HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine

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A R T I C L E   I N F O

Article history:
Received 9 July 2008
Received in revised form 4 September 2008
Accepted 26 September 2008
Available online 16 October 2008

Keywords:
Human Papillomavirus
Humoral immunity
Immune memory

A B S T R A C T

The efficacy of the quadrivalent Human Papillomavirus (HPV) vaccine is thought to be mediated by humoral immunity. We evaluated the correlation between quadrivalent HPV vaccine-induced serum anti-HPV responses and efficacy. 17,622 women were vaccinated at day 1, and months 2 and 6. At day 1 and at 6–12 months intervals for up to 48 months, subjects underwent Papanicolaou and genital HPV testing. No immune correlate of protection could be found due to low number of cases. Although 40% of vaccine subjects were anti-HPV 18 seronegative at end-of-study, efficacy against HPV 18-related disease remained high (98.4%; 95% CI: 90.5–100.0) despite high attack rates in the placebo group. These results suggest vaccine-induced protection via immune memory, or lower than detectable HPV 18 antibody titers.
1. Introduction

The lifetime risk of infection with the Human Papillomavirus (HPV) exceeds 50% [1,2]. HPV infection can cause epithelial dysplasia and cancer of the cervix, a significant proportion of cancers of the genitalia (both genders), anal canal, and the oropharynx, as well as benign tumors of the genitalia (condyloma acuminata) and the larynx (recurrent respiratory papillomatosis [RRP]) [3–8].

HPV types are defined by sequence variation in the gene encoding the L1 protein, the major constituent of the viral capsid. Over 40 different HPV types are known to infect cervical, anogenital, and oropharyngeal epithelia. These types are divided into two groups: (a) high-risk HPV types that can cause cancer; and (b) low-risk HPV types that rarely cause cancer, but commonly cause dysplastic lesions. Among the high-risk HPV types, HPV 16 and HPV 18 cause approximately 70% of cervical and anal cancer cases [9], and over 80% of HPV-related external genital and oropharyngeal cancer cases. HPV 6 and HPV 11 are low-risk HPV types that cause approximately 90% of all genital wart cases [10] and virtually all RRP cases [11].

A prophylactic vaccine targeting HPV types 6, 11, 16, and 18 has been developed, and is currently available in many countries. This vaccine contains L1 proteins of the 4 vaccine HPV types arranged as 4 separate species of virus-like particles (VLPs) adsorbed onto amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant. In studies, prophylactic administration of this vaccine to 16–26-year-old women was 96–100% effective in preventing HPV 16- and HPV 18-related cervical squamous cell cancer, cervical adenocarcinoma, vulvar cancer, and vaginal cancer (based on a demonstration of efficacy against HPV 16- and HPV 18-related cervical intraepithelial neoplasia [CIN] grade 3, cervical adenocarcinoma in situ [AIS], vulvar intraepithelial neoplasia [VIN] grades 2/3, and vaginal intraepithelial neoplasia [VaIN] grades 2/3, respectively) [12–15]. The vaccine was 98–100% effective in preventing HPV 6- and HPV 11-related genital warts and CIN.

Because HPV infection is sexually transmitted, men and women remain at risk of infection as long as they are sexually active. Thus, to be maximally effective, prophylactic HPV vaccines should induce long-lived protective efficacy (i.e., at least 10 years, preferably lifelong). In clinical trials, sustained protective efficacy was observed through at least 5 years following vaccination onset [16]. Ongoing studies are evaluating the longer-term effectiveness of the vaccine.

To date, an immune marker that can identify vaccinated subjects who are protected from acquisition of infection with types targeted by the vaccine has not been identified. Such a marker would be useful in defining the duration of vaccine-induced protective efficacy (and the timing of administration of a booster dose of vaccine, if needed). An immune marker would also simplify the bridging of protective efficacy of the quadrivalent HPV vaccine to new populations and to new formulations. Additionally, an immune marker would aid in the evaluation of follow-on multivalent vaccines.

Preclinical studies have suggested that the protective efficacy of the quadrivalent HPV vaccine is mediated by anti-HPV L1 humoral responses [17–19]. Administration of L1 VLP vaccines targeting animal papillomaviruses prevents infection and disease and is accompanied by induction of anti-L1 neutralizing antibodies. Transfer of serum from vaccinated animals to unvaccinated animals protected the unvaccinated animals from acquisition of infection and disease following a virus challenge. On the basis of these findings, Phase II and Phase III clinical trials of the quadrivalent HPV vaccine in young–adult women have focused on measurement of serum anti-HPV L1 responses shortly after completion of the 3-dose vaccination regimen and for up to 4.5 years thereafter. To define a candidate immune correlate of vaccine efficacy, an evaluation of the correlation between vaccine-induced serum anti-HPV responses and the vaccine’s protective efficacy was conducted. We evaluated this correlation among 17,622 young adult women enrolled in efficacy studies of the quadrivalent HPV vaccine.

2. Materials and methods

2.1. Design of the phase III clinical trials

Protocols 013 (NCT00092521) and 015 (NCT00092534) (termed FUTURE I and FUTURE II, respectively) were phase III, randomized, double-blind, placebo-controlled clinical trials designed to investigate the prophylactic efficacy of the quadrivalent (types 6, 11, 16, 18) HPV L1 VLP vaccine (GARDASIL™/SILGARD™, Merck and Co., Inc., Whitehouse Station, NJ) on HPV 6/11/16/18-related CIN, AIS, or cervical cancer (protocol 013 co-primary endpoint); HPV 6/11/16/18-related condylomata acuminata, VIN, VaIN, vulvar cancer, or vaginal cancer (protocol 013 co-primary endpoint), and HPV 16/18-related CIN 2/3, AIS, or cervical cancer (protocol 015 primary endpoint) [12,15].

Between December 2001 and May 2003, 17,622 15–26-year-old women were enrolled in the two trials (17,599 received at least 1 dose of vaccine or placebo). The trials enrolled women who reported 0–4 lifetime sexual partners at day 1. Enrolled subjects with clinical evidence of genital HPV disease at day 1 were discontinued from the study prior to randomization. Subjects received intramuscular injections of quadrivalent HPV vaccine or visually indistinguishable placebo at enrollment (day 1), month 2, and month 6. Each protocol was approved by the institutional review boards (ethical review committees) at participating centers and informed consent was received from all subjects enrolled. The designs of protocols 013 and 015 are described elsewhere [15,20].

2.2. Study vaccine

The quadrivalent vaccine consisted of a mixture of four recombinant HPV type-specific VLPs composed of full-length L1 major capsid proteins of HPV types 6, 11, 16 and 18 synthesized in Saccharomyces cerevisiae [21–23]. The vaccine is comprised of 20 μg of HPV 6 VLP, 40 μg of HPV 11 VLP, 40 μg of HPV 16 VLP and 20 μg of HPV 18 VLP, formulated with 225 μg of amorphous aluminum hydroxyphosphate sulfate adjuvant. The placebo contained the same adjuvant and was visually indistinguishable from vaccine.

2.3. Clinical follow-up and laboratory testing

Examination for the presence of genital warts and vulvar and vaginal lesions was performed at enrollment (day 1), month 3 (protocol 013 only), and months 7, 12, 24, 36, and 48 (also at months 18 and 30 for protocol 013). ThinPrep™ (Cytyc, Boxborough MA, USA) cytology specimens for Pap testing were collected at enrollment (day 1), month 7, and at 6–12-month intervals thereafter. Cytology specimens were classified using The Bethesda System-2001 [24]. Procedures for algorithm-based cytology, colposcopy and biopsy referral have been described previously [12,15]. Biopsy material was first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN), and then read for endpoint determination by a blinded panel of four pathologists as described previously.

Blood samples were obtained at enrollment (day 1) for anti-HPV serology testing for HPV types 6, 11, 16, and 18 using competitive Lumineux-based immunoassays (cLIA; developed by Merck Research Laboratories, West Point, PA, using technology from the Lumineux Corporation, Austin, TX) [25]. Dilution-corrected serostatus cutoffs were 20 mMU/mL for HPV 6, 16 mMU/mL for HPV 11, 20 mMU/mL...
2.4. Statistical analyses

Analyses were conducted in a per-protocol population. Subjects included in this population received all three doses of vaccine or placebo within 1 year, were seronegative at day 1 and PCR negative from day 1 through month 7 to relevant vaccine HPV types (subjects could be positive for one type and be counted in the per-protocol population of another vaccine HPV type if they were naïve to that type). Subjects did not deviate from the protocol; follow-up began 1 month post-dose 3 (month 7).

Vaccine efficacy (defined as $[1 - \text{relative risk}] \times 100\%$) and the corresponding 95% confidence intervals were estimated using an exact procedure which accounted for the amount of follow-up (i.e., person-time at risk) in the vaccine and placebo groups. Subjects were pooled across the studies by vaccination group (vaccine or placebo) for analysis. Some efficacy endpoints in this report are composite endpoints, including more than one lesion type and/or more than one HPV type. If a subject met the criteria for one or more of the components of a composite endpoint, she was counted as a case for the composite endpoint once and only once.

3. Results

Of the 17,622 women who were randomized in protocols 013 and 015, 17,599 were allocated and received either vaccine or placebo (8799 were allocated to vaccine, and 8800 were allocated to placebo) (Fig. 1). Approximately, 97% of those subjects who received either vaccine or placebo received all three doses and completed the vaccination phase. At the time of this report 93% of vaccine recipients and 84% of placebo recipients had completed follow-up in the study in which they were enrolled. Data represent a mean follow-up time of 44 months (protocols 013 and 015 were closed early in order to vaccinate the placebo population).

Quadrivalent HPV vaccine elicited a strong immunologic response against all vaccine HPV types in subjects who were included in the per-protocol immunogenicity population (Table 1). Geometric mean titers (GMT) reached a peak at month 7, and declined thereafter as expected. Over 99% of subjects seroconverted for vaccine HPV types by month 7. Variability was seen in the percentage of subjects who were seropositive for vaccine HPV types at end-of-study; a lower percentage of subjects were seropositive for HPV18 at end-of-study, when compared to other vaccine HPV types.

At the end-of-study for protocols 013 and 015, efficacy of the quadrivalent HPV vaccine against any grade CIN or AIS related to HPV 6, 11, 16, or 18 in the per-protocol efficacy population was 96.0% (95% CI: 92.2–98.2) (Table 2). This efficacy is illustrated in a significant difference in the time to any grade CIN or AIS as seen in Fig. 2. Nine cases of any grade CIN were seen among subjects who received quadrivalent HPV vaccine versus 222 cases in those subjects receiving placebo. Vaccine efficacy against CIN 2 or worse related to HPV 6, 11, 16, or 18 in the per-protocol population was
Table 1

Anti-HPV 6, 11, 16 and 18 serologic responses by study time point among 16–26-year-old women who received quadrivalent HPV vaccine.a.

<table>
<thead>
<tr>
<th>Quadrivalent HPV vaccine (N = 8787)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Anti-HPV 6</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Month 7</td>
</tr>
<tr>
<td>Month 24</td>
</tr>
<tr>
<td>End-of-studyb</td>
</tr>
<tr>
<td>Anti-HPV 11</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Month 7</td>
</tr>
<tr>
<td>Month 24</td>
</tr>
<tr>
<td>End-of-studyb</td>
</tr>
<tr>
<td>Anti-HPV 16</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Month 7</td>
</tr>
<tr>
<td>Month 24</td>
</tr>
<tr>
<td>End-of-studyb</td>
</tr>
<tr>
<td>Anti-HPV 18</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Month 7</td>
</tr>
<tr>
<td>Month 24</td>
</tr>
<tr>
<td>End-of-studyb</td>
</tr>
</tbody>
</table>

N = number of subjects that were randomized to the respective vaccination group who received at least 1 injection and had non-missing data; n = number of evaluable subjects with serology data at relevant time point; m = number of seropositive subjects (subject is defined as seropositive to HPV 6, 11, 16 or 18 if her corresponding anti-HPV cLIA was ≥20 mMU/mL, 16 mMU/mL, 20 mMU/mL, or 24 mMU/mL, respectively); CI = confidence interval; GMT = geometric mean titer; mMU = milli Merck units.

a The per-protocol immunogenicity population includes all subjects who were not general protocol violators, received all three vaccinations within acceptable day ranges, were seronegative at day 1 and PCR negative day 1 through month 7 for the relevant HPV type(s), and had a month 7 serum sample collected within an acceptable day range.

Table 2

Efficacy against HPV 6/11/16/18-related CIN (any grade) or AIS at end-of-study, by severity. Per-protocol efficacy population of protocols 013 and 015 at end-of-study.

<table>
<thead>
<tr>
<th>Quadrivalent HPV vaccine (N = 8799)</th>
<th>Placebo (N = 8800)</th>
<th>Observed efficacy (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Cases</td>
<td>Rate</td>
<td>n</td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>------</td>
<td>---</td>
</tr>
<tr>
<td>CIN (any grade) or AIS</td>
<td>7629</td>
<td>9</td>
<td>0.0</td>
</tr>
<tr>
<td>By lesion severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN 1</td>
<td>7629</td>
<td>7</td>
<td>0.0</td>
</tr>
<tr>
<td>CIN 2 or worse</td>
<td>7629</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>CIN 2</td>
<td>7629</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>CIN 3 or worse</td>
<td>7629</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>CIN 3</td>
<td>7629</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>AIS</td>
<td>7629</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>By HPV type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV 6</td>
<td>6688</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>HPV 11</td>
<td>6688</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>HPV 16</td>
<td>6448</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>HPV 18</td>
<td>7158</td>
<td>1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Subjects are counted once in each applicable endpoint category; a subject may appear in more than one category.

N = number of subjects randomized to the respective vaccination group who received at least 1 injection; n = number of subjects evaluable, i.e., number of subjects in the given population who also have at least one follow-up visit; CI = confidence interval; CIN = cervical intraepithelial neoplasia; AIS = adenocarcinoma in situ; rate = incidence rate per 100 person years at risk.
Fig. 2. Time to HPV 6 (A), HPV 11 (B), HPV 16 (C), HPV 18 (D) and HPV 6/11/16/18-related (E) CIN (any grade) or AIS in the per-protocol efficacy population of protocol 013 and protocol 015 at end-of-study. *Case counting started at month 7. Vaccine lines along the horizontal axis are not visible.

4. Discussion

The hallmark of prophylactic vaccine efficacy is disease prevention. Accordingly, the WHO’s recommended clinical endpoints for determining the efficacy of prophylactic HPV vaccines are disease endpoints [26]. However, the significance of vaccine immunogenicity becomes more apparent after efficacy against clinical disease endpoints has been established. Analyzing the immune response to
vaccination with the quadrivalent HPV vaccine was useful in bridging the response from girls and women 16–23 years old to those too young to be ethically followed for disease endpoints [27]. However, no immune correlate of protection for prevention of HPV-related disease has been identified to date in any HPV vaccine or natural history study. Immunological correlates of protection from HPV-related disease could be valuable in defining the antibody levels needed for protection from vaccine HPV types, as well as the length of protection, and whether a booster dose is needed.

Taking into account the trials reported herein, and although data are not available for all subjects, we found little correlation between month 7 antibody levels and the chances of becoming a case of vaccine HPV type-related disease. This is in part due to few cases of vaccine HPV type-related disease among vaccinated subjects, which are needed to accurately determine an immunological correlate of protection. In addition, not all month 7 antibody titers were available for subjects that did become a case of vaccine-type related disease. However, the data presented show no evidence of vaccine HPV type-related breakthrough disease due to waning antibody levels or seropositivity (particularly important in the case of HPV 18 immunogenicity).

A robust neutralizing antibody response to all four vaccine HPV types was seen at month 7; ≥99% of previously naïve subjects seroconverted for all four HPV types. Twenty-four months after vaccination, measurable neutralizing antibodies against HPV 6, 11, and 16 were observed in more than 95% of vaccinated subjects. In contrast, neutralizing antibodies against the HI84 epitope of HPV 18 were measurable in 71.6% of vaccinated subjects at month 24, and in 60% of subjects by end-of-study (average follow-up of 44 months). Whereas seropositivity percentages seem to be lower for HPV 18 at month 24 and at end-of-study than for the other three vaccine HPV types, analysis of the cases of HPV 6/11/16/18-related disease among vaccinated subjects during the trials provides important insight. Only one of these cases of CIN was related to HPV 18, and this case was confounded by the presence of HPV 56. While HPV 18 neutralizing antibody levels are observed to be diminishing, it is clear that these lower levels are not leading to breakthrough disease. This suggests that even in the HPV 18 seronegative subjects (39.7% of all subjects at end-of-study), vaccine efficacy remains near 60% of subjects by end-of-study (average follow-up of 44 months). Whereas seropositivity percentages seem to be lower for HPV 18 at month 24 and at end-of-study than for the other three vaccine HPV types, analysis of the cases of HPV 6/11/16/18-related disease among vaccinated subjects during the trials provides important insight. Only one of these cases of CIN was related to HPV 18, and this case was confounded by the presence of HPV 56. While HPV 18 neutralizing antibody levels are observed to be diminishing, it is clear that these lower levels are not leading to breakthrough disease. This suggests that even in the HPV 18 seronegative subjects (39.7% of all subjects at end-of-study), vaccine efficacy remains near 60% of subjects by end-of-study, possibly due to immune memory.

We have measured and presented data concerning vaccine HPV type-specific neutralizing antibody titers; those which are able to
neutralize HPV 6, 11, 16, and 18 inhibit infection of basal epithelial cells [28,29]. While vaccination with the quadrivalent HPV vaccine invariably results in the production of a plethora of HPV-specific antibodies, not all of these antibodies are capable of virus neutralization. The measurement of all HPV-specific antibodies produced in response to vaccination (both neutralizing and non-neutralizing) while a surrogate of total (potentially non-specific) antibody response, is therefore less informative about the direct inhibitory effect of vaccination on vaccine HPV types. However, the interpretation of specific HPV-related neutralizing antibody titers must be viewed against the background of the cLIA assay. While post-vaccination sera are analyzed for antibodies directed against the most dominant known neutralizing epitopes (H6.M48, K11.B2, H16.V5, and H18.J4; for HPV types 6, 11, 16, and 18, respectively), it is possible that antibodies specific for other neutralizing epitopes exist that will not be accounted for by the current cLIA assay. This could lead to an underestimation of neutralizing antibody titers directed against vaccine HPV types.

Efficacy against HPV 18-related disease is of crucial importance. HPV 18 not only causes some 20% of cervical cancer and HPV-related vulvar cancer, it is also the predominant HPV type leading to adenocarcinoma of the cervix, a disease with increasing incidence and an aggressive course, especially in younger women. Cytologic screening often fails to detect its precursors due to atypical cellular patterns and being in the endocervical canal and out of range of colposcopy. The mechanism for protection among subjects who become nominally anti-HPV 18 seronegative several years after vaccination remains to be determined. Olsson et al. have demonstrated that subjects vaccinated with the quadrivalent HPV vaccine are able to generate an anamnestic response upon further exposure to HPV vaccine (HPV VLPs), even among subjects who had become HPV seronegative before antigen challenge [30]. Support is lent by data demonstrating heightened antibody responses among women receiving quadrivalent HPV vaccine who were seropositive for vaccine HPV types at enrollment, as recently published by Giuliano et al. [31]. Immune memory is likely central to the efficacy against HPV 18-related disease among recipients of the quadrivalent HPV vaccine, despite antibody titers which decline after vaccination. Protection through anamnestic responses has also been observed in the case of Hepatitis B virus (HBV) vaccine. Like HPV, HBV is a sexually transmitted infection (as well as a blood-borne virus in contradistinction to HPV) that can persist and may lead to hepato-cellular cancer. Among recipients of the HBV vaccine, efficacy persists in the absence of detectable antibody titers, indicating the importance of immune memory. Moreover, on exposure to natural virus (e.g. needlestick accidents) an anamnestic response is seen, with no disease breakthrough. Data suggest that the quadrivalent HPV vaccine is able to largely prevent disease related to vaccine HPV types (e.g. nascent infection) through the generation of a timely anamnestic response after exposure to a fourth vaccine dose. Whether natural infection in the genital tract or other mucosal surfaces result in an anamnestic response is yet to be demonstrated. This supposition is plausible given that papillomaviruses will attach but not penetrate host cells for a significant period of time, and that virus bound in this way appears susceptible to inactivation by neutralizing antibodies [32].

In summary, we have shown continued immunogenicity and efficacy for vaccine HPV types 44 months after vaccination. While no immune correlate of protection from HPV 6/11/16/18-related disease can yet be determined, it is unlikely that waning of antibody titers is responsible for disease breakthrough in subjects who have received quadrivalent HPV vaccine. Moreover, it is probable that the anamnestic response plays a key role in protection from infection and disease in those subjects who have low or no detectable HPV 6/11/16/18 antibody levels years after primary vaccination.

Acknowledgements

The authors wish to thank Margaret James, Carolyn Maass, Kathryn McCarroll, Kathy Harkins, and MaryAnne Rutkowski for help with statistical programming and analysis.

Conflict of interest information: NM has received lecture fees, advisory board fees, and consultancy fees from Merck and Sanofi Pasteur MSD. SEO has received lecture fees from Merck. MHA has received lecture fees and grant support from Merck. OEI has received lecture fees from Merck and GlaxoSmithKline. CMW has received funding through her institution to conduct HPV vaccine studies for GlaxoSmithKline. KA has received consultancy and advisory board fees. XB has received lecture fees from Merck and GlaxoSmithKline, and has received funding through his institution to conduct HPV vaccine studies GlaxoSmithKline. JP has received consultancy fees, advisory board fees, and lecture fees from Merck. JD has received consultancy fees, lecture fees, and research grants from Merck and Sanofi Pasteur MSD. SL has received lecture fees from Merck and Sanofi Pasteur MSD. EJ has received lecture fees from Merck, Sanofi Pasteur MSD and GlaxoSmithKline. SKK has received consultancy fees, and has received funding through her institution to conduct HPV vaccine studies for Sanofi Pasteur MSD and Digene. SMG has received advisory board fees and grant support from Commonwealth Serum Laboratories (CSL) and GlaxoSmithKline, lecture fees from Merck, and funding through her institution to conduct HPV vaccine studies for GSK. DGF has received consultancy fees and funding through his institution to conduct HPV vaccine studies for GlaxoSmithKline, and lecture fees and consultancy fees from Merck. KS has received consultancy fees from Merck. SM has received lecture fees and advisory board fees from Merck. GP has received lecture fees and consultancy fees from Merck and Sanofi Pasteur MSD. DRB has received lecture fees, advisory board fees, and intellectual property fees. MS has received lecture fees and grant support from Merck. Additionally, SEO, CMW, MHA, OEI, GWKT, XB, JP, JD, EHT, SL, EJ, SKK, GP, SMG, DGF, KS, MS, LK, and DRB have received funding through their institutions to conduct HPV vaccine studies for Merck. FJT, CR, AT, JB, LCL, KEDG, SV, SL, TMH, RH, and EB are employees of Merck and potentially own stock and/or stock options in the company. Role of the funding source: The studies were designed by the sponsor (Merck and Co., Inc.) in collaboration with external investigators and an external data and safety monitoring board. The sponsor collated the data, monitored the conduct of the study, performed the statistical analysis and coordinated the writing of the manuscript with all authors. The authors were actively involved in the collection, analysis or interpretation of the data, the revising of the manuscript for intellectual content, and approved the final manuscript.

References


Christensen ND, Cladel NM, Reed CA. Postattachment neutralization of papillomaviruses by monoclonal and polyclonal antibodies. Virology 1995;207(Febrary(1)):136–42.