

Comparison of the Immunogenicity and Reactogenicity of Cervarix and Gardasil Human Papillomavirus Vaccines in HIV-Infected Adults: A Randomized, Double-Blind Clinical Trial

Lars Toft,¹ Merete Storgaard,¹ Martin Müller,² Peter Sehr,³ Jesper Bonde,^{4,5} Martin Tolstrup,¹ Lars Østergaard,¹ and Ole S. Søgaard¹

¹Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark; ²Research Program Infection and Cancer, German Cancer Research Center; ³Chemical Biology Core Facility, European Molecular Biology Laboratory, Heidelberg, Germany; and ⁴Department of Pathology and ⁵Clinical Research Centre, Copenhagen University Hospital Hvidovre, Copenhagen, Denmark

Background. We compared the immunogenicity and reactogenicity of Cervarix or Gardasil human papilloma-virus (HPV) vaccines in adults infected with the human immunodeficiency virus (HIV).

Methods. This was a double-blind, controlled trial randomizing HIV-positive adults to receive 3 doses of Cervarix or Gardasil at 0, 1.5, and 6 months. Immunogenicity was evaluated for up to 12 months. Neutralizing anti-HPV-16/18 antibodies were measured by pseudovirion-based neutralization assay. Laboratory tests and diary cards were used for safety assessment. The HPV-DNA status of the participants was determined before and after immunization.

Results. Ninety-two participants were included in the study. Anti-HPV-18 antibody titers were higher in the Cervarix group compared with the Gardasil group at 7 and 12 months. No significant differences in anti-HPV-16 antibody titers were found among vaccine groups. Among Cervarix vaccinees, women had higher anti-HPV-16/18 antibody titers compared to men. No sex-specific differences in antibody titers were found in the Gardasil group. Mild injection site reactions were more common in the Cervarix group than in the Gardasil group (91.1% vs 69.6%; $P = .02$). No serious adverse events occurred.

Conclusions. Both vaccines were immunogenic and well tolerated. Compared with Gardasil, Cervarix induced superior vaccine responses among HIV-infected women, whereas in HIV-infected men the difference in immunogenicity was less pronounced.

Clinical Trials Registration. NCT01386164.

Keywords. HPV vaccines; HIV; neutralizing antibodies; serology; safety; Gardasil; Cervarix.

Persistent infection with oncogenic human papilloma-virus (HPV) genotypes cause approximately 600 000 cancers worldwide every year, including cervical cancer, anal cancer, vulvar and vaginal cancers, penile cancer, and certain oropharyngeal cancers [1–3]. Most

HPV-associated cancers are caused by genotypes 16 and 18 [4, 5] and the 2 commercially available HPV vaccines, Cervarix (GlaxoSmithKline) and Gardasil (Merck), both protect against these oncogenic HPV types. Gardasil additionally covers HPV-6 and -11, the major causative agents of genital warts [6–8].

People infected with human immunodeficiency virus (HIV) have a highly increased risk of developing HPV-associated cancers [9]. Despite the increasing use of highly active antiretroviral therapy (HAART) as routine treatment in HIV-infected individuals and establishment of screening programs for precancerous cervical lesions, the incidence of cervical cancer among HIV-infected women has not declined in recent years [10]. Additionally, the incidence of anal cancer is steadily

Received 8 October 2013; accepted 11 November 2013; electronically published 23 November 2013.

Correspondence: Lars Toft, MD, Department of Infectious Diseases, Aarhus University Hospital, Skejby, 8200 Aarhus, Denmark (larsnise@rm.dk).

The Journal of Infectious Diseases 2014;209:1165–73

© The Author 2013. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/infdis/jit657

increasing in HIV-infected men and women [11–13] and some countries, including the United States and Australia, now recommend routine HPV vaccination of HIV-infected individuals. However, little is known about immunity induced by HPV vaccination in HIV-infected adults.

Both HPV vaccines are based on virus-like particles using type-specific HPV L1 capsid protein as vaccine antigen. Cervarix is formulated with an aluminum salt- and Toll-like receptor (TLR) 4 agonist-based adjuvant system called AS04, and Gardasil is formulated with amorphous aluminum hydroxyphosphate sulfate as adjuvant [7, 14]. The vaccines are best administered before sexual debut, and vaccination does not clear existing HPV infections [15]. However, recent trials show that adults also benefit from vaccination if they have no preexisting infection with vaccine-type HPVs [16, 17] and that vaccination reduces the risk of subsequent HPV-associated disease among people treated surgically for precancerous cervical and anal lesions [18, 19].

The exact mechanisms of vaccine-induced protection have not been established, but neutralizing antibodies are believed to be of crucial importance [20–22], and higher antibody titers following immunization may be a surrogate marker for prolonged protection [23, 24]. In healthy women, Cervarix was shown to induce higher antibody titers against HPV-16 and -18 compared to Gardasil [23, 24].

Both vaccines are reportedly safe and immunogenic in HIV-infected individuals with high seroconversion rates, albeit lower antibody titers than in age-matched HIV-negative persons [25–29]. To our knowledge, no studies have reported comparative immunogenicity and safety data on Cervarix and Gardasil in HIV-infected persons, and no efficacy studies have been published using either vaccine. We and others have shown that TLR9-adjuvanted vaccines induce superior antibody responses in HIV-infected individuals [30, 31]. Thus, the TLR4-directed adjuvant in Cervarix may be particularly useful in this population, but sex-specific differences in TLR signaling and altered TLR4 responsiveness in HIV-infected individuals may affect vaccine responses [32, 33].

This study was undertaken to compare the immunogenicity and reactogenicity of Cervarix and Gardasil in HIV-infected adult men and women.

METHODS

Study Design

This was an investigator-initiated, randomized, double-blind, head-to-head trial randomizing persons with HIV to vaccination with either Cervarix or Gardasil. The study protocol was approved by the Danish Medicines Agency, the Regional Ethical Committee, and the Danish Data Protection Agency. The study was monitored for regulatory compliance and data quality by the Division of Good Clinical Practice at Aarhus University Hospital.

Setting and Participants

The study was conducted at the Department of Infectious Diseases, Aarhus University Hospital, Denmark. HIV-positive patients seen at the outpatient clinic during 2011 were invited by letter to participate. Consenting HIV-seropositive volunteers 18 years or older were eligible for enrollment. We excluded individuals who (1) had previously been vaccinated against HPV, (2) had a history of malignancy or autoimmune disease, (3) had received systemic immunosuppressive treatment, (4) had previously had an allergic reaction to vaccination, (5) were pregnant, breastfeeding, or unwilling to use reliable contraception methods for the duration of the trial, and/or (6) had an HIV RNA level of >200 copies/mL if receiving antiretroviral treatment.

Randomization and Intervention

Participants were stratified according to use of HAART and sex and thereafter randomized 1:1 in blocks of 4 to receive Cervarix or Gardasil. Random allocation sequences were computer-generated by the hospital pharmacy. Participants were assigned their study identification number according to the chronological order in which they were enrolled. Participants and investigators were masked to the assigned vaccine throughout the study.

Prefilled vaccines were provided by the hospital pharmacy. Participants received vaccination with either Cervarix or Gardasil at months 0, 1.5, and 6 and they were seen at month 7 and 12 for immunogenicity and safety follow-up. Trained study nurses administered all vaccines by intramuscular injection into the left or right arm. Before any trial procedures, blood samples were collected for immunogenicity measurements and safety laboratory tests, including HIV RNA, CD4⁺ cell count, hemoglobin, erythrocyte volume fraction, white blood cell count, alkaline phosphatase, alanine aminotransferase, and creatinine parameters.

Assessment of Neutralizing Antibodies

Serum samples were heat-inactivated for 60 minutes at 56°C and stored at –80°C before shipment for blinded analysis at the joint Chemical Biology Core Facility of the German Cancer Research Center and the European Molecular Biology Laboratory, Heidelberg, Germany, using a pseudovirion-based neutralization assay (PBNA). Pseudovirions (PSVs) were prepared and closely quality-monitored as previously described [34–36]. All liquid handling and plate reading was done using automated devices and plate readers (PerkinElmer) [37]. Serum samples were diluted 10 times in 3-fold increments in complete Dulbecco's modified Eagle medium cell culture medium in 384-well polypropylene plates to achieve a final serum dilution in the PBNA of 1:40 to 1:787 320. Two microliters of diluted serum and 18 µL PSVs diluted in cell culture medium was mixed in 384-well assay plates. After 1 hour of incubation at room temperature, 20 µL HeLa T cells was added at a final density of 1500 cells/well and

incubated for 2 days at 37°C/5% CO₂. Finally, 20 µL/well glow substrate solution (PJK) was added and the luminescence read in an Envision plate reader. Triplicate plates originating from the same serum dilution were used for each HPV type. Inhibition (%) was calculated by normalization of the luminescence to the mean of the negative control wells on each plate. The median of the triplicate values was used for curve fitting, and the calculation of the effective dilution giving a 50% inhibition value for each serum was performed in ActivityBase (IDBS).

Assessment of HPV DNA Status

Anal and cervical swabs for HPV DNA testing were collected at baseline and at month 7 by inserting a Regular FLOQSwab (Copan Diagnostics) into the anal canal or the cervix uteri. Swabs were placed into tubes containing UTM-RT transport medium (Copan Diagnostics). One milliliter of each sample was spun down (5 minutes at 14 000 rpm), supernatants were removed, and cell pellets were resuspended in 180 µL phosphate-buffered saline and 20 µL Proteinase K (Roche Diagnostics). Samples were mixed and incubated for 1 hour at 56°C and 1 hour at 90°C. HPV DNA was purified using a MagNA Pure LC 96 and MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Switzerland). Five microliters of purified DNA was used for the PCR amplification in concordance with the manufacturer's specifications with subsequent microarray detection for any of 35 defined HPV genotypes included in the Genomica CLART HPV2 Genotyping micro array (Genomica).

Assessment of Safety

Participants were observed for 30 minutes after each immunization to evaluate immediate adverse events. Solicited adverse events occurring during the first 4 days after each immunization (injection site pain, swelling, erythema, fever, headache, nausea, myalgia, arthralgia and rash) and other signs of illness and/or changes in medication occurring within 15 days after immunization were recorded on diary cards. Solicited and unsolicited adverse events, as well as laboratory tests, were graded according to the common toxicity criteria version 2.0. All solicited local (injection site) and influenza-like (fever, arthralgia, chills, and fatigue) reactions were considered causally related to vaccination. Other adverse events were evaluated by the primary investigator and graded as "unlikely" or "probably" related to the study vaccines.

Statistical Analysis

The primary objective was to compare the serological anti-HPV-16 and -18 neutralizing antibody titers among vaccine groups at month 7. Secondary objectives were to compare antibody titers at month 12 and to stratify vaccine responses by baseline HPV serology among HPV DNA-negative participants. We powered our trial to detect a ≥35% difference in mean geometric mean titers (GMTs) between the 2 vaccine

groups at month 7. Setting type I error probability (α) to .05 (2-sided), type II error probability (β) to .10 (power = 1- β = .90), and assuming a standard deviation of 50% of the mean GMT, we found that 43 patients per group were required to detect this difference.

Continuous variables were summarized using medians with interquartile ranges. Dichotomous and categorical variables were summarized using numbers and percentages. We conducted per-protocol analyses of all randomized subjects for whom immunogenicity data were available at baseline and a subsequent timepoint.

We calculated crude GMTs on the primary endpoints. An adjusted estimate was obtained using a multivariate linear regression that included the following variables: current smoker, baseline CD4⁺ cell count, current use of HAART (yes or no), body mass index, sex, age, type-specific baseline HPV antibody titer, and HPV DNA status. Finally, as the AS04 adjuvant may have differential effects in men and women, we conducted the above-mentioned analysis stratified on sex. Incident and persistent HPV-16 and -18 infection was summarized according to vaccine group.

We used the χ^2 test to compare GMTs and the proportion of adverse events between the vaccine groups. We used 2-way *t* test to analyze for baseline group differences and temporal changes in CD4⁺ cell count and log-transformed HIV RNA from baseline, and performed subanalyses of the 2 variables stratified according to HAART use. We used Stata software, version 12.2 (StataCorp), for statistical analyses.

RESULTS

Study Population

Of the 512 individuals invited to participate, 121 signed up and were screened for eligibility, and 92 of these were subsequently enrolled in the trial between 10 August and 2 November 2011 (Figure 1). The trial ended on 3 December 2012 when the last participant was seen for follow-up. Two participants did not complete the 3-dose vaccination series: 1 withdrew her consent and 1 was inappropriately enrolled (medical condition incompatible with intramuscular injections). One was lost to follow-up and missed the 12-month visit. The Gardasil group had a higher proportion of smokers than the Cervarix group (16 vs 6; *P* = .026). The 2 groups were similar in immune status and all other baseline characteristics at the time of inclusion in the study (Table 1). Baseline characteristics did not differ significantly between men and women.

Immunogenicity

Primary endpoints are shown in Figure 2. Both vaccines increased HPV-16 GMTs from baseline to 7 and 12 months, and no significant differences in anti-HPV-16 antibody titers were found between vaccine groups. Anti-HPV-18 GMTs were

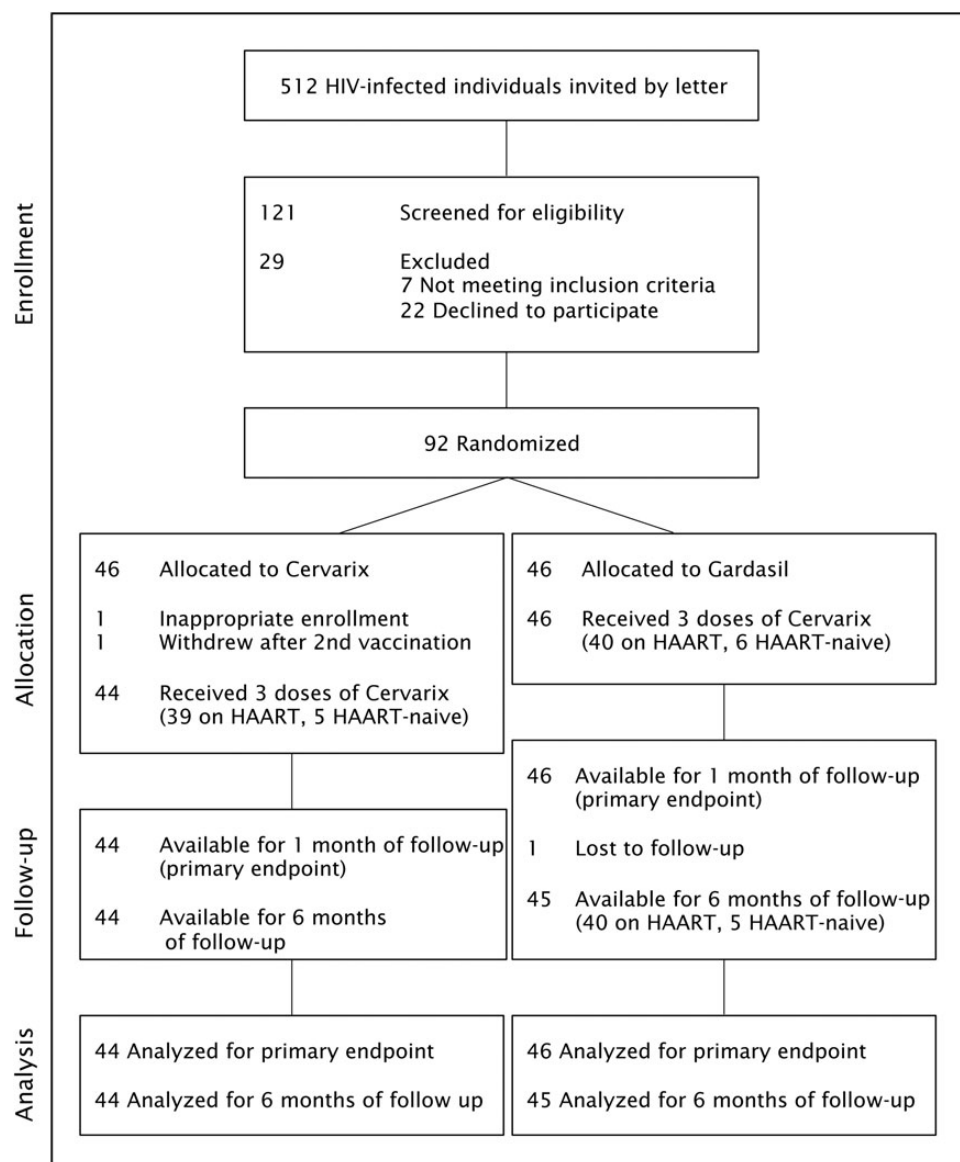


Figure 1. Disposition of study participants. Abbreviations: HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

higher in the Cervarix group compared with the Gardasil group at 7 and 12 months. The GMT ratio was 4.31 at 7 months (95% confidence interval [CI], 2.21–8.40) and 4.15 at 12 months (95% CI, 1.95–8.84). Adjustment for potential confounders (current smoker, baseline CD4⁺ cell count, current use of HAART, body mass index, sex, age, type-specific baseline HPV antibody titer, and HPV DNA status) did not affect the results.

An analysis of antibody titers according to baseline serostatus and anogenital DNA status is shown in Table 2. Again, we found no significant differences in anti-HPV-16 antibody titers between vaccine groups. Among those seronegative and HPV-18 DNA negative at baseline, anti-HPV-18 antibody titers were higher in the Cervarix group than in the Gardasil

group. The GMT ratio was 7.60 at 7 months (95% CI, 2.56–22.5) and 8.90 at 12 months (95% CI, 2.56–31.0).

Results of an analysis stratifying antibody responses by sex are shown in Figure 3. Among participants vaccinated with Cervarix, women had 3.16-fold (95% CI, 1.56- to 6.40-fold) higher anti-HPV-16 antibody titers at 7 months, and 4.43-fold (95% CI, 1.85- to 10.6-fold) higher anti-HPV-16 antibody titers at 12 months when compared to men. Anti-HPV-18 antibody titers in the Cervarix group were 2.07-fold (95% CI, .88- to 4.85-fold) and 3.57-fold (95% CI, 1.36- to 9.34-fold) higher among female compared to male participants. We found no differences in antibody titers between men and women in the Gardasil group.

Table 1. Baseline Characteristics of the Study Population

| Characteristic | Cervarix Group (n = 45) | Gardasil Group (n = 46) |
|--|----------------------------|----------------------------|
| Sex | | |
| Male | 30 (66.7) | 31 (67.4) |
| Female | 15 (33.3) | 15 (32.6) |
| Race | | |
| White | 38 (84.4) | 37 (80.4) |
| Other | 7 (15.6) | 9 (19.6) |
| Age, y, median (IQR) | 47.0 (38.6–54.2) | 44.5 (38.2–51.9) |
| Body mass index, kg/m ² , median (IQR) | 23.8 (21.8–26.7) | 24.1 (21.0–26.4) |
| Current smoker | 6 (13.3) | 16 (34.8) |
| CD4 ⁺ cell count, cells/ μ L, median (IQR) | 600 (470–750) | 585 (440–760) |
| Undergoing HAART | | |
| Yes | 40 (88.9) | 40 (87.0) |
| No | 5 (11.1) | 6 (13.0) |
| HIV RNA level, log₁₀ copies/mL, median (IQR) | | |
| In patients receiving HAART | 1.28 | 1.28 |
| In patients not receiving HAART | 4.97 (4.81–5.32) | 4.58 (3.85–5.22) |
| HPV serostatus and HPV DNA status^a | | |
| HPV-16 | | |
| Seronegative, HPV DNA negative | 22 (51.2) | 19 (44.2) |
| Seronegative, HPV DNA positive | 0 | 2 (4.6) |
| Seropositive, HPV DNA negative | 17 (39.5) | 15 (34.9) |
| Seropositive, HPV DNA positive | 4 (9.3) | 7 (16.3) |
| HPV-18 | | |
| Seronegative, HPV DNA negative | 22 (51.2) | 18 (41.9) |
| Seronegative, HPV DNA positive | 0 | 1 (2.3) |
| Seropositive, HPV DNA negative | 19 (44.2) | 20 (46.5) |
| Seropositive, HPV DNA positive | 2 (4.6) | 4 (9.3) |
| HPV-16 and -18 | | |
| Both seronegative, HPV DNA negative | 16 (37.2) | 15 (34.9) |
| Both seronegative, HPV DNA positive | 0 | 0 |
| Both seropositive, HPV DNA negative | 13 (30.2) | 12 (27.9) |
| Both seropositive, HPV DNA positive | 1 (2.3) | 2 (4.6) |

Data are presented as No. (%) of patients unless otherwise indicated.

Abbreviations: HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range.

^a Two participants in the Cervarix group and 3 participants in the Gardasil group with inconclusive baseline HPV DNA status are not included.

Among female participants, anti-HPV-16 GMTs were 2.98-fold (95% CI, .76-fold to 11.6-fold) and 3.90-fold (95% CI, 1.05- to 14.6-fold) higher in the Cervarix compared to the

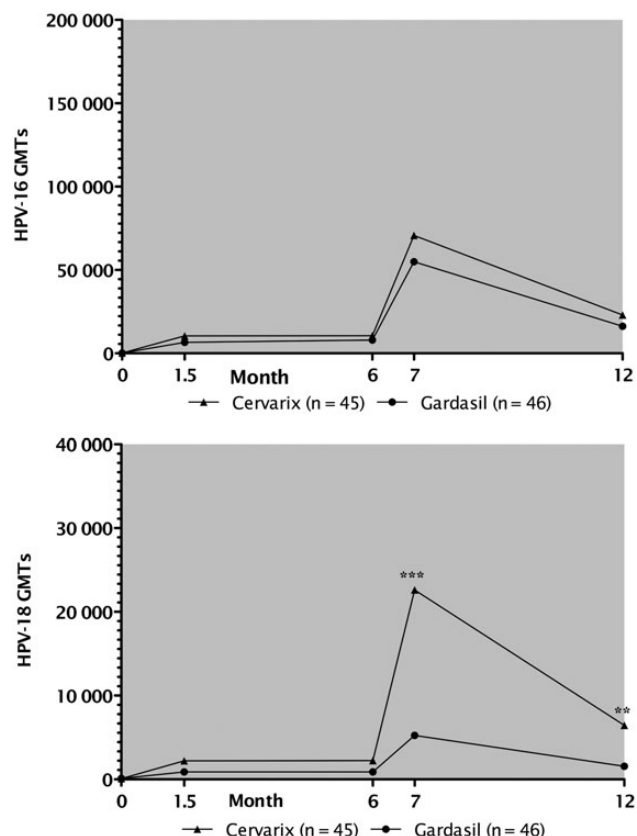


Figure 2. Human papillomavirus (HPV) serotype-specific neutralizing antibody geometric mean titers (GMTs). HPV serotype-specific neutralizing antibody GMTs were computed using the log₁₀ transformation of ED₅₀ values for anti-HPV-16 and HPV-18 serum neutralizing antibodies as measured by pseudovirus neutralization assay at months 0, 1.5, 6, 7, and 12. ***P* < .001, ****P* < .0001. One study participant from the Cervarix group withdrew after the second visit, and 1 study participant from the Gardasil group was lost to follow-up after the fourth visit. Abbreviation: ED₅₀, effective dilution giving a 50% inhibition.

Gardasil group at 7 and 12 months, and anti-HPV-18 GMTs were 3.93-fold (95% CI, 1.02- to 15.2-fold) and 6.02-fold (1.05- to 14.6-fold) higher in the Cervarix compared to the Gardasil group at 7 and 12 months, respectively.

Among male participants, We found no significant differences in anti-HPV-16 GMTs between the vaccine groups. Anti-HPV-18 titers were 4.54-fold (95% CI, 2.15- to 9.59-fold) and 3.55-fold (95% CI, 1.48- to 8.53-fold) higher in the Cervarix group than in the Gardasil group at 7 and 12 months, respectively. Adjustment for potential confounders (current smoker, baseline CD4⁺ cell count, current use of HAART, body mass index, sex, age, type-specific baseline HPV antibody titer, and HPV-DNA status) did not affect sex-specific results.

Anogenital HPV DNA Detection at 7 Months

Eighty participants had available anogenital HPV DNA detection at both baseline and 7 months. Table 3 summarizes

Table 2. Geometric Mean Antibody Titers (GMTs) and GMT Ratios According to Baseline Seropositivity and Anogenital Human Papillomavirus DNA Detection

| HPV Type | Baseline HPV Serostatus and DNA Status | Month | Cervarix Group | | | Gardasil Group | | | GMT Ratio | (95% CI) |
|----------|--|-------|----------------|---------|------------------|----------------|--------|------------------|-----------|-------------|
| | | | No. | GMT | (95% CI) | No. | GMT | (95% CI) | | |
| HPV-16 | Seronegative, HPV DNA negative | 0 | 22 | 40 | | 19 | 40 | | 1 | |
| | | 7 | 21 | 59 112 | (33 571–104 084) | 19 | 46 906 | (17 126–128 473) | 1.26 | (.42–3.74) |
| | | 12 | 21 | 14 027 | (6768–29 068) | 19 | 9363 | (3911–22 415) | 1.50 | (.50–4.46) |
| | Seronegative, HPV DNA positive | 0 | 0 | | | 2 | 40 | ^a | | |
| | | 7 | 0 | | | 2 | 93 422 | | | |
| | | 12 | 0 | | | 2 | 44 848 | | | |
| | Seropositive, HPV DNA negative | 0 | 17 | 500 | (155–1616) | 15 | 456 | (190–1090) | 1.10 | (.26–4.60) |
| | | 7 | 17 | 121 869 | (74 049–200 572) | 15 | 76 465 | (41 846–139 725) | 1.59 | (.76–3.35) |
| | | 12 | 17 | 54 980 | (30 704–98 447) | 14 | 25 589 | (10 670–61 367) | 2.15 | (.81–5.68) |
| HPV-18 | Seronegative, HPV DNA negative | 0 | 22 | 40 | | 18 | 40 | | 1 | |
| | | 7 | 21 | 24 368 | (11 559–51 374) | 18 | 3208 | (1371–7507) | 7.60 | (2.56–22.5) |
| | | 12 | 21 | 6135 | (2588–14 544) | 17 | 689 | (264–1801) | 8.90 | (2.56–31.0) |
| | Seronegative, HPV DNA positive | 0 | 0 | | | 1 | 40 | ^a | | |
| | | 7 | 0 | | | 1 | 347 | | | |
| | | 12 | 0 | | | 1 | 64 | | | |
| | Seropositive, HPV DNA negative | 0 | 19 | 179 | (95–337) | 20 | 262 | (146–470) | 0.68 | (.30–1.57) |
| | | 7 | 19 | 21 837 | (12 756–37 383) | 20 | 9342 | (3997–21 834) | 2.34 | (.87–6.25) |
| | | 12 | 19 | 6822 | (3569–13 040) | 20 | 3385 | (1407–8143) | 2.02 | (.70–5.84) |
| | Seropositive, HPV-DNA positive | 0 | 2 | 7290 | ^a | 4 | 640 | ^a | | |
| | | 7 | 2 | 19 149 | | 4 | 10 965 | | | |
| | | 12 | 2 | 13 432 | | 4 | 5844 | | | |

GMTs were computed using the log₁₀ transformation of titers. GMT ratio is Cervarix GMT divided by Gardasil GMT. Two participants in the Cervarix group and 3 participants in the Gardasil group with inconclusive baseline HPV DNA status were not included in the analysis.

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HPV, human papillomavirus.

^a CIs not reported for subgroups with <5 participants.

^b GMT ratios not calculated if 1 or more subgroups had <5 participants.

incident and persistent HPV-16 and -18 infection. All incident HPV-16/18 infections occurred among male participants.

Safety

No serious adverse events were detected in this study. Both vaccines were generally well tolerated and very few mild systemic reactions were observed (Table 4). Injection site reactions were more common in the Cervarix group than in the Gardasil group (91.1% vs 69.6%; $P = .02$). No sex-related differences in injection site reactions were detected (data not shown).

DISCUSSION

In the present study, we found that Cervarix was significantly more immunogenic than Gardasil in HIV-infected women. However, this difference in immunogenicity was less pronounced in HIV-infected men where the 2 vaccines induced

similar antibody responses against HPV-16 but higher anti-HPV-18 antibodies for Cervarix than Gardasil. Mild injection site reactions were more common in the Cervarix group, but the overall reactogenicity of both vaccines was acceptable for both patients and physicians. Collectively, our findings suggest that Cervarix is superior to Gardasil in terms of inducing protective immunity against oncogenic HPV-16 and HPV-18 infection in HIV-infected women. However, whether this difference translates into enhanced or prolonged protection against cervical cancer is still unknown.

To our knowledge, this is the first clinical trial comparing immunogenicity data of Cervarix and Gardasil in HIV-infected individuals. One strength of the study was that all participants and investigators were masked to the assigned test drugs throughout the trial, leading to unbiased safety and immunogenicity measurements. The study was adequately powered to detect a 35% difference in GMTs between the vaccine groups,

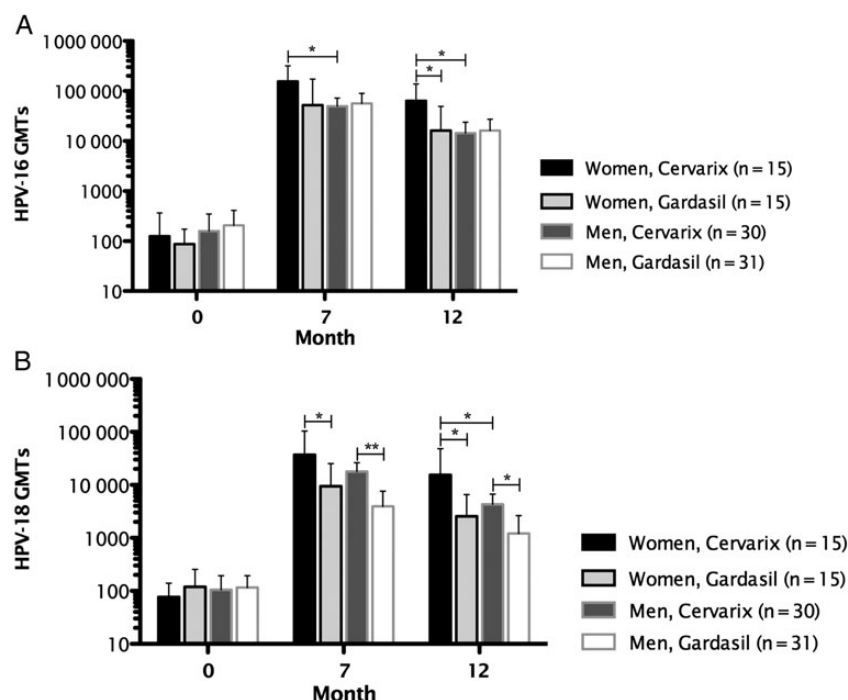


Figure 3. Human papillomavirus (HPV) serotype-specific neutralizing antibody geometric mean titers (GMTs) stratified according to sex. GMTs were computed using the \log_{10} transformation of ED_{50} values for anti-HPV-16 and anti-HPV-18 serum neutralizing antibodies measured by pseudovirion neutralization assay at months 0, 7, and 12. * $P < .05$; ** $P < .001$. One woman from the Cervarix group withdrew before month 7, and 1 man from the Gardasil group was lost to follow-up before month 12. Abbreviation: ED_{50} , effective dilution giving a 50% inhibition.

and it is unlikely that the observed differences in antibody titers were due to chance. The randomization was considered successful because the 2 vaccine groups were comparable in size

Table 3. Week 28 Human Papillomavirus DNA at 7 Months, According to Baseline Results

| Result at 7 mo | Cervarix Group, Proportion (%) of Participants | Gardasil Group, Proportion (%) of Participants |
|------------------------------------|--|--|
| HPV-16 DNA detected at 7 mo | | |
| All | 4/39 (10) | 8/41 (20) |
| Persistent infection | 2/4 (50) | 7/9 (78) |
| Incident infection | 2/35 (6) | 1/32 (3) |
| HPV-18 DNA detected at 7 mo | | |
| All | 3/39 (8) | 4/41 (10) |
| Persistent infection | 1/2 (50) | 3/5 (60) |
| Incident infection | 2/38 (5) | 1/36 (3) |

Results presented for those with known baseline HPV DNA status. Six HPV DNA tests were either missing or inconclusive at 7 months. Persistent infection is the number with DNA detected at 7 months among those with DNA of the same type detected at study entry. Incident infection is the number with DNA detected at 7 months among those with DNA of that type not detected at entry.

Abbreviation: HPV, human papillomavirus.

and most baseline characteristics. The study was conducted at a single trial site, minimizing variations in trial procedures and blood sample analyses.

The study also had several limitations. First, the study was not powered to compare the 2 vaccines on clinical endpoints and, with only 15 female subjects per vaccine group, it was not powered to detect sex-related differences. Serologic correlates have not been established for protection against HPV infection, and the significance of the magnitude of neutralizing antibody titers remains elusive. We did not have long-term follow-up and cannot predict decreases in antibody responses over time. Finally, we had no HIV-negative controls and thus we cannot directly compare immunogenicity of the HPV vaccines in HIV-infected individuals to that in the general population.

A plausible explanation for the difference in immunogenicity between Gardasil and Cervarix could be the vaccine adjuvants, Cervarix being formulated with the highly immunogenic TLR4-stimulating AS04 adjuvant system. The overall difference in antibody titers was significant for HPV-18 but not for HPV-16, perhaps owing to the fact that Gardasil contains twice as much HPV-16 antigen as Cervarix (40 μ g vs 20 μ g, respectively) whereas both vaccines contain 20 μ g of HPV-18 antigen.

The PBNA used in this study has increased sensitivity compared to the traditional neutralization assays [37]. Accordingly, we report higher titers than previously found among healthy

Table 4. Injection-Related Adverse Events

| Adverse Event | First Vaccination | | | Second Vaccination | | | Third Vaccination | | |
|--------------------------------------|-------------------------|-------------------------|---------|-------------------------|-------------------------|---------|-------------------------|-------------------------|---------|
| | No. (%) of Patients | | P Value | No. (%) of Patients | | P Value | No. (%) of Patients | | P Value |
| | Cervarix Group (n = 45) | Gardasil Group (n = 46) | | Cervarix Group (n = 45) | Gardasil Group (n = 46) | | Cervarix Group (n = 44) | Gardasil Group (n = 46) | |
| At least 1 adverse event | 38 (84.4) | 25 (54.3) | .002 | 23 (52.3) | 13 (28.2) | .02 | 27 (61.4) | 18 (39.1) | .04 |
| Injection site pain | 38 (84.4) | 15 (32.6) | <.001 | 22 (50.0) | 9 (19.6) | .002 | 25 (56.8) | 17 (37.0) | ns |
| Injection site swelling | 10 (22.2) | 3 (6.5) | .04 | 9 (20.5) | 2 (4.3) | .03 | 9 (20.5) | 4 (8.7) | ns |
| Injection site erythema | 4 (8.9) | 5 (10.9) | ns | 7 (15.9) | 2 (4.3) | ns | 8 (18.2) | 2 (4.3) | <.05 |
| Influenza-like symptoms ^a | 6 (13.3) | 4 (8.7) | ns | 0 | 2 (4.3) | ns | 0 | 0 | |
| Headache | 1 (2.2) | 3 (6.5) | ns | 0 | 2 (4.3) | ns | 0 | 0 | |
| Nausea | 1 (2.2) | 1 (2.2) | ns | 0 | 0 | | 0 | 1 | ns |

Solicited adverse events occurring during the first 4 days after each immunization. *P* Values for "At least 1 adverse event" and "Injection site pain" were calculated using χ^2 test. Fischer's exact test was used for calculating the *P* Values in "Injection site swelling", "Injection site erythema", "influenza-like symptoms", "headache" and "nausea" due to small numbers of events.

Abbreviation: ns, not significant (*P* value $\geq .05$).

^a Influenza-like symptoms included pyrexia, arthralgia, chills, and fatigue.

women [23]. The Cervarix/Gardasil antibody ratios among HIV-infected women were comparable to those reported by Einstein [23]. Until universal standards for HPV serology are implemented, absolute comparison of neutralizing titers obtained in different studies remains difficult. We cannot compare the antibody titers to the available immunogenicity data of Gardasil in HIV-infected individuals. We specifically tested for antibodies with neutralizing activity giving a functional titer, whereas the previously published studies have used either a competitive Luminex-based immunoassay, presenting data in milli-Merck units per milliliter [25–28], or an enzyme-linked immunosorbent assay [29].

Sex differences in humoral responses to viral vaccinations in particular have been reported in numerous studies and several potential mechanistic explanations have been hypothesized, that is, gonadal hormone levels and yet-undefined antigen-specific interactions with the immune system [38, 39]. It was surprising, however, that the observed sex-related differences in antibody titers were only found among those vaccinated with Cervarix. Some data suggest that AS04-adjuvanted vaccines could have increased effect on women; for example, AS04-adjuvanted genital herpes vaccination reduced the rate of acquisition of genital herpes in women but had no effect in men [40]. AS04 contains aTLR4-stimulating adjuvant (monophosphoryl lipid A), and TLR4 signaling pathways are altered by HIV infection [33]. Sex-specific differences in TLR-induced cytokine production in HIV-infected individuals have previously been described, women showing higher responses than men [41]. Furthermore, TLR4 expression and responsiveness is increased following administration of exogenous estradiol in rodents [32], thus suggesting potential mechanisms behind the observed differences in our study. Also, we cannot rule out that

the observed sex differences may be partially related to HIV infection. Several studies have indicated that men and women respond differently to both HIV infection and antiretroviral therapy [42, 43]. Our study emphasizes the need to carefully design and interpret clinical studies with enhanced focus on differences between male and female participants.

Ultimately, this study supports the establishment of efficacy trials to determine the impact of routine HPV vaccination on the incidence of HPV-related cancers in HIV-infected adults, as a supplement to cervical and anal cancer screening programs.

Notes

Acknowledgments. We thank the participants for their involvement in the trial. We also thank the study nurses, Iben Loftheim and Inge Arbs, for their important job vaccinating the participants, and lab technician Lene Svinth-Johnke for the excellent job handling all the samples.

Financial support. This work was supported by Aarhus University, Henrik Henriksen's Foundation, The Hede Nielsen Family Foundation, Aase and Ejnar Danielsen's Foundation, Jørgen Holm and Wife's Foundation, Lykfeldt and Wife's Foundation, and the Danish Medical Association.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

- Munoz N, Castellsague X, de Gonzalez AB, et al. Chapter 1: HPV in the etiology of human cancer. *Vaccine* **2006**; 24(suppl 3):S3/1–10.
- Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* **1999**; 189:12–9.
- Forman D, de Martel C, Lacey CJ, et al. Global burden of human papillomavirus and related diseases. *Vaccine* **2012**; 30(suppl 5):F12–23.
- Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer* **2009**; 124:2375–83.

5. Insinga RP, Liaw KL, Johnson LG, Madeleine MM. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev* **2008**; 17:1611–22.
6. Parkin DM, Bray F. Chapter 2: the burden of HPV-related cancers. *Vaccine* **2006**; 24(suppl 3):S3/11–25.
7. Barr E, Tammis G. Quadrivalent human papillomavirus vaccine. *Clin Infect Dis* **2007**; 45:609–7.
8. Keam SJ, Harper DM. Human papillomavirus types 16 and 18 vaccine (recombinant, AS04 adjuvanted, adsorbed) [Cervarix]. *Drugs* **2008**; 68:359–72.
9. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* **2007**; 370:59–67.
10. Palefsky J. Human papillomavirus-related disease in people with HIV. *Curr Opin HIV AIDS* **2009**; 4:52–6.
11. Patel P, Hanson DL, Sullivan PS, et al. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992–2003. *Ann Intern Med* **2008**; 148:728–36.
12. Piketty C, Selinger-Leneman H, Grabar S, et al. Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *AIDS* **2008**; 22:1203–11.
13. Sunesen KG, Norgaard M, Thorlacius-Ussing O, Laurberg S. Immunosuppressive disorders and risk of anal squamous cell carcinoma: a nationwide cohort study in Denmark, 1978–2005. *Int J Cancer* **2010**; 127:675–84.
14. Didierlaurent AM, Morel S, Lockman L, et al. AS04, an aluminum salt-and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. *J Immunol* **2009**; 183:6186–97.
15. Hildesheim A, Herrero R, Wacholder S, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. *JAMA* **2007**; 298:743–53.
16. Castellsague X, Munoz N, Pitisuttithum P, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. *Br J Cancer* **2011**; 105:28–37.
17. Olsson SE, Kjaer SK, Sigurdsson K, et al. Evaluation of quadrivalent HPV 6/11/16/18 vaccine efficacy against cervical and anogenital disease in subjects with serological evidence of prior vaccine type HPV infection. *Hum Vaccin* **2009**; 5:696–704.
18. Joura EA, Garland SM, Paavonen J, et al. Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data. *BMJ* **2012**; 344:e1401.
19. Swedish KA, Factor SH, Goldstone SE. Prevention of recurrent high-grade anal neoplasia with quadrivalent human papillomavirus vaccination of men who have sex with men: a nonconcurrent cohort study. *Clin Infect Dis* **2012**; 54:891–8.
20. Frazer IH. Measuring serum antibody to human papillomavirus following infection or vaccination. *Gynecol Oncol* **2010**; 118:S8–11.
21. Stanley M. Potential mechanisms for HPV vaccine-induced long-term protection. *Gynecol Oncol* **2010**; 118:S2–7.
22. Dessy FJ, Giannini SL, Bougelet CA, et al. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. *Hum Vaccin* **2008**; 4:425–34.
23. Einstein MH, Baron M, Levin MJ, et al. Comparison of the immunogenicity and safety of Cervarix and Gardasil human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18–45 years. *Hum Vaccin* **2009**; 5:705–19.
24. Einstein MH, Baron M, Levin MJ, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 vaccine and HPV-6/11/16/18 vaccine: follow-up from months 12–24 in a phase III randomized study of healthy women aged 18–45 years. *Hum Vaccin* **2011**; 7:1343–58.
25. Levin MJ, Moscicki AB, Song LY, et al. Safety and immunogenicity of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine in HIV-infected children 7 to 12 years old. *J Acquir Immune Defic Syndr* **2010**; 55:197–204.
26. Weinberg A, Song LY, Saah A, et al. Humoral, mucosal, and cell-mediated immunity against vaccine and nonvaccine genotypes after administration of quadrivalent human papillomavirus vaccine to HIV-infected children. *J Infect Dis* **2012**; 206:1309–18.
27. Wilkin T, Lee JY, Lensing SY, et al. Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. *J Infect Dis* **2010**; 202:1246–53.
28. Kahn JA, Xu J, Kapogiannis BG, et al. Immunogenicity and safety of the human papillomavirus-6, -11, -16, -18 vaccine in HIV-infected young women. *Clin Infect Dis* **2013**; 57:735–44.
29. Denny L, Hendricks B, Gordon C, et al. Safety and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in HIV-positive women in South Africa: a partially-blind randomised placebo-controlled study. *Vaccine* **2013**; 31:5745–53.
30. Cooper CL, Davis HL, Angel JB, et al. CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults. *AIDS* **2005**; 19:1473–9.
31. Sogaard OS, Lohse N, Harboe ZB, et al. Improving the immunogenicity of pneumococcal conjugate vaccine in HIV-infected adults with a toll-like receptor 9 agonist adjuvant: a randomized, controlled trial. *Clin Infect Dis* **2010**; 51:42–50.
32. Rettew JA, Huet YM, Marriott I. Estrogens augment cell surface TLR4 expression on murine macrophages and regulate sepsis susceptibility in vivo. *J Endocrinol* **2009**; 150:3877–84.
33. Nicol MQ, Mathys JM, Pereira A, et al. Human immunodeficiency virus infection alters tumor necrosis factor alpha production via Toll-like receptor-dependent pathways in alveolar macrophages and U1 cells. *J Virol* **2008**; 82:7790–8.
34. Seitz H, Schmitt M, Bohmer G, et al. Natural variants in the major neutralizing epitope of human papillomavirus minor capsid protein L2. *Int J Cancer* **2013**; 132:E139–48.
35. Castro F, Dirks WG, Fahnrich S, et al. High-throughput SNP-based authentication of human cell lines. *Int J Cancer* **2013**; 132:308–14.
36. Schmitt M, Pawlita M. High-throughput detection and multiplex identification of cell contaminations. *Nucleic Acids Res* **2009**; 37:e119.
37. Sehr P, Rubio I, Seitz H, et al. High-throughput pseudovirion-based neutralization assay for analysis of natural and vaccine-induced antibodies against human papillomaviruses. *PLoS One* **2013**; 8:e75677.
38. Green MS, Shohat T, Lerman Y, et al. Sex differences in the humoral antibody response to live measles vaccine in young adults. *Int J Epidemiol* **1994**; 23:1078–81.
39. Klein SL. Sex differences in prophylaxis and therapeutic treatments for viral diseases. In: *Handbook of experimental pharmacology*. Rosenthal, Walter, eds. New York: Springer, **2012**; 499–522.
40. Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med* **2002**; 347:1652–61.
41. Meier A, Chang JJ, Chan ES, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med* **2009**; 15:955–9.
42. Moore AL, Mocroft A, Madge S, et al. Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. *J Acquir Immune Defic Syndr* **2001**; 26:159–63.
43. Maman D, Pujades-Rodriguez M, Subtil F, et al. Gender differences in immune reconstitution: a multicentric cohort analysis in sub-Saharan Africa. *PLoS One* **2012**; 7:e31078.