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EXPERT REVIEWS

9-Valent HPV vaccine for cancers, pre-cancers and genital warts related to HPV

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Human papillomavirus (HPV) is the causative agent of nearly all cervical cancer cases as well as a substantial proportion of anal, vulvar, vaginal, penile and oropharyngeal cancers, making it responsible for approximately 5% of the global cancer burden. The first-generation HPV vaccines that is, quadrivalent HPV type 6/11/16/18 vaccine and bivalent HPV type 16/18 vaccine were licensed in 2006 and 2007, respectively. A second-generation 9-valent HPV type 6/11/16/18/31/33/45/52/58 vaccine with broader cancer coverage was initiated even before the first vaccines were approved. By preventing HPV infection and disease due to HPV31/33/45/52/58, the 9vHPV vaccine has the potential to increase prevention of cervical cancer from 70 to 90%. In addition, the 9vHPV vaccine has the potential to prevent 85–95% of HPV-related vulvar, vaginal and anal cancers. Overall, the 9vHPV vaccine addresses a significant unmet medical need, although further health economics and implementation research is needed.

Keywords: cancer prevention • genital warts • human papillomavirus • pre cancers • prophylactic vaccine

First-generation HPV vaccines

Human papillomavirus (HPV) is a family of over 150 related, non-enveloped, epitheliotropic DNA viruses [1]. Twelve types of HPV (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) have been recognized as oncogenic by the International Agency for Research on Cancer [2]. HPV is the causative agent of nearly all cervical cancer cases, as well as a substantial proportion of anal, vulvar, vaginal, penile and oropharyngeal cancers [3]; thus HPV is responsible for approximately 5% of the global cancer burden [4]. Mature viral particles are composed of 72 pentamers of L1 proteins arranged in icosahedral symmetry. Prophylactic HPV vaccines have been developed based on recombinant L1 proteins that self-assemble in virus-like particles (VLP) [5]. The first-generation HPV vaccines, including the quadrivalent HPV6/11/16/18 L1 VLP (qHPV) and bivalent HPV16/18 L1 VLP (bHPV) vaccines, address oncogenic HPV types 16 and 18, which were recognized as causing most cervical cancer cases worldwide [6]. In clinical studies, both vaccines were shown to prevent cervical dysplasia related to oncogenic HPV types 16 and 18 [7–9]. In clinical studies, the qHPV vaccine was also shown to prevent vulvar, vaginal and anal dysplasia related to HPV types 16 and 18 and anogenital warts related to HPV types 6 and 11 [7,10–12].

Since 2006, these two HPV vaccines have been widely licensed (in >100 countries) [13]. By 2014, HPV vaccination had been included in the national immunization programs of at least 57 countries [14]. Real world data collected in the 10 years since licensure in several countries have shown marked reductions in the prevalence of HPV vaccine-type related infection [15–19] and the incidence of high-grade cervical abnormalities [20–26] and of genital warts [17,25,27–35]. Analyses of clinical trial and post-licensure data reinforce the favorable safety profile of these two HPV vaccines [36–44].

Given the high amino acid homology between the L1 capsid proteins of phylogenetically related HPV types [45,46], it was hypothesized that vaccination with bHPV and qHPV vaccines may generate cross-reactive antibodies that may be able to bind and possibly neutralize virions of HPV types closely related to HPV16 and/or HPV18 that are not

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Table 1. Estimated type contribution for certain HPV-related cancer & disease cases.

	HPV 6, 11, 16 and 18	HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58
Cervical cancer cases	70% [52]	90% [52]
CIN 2/3	50% [50]	80% [50]
CIN 1	25% [50]	50% [50]
Vulvar cancer cases†	80% [54]	90% [54]
VIN 2/3	80% [54]	95% [54]
Vaginal cancer cases†	65% [56]	85% [56]
VaIN 2/3	65% [56]	80% [56]
Anal cancer cases†	85% [55]	95% [55]
AIN 2/3	80% [55]	85% [55]
Genital warts	90% [86]	90% [86]

†Not all pre-cancers and lesions are related to HPV. Approximately 30% of vulvar cancers, 70–75% of vaginal cancers and 90–95% of anal cancers are related to HPV. Data shown are for HPV-related cancers.

represented in the vaccines, which in turn may prevent infection and disease associated with these other types (cross-protection). Partial cross-protection against other phylogenetically related oncogenic HPV types had been reported for both the qHPV and bHPV vaccines in clinical trials and in real world public health programs where high coverage has occurred. However, the observed cross-protection is only partial, and its extent, duration and public health significance remain to be demonstrated [5,16,47,48].

Addressing an unmet medical need – rationale for a second-generation, multivalent HPV vaccine

During the clinical development of the qHPV and bHPV vaccines, it became known that oncogenic HPV types 31/33/45/52/58 represented the next five most frequent types causing cervical cancers worldwide [49]. Therefore, a program to develop a 9-valent HPV (9vHPV) vaccine with higher cervical cancer coverage including the types already covered by existing vaccines, as well as these five additional types, was initiated even before the first licensure of an HPV vaccine in 2006.

In parallel, several researchers began estimating the contribution of the nine HPV types contained in the vaccine (HPV6/11/16/18/31/33/45/52/59) to anogenital disease, in an attempt to estimate the potential impact of this vaccine. This included analyses of low- and high-grade cervical disease (cervical intraepithelial neoplasia grade 1 [CIN1] and grade 2/3 [CIN2/3], respectively, and adenocarcinoma *in situ* [AIS]). A study of 10,656 women aged 15–45 years across 27 countries showed approximately 85% or more of CIN3 and AIS, and approximately 50% of CIN1 lesions were attributed to HPV6/11/16/18/31/33/45/52/58 [50]. Though there was some regional variance observed for the contribution of HPV16/18 and HPV31/33/45/52/58 to cervical disease, when considering the 9vHPV types together, the majority (71–85%) of CIN2/3 in the regions studied (North America, Latin America, Europe, Asia, and Oceania) were attributed to the 9vHPV types [51]. In a population-based study of 5378 cervical lesions conducted by the CDC in five catchment areas across the USA among women aged 21–39 years, the attribution to CIN2/3 for the high-risk 9vHPV vaccine types was 75% (65.3% for CIN2 and 86.2% for CIN3/AIS).

Epidemiological studies conducted since initial licensure of the qHPV and bHPV vaccines have demonstrated that HPV16 and HPV18 cause approximately 70% of cervical cancers worldwide and HPV types 31/33/45/52/58 cause approximately an additional 20% of cervical cancers worldwide [52,53]. These estimates have minimal regional variation; a worldwide study of 8977 HPV-positive invasive cervical cancers [53] reported that approximately 96, 88, 88, 91, 86 and 87% of the HPV-positive cervical cancers in North America, Central South America, Europe, Asia, Oceania and Africa, respectively, were attributed to the high-risk 9vHPV vaccine types. Regional assessment provides useful information given that most cervical cancer cases (~85%) occur in less developed regions [3].

Additional epidemiological studies conducted to assess HPV type attribution in non-cervical cancers showed that the 9vHPV vaccine had the potential to prevent 85–95% of HPV-related vulvar, vaginal and anal cancers (Table 1) [54–56] and 80–95% of their associated high-grade lesions. Compared to the qHPV vaccine, this represents an additional 10–20% protection against these cancers, plus an additional 5–15% protection against the high-grade lesions associated with these cancers [54–56].

By preventing HPV infection and disease due to HPV31/33/45/52/58, the 9vHPV vaccine has the potential to increase prevention of cervical cancer from 70 to 90%, high-grade cervical dysplasia from 50 to 80%, while also providing additional protection against HPV-related vulvar, vaginal and anal cancers. Overall, the 9vHPV vaccine has the potential to address a significant unmet medical need.

The 9vHPV vaccine

Dose selection

Adding more antigen types to an existing vaccine can potentially impact the vaccine's immunogenicity and safety. In particular, immune interference can be an obstacle in the development of a multivalent vaccine (e.g., immune interference was observed in two Phase I/II studies that compared the immunogenicity of an investigational tetravalent HPV

Table 2. Vaccine candidates assessed in three Phase II immunogenicity and safety studies to select a dose formulation for Merck's second-generation HPV vaccine [58].

Study	Design	Vaccine formulations								Dates	Outcome
		Vaccine	*Antigen amount: original HPV types (μg)*				*Total antigen amount: all new HPV types[†] (μg)*	*AAHS (μg)*	*AAHS/ antigen ratio*		
			6	*11*	*16*	*18*					
1	3-dose formulations (low, mid, high) of 8vHPV vaccine versus qHPV vaccine (control)	qHPV	20	40	40	20	0	225	1.88	Dec 2005 to Aug 2007	Not selected for Phase III evaluation
		8vlow	20	40	40	20	20	225	1.61		
		8vmid	20	40	40	20	80	280	1.40		
		8vhigh	20	40	40	20	160	395	1.41		
2	3-dose formulations (low, mid, high) of 9vHPV vaccine versus qHPV vaccine (control)	qHPV	20	40	40	20	0	225	1.88	Sep 2007 to Mar 2013[‡]	Mid-dose selected for Phase III evaluation
		9vlow	20	40	40	20	100	500	2.27		
		9vmid	30	40	60	40	100	500	1.85		
		9vhigh	30	40	80	55	150	500	1.41		
3	qHPV vaccine + 5vHPV vaccine versus 4vHPV vaccine + placebo (control)	qHPV	20	40	40	20	0	225	1.88	Oct 2007 to May 2009	Not selected for Phase III evaluation
		placebo	0	0	0	0	0	225	–		
		qHPV	20	40	40	20	0	225	1.88		
		5v	0	0	0	0	150	225	1.50		

Study 1: Protocol V502-001 [87]; Study 2: Protocol V503-001 [61]; Study 3: Protocol V504-001 [88]; 8vHPV vaccine: 8-valent HPV types 6/11/16/18/31/45/52/58 vaccine; 9vHPV vaccine: 9-valent HPV Types 6/11/16/18/31/33/45/52/58 vaccine; 5vHPV vaccine: 5-valent HPV types 31/33/45/52/58 vaccine; AAHS: Amorphous aluminum hydroxyphosphate sulfate (adjuvant)

[†]Total antigen amount including all new types (equally divided between the types).

[‡]Study 2 was the Phase II portion of Protocol V503-001, a Phase II/III study with interim analysis for dose selection conducted in 2008 prior to initiation of Phase III. The final analysis for the study was conducted after unblinding of the study database in 2013.

type 16/18/31/45 vaccine with the licensed bHPV vaccine [57]. This immune interference could not be overcome). As described below, a comprehensive approach was necessary to address this difficulty and select a vaccine candidate.

To select a dose formulation for a second-generation HPV vaccine, three Phase II studies were conducted evaluating immune interference of seven multivalent HPV vaccine formulations (Table 2) [58]. In summary, all vaccines were administered as a 3-dose regimen at day 1, month 2 and month 6. The key criteria for selection of a vaccine candidate were: non-inferior anti-HPV6/11/16/18 geometric mean titers (GMTs) at month 7 compared with the qHPV vaccine (defined as the lower bound of the 95% CI of the GMT ratio [vaccine candidate/control] being greater than 0.5); and no negative trend in anti-HPV6/11/16/18 GMTs at month 7 across all four vaccine types compared with the qHPV vaccine. The selected vaccine candidate was also to generate robust antibody responses to the additional HPV types and be generally well tolerated. In the first study (Study 1), subjects received one of the three-dose formulations of an 8-valent HPV6/11/16/18/31/45/52/58 (8vHPV) vaccine or qHPV vaccine (control). As seen in (Table 2), the three-dose formulations of the 8vHPV vaccine differed by the amount of HPV31/45/52/58 antigens; they had the same amount of HPV6/11/16/18 antigen as the qHPV vaccine, but lower adjuvant to antigen ratio than the qHPV vaccine. The study demonstrated a trend for lower antibody responses for all four HPV types common to the 8vHPV vaccine and qHPV vaccine (HPV6/11/16/18) (Figure 1). Therefore, the 8vHPV vaccine was not developed further.

In an attempt to overcome this immune interference, two additional studies (Studies 2 and 3) were conducted subsequently. Study 2 represented the Phase II part of a Phase II/III dose-ranging, efficacy, immunogenicity and safety study, Protocol V503-001, described below. In Study 2, subjects received one of three dose formulations of a 9vHPV vaccine (termed low-, mid- and high-dose formulations) or qHPV vaccine (control); the low-dose 9vHPV vaccine had increased adjuvant to antigen ratio and the same dose of HPV6/11/16/18 antigens compared with qHPV vaccine; the mid-dose 9vHPV vaccine had the same adjuvant to antigen ratio and increased doses of HPV antigens compared with the qHPV vaccine; and the high-dose 9vHPV vaccine had increased amounts of antigen compared with the mid-dose 9vHPV vaccine (Table 2). This study demonstrated that the immune interference was largely overcome with the mid- and high-dose formulations of the 9vHPV vaccine (Figure 1).

In Study 3, the subjects concomitantly received qHPV vaccine and a 5-valent HPV31/33/45/52/58 (5vHPV) vaccine

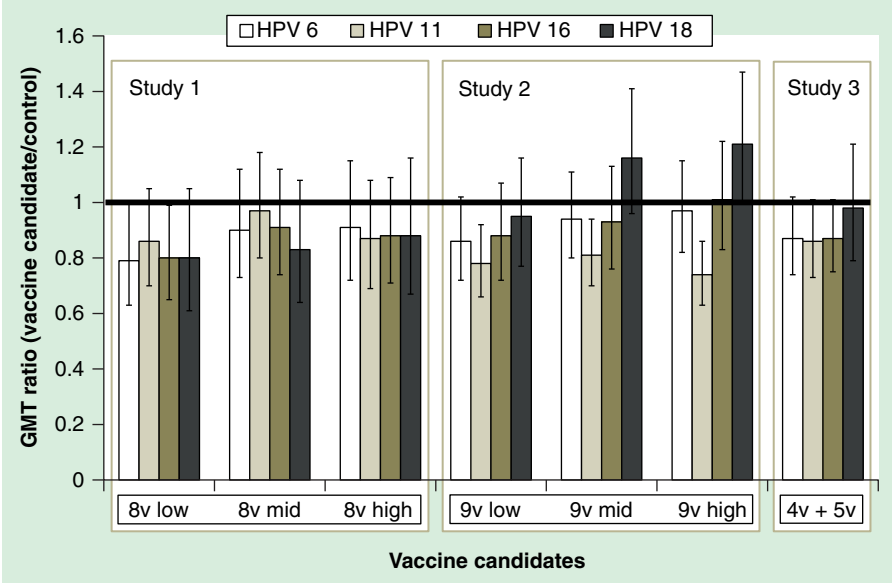

Figure 1. Immunogenicity comparison between vaccine candidates tested in Phase II and controls. Anti-HPV6, 11, 16 and 18 GMT ratios (vaccine candidates versus controls) are shown for all seven vaccine candidates tested in Phase II studies. The error bars represent the corresponding 95% confidence interval. Immunogenicity was tested at month 7 (1 month post-dose 3) for 8-valent HPV vaccine and 5-valent HPV vaccine and at month 3 (1 month post-dose 2) for 9-valent HPV vaccine [58].

or qHPV vaccine plus placebo (control) (Table 2). This study demonstrated a trend for lower antibody responses for HPV6/11/16/18 in the investigational group compared with the control group (Figure 1). Therefore, the 5vHPV vaccine was not developed further.

The dose selection for the new types was based on the initial study of the 8vHPV vaccine (Study 1). The three-dose formulations of the 8vHPV vaccine tested contained different amounts of antigen for the new types (5, 20 or 40 µg each). All three doses were highly immunogenic (over 97% subjects seroconverted at month 7, and the GMT response was dose dependent) [58]. The doses tested in studies 2 and 3 were selected based on these initial results (in study 2, 20 µg for each of the new types in low- and mid-dose formulations; 30 µg for each of the new types for the high-dose formulation; in study 3, 30 µg for each of the new types). These two doses provided similar immunogenicity in study 2. All vaccine candidates assessed in studies 1, 2 and 3 were generally well tolerated. Based on these results, the mid-dose formulation was selected for Phase III evaluation.

Each dose of qHPV vaccine contains 20, 40, 40 and 20µg of HPV6/11/16/18 VLPs, respectively, and 225 µg of amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant (AAHS substantially enhances HPV vaccine immunogenicity [59,60]). Each 0.5ml dose of the mid-dose formulation of 9vHPV vaccine contains 30, 40, 60, 40, 20, 20, 20, 20 and 20 µg of HPV6/11/16/18/31/33/45/52/58 VLPs, respectively, and 500 µg of AAHS. The amount of AAHS in the 9vHPV vaccine is the same as that used in Recombivax HB (hepatitis B vaccine [recombinant]; Merck & Co, Kenilworth, NJ) (also known as HBvaxPRO in some countries), a recombinant protein-based vaccine licensed in many countries to prevent infection with hepatitis B virus, another oncogenic DNA virus. Recombivax HB has been widely administered to infants, adolescents and adults and was found to be effective and have an acceptable safety profile.

Pivotal efficacy, immunogenicity & safety study in young women 16–26 years of age – Protocol V503-001 [61]

Study design

Protocol V503-001 was a double-blinded (with in-house blinding), controlled with qHPV vaccine, dose-ranging, efficacy, immunogenicity and safety study of the 9vHPV vaccine. The study used a seamless Phase II/III adaptive design, which allowed prompt progression from Phase II dose selection to Phase III efficacy evaluation following dose selection. Implementation of this adaptive study design and associated challenges have been described [62]. Subjects were enrolled in two parts (Part A and B) (Figure 2).

- Approximately 1240 subjects were to be enrolled in Part A and randomized in equal numbers to receive one of three dose formulations of 9vHPV vaccine (termed low-, mid- and high-dose 9vHPV vaccine) or the comparator qHPV vaccine. All vaccines were administered as a 3-dose regimen (day 1, month 2, month 6). The mid-dose formulation was selected based on interim immunogenicity and safety analyses for Phase III evaluation of the 9vHPV vaccine (for convenience, this Phase II evaluation was named Study 2 in the dose selection section).
- After selection of a 9vHPV vaccine dose formulation, approximately 13,380 subjects were to be enrolled in Part B and randomized in equal numbers to receive either the selected dose formulation of 9vHPV vaccine or the comparator qHPV vaccine. The 9vHPV vaccine and qHPV vaccine were administered at day 1, month 2 and month 6. Subjects enrolled in Part A received low- or high-dose 9vHPV vaccine and completed the study at month 7. Subjects enrolled in Part A who received mid-dose 9vHPV vaccine or qHPV vaccine, and all subjects enrolled in Part B were eligible to participate in the follow-up for efficacy and immunogenicity for up to 54 months.

As the qHPV and bHPV vaccines prevent pre-cancers due to HPV16 and 18 and are available and recommended in many countries, using a placebo comparator to assess the clinical efficacy of the 9vHPV vaccine was not deemed acceptable for

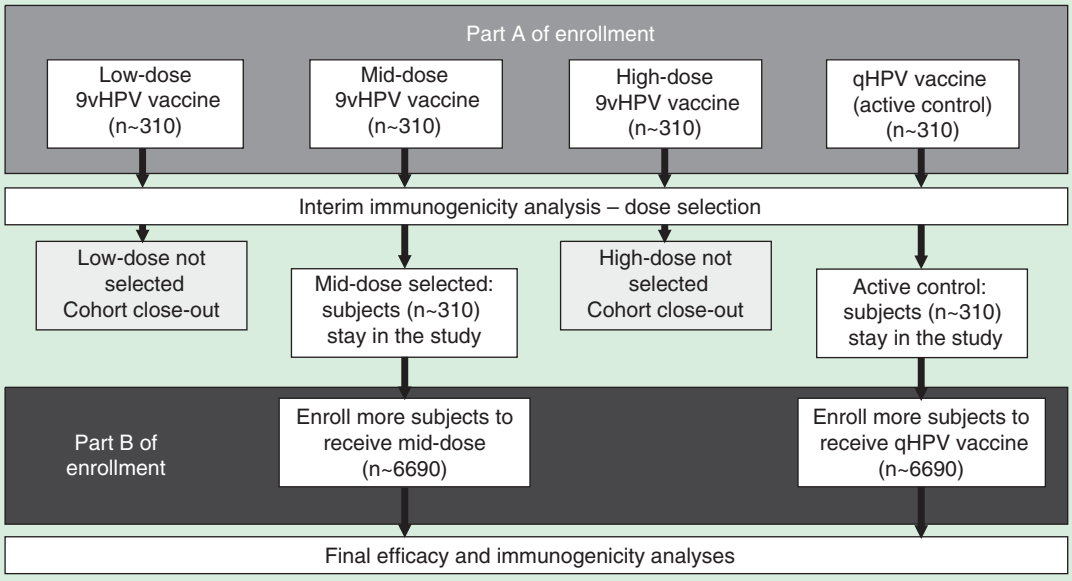

Figure 2. Seamless Phase II/III adaptive design. Protocol V503-001 was enrolled in two parts. Subjects enrolled in part A were equally randomized to 3-dose formulations of 9vHPV vaccine or qHPV vaccine (control) and received a 3-dose regimen (at day 1, month 2 and month 6) of the corresponding vaccine. Selection of a vaccine candidate was based on post-dose 2 immunogenicity analyses. The mid-dose 9vHPV vaccine was selected for evaluation in Phase III. Subjects in the low-dose and high-dose 9vHPV vaccine cohorts completed the study at month 7. Subjects in the mid-dose cohort 9vHPV vaccine and qHPV vaccine cohort remained in the study for efficacy evaluation and additional subjects were enrolled in part B and equally randomized to mid-dose 9vHPV vaccine or qHPV vaccine. The numbers of subjects denote enrollment targets.

ethical reasons. Thus, the qHPV vaccine was used as an active comparator [62–64]. The qHPV vaccine is highly efficacious in preventing infection and disease related to HPV6, 11, 16 and 18 and the same was anticipated for the 9vHPV vaccine. Thus, the incidence of disease endpoints related to these HPV types was expected to be very low in both vaccination groups, thereby precluding a direct comparison between the two vaccines for efficacy endpoints associated with HPV6, 11, 16 and 18. For this reason, the two vaccines were compared using immunogenicity data for these four HPV types. The Phase III study of the 9vHPV vaccine was designed to demonstrate that the 9vHPV vaccine provides: an antibody response to HPV6, 11, 16 and 18 that is non-inferior to the qHPV vaccine (defined as the lower bound of the 95% CI of the GMT ratio [9vHPV/qHPV] being greater than 0.67); and protection against infection and disease caused by the new HPV types (31/33/45/52/58). The primary efficacy analysis was based on the composite endpoint of HPV31/33/45/52/58-related high-grade cervical, vulvar and vaginal disease. A key secondary efficacy analysis was based on the composite endpoint of HPV31/33/45/52/58-related 6-month persistent infection.

Follow-up

For efficacy evaluation, pelvic samples (Pap tests, genital swabs) were collected and external genital examination was conducted every 6 months for assessment of efficacy. Pap test results were used to identify subjects with potential cervical HPV disease; cervical, vaginal and external genital tissue specimens were collected in such subjects according to the protocol-mandated triage. Overall, the methods used for evaluation of efficacy were consistent with those used in the efficacy studies to support the licensure of the qHPV vaccine [63,64]. Specifically, the 9vHPV vaccine and the qHPV vaccine clinical programs used the same eligibility criteria, the same pathology panel for adjudication of lesions in tissue samples collected during the studies, the same PCR assays for detection of type-specific HPV DNA in gynecological swabs and tissue samples. Details on the methods for efficacy evaluation have been reported [63,64].

For evaluation of immunogenicity, serum was collected at day 1 (pre-vaccination) and month 7 for the primary immunogenicity assessment. Persistence of antibody response was also assessed at later time points [63]. The 9vHPV vaccine and qHPV vaccine programs used different immunoassays for assessment of anti-HPV antibody responses. The qHPV vaccine program used the HPV-4 competitive Luminex Immunoassay (cLIA) which measures antibody responses to HPV6, 11, 16 and 18 [65,66]. The 9vHPV vaccine program used the HPV-9 cLIA which measures antibody responses to HPV6, 11, 16, 18, 31, 33, 45, 52 and 58 [67]. Both assays measure HPV type-specific neutralizing antibody levels and are based on the same principle: antiserum from vaccines competes with fluorescently labeled monoclonal antibodies binding to HPV type-specific neutralizing epitopes. The same monoclonal antibodies to HPV6, 11, 16 and 18 are used by both assays. Additional HPV type-specific monoclonal antibodies (to HPV 31, 33, 45, 52 and 58) were developed for the HPV-9 cLIA [45].

Table 3. Vaccine efficacy for HPV31, 33, 45, 52 or 58-related infection or disease in the per-protocol population[†].

Endpoint	9vHPV vaccine (n = 7099)		qHPV vaccine (n = 7105)		Risk reduction (95% CI)
	# cases/total #	Rate[‡]	# cases/total #	Rate[‡]	
High-grade cervical, vulvar, and vaginal disease[§]					
Related to HPV31, 33, 45, 52, or 58	1/6,016	0.1	30/6,017	1.6	96.7 (80.9, 99.8)
Related to HPV31	0/5,308	0.0	7/5,252	0.4	100 (40.1, 100)
Related to HPV33	0/5,624	0.0	7/5,628	0.4	100 (39.3, 100)
Related to HPV45	0/5,724	0.0	2/5,724	0.1	100 (−246.8, 100)
Related to HPV52	0/5,320	0.0	11/5,216	0.7	100 (67.3, 100)
Related to HPV58	1/5,361	0.1	6/5,340	0.4	83.4 (−23.9, 99.3)
Persistent infection ≥6 months' duration[¶]					
Related to HPV31, 33, 45, 52, or 58	35/5,939	2.1	810/5,953	52.4	96.0 (94.4 to 97.2)
Related to HPV31	7/5,251	0.5	150/5,198	10.5	95.5 (90.7, 97.9)
Related to HPV33	1/5,553	0.1	106/5,560	6.9	99.1 (95.2, 100)
Related to HPV45	4/5,649	0.3	124/5,658	7.9	96.8 (92.1, 98.9)
Related to HPV52	11/5,263	0.7	387/5,160	27.9	97.3 (95.3, 98.7)
Related to HPV58	12/5,297	0.8	225/5,284	15.6	94.8 (91.0, 97.1)

N: number of subjects who received at least one dose of vaccine.
cases/total: number of subjects with an endpoint among the subjects in the per protocol efficacy population who had at least one follow-up for the endpoint being analyzed.
Data current though visits that occurred before or on 10 April 2013 (maximum follow-up time: 64 months after vaccination dose 3; median: 40 months).
[†]The per-protocol efficacy population consisted of participants who received all three doses of vaccine within 1 year, were HPV-uninfected (i.e., were seronegative at day 1 and had negative results on PCR assays for all HPV types tested from day 1 through month 7) to the vaccine HPV type being analyzed, and had no protocol violations.
[‡]Rate is the estimated number of cases per 1000 person-years.
[§]This category includes high-grade cervical epithelial neoplasia, adenocarcinoma *in situ*, cervical cancer, high-grade vulvar intraepithelial neoplasia, high-grade vaginal intraepithelial neoplasia, vulvar cancer and vaginal cancer.
[¶]Persistent infection was defined as detection of the same HPV type in a genital swab or tissue specimen collected on two or more consecutive visits, with an interval of at least 6 months (±1 month) between the visits.
From The New England Journal of Medicine, Joura EA, Giuliano AR, Iversen OE et al., A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women, 372:711-723. Copyright © 2015 Massachusetts Medical Society.Reproduced with permission from Massachusetts Medical Society [63].

All subjects were evaluated for injection-site and systemic adverse events (AEs) for 15 days following any vaccination using vaccination report card-based surveillance [63,64]. Serious AEs were monitored from enrollment through 6 months following the third vaccination regardless of causality. Serious vaccine-related AEs and AEs resulting in death were monitored for the entire duration of the study.

Analysis populations

HPV is primarily acquired through sexual activity. The study enrolled a population of young women 16–26 years of age at risk for HPV infection (i.e., sexually active). Similar to efficacy studies in the qHPV vaccine clinical program, women were enrolled in this study regardless of their day 1 HPV status or Pap test result. Since HPV L1 VLP vaccines have no therapeutic activity, vaccine efficacy was assessed in a susceptible population of subjects who were seronegative and PCR-negative at day 1 and remained PCR-negative through 1 month post dose 3 for the HPV type(s) being analyzed, received all three doses of vaccine within 1 year, and did not violate the protocol (per-protocol efficacy [PPE] population) [63,64]. Vaccine efficacy was also assessed in a modified intention-to-treat population. Type-specific immunogenicity was analyzed in per-protocol immunogenicity (PPI) cohorts defined as subjects eligible for the PPE cohorts and who were vaccinated and had their month 7 serum samples collected within pre-defined time intervals [63,64].

Efficacy, immunogenicity & safety results

The study used a fixed-event design: the primary efficacy analyses were conducted after at least 30 primary efficacy endpoints accrued. The analyses summarized below are current through visits that occurred before or on 10 April 2013, representing an efficacy assessment up to 64 months post-dose 3 (median: 40 months post-dose 3).

Efficacy (HPV31, 33, 45, 52, 58)

In the PPE population, the incidence rate of high-grade cervical, vulvar and vaginal disease related to HPV31, 33, 45,

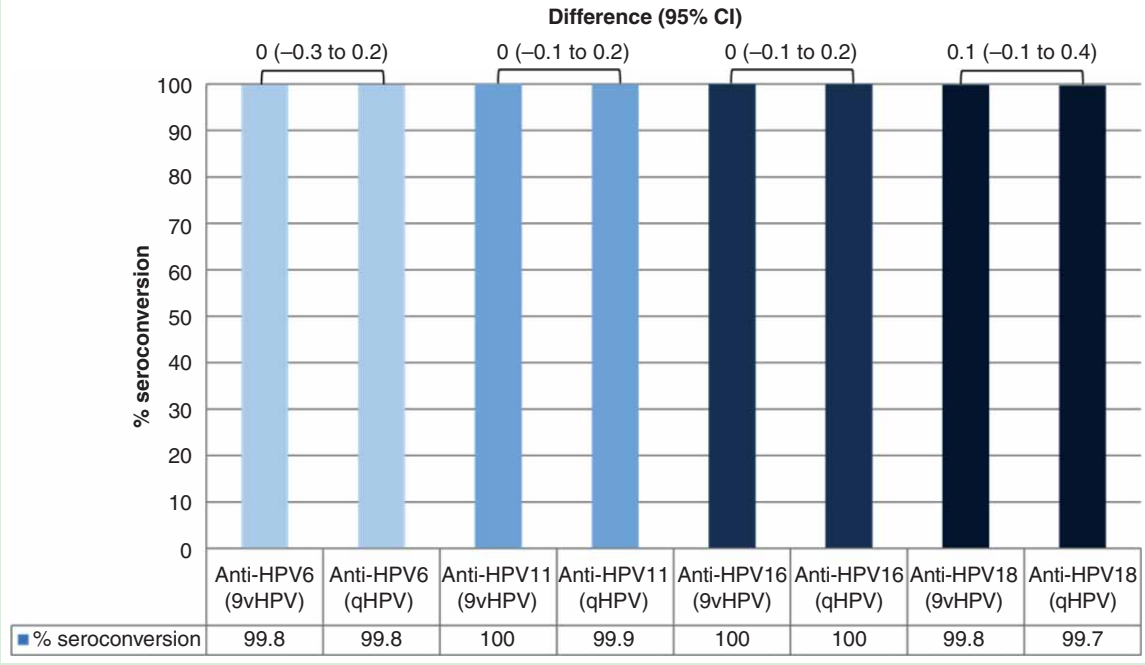

Figure 3. Anti-HPV 6, 11, 16 and 18 seroconversion rates at 1 month post-dose 3 for the 9vHPV and qHPV vaccines in women aged 16–26 years. Non-inferiority analyses in the per-protocol immunogenicity population [63].

52 and 58 was 0.1 per 1000 person-years in the 9vHPV group and 1.6 per 1000 person-years in the qHPV group (1 case versus 30 cases) representing a vaccine efficacy of 96.7% (95% CI, 80.9 to 99.8) (Table 3). The primary efficacy hypothesis (to demonstrate that the lower bound of the 95% CI of 9vHPV vaccine versus qHPV vaccine efficacy to prevent the composite endpoint of HPV31/33/45/52/58-related high-grade cervical, vulvar and vaginal disease was greater than 25%) was verified. The single case in the 9vHPV vaccine group was an HPV58-positive CIN2. This subject was positive by PCR testing to HPV56 at baseline and in all specimens obtained between day 1 and the time of diagnosis, with HPV58 DNA detected only at the time of diagnosis. The incidence of persistent infection (i.e., lasting ≥6 months) related to HPV31, 33, 45, 52 and 58 in the PPE population was 2.1 per 1000 person-years in the 9vHPV group and 52.4 per 1000 person-years in the qHPV group (35 versus 810 cases) representing a vaccine efficacy of 96.0% (95% CI: 94.4 to 97.2) (Table 3). The secondary efficacy hypothesis (to demonstrate that the lower bound of the 95% CI of 9vHPV vaccine versus qHPV vaccine efficacy to prevent HPV31/33/45/52/58-related 6-month persistent infection was greater than 25%) was verified. The 9vHPV vaccine prevented persistent infection and high-grade disease associated with each of the five HPV types.

Immunogenicity & incidence of disease (HPV6, 11, 16, 18)

Per-protocol analyses of immunogenicity showed that nearly 100% of subjects seroconverted for HPV6, 11, 16 and 18 at 4 weeks following vaccination dose 3 (Figure 3). The fold difference between GMT ratios (9vHPV/qHPV) for the four HPV types ranged from 0.80 to 1.19 (Figure 4). Non-inferiority of GMT in the 9vHPV group compared with qHPV group (defined as the lower bound of the 95% CI of the GMT ratio being greater than 0.67) was demonstrated for all four HPV types. Efficacy of 9vHPV vaccine against persistent infection and disease related to HPV6/11/16/18 was inferred based on demonstration of non-inferior immunogenicity compared with qHPV vaccine. Supportive analyses were conducted based on the incidence of persistent infection and disease related to HPV6/11/16/18 (Table 4). As anticipated, rates of infection or disease due to HPV6, 11, 16 or 18 were low in both vaccination groups (e.g., 1/5823 and 1/5832 for HPV6/11/16/18-related high-grade cervical disease in the 9vHPV and qHPV vaccine groups, respectively). Rates of 6-month persistent infection and cervical disease were similar in the two vaccination groups, with 95% CIs largely overlapping. Of note, in historical cohorts of the qHPV vaccine clinical program, the number of cases of HPV6/11/16/18-related high-grade cervical disease was 2/7864 in the qHPV vaccine cohort and 110/7865 in the placebo cohort in analyses conducted after 42 months of mean follow-up post-dose 1 [68]. Even though these analyses are from a different clinical program, they represent a relevant measure of incidence of disease in an unvaccinated population (placebo cohort) since the 9vHPV and qHPV vaccine programs used the same eligibility criteria, analysis populations and methods for endpoint assessment.

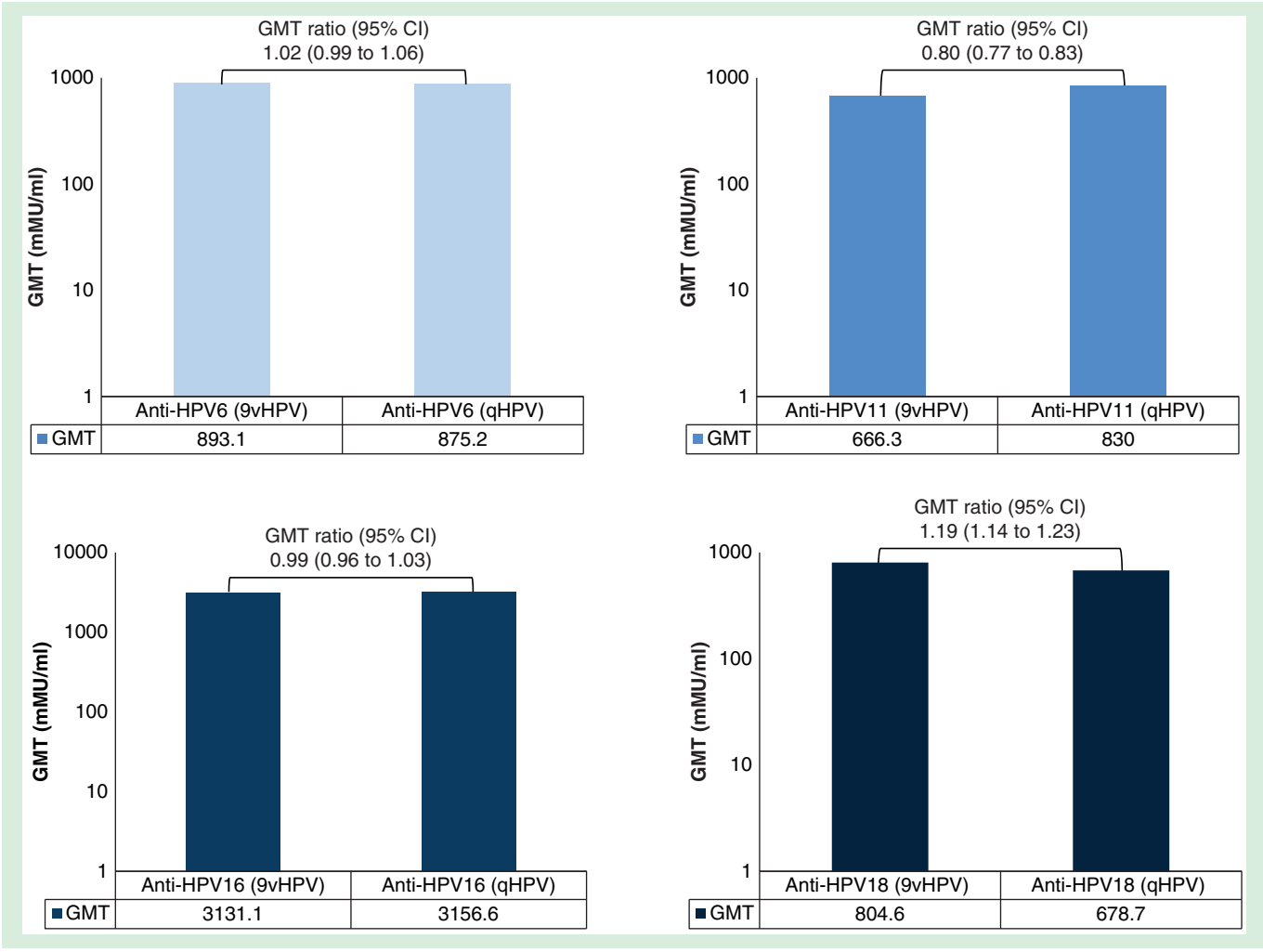

Figure 4. Ratio of geometric mean titers (GMTs) at 1 month post-dose 3 for the 9vHPV and qHPV vaccines in women aged 16–26 years. Non-inferiority analyses in the per-protocol immunogenicity population [63].

Safety

Administration of a 3-dose regimen of 9vHPV vaccine was generally well tolerated. As seen in reference [63]: few subjects discontinued vaccination due to an AE; the proportions of subjects with serious AEs were comparable between the two vaccine groups; (iii) 10 subjects died during the study (five in each vaccine group), none of the deaths were considered vaccine-related. As shown in (Table 5), the two vaccines had similar AE profiles; injection-site AEs were more common in the 9vHPV vaccine group than in the qHPV vaccine group. Injection-site AEs were mostly mild to moderate in intensity. A total of 2321 subjects (1192 in the 9vHPV vaccine group, 1129 in the qHPV vaccine group) became pregnant during the study [63]. The proportions of pregnancies with adverse outcomes were comparable between the two groups. Congenital anomalies were detected in 41 pregnancies (20 in the 9vHPV vaccine group and 21 in the qHPV vaccine group). No congenital anomaly was reported in pregnancies with an estimated date of conception within 30 days before or after a vaccination with 9vHPV vaccine.

Additional analyses

Modified intention-to-treat (mITT) analyses have also been presented [63]. These analyses consider all study participants who received at least one vaccination and for whom there was at least one measurement of efficacy for the corresponding endpoint. Such analyses include subjects who are infected and subjects who are not infected at baseline with the HPV type being analyzed. Therefore, results from these analyses represent a mixture of prophylactic and therapeutic efficacy. However, the 9vHPV vaccine is a prophylactic vaccine and it has no therapeutic activity (i.e., vaccination will not cause existing infection or disease to disappear). Most disease cases in the mITT population came from subjects who were already infected at study enrollment. Only the first occurrence of an endpoint (most likely due to the HPV type that subject was infected with at baseline) is considered in the mITT analyses, not overall protection against all vaccine HPV types. The mITT analyses, therefore, support that the 9vHPV vaccine has no therapeutic activity, but they cannot be used to

Table 4. Incidence of HPV6, 11, 16, and 18-related persistent infection and cervical disease in the per protocol population[†].

Endpoint	9vHPV vaccine (n = 7099)		qHPV vaccine (n = 7105)		
	# cases/total #	Rate[‡] (95% CI)	# cases/total #	Rate[‡] (95% CI)	Risk reduction (95% CI)
HPV 6/11/16/18-related					
Persistent infection[§]	59/5812	3.6 (2.8, 4.7)	80/5,830	5.0 (3.9, 6.2)	26.4 (−4.3, 47.5)
Cervical disease	1/5823	0.1 (0.0, 0.3)	3/5,832	0.2 (0.0, 0.5)	66.5 (−203.4, 98.7)
High-grade cervical disease[¶]	1/5823	0.1 (0.0, 0.3)	1/5,832	0.1 (0.0, 0.3)	−0.4 (≤−999, 97.4)

N: number of subjects who received at least one dose of vaccine.
#cases/total: number of subjects with an endpoint among the subjects in the per protocol efficacy population who had at least one follow-up for the endpoint being analyzed.
Data current though visits that occurred before or on 10 April 2013 (maximum follow-up time: 64 months after vaccination dose 3; median: 40 months).
[†]The per-protocol efficacy population consisted of participants who received all three doses of vaccine within 1 year, were HPV-uninfected (i.e., were seronegative at day 1 and had negative results on PCR assays for all HPV types tested from day 1 through month 7) to the vaccine HPV type being analyzed, and had no protocol violations.
[‡]Rate is the estimated number of cases per 1000 person-years.
[§]Persistent infection was defined as detection of the same HPV type in a genital swab or tissue specimen collected on two or more consecutive visits, with an interval of at least 6 months (±1 month) between the visits.
[¶]This category includes high-grade cervical epithelial neoplasia, adenocarcinoma in situ, cervical cancer.
From The New England Journal of Medicine, Joura EA, Giuliano AR, Iversen OE et al., A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women, 372:711-723.

infer overall prophylactic benefit of the 9vHPV vaccine or lack thereof in mITT populations. Overall, the mITT analyses provide confounded estimates of efficacy that largely reflect the composition of the study population (i.e., rates of HPV infection at baseline) rather vaccine efficacy and cannot be extrapolated to real-life situations.

Immunogenicity bridging from young women 16–26 years of age to girls & boys 9–15 years of age – Protocol V503-002 [69]

Prophylactic HPV vaccination should be provided prior to exposure to HPV (i.e., prior to starting sexual activity). The median age of sexual debut is in the late teens (15–19 years) in most countries [70]. Thus, preadolescents and young adolescents (girls and boys 9–15 years of age) represent the ideal target population for HPV vaccination. By 2014, licensed HPV vaccines were introduced in the national vaccination programs of at least 57 countries [14]. Although the age range of the primary target group varies by country, all these programs target preadolescents and young adolescents [13,14]. However, efficacy studies cannot be conducted in young adolescents because they are not generally exposed to HPV. Therefore, 9vHPV vaccine efficacy findings in young women 16–26 years of age were extended to girls and boys 9–15 years of age based on the demonstration of non-inferior immunogenicity. The same approach was previously used in studies to support the licensure of the first-generation HPV vaccines in young adolescents and is consistent with the guidelines from the World Health Organization on HPV vaccine development [5,71–73]. The design and results of this study (protocol V503-002) have been reported [74]. Briefly, all subjects received a 3-dose regimen of 9vHPV vaccine (day 1, month 2, month 6) and were assessed for immunogenicity by HPV-9 cLIA at month 7 (4 weeks following dose 3). Figure 5 displays anti-HPV GMTs at month 7 for all nine vaccine types in girls, boys and young women. GMTs were higher in girls and boys than in women and non-inferiority (defined as the lower bound of the 95% CI of the GMT ratio [girls/women or boys/women] being greater than 0.67) was demonstrated for all nine vaccine HPV types for both girls and boys [74]. Based on these results, efficacy of 9vHPV vaccine in girls and boys 9–15 years of age was inferred.

Protocol V503-003 [75] *– immunogenicity bridging from young women 16–26 years of age to young men 16–26 years of age*

A study (protocol V503-003) was conducted to evaluate the immunogenicity of a 3-dose regimen of the 9vHPV vaccine in young men 16–26 years of age with a comparison to young women 16–26 years of age. Per protocol, immunogenicity was to be analyzed separately in heterosexual men (HM) and men having sex with men (MSM). The results of that study have been presented at the Eurogin 2015 conference [76]. Briefly, over 99.5% of subjects seroconverted to all nine HPV types at month 7, and month 7 GMTs were non-inferior in HM compared with women (i.e., the lower bound of the 95% CI of the GMT ratio [HM/women] being greater than 0.67). A protocol-specified secondary analysis showed that month 7 GMTs were lower in men having sex with men (MSM) than in heterosexual men; of relevant note, in the qHPV vaccine clinical program, month 7 GMTs following qHPV vaccination were also lower in MSM compared with HM, and the qHPV vaccine was highly efficacious in preventing infection and disease due to vaccine HPV types in

Table 5. Vaccine-related injection-site & systemic adverse events (incidence ≥2% in at least one vaccination group.

	9vHPV vaccine (n = 7,071)		qHPV vaccine (n = 7,078)		Difference in % versus qHPV vaccine estimate (95% CI)
	Count	(%)	Count	(%)	
With one or more adverse events[†]	6640	(93.9)	6,419	(90.7)	
Injection-site event[‡]	6414	(90.7)	6,012	(84.9)	
Pain	6356	(89.9)	5,910	(83.5)	6.4 (5.3, 7.5)
Swelling	2830	(40.0)	2,035	(28.8)	11.3 (9.7, 12.8)
Erythema	2407	(34.0)	1,810	(25.6)	8.5 (7.0, 10.0)
Pruritus	388	(5.5)	282	(4.0)	
Vaccine-related systemic event[†]	2.086	(29.5)	1,929	(27.3)	
Headache	1031	(14.6)	969	(13.7)	
Pyrexia	357	(5.0)	301	(4.3)	
Nausea	311	(4.4)	261	(3.7)	
Dizziness	211	(3.0)	197	(2.8)	
Fatigue	166	(2.3)	150	(2.1)	

The summaries provided are counts of subjects and the percents were calculated relative to the number of subjects as-treated.
Estimates of the difference in % and corresponding 95% CI are provided for the events solicited on the vaccination report card.
N: Number of subjects as-treated who received at least 1 dose of the indicated vaccine and had at least one follow-up visit for adverse events.
[†]Days 1–15 following any vaccination visit.
[‡]Days 1–5 following any vaccination visit.
From The New England Journal of Medicine, Joura EA, Giuliano AR, Iversen OE *et al.*, A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women, 372:711-723. Copyright © 2015 Massachusetts Medical Society.
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both MSM and HM [11,12,77]. Based on these results, efficacy of the 9vHPV vaccine in men 16–26 years of age was inferred.

Licensure & recommendation status

The 9vHPV vaccine was licensed in the USA in December 2014, in Canada in February 2014 and in the EU and Australia in June 2015 under the trade name Gardasil 9 (Merck & Co, Inc., Kenilworth, NJ) for use in both males and females. The upper age range of licensure differs between these countries; for instance, the vaccine is licensed in the USA in women up to age 26 years, in Australia and Canada up to age 45 years, and in the EU without upper age limit. In February 2015, the Advisory Committee on Immunization Practices (ACIP) included the 9vHPV vaccine in its recommendation for routine HPV vaccination of girls and boys at age 11 or 12 years and catch-up vaccination in females 13–26 years, males 13–21 years not vaccinated previously, and MSM and immunocompromised persons (including those with HIV infection) through age 26 years [78]. The ACIP stated that if vaccination providers do not know or do not have available the HPV vaccine previously administered or are in settings transitioning to 9vHPV vaccine, the 9vHPV vaccine may be used for continuing or completing a series started with a different HPV vaccine [78]. The ACIP noted that introduction of the 9vHPV vaccine was cost-saving when compared to qHPV vaccine in both sexes in a cost–effectiveness model [78].

Expert commentary

The 9vHPV vaccine has the potential to prevent approximately 90% anogenital cancers caused by HPV, which represent an annual burden of disease of nearly 600,000 cases worldwide [3]. Therefore, the 9vHPV vaccine could be beneficial in countries without organized cervical cancer screening programs. It could also have a substantial impact in countries with an organized cervical cancer screening program. The 9vHPV vaccine has the potential to prevent approximately 50% of low-grade dysplasia and 80% high-grade cervical dysplasia. Diagnoses of CIN1, CIN2, CIN3 and AIS require colposcopy and biopsy. CIN2/3 and AIS are generally treated by excision. While surgery is often curative, it may heighten the risk of premature delivery [79]. After surgery, women remain at risk of recurrent CIN and vulvar and vaginal pre-cancers and cancers [80]. Colposcopy, biopsy and excisional surgery of the cervix represent a substantial economic burden [81]. Overall, by prevention of a large number of CIN occurrences, 9vHPV vaccination could eliminate a substantial number of invasive procedures associated with CIN treatment, thereby potentially reducing healthcare utilization and the resulting impact on quality of life, as well as potential risks associated with the procedures. The 9vHPV vaccine also has the potential to prevent 85–95% of HPV-related non-cervical anogenital cancers (for which screening programs are not generally in place) and 90% of anogenital warts. Overall, the 9vHPV vaccine could represent an important medical advance in both developed and developing countries.

Improvement in implementation of HPV vaccination is needed to ensure that the full health benefit of 9vHPV vaccine is achieved. By 2014, licensed HPV vaccines were introduced in the national vaccination programs of at least 57 countries [14]. Vaccination of boys and men is

recommended in four countries (Australia, Austria, Canada, USA) [14,82]. Although the age range of the primary target group varies by country, all these programs target pre-adolescents and young adolescents [13,14]. However, providing vaccinations to that population is challenging because they make infrequent healthcare visits. Countries with school-based vaccination programs have achieved high vaccine coverage; however, vaccine uptake has remained low in many countries [14,83]. Also, additional efforts are warranted to expand vaccine programs to males. Several approaches have been proposed to improve HPV vaccination coverage. For instance, concomitant administration of HPV vaccines with other vaccines routinely recommended in preadolescents and young adolescents could facilitate adherence to recommended vaccination regimens [83]. Also, while the 9vHPV vaccine development was ongoing, public health authorities in several regions became interested in comparing 2-dose with 3-dose regimens for both of the marketed vaccines. This interest is based on expectations that a schedule with fewer doses in younger cohorts may positively impact vaccination programs by increasing acceptability and compliance while reducing costs [83]. Studies to compare the immunogenicity of 2- and 3-dose regimens of the first-generation vaccines were conducted and supported the introduction of 2-dose regimens in young adolescents in several countries [84]. In October 2014, the WHO recommended a 2-dose schedule with a 6-month interval between doses for girls younger than 15 years, while retaining the 3-dose schedule for girls 15 years and older [85]. Like the first-generation HPV vaccines, the 9vHPV vaccine was developed as a 3-dose regimen vaccine administered at 0, 2 and 6 months. A study to compare 2- and 3-dose regimens of 9vHPV vaccine is currently underway.

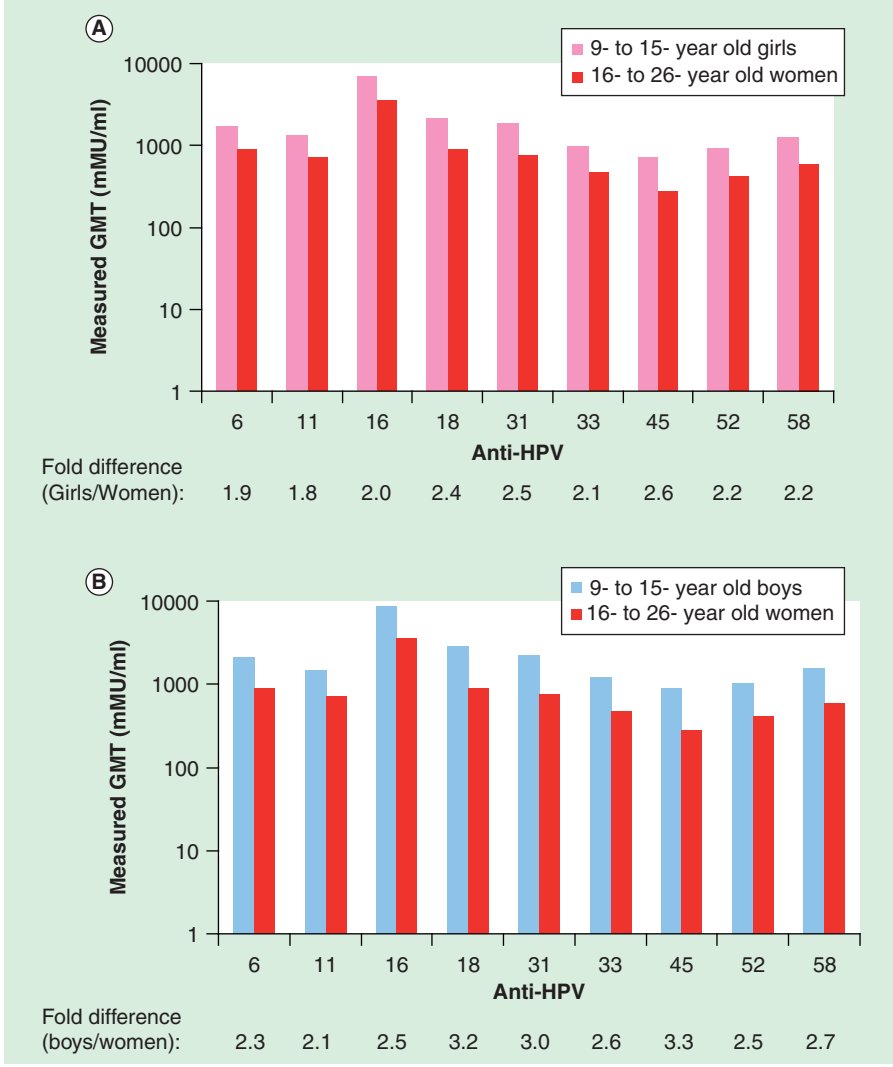

Figure 5. Ratio of geometric mean titers (GMTs) at 1 month post-dose 3 of 9vHPV vaccine for girls aged 9–15 years versus women aged 16–26 years **(A)** or boys aged 9–15 years versus women aged 16–26 years **(B)** vaccines. Non-inferiority analyses were conducted in the per-protocol immunogenicity population [74].

Five-year view

It is anticipated that similar to the first-generation HPV vaccines, the 9vHPV vaccine will be licensed and recommended in many countries. Extensions of ongoing clinical studies to provide longer term immunogenicity and effectiveness, as well as real world epidemiological studies to assess vaccine impact on prevalence of HPV infection and burden of disease will provide further information in support of vaccine safety and effectiveness. Similar to the first-generation HPV vaccines, the 9vHPV vaccine should eventually become part of the national vaccination program in many countries. Implementation of a 2-dose regimen of the 9vHPV vaccine in pre-adolescents and young adolescents should help broaden adoption of the vaccine. Overall, coverage should increase as a result of more generalized vaccination of pre-adolescents and young adolescents, inclusion of males in more national vaccination programs, broader availability of HPV vaccines in mid- and low-income countries and inclusion of GAVI-eligible countries.

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Key issues

- The 9vHPV vaccine covers the same oncogenic HPV types (HPV 16 and 18, responsible for 70% of cervical cases worldwide) as the prophylactic HPV vaccines already available plus the five next common cervical cancer causing types (HPV 31, 33, 45, 52, 58), with the potential to prevent nearly 90% cervical cancer cases worldwide.
- The 9vHPV vaccine has the potential to prevent 80% cases of high-grade cervical dysplasia, which could result in substantial decrease of invasive procedures used to treat these lesions in countries with organized cervical cancer screening programs.
- The 9vHPV vaccine has the potential to prevent 85–95% of HPV-related vulvar, vaginal and anal cancers worldwide; there is no widely implemented screening program for these types of cancers.
- Similar to the qHPV vaccine, the 9vHPV vaccine prevents genital warts caused by HPV 6 and 11 (the HPV types responsible for 90% genital warts).
- In a Phase III clinical study conducted in sexually active young women aged 16–26 years, the 9vHPV vaccine prevented infection, high-grade intraepithelial neoplasia (precancers) and genital warts caused by vaccine HPV types.
- Efficacy findings in young women were extended to other demographic groups (girls and boys aged 9–15 years, men aged 16–26 years) based on the demonstration of non-inferior immunogenicity.
- In clinical studies, the adverse event profile of the 9vHPV vaccine was comparable with that of the qHPV vaccine. Injection-site adverse events were more common with the 9vHPV vaccine and were mostly mild to moderate in intensity.
- The 9vHPV vaccine was licensed in the USA in December 2014, Canada in February 2015, and the EU and Australia in June 2015. In February 2015, the Advisory Committee on Immunization Practices included the 9vHPV vaccine in its recommendations for HPV vaccination; the Advisory Committee on Immunization Practices recommended routine vaccination of girls and boys at age 11 or 12 years and catch-up vaccination for males and females up to 21 and 26 years old, respectively; vaccination was also recommended up to 26 years for men having sex with men and immunocompromised persons (including those with HIV infection) if not vaccinated previously.

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