Published in final edited form as:

Vaccine. 2009 September 18; 27(41): 5612–5619. doi:10.1016/j.vaccine.2009.07.027.

Longer-term efficacy of a prophylactic monovalent human papillomavirus type 16 vaccine

Ali Rowhani-Rahbar^a, Constance Mao^b, James P. Hughes^c, Frances B. Alvarez^d, Janine T. Bryan^d, Hawes Stephen E.^a, Noel S. Weiss^a, and Laura A. Koutsky^a

^aDepartment of Epidemiology, University of Washington, Seattle, WA, USA

^bDepartment of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA

^cDepartment of Biostatistics, University of Washington, Seattle, WA, USA

^dMerck Research Laboratories, West Point, PA, USA

Abstract

We conducted an extended follow-up study (March 2006 – May 2008) to assess the longer-term efficacy of a prophylactic monovalent human papillomavirus (HPV) type 16 L1 virus-like particle vaccine in women (n = 290) who had enrolled in a randomized controlled trial of this vaccine during October 1998 – November 1999 in Seattle and remained HPV-16 DNA negative during the course of that trial. During the extended follow-up period, none of the vaccine recipients was found to be infected with HPV-16 or developed HPV-16-related cervical lesions; among placebo recipients, 6 women were found to be infected with HPV-16 (vaccine efficacy [VE] = 100%; 95% confidence interval [CI]: 29% – 100%) and 3 women developed HPV-16-related cervical lesions (VE = 100%; 95% CI: <0% – 100%). Approximately 86% of vaccine recipients remained HPV-16 competitive Luminex immunoassay seropositive at an average of 8.5 years of follow-up. During the combined original trial and extended follow-up period, 20 and 22 women developed any cervical lesion regardless of HPV type among the vaccine and placebo recipients, respectively (VE = 15%; 95% CI: <0% – 56%). The results suggest that this monovalent HPV-16 vaccine remains efficacious through 8.5 years after its administration.

Keywords

Human papillomavirus type 16; Prophylactic vaccines; Efficacy

1. Introduction

Infection with high-risk types of genital human papillomavirus (HPV) is now considered to be a necessary cause of cervical cancer.[1,2] Evidence of this causal link has culminated in the

© 2009 Elsevier Ltd. All rights reserved.

Corresponding author Ali Rowhani-Rahbar, University of Washington HPV Research Group, 155 NE 100th Street, Suite 510, Seattle, WA 98115, Phone: +1 650 721 6727, Fax: +1 650 736 2239, rowhani@u.washington.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest information

The University of Washington has received funding from Merck to support HPV vaccine research conducted by CM and LAK. FBA and JTB are employees of Merck.

development and testing of prophylactic HPV L1 virus-like particle (VLP) vaccines as a critical means for cervical cancer prevention. These vaccines are composed of HPV type-specific L1 proteins that self-assemble into noninfectious recombinant VLPs.[3] Three prophylactic HPV L1 VLP vaccines which have been tested in randomized controlled trials to date include a monovalent HPV-16 vaccine, a quadrivalent HPV-6/11/16/18 vaccine (Gardasil®), both developed by Merck & Co., Inc., and a bivalent HPV-16/18 vaccine (Cervarix®) developed by GlaxoSmithKline Biologicals. The trials have consistently shown that these vaccines are remarkably efficacious in preventing infection and cervical lesions caused by the vaccine-HPV types, at least for a few years.[4–17]

On June 8, 2006, The United States (U.S.) Food and Drug Administration (FDA) announced the approval of the quadrivalent HPV-6/11/16/18 vaccine for females between the ages of 9 and 26.[18] Since the main target group of prophylactic HPV vaccines is children before sexual debut, it is important to assess whether such vaccines can confer protection for more than a few years. Furthermore, it has been shown that decision analytic models for determining the public health impact and cost-effectiveness of HPV vaccination programs are sensitive to the duration of vaccine-induced immunity.[19–32] The duration of vaccine-induced immunity is generally assumed to range from 10 years to life-long in these studies.

Unfortunately, empirical evidence for this key assumption is lacking. It is currently unknown whether prophylactic HPV vaccines remain effective for more than a few years after vaccination. If these vaccines do not show durable protection, boosting is a possibility; however, the need for frequent vaccination throughout the adult life may be unacceptable from both individual and public health perspectives. Programmatic and cost implications of trying to achieve wide coverage for repeated boosting will be overwhelming, particularly in developing countries, where these vaccines could have their highest impact.[33]

We conducted an extended follow-up study to assess the longer-term efficacy of a monovalent HPV-16 vaccine against HPV-16 infection and HPV-16-related cervical intraepithelial neoplasia (CIN) by enrolling a portion of women who had participated in a randomized controlled trial of this vaccine 8.5 years earlier.

2. Materials and methods

2.1. Study population

Between October 1998 and November 1999, 2,391 women participated in a multi-center double-blind phase IIb randomized controlled trial of a monovalent HPV-16 vaccine in the U.S. Unmarried healthy women between the ages of 16 to 23 who were not pregnant and did not expect to get pregnant through month 7 of the original trial were eligible for participation. The monovalent HPV-16 vaccine was administered in a three-dose regimen (i.e., day 1, month 2, and month 6). The original trial ended when women completed 48 months of follow-up post-enrollment in January 2004. The monovalent HPV-16 vaccine was found to be highly efficacious against HPV-16 persistent infection (vaccine efficacy [VE] = 94%; 95% confidence interval [CI]: 88% – 98%) and HPV-16-related CIN (VE = 100%; 95% CI: 84% – 100%). Details of that study can be found elsewhere.[10] This is the original trial which gave rise to the participants in the current study.

Of 2,391 women who took part in the original trial, 500 were enrolled in Seattle. Half of these women received the vaccine and the other half received placebo, and all became unblinded to their treatment assignment after completion of the original trial. Beginning in February 2006, all of the 500 women were offered participation in a new extended follow-up study conducted in Seattle, with three visits occurring every six months, to assess the longer-term efficacy of

the monovalent HPV-16 vaccine. The institutional review board of the University of Washington approved the study and informed consent was obtained from all participants.

The contact information that we had for these women was used to send out a letter offering participation. Updated contact information was sought through the University of Washington's Alumni Association, the contact person(s) listed by the participant at her last visit in the original trial, and public records. We used public records such as national telephone directories, People Search, and Accurint®, the locate-and-research tool of LexisNexis®.[34,35] The letter explained how we obtained the subject's address and clearly stated that there was no obligation on the part of the subject to participate in this study. Women who received the letter and were interested were asked to call the clinic to make their first appointment for collection of specimens by the study clinicians. The study assistant called or sent an e-mail to women who did not call within two weeks after the letters were sent out in order to find out if they were interested in participation.

After the quadrivalent HPV-6/11/16/18 vaccine was licensed in the U.S. in 2006, we started offering the three-dose vaccination series to all women who had participated in the monovalent HPV-16 vaccine trial, according to the protocol. Participants who declined receipt of the quadrivalent HPV-6/11/16/18 vaccine were informed that they may be able to receive it independent of this study since it was commercially available in the U.S. They were informed, however, that they may not be eligible to receive the vaccine outside of this study once they became older than the upper age limit prescribed in the label for this vaccine.

2.2. Collection of specimens

A self-administered questionnaire was used to ascertain gynecologic and medical history prior to each study visit during the extended follow-up period. Participants who indicated an abnormal Pap test since their last visit in the original trial signed a medical release for these records. For participants who had undergone colposcopy, biopsy or definitive therapy procedures since their last visit in the original trial (n = 23), the operative reports, results of the biopsy, loop electrosurgical excision procedure (LEEP) specimens, hematoxylin and eosin slides, and the specimen blocks were obtained (n = 19).

A complete pelvic examination was performed and gynecologic specimens were collected. These included labial, vulvar, lateral vaginal, ecto- and endocervical, perineal, and perianal specimens collected using a Dacron swab for detection of HPV-16 DNA. Cervical samples for Pap test (ThinPrep, Cytec, Boxborough, MA) were collected. Cytology specimens were evaluated using the Bethesda System 2001.[36] For Pap tests showing atypical squamous cells of undetermined significance (ASC-US), reflex HPV testing was performed through the residual ThinPrep™ material using the Digene Hybrid Capture II high-risk probe (Qiagen, Gaithersburg, MD). Participants whose probe was positive were referred for colposcopy. Women with Pap tests showing low-grade squamous intraepithelial neoplasia (LSIL), high-grade squamous intraepithelial neoplasia (HSIL), atypical glandular cells (AGC), or atypical squamous cells, cannot exclude high-grade intraepithelial lesion (ASC-H) were referred for colposcopy at any time during the study. When a Pap test result was received, a study clinician would call the participant to explain the abnormal results and offer further follow-up with colposcopy.

All participants with colposcopic abnormalities underwent biopsy. In addition, a swab specimen from the site of the biopsy was taken for HPV-16 DNA detection after the biopsy specimen had been collected. An endocervical curettage (ECC) was performed if clinically indicated. Participants who underwent colposcopy and biopsy were asked to read and sign a separate consent form for this procedure. Women with CIN-2 or CIN-3, precursor lesions to cervical cancer indicating moderate and severe dysplasia, were offered definitive therapy or

treatment by LEEP. Cervical biopsy (as well as ECC and LEEP, if applicable) specimens were shipped to the study-designated pathology laboratory. Clinical management for participants was based on these results. Afterwards, the same slides were reviewed for endpoint adjudication by an independent Pathology Panel consisting of four gynecologic pathologists. The Pathology Panel results were used for case definition in the analysis.

Ten milliliter (mL) of blood was drawn for detection and measurement of serum HPV-16 antibody. Blood was drawn by a certified phlebotomist in a blood drawing chair or while the patient was lying down on an exam table. The blood specimens were centrifuged for 20 minutes. The vials containing sera were stored in a -20°C freezer until shipment to the study-designated laboratory.

2.3. Laboratory methods

Genital swabs and tissue specimens were tested for the presence of HPV-16 DNA using multiplex polymerase chain reaction (PCR) analysis. Multiplex PCR allowed the simultaneous detection of three gene products (i.e., L1, E6, and E7) in one reaction using HPV-16 specific primers. A specimen was called positive when two or three genes were positive or when the same single gene scored positive on consecutive tests. All specimens that were found to be infected in this study tested positive for at least two genes. Blood samples were tested for the presence of anti-HPV-16 immunoglobulin G (IgG) using a competitive Luminex-based immunoassay (cLIA). This test has been developed by Merck Research Laboratories, West Point, PA using technology from the Luminex Corporation, Austin, TX. The positivity cutoff point for the HPV-16 cLIA is 20 milli-Merck units per milliliter (mMU/mL).

2.4. Case definition

Two main endpoints were defined. HPV-16 infection was defined as detection of HPV-16 DNA in cervical or genital specimens. HPV-16-related CIN was defined based on the following criteria: 1) a histological diagnosis of CIN by the Pathology Panel review; and 2) an HPV-16 DNA positive result of the same biopsy tissue or the swab specimen collected from the biopsy site. The primary aim was to assess vaccine efficacy against incident HPV-16 infection and HPV-16-related cervical lesions during the extended follow-up period (i.e., since the end of the original trial). The secondary aim was to assess vaccine efficacy against incident HPV-16 infection and HPV-16-related cervical lesions during the entire project period (i.e., the combined original trial and extended follow-up period).

2.5. Statistical analysis

Assuming a true vaccine efficacy of 85%, an event rate of 5.8 per 100 woman-years in the placebo group observed in the original trial, and accounting for lack of data on HPV-16 status during the gap period, we estimated that we would achieve 80% power to reject the null hypothesis of vaccine efficacy = 0% against incident HPV-16 infection during the extended follow-up period by enrolling 375 women who had participated in the original trial. Assuming a true vaccine efficacy of 90% and an event rate of 1.1 per 100 woman-years in the placebo group observed in the original trial, we estimated that we would achieve 80% power to reject the null hypothesis of vaccine efficacy = 0% against HPV-16-related cervical lesions during the extended follow-up period by enrolling 375 women who had participated in the original trial.

Three separate study populations were defined for assessing vaccine efficacy. The primary efficacy analysis was conducted in the per-protocol susceptible population, defined as participants who were HPV-16 DNA negative and HPV-16 seronegative at day 1 visit in the original trial, remained HPV-16 DNA negative through one month after administration of the third dose of the vaccine or placebo, received three doses of vaccine or placebo within one

year, and did not have any protocol violations during the original trial. Protocol violations included engagement in sexual intercourse within 48 hours of either the first or month 7 visits, receiving nonstudy vaccine, receiving immunosuppressive agents or immunoglobulin, enrollment in another study of an investigational agent, or a month 7 visit outside the range (i.e., 14 – 72 days after the third dose of the vaccine).

Two additional study populations were defined for assessing vaccine efficacy. Unrestricted susceptible population included all participants who were HPV-16 DNA negative and HPV-16 seronegative at day 1 visit in the original trial. Intention-to-treat population included all participants who had undergone randomization, regardless of their baseline HPV status or evidence of HPV-16-related anogenital disease. Since the intention-to-treat population included participants who were HPV-16 DNA positive and/or HPV-16 seropositive at enrollment in the original trial, this population was only used for assessing vaccine efficacy against the development of HPV-16-related CIN in the current study.

Vaccine efficacy was defined as one minus the ratio of the incidence rate of an endpoint in the vaccine group divided by that in the placebo group (i.e., rate ratio) adjusted for age and race. Adjusted rate ratios and corresponding 95% CIs were obtained through an exact Poisson regression model. In the analysis of the extended follow-up period, the case ascertainment started the day after the last follow-up day for an endpoint in the original trial period. All participants who had been found to be infected with HPV-16 at any point during the original trial period were excluded from the analysis of the extended follow-up period. To provide information on the broadest impact of vaccination, we also calculated vaccine efficacy against all cervical lesions regardless of HPV type in the intention-to-treat population during the entire project period.

In the analysis of the entire project period, the case ascertainment started after month 7 in the per-protocol susceptible population and after day 1 in the unrestricted susceptible population and intention-to-treat population. In all analyses, the follow-up time ended at the time of an endpoint event or at the last visit, whichever came first. The follow-up of women was censored when they received the quadrivalent HPV-6/11/16/18 vaccine. Of note, 70 women in the vaccine group and 82 women in the placebo group received the quadrivalent HPV-6/11/16/18 vaccine.

Geometric mean titers of serum HPV-16 antibody in the per-protocol susceptible population were calculated and illustrated over the entire project period using a longitudinal plot. The proportion of participants who were HPV-16 seropositive was calculated and compared by the vaccination status and at each selected time point. All analyses were conducted using Stata 10 (Stata Corporation, College Station, TX).

3. Results

Of 500 women who had been invited to participate in this study, 68 women received our invitation but chose not to participate. We were not able to locate 141 women. A total of 291 (58%) women were enrolled between March 2006 and May 2008. One woman experienced a syncopal episode immediately after blood draw and the decision was made to discontinue the examination. Final analyses included 290 participants. Participants and nonparticipants did not differ with regard to age at enrollment in the original trial, race, age at first sexual intercourse, lifetime number of male sex partners at enrollment in the original trial, and number of new male sex partners during the original trial period (Table 1). Participants provided at least one visit for a total of 591 visits during the extended follow-up period. The mean follow-up time since enrollment in the original trial was 8.5 years (range: 7.2 – 9.5 years). The number of participants in the extended follow-up period based on timing of their visit relative to

enrollment in the original trial is shown in Table 2. A total of 114 out of 290 women (39%) completed the third visit; the majority of visits ($419/591 = 71\%$) occurred approximately between the 8th and 9th year of the follow-up.

Of 290 women, 148 were in the vaccine group and 142 were in the placebo group. The flow diagram shows women included in the per-protocol susceptible population, unrestricted susceptible population, and intention-to-treat population for the analysis of the extended follow-up period and the entire project period separately (Figure 1). Demographic and sexual characteristics of the two treatment arms were balanced at the enrollment visit of the extended follow-up period (Table 3). The two treatment arms were also balanced with regard to the outcome of pregnancy and development of new medical conditions following enrollment in the original trial (data not shown).

During the extended follow-up period, no woman was found to be infected with HPV-16 in the vaccine group and six women were found to be infected with HPV-16 in the placebo group (VE = 100%; 95% CI: 29% – 100%). Two women were found to be infected with HPV-16 at their first study visit. These two women provided only one visit. One woman was found to be infected with HPV-16 at her first and second study visits. This woman provided only two visits. Two women were found to be infected with HPV-16 at their first study visit but were not found to be infected with HPV-16 at either their second or third study visits. One woman was found to be infected with HPV-16 at her second study visit but was not found to be infected with HPV-16 at her first or third study visits. These six women belonged to the per-protocol susceptible population; consequently, they were included in unrestricted susceptible population as well.

During the extended follow-up period, no woman developed HPV-16-related CIN in the vaccine group and three women developed HPV-16-related CIN in the placebo group (VE = 100%; 95% CI: <0% – 100%). One woman developed HPV-16-related CIN-3 and two other women developed HPV-16-related CIN-2. These three women belonged to the per-protocol susceptible population; consequently, they were included in unrestricted susceptible and intention-to-treat populations as well. Table 4 provides vaccine efficacy estimates against HPV-16 infection and HPV-16-related CIN during the extended follow-up period and the entire project period in each study population. The table also provides vaccine efficacy estimates against all cervical lesions regardless of HPV type in the intention-to-treat population during the entire project period.

Immunogenicity analysis showed that serum HPV-16 antibody levels had only slightly decreased since month 48 post-enrollment in the original trial among the vaccine recipients (Figure 2). At an average of 8.5 years of follow-up, the geometric mean titer of serum HPV-16 antibody was significantly higher in the vaccine group (71.7 mMU/mL; 95% CI: 55.7 – 92.3) than in the placebo group (13.7 mMU/mL; 95% CI: 11.7 – 16.1) and a significantly higher proportion of vaccine recipients ($69/80 = 86\%$; 95% CI: 77% – 93%) than placebo recipients ($7/78 = 9\%$; 95% CI: 4% – 18%) were HPV-16 seropositive.

4. Discussion

To our knowledge, this study provides information on efficacy and immunogenicity after the longest duration of follow-up for any prophylactic HPV L1 VLP vaccine. The results of this study suggest that 8.5 years after its administration, the monovalent HPV-16 vaccine remains efficacious and immunogenic. During the extended follow-up period, all cases of incident HPV-16 infection and HPV-16-related cervical lesions developed in the placebo group. The geometric mean titer of serum HPV-16 antibody among the vaccine recipients had only slightly declined since the end of the original trial and remained significantly higher than that among

the placebo recipients. None of the vaccine recipients who were HPV-16 seronegative were found to be infected with HPV-16 during the extended follow-up period.

One of the populations in which the monovalent HPV-16 vaccine was found to be efficacious in this study was the unrestricted susceptible population. This group of participants may closely reflect an HPV-16-naïve population (e.g., adolescent girls) who may not strictly comply with a three-dose regimen or the vaccination protocol. The demonstrated reduced vaccine efficacy against HPV-16-related cervical lesions in the intention-to-treat population during the entire project period is consistent with the findings of phase III trials of the quadrivalent HPV-6/11/16/18 vaccine.[12,14] This finding is not surprising as the intention-to-treat population included women who were already infected with HPV-16 at the time of enrollment. The HPV L1 VLP vaccines are prophylactic, not therapeutic; there is no evidence that the administration of these vaccines can alter the course of infection or development of cervical lesions among individuals who are already infected with HPV.[37]

The prophylactic HPV L1 VLP vaccines have been shown to induce strong humoral immune responses.[9,10,12,38] The results of the original trial of the monovalent HPV-16 vaccine indicated that following a three-dose vaccination regimen, all vaccine recipients seroconverted; serum HPV-16 antibody levels peaked at month 7, waned over time, reached a plateau at approximately month 30, and remained stable through month 48 while exceeding antibody levels in the placebo group by several fold.[10] Using the results of the original trial, mathematical modeling has been utilized to predict the long-term antibody response to the monovalent HPV-16 vaccine. These mathematical models have indicated that almost life-long persistence of serum HPV-16 antibody following vaccination is expected at levels above those in natural HPV-16 infection in 76% of women.[39] Findings of the current study provide empirical evidence on longevity of antibody response 8.5 years after administration of this vaccine.

The minimum antibody level required to maintain protective efficacy for prophylactic HPV L1 VLP vaccines remains to be identified. In the meantime, it is reassuring that none of the vaccine recipients in this study developed breakthrough HPV-16 infection during the extended follow-up period. An antigen challenge of the quadrivalent HPV-6/11/16/18 vaccine delivered at year 5 after administration of that vaccine has been shown to result in an anamnestic response which indicates induction of robust immune memory following vaccination.[40]

This study was subject to some limitations. First, while we utilized a comprehensive and extensive search strategy to find and invite all women who had participated in the original trial, the participation proportion was approximately 60%, falling short of our anticipated participation proportion of 75%. The small sample size resulted in some imprecision, particularly in vaccine efficacy estimates against HPV-16-related cervical lesions during the extended follow-up period. Second, the presence of a gap between the original trial period and the first visit during the extended follow-up period introduced uncertainty about the *timing* of incident HPV-16 infection. Placebo recipients who were found to be infected with HPV-16 at their first visit during the extended follow-up period must have developed this infection at some point during the gap period. Thus, the incidence rate of HPV-16 infection in the placebo group reported here is almost certainly an underestimation of the true rate, given that we have slightly overestimated the person-time of risk of acquiring HPV-16.

Third, the presence of the gap period introduced uncertainty about the HPV-16 *status* of participants during this time. It is possible that some women who were found to be uninfected with HPV-16 at their first visit during the follow-up period had become infected and subsequently suppressed their infection during the gap period, resulting in an underestimation of HPV-16 incidence rate in the vaccine group, the placebo group, or both, in this study. Fourth,

women entered the analysis of the extended follow-up period provided that they were not infected with HPV-16 during the original trial period. Such conditioning on a post-randomization event (here: lack of HPV-16 infection in the original trial period) can result in the creation of the two so-called “improper” subgroups who may not be fully comparable. [41] This bias is known as post-treatment selection bias. We do not believe that this bias has had an important impact on the results of this study; we found sexual characteristics and history of sexually transmitted infections of the two study groups during the gap period ascertained at the first visit during the extended follow-up period to be balanced. Finally, since women participating in the original trial had received the monovalent HPV-16 vaccine, we could not assess the longer-term efficacy against other HPV types such as those included in the quadrivalent HPV-6/11/16/18 vaccine.

HPV-16 is estimated to cause over 50% of all cervical cancer cases in the U.S. and worldwide. [42] The quadrivalent HPV-6/11/16/18 vaccine has the same HPV-16 antigen component as the monovalent HPV-16 vaccine. A long-lasting high-level efficacy of this vaccine may translate to prevention of a substantial number of cervical cancer cases in the U.S. and around the world. The ongoing population-based long-term follow-up studies conducted in Nordic countries should provide valuable information on the durability of protection conferred by prophylactic HPV L1 VLP vaccines.[43,44]

Acknowledgements

The authors wish to thank Lauren Asaba, Amanda Chong-Tim, Nancy Dorn, Amy Garechana, Preetma Kooner, Alexandra Kurhan, Meagan Lehr, Laura Merrell, Sandra O'Reilly, and Dana Varon for their dedication throughout this project.

References

1. Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. *J Natl Cancer Inst Monogr* 2003;31:3–13. [PubMed: 12807939]
2. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;189(1):12–19. [PubMed: 10451482]
3. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. *Vaccine* 2006;24:S3/114–S3/121. [PubMed: 16949998]
4. La Torre G, de Waure C, Chiaradia G, Mannocci A, Ricciardi W. HPV vaccine efficacy in preventing persistent cervical HPV infection: a systematic review and meta-analysis. *Vaccine* 2007;25(50):8352–8358. [PubMed: 17996990]
5. Rambout L, Hopkins L, Hutton B, Fergusson D. Prophylactic vaccination against human papillomavirus infection and disease in women: a systematic review of randomized controlled trials. *CMAJ* 2007;177(5):469–479. [PubMed: 17671238]
6. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347(21):1645–1651. [PubMed: 12444178]
7. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004;364(9447):1757–1765. [PubMed: 15541448]
8. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005;6(5):271–278. [PubMed: 15863374]
9. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human

- papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006;367(9518):1247–1255. [PubMed: 16631880]
10. Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2006;107(1):18–27. [PubMed: 16394035]
 11. Villa LL, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer* 2006;95(11):1459–1466. [PubMed: 17117182]
 12. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007;356(19):1915–1927. [PubMed: 17494925]
 13. The FUTURE II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. *Lancet* 2007;369(9576):1861–1868. [PubMed: 17544766]
 14. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007;356(19):1928–1943. [PubMed: 17494926]
 15. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CM, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369(9580):2161–2170. [PubMed: 17602732]
 16. Perez G, Lazcano-Ponce E, Hernandez-Avila M, Garcia PJ, Munoz N, Villa LL, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) L1 virus-like-particle vaccine in Latin American women. *Int J Cancer* 2008;122(6):1311–1318. [PubMed: 18000825]
 17. Gall, S.; Teixeira, J.; Wheeler, C.; Naud, P.; Harper, DM.; Franco, E., et al. Substantial impact on precancerous lesions and HPV infections through 5.5 years in women vaccinated with the HPV-16/18 L1 VLP AS04 candidate vaccine. American Association for Cancer Research Annual Meeting; Apr 14–18; Los Angeles, CA, USA. 2007. 2007
 18. Food and Drug Administration. Available at <http://www.fda.gov/bbs/topics/NEWS/2006/NEW01385.html>
 19. Kim JJ, Kobus KE, Diaz M, O'Shea M, Van Minh H, Goldie SJ. Exploring the cost-effectiveness of HPV vaccination in Vietnam: insights for evidence-based cervical cancer prevention policy. *Vaccine* 2008;26(32):4015–4024. [PubMed: 18602731]
 20. Goldie SJ, Kohli M, Grima D, Weinstein MC, Wright TC, Bosch FX, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. *J Natl Cancer Inst* 2004;96(8):604–615. [PubMed: 15100338]
 21. Goldie SJ, Grima D, Kohli M, Wright TC, Weinstein M, Franco E. A comprehensive natural history model of HPV infection and cervical cancer to estimate the clinical impact of a prophylactic HPV-16/18 vaccine. *Int J Cancer* 2003;106(6):896–904. [PubMed: 12918067]
 22. Goldie SJ. Health economics and cervical cancer prevention: a global perspective. *Virus Res* 2002;89(2):301–309. [PubMed: 12445670]
 23. Garnett GP, Kim JJ, French K, Goldie SJ. Chapter21: Modelling the impact of HPV vaccines on cervical cancer and screening programmes. *Vaccine* 2006;24:S3/178–S3/186.
 24. Diaz M, Kim JJ, Albero G, de Sanjose S, Clifford G, Bosch FX, et al. Health and economic impact of HPV 16 and 18 vaccination and cervical cancer screening in India. *Br J Cancer* 2008;99(2):230–238. [PubMed: 18612311]
 25. Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett GP. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. *PLoS Med* 2006;3(5):e138. [PubMed: 16573364]
 26. Kulasingam SL, Myers ER. Potential health and economic impact of adding a human papillomavirus vaccine to screening programs. *JAMA* 2003;290(6):781–789. [PubMed: 12915431]
 27. Sanders GD, Taira AV. Cost-effectiveness of a potential vaccine for human papillomavirus. *Emerg Infect Dis* 2003;9(1):37–48. [PubMed: 12533280]

28. Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. *Emerg Infect Dis* 2004;10(11):1915–1923. [PubMed: 15550200]
29. Elbasha EH, Dasbach EJ, Insinga RP. Model for assessing human papillomavirus vaccination strategies. *Emerg Infect Dis* 2007;13(1):28–41. [PubMed: 17370513]
30. Brisson M, Van de Velde N, De Wals P, Boily MC. Estimating the number needed to vaccinate to prevent diseases and death related to human papillomavirus infection. *CMAJ* 2007;177(5):464–468. [PubMed: 17709404]
31. Brisson M, Van de Velde N, De Wals P, Boily MC. The potential cost-effectiveness of prophylactic human papillomavirus vaccines in Canada. *Vaccine* 2007;25(29):5399–5408. [PubMed: 17561316]
32. Van de Velde N, Brisson M, Boily MC. Modeling human papillomavirus vaccine effectiveness: quantifying the impact of parameter uncertainty. *Am J Epidemiol* 2007;165(7):762–775. [PubMed: 17276976]
33. Jacob M, Bradley J, Barone MA. Human papillomavirus vaccines: what does the future hold for preventing cervical cancer in resource-poor settings through immunization programs? *Sex Transm Dis* 2005;32(10):635–640. [PubMed: 16205306]
34. People Search. Available at: <http://www.peoplesearch.com/>
35. Accurint. Available at: <http://www.accurint.com/>
36. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002;287(16):2114–2119. [PubMed: 11966386]
37. Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. *JAMA* 2007;298(7):743–753. [PubMed: 17699008]
38. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine* 2006;24(27–28):5571–5583. [PubMed: 16753240]
39. Fraser C, Tomassini JE, Xi L, Golm G, Watson M, Giuliano AR, et al. Modeling the long-term antibody response of a human papillomavirus (HPV) virus-like particle (VLP) type 16 prophylactic vaccine. *Vaccine* 2007;25(21):4324–4333. [PubMed: 17445955]
40. Olsson SE, Villa LL, Costa RL, Petta CA, Andrade RP, Malm C, et al. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine* 2007;25(26):4931–4939. [PubMed: 17499406]
41. Hudgens MG, Hoering A, Self SG. On the analysis of viral load endpoints in HIV vaccine trials. *Stat Med* 2003;22(14):2281–2298. [PubMed: 12854093]
42. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003;88(1):63–73. [PubMed: 12556961]
43. Lehtinen M, Apter D, Dubin G, Kosunen E, Isaksson R, Korpivaara EL, et al. Enrolment of 22,000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. *Int J STD AIDS* 2006;17(8):517–521. [PubMed: 16925896]
44. Lehtinen M, Idanpaan-Heikkilä I, Lunnas T, Palmroth J, Barr E, Cacciatore R, et al. Population-based enrolment of adolescents in a long-term follow-up trial of human papillomavirus vaccine efficacy. *Int J STD AIDS* 2006;17(4):237–246. [PubMed: 16595046]

Figure 1.

Study participants flow diagram. HPV: human papillomavirus; PSP: per-protocol susceptible population; USP: unrestricted susceptible population; ITT: intention-to-treat population

Numbers
Vaccine
Placebo

93 93 93 93 91 91 91
96 96 96 95 92 92 89

55 80 65
64 78 63

Figure 2.
Levels of serum HPV-16 antibody in the per-protocol susceptible population over the entire project period

Table 1

Characteristics of participants and nonparticipants in the extended follow-up study

Characteristic	Participants	Nonparticipants	p ^a
	n = 290	n = 210	
Mean age at enrollment in the original trial (range), yr	20.4 (17–23)	20.2 (18–23)	0.17
Race/ethnicity, n (%)			
Asian	31 (10.7)	23 (10.9)	0.32
African-American	10 (3.5)	8 (3.8)	
Hispanic American	9 (3.1)	3 (1.4)	
Native American	5 (1.7)	0 (0)	
White	226 (77.9)	169 (80.5)	
Other	9 (3.1)	7 (3.3)	
Mean age at first sexual intercourse (range), yr	17.3 (12.0–22.0)	17.2 (13.0–23.0)	0.66
Lifetime number (%) of male sex partners at enrollment in the original trial			
1	89 (30.7)	59 (28.1)	0.75
2	73 (25.2)	49 (23.3)	
3	45 (15.5)	39 (18.6)	
> 3	83 (28.6)	63 (30.0)	
Number (%) of new male sex partners during the original trial period			
0	195 (67.2)	122 (58.1)	0.15
1	76 (26.2)	70 (33.3)	
2	17 (5.9)	13 (6.2)	
3	1 (0.3)	4 (1.9)	
> 3	1 (0.3)	1 (0.5)	

^aFrom t-test or chi-square test

Table 2
Number of participants in the extended follow-up period based on timing of their visit relative to enrollment in the original trial

	Month			
	80–86 (n = 12)	87–93 (n = 115)	94–100 (n = 210)	101–107 (n = 209)
First visit (n = 290)	12	99	87	81
Second visit (n = 187)	0	15	103	54
Third visit (n = 114)	0	1	20	74
				108–115 (n = 45)
				11
				15
				19

Table 3

Characteristics of the participants by vaccination status at enrollment in the extended follow-up period

Characteristic	Vaccine Group	Placebo Group
	n = 148	n = 142
Mean age (range), yr	28.9 (23.3–32.7)	28.5 (21.4–32.4)
Race/ethnicity, n (%)		
Asian	14 (9.5)	17 (12.0)
African-American	3 (2.0)	7 (4.9)
Hispanic American	2 (1.3)	7 (4.9)
Native American	2 (1.3)	3 (2.1)
White	122 (82.4)	104 (73.2)
Other	5 (3.4)	4 (2.8)
Mean age at first sexual intercourse (range), yr	17.5 (14.0–22.0)	17.1 (12.0–22.0)
Lifetime number (%) of male sex partners		
1	5 (3.5)	6 (4.3)
2	14 (9.7)	13 (9.3)
3	14 (9.7)	14 (10.0)
> 3	112 (77.2)	107 (76.4)
Number (%) of new male sex partners after the end of the original trial		
0	55 (37.9)	57 (41.0)
1	29 (20.0)	18 (12.9)
2	15 (10.3)	26 (18.7)
3	15 (10.3)	9 (6.5)
> 3	31 (21.4)	29 (20.9)
Current average number (%) of acts of intercourse per month		
0	20 (14.1)	18 (13.9)
1	8 (5.6)	10 (7.7)
2	8 (5.6)	7 (5.4)
3	8 (5.6)	5 (3.9)
> 3	98 (69.0)	89 (69.0)
Number (%) of women with selected STIs		
Bacterial vaginosis	17 (11.5)	14 (9.9)
Chlamydia	3 (2.1)	2 (1.4)
Gonorrhea	1 (0.7)	0 (0)
Trichomoniasis	1 (0.7)	1 (0.7)
Pap test diagnosis at enrollment in the extended follow-up period		
Unsatisfactory	3 (2.0)	3 (2.1)
Satisfactory	145 (98.0)	139 (97.9)
Normal	133 (91.7)	126 (90.6)
ASC-US	7 (4.8)	8 (5.7)
LSIL	5 (3.4)	5 (3.6)
HSIL	0 (0)	0 (0)

Characteristic	Vaccine Group	Placebo Group
	n = 148	n = 142

STIs = sexually transmitted infections; ASC-US = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesions; HSIL = high-grade squamous intraepithelial lesions

Table 4
Efficacy against HPV-16 infection, HPV-16-related cervical lesions, and all cervical lesions regardless of HPV type

Endpoint	Vaccine Group				Placebo Group				Efficacy ^b , % (95% CI)
	No. of Subjects	No. of Cases	Woman-years	Rate ^a	No. of Subjects	No. of Cases	Woman-years	Rate ^a	
Extended follow-up period									
HPV-16 infection									
Per-protocol	113	0	516.9	0	103	6	454.0	1.3	100 (29–100)
Unrestricted	123	0	584.9	0	106	6	470.9	1.3	100 (33–100)
HPV-16-related cervical lesions									
Per-protocol									
CIN-1 or worse	113	0	516.9	0	103	3	457.8	0.7	100 (<0–100)
CIN-2 or worse	113	0	516.9	0	103	3	457.8	0.7	100 (<0–100)
Unrestricted									
CIN-1 or worse	123	0	584.9	0	106	3	474.6	0.6	100 (<0–100)
CIN-2 or worse	123	0	584.9	0	106	3	474.6	0.6	100 (<0–100)
Intention-to-treat									
CIN-1 or worse	134	0	634.8	0	113	3	507.5	0.6	100 (<0–100)
CIN-2 or worse	134	0	634.8	0	113	3	507.5	0.6	100 (<0–100)
Entire project period									
HPV-16 infection									
Per-protocol	114	1	902.5	0.1	118	21	832.2	2.5	96 (27–100)
Unrestricted	126	3	1,053.9	0.3	127	27	929.3	2.9	90 (31–99)
HPV-16-related cervical lesions									
Per-protocol									
CIN-1 or worse	114	0	909.8	0	118	8	884.1	0.9	100 (41–100)
CIN-2 or worse	114	0	909.8	0	118	7	891.0	0.8	100 (29–100)
Unrestricted									
CIN-1 or worse	126	0	1,078.00	0	127	9	1,014.20	0.9	100 (50–100)
CIN-2 or worse	126	0	1,078.00	0	127	8	1,021.10	0.8	100 (41–100)
Intention-to-treat									
CIN-1 or worse	148	4	1,240.50	0.3	142	10	1,142.60	0.9	64 (–27–92)

Endpoint	Vaccine Group				Placebo Group				Efficacy ^b , % (95% CI)
	No. of Subjects	No. of Cases	Woman-years	Rate ^a	No. of Subjects	No. of Cases	Woman-years	Rate ^a	
CIN-2 or worse	148	3	1,246.80	0.2	142	8	1,156.50	0.7	64 (-51-94)
<i>All cervical lesions regardless of HPV type</i>									
Intention-to-treat									
CIN-1 or worse	148	20	1,169.80	1.7	142	22	1,086.70	2.0	15 (-68-56)
CIN-2 or worse	148	8	1,225.80	0.7	142	12	1,137.80	1.1	33 (-79-77)

HPV: human papillomavirus; CI: confidence interval; CIN: cervical intraepithelial neoplasia

^a Per 100 woman-years

^b Adjusted for age and race