Noninferiority of Antibody Response to Human Papillomavirus Type 16 in Subjects Vaccinated with Monovalent and Quadrivalent L1 Virus-Like Particle Vaccines[∇]

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The incorporation of multiple antigens into a single human papillomavirus (HPV) vaccine may induce immune interference. To evaluate whether interference occurs when HPV type 16 (HPV16) virus-like particles are combined in a multivalent vaccine, we conducted a study to evaluate anti-HPV16 responses among subjects receiving three-dose regimens of either a monovalent HPV16 vaccine or a quadrivalent HPV (types 6, 11, 16, and 18) vaccine.

Natural history studies conducted over the past 20 years have demonstrated that human papillomavirus (HPV) infection is a necessary prerequisite for the development of cervical cancer (9). These studies have also demonstrated that among the 40 genital HPV types, only a subset of approximately 15 HPV types are oncogenic. Among these types, HPV type 16 (HPV16) is responsible for more than 50% of cervical cancers and an even higher proportion of HPV-related vulvar and vaginal cancers (1). Of all HPV types with a tropism for genital tissues, HPV16 is the most likely to persist and result in abnormal Pap test results and cervical dysplastic lesions (5).

While combining several antigens into one vaccine is an efficient means to broaden the vaccine's coverage, such combination may result in immune interference, defined as the reduction in the immunogenicity of a vaccine antigen when it is administered as a component of a vaccine that includes multiple vaccine antigens. There are no physicochemical tests that can be used to accurately predict whether combining several immunogenic antigens into a single vaccine will result in interference. Thus, for the HPV vaccine candidates, determining the impact of combining HPV virus-like particles (VLPs) targeting oncogenic HPV types into a single vaccine on the immunogenicity of each VLP required a clinical evaluation.

Here, we present the results of a study that compared anti-HPV16 responses following the administration of three-dose regimens of either an HPV16 vaccine or the quadrivalent HPV (types 6, 11, 16, and 18) vaccine.

The results presented in this report are representative of data from 3,882 subjects who were enrolled in Merck HPV vaccine protocol 012 between 30 May 2002 and 30 June 2004. Protocol 012 was part of a larger, randomized, double-blind (operating under in-house blinding procedures), placebo-controlled, multicenter study enrolling 5,455 subjects to evaluate the efficacy of a quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine (GARDASIL; Merck and Co., Inc.) in 16- to 23-year-old women (protocol 013; FUTURE I) (3a). Subjects participating in protocol 013 were enrolled in the context of two separate immunogenicity substudies: a concomitant hepatitis B vaccine (recombinant) administration substudy (protocol 011, to be reported separately elsewhere) and a monovalent HPV16 vaccine immunobridging substudy (protocol 012), the results of which are presented herein (Fig. 1). Protocol 012 randomized subjects in a 6:1:6 ratio to receive the quadrivalent HPV vaccine, the HPV16 vaccine, or a placebo, respectively.

Subjects must have reported zero to four lifetime sex partners, no prior history of clinical HPV disease (e.g., cervical intraepithelial neoplasia or genital warts), and no allergy to
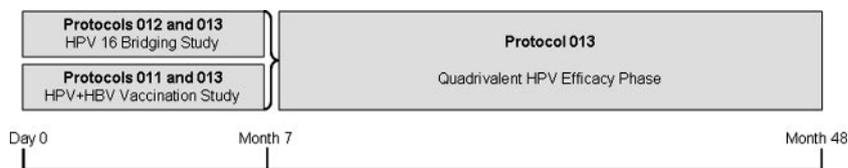
FIG. 1. Schematic of substudies 011 and 012 in the context of the larger efficacy study 013. HBV, hepatitis B virus.

vaccine components at day 1 to be enrolled. Subjects were enrolled regardless of day-1 HPV status or Pap test results. All eligible subjects received intramuscular (deltoid) injections of the quadrivalent HPV vaccine, the monovalent HPV16 vaccine, or a placebo upon enrollment (day 1) and at months 2 and 6. Subjects underwent cervicovaginal sampling for the detection of HPV infection, as well as serology testing. To improve precision, subjects were included in the primary immunogenicity analyses only if they did not have protocol violations that may have influenced vaccine-induced immune responses.

The quadrivalent HPV vaccine and the visually indistinguishable aluminum-containing placebo have been described previously (8). Allocation schedules were generated by a computer program using a blocking factor of 13. Subjects were randomized in a 6:1:6 ratio to receive the quadrivalent HPV vaccine, the HPV16 vaccine, or the placebo, respectively, at each study site. An interactive voice response system was used to allocate subjects.

Blood samples were obtained upon enrollment and at month 7 for serology testing for anti-HPV6, anti-HPV11, anti-HPV16, and anti-HPV18 with a competitive immunoassay developed by Merck Research Laboratories using technology from the Luminex Corporation, Austin, TX (2). At each study visit, all examinations and specimen collections took place prior to vaccination. Temperature and weight measurements and a serum or urine sample for pregnancy testing (using the human β chorionic gonadotropin test) were obtained prior to each injection. If the subject had a temperature of $\geq100°$F or $\geq37.8°$C (measured orally) within 24 h prior to a scheduled vaccine dose, the injection was postponed.

Comparisons of the degrees of immunogenicity of the quadrivalent HPV vaccine and the monovalent HPV16 vaccine were carried out via a competitive luminescence immunoassay (cLIA) (2). This assay was used to evaluate the serological responses to the vaccines, to measure HPV infection-induced antibodies, and to exclude subjects with evidence of a present or past HPV infection from the primary analysis. For the assessment of immunogenicity, the end points of interest were (i) the anti-HPV16 geometric mean titer (GMT) at week 4 post-dose 3 and (ii) the percentage of subjects who seroconverted for HPV16 by week 4 post-dose 3. As the HPV L1 VLP vaccines are intended to be prophylactic, the primary immunogenicity analysis was conducted with data from the per-protocol immunogenicity population. This population included subjects who were HPV16 seronegative by cLIA at day 1 and PCR negative for HPV16 (as determined by testing swab and biopsy specimens) from enrollment through month 7.

The first primary hypothesis addressed the similarity (noninferiority) of HPV16 immune responses to the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine and the monovalent HPV16 L1 VLP vaccine as measured by anti-HPV16 GMTs at week 4 post-dose 3. This hypothesis was addressed by an analysis-of-variance model, which involved modeling the natural log of the post-dose 3 anti-HPV16 levels of the subjects as a function of study country and vaccination group, both of which were fixed effects. The antilogs of (i) the estimated treatment effect and (ii) its confidence interval were utilized to report the estimated GMT and the 95% confidence interval for the GMT, respectively. This methodology tested a null hypothesis of a 0.5-fold difference or less in GMTs against the alternative hypothesis of a >0.5-fold difference in GMTs. The second primary immunogenicity hypothesis addressed the similarity of anti-HPV16 responses to the quadrivalent HPV vaccine and the monovalent HPV16 vaccine as measured by the percentage of subjects who seroconverted for HPV16 by week 4 post-dose 3 (seroconversion was defined as a change in serostatus from seronegative to seropositive, where the cLIA cutoff for determining serostatus was 20 milli-Merck units [mMU] of anti-HPV16/ml of serum; a subject with a cLIA titer at or above the serostatus cutoff was considered to be seropositive). This hypothesis was addressed by a one-sided test of noninferiority conducted at the 0.025 level (P). In order for the null hypothesis to be rejected, the lower bound of the 95% confidence interval for the difference between the quadrivalent vaccine group and the monovalent HPV16 vaccine group in the percentage of subjects who seroconverted had to be greater than -0.05. The test was based on the method of Miettinen and Nurminen (4) for testing the equivalence of two proportions, which allows for stratification by study size. The success of the study required both primary immunogenicity hypotheses to be successful, and therefore, no multiplicity adjustments were required.

An audit conducted by Merck Research Laboratories concluded that there was a deviation from the standard operating procedure for the testing of a subset of serum samples. Serum samples accounting for approximately 0.1% of day-1 serology results and 0.4% of postvaccination serology results were determined not to have been tested according to the standard operating procedure corresponding to the vaccination group. All day-1 serum samples which were tested out of compliance with the standard operating procedure for the actual clinical material received (i.e., the HPV standard operating procedure) were reanalyzed. The remaining nonconformant test results were removed from the database.

Of the 3,882 subjects enrolled, 3,875 received at least one dose of a vaccine or the placebo. A total of 95% of subjects in the quadrivalent HPV vaccine group and 96% of subjects in the HPV16 vaccine group received all three doses of the vaccine. A total of 211 subjects discontinued study participation during the vaccination period. The percentages of subjects discontinuing treatment in each category were comparable among the three vaccination groups.
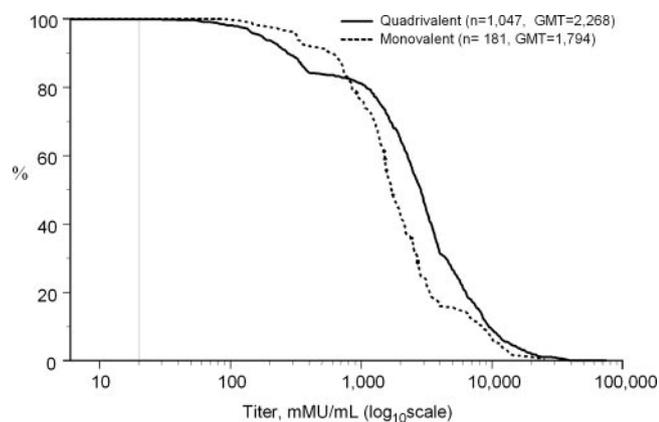
FIG. 2. Reverse cumulative distributions of anti-HPV16 levels in HPV vaccination groups at month 7 as determined by cLIA. The per-protocol immunogenicity population included all subjects who were not general protocol violators, received all three vaccinations within acceptable day ranges, were seronegative for HPV16 at day 1 and PCR negative from day 1 through month 7, and had a month 7 serum sample collected within an acceptable day range. The vertical line at 20 mMU/ml represents the seroconversion cutoff value for anti-HPV16. n, number of subjects contributing to the analysis.

Each subject serum sample was tested initially at a 1:4 dilution. As can be seen when the results are stratified by the percentages of seroconverters corresponding to certain GMTs, HPV16 titers in samples from the HPV16 monovalent vaccine group and those from the quadrivalent HPV vaccine group were comparable (Fig. 2). The observed GMT at month 7 was 2,310 mMU/ml (confidence interval, 2,139.9 to 2,493.9 mMU/ml) for subjects receiving the quadrivalent HPV vaccine and 1,701 mMU/ml (confidence interval, 1,461.7 to 1,980.6 mMU/ml) for subjects receiving the monovalent HPV16 vaccine (compared to a median of 28 mMU/ml for placebo-treated subjects who were positive for HPV16 at month 7 [$n = 17$]). If a result in the quantifiable range could not be obtained, the sample was retested at dilutions of 1:40 and 1:400 and potentially at 1:4,000 if further dilution was required. Subjects who received the placebo and were included in the per-protocol analysis did not have detectable levels of anti-HPV16 at month

7. The observed HPV16 seroconversion rate for subjects receiving the quadrivalent HPV vaccine was 99.8% (confidence interval, 99.3% to 100%) compared to 100% (confidence interval, 98% to 100%) for those subjects receiving the HPV16 monovalent vaccine.

To address the primary analysis of immunogenicity, an analysis was conducted using a model that accounted for the country of origin and the vaccination group. In this analysis, anti-HPV16 GMTs at week 4 post-dose 3 (as estimated using this model) and the percentages of subjects who seroconverted by week 4 post-dose 3 (as estimated using this model) in the two vaccination groups were compared (Table 1). The statistical criterion for noninferiority with respect to GMT required that the lower bound of the 95% confidence interval for the difference (n-fold) in anti-HPV16 GMTs (the ratio of the GMT for the quadrivalent vaccine group to the GMT for the monovalent vaccine group) exclude a decrease of 2-fold or more. By using this statistical criterion, along with the statistical criterion of noninferiority for seroconversion (Table 1), the noninferiority of the anti-HPV16 GMT response in the quadrivalent HPV vaccine group relative to that in the monovalent HPV16 vaccine group was established. Additionally, immune responses to the quadrivalent HPV vaccine in these trials were comparable across cohorts of subjects with different baseline covariates of immunogenicity, such as race and religion (A. R. Giuliano, E. Lazcano-Ponce, L. L. Villa, T. Nolan, C. Marchant, D. Radley, G. Golm, K. McCarroll, M. T. Esser, S. Vuocolo, and E. Barr, submitted for publication).

Recent vaccines directed against *Streptococcus pneumoniae* have been engineered to include more than 20 different capsular polysaccharide types. However, the combination of multiple antigens into a single vaccine presumes that the administration of the antigens in combination will not reduce the efficacy, safety, and/or immunogenicity of the combined vaccine compared to those of each individual antigen vaccine (3). Caveats such as these are underscored by studies that have shown immune interference when effective vaccines are combined into one product (7). Another concern about combination vaccines is that naturally occurring immune responses to closely related viruses may interfere with protection against the primary intended antigen. Vaccination against herpes simplex

TABLE 1. Analysis of noninferiority as measured by GMTs and seroconversion in subjects who received the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine and subjects who received the monovalent HPV16 L1 VLP vaccine[a]

Group[b]	Vaccine	No. of subjects overall	No. of subjects contributing to the analysis	Noninferiority by:	
				GMT (mMU/ml)[c]	Seroconversion (%)[d]
A	Quadrivalent	1,783	1,047	2,030.4	99.8
B	Monovalent	304	181	1,911.0	100.0

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

[b] Subjects who received at least one injection were randomized to the quadrivalent and monovalent vaccination groups.

[c] The estimated difference (n-fold) between the GMTs of groups A and B was 1.06 (95% confidence interval, 0.84 to 1.35). The P value for noninferiority was <0.001. For the null hypothesis that the ratio of the group A GMT to the group B GMT is ≤0.5, a P value of <0.025 supported a conclusion that the anti-HPV16 response in the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine group was noninferior to the response in the monovalent HPV16 L1 VLP vaccine group. The estimated GMT, difference (n-fold), associated confidence intervals, and P values were based on a statistical analysis model adjusting for country.

[d] The estimated percentage point difference between group A and B seroconversion rates was −0.02 (95% confidence interval, −0.7 to 2.0). The P value for noninferiority was <0.001. For the null hypothesis that the percentage of group A seroconverters minus the percentage of group B seroconverters is ≤−0.05, a P value of <0.025 supported a conclusion that the anti-HPV16 seroconversion rate in the quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine group was noninferior to that in the monovalent HPV16 L1 VLP vaccine group.

virus type 2 (HSV-2) provides a potential example of such unexpected findings. Despite the generation of antibody responses against HSV-2 in both genders, the vaccine was not efficacious in women who were seropositive for HSV-1 and seronegative for HSV-2 at the baseline. Further analysis showed that the vaccine was efficacious only in women who were seronegative for both HSV-1 and HSV-2 and that it was not efficacious in men regardless of their serological status (6). The mechanisms by which preexisting antibodies against heterologous but related antigens interfere with vaccine-induced immunity are not understood.

Data in this report elaborate on the immunogenicity of the quadrivalent HPV vaccine by bridging data from the monovalent HPV16 vaccine to the quadrivalent HPV vaccine. Immunization with the quadrivalent HPV vaccine was found to induce immune responses against HPV16 (as measured by both anti-HPV16 antibody titers at week 4 post-dose 3 and the percentage of subjects who seroconverted for HPV16 by week 4 post-dose 3) that were at least as good as those induced by the monovalent HPV16 vaccine. This result helps to mitigate the theoretical concern that the inclusion of several antigens in an HPV vaccine will result in a reduction of the immune response to one or all of the component antigens.

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