in males 20–24 years of age and in females 16–24 years of age. The median time between incident HPV 6 or HPV 11 infection and detection of genital warts is as little as 3 months, explaining their relative early occurrence in life [35].

1.5. HPV disease burden in men

The incidence of HPV infection among males is high and leads to a substantial disease burden in males, including that related to anal intraepithelial neoplasia (AIN) and penile intraepithelial neoplasia (PIN). Rates of HPV-associated lesions are higher in male sexual partners of females with CIN [36]. There is no established screening program for HPV infection and related diseases in males and screening for HPV infection in men is not recommended, despite a high incidence of infection as demonstrated by several studies conducted in both developed and developing countries.

According to the American Cancer Society, about 1900 men will be diagnosed with anal cancer in the United States in 2007 [37]. Fritsch and coworkers have shown that approximately 58% of anal cancers among heterosexual men, and 100% among homosexual men were positive for high-risk HPV DNA. The predominant types in invasive anal cancer were HPV Types 16 (87%), 18 (7%), 31 (1%), or 33 (6%). Other types were present in 2% of cases [38]. Anal Pap smears are available; however, the Center for Disease Control (CDC) does not recommend anal Pap tests because there is not enough research to show that removing abnormal anal cells actually prevents anal cancer from developing in the future [37].

Despite its relative rarity in developed countries, SCC of the penis is one of the most common genitourinary cancers in many developing countries of Asia, Africa, and South America. In Puerto Rico it represents almost 20% of all cancers in males [39]. In a collective review of 357 cases of SCC of the penis reported from 1983 to January 1993 from North and South America, Europe, and Asia, up to 77% of 39 cases of PIN/carcinoma in situ and 318 cases of invasive SCC contained HPV Types 11, 16, 18, or 30 [39]. HPV Types 16 and 18, alone or in combination, were detected in 83% of the 178 cases that tested positive for HPV. In a study conducted in Northern Thailand, the most prevalent genotype among 88 specimens of penile tissue (65 malignant, 1 pre-malignant, and 22 benign cases) was HPV 18 (55.4%) followed by HPV 6 (43.1%) [40]. Treatment of the primary lesion in SCC of the penis depends on the stage of the disease. Partial or total amputation has offered satisfactory results for local control of deeply invasive disease. This radical approach may have a local recurrence rate of up to 19% [39].

1.6. Head and neck cancers

In 2000, head and neck malignancies were ranked as the 8th leading cause of cancer death worldwide. Approximately 481,100 new cases developed, and 320,000 persons died of this disease [41]. HPV is associated with some of these cancers. In an analysis of 5046 head and neck SCC specimens from 60 studies worldwide,

overall HPV prevalence was 25.9% [42]. HPV prevalence was significantly higher in oropharyngeal SCCs (35.6%) than oral SCCs (23.5%) or laryngeal SCCs (24.0%). HPV 16 accounted for a larger majority of HPV-positive oropharyngeal SCCs (86.7%), compared with HPV-positive oral SCCs (68.2%) and laryngeal SCCs (69.2%). Aside from HPV 16 and HPV 18, other oncogenic HPV types were rarely detected in head and neck SCCs [42]. There is an increased risk of head and neck cancer in patients with a primary diagnosis of cervical carcinoma in situ and invasive cervical and anal cancer [41].

In the United States, oral SCCs are the 8th most common cancer among men and the 14th most common among women [43]. The proportion of oral SCCs that are potentially HPV-related increased in the United States from 1973 to 2004 [43]. In Sweden, the proportion of HPV DNA positive tonsil tumors increased from 28% in the 1970s to 68% in the 2000s [44]. Patients with HPV DNA positive oral SCCs tend to be younger and are less likely to have a history of tobacco or alcohol use than those with HPV DNA negative tumors [43].

1.7. Recurrent respiratory papillomatosis

HPV also causes recurrent respiratory papillomatosis (RRP). RRP is a rare disease that occurs in both children and adults [25]. The incidence of RRP in the United States is estimated at 4.3 per 100,000 children and 1.8 per 100,000 adults [45]. RRP is the most common benign neoplasm to affect the larynx in children (~6000 active cases of RRP in 1995 with >2300 new cases) [45]. The strongest risk factor for juvenile RRP is a positive maternal history of genital warts [46]. RRP is due to infection of the upper airway with HPV Types 6 and/or 11. HPV 11 infections are associated with disease severity contributing to frequent surgeries, additional adjuvant therapies, and co-morbidities (i.e., tracheal and pulmonary airway disease) requiring tracheostomy [45]. Common pediatric respiratory disorders may be erroneously diagnosed before the recognition of RRP. Among them are recurrent croup, asthma, laryngeal hemangioma, and tracheomalacia [45]. Treatment of pediatric RRP depends on the degree of airway involvement. Tracheostomy may be warranted in the face of acute respiratory distress. Additional interventions include CO₂ laser excision or papilloma excision via direct laryngoscopy. Children experience considerable morbidity, as the papillomas grow relentlessly, despite thorough surgical excision. Juvenile RRP treatment may require up to 100 procedures to reduce the size of the lesions [46].

2. Transmission

HPV infection is widespread and there are limited ways to prevent transmission. Total abstinence from all genital contact is the most effective method for preventing HPV infection [47]. Lifetime mutual monogamy is also an effective HPV prevention strategy; however, a monogamous person is at risk from his/her non-monogamous partner. In a study of 242 monogamous females, the risk of acquiring HPV was 46% at 3 years after first intercourse [48].

Although essential for the prevention of other sexually transmitted diseases (STDs), condom use reduces, but does not eliminate, the risk of HPV infection [49,50]. The HPV types associated with anogenital disease are primarily transmitted through any activity that involves genital-skin or oral mucosa contact. This can include genital-to-genital, manual-to-genital, or oral-to-genital contact [23,49,51]. Transmission of at least some types of anogenital HPV infections is not limited to penetrative sexual intercourse [49]. The 24-month cumulative incidence of HPV infection among virgins before initiation of penetrative sexual intercourse was 15.3%. Trans-

mission of HPV occurs through shedding of upper layer cells loaded with HPV virions [52]. HPV can also be transmitted via non-sexual routes: mother to newborn (vertical transmission) [53] and fomites (e.g., undergarments, surgical gloves, biopsy forceps) [54,55].

The importance of education in the prevention of STDs is widely recognized [56]. Many studies of adolescent and young adult females find a considerable lack of knowledge regarding HPV and the potential consequences of infection [57–62]. Most infected individuals are asymptomatic and are unaware that they are spreading the virus. While a healthy immune system suppresses the virus, it is difficult to predict when HPV is no longer contagious. Furthermore, there is no consensus on whether the virus is eliminated from the body or remains at an undetectable level [62]. Males and females both acquire and transmit HPV, including high-risk types, even when they do not have visible signs of disease, thus leading to a substantial disease burden in both men and women.

3. Economic burden of HPV-associated disease

HPV-related diseases have a high economic and societal burden in both men and women. Studies in the United States show that the average cost of CIN per episode of care is \$1709 (CIN 1 = \$1026; CIN 2 = \$1300; and CIN 3 = \$3235) [63]. These costs are significant when compared with those of other common diseases (diabetes annual chronic maintenance is \$1541, essential hypertension annual chronic maintenance is \$739, and severe osteoarthritis annual chronic maintenance is \$1919) [64].

The financial burden of low-grade cervical lesions, including those caused by HPV Types 6 and 11, is high, because these lesions lead to frequent follow-up visits and, at times, interventions. In the United States, approximately 110,000–160,000 cases of CIN 1 each year are caused by HPV Types 6 and 11. At a cost of \$1026 per case for a confirmed and treated case of CIN 1, this translates to a cost of HPV 6- and 11-related CIN 1 of \$113 million to \$164 million [63,65].

Economic costs of high-grade cervical lesions are high, since treatment includes either ablative or excisional procedures with long-term follow-up. According to the World Health Organization, it has been estimated that in the United States the cost of cervical cancer screening, follow-up, and treatment is approximately \$5 billion per year [27]. In an observational cohort study using 1997–2002 administrative and laboratory records from 103,476 women enrolled in a United States health plan, the overall annual cervical cancer prevention and treatment costs were \$26,415 per 1000 patients [63]. Routine cervical cancer screening comprised nearly two thirds of the total annual cervical HPV-related health care costs, with 10% of expenditures dedicated to the treatment of invasive cervical cancer, 17% to the management of cervical precancers, and 9% attributed to dealing with false-positive Pap test results [63]. Per-case treatment cost in the United States (in 2002 dollars) for invasive cervical cancer have been estimated at \$20,518 for Stage I disease, \$31,477 for Stage II–III disease, and \$46,839 for Stage IV disease [63].

The annual total cost of head and neck malignancies may exceed \$1.6 billion in the United States [66]. Per individual, juvenile RRP treatment may require up to 100 procedures to reduce the size of the lesions. Total lifetime cost to treat RRP ranges from \$60,000 to \$470,000 [46]. In the United States, the cost for juvenile RRP is estimated to be between \$53 million and \$163 million. The cost for adult RRP is estimated at \$64 million [66].

Diagnosis of genital warts carries a high and immediate financial burden, given the frequency of the disease, the short time from infection to disease, the high cost of some treatments, and the high rate of recurrence. In one United States study, the direct costs associated with anogenital warts (including recurrence) were esti-

mated at \$167 million [67]. Associated costs to health plans were highest among females 20–24 years old and males 25–29 years old [68]. Treatment for individual genital wart episodes involved 3.1 physician visits and incurred costs of \$436. These data may be underrepresented, as individuals may elect to seek treatment outside of their health plan because of the stigma associated with genital warts [68]. Costs range from approximately \$275 to \$425 for 1 course of podofilox, surgical excision, or laser treatment [69]. Costs range from approximately \$950 to \$1650 for 1 course of cryotherapy, trichloroacetic acid, imiquimod, or podophyllum resin [69].

The cost of treatment for genital warts does not necessarily reflect its level of effectiveness [70]. Cryotherapy (United States data) costs \$268–415 per successful treatment course with a 60–90% clearance rate. Imiquimod costs \$607–649 per successful treatment course with a 30–50% clearance rate. Interferon costs \$2744–5803 per successful treatment course with 20–60% clearance rate. In another United States study conducted by the CDC in 2004, the cost of anogenital warts in the United States was estimated to be \$200 million. This figure is based on an assumed annual incidence of 375,000 cases receiving treatment, at a cost of \$530 per case [63]. Using a large claims database from a set of United States health plans, a prevalence-based analysis of the economic burden of anogenital warts conducted in 2000 estimated annual direct medical costs of \$775 per 1000 population [63]. Extrapolated to the United States population, this would translate to annual direct medical costs due to anogenital warts of \$225 million, similar to the estimate from the 2004 CDC study. However, this result may be conservative if a portion of enrollees received care in settings outside of their health plan [63]. The estimated cost for managing 76,457 incident cases of genital warts in the United Kingdom was approximately £10.1 million. The cost for the 55,657 cases of recurrent or persistent episodes of genital warts was estimated to be £12.3 million [71].

The number of visits needed after diagnosis of an HPV lesion also costs the patient in terms of missed hours of work. In the United States, CIN episodes require, on average, 7.2 visits (range 6.6 for CIN 1 to 8.1 for CIN 3) and a follow-up duration of nearly 20 months [63]. Episodes of genital warts involve 3.1 physician office visits on average, with costs of \$436 [68].

4. Prophylactic HPV vaccines

New vaccine antigen discoveries and technologies have allowed for the development of highly immunogenic prophylactic HPV vaccines. Animal models have shown that papillomavirus infection can be blocked by a humoral immune response. This suggests that antibodies play a role in neutralizing infection, providing the rationale for a prophylactic vaccine approach [72]. Technical advances in the early 1990s demonstrated that 3-dimensional structures highly similar to papillomavirus particles could be produced by the expression of HPV viral capsid proteins (L1 and L2). These DNA-free VLPs are empty capsids and contain no oncogenic or infectious materials [73].

Understanding the way in which L1 VLP vaccines can prevent HPV-related disease has progressed considerably. Animal studies have shown that L1 VLP vaccines trigger high humoral responses and can prevent HPV infection and disease [74–77]. VLPs are highly immunogenic. Since L1 VLPs closely resemble the conformation of authentic virions, their administration is aimed to rapidly induce high levels of papillomavirus-neutralizing IgG antibodies specific to L1 and to produce neutralizing IgG antibodies each time the virus is encountered (i.e., immune memory) [73]. Components of L1 VLP vaccines mimic the virus and stimulate immunity by inducing the

production of humoral antibodies that target a key protein virus shell that is expressed prior to virus penetration of host cells [72,78].

L1 vaccines may also trigger some cell-mediated immune response, but this is thought to be less relevant, since L1 proteins are only minimally expressed in infected basal epithelial cells of benign lesions or in abnormal proliferative cells of premalignant or malignant lesions [29,78–80]. Consequently, humoral response is generally thought to be more relevant than cell-mediated response for prophylactic HPV vaccines [29,80].

5. Program objectives—GARDASIL® quadrivalent HPV (types 6, 11, 16, 18) vaccine

5.1. Vaccine description

The quadrivalent HPV vaccine (HPV 6/11/16/18 vaccine) bears the trade name GARDASIL® (Merck and Co., Inc., Whitehouse Station, NJ) and contains VLPs of the L1 protein of HPV types 6, 11, 16, and 18. HPV 6/11/16/18 vaccine is synthesized in *Saccharomyces cerevisiae* and adsorbed on amorphous aluminum hydroxyphosphate sulfate adjuvant. The L1 proteins are the same size and length of the natural L1 protein (i.e., they are not truncated).

5.2. Efficacy in women aged 16–26—overview

The primary objectives of the HPV 6/11/16/18 vaccine clinical program were to demonstrate that vaccination reduces the incidence of HPV 6, 11, 16, and 18-related CIN, AIS or cervical cancer, and HPV 6, 11, 16, and 18-related external genital lesions (condyloma, VIN 1–3 and VaIN 1–3) among women who were naïve to the respective vaccine HPV type pre-vaccination.

In June 2006, the HPV 6/11/16/18 vaccine was licensed in the United States, and subsequently in the European Union (September 2006), both following expedited review. The vaccine has since been approved in 100 countries and over 26 million doses have been distributed as of March 31, 2008. The licensure was primarily based on a demonstration that the vaccine prevented HPV 16- and 18-related high-grade precancerous lesions in subjects who were naïve to HPV 16 and/or 18 at the time of administration of the first dose. Both infection and CIN 1 tend to resolve spontaneously, though infection remains asymptomatic whereas CIN 1 is typically detected at screening and usually leads to follow-up. In contrast, CIN 3 and AIS are the immediate and obligate precursors of squamous-cell and adeno-carcinoma of the cervix, respectively. CIN 2 is also considered to be high grade, though spontaneous regression is more common than for CIN 3 [81]. Thus, as a requirement for licensure, the United States Food and Drug Administration and the World Health Organization [82] required that HPV vaccines demonstrate a reduction in the incidence of CIN 2/3 or AIS caused by vaccine HPV types.

Included in the application for licensure were efficacy summaries that integrated data from 3 separate efficacy/immunogenicity trials of the HPV 6/11/16/18 vaccine: Protocol V501-007 [NCT00365716], V501-013 [NCT00092521], and V501-015 [NCT00092534], which were similar in design and infrastructure and which mandated similar rigorous procedures for the collection of CIN, cervical cancer, and external genital lesion data [83–85]. Also included in the integrated summaries of efficacy was data from a proof of concept Phase IIa study (Protocol 005, NCT00365378) whereby subjects were randomized to either the HPV 16 vaccine component of the quadrivalent vaccine, or placebo [86]. Protocol 007 was a Phase IIb study. Protocols 013 and 015 were Phase III studies and are also referred to as FUTURE I and II (Females United to Unilaterally Reduce Endo/Ectocervical

Disease) and will be referred to as such throughout this review. All were double-blind studies (with sponsor blinding). In each study, subjects were randomized in a 1:1 ratio to receive vaccine or placebo at Day 1, Month 2, and Month 6. Although the visit schedule varied somewhat among the studies, subjects in all studies returned to the study site for collection of specimens, gynecologic examinations, and/or Pap tests at follow-up visits post-dose 3. In Protocols 005, 007, and FUTURE I, the follow-up visits occurred approximately every 6 months, whereas in FUTURE II, the follow-up visits occurred approximately every 12 months. However, in all studies, the first detection of atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL) prompted an interim visit 6 months after the visit in which ASC-US or LSIL was detected.

In all studies, procedures performed at both scheduled and unscheduled visits provided efficacy data. The procedures performed at scheduled visits included serum sample collection at enrollment, collection of cervicovaginal specimens at enrollment and Month 7 (and Month 3 in FUTURE I) and Pap testing at Month 7 and all subsequent visits. The procedures that were typically performed at unscheduled visits were repeat Pap tests, colposcopy, and biopsy. In each of the protocols, the results of the Pap tests performed at Month 7 and subsequent visits were used to identify subjects with HPV disease. Protocol-specified guidelines, which were mandatory in FUTURE I and FUTURE II but not in Protocol 005 or 007, were used to triage subjects with Pap abnormalities to colposcopy. If a lesion suspected to be HPV-related was observed on colposcopy, it was biopsied.

A panel consisting of 4 pathologists reviewed all cervical biopsy slides and adjudicated all cervical pathology for the purpose of providing the official pathologic diagnosis for the analysis of vaccine efficacy. The pathology panel followed established guidelines for review of the slides, and the membership of the pathology panel remained the same for the duration of all studies included in these analyses. Each pathology panel member independently reviewed the slides, blinded to treatment and the subject's HPV PCR status, to formulate a consensus. The consensus diagnosis of this panel represented the final diagnosis for study purposes. A recent study published in *Am J Surg Pathol*, was the first to describe the inter- and intra-observer agreement of CIN 2/3 diagnoses among Merck's panel of gynecologic pathologists [87]. Substantial inter-observer agreement was observed (weighted kappa = 0.765–0.865). Agreement with weighted kappa = 0.779–0.887 was observed between the individual panelists and the gold standard, which is almost perfect agreement by Landis-defined categories. These data indicate that the interpretation of histologic endpoints used in the HPV 6/11/16/18 vaccine clinical trial program is highly valid and reliable.

FUTURE I was designed for maximal ascertainment of HPV disease and required stringent criteria for disease determination and follow-up, including more frequent protocol-specified examinations and screening, and more aggressive colposcopy triage for suspected disease. In contrast to FUTURE I, FUTURE II followed a clinical protocol that was based on Pap screening intervals and management algorithms that constituted the standard-of-care in different communities [88,89]. In FUTURE II, Pap tests and HPV swabs were taken at Day 1, Months 7 and 12, and in 12-month intervals thereafter. Thus FUTURE II provided a more “real-world” scenario in which to evaluate the efficacy of HPV 6/11/16/18 vaccine.

The studies enrolled subjects regardless of their baseline HPV status as determined by both PCR and serology. All lesions deemed possibly, probably, or definitely HPV-related or whose clinical diagnosis was unknown required biopsy for confirmatory pathologic diagnosis.

Type-specific HPV detection was performed using highly sensitive PCR techniques [83,86,90]. A competitive Luminex Immunoassay (cLIA) was used for detection of serum anti-HPV responses that compete against monoclonal antibodies for binding to epitopes critical for virus entry (i.e., antibodies with neutralizing potential) [91]. The L1 proteins of HPV 6, 11, 16, and 18 share significant homology at the amino acid level (64–92%). Likewise, HPV 18 and HPV 45 share 88% homology at the amino acid level. Therefore, in an assay which measures total IgG, heterologous anti-HPV 11 antibodies would likely bind HPV 6 VLPs in addition to the homologous anti-HPV 6 VLP antibodies. Likewise, heterologous anti-HPV 18 antibodies would likely bind HPV 45 VLPs in addition to the homologous anti-HPV 18 VLP. This crossreactivity would prevent the ability to measure HPV 6, 11, 16, and 18 type-specific seroconversion and antibody titers. Therefore, a competitive Luminex immunoassay was developed to monitor a single, type-specific, neutralizing epitope for each of HPV 6, 11, 16, and 18. The four mAbs which were chosen, H6.M48 for HPV 6, K11.B2 for HPV 11, H16.V5 for HPV 16, and H18.J4 for HPV 18 [91–94] have been shown to recognize neutralizing epitopes on the associated HPV VLPs. In the cLIA, only a subpopulation of the total immune response is evaluated. Therefore, the antibody titers that are reported in the cLIA assay represent only a portion of the neutralizing antibodies that are generated through vaccination.

5.3. Populations for prophylactic efficacy analyses

5.3.1. Per-protocol

The primary analyses for vaccine efficacy were done in 4 type-specific per-protocol susceptible populations. This included subjects who: (1) received all 3 vaccinations within 1 year; (2) were seronegative and PCR-negative at Day 1 and PCR-negative through 1 month post-dose 3 to the appropriate vaccine HPV type; and (3) generally did not deviate from the protocol. Ascertainment of endpoints began 1 month after the third dose. Women with abnormal cytology at enrollment were not excluded from this population. It is important to note that as the vaccine contains 4 HPV types, there were 4 per-protocol populations, one per type, as outlined in Fig. 1. Thus a woman who was seronegative to HPV type 6 on Day 1 and who was also HPV 6 DNA negative by PCR Day 1 through 1 month

post-dose 3, would be in the per-protocol population for HPV 6. She may have been infected with one or more of the other 3 vaccine HPV types pre-vaccination, or at any time during the study. The intersection of the 4 per-protocol populations represents the women who remained naïve to all of the 4 types through completion of the vaccination regimen. As the trials measure type-specific and composite endpoints, it is also important to note that a per-protocol analysis of HPV 6, 11, 16, and 18-related endpoints is a combination of the 4 type-specific per-protocol populations. It does not mean that a woman had to be naïve to all of the 4 vaccine HPV types.

5.3.2. Unrestricted susceptible

The unrestricted susceptible population is less stringent than the per-protocol. It is also type-specific as described above. To be in this analysis, a woman had to be seronegative and DNA negative to a specific HPV type at Day 1 only. She may have become infected with the HPV type to which she was naïve before completion of the 3 dose series. Women who received less than 3 doses were also included, though it should be noted that most participants eventually received all 3 doses. Protocol violators were included. As in the per-protocol population, women with abnormal cytology at enrollment were not excluded from this population.

5.4. Baseline characteristics of the study population

The highest overall enrollment came from Denmark, Brazil, and the United States. FUTURE I was more heavily enrolled in Latin America and North America than was FUTURE II, which was primarily enrolled in the Nordic countries of Europe. The mean age of the participants in all studies was approximately 20 years. There was one 13-year old, and three 15-year-olds. The remainder was aged 16–26 years. The majority of subjects were sexually active (5.9% were virgins) with a median of 2 lifetime sexual partners (females with more than 4 lifetime sexual partners were excluded). The mean age at sexual debut was 17 years. Twenty-three percent of subjects reported having a past pregnancy, with the percentages being higher in Latin America and Asia Pacific. There was some geographic variation in the use of hormonal contraceptives, with 58% of the total subjects using oral contraceptives.

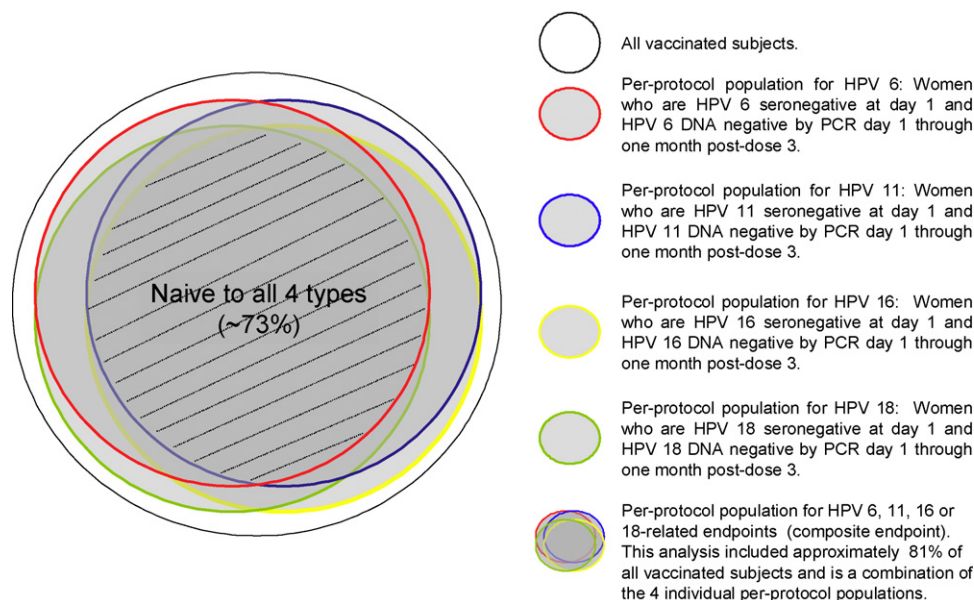


Fig. 1. Description of the type-specific per-protocol susceptible population.

Subjects were required to have no history of abnormal Pap tests prior to enrollment, but those with unrecognized disease or those who had never undergone screening were not excluded from participation. Although some were found to have LSIL or HSIL at enrollment, most of these were low-grade abnormalities (ASC-US or LSIL; less than 1% HSIL). Four percent of subjects were positive for Chlamydia at enrollment. This appeared to be higher in Latin-American subjects, although the reason for this is not clear. Across the studies, ~73% of enrolled subjects were naïve to all of the 4 HPV types included in the vaccine.

5.5. Prophylactic efficacy in young adult women

Prophylactic efficacy against squamous cell cervical carcinoma (via CIN 3 surrogate), cervical adenocarcinoma (via AIS surrogate) and HPV-related vulvar and vaginal cancer (via VIN and VaIN 2/3 surrogates) is shown in Table 1 [84,95,96]. The analyses represent approximately 3 years of follow-up. For subjects who were naïve to HPV 16 or HPV 18 through completion of the vaccination regimen, HPV 6/11/16/18 vaccine provided 98–100% efficacy against HPV 16- and 18-related FIGO stage 0 squamous cell carcinoma (CIN 3) and adenocarcinoma (AIS) and 100% for VIN 2/3 and VaIN 2/3. To date, the HPV 6/11/16/18 vaccine is the only HPV vaccine to demonstrate efficacy against HPV 18 related CIN 2/3 and AIS. Prophylactic efficacy against HPV 6, 11, 16, and 18-related CIN 1 and vulvar and vaginal condyloma (FUTURE I study data shown) was up to 100%.

As stated above, a woman who was included in the per-protocol population for HPV 6, may have been infected with HPV 11, 16, or 18 either before or during the course of the study. As efficacy for HPV 6-associated endpoints was high, it can be inferred that infection with other HPV types does not impact vaccine efficacy for HPV 6-related disease. To further illustrate this point, a recent publication assessed whether vaccination of women who are already infected with 1–3 vaccine types are protected against disease caused by the remaining vaccine HPV types. In FUTURE I and FUTURE II combined,

19.8% of the study participants were seropositive for HPV 6, 11, 16, and/or 18; 14.9% were PCR positive; and 26.8% were positive by either PCR or serological analysis (2368 vaccine recipients [26.9%] and 2354 placebo recipients [26.7%]). In this sub-population, vaccination was 100% effective (95% confidence interval [CI], 79%–100%) in preventing incident CIN 2/3 or AIS caused by the HPV type or types for which the women were negative at enrollment. Efficacy for preventing condyloma, VIN 1–3 or VaIN 1–3 was 94% (95% CI, 81%–99%). These results support vaccination of the general population without prescreening for HPV infection.

Due to the high efficacy seen in FUTURE I and II, the independent Data and Safety Monitoring Board (DSMB) of these studies recommended vaccination of women in the placebo group earlier than planned. The final end-of-study data with up to 4 years of follow-up (data not published) showed similar high vaccine efficacy.

5.6. Immune response in young adult women

The immune response required for protection varies for different pathogens. Acute pathogens, such as influenza virus, cause high levels of viremia and a rapid immune response. This response is characterized by activation of T cells and B cells with a corresponding high level antibody response that is necessary to control amplification of the pathogen [97]. HPV is not an acute pathogen. Once a persistent HPV infection is established, the time to progression is measured in months to years, not days or weeks. The time to progression depends on a number of factors, some of which have yet to be defined [98,99].

The natural course of HPV infection results in an evasion of the immune system. HPV replication and release do not cause cell death that normally would trigger an innate immune response. Thus, there is little or no generation of inflammatory cytokines important for dendritic cell activation [100]. Unlike natural infection with HPV, vaccination with L1 VLPs elicits a robust immune response [100].

Table 1
Summary of prophylactic vaccine efficacy (adapted from references [84,95,96])

Endpoint ^a	Vaccine			Placebo			Efficacy (%)	95% CI
	n	Cases	Rate ^b	n	Cases	Rate ^b		
(A) Per-protocol susceptible population								
HPV 16 or 18-related endpoints								
CIN 2	8579	0	0	8550	56	0.3	100	(93–100)
CIN 3	8579	1	<0.1	8550	51	0.2	98	(89–100)
AIS	8579	0	0	8550	7	<0.1	100	(31–100)
VIN 2/3	7811	0	0	7785	8	0.04	100	(42–100)
VaIN 2/3	7811	0	0	7785	7	0.04	100	(31–100)
HPV 6, 11, 16 or 18-related endpoints								
CIN 1	2241	0	0	2258	49	0.9	100	(92–100)
Condyloma	2261	0	0	2279	48	0.9	100	(92–100)
Vulvar condyloma	2261	0	0	2279	47	0.8	100	(92–100)
Vaginal condyloma	2261	0	0	2279	6	0.1	100	(14–100)
(B) Unrestricted susceptible population								
HPV 16 or 18-related endpoints								
CIN 2	9729	1	<0.01	9737	77	0.3	99	(93–100)
CIN 3	9729	2	<0.01	9737	75	0.3	97	(90–100)
AIS	9729	0	0	9737	10	<0.1	100	(55–100)
VIN 2/3	8757	1	0	8774	20	0.08	95	(69–100)
VaIN 2/3	8757	0	0	8774	9	0.03	100	(49–100)
HPV 6, 11, 16 or 18-related endpoints								
CIN 1	2667	2	0	2684	68	0.9	97	(89–100)
Condyloma	2667	3	<0.1	2684	67	0.9	96	(86–99)
Vulvar condyloma	2667	2	<0.1	2684	65	0.8	97	(89–100)
Vaginal condyloma	2667	1	<0.1	2684	8	0.1	87	(6–100)

^a A subject is counted only once within each applicable row. Some subjects are counted in more than one row.

^b Cases per 100 Person-Years at Risk.

Because of the lack of disease breakthrough to date with L1 VLP HPV vaccines, it has not been possible to establish a minimum level of antibodies needed to protect against clinical disease caused by HPV 6, 11, 16, and/or 18, nor to identify all the epitopes and antibodies actually involved in neutralization and how these vary with the conformation of the antigen. Because there is not a known correlate of protection, the interpretation of achieving a certain antibody level in response to HPV vaccination is not clear. In spite of the inherent limitations in using the immune response as a predictor of vaccine efficacy, the HPV 6/11/16/18 vaccine is being used in diverse settings worldwide, thus an evaluation to determine the impact of baseline covariates on the immune response was performed using a combined database of 6 Phase 2/3 trials [101]. The analysis included virginal 9–15-year-old girls, virginal 9–15-year-old boys, and 16–26-year-old young women, most of whom were sexually active. The two main factors which impacted the immune response were Day 1 serostatus and age at vaccination initiation. GMTs were higher in women who were seropositive at enrollment (resulting from exposure to HPV before enrollment) than the levels observed in control subjects. Fig. 2 summarizes the serum anti-HPV 6, 11, 16, and 18 responses 1 month after the completion of the vaccination regimen in girls and women, stratified by age at enrollment. For each of the 4 HPV types, Month 7 GMTs decreased as the age at first vaccination increased. The declines in Month 7 anti-HPV GMTs were steepest over the range of 9–15 years old at the initiation of vaccination.

Numeric differences in GMTs (as measured 1 month post-dose 3) were observed among subpopulations defined by race/ethnicity; however, no consistent pattern was observed across all vaccine types. North America subjects tended to have higher GMTs than Latin America, Asia, and European subjects. For each of the 4 HPV vaccine types, there were no significant differences in GMTs (as measured 1 month post-dose 3) between subjects who used hormonal contraceptives during the vaccination period and those who did not. Smoking status pre-vaccination also did not impact vaccine-induced immune response.

5.7. The role of immune memory

For vaccines, the demonstration of immune memory is the hallmark of long-term protection. Immune memory has been suspected to play a role in natural immunity to HPV 16 [102]. Immune memory is characterized by the ability to respond specifically and more rapidly upon a subsequent encounter with a pathogen or antigen. The aim of a prophylactic vaccine is to initiate a response to an antigen to which the immune system is naïve in order to prepare the immune system for subsequent exposure to the pathogen [103]. Adequate activation of adaptive immune memory culminates in the production and preservation of a memory pool of lymphocytes [103]. Prophylactic vaccination leads to long-term memory cell maintenance and protective efficacy. Humoral immunity prevents re-infection (and, therefore, provides long-term duration of protection) through the generation of highly specific immune memory cells. Immune memory yields a rapid and robust immune response upon repeated exposure to the specific pathogen or antigen [97].

Demonstration of an anamnestic response in vaccinees after an antigen challenge confirms the presence of immune memory. One study of the HPV 6/11/16/18 vaccine among a total of 552 women between the ages of 16–23 demonstrated that the vaccine induced an anamnestic response and had high efficacy through 5 years of follow-up (Fig. 3) [104]. The majority of subjects who were given a challenge dose of vaccine at Month 60 had anti-HPV levels, as measured 1 month post-challenge, that were above the levels that they achieved post-dose 3. An immune response typical of immune memory was observed amongst subjects who had initially received

a three dose regimen and who were seronegative by Month 60, 75% (6/8), 86% (6/7), 100% (1/1), and 97% (29/30) became seropositive to HPV 6, 11, 16, and 18 at 1 month post-challenge, respectively. Conversely, women who have been infected in the past and have cleared their infection mount an anamnestic response when administered quadrivalent HPV vaccine, pointing to the immunological similarity of the vaccine with the native virion [105].

5.8. Immunogenicity bridging

A Phase III immunogenicity study was conducted in young adolescents (both male and female) from 10 to 15 years of age [106]. This non-inferiority immunogenicity study was designed to bridge the efficacy findings in young women to preadolescent and adolescent girls and boys, as efficacy studies in younger adolescents are not feasible. This study enrolled 506 girls and 510 boys 10–15 years of age and 513 older adolescent and young adult females (16–23 years of age). The study included an assessment of immune response and safety.

The primary end point was an assessment of serum neutralizing antibodies to HPV 6, 11, 16, and 18 L1 proteins at 1 month post-dose 3. Prespecified seroconversion thresholds were ≥ 20 mMU/mL for HPV 6 and HPV 16, ≥ 16 mMU/mL for HPV 11, and ≥ 24 mMU/mL for HPV 18. Subjects in the study were followed for 14 days after each injection, and body temperature and injection site reactogenicity was assessed within 5 days after each injection. Serious vaccine-related adverse event information was collected throughout the study.

One month after a 3-dose vaccination regimen, the neutralizing antibody responses in boys and girls were statistically non-inferior ($p < 0.001$) and observationally higher (1.7–2.7-fold) than those observed in 16–23-year-old females. Seroconversion for each of the 4 vaccine HPV types was achieved in >99% of participants in the study. Seroconversion to anti-HPV 6, 11, and 16 was observed in 100% of subjects, regardless of age or gender. Rates of seroconversion to anti-HPV 18 were 100% in girls 10–15 years of age, 99.7% in boys 10–15 years of age, and 99.1% in women 16–23 years of age. It appears from these data that boys had a higher immune response than girls and young adult females, though the reason for this is unclear.

Vaccination was generally well tolerated. The incidence of all systemic adverse events and vaccine-related systemic events was similar among groups. Discontinuations and serious adverse events were rare. Although significantly more girls and boys experienced fevers than 16–23-year-old females, most (96.4%) fevers were low grade and resolved rapidly.

A second Phase III safety and immunogenicity study of HPV 6/11/16/18 vaccine enrolled female and male subjects 9–15 years of age [107]. This study was unique in that the safety comparator for the HPV 6/11/16/18 vaccine was a non-aluminum-containing saline placebo whereas, all other studies to date have compared the vaccine with aluminum-containing placebo. Safety, serum neutralizing antibody levels, and seroconversion rates were assessed. The immune response to vaccination among boys and girls 9–15 years of age was persistent through at least 1 year post-dose 3. An expected drop in anti-HPV responses was observed between Month 7 (1 month post-dose 3) and Month 18. In women aged 16–23, vaccine-induced anti-HPV responses have been shown to decline postvaccination, plateau between Months 18 and 24 and remain stable through at least 5 years [105,108].

In the adolescent study, HPV 6/11/16/18 vaccine was generally safe and well tolerated. A larger proportion of subjects who received the quadrivalent vaccine experienced injection site adverse experiences compared with placebo subjects. Few subjects discontinued vaccination because of an adverse experience.

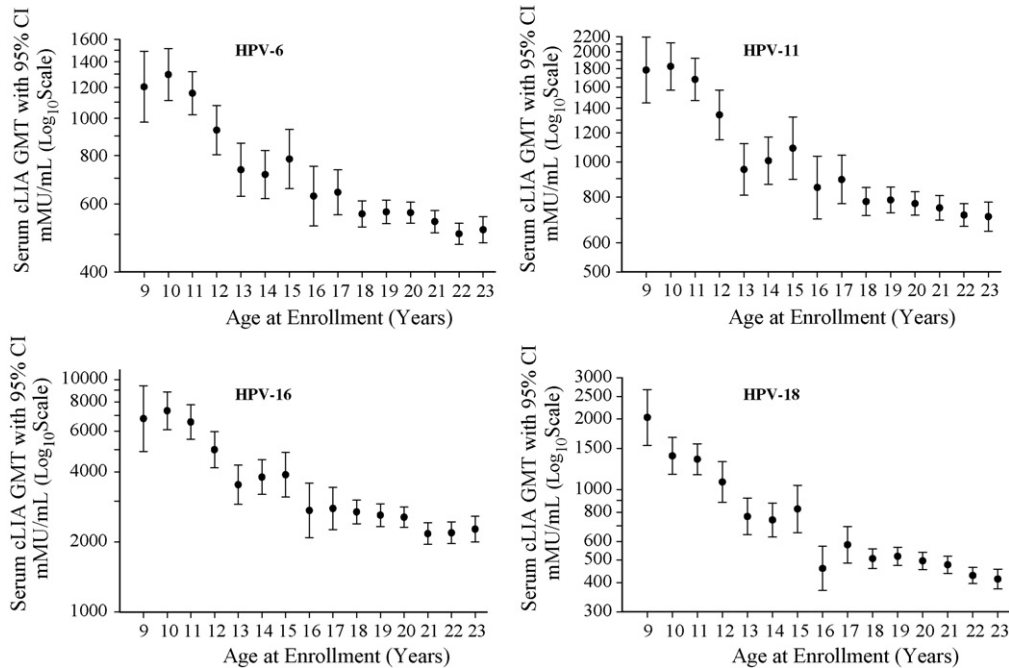


Fig. 2. Anti-HPV-6, -11, -16, and -18 responses 1 month after the completion of the vaccination regimen in girls and women, stratified by age at enrollment (adapted from reference [101]).

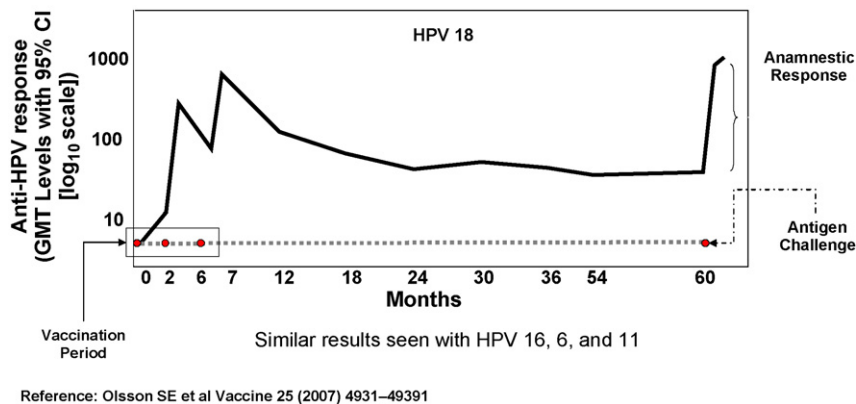
5.9. Cross-protection

The 18 HPV types that can cause cervical cancer are organized into 5 species based on L1 protein gene homology. HPV 16 and HPV 18 are prototype members of the A9 and A7 species of oncogenic HPVs, respectively. The HPV 16 and HPV 18 L1 proteins share varying degrees of homology with respective species members (A9: HPV 31, 33, 35, 52, 58; A7: HPV 39, 45, 59). Non-vaccine members of the A9 and A7 species are responsible for up to 20% of all cervical cancers, and an even larger proportion of CIN lesions. The remaining 3 oncogenic HPV Species (A5 [prototype HPV 51], A6 [prototype HPV 56], and A11 [prototype HPV 73]) include HPV types that rarely cause cancer, but commonly cause CIN. Thus, A5, A6, A7, A9, and A11 species members (other than HPV 16 and HPV 18) are responsible for a substantial proportion of the overall burden of clinical HPV disease.

Anti-HPV 16 and anti-HPV 18 generated by HPV 6/11/16/18 vaccine may be able to neutralize infection and prevent disease caused

by related HPV types. A key objective of the clinical program is to determine whether the vaccine’s prophylactic efficacy extends to HPV types whose L1 proteins share $\geq 80\%$ homology (at the amino acid level) with HPV 16 or HPV 18 and are individually responsible for $\geq 2\%$ of cervical cancers. This capability could increase the expected cancer reduction of this vaccine.

A pre-specified evaluation of the impact of the vaccine on the rates of infection and disease associated with these 10 non-vaccine HPV types was conducted in a population that was, pre-vaccination, seronegative and PCR negative to all of HPV 6, 11, 16, and 18 and PCR negative to all of 10 non-vaccine HPV types for which testing was available (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59). They were also required to have normal Pap test at the enrollment visit. It should be noted that as over 40 HPV types are known to infect the anogenital tract, this analysis only approximates vaccination of HPV-naïve females [109]. The first analysis of cross protection was presented at the 47th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy. Within approximately 3 years of



Reference: Olsson SE et al Vaccine 25 (2007) 4931–49391

Fig. 3. Persistence of anti-HPV 18 responses following a three-dose regimen of HPV 6/11/16/16 administered at Day 1, Month 2 and Month 6, followed by an antigen challenge at Month 60 (adapted from reference [104]).

Table 2
Human papillomavirus (HPV) vaccine-related injection site and systemic adverse events (adapted from reference [110])

Adverse experience	HPV vaccine (n = 5088) (%)	Aluminum-containing placebo (n = 3470) (%)	Saline placebo (n = 320) (%)	All placebo recipients (n = 3790) (%)
Injection site (1–5 days after vaccination)				
Pain	83.9	75.4	48.6	–
Swelling	25.4	15.8	7.3	–
Erythema	24.6	18.4	12.1	–
Pruritus	3.1	2.8	0.6	–
Systemic (1–15 days after vaccination) ^a				
Fever	10.3	–	–	8.6
Nausea	4.2	–	–	4.1
Dizziness	2.8	–	–	2.6

Note: The vaccine-related adverse experiences that were observed among recipients of quadrivalent HPV (6/11/16/18) vaccine were at a frequency of at least 1.0% and also at a greater frequency than that observed among placebo recipients.

^a The most frequently reported serious systemic adverse experiences (SAE) among quadrivalent vaccine recipients regardless of causality were headache (0.03% vaccine vs. 0.02% placebo); gastroenteritis (0.03% vaccine vs. 0.01% placebo); appendicitis (0.03% vaccine vs. 0.01% placebo); pelvic inflammatory disease (0.02% vaccine vs. 0.02% placebo) and urinary tract infection (0.02% vaccine vs. 0.02% placebo).

follow-up, combined efficacy for CIN 2/3 or AIS caused by all 10 non-vaccine HPV types was 38% (95% CI: 6, 60). The final end-of study data with approximately 3.6 years of follow-up (presented at the 24th International Papillomavirus Conference (IPC) in November 2007) showed similar high vaccine efficacy.

5.10. Safety

Safety data have been reported in all studies of quadrivalent HPV vaccine [84,85,105–107]. This includes data on pregnancy outcomes, as well as all serious adverse events, and systemic adverse events, categorized by organ system. The HPV vaccine is categorized by the United States Food and Drug Administration as a pregnancy Category B medication. This is the first vaccine designated as pregnancy category B; however, vaccination is not recommended during pregnancy.

Safety data have also been combined across five clinical trials, four of which were placebo controlled [110]. In all except one of the clinical trials, safety was assessed with vaccination report card-aided surveillance for 14 days after each injection of quadrivalent vaccine or placebo. Table 2 displays the proportion of subjects in the vaccine (n = 5088), adjuvant-containing placebo (n = 3470), and saline placebo groups (n = 320) who reported an injection site adverse event. This table includes those subjects who used vaccination report card-aided surveillance. Compared with the saline placebo group, administration of the vaccine and adjuvant-containing placebo was associated with higher incidences of

injection site adverse events. Subjects who received vaccine were more likely to report fever than were subjects in the placebo group.

Among all vaccinated subjects (i.e., those who received either placebo or quadrivalent HPV vaccine) with safety information, 206/21,464 reported a serious systemic adverse event [110]. The most frequently reported serious systemic adverse events among quadrivalent HPV vaccine recipients, regardless of causality, were headache (0.03% of vaccine recipients vs. 0.02% of placebo recipients), gastroenteritis (0.03% vs. 0.01%), appendicitis (0.03% vs. 0.01%), pelvic inflammatory disease (0.02% vs. 0.02%), and urinary tract infection (0.02% vs. 0.02%). During all of the clinical studies combined, 18 deaths were reported (11 vaccine recipients vs. 7 placebo recipients) [95]. None of the deaths were judged to be vaccine or placebo related. The most common cause of death was motor vehicle accident (four vaccine vs. three placebo), followed by overdose or suicide (two vaccine vs. two placebo), and pulmonary embolus or deep vein thrombosis (one vaccine vs. one placebo). There were two cases of sepsis, one case of pancreatic cancer, and one case of arrhythmia in the group that received quadrivalent vaccine, and one case of asphyxia in the placebo group [95].

5.11. Studies in women aged 26–45

The risk for HPV infection is lifelong and older adults remain at risk for HPV disease. Following an age-associated period of general decline in HPV, there is an increased prevalence of HPV infection in females after menopause [111]. In Chile, Colombia, and Mexico,

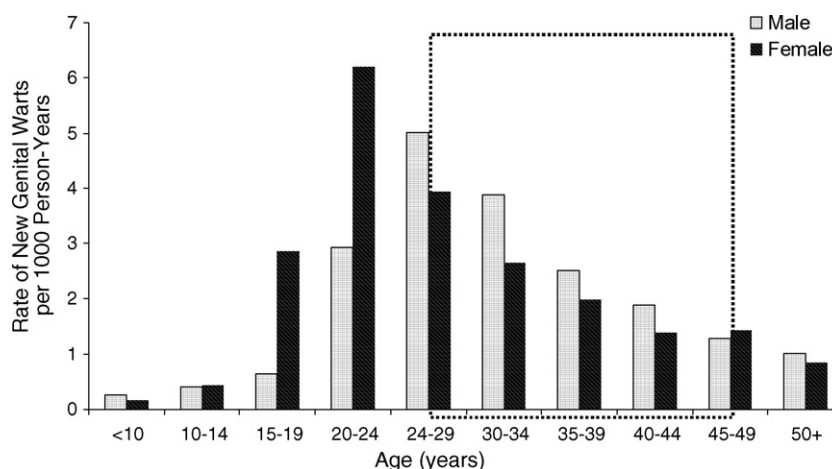


Fig. 4. Incidence of new genital warts by age in the United States (adapted from reference [68]).

although the first peak in prevalence is in younger women, a second peak has been observed among older women [112]. In Costa Rica, there is a U-shaped prevalence curve, with younger and older women having higher HPV prevalence than middle-aged women [113]. In a Canadian population of 955 women, HPV was highest in women aged 20–24 years, progressively declining until age 44 years, increasing significantly higher ($P=0.01$) among women ages 45–49 years [114].

This second prevalence peak in older women may result from reinfection with HPV or from reactivation of a latent HPV infection [111]. Immunity tends to decline with age [115] and risk of infection leading to cancer increases with age [116]. Fig. 4 shows the incidence of new genital warts by age in the United States [68]. As shown in Fig. 4, a substantial proportion of new cases of genital warts in women occurred in those above 24 years of age.

Social changes in many countries over the past 30 years such as the delay in first marriage and increasing divorce rates have further increased the risk of HPV infection among women in their late 20s, 30s, and 40s. There is currently an ongoing international, placebo-controlled, multi-center study of HPV 6/11/16/18 vaccine in 24–45-year-old women (termed FUTURE III). FUTURE III was designed to extend the efficacy findings in preventing HPV-related diseases in 16–26-year-old women to those through age 45. Because it is an extension study, the pre-specified endpoint is a composite of persistent infection and cervical disease. This is the first efficacy study of any HPV vaccine in adult women.

The study enrolled 3819 women with no history of cervical disease in the past 5 years, LEEP, hysterectomy, or genital warts. They received the same dosing regimen of HPV 6/11/16/18 vaccine or placebo as described for the younger cohorts. Pap testing, genital inspection and cervicovaginal sampling are being conducted every 6 months. Analyses include demographics, primary efficacy, tolerability, and immunogenicity. Data presented at the 24th International Papillomavirus Conference (IPC) in November 2007 has shown that HPV 6/11/16/18 vaccine significantly reduced the rate of infection and of disease due to HPV 6, 11, 16, and 18 in women up to the age of 45 who were naïve to the relevant HPV type.

5.12. Efficacy studies in men

There is an ongoing study to evaluate the efficacy of HPV 6/11/16/18 vaccine in young men. The primary efficacy objective is to demonstrate that HPV 6/11/16/18 vaccine reduces the combined incidence of HPV 6-, 11-, 16-, or 18-related genital warts, penile/perianal/perineal intraepithelial neoplasia, and penile, perianal, or perineal cancer in young men who are naïve to the relevant HPV type, compared with placebo. Included is a substudy in men who have sex with men (MSM). The MSM substudy efficacy objective is to investigate the impact of HPV 6/11/16/18 vaccine on the combined incidence of HPV 6-, 11-, 16-, or 18-related AIN or anal cancer in MSM who are naïve to the relevant HPV type. This randomized, double-blind placebo-controlled study of approximately 4000 men includes 16–26-year-olds with 0–5 lifetime partners. At regular intervals, genital sampling for HPV infection and inspection for genital warts are conducted. Participants in the MSM substudy will undergo anal Pap test/HPV testing at 6-month intervals with referral to high-resolution anoscopy for abnormalities. Subjects will be followed for approximately 3 years.

5.13. Pilot program: oropharyngeal cancer

There is currently a pilot program to evaluate methods for measurement of oropharyngeal HPV infection. The planned pilot study will be designed to evaluate methods of oral sample collection and methods of sample purification for evaluation of oral HPV

infection. Given the reported increased prevalence of oral HPV in HIV-infected individuals, HIV positive males age 18 and older will be randomized to one of several collection method groups. Written consent will be obtained from each subject prior to any study procedures being performed. HPV-related analyses will include type-specific PCR and line blot assays in oral specimens. If the pilot study is successful there is the potential to conduct natural history and Phase III studies in this area.

5.14. Long-term follow-up

Follow-up via the Nordic Cancer Registry program is an important and unique aspect of the clinical program for the HPV 6/11/16/18 vaccine. This will allow follow-up for at least 10 years post-licensure and will evaluate long-term vaccine effectiveness and safety, immunogenicity, and economic impact of combining vaccination with less intense screening programs. Overall, nearly 5500 Nordic females enrolled in FUTURE II will be followed via this registry. These subjects provide a “sentinel cohort” of the vaccine’s effects, since they have been vaccinated several years prior to the vaccine becoming available to the public.

In addition, the adolescent study protocol 018 was extended to provide 10-year immunogenicity, safety, and effectiveness data for the HPV 6/11/16/18 vaccine among those adolescents who were vaccinated between the ages of 9 and 18. All subjects who received 3 doses of vaccine will be eligible to participate in the extension. Serum samples will be collected and a physical exam will be performed annually until the age of 16. Starting at age 16, twice yearly visits will include the collection of sexual history and genital clinical specimens. The proposed study will follow subjects over 10 year with an interim analysis conducted at 5.5 years post-dose 3.

Vaccine induced anti-HPV responses will be described by age and gender. The incidence of HPV 6-, 11-, 16-, and 18-related CIN or cervical cancer, and HPV 6-, 11-, 16-, and 18-related external genital lesions (condyloma, VIN1–3, and VaIN 1–3) in females will be estimated and observationally compared to the incidence of the same outcomes among placebo subjects in previous Phase II/III trials. The incidence of HPV 6, 11, 16, and 18-related persistent infection and penile/perineal/perianal disease in males will be estimated and observationally compared to the incidence of the same outcomes among placebo subjects in aforementioned adult male study. If observed, the relationship between breakthrough disease and antibody levels, and risk factors for vaccine failure will be examined. Subjects will be monitored for serious adverse experiences (vaccine or procedure-related). All pregnancies will be followed for outcome. This planned 10-year study will provide the first long-term data among adolescents for any HPV vaccine.

6. Summary

HPV-related cancers, low-grade neoplasia, genital warts, and RRP constitute a substantial public health burden. A prophylactic quadrivalent HPV 6/11/16/18 vaccine is highly effective in reducing the risk of HPV 6-, 11-, 16-, and 18-associated anogenital diseases. There are ongoing studies to evaluate vaccine efficacy in men and adult women. Planned long-term efficacy and safety evaluations, as well as programs to evaluate vaccine impact on oropharyngeal cancer are also underway. Immunization with this vaccine holds promise for reducing the overall burden of clinical HPV disease.

Conflict of interest

E.B. and H.S. are employed by Merck and potentially hold stock and/or stock options in the company. Merck funded this study in its entirety.

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