Prophylactic HPV Vaccines

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Abstract: Cervical cancer and its precursor intra-epithelial lesions are linked to infection by a subset of human papillomavirus (HPV) types, the so-called "high-risk" HPVs, the most prevalent being HPV16 and HPV18. Two prophylactic vaccines containing combinations of the major capsid protein (L1) of HPV16 and HPV18 have been shown to efficiently prevent infection by inducing capsid-specific neutralizing antibodies. Since the year 2006 these vaccines have been implemented in many countries in the hope that the incidence of cervical cancer will be drastically reduced in coming decades. Nevertheless, the real efficacy of the present HPV vaccines in preventing cervical cancer is not known. This review summarizes the clinical studies that led to assess the prophylactic power of these vaccines and discusses some open questions and controversy on cervical cancer prevention in relation with the HPV vaccine.

INTRODUCTION

Human papillomaviruses (HPVs) are small non-enveloped viruses (Fig. **1**) with a genome consisting of a double-stranded circular DNA molecule, which has been divided into a non-coding regulatory region, an early region encoding a few proteins with viral replicating as well as cell proliferating and transforming functions, and a late region encoding the structural L1 and L2 proteins [1]. Over 100 HPV genotypes have been identified so far divided into two groups according to their cutaneous or mucosal tropism [2]. The later are of high clinical relevance because they cause various types of neoplasia: (i) benign genital warts, low-grade cervical epithelial proliferation and recurrent respiratory papillomatosis (RRP), which are caused by the so-called "low-risk" or non-oncogenic HPV types, the most prevalent being HPV6 and HPV11; and (ii) low- and high-grade epithelial abnormalities (cervical intraepithelial neoplasia, CIN) that may progress to cervical and anogenital cancers, which are caused by the so-called "high-risk" or oncogenic HPVs, the most prevalent of which are HPV16 and HPV18 [3].

Genital HPVs are the most common sexually transmitted infectious agents worldwide. HPV infection is asymptomatic, although it can be associated with mild cytologic abnormalities, and in most cases is a transient event. It has been estimated that 70% of new infections clear within one year and 90% within two years [4], while in 10% of cases infection persists. Average duration of infection with high-risk HPVs is approximately 8 months and is longer than persistent infection with low-risk HPVs. Prospective studies indicate that women with persistent infection with oncogenic HPV types are at significantly higher risk of developing precancerous CIN and eventually cancer compared with women who are transiently positive for the same virus types [5].

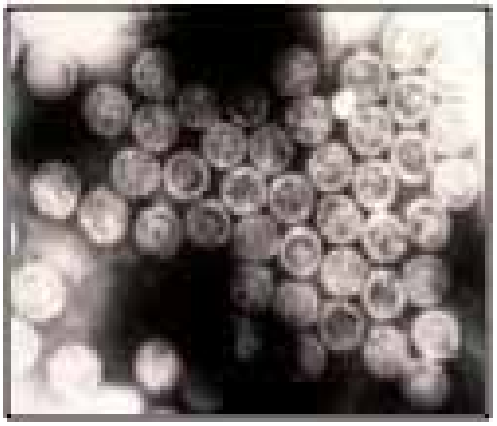

Fig. (1). HPV viral particles isolated from a cervical intraepithelial lesion. (Courtesy of IARC) [68].

Transmission of genital HPVs occurs primarily by sexual intercourse. HPV infection occurs soon after onset of sexual activity. An estimated 6.2 million new HPV infections occur yearly in the United States, of which 74% are in women aged 15-24 years. In a study on HPV prevalence in this country among women aged 14-59 years the overall prevalence of HPV (any type) was 26.8%, with nearly half of the cases occurring between ages 20-24 years [6]. Surprisingly, combined prevalence of the four types included in the vaccine Gardasil (HPV6, HPV11, HPV16, HPV18) was lower than expected at 3.4%. Theoretical estimates indicate that over 80% of sexually active women will have suffered a genital HPV infection by age 50 years. HPV infection is also common among men, with a prevalence of more than 20%

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among heterosexual men [7]. HPV16 and HPV18 account for about 70% of cervical cancer cases [8] and oropharyngeal cancers [9], and HPV-6 and HPV11 are associated with 90% of anogenital warts (condiloma) [10]. HPV viral load can determine progression through CIN of advanced grade and persistence of such premalignant lesions [11]. Further, co-infection with other sexually transmitted pathogens, such as HIV and HHV-2, facilitates progression to cervical cancer by HPV.

Since the 1950's mortality caused by cervical cancer in developed countries has decreased by about 60-70% and this tendency continues to fall by nearly 4% a year [12]. Such strong reduction has been due to widespread implementation of Papanicolaou (Pap smear) screening. However, such reductions are seen with organized population-based screening, and not with opportunistic unorganized screening. Besides Pap testing, several prospective studies have shown that condoms help prevent HPV infection. For example, a study among sexually active college women showed that consistent and correct condom use reduced HPV infection by 70% [13]. Nevertheless, in the United States during 2008 an estimated 11,070 new cases of cervical cancer were diagnosed and 3870 women died of cervical cancer during 2008 [12]. Cervical cancer is a leading cause of dead worldwide with half a million new cases and half this number deaths yearly [14].

In the year 2006, the US Food and Drug Administration licensed the quadrivalent HPV vaccine by Merck and Co., Inc. Using recombinant technology the L1 major capsid protein of HPV is produced in the yeast *Saccharomyces cerevisiae.* This protein has the capacity to self-assemble into structures called virus-like particles (VLPs), which resemble the HPV virions but contain no viral genome. Applied intramuscularly the VLPs in the vaccine elicit protective immune responses by inducing capsid-specific neutralizing antibodies that are also present at mucosal surfaces, so that if exposure to the virus occurs these antibodies will coat the virus and prevent it from entering the cells. In the year 2008 a bivalent vaccine directed against HPV16 and HPV18 by GlaxoSmithKline was approved for commercialization (Table **1**). These vaccines appear to confer a high degree of protection against the HPV types they include. However, the efficacy of these vaccines in preventing cervical cancer remains unclear. Therefore, long-term follow-up studies are needed to evaluate the prophylactic effectiveness of the current HPV vaccines.

DEVELOPMENT OF PROPHYLACTIC VACCINES AGAINST HPV

Because HPV virions are very difficult to obtain in cell culture systems *in vitro* [15], a vaccine made from attenuated virus has not been feasible. Thus, vaccines preventing infection by HPV have been obtained based on the induction of neutralizing antibodies against conformational epitopes of the L1 protein, which block virus entry into cervical keratinocytes. A vaccine based on the HPV16 $L2^{108\text{-}120}$ synthetic peptide showed cross-reactive antibody response to HPV1, 11, 16 and 18 when administered intranasally to mice [16]. In a phase I placebo-controlled clinical trial this peptide was administered intranasally to 13 healthy volunteers at 0.1 mg (n=5) or 0.5 mg (n=5) doses of peptide or placebo (n=3) without adjuvant. Inoculation induced anti-L2 antibodies binding to both HPV16 and HPV52 L1/L2-capsids in 4 out of 5 subjects receiving the highest dose of peptide [17] with no secondary effects.

However, peptide vaccines were soon relegated to a secondary place by the success of L1 VLP vaccines (Table **1**). In contrast to denatured L1 protein, VLPs retain conformational epitopes and hence the ability to induce neutralizing antibodies (IgG1 subclass) against the virus [18, 19]. Further, L1 VLPs of HPV16 are internalized by DCs that become activated and induce potent B and T cell responses against this virus [20, 21]. Studies with rabbits showed effective protection by VLPs against infection by the cottontail rabbit papillomavirus (CRPV) [22, 23] Further studies have shown that HPV VLPs stimulate humoral and cellular immune responses in Rhesus macaques vaccinated with either L1 VLPs or VLPs harboring fragments from proteins of the simian or HIV [24]. Since then, a wealth of studies using VLPs based on L1 alone or L1 and L2 of genital HPV produced in several systems have shown their potential as prophylactic vaccines [25].

CLINICAL STUDIES WITH MONOVALENT AND QUADRIVALENT VACCINES

Two phase II studies, one with a monovalent HPV-16 vaccine (protocol 005) and the other with a quadrivalent HPV6/11/16/18 vaccine (protocol 007), evaluated the

Table 1. HPV Vaccines

Vaccine	Production System	HPV Types	L1 Protein /Dose	Adjuvant	Administration Schedule
Quadrivalent vaccine Commercial name: Gardasil™ Manufacturer: Merck Sharp & Dohme	Yeast (S. cerevisiae) transformed with an L1-expressing plasmid	6/11/16/18	20/40/40/20 μg	225 μg aluminum hydroxyphosphate sulfate	0, 2, 6 months
Bivalent vaccine Commercial name: Cervarix™ Manufacturer: GlaxoSmithkline	Insect cell line (Trichoplusia ni) infected with L1 recombinant baculovirus	16/18	20/20 μg	500 μg aluminum hydroxide, 50 μg 3-O-deacylated-4'-monophosphoryl lipid A	0, 1, 6 months

efficacy of these vaccines taking persistent infection as end point. Two phase III studies of the quadrivalent HPV vaccine (protocols 013 and 015) evaluated the efficacy of the vaccine on precancerous lesions. Table **2** summarizes results of clinical studies with L1 VLP-based vaccines reported to date.

These studies used recombinant L1 VLPs of HPV16 (high risk) and HPV11 (low risk) produced in insect cells [26, 27] and yeast [28, 29], showed seroconversion in most vaccinated individuals and protection against infection over a follow-up period of two years. Titers of anti-L1 antibody achieved (predominantly IgG1) were higher than those seen in natural infection. The neutralizing nature of the antibodies was shown in a neutralizing assay using HPV16 pseudovirions [30] in the study of Harro *et al.*, while Evans *et al.* used an assay based on RT-PCR as described previously [31].

Harro *et al.* reported a double blind, placebo-controlled, dose-escalation trial to evaluate the safety and immunogenicity of a HPV16 L1 VLP vaccine in healthy adults. Various formulations were tested: one with no adjuvant, another with MF59 or alum as adjuvant. All of them were well tolerated. The intensity of the humoral response was dose-dependent only when the vaccine was administered without or with MF59 adjuvant and dose-independent when it was administered with aluminum hydroxy-phosphate sulphate as adjuvant [26]. The majority of vaccine recipients showed antibody titers 40-fold higher than those seen in natural infection. In addition, cross-reactivity against HPV18, 31 and 53 was observed in women vaccinated with HPV16 VLPs [32], although the relevance of such cross-reaction in terms of protection against those viruses remains to be determined. The HPV11 L1 vaccine tested in a phase I study by Evans *et al.* was also well tolerated and was able to induce neutralizing antibodies as well as lymphoproliferation in PBMC of vaccinated individuals upon stimulation with heterologous L1 antigens from HPV6 and HPV16, indicating that T cell helper epitopes are conserved across HPV types.

The phase II study reported by Koutsky *et al.* [28] (Protocol 005) used VLPs made of recombinant HPV16 L1 produced in the yeast *S. cerevisiae*, administered without preservative or with aluminum hydroxyphosphate and sulphate as adjuvant. The study was a double blind, placebo-controlled trial enrolling 2392 young women (aged 16-23 years) from 16 centers in the United States between October 1998 and November 1999, negative for HPV16 DNA and specific antibodies. Study participants were randomized to receive three doses of either placebo (1198 participants) or the vaccine (40 μg/dose) im. The primary end point was persistent HPV16 infection, defined as the detection of HPV-16 DNA in samples obtained at two or more time points. Seroconversion was observed in virtually all cases (99.7%) and the antibody titers were nearly 60-fold higher than those seen in persons infected with HPV16. During the follow-up period (a mean of 17.4 months) the incidence of persistent infection in the vaccinated group was zero, in contrast to 3.8% (woman-years at risk) in the placebo control group, with a total of 9 cases of HPV16-related CIN in this group. A follow-up study 3.5 years after vaccination showed the vaccine to provide 100% protection against HPV16–related CIN II or higher (CIN II+); efficacy against persistent infection decreased slightly to 94% (95% CI, 88%-98%) [33].

A subsequent phase II randomized double-blind, placebo-controlled study (Protocol 007) assessed the efficacy of the quadrivalent HPV-6/11/16/18 vaccine (Gardasil™) [34]. Primary endpoint was persistent infection with HPV6, 11, 16 or 18, CIN, cervical cancer or external genital disease caused by the HPV types included in the vaccine. Study participants were randomized to receive three doses of vaccine (277 women) or placebo (275). Persistent infection or disease associated with HPV6, 11, 16 or 18 decreased by 90% (95% CI, 71%-97%, P<.0001) in the group receiving vaccine as compared with the placebo group. Further analyses including two additional years follow-up were published in 2007 known as Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) I [35] and II [36].

In the FUTURE I study, a randomized double-blind, placebo controlled trial, 5455 participant women aged 16-24 years in 16 countries were selected from January 2002 through March 2003. The participants were randomized to receive vaccine (2723 women) or placebo (2732 women). Follow-up was for an average of 3 years after first vaccine dose. Composite end points were the incidence of genital warts, VIN or CIN, and the incidence of CIN, adenocarcinoma in situ or cancer associated with HPV-6/11/16 or 18. In the per-protocol susceptible population (PPSP) (those participants who completed the vaccination regimen, did not violate protocol, were seronegative, and had negative PCR results for the HPV strains included in the vaccine through 1 month after the third vaccine dose), vaccine efficacy was 100% (95% CI, 94%-100%) for each of the composite end points. In an unrestricted susceptible population (URSP) that included all women who were seronegative and had negative PCR results at baseline, some of whom might have violated the protocol, vaccine efficacy was 98% (95% CI, 92%-100%) against CIN and 95% (95% CI, 87%-99%) against vaginal and external anogenital lesions (Table **3**). In an intention-to-treat population (ITTP) that included all participants who had undergone randomization regardless of their baseline HPV status, efficacy was 55% (95% CI, 40%-66%) against cervical lesions and 73% (95% CI, 58%-83%) against vaginal and external anogenital lesions. A second ITTP analysis was done to evaluate lesions associated with any HPV type; vaccine efficacy was found to be 20% (95% CI, 8%-31%) against CIN I, 7.8% (95% CI, <0-27.2%) against CIN II, and 34% (95% CI, 15%-49%) against vaginal and external anogenital lesions (Table **3**). This study concluded that the quadrivalent vaccine significantly reduced the incidence of HPV-associated anogenital diseases [35].

The FUTURE II study examined vaccine efficacy against high-grade cervical lesions associated with HPV-16 and HPV18 [36] (Table **3**). The study was randomized, double-blind and placebo controlled. A total of 12167 participant women in 13 countries, aged 15-26 years, enrolled from June 2002 through May 2003, were assigned to receive three doses of vaccine (6087 women) or placebo (6080 women). The primary end point was CIN II+, adenocarcinoma in situ, or cervical cancer related to HPV-16 or 18. Results were reported for 5305 women in the vaccine group and 5260 women in the placebo group, who were followed for an average of 3 years after receiving the first dose of vaccine or

Table 2. Summary Results of Clinical Studies Carried out with Prophylactic Vaccines Against HPV

Composition / System of Production / Adjuvant	Via	Phase of Study	Size and Characteristics of the Sample	Clinical Response	Refs.
$L2^{108-120}$ synthetic peptide from HPV 16 with no adjuvant	IN	I	Placebo-controlled trial. 13 womwn Vaccine group: 0.1 mg (n=5) or 0.5 mg (n=5) doses of peptide / Placebo: n=3	Seroconversion in 4 out of 5 subjects receiving the highest dose of peptide No response in the 0.1 mg group	(a)
L1 VLPs from HPV 16 Produced in yeast Adjuvant: aluminum hydroxy phosphate sulphate	IM	II	Double blind, placebo-controlled trial 2392 Women aged 16-23 years, negative for HPV16 DNA. Vaccine: n=1194 / Placebo: n=1198 PEP: Persistent HPV16 infection Mean FUP: 17.4 months.	Vaccine well tolerated. Seroconversion in 99.7% of cases. No persistent HPV16 infections in the vaccine group 3.8% persistent HPV16 infections in the control group	(b)
L1 VLPs from HPV 6, 11, 16 and 18 Produced in yeast Adjuvant: aluminum hydroxy phosphate sulphate (Gardasil™, Merck & Co.)	IM	II	Randomized double-blind, placebo-controlled study (Vaccine: n=277 / Placebo: n=275) PEP: persistent infection with HPV6, 11, 16 or 18, CIN, cervical cancer or external genital disease caused by the HPV types included in the vaccine. FUP: 36 months	Persistent infection or disease associated with HPV6, 11, 16 or 18 decreased by 90% in women receiving vaccine)	(c)
	IM	III	Randomized, double-blind and placebo controlled study (FUTURE Istudy) 12167 women aged 15-26 years, in 13 countries, were distributed randomly to receive 3 doses of vaccine or placebo. Results reported for n=5305 (vaccine group) and n= 5260 (placebo group) PEP: CIN II or 3, adenocarcinoma in situ, or cervical cancer related to HPV16 or 18 FUP: 3 years from first dose	Vaccine efficacy for prevention of the primary end point was 98% in the per-protocol susceptible population and 44% in an intention-to-treat group 21 cases of CINII / III or 18 in the control group (placebo)	(d)
	IM	III	Randomized, double-blind and placebo controlled study 5455 women aged 16-24 years, were distributed randomly to receive 3 doses of vaccine or placebo. Results reported for n=2723 (vaccine group) and n= 2732 (placebo group) PEP: composite incidence of genital warts, VIN or CIN, and the incidence of CIN, adenocarcinoma in situ or cancer associated with HPV6, 11, 16 or 18 FUP: 3 years from first dose	Vaccine efficacy was 100% for each of the composite end points 21 cases of CINII / III or 18 in the control group (placebo)	(e)
L1 VLPs from HPV 16 and 18 Produced in baculovirus Adjuvant: AS04 (aluminum hydroxide plus monophosphoryl lipid A) (Cervarix™, GlaxoSmith-Kline)	IM	III	Randomized, double-blind, placebo controlled trial 1,113 women aged 15-25 years PEP: HPV infection assessed by cervical cytology and self-obtained cervicovaginal samplesFUP: max. 27 months	Vaccine efficacy was 91.6% against incident infection and 100% against persistent infection with HPV16 and 18	(f)
			Extended follow up study of the same trial: 4.5 years from first dose (HPV 007 study) Included only subjects that received all three doses of bivalent vaccine (n=393) or placebo (n=383) PEP: HPV infection assessed by HPV DNA detection in cervical samples, and cervical cytology yearly	Seropositivity for HPV16 and 18 antibodies was maintained during the follow-up period in 98% of vaccinated women Vaccine efficacy was 96.9% against incident infection, 100% against persistent infection, and 100% against CIN associated with both HPV types	(g)

Table 2. contd….

Composition / System of Production / Adjuvant	Via	Phase of Study	Size and Characteristics of the Sample	Clinical Response	Refs.
L1 VLPs from HPV 16 and 18 Produced in baculovirus Adjuvant: AS04 (aluminum hydroxide plus monophosphoryl lipid A) (Cervarix™, GlaxoSmith-Kline)	IM	III	Double blind, placebo-controlled trial (PATRICIA study) 18,525 women, 15-25 years of age, randomly assigned to receive HPV vaccine (n=9,258) or a control hepatitis A vaccine (n=9,267) PEP: vaccine efficacy against CIN II or higher associated with HPV16 or 18 FUP: interim analysis at 15 months	Vaccine efficacy was 90.4% 23 cases of CIN II+ were detected: 2 in the HPV16/18/AS04 vaccine group and 21 in the control group	(h)
	IM	III	Masked, community-based, randomized trial, conducted in Costa Rica 2189 women (aged 18-25 years) positive for HPV DNA at enrollment. Vaccine n=1088, Control n=1101 PEP: viral clearance FUP: 12 months	There was no evidence of increased viral clearance at 6 or 12 months in the group that received HPV vaccine compared with the control group	(i)

Abbreviations: FUP, follow-up period; IM, intramuscular; IN, intranasal; PEP, primary end point.
(a) [17]
(b) [28]
(c) [34]
(d) [36]
(e) [35]
(f) [44]
(g) [45]
(h) [46]
(i) [48]

placebo. In the PPSP, vaccine efficacy for prevention of the primary end point was 98% (95% CI, 86%-100%) and in an ITTP group of the complete randomized population (women with or without previous infection) it was 44% (95% CI, 26%-58%) (Table **3**). In a second ITTP analysis the estimated vaccine efficacy for all high-grade lesions irrespective of HPV type efficacy was 17% (95% CI, 1%-31%). Therefore, there is no evidence of protection against disease caused by HPV types for which the participant women were positive at enrollment.

The published efficacy data of the quadrivalent HPV6/11/16/18 vaccine summarized in Table **3** can be considered at two levels:

1) Efficacy against pre-cancer (CIN) lesions. Data from the two main studies indicate that for the population aged 15 to 26 years a reduction in the number of cases of pre-cancer lesions CIN I+ and CIN II+ of 17% and 7.8%, respectively, can be expected. For girls aged 12 to 14 years there are no direct reports on efficacy. However, for the population of girls and women vaccinated before the onset of sexual activity reductions of 16.9% and 27% were reported (Table **3**). The retrospective subgroup analysis reporting a 46.1% efficacy cannot be considered, as it has not been documented properly. The fact that this estimate is lower than the expected 70% is explained in the same report by baseline high-risk HPV infections that were not detected by Pap- test or the current HPV testing methods [37]. However, this is incongruent with the fact that the explored group was a sexually naïve population in which no infection should be expected.

2) Efficacy against cervical cancer [38-40]. A comparative study on HPV type distribution among high-grade cervical lesions and cervical cancer showed that HPV16 and HPV18 are associated with 25% of CIN I and 52% of CIN II and III lesions, and with 70% of cervical cancers [40]. Based on this, it has been assumed that the implementation of the HPV-16 and HPV-18 vaccines should reduce in 70% the number of cervical cancer cases. At present it is not possible to document this assumption with available data from clinical trials, as long-term studies are required. Indeed, in the FUTURE I study efficacy in the ITT-population showed a tendency to diminish in cervical lesions of higher grades (see Table **3**, FUTURE I, end point CIN I+ vs. CIN II+, 20% and 7.8% efficacy, respectively). In the FUTURE II study, an efficacy of 98% against CIN II+ lesions associated with HPV16 and HPV18 was reported in the population vaccinated before the onset of sexual activity that was negative for these virus types. As indicated, the degree and duration of such protection remains to be determined in ongoing studies.

CLINICAL STUDIES WITH THE BIVALENT VACCINE

A bivalent vaccine that contains L1 VLPs of HPV types 16 and 18 produced in a baculovirus system and AS04 as adjuvant. The AS04 adjuvant is formulated with 3-O-desacyl-4'monophosphoryl lipid A (MPL) and aluminum salts, which enhances the initiation of the immune response by activating innate immunity. This new adjuvant seems to be safe, according to an integrated analysis of individual data of the HPV-16/18 vaccine trials (n=39,160) for the relative risk of experiencing any autoimmune event [41-43]. The HPV16/18/AS04 vaccine was tested in a multicenter double-blind, randomized, placebo controlled trial [44] in which a total of 1,113 women (15-25 years of age) were randomized

Table 3. Summary of Reported Efficacy Results Obtained in Clinical Studies Carried out with GARDASIL®

Study	Population	End point	Efficacy (95% CI)	Ref.
Population: All women of age 15-26 years who had undergone randomization (those with or without previous infection) Vaccine efficacy against all high-grade cervical lesions, regardless of causal HPV type				
Future I	ITTP: All women of age 16-24 years	CIN I +	20% (8-31)	(a)
	ITTP: All women of age 16-24 years	CIN II +	7.8% (<0-27.2)	(b)
Future II	ITTP: All women of age 15-26 years	CIN II +	17% (1-31)	(c)
Population: All women of age 15-26 years Vaccine efficacy restricted to the prevention of primary composite end point (cervical intraepithelial neoplasia grade 2 or 3, adenocarcinoma in situ, or cervical cancer related to HPV-16 or HPV-18)				
Future II	ITTP: All women of age 15-26 years	CIN II/III + HPV16/18 ca.	44% (26-58)	(c)
Population: Women who had not been previously infected with HPV-16 or HPV-18 Vaccine efficacy against cervical intraepithelial neoplasia related to any HPV type				
Future II	PPSP: HPV 16 and 18 negative up to 7 months, no protocol violations	CIN II +	NR	(c)
	URSP: HPV16 and 18 negative at enrollment, protocol violations possible	CIN II +	27% (4-44)	(c)
Future I and Future II and Phase II study	PPSP: HPV-6, -11, -16 and -18 negative at enrollment, remained HPV DNA-negative through 1 month after the administration of the third dose of vaccine or placebo (month 7)	CIN II +	16.9% (<0-39.8)	(d)
Future I and Future II	RMITT-2: HPV6, 11, 16 and 18 negative at enrollment, and normal PAP-test, at least one vaccine dose	CIN II +	37.9% (13.2-55.9)	(e, f)
Population: Women who had not been previously infected with HPV-16 or HPV-18 Vaccine efficacy against cervical intraepithelial neoplasia related to HPV-16 and/or HPV-18				
Future II	PPSP: HPV-16 and -18 negative until 7th month, no protocol violations	Only HPV-16 and/or HPV-18-associated CIN II	98% (86-100)	(c)
	URSP: HPV-16 and -18 negative at enrollment, protocol violations possible		95% (85-99)	(c)
Retrospective analysis				
Future I and Future II and others	RMITT-2 new: HPV6, 11, 16 and 18 negative at enrollment, and normal PAP-test, and negative for another 10 high-risk HPV types tested	CIN II + Due to any HPV type	46.1%	(b)

CI: Confidence Interval
FUTURE = Females United To Unilaterally Reduce Endo/Ectocervical Disease
ITTP: Intention-to-treat population
NR: Not reported
PPSP: Per-protocol susceptible population
RMITT: Restricted modified intention to treat population
URSP: Unrestricted susceptible population
(a) [35]
(b) [37]
(c) [36]
(d) [38]
(e) [39]
(f) [40]

to receive three doses of vaccine or placebo. Primary end point was HPV infection assessed by cervical cytology and self-obtained cervicovaginal samples for up to 27 months. The authors reported that the vaccine was well tolerated and highly immunogenic and that its efficacy was 91.6% against incident infection and 100% against persistent infection with HPV16 and 18. In the ITTP analyses, efficacy was 95.1% against persistent infection and 92.2% against abnormal cytology associated with these virus types. In a follow-up study of this trial (HPV-007 study), the vaccine was shown to provide 100% protection over a period of 4.5 years [45]. This study included only the subset of women who originally received all three doses of bivalent vaccine (n=393) or placebo (n=383). End point was HPV infection assessed by

HPV DNA detection in cervical samples as well as yearly cervical cytology. The study showed that seropositivity for HPV-16 and HPV-18 antibodies was maintained during the follow-up period in 98% of vaccinated women. Vaccine efficacy was 96.9% against incident infection, 100% against persistent infection (12 month definition), and 100% against CIN associated with both HPV types included in the vaccine. Evidence of protection against incident infection with HPV31 and 45 was also reported.

A larger phase III multicenter, international study was initiated to assess efficacy of the HPV16/18/AS04 vaccine against infection with these HPV types (PATRICIA study). After excluding 119 women because they presented high-grade or missing cytology results, a total of 18,525 women, 15-25 years of age, were randomly assigned to receive HPV vaccine (9,258 women) or a control hepatitis A vaccine (9,267 women). The vaccinated population under study included women who had prevalent infection with oncogenic HPV types, as well as low-grade cytological abnormalities at study entry and who received at least one dose of the vaccine. The primary end point was vaccine efficacy against CIN II+ associated with HPV16 or HPV18 infection assessed in women who were seronegative and HPV negative at entering the trial. An interim analysis for efficacy was performed at approximately 15 months after the first vaccine dose, when 23 cases of CIN II+ with HPV16 or HPV18 DNA in the lesion were detected [46]. Of these, 2 were recorded in the HPV16/18/AS04 vaccine group and 21 in the control group. Thus, vaccine efficacy against CIN II+ containing HPV16 or HPV18 DNA was 90.4%. A later phase III study, non-randomized, open-label and age-stratified (age 15-25 years compared to 26-55 years), showed high level of total HPV-16/18 IgG antibodies in the serum and at the cervix up to 24 months after the first dose of vaccine [47]. Regardless of age, the levels of HPV-16 and HPV-18 antibodies in the cervicovaginal secretions were highly correlated with antibody levels detected in serum samples, indicating that the HPV16/18-specific antibodies transude to the cervical epithelium and confer site-specific immunity.

In yet another phase III study, a masked, community-based, randomized trial was conducted in Costa Rica. This study included 2189 women (aged 18-25 years) positive for HPV DNA at enrollment, who were randomly distributed to receive three doses of the bivalent HPV16/18/AS04 vaccine (1088 women) or control hepatitis A vaccine (1101 women). Endpoint was viral clearance assessed by detection of HPV DNA by a molecular hybridization assay and by PCR. It was found that in women positive for HPV, vaccination does not facilitate clearance of the virus: HPV16/18 clearance rates at 12 months were 48.8% in the HPV vaccine group and 49.8% in the control group. Hence, it was concluded that the vaccine should not be used to treat prevalent infections [48].

DURATION OF PROTECTION

Long-term duration of protection by HPV vaccines is a crucial parameter that remains to be determined, since observations in published reports are limited to 5 years after vaccination. The fact that no minimum protective titer has been determined yet adds uncertainty to the actual protection provided by these vaccines. In vaccinated women antibody titers against HPV16 L1 decline gradually after the third dose and seem to reach a plateau by 24 months. By 36 months geometric mean titers in the vaccine group were higher than those in women of the placebo group who were HPV-16 seropositive at enrollment, suggesting that vaccination elicits antibody titers higher than those induced by natural infection [49]. Immunogenicity studies in female children aged 9 to 15 years appear to indicate that serologic responses to HPV are comparable to those of women aged 16 to 26 years [50-52].

SAFETY

Available safety data from clinical trials include 11,778 vaccine recipients and 9686 placebo recipients aged 9 to 26 years, and an additional group of 5088 vaccine and 3790 placebo recipients [52, 53]. Report cards for 14 days after each injection were used. Pain at injection site was the most common adverse event and was reported by 83.9% of vaccine recipients and by 75.4% receiving aluminum placebo and 48.6% receiving saline placebo; swelling and erythema were also reported each by 25% of participants. Systemic adverse effects in both vaccine and control groups included fever (13%) nausea (6.7%), dizziness (4%) and diarrhea (3.6%). Serious adverse events, such as hypertension, bronchospasm, gastroenteritis, vaginal hemorrhage, headaches and rigidity occurred in about 0.1% of participants. Further, 10 vaccine and 7 placebo recipients died during the clinical trials; however, none of these deaths was considered to be vaccine related [52, 53]. Besides this data, in the United States the Food and Drug Administration (FDA) produced through the Vaccine Adverse Event Reporting System (VAERS) nearly 8900 reports from 2006 through 2008 [52, 54, 55]. In this period there were 38 Guillain-Barre syndrom (GBS) reports, a serious illness of the nervous system that can result in paralysis. The Center for Disease Control (CDC) pointed out that because GBS occurs at a rate of 1-2/100,000 person years, it is likely that some cases will occur after vaccination but will not be due to vaccination [56]. In addition, 18 people died after receiving the vaccine. While the deaths were quite possibly not linked to the vaccine, eleven of them occurred less than a week after receiving the vaccine, and seven in less than two days. The most common diagnosed cause (~25%) was blood clotting. Further causes were myocarditis, arrhythmia and meningitis.

At present, the HPV vaccines are not licensed for children younger than 9 years or women older than 26. The vaccine is contraindicated in people with hypersensitivity to yeast or any component included in the vaccine. It should not be administered to patients with moderate to severe diseases, and is not recommended the administration to pregnant women, although studies in rats showed no evidence of impaired fertility or harm to the fetus. However, it is unknown whether Gardasil may have long term effects on fertility. The vaccine is licensed to be administered to lactating women, although it is unknown whether vaccine antigens or antibodies induced by the vaccine are excreted in human milk, and a three-fold higher number of breastfeeding infants (n=6) whose mothers received Gardasil had acute respiratory illnesses within 30 days as compared to the

placebo group, as reported by the manufacturer to the FDA [52, 54, 55]. Vaccinees should be observed for syncope for 15 minutes after vaccine administration.

When the FDA fast-tracked Gardasil, Merck and Co. Inc. agreed to conduct a safety surveillance study including 44,000 vaccinated subjects, including a number of children aged 11-12 years, who would be followed for 60 days for assessment of short-term safety (i.e., emergency room visits, hospitalizations, and deaths), as well as an additional 6 months subsequent to vaccination for new autoimmune disorders, rheumatic conditions, or thyroiditis. The final study report should be submitted by September 2009 when the vaccine will be fully evaluated for safety [57].

COST-EFFECTIVENESS CALCULATIONS

Before long-term data about the impact of HPV vaccination on the rate of cervical cancer become available, which will take several decades, mathematical models can help estimate cost-effectiveness ratios and hence the magnitude of the benefit of vaccination. In these models benefit is usually expressed as quality-adjusted life-years (QALYs) gained. QALY is a measurement unit of the increase in years of life due to a public health intervention considering its effect on life quality. It is calculated correcting life expectancy with a quality index (Q factor), which ranges from 1 (best quality) to 0 (dead).

Several mathematic models have been applied to extend previous studies on HPV vaccination and examine the prospective clinical benefit, defined as increased life expectancy. In one of them [58], the authors considered a hypothetical sample population of 12 year-old girls in the United States, assuming a vaccination rate of 70% against high-risk HPV and a comparable regime of Pap-tests for vaccinated and non-vaccinated. According to this study, it would be necessary to vaccinate 600 girls to prevent a single case of cervical cancer. The cost of prophylactic vaccination would be higher than the current follow-up and treatment protocols, but life expectancy would increase by 2.8 days. This should be compared with increases of 2.7, 3.0 and 3.3 calculated for measles, mumps and pertussis vaccines, respectively, albeit these at much lower cost. In another study [59], it was concluded that the most cost-effective strategy would be vaccination of 12 year-old girls followed by Pap-tests every two years after the age of 24. Other authors suggest that comparable but more cost-effective results would be obtained with vaccination of 12 year-olds followed by Pap-test every three years starting at the age of 25 [60]. These studies assume a three-dose vaccination protocol with booster every 10 years.

A 75% reduction in cervical cancer at a cost of $3,000 (year 2005 dollars) per (QALY) has been estimated assuming: (i) vaccination at age 12 years or younger with the HPV6/11/16/18 vaccine (average cost of $360 per vaccination series), (ii) 90% efficacy against infection, (iii) 100% efficacy against diseases attributable to the targeted HPV types, (iv) lifelong duration of protection, and (v) 70% vaccine coverage [61]. In yet another study, assuming 100% vaccine coverage, 90% vaccine efficacy against HPV 16/18, lifetime duration of protection, and a cost of $377 per vaccine series an estimated 58% reduction was achieved in the lifetime risk for cervical cancer for the vaccinated cohort at a cost of $24,300 (year 2002 dollars) [60].

However, more recent calculations also assuming vaccination with the HPV6/11/16/18 vaccine at age 12 years against Pap-test alone, and assuming lifelong duration of protection, predict costs of about $43,600 per QALY [62], still under the threshold of $50,000 per QALY gained, above which a public health intervention is considered not to be attractive [62]. However, the cost-effectiveness ratio for extending vaccination as a temporary catch-up program to the age of 21 years was $120,400 per QALY, and to the age of 26 years was $152,700 per QALY, even though the benefit on reduction of the incidence of genital warts due to the additional vaccination against HPV6 and HPV11 was considered. In this model, the results were sensitive to the duration of vaccine-induced immunity, so that if immunity waned after 10 years, the cost of vaccination of preadolescent girls exceeded $140,000 per QALY, and catch-up strategies were less cost-effective than screening alone. A reduction in the risk of disease caused by infection with high-risk HPV types not included in the vaccine (cross-reactivity) would have little consequence on the cost-effectiveness ratio. In contrast, an increase of 5% or higher in the rate of infections with these virus types (replacement) would make the vaccine cost-ineffective. Thus, the cost-effectiveness of the vaccine will depend on the duration of vaccine-induced protection and on the degree of replacement of the vaccine-targeted HPV types with other high-risk types.

Women in the United States are screened frequently for early detection of cervical dysplasia. Approximately 50 million Pap tests are performed every year in this country with a cost of over four billion dollars a year. HPV-vaccinees should still have Pap screenings regularly, so the vaccine would not have effect on Pap screening costs nor eliminates the need for HPV screenings as the vaccine will not protect against diseases caused by all other high-risk HPV types [55].

DISCUSSION

The bivalent and quadrivalent vaccines are thought to be effective in protecting against HPV16 and 18 infection and the precancerous lesions that they cause. On June 2006, the US Food and Drug Administration licensed the quadrivalent HPV vaccine by Merck and Co., Inc. The bivalent vaccine (Cervarix™) was approved during the year 2008. The designated target group for immunization with HPV vaccines is peri-pubertal females. It will be decades before the vaccinated population reaches the age at which women most frequently get cervical cancer (mid to end forties). This means that it will be a long time before we know the vaccine's real efficacy to prevent cervical cancer.

Ongoing follow-up studies should provide information as to the suitability of the three-dose vaccination protocol and extent of the immune response, the duration of protection against HPV infection, the possibility that these vaccines induce cross-protection against other oncogenic HPV types, and the protection against cervical cancer induced by the vaccines in broad populations and age ranges of women. Duration of protection due to the vaccine in the long term is

still an unknown variable that will determine the age to start with Pap-tests and their frequency. Another point to clarify how the other oncogenic genital HPVs will behave after protection against HPV16 and HPV18 has been achieved, since it cannot be ruled out that these will evolve to occupy the niche left by the viral types targeted by the vaccines (replacement) [63, 64].

There are several factors that may restrict the impact of these vaccines on cervical cancer. The first, and most important, is their elevated cost. Gardasil is the most expensive vaccine ever recommended by the FDA. It has been estimated that this vaccine alone will be more expensive than all other childhood vaccines put together (John Schiller, National Cancer Institute) [65]. The current market price of the vaccine (3 doses) is $360 (year 2009 dollars); however, vaccine administration, including physician's visits, increases actual cost. In addition, the costs of vaccination are added to those for Pap tests and HPV diagnostics, which need to be applied also to the vaccinated population. The most recent calculations of cost-effectiveness in terms of cost per QALY gained under a variety of combinations of assumptions reveal that the HPV-16 and HPV18 vaccine may be expected to be economically attractive (at a cost below $50,000 per QALY) only if the vaccine induces life-long immunity and if high-coverage is achieved in the primary target group of girls aged 12 years. If immunity lasted 10 years the vaccination of preadolescent girls exceeded $140,000 per QALY. If a booster was required to maintain lifelong immunity, and there was inconsistency in vaccination coverage and screening the cost per QALY would be above $200,000.

There are two obvious uncertainties in these calculations: the duration of immunity and the actual vaccine efficacy. Immunologic data provided evidence for a strong initial immune response with antibody levels superior to those after natural infection [33,45]. However, data in published reports is limited to 5 years after vaccination and a longer period will be needed to determine this critical parameter. Therefore, it is still too early to assume that the efficacy of 98% against CIN II+ lesions associated with HPV-16 and HPV-18 (Table **3**) can be extrapolated to the same degree of protection against cervical cancer. The estimation of the benefit of vaccination is complicated by the efficacy of the extensive prevention programs (cytology-based screening) implemented since many years in developed countries. According to estimates of the American Cancer Society, the number of cervical cancer cases declined 74% between 1955 and 1992, and the rate continues to decrease yearly. With these statistics in mind, there is controversy in the United States [54] as well as in European countries like Germany [66] and Sweden [67], as to whether vaccination of children will provide a reasonable benefit preventing cervical cancer, if it will be cost-effective, and if it will be free of unforeseen secondary effects which should be addressed in further studies.

Apart from preadolescent girls, other groups (e.g. young men or sexually active women of all ages) may benefit from vaccination, since it would help prevent more efficiently high-risk HPV propagation, and because these viruses are also associated with cancer of the penis, as well as anal and head and neck cancers, which affect both females and males. However, the cost effectiveness of such interventions would be well above the baseline calculations of current models [62]. An important factor for the success of prophylactic vaccination will be the way the population accepts the vaccine, since there could be some conflict, as no precedent exists for vaccinating 10-12 year old children against a sexually transmitted disease. This factor might be of high relevance, as it has been estimated in cost calculation models that if 5% of women were neither screened nor vaccinated, all strategies that involved a catch-up program would exceed $100,000 per QALY, and catch-up to 26 years of age would exceed $200,000 per QALY [62]. In addition, vaccination might lead to the misinterpretation that screening is no longer necessary, which would introduce another variable that will need to be considered in future cost evaluations.

In developing countries, where cervical cancer is still the most frequent cancer in women, the elevated cost of the HPV vaccines at present restricts their implementation. Cost-effectiveness studies have not been applied to the specific parameters of these countries. However, extrapolating recent studies modeling HPV vaccination cost-effectiveness in the United States, it can be predicted that the calculated costs per QALY gained in the different scenarios are not affordable at present vaccine prices ($120, year 2009 dollars), even under the most favorable conditions, i.e. if high coverage could be achieved in the primary target group of 12-year-old girls and if vaccine-induced immunity lasted lifelong. Nevertheless, since in general developing countries do not have extensive secondary-prevention programs, implementation of HPV vaccines could be the best intervention against HPV-associated disease and also cost-effective, provided that vaccine efficacy and long-lasting immunity are confirmed, and that the prices of the vaccines are reduced in these countries.

In conclusion, the impact of HPV vaccination on the rate of cervical cancer will not be perceptible for decades. In the mean time, decisions on the way vaccine programs are applied will depend on future studies reporting intermediate data on duration of protection, efficacy and cost effectiveness. Nonetheless, these vaccines are expected to reduce but not eliminate the risk of cervical cancer since they only target two of the oncogenic genital HPV types. Studies with multivalent vaccines would be necessary to extend the degree of protection of the vaccine.

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