Prevention of persistent human papillomavirus (HPV) infection by a HPV 16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica

Rolando Herrero[1,2]
Sholom Wacholder[3]
Ana C Rodríguez[1]
Diane Solomon[3]
Paula González[1]
Aimee R. Kreimer[3]
Carolina Porras[1]
John Schussler[5]
Silvia Jiménez[1]
Mark E Sherman[3]
Wim Quint[6]
John T Schiller[7]
Douglas R Lowy[7]
Mark Schiffman[3]
Allan Hildesheim[3]

For the Costa Rica Vaccine Trial (CVT) Group

[1] Proyecto Epidemiológico Guanacaste, Fundación INCIENSA, Costa Rica
[2] Early Detection and Prevention Section, International Agency for Research on Cancer
[3] Division of Cancer Epidemiology and Genetics, National Cancer Institute, USA
[4] Division of Cancer Prevention and Control, National Cancer Institute, USA
[5] Information Management Systems (IMS), MD USA [6] DDL Diagnostic Laboratory, The Netherlands
[7] Center for Cancer Research, National Cancer institute, USA

The study is registered at Clinicaltrials.gov (NCT00128661)

Correspondence to: Rolando Herrero, M.D., Ph.D., Early Detection and Prevention

Section, International Agency for Research on Cancer, 150 Cours Albert Thomas 69372

Lyon, France, email: herreror@iarc.fr

Running title: HPV 16/18 vaccine efficacy in Guanacaste

Word count: 4109, 1 figure, 8 tables, 9 supplemental tables

Keywords: clinical trial, HPV vaccine, Guanacaste, age, cross-protection

1

Funding/Support and Financial Disclosures

Abstract

Target groups for HPV vaccination are controversial. We evaluated vaccine efficacy (VE) against 1-year persistent infection, stratified by age and sexual behavior. We randomized 7,466 healthy women 18-25y to HPV16/18 or Hepatitis A vaccine (follow-up=50.4 months). According-to-protocol (ATP) cohorts included compliant HPV-negative women; intention-to-treat (ITT) included all randomized women. ATP VE was 90.9% (95%CI=82.0, 95.9) against HPV16/18 infections, 44.5% against HPV31/33/45 (95%CI=17.5, 63.1) and 12.4% (95% CI=-3.2, 25.6) against any oncogenic infection. Overall ITT VE against HPV16/18 infections was 49.0%, but ATP and ITT VE almost reached 100% in year 4 of follow-up. ATP efficacy against HPV16/18 was similar by age, but ITT VE was highest among youngest women (68.9% among 18-19; 21.8% among 24-25 years olds) and 79.8% among virgins. Among previously unexposed women, vaccination is highly efficacious against HPV16/18 and partially against HPV31/33/45. Vaccination is most effective before initiating sexual activity, with programmatic and individual decision implications.

3

Statement of significance

In an independent trial of the bivalent ASO4-adjuvanted HPV 16/18 vaccine (Cervarix) conducted among young women in Costa Rica, we confirmed the high efficacy against HPV 16/18 persistent infection and partial cross-protection against HPV 31/33/45. Furthermore, efficacy data suggests that the benefit of HPV vaccination is maximal when the vaccine is given to young women before initiation of sexual activity.

4

Introduction

HPV vaccines have enormous potential for cervical cancer (CC) control, and developed countries are vaccinating adolescent girls to prevent cervical neoplasia. However, the worldwide CC burden (500,000 cases annually) will only decline with high vaccination coverage in developing countries, where most cases (85%) occur (1). In this context, target ages and population groups to maximize reduction in morbidity, treatment and mortality are still controversial.

The two vaccines based on L1-virus-like-particles licensed worldwide, quadrivalent Gardasil[®] (2,3) and bivalent Cervarix[®], (4,5) are highly protective against cervical neoplasia caused by vaccine HPV types 16 and 18 among women without current or past infection with these types. There is also evidence of limited cross-protection against HPV31, 33, 45 and possibly others (5-7). However, vaccination does not increase clearance or decrease progression of established infections (8,9).

The ultimate goal is CC prevention, but trials with that endpoint are impractical. The choice of trial endpoints has been intensively debated (10). Regulatory authorities required histopathological outcomes, namely cervical intraepithelial neoplasia grade 2 or greater (CIN2+) (in effect mainly CIN2), as cancer surrogates in licensure trials.

Although HPV infection is a necessary cause of CC, acute infection is extremely common and usually clears within months (11). Persistent oncogenic HPV infection, which is less frequent, is a much better endpoint than incident infection and, in some respects, a better

5

surrogate marker than CIN2, because infection can be measured with high reproducibility (12), while CIN2 is subject to significant histologic misclassification (13). Also, attribution of the HPV type that caused a CIN2+ is difficult when multiple-type infections are present, as is common (14).

Evaluating vaccine efficacy and potential impact in population subgroups can assure maximum benefit from high-cost programs in different settings. Developing countries with limited resources are considering whether investment in this preventive measure is worthwhile. In developed countries, benefit is uncertain for older women born in earlier cohorts and those who miss vaccination as adolescents, particularly in the US, where vaccine uptake in adolescents is limited (15).

We report here on efficacy of an HPV16/18 ASO4-adjuvanted vaccine (Cervarix[®], Glaxo SmithKline Biologicals) in a large community-based clinical trial in a high incidence area of Costa Rica (16), with 1 year persistence of a cervical HPV infection as endpoint, including estimates of vaccine efficacy by age, sexual behavior and previous exposure to individual HPV types. We present results for both ITT cohorts, reflecting real world efficacy, and ATP cohorts, as a proxy for an ideal where women are fully vaccinated before exposure, so they can receive maximum benefit.

6

Results

Figure 1 presents the CONSORT diagram. Of nearly 25,000 women screened, 7,466
women were randomized, with 3,727 in the vaccine arm and 3,739 in the control arm (ITT
cohort for HPV 16/18). The ATP cohort comprised 2,635 and 2,677 women in the vaccine
and control arm, respectively. The 7,466 women represented 59.1% of 12,624 potentially
eligible women (considering those with recruitment deferred beyond the enrollment period for
different reasons as non-eligible) and 30.5% of all 24,467 women screened from the census

Age, study clinic, presence and number of individual HPV types detected and baseline
cytology were similar in the two arms (Supplemental Table 1). As noted before (8), HPV16
was more common at baseline in vaccine than control arm (6.0% vs. 7.1%, p=0.05). For
this analysis, women in the ATP cohort for HPV 16/18 had accumulated 10,268 and
10,472 person years in the vaccine and control arm, respectively, with a median follow-up
time of 50.4 months. Total follow-up time, number of visits, maximum time between tests,
and number of annual, semi-annual or colposcopy visits were similar by arm (data not
shown). More than 90% of eligible women attended their corresponding visits and
provided specimens.

Estimated vaccine efficacy against HPV16/18 was 90.9% (95%CI=82.0, 95.9) in the ATP
cohort and 49.0% (95%CI=38.1, 58.1) in the ITT cohort (Table 1). The efficacy against
HPV31/33/45, for which previous evidence of protection exists, was 44.5% (95%CI= 17.5,
63.1) in the ATP cohort. Efficacies against other oncogenic types combined were not
significant. The overall efficacy against all oncogenic types was approximately 10% in

7

both the ATP and ITT analyses. Considering individual A9-species HPV types in ATP cohorts, protection against target HPV16 was 86.5% (95%CI=72.9, 94.0), with significant cross-protection against HPV31 (45.7%, 95%CI=8.2, 68.6). There was a non-significant cross-protection (37.3%, 95%CI=-51.4, 75.3) against HPV33, but not against other types in this species (Supplemental Table 2). In A7-species, efficacy against HPV18 persistent infection was 100% (95%CI=90.7, 100.0), with non-significant cross protection for closely related HPV 45 (52.0%, 95%CI=-9.8, 80.4). The other types in this species had non-significant negative estimates of efficacy. In species A5 and A6, the only noteworthy finding was an increase in persistent infection with HPV 51 (A5) in the HPV arm (VE: -63.9% (95%CI=-150.7, -8.2).

Table 2 presents ATP efficacy against HPV 16 by baseline HPV 16 serology status. Rates of 'breakthrough' persistent infections in the HPV arm were higher among seropositives than seronegatives, even though in the control arm, the rate of infections was lower in the seropositives. Thus, efficacy was over 90% among HPV16 seronegative women, but only 50% among the seropositives. Interestingly, efficacy against HPV16 was similar among HPV18 seronegative and seropositive women.

Efficacy in the ATP cohort was similar regardless of vaccination age (p for trend 0.362 (Table 3); however, in the ITT cohort, VE declined from 68.9% (95%CI=53.1, 79.9) for women 18-19 years old to 21.8% (95% CI=-16.9, 47.9) among 24-25 years olds (p for trend=0.005). Corresponding rate reductions per 100 women vaccinated declined from 5.2 (95% CI=3.6, 6.6) to 1.6 (95% CI=-1.0, 4.0). Similarly, in the ITT cohort, efficacy was highest among virgins at enrollment (79.8%, 95%CI= 44.9, 94.1), with decreasing efficacy

8

with increasing time since first sexual intercourse (Table 4) and increasing number of

sexual partners (Table 5). When considering stratification of the ITT results by time since

first sexual intercourse and number of sexual partners, virgins, despite high vaccine

efficacy, had a lower rate reduction than sexually active women, because they can only

contribute outcomes after initiation of sexual activity and therefore have less observation

time. Among sexually active women, rate reductions, like vaccine efficacy, declined with

time since first sexual intercourse. On the other hand, rate reductions increased with the

number of sexual partners despite declining vaccine efficacy, as a consequence of the

higher attack rate with increasing number of partners.

We also investigated VE according to time between vaccination and incidence of

persistent infections (Table 6). In the ATP analysis, VE against HPV 16/18 increased with

time since enrollment to 100% after 34 months. In the ITT analysis, efficacy also

increased with follow-up from only 16% in the first two years to over 90% after 46 months.

A similar effect was observed when considering efficacy against HPV 31, 33 and 45

combined, with ATP efficacy going from 41.7% (95%CI=-31.3, 75.4) 10-22 months after

vaccination to 57.6% (95%CI=-31.9. 88.5) after 46 months. In the ITT analysis,

corresponding VE went from -19.4% (95%CI= -64.9, 13.3) to 53.1% (95%CI=8.0, 77.1).

In an effort to compare the VE to prevent 12-month persistent infections with VE to

prevent 6-month persistent infections, we also calculated VE against that outcome,

including the same stratified analyses (Supplemental tables 3-9). The results were very

similar although there is more statistical power. In this context, it is noteworthy that HPV

9

31 was no longer the only non-vaccine HPV type with significant protection. The VE to prevent 6-month infection with HPV 45 was 73.0% (95%CI=45.3, 87.8)

Among women who were HPV DNA positive at enrollment, we did not detect significant efficacy against persistent infection with any of the HPV types investigated (supplemental table 10).

We also analyzed the 600 subjects excluded from ATP because they received at least one of the 3 vaccine doses outside the ATP windows. Estimates of VE against HPV16/18 using similar exclusion criteria as those for the ATP analysis and among all women (ITT) produced results similar to the respective analyses among women who received their 3 doses within the windows (supplemental table 11).

Discussion

Results from this independent trial support the strong protective effect of Cervarix against 12-month HPV16/18 persistent infections in the ATP cohort (5). Protection was close to 90% against these two types which are responsible for about 70% of cervical cancers (17). In addition, we observed nearly 50% cross protection against HPV 31/33/45, associated with about 10% of cancers. The VE against HPV16/18 was only 50% when considering all vaccinated women (ITT), and just 12% when considering persistent infections with any oncogenic HPV type, even in ATP cohorts.

10

For these analyses, we chose the surrogate outcome of persistent infection, which is highly reproducible (18), unlike histopathologic endpoints emphasized in previous reports. In previous work we have reported from Guanacaste, we compared the relative reproducibility and validity of CIN2 and CIN3 diagnoses by comparing community pathologists' diagnoses with 2 independent reviewers from the United States (total, n = 357). Two review pathologists agreed with 84% and 81%, respectively, of initial diagnoses of CIN3 compared with 13% and 31% of CIN2. Although CIN3 is a substantially more reproducible diagnosis than CIN2, the latter constitutes an important fraction of lesions in reported clinical trials (13). In addition, the virologic outcome provides direct assessment of causality in the presence of multiple infections and has a relatively high positive predictive value for subsequent development of lesions (19). The ITT analyses incorporate the reality of incomplete vaccination in mostly sexually active adults, and can be extrapolated to other populations of similar age, sexual behavior and compliance. In contrast, most women in the ATP analyses are probably naïve to HPV infection, allowing extrapolation to women vaccinated before sexual debut and who comply with vaccination regimens.

We observed statistically significant cross-protection against HPV31/33/45 as a group. There was no apparent efficacy against the very common persistent infections with HPV types other than HPV16, 18, 31, 33, and 45, an association that attenuated the overall efficacy against persistent infections with all oncogenic types down to 12%. The nominally significant deleterious effect on HPV51 may be a chance finding among many comparisons made, and was not observed in the other large Cervarix trials (20). The 4-year follow up of our study was too short to see whether other HPV types replace vaccine

11

types in vaccinated cohorts. Natural history data do not indicate that one HPV type modifies the epidemiology of the other (21,22), but we did not investigate whether the presence of a non-vaccine type modifies the vaccine's protection against infection with HPV16 or HPV18.

Inclusion of women regardless of serostatus, which is imperfectly measured, allowed us to observe the full impact of the vaccine in a population including presumably immune women. The ATP VE against HPV16 among women seronegative for HPV16 was 92.2%, about twice as high as in seropositives. The attack rate of persistent infection was lower in seropositives than seronegatives in the control arm, likely reflecting natural protection by serum antibodies and possibly other immune mechanisms (23), or reduced exposure a few years after initiation of sexual activity. The higher attack rate of persistent infection among seropositives than seronegatives in the vaccine arm may reflect high proportions of missed infections (possibly due to inadequate sampling of the genital tract, missed test results or latent infections) in women who do not benefit from vaccination because they were infected before baseline. The absence of reduction in efficacy against HPV16 persistent infection among HPV18 seropositives suggests that immune protection, rather than other correlates of sexual activity associated with antibody levels, explains the effect.

Similar efficacy against persistent infection with HPV16/18 in ATP analysis by age indicates that the vaccine is effective at protecting against new infections in unexposed women independent of age. The strong decline in efficacy from 68% at ages 18-19 to 21% at ages 24-25 in the ITT cohort probably reflects that in the latter, there is a significantly larger fraction of women who have initiated sexual activity prior to vaccination

12

and have been exposed to HPV. Rate reductions also clearly decline with age and years since first intercourse, with the exception of virgins who do not contribute time at risk until they start sexual activity. It should be noted however, that the reduction in vaccine efficacy and rate reductions is only present in the age group 24-25. Interestingly, rate reductions tend to be higher among women with more partners (as their attack rate is higher) despite lower vaccine efficacy. These results indicate that both susceptibility and rates of transmission are important parameters when assessing the potential impact of prophylactic vaccines and have implications for vaccination efforts and screening policy. In the absence of a test to determine expected benefit of an individual woman, age appears to be clearly a criterion to consider for definition of target groups for vaccination. The declines in estimates of VE seen with increasing age and time since sexual debut suggest that many infections that could eventually progress to cancer occur early, and can only be prevented with adolescent vaccination.

The observation that vaccination did not substantially reduce oncogenic infections has implications for screening programs, because the positive predictive value of the tests will likely be reduced, as many of the persistent infections by non-vaccine HPV types are unlikely to progress to significant lesions. The lack of reduction in infections with the lesser oncogenic types can lead to more diagnostic and therapeutic procedures than necessary in vaccinated cohorts.

Interestingly, we noted that VE against HPV 16 and HPV 18 tended to increase with time since vaccination, to 100% in the ATP cohort and to almost 95% in the ITT cohort, with a similar effect for the combined outcome of HPV 31, 33 and 45 (to a maximum close to

13

60%). One possible explanation of increasing efficacy against persistent HPV16/18 infections with time since vaccination in the ATP cohort is waning influence of false-negative baseline HPV DNA results, for which efficacy is zero or low. This interpretation is supported by the reduced VE against HPV 16 observed among women who were seropositive for anti-HPV 16 antibodies. Similarly, the likely explanation in the ITT cohort is waning influence of baseline prevalent infections. In ITT, increased VE with time since vaccination reflects protection against new infections, but the impact of this protection in the out years needs to be interpreted in the context of the fact that exposure tends to be higher early on after initiation of sexual activity, with reduced exposure typically seen with increasing age/time.

Most of the findings, including the stratified analyses were similar using a 6-month HPV persistence endpoint, with the advantage that the number of endpoints was larger, indicating that 6-month persistence could serve as an adequate surrogate endpoint in HPV vaccine trials, particularly for the evaluation of HPV types that occur with lower frequency or have lower vaccine efficacies that require larger sample sizes to achieve statistical significance.   In this study, for example, VE against HPV 45 was not significant using the 12-month endpoint but was clearly so with the 6-month endpoint.

This analysis has some limitations and strengths. One of the limitations is that we had a relatively small sample size to accurately assess the lower efficacy of individual non-vaccine HPV types, as has been the case with other clinical trials of Cervarix (5). Those multicentric trials as well as those reported for Gardasil recruited smaller number of women in multiple research centers. In contrast, the Costa Rica HPV trial was conducted

14

in a homogeneous population of young women at high risk of HPV infection. In this context, the results can be extrapolated to similar groups of women in areas of high HPV prevalence. It should be noted however, that high prevalence of HPV in young women is very common in most areas of the world, particularly those where the vaccine is been considered to control the cervical cancer problem. Differences in sexual practices, in particular the distribution of age at first intercourse in the population should be taken into account when designing HPV vaccination programs One of the strengths of this study is that it is a large trial in a stable community, which will allow long term follow-up up of these cohorts. Moreover, the fact that the results of this trial are very similar to those obtained in the multicentric trials points to the generalizability of vaccine efficacy results. The fact that participation rates at enrollment were limited could also affect the external validity of the results, but not the internal validity of the randomized trial. We used virological outcomes, which have some advantages as they are highly reproducible and do not present problems for causality assessment in the presence of multiple infections. However, the clinical significance of virological outcomes, particularly for non vaccine types is still under active debate.

In conclusion, the clear benefit of Cervarix against persistent HPV16/18 infections observed among unexposed women decreases with age and sexual experience. These findings, together with extensive data indicating that HPV is acquired early on after sexual debut (24,25) and the possibility of natural immunity (23) suggest limited value, in general, for vaccination beyond a few years after adolescence in areas of high prevalence of HPV infection and high risk of cervical cancer. Efforts that focus vaccination on women before sexual debut may be most effective at reaching the most vulnerable groups.

15

Materials and Methods

Design, subjects and procedures

This analysis presents a double-blind randomized controlled trial of Cervarix among healthy women 18-25 years old. Detailed methods have been published (16).

Potential participants from a census were invited (June 2004-December 2005). After signing informed consent, an interview, medical history, physical exam and pregnancy test were conducted. For eligibility, women had to be healthy, not pregnant, not breastfeeding and using contraception during the vaccination period. Main exclusion criteria were chronic diseases, history of reactions to vaccines, and history of hepatitis A or vaccination against it. Women were recruited and randomized regardless of past sexual behavior, HPV status or cytology.

A pelvic exam was performed on sexually experienced women. Exfoliated cells for cytology, HPV DNA, CT DNA, GC DNA and other testing were collected with a Cervex brush by firmly rotating the brush 5 times 360° around the cervical os. In women whose cervix exhibited extensive ectopy, the cervex brushing was also used on the ectocervix to insure sampling of the squamo-columnar junction. Blood was collected from all participants (16).

Randomization and vaccines

16

Participants were randomized with equal chance to Cervarix® or Hepatitis A vaccine.

Each dose of the HPV16/18 vaccine contained HPV16 and HPV18 L1 virus-like-particle

(20 µg of each) adjuvanted with 50 µg 3-O-desacyl-4´-monophosphoryl lipid A and 0·5 mg

aluminum hydroxide. Each dose of the control hepatitis A vaccine contained 720 ELISA

units (EU) of inactivated hepatitis A antigen and 0·5 mg aluminum hydroxide. Both were

formulated in 0.5 ml doses with identical packaging and appearance to assure blinding.

Vaccination schedule consisted of 3 doses at 0, 1 and 6 months. Desirable windows for

vaccination defined periods beyond which the corresponding dose was not administered

(16). At 6 months, sexually active women self-collected vaginal cells for HPV testing, with

results comparable to clinician-collected specimens (8).

Follow-up

Each participant was scheduled for 4 annual follow-up examinations. Cytology was

classified using the Bethesda system (26). Women with LSIL or HPV positive ASC-US

were followed semi-annually for safety until obtaining 3 normal results. A repeat LSIL,

HPV positive ASC-US, a single ASC-H, HSIL+ or glandular abnormalities prompted

colposcopy and treatment as needed. Unsatisfactory cytology was managed as LSIL.

The study was approved and supervised by the IRBs of the Instituto Costarricense de

Investigación y Enseñanza en Nutrición y Salud (INCIENSA) in Costa Rica and the NCI in

the US.

Safety monitoring

All participants were observed 30-60 minutes following vaccination. Adverse event and pregnancy information was actively collected during follow-up. An independent data and safety monitoring board (DSMB) met regularly to examine unblinded adverse event (most recent meeting February, 2011), and repeatedly recommended trial continuation. The study is still blinded and investigators had no access to unmasked data by arm; therefore, no safety data are presented in this report. However, two published reports on pregnancies and autoimmune conditions have included safety data from this study (27,28).

HPV DNA and antibody testing

Broad spectrum PCR-based HPV DNA testing was performed at DDL Diagnostic Laboratory, based on amplification and probe hybridization using the SPF_{10} HPV DNA enzyme immunoassay (DEIA) system followed by typing using the $LiPA_{25}$ version 1 line detection system as described (29,30). To ensure that HPV16 and HPV18 infections were not missed, all specimens positive for HPV DNA using SPF_{10} DEIA but negative for HPV16 or HPV18 by $LiPA_{25}$ were also tested with type-specific primers/probes for the presence of HPV16 and HPV18 DNA (30,31).

ELISA was used for the detection and quantification of IgG antibodies against HPV16 and 18 separately by GSK as described (32).

Statistical analysis

18

Results presented are post-licensure analyses, conducted by an external group (Information Management Systems, IMS) under the direction of the investigators, who remain masked to individuals' randomizations.

We defined persistence as detection of same-type HPV in samples collected at two visits, at least 10 months apart (minimum required for two consecutive annual visits), without intervening negatives. Similarly, 6-months persistence was calculated as detection of same-type HPV in samples collected at two visits, at least 4 months apart (minimum required for two consecutive semi-annual visits). There were a total of 2,668 oncogenic infections with 10+ months between first detection and last detection, of which 496 (18.6%) are not counted as persistent due to intervening negatives.

We defined different cohorts for each endpoint of HPV infection. According-to-protocol (ATP) cohorts include women who received 3 doses within protocol-defined windows, were protocol-compliant during vaccination, had no biopsy/treatment before the 6 months visit, and were HPV DNA-negative by PCR for the corresponding HPV type at enrollment and the 6-month visit (when receiving third dose) (2,635 women in the HPV vaccine arm and 2,677 in the control arm) (16). The intention-to-treat (ITT) cohorts include all randomized women, regardless of compliance or enrollment infection (3,727 in the HPV arm and 3,739 in the control arm) .

19

Balance by arm overall and within subgroups was evaluated by exact binomial test when the number of women was ≤30 and by the analogous normal approximation to the binomial test when the total was >30.

Vaccine efficacy (VE) is the percentage reduction in endpoint related to vaccine administration, estimated as the complement of the ratio of the cumulative attack rates (AR) in the HPV and control arms. The AR is the percentage of women in the cohort who experience the endpoint. The confidence interval for VE is derived from the corresponding confidence interval for the risk ratio. The exact conditional test was used for analyses of VE. The analytical unit for all analyses is the woman rather than the infection because our principal interest is to determine the proportion of women protected against persistent HPV infections with the potential to cause cancer in the woman.

We used the difference between the ARs in the vaccinated and control arms to address the question of absolute impact of vaccination overall and in subgroups. The confidence interval for the difference was calculated based on the exact test.

The primary objective in our pre-specified plan was to evaluate VE against 1-year persistent infection with HPV16 and/or HPV18 (HPV 16/18). We evaluated cross-protection against HPV31/33/45, for individual oncogenic HPV types, and for all oncogenic types combined. 6-month persistent infection was also evaluated in secondary analyses. In addition, stratified VE was calculated by enrollment covariates (age, age at first intercourse, time since first intercourse, number of sexual partners, HPV DNA and antibody status).

20

Oncogenic HPV types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68-73 (68-73 cannot be differentiated with genotyping method employed). PCR results from all visits of a participant were included in the analyses (annual, semi-annual and colposcopy).

More than 600 women received their 3 doses outside the strict ATP windows. Separate cohorts were defined to analyze efficacy in this subgroup using similar criteria for ATP and ITT as described above.

Our results are based on an event-triggered statistical analysis plan (SAP) approved by US FDA. The SAP specifies a one-sided α-level of 0.001 for this "interim" analysis of persistent HPV-16/18 infections. Results in this paper provide the most up-to-date available data from the latest data freeze of 21 June 2010. A previously published abstract for the International Papillomavirus Conference (IPC) held in July 2010 included analysis of persistent HPV-16/18 infections from an earlier (1 Jan 2010) data freeze; the P-value was $<10^{-10}$ in the ATP cohort. For regulatory purposes, we consider that the two freezes constitute two separate interim analyses, leaving 0.023 (=0.025-0.001*2) as the one-sided α level when we perform our final analysis. Only the analysis of persistent HPV-16/18 infections entails α spending according to the SAP. Other analyses are exploratory in the SAP and do not require adjustment.

**Names and Affiliations of investigators in the Costa Rica Vaccine Trial (CVT) group
are as follows:**

Proyecto Epidemiológico Guanacaste, Fundación INCIENSA, San José, Costa Rica

Mario Alfaro (Cytopathologist)

Manuel Barrantes (Field Supervisor)

M. Concepción Bratti (co-Investigator)

Fernando Cárdenas (General Field Supervisor)

Bernal Cortés (Specimen and Repository Manager)

Albert Espinoza (Head, Coding and Data Entry)

Paula González (co-Investigator)

Diego Guillén (Pathologist)

Rolando Herrero (co-Principal Investigator)

Silvia E. Jiménez (Trial Coordinator)

Jorge Morales (Colposcopist)

Lidia Ana Morera (Head Study Nurse)

Elmer Pérez (Field Supervisor)

Carolina Porras (co-Investigator)

Ana Cecilia Rodríguez (co-Investigator)

Libia Rivas (Clinician´s coordinator)

Luis Villegas (Colposcopist)

University of Costa Rica, San José, Costa Rica

Ivannia Atmella (Microbiologist, Immunology Laboratory)

José Bonilla (Head, HPV Immunology Laboratory)

Enrique Freer (Director, HPV Diagnostics Laboratory)

Alfonso García-Piñeres (Immunologist)

Margarita Ramírez (Microbiologist, Immunology Laboratory)

Sandra Silva (Head Microbiologist, HPV Diagnostics Laboratory)

United States National Cancer Institute, Bethesda, MD, USA

Allan Hildesheim (co-Principal Investigator & NCI co-Project Officer)

Aimee R. Kreimer (Investigator)

Douglas R. Lowy (HPV Virologist)

Nora Macklin (Trial Coordinator)

Mark Schiffman (Medical Monitor & NCI co-Project Officer)

John T. Schiller (HPV Virologist)

Mark Sherman (QC Pathologist)

Diane Solomon (Medical Monitor & QC Pathologist)

Sholom Wacholder (Statistician)

23

SAIC, NCI-Frederick, Frederick, MD, USA

Ligia Pinto (Head, HPV Immunology Laboratory)

Troy Kemp (Scientist, HPV Immunology Laboratory)

Women's and Infants' Hospital, Providence, RI, USA

Claire Eklund (QC Cytology)

Martha Hutchinson (QC Cytology)

Georgetown University, Washington DC

Mary Sidawy (Histopathologist)

DDL Diagnostic Laboratory, The Netherlands

Wim Quint (Virologist, HPV DNA Testing)

Leen-Jan van Doorn (HPV DNA Testing)

Acknowledgements

24

25

Cervarix is a registered trade mark of the Glaxo Smith Kline Biologicals group of companies.

Gardasil is a registered trade mark of Merck and Co. Inc.

Registered with clinicaltrials.gov: NCT00128661.

26

Table 1. Vaccine efficacy against 1 year persistence of different combinations of HPV types.

HPV Type	Arm	ATP analysis[a]					ITT analysis[b]				
		Number of women in ATP cohort	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Efficacy (95% CI)	Number of women in ITT cohort	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)
HPV 16, 18	Vaccine	2635	8	0.3 (0.1, 0.6)	3.0 (2.5, 3.3)	90.9%[c] (82.0, 95.9)	3727	153	4.1 (3.5, 4.8)	3.9 (2.9, 5.0)	49.0%[d] (38.1, 58.1)
	Control	2677	89	3.3 (2.7, 4.1)			3739	301	8.1 (7.2, 9.0)		
HPV 31, 33, 45	Vaccine	2642	37	1.4 (1.0, 1.9)	1.1 (0.4, 1.8)	44.5% (17.5, 63.1)	3727	150	4.0 (3.4, 4.7)	0.7 (-0.2, 1.7)	15.5% (-5.0, 32.0)
	Control	2695	68	2.5 (2.0, 3.2)			3739	178	4.8 (4.1, 5.5)		
Other oncogenic types	Vaccine	2643	230	8.7 (7.7, 9.8)	-1.0 (-2.6, 0.5)	-13.4 (-36.9, 6.0)	3727	559	15.0 (13.9, 16.2)	-0.2 (-2.0, 1.5)	-1.4% (-14.1, 9.8)
	Control	2697	207	7.7 (6.7, 8.7)			3739	553	14.8 (13.7, 16.0)		
Any Oncogenic Type	Vaccine	2643	267	10.1 (9.0, 11.3)	1.4 (-0.3, 3.2)	12.4% (-3.2, 25.6)	3727	764	20.5 (19.2, 21.8)	2.6 (0.5, 4.7)	11.3% (2.2, 19.5)
	Control	2697	311	11.5 (10.4, 12.8)			3739	864	23.1 (21.8, 24.5)		

----
[a]ATP (according to protocol) cohort includes women who received all 3 doses within protocol – defined windows, complied with the protocol during the vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month visit and were HPV DNA negative (by PCR) for at least one of the HPV types in the endpoint at enrollment and at the 6-month visit.
[b] ITT (intention to treat) cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.
[c]One-sided P-value for test of vaccine efficacy equals zero against the alternative that vaccine efficacy is greater than 0 is less than 10^{-17}.
[d]One-sided P-value for test of vaccine efficacy equals zero against the alternative that vaccine efficacy is greater than 0 is less than 10^{-11}.

27

Table 2. Vaccine efficacy (ATP) against 1 year persistence with HPV 16 stratified by HPV 16 and HPV 18 serology at enrollment

HPV Serology	Arm	Number of women	Number of women with events	Rate per 100 women	(95% CI)	Rate reduction/ 100 women	(95% CI)	Vaccine Efficacy	(95% CI)
HPV 16 serology Negative	Vaccine	1875	4	0.2	(0.1, 0.5)	2.5	(2.0, 2.8)	92.2%	(80.3, 97.6)
	Control	1856	51	2.7	(2.1, 3.6)				
HPV 16 serology Positive	Vaccine	558	4	0.7	(0.2, 1.7)	0.7	(-0.5, 1.7)	50.6%	(-63.3, 87.0)
	Control	551	8	1.5	(0.7, 2.7)				
HPV 18 serology negative	Vaccine	1853	4	0.2	(0.1, 0.5)	2.2	(1.6, 2.4)	90.9%	(76.7, 97.2)
	Control	1854	44	2.4	(1.8, 3.1)				
HPV 18 serology positive	Vaccine	563	3	0.5	(0.1, 1.4)	2.1	(0.6, 2.9)	79.4%	(33.5, 95.3)
	Control	541	14	2.6	(1.5, 4.2)				

The stratification by HPV 16 serology excludes 31 and 45 subjects without HPV 16 serology results from the vaccine and control arm, respectively
The stratification by HPV 18 serology excludes 48 and 57 subjects without HPV 18 serology from the vaccine and control arm, respectively.

Table 3. Vaccine efficacy against 1 year persistence with HPV 16/18 stratified by age at enrollment

		ATP analysis[a]					ITT analysis[b]				
Age	Arm	Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)	Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)
18-19 years	Vaccine	825	1	0.1 (0.0, 0.6)	2.9 (2.0, 3.1)	95.9 (78.5, 99.8)	1193	28	2.3 (1.6, 3.3)	5.2 (3.6, 6.6)	68.9% (53.1, 79.9)
	Control	870	26	3.0 (2.0, 4.3)			1244	94	7.6 (6.2, 9.1)		
20-21 years	Vaccine	659	3	0.5 (0.1, 1.2)	2.9 (1.6, 3.6)	86.6 (59.2, 96.8)	946	46	4.9 (3.6, 6.4)	3.6 (1.3, 5.8)	42.8% (17.9, 60.6)
	Control	649	22	3.4 (2.2, 5.0)			905	77	8.5 (6.8, 10.5)		
22-23 years	Vaccine	588	1	0.2 (0.0, 0.8)	3.8 (2.7, 4.1)	95.7 (77.4, 99.8)	818	36	4.4 (3.1, 6.0)	4.7 (2.2, 6.9)	51.5% (28.4, 67.7)
	Control	625	25	4.0 (2.7, 5.8)			848	77	9.1 (7.3, 11.2)		
24-25 years	Vaccine	563	3	0.5 (0.1, 1.4)	2.5 (1.0, 3.3)	82.2 (43.9, 95.9)	770	43	5.6 (4.1, 7.4)	1.6 (-1.0, 4.0)	21.8% (-16.9, 47.9)
	Control	533	16	3.0 (1.8, 4.7)			742	53	7.1 (5.5, 9.2)		

----
[a]ATP (according to protocol) cohort includes women who received all 3 doses within protocol – defined windows, complied with the protocol during the vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month visit and were HPV DNA negative (by PCR) for at least one of the HPV types in the endpoint at enrollment and at the 6-month visit.
[b]ITT (intention to treat) cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

Table 4. ATP and ITT efficacy estimates against HPV 16/18 by time since first sexual intercourse at enrollment

Time since first sex	Arm	ATP analysis[a]					ITT analysis[b]				
		Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)	Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)
Virgin	Vaccine	566	1	0.2 (0.0, 0.9)	2.6 (1.4, 2.9)	93.6% (64.8, 99.7)	773	4	0.5 (0.2, 1.2)	2.0 (0.9, 2.7)	79.8% (44.9, 94.1)
	Control	615	17	2.8 (1.7, 4.3)			819	21	2.6 (1.6, 3.8)		
<2 years	Vaccine	227	1	0.4 (0.0, 2.2)	4.5 (1.7, 5.3)	91.0% (48.3, 99.6)	352	12	3.4 (1.9, 5.7)	7.5 (3.7, 10.4)	68.7% (41.2, 84.3)
	Control	244	12	4.9 (2.7, 8.2)			349	38	10.9 (7.9, 14.5)		
2 years	Vaccine	233	0	0.0 (0.0, 1.3)	4.1 (1.8, 4.1)	100.0% (62.5, 100.0)	335	19	5.7 (3.6, 8.6)	7.3 (2.7, 11.2)	56.1% (25.2, 75.0)
	Control	221	9	4.1 (2.0, 7.3)			325	42	12.9 (9.6, 16.9)		
3 years	Vaccine	279	0	0.0 (0.0, 1.1)	5.1 (3.1, 5.1)	100.0% (76.2,100.0)	395	19	4.8 (3.0, 7.3)	5.8 (2.0, 9.1)	54.9% (23.1, 74.3)
	Control	256	13	5.1 (2.9, 8.3)			394	42	10.7 (7.9, 14.0)		
4+ years	Vaccine	1330	6	0.5 (0.2, 0.9)	2.4 (1.6, 2.9)	84.1% (64.2, 93.9)	1872	99	5.3 (4.3,6.4)	3.2 (1.6, 4.8)	38.0% (20.4, 51.9)
	Control	1341	38	2.8 (2.0, 3.8)			1852	158	8.5 (7.3, 9.9)		

----
[a]ATP (according to protocol) cohort includes women who received all 3 doses within protocol – defined windows, complied with the protocol during the vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month visit and were HPV DNA negative (by PCR) for at least one of the HPV types in the endpoint at enrollment and at the 6-month visit.
[b] ITT (intention to treat) cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

Table 5. ATP and ITT efficacy estimates against HPV 16/18 by number of sexual partners at enrollment

Number of sex partners	Arm	ATP analysis[a]					ITT analysis [b]				
		Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)	Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)
Virgin	Vaccine	566	1	0.2 (0.0, 0.9)	2.6 (1.4, 2.9)	93.6% (64.8, 99.7)	773	4	0.5 (0.2, 1.2)	2.0 (0.9, 2.7)	79.8% (44.9, 94.1)
	Control	615	17	2.8 (1.7, 4.3)			819	21	2.6 (1.6, 3.8)		
1 partner	Vaccine	904	3	0.3 (0.1, 0.9)	2.6 (1.6, 3.1)	88.8% (66.5, 97.3)	1237	40	3.2 (2.4, 4.3)	3.4 (1.7, 4.9)	51.1% (28.9, 66.7)
	Control	915	27	3.0 (2.0,4.2)			1256	83	6.6 (5.3, 8.1)		
2 partners	Vaccine	544	1	0.2 (0.0, 0.9)	3.1 (1.8, 3.4)	94.4% (69.1, 99.7)	777	38	4.9 (3.5, 6.6)	5.9 (3.1, 8.3)	54.5% (33.5, 69.3)
	Control	519	17	3.3 (2.0, 5.1)			753	81	10.8 (8.7, 13.1)		
3+ partners	Vaccine	621	3	0.5 (0.1, 1.3)	4.0 (2.5, 4.7)	89.2% (67.9, 97.4)	940	71	7.6 (6.0, 9.4)	5.2 (2.3, 7.9)	40.7% (20.4, 56.0)
	Control	628	28	4.5 (3.0, 6.3)			911	116	12.7 (10.7, 15.0)		

----
[a]ATP (according to protocol) cohort includes women who received all 3 doses within protocol – defined windows, complied with the protocol during the vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month visit and were HPV DNA negative (by PCR) for at least one of the HPV types in the endpoint at enrollment and at the 6-month visit.
[b] ITT (intention to treat) cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

31

Table 6. Rates of persistent infection with HPV 16/ 18 and vaccine efficacy (ATP and ITT) against HPV 16/18 by time since enrollment

Time since enroll.	Arm	ATP analysis[a]					ITT analysis[b]				
		Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)	Number of women	Number of women with events	Rate per 100 women (95% CI)	Rate reduction/ 100 women (95% CI)	Vaccine Efficacy (95% CI)
10-22 mo	Vaccine	1599	5	0.3 (0.1, 0.7)	0.8 (0.2, 1.2)	71.2% (25.6, 90.5)	3056	115	3.8 (3.1, 4.5)	0.7 (-0.3, 1.7)	15.6% (-8.1, 34.2)
	Control	1655	18	1.1 (0.7, 1.7)			3071	137	4.5 (3.8, 5.2)		
22-34 mo	Vaccine	2190	3	0.1 (0.0, 0.4)	1.6 (1.1, 1.8)	91.9% (76.6, 98.0)	2870	25	0.9 (0.6, 1.3)	1.3 (0.7, 1.8)	59.7% (36.5, 75.0)
	Control	2239	38	1.7 (1.2, 2.3)			2913	63	2.2 (1.7, 2.7)		
34-46 mo	Vaccine	1258	0	0.0 (0.0, 0.2)	1.4 (0.9, 1.4)	100.0% (81.0, 100.0)	3031	11	0.4 (0.2, 0.6)	1.8 (1.4, 2.2)	83.5% (69.6, 91.7)
	Control	1240	17	1.4 (0.8, 2.1)			3001	66	2.2 (1.7, 2.8)		
46+ mo	Vaccine	973	0	0.0 (0.0, 0.3)	1.6 (1.0, 1.6)	100.0% (78.6, 100.0)	2101	2	0.1 (0.0, 0.3)	1.6 (1.2, 1.7)	94.3% (80.1, 99.1)
	Control	1011	16	1.6 (0.9, 2.5)			2083	35	1.7 (1.2, 2.3)		

----
[a]ATP (according to protocol) cohort includes women who received all 3 doses within protocol – defined windows, complied with the protocol during the vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month visit and were HPV DNA negative (by PCR) for at least one of the HPV types in the endpoint at enrollment and at the 6-month visit.
[b] ITT (intention to treat) cohorts include all women randomized and vaccinated, regardless of prevalence of infection and follow-up visits.

1   **References**

2

3   1. Arbyn M, Castellsague X, de Sanjose S, Bruni L, Saraiya M, Bray F, et al.

4   Worldwide burden of cervical cancer in 2008. Ann.Oncol. 2011 (epub ahead

5   of print)

6   2. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A

7   controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med

8   2002;347**:**1645-51.

9   3. Munoz N, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M,

10   Wheeler CM, et al. Impact of human papillomavirus (HPV)-6/11/16/18

11   vaccine on all HPV-associated genital diseases in young women. J Natl

12   Cancer Inst 2010;102**:**325-39.

13   4. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CM, et al.

14   Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine

15   against infection with human papillomavirus types 16 and 18 in young

16   women: an interim analysis of a phase III double-blind, randomised

17   controlled trial. Lancet 2007;369**:**2161-70.

18   5. Paavonen J, Naud P, Salmeron J, Wheeler CM, Chow SN, Apter D, et al.

19   Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine

20   against cervical infection and precancer caused by oncogenic HPV types

33

21   (PATRICIA): final analysis of a double-blind, randomised study in young

22   women. Lancet 2009;374**:**301-14.

23   6.   Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M,

24   Wheeler CM, et al. The impact of quadrivalent human papillomavirus (HPV;

25   types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and

26   disease due to oncogenic nonvaccine HPV types in generally HPV-naive

27   women aged 16-26 years. J Infect.Dis. 2009;199**:**926-35.

28   7.   Herrero R. Human papillomavirus (HPV) vaccines: limited cross-protection

29   against additional HPV types. J Infect.Dis. 2009;199**:**919-22.

30   8.   Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti

31   MC, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine

32   among young women with preexisting infection: a randomized trial. JAMA

33   2007;298**:**743-53.

34   9.   VRBPAC Background Document: Gardasil™ HPV Quadrivalent Vaccine:

35   May 18, 2006 VRBPAC Meeting

36   (http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4222B3.pdf).

37   10. Chang Y, Brewer NT, Rinas AC, Schmitt K, Smith JS. Evaluating the impact

38   of human papillomavirus vaccines. Vaccine 2009;27**:**4355-62.

39   11. Rodriguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle

40   PE, et al. Rapid clearance of human papillomavirus and implications for

41   clinical focus on persistent infections. J Natl Cancer Inst 2008;100**:**513-7.

42   12. Castle PE, Porras C, Quint WG, Rodriguez AC, Schiffman M, Gravitt PE, et

43   al. Comparison of two PCR-based human papillomavirus genotyping

44   methods. J Clin Microbiol. 2008;46**:**3437-45.

45   13. Carreon JD, Sherman ME, Guillen D, Solomon D, Herrero R, Jeronimo J, et

46   al. CIN2 is a much less reproducible and less valid diagnosis than CIN3:

47   results from a histological review of population-based cervical samples. Int J

48   Gynecol Pathol. 2007;26**:**441-6.

49   14. Jenkins D. A review of cross-protection against oncogenic HPV by an HPV-

50   16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological

51   and clinical endpoints and implications for mass vaccination in cervical

52   cancer prevention. Gynecol Oncol 2008;110**:**S18-S25.

53   15. National, state, and local area vaccination coverage among adolescents

54   aged 13-17 years --- United States, 2009. MMWR Morb.Mortal.Wkly.Rep.

55   2010;59**:**1018-23.

56   16. Herrero R, Hildesheim A, Rodriguez AC, Wacholder S, Bratti C, Solomon D,

57   et al. Rationale and design of a community-based double-blind randomized

35

58   clinical trial of an HPV 16 and 18 vaccine in Guanacaste, Costa Rica.

59   Vaccine 2008;26**:**4795-808.

60   17. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE,

61   Lloveras B, et al. Human papillomavirus genotype attribution in invasive

62   cervical cancer: a retrospective cross-sectional worldwide study. Lancet

63   Oncol 2010;11**:**1048-56.

64   18. Castle PE, Wheeler CM, Solomon D, Schiffman M, Peyton CL.

65   Interlaboratory reliability of Hybrid Capture 2. Am J Clin Pathol.

66   2004;122**:**238-45.

67   19. Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, Alfaro M, et

68   al. Short term persistence of human papillomavirus and risk of cervical

69   precancer and cancer: population based cohort study. BMJ

70   2009;339**:**b2569.

71   20. VRBPAC Briefing Document. CERVARIX. Human Papillomavirus Bivalent

72   (Types 16 and 18) Vaccine, Recombinant. Vaccines and Related Biological

73   Products Advisory Committee (VRBPAC). Briefing Document. September 9,

74   2009.

75   http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMat

76   erials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProd

77   uctsAdvisoryCommittee.

78   21. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-

79   year prospective study of human papillomavirus persistence among women

80   with a cytological diagnosis of atypical squamous cells of undetermined

81   significance or low-grade squamous intraepithelial lesion. J Infect.Dis.

82   2007;195**:**1582-9.

83   22. Chaturvedi AK, Katki HA, Hildesheim A, Rodriguez AC, Quint W, Schiffman

84   M, et al. Human papillomavirus infection with multiple types: pattern of

85   coinfection and risk of cervical disease. J Infect.Dis. 2011;203**:**910-20.

86

87   23. Safaeian M, Porras C, Schiffman M, Rodriguez AC, Wacholder S, Gonzalez

88   P, et al. Epidemiological study of anti-HPV16/18 seropositivity and

89   subsequent risk of HPV16 and -18 infections. J Natl Cancer Inst

90   2010;102**:**1653-62.

91   24. Winer RL, Hughes JP, Feng Q, O'Reilly S, Kiviat NB, Koutsky LA.

92   Comparison of incident cervical and vulvar/vaginal human papillomavirus

93   infections in newly sexually active young women. J Infect.Dis.

94   2009;199**:**815-8.32.

95   25. Rodriguez AC, Burk R, Herrero R, Hildesheim A, Bratti C, Sherman

96   ME, et al. The natural history of human papillomavirus infection and cervical

37

97   intraepithelial neoplasia among young women in the Guanacaste cohort

98   shortly after initiation of sexual life. Sex Transm.Dis. 2007;34**:**494-502

99   26. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The

100   2001 Bethesda System: terminology for reporting results of cervical

101   cytology. JAMA 2002;287**:**2114-9.

102   27. Wacholder S, Chen BE, Wilcox A, Macones G, Gonzalez P, Befano B, et al.

103   Risk of miscarriage with bivalent vaccine against human papillomavirus

104   (HPV) types 16 and 18: pooled analysis of two randomised controlled trials.

105   BMJ 2010;340**:**c712.

106   28. Verstraeten T, Descamps D, David MP, Zahaf T, Hardt K, Izurieta P, et al.

107   Analysis of adverse events of potential autoimmune aetiology in a large

108   integrated safety database of AS04 adjuvanted vaccines. Vaccine

109   2008;26**:**6630-8.

110   29. Kleter B, Van Doorn LJ, ter Schegeget J, Schrauwen L, van Krimpen K,

111   Burger M, et al. Novel short-fragment PCR assay for highly sensitive broad-

112   spectrum detection of anogenital human papillomaviruses. Am J Pathol.

113   1998;153**:**1731-9.

114   30. Kleter B, Van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter

115   Schegget J, et al. Development and clinical evaluation of a highly sensitive

38

116   PCR-reverse hybridization line probe assay for detection and identification

117   of anogenital human papillomavirus. J Clin Microbiol. 1999;37**:**2508-17.

118 31. Van Doorn LJ, Molijn A, Kleter B, Quint W, Colau B. Highly effective

119   detection of human papillomavirus 16 and 18 DNA by a testing algorithm

120   combining broad-spectrum and type-specific PCR. J Clin Microbiol.

121   2006;44**:**3292-8.

122 32. Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MP, Poncelet SM, et

123   al. Correlation between direct ELISA, single epitope-based inhibition ELISA

124   and pseudovirion-based neutralization assay for measuring anti-HPV-16

125   and anti-HPV-18 antibody response after vaccination with the AS04-

126   adjuvanted HPV-16/18 cervical cancer vaccine. Hum.Vaccin. 2008;4**:**425-

127   34.

128

129

39

130   **List of figure and table legends**

131   Figure 1 legend

132   HPV=human papillomavirus. CIN2+=cervical intraepithelial neoplasia grade 2 or

133   higher. LEEP=loop electrosurgical excision procedure. *Four women

134   received discordant vaccines (one woman was enrolled twice and received

135   three doses of each vaccine and three women received two doses of one

136   vaccine and one dose of the other vaccine). For the aim of this analysis, the

137   women were assigned to the group for which the first dose was given.

138   Table 1 legend

139

140   [a]ATP (according to protocol) cohort includes women who received all 3 doses

141   within protocol – defined windows, complied with the protocol during the

142   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

143   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

144   the endpoint at enrollment and at the 6-month visit.

145   [b] ITT (intention to treat) cohorts include all women randomized and vaccinated,

146   regardless of prevalence of infection and follow-up visits.

147   [c]One-sided P-value for test of vaccine efficacy equals zero against the

148   alternative that vaccine efficacy is greater than 0 is less than 10^{-17}.

149   [d]One-sided P-value for test of vaccine efficacy equals zero against the

150   alternative that vaccine efficacy is greater than 0 is less than 10^{-11}.

40

151   gend

152   Table 2 legend

153   [a]ATP (according to protocol) cohort includes women who received all 3 doses

154   within protocol – defined windows, complied with the protocol during the

155   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

156   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

157   the endpoint at enrollment and at the 6-month visit. The stratification by HPV 16

158   serology excludes 31 and 45 subjects without HPV 16 serology results from the

159   vaccine and control arm, respectively

160   The stratification by HPV 18 serology excludes 48 and 57 subjects without HPV

161   18 serology from the vaccine and control arm, respectively.

162

163   Table 3 legend

164

165   [a]ATP (according to protocol) cohort includes women who received all 3 doses

166   within protocol – defined windows, complied with the protocol during the

167   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

168   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

169   the endpoint at enrollment and at the 6-month visit.

170   [b]ITT (intention to treat) cohorts include all women randomized and vaccinated,

171   regardless of prevalence of infection and follow-up visits.

172

41

173   Table 4 legend

174   [a]ATP (according to protocol) cohort includes women who received all 3 doses

175   within protocol – defined windows, complied with the protocol during the

176   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

177   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

178   the endpoint at enrollment and at the 6-month visit.

179   [b]ITT (intention to treat) cohorts include all women randomized and vaccinated,

180   regardless of prevalence of infection and follow-up visits.

181

182   Table 5 legend

183

184   [a]ATP (according to protocol) cohort includes women who received all 3 doses

185   within protocol – defined windows, complied with the protocol during the

186   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

187   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

188   the endpoint at enrollment and at the 6-month visit.

189   [b]ITT (intention to treat) cohorts include all women randomized and vaccinated,

190   regardless of prevalence of infection and follow-up visits.

191

192
193   Table 6 legend

194

195   [a]ATP (according to protocol) cohort includes women who received all 3 doses

196   within protocol – defined windows, complied with the protocol during the

197   vaccination period did not have a biopsy or treatment (LEEP) prior to the 6-month

198   visit and were HPV DNA negative (by PCR) for at least one of the HPV types in

199   the endpoint at enrollment and at the 6-month visit.

200   [b]ITT (intention to treat) cohorts include all women randomized and vaccinated,

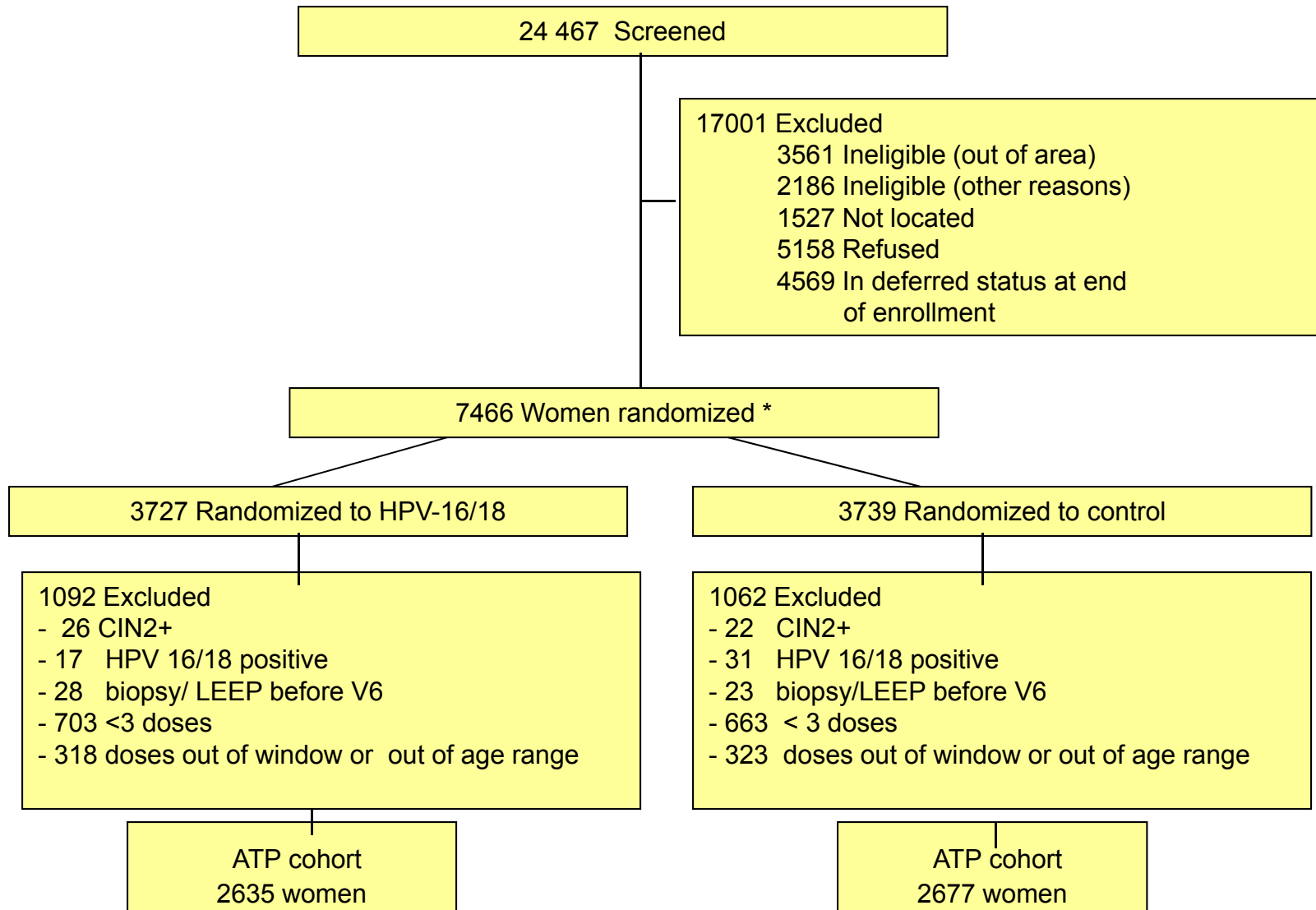201   regardless of prevalence of infection and follow-up visits.

202

43

Figure 1. Trial profile

```
                        ┌──────────────────────────┐
                        │   24 467  Screened       │
                        └──────────────────────────┘
                                     │
                                     │      ┌─────────────────────────────────────┐
                                     │      │ 17001 Excluded                      │
                                     │      │    3561 Ineligible (out of area)    │
                                     ├──────│    2186 Ineligible (other reasons)  │
                                     │      │    1527 Not located                 │
                                     │      │    5158 Refused                     │
                                     │      │    4569 In deferred status at end   │
                                     │      │         of enrollment               │
                                     │      └─────────────────────────────────────┘
                                     │
                        ┌──────────────────────────┐
                        │ 7466 Women randomized *  │
                        └──────────────────────────┘
```

3727 Randomized to HPV-16/18	3739 Randomized to control
1092 Excluded	1062 Excluded
- 26 CIN2+	- 22 CIN2+
- 17 HPV 16/18 positive	- 31 HPV 16/18 positive
- 28 biopsy/ LEEP before V6	- 23 biopsy/LEEP before V6
- 703 <3 doses	- 663 < 3 doses
- 318 doses out of window or out of age range	- 323 doses out of window or out of age range
ATP cohort 2635 women	ATP cohort 2677 women

HPV=human papillomavirus. CIN2+=cervical intraepithelial neoplasia grade 2 or higher. LEEP=loop electrosurgical excision procedure.
*Four women received discordant vaccines (one woman was enrolled twice and received three doses of each vaccine and three women received two doses of one vaccine and one dose of the other vaccine). For the aim of this analysis, the women were assigned to the group for which the first dose was given.

AAGR American Association for Cancer Research

CANCER DISCOVERY

Prevention of persistent human papillomavirus (HPV) infection by a HPV 16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica

Rolando Herrero, Sholom Wacholder, Ana Cecilia Rodríguez, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/2159-8290.CD-11-0131
Supplementary Material	Access the most recent supplemental material at: http://cancerdiscovery.aacrjournals.org/content/suppl/2011/10/16/2159-8290.CD-11-0131.DC2 http://cancerdiscovery.aacrjournals.org/content/suppl/2011/09/01/2159-8290.CD-11-0131.DC1
Author Manuscript	Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerdiscovery.aacrjournals.org/content/early/2011/08/30/2159-8290.CD-11-0131. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.