ORIGINAL ARTICLE

Noninferiority of One HPV Vaccine Dose to Two Doses

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ABSTRACT

BACKGROUND

Multidose human papillomavirus (HPV) vaccination is efficacious, yet the vaccine has been underused globally. Emerging data suggest that a single dose may provide protection. Whether a single dose of HPV vaccine would provide similar protection to two doses is uncertain.

METHODS

In this trial, we assessed whether one dose of an HPV vaccine was noninferior to two doses. Girls 12 to 16 years of age were randomly assigned, in a 1:1:1:1 ratio, to receive one or two doses of a bivalent HPV vaccine or one or two doses of a nonavalent HPV vaccine. The primary end point was new HPV type 16 or 18 infection occurring from month 12 to month 60 and persisting for at least 6 months. The prespecified noninferiority margin was 1.25 infections per 100 participants. We also assessed vaccine effectiveness by comparing HPV16 or HPV18 infection among the trial participants with that among girls and women enrolled in a nonrandomized survey.

RESULTS

A total of 20,330 participants were enrolled and underwent randomization, and 3005 unvaccinated participants were enrolled in the survey. The noninferiority analysis showed that one vaccine dose was noninferior to two doses in preventing HPV16 or HPV18 infection. The rate difference between one and two doses of the bivalent vaccine was −0.13 infections per 100 participants (95% confidence interval [CI], −0.45 to 0.15; P<0.001 for noninferiority), and the difference between one and two doses of the nonavalent vaccine was 0.21 infections per 100 participants (95% CI, −0.09 to 0.51; P<0.001 for noninferiority). The vaccine effectiveness was at least 97% in each of the four trial groups. No safety concerns were identified.

CONCLUSIONS

One dose of either a bivalent or nonavalent HPV vaccine provided protection against HPV16 or HPV18 infection and was not inferior to two doses. (Funded by the National Cancer Institute and others; ESCUDDO ClinicalTrials.gov number, NCT03180034.)

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Persistent human papillomavirus (HPV) infection can cause cervical cancer, and 77% of the global burden of cervical cancer is attributable to HPV types 16 and 18.[1] HPV vaccination could prevent most cervical cancers, but access remains inadequate: nearly 20 years after recommendation by the World Health Organization (WHO), only 27% of adolescent girls worldwide have been vaccinated.[2] Countries where vaccination is not yet available or where access is limited bear 90% of the burden of cervical cancer and related deaths because most women in these countries also lack access to cervical cancer screening and treatment.[3]

In a post hoc analysis in the Costa Rica HPV Vaccine Trial,[4] we found that protection against persistent HPV16 or HPV18 infection among women in a randomized population who received three doses of a bivalent vaccine was similar to that among women in a nonrandomized population who had received one dose, despite lower levels of antibodies among those who received one dose; the antibody levels in both groups remained protective a decade after vaccination.[5] Additional nonrandomized data from India[6] and a randomized, controlled efficacy trial in Kenya[7] showed a high efficacy for a single dose of HPV vaccine. Sustained immune responses were observed in these studies, as well as in a trial conducted in Tanzania.[8]

The double-blind, randomized, controlled ESCUDDO trial[9,10] evaluated the noninferiority of one dose of a bivalent or nonavalent HPV vaccine to the respective two-dose regimens in the prevention of cervicovaginal HPV16 or HPV18 infection over a period of 5 years. The trial also used a survey of unvaccinated participants to assess vaccine effectiveness. The bivalent and nonavalent vaccines were chosen because they are approved by the Food and Drug Administration and prequalified by the WHO but differ in valency, adjuvant, and protection against different HPV types.

Methods

Trial Design and Participants

The trial was approved and supervised by research ethics committees in Costa Rica and the United States. The primary research ethics committee was the committee in Costa Rica. The trial was supervised first by the committee of the Costa Rican Clinical Research Institute and then by the committee of the Hospital Clínica Bíblica. Written assent was obtained from participants younger than 18 years of age, and written consent was obtained from their parents or guardians. Participants 18 years of age or older provided written informed consent. The funders had no role in the design of the trial, the collection and analysis of the data, the preparation and content of the manuscript, or the decision to submit the manuscript for publication.

Participants 12 to 16 years of age from more than 200 districts in Costa Rica were enrolled for the randomized portion of the trial[9] from November 29, 2017, to February 28, 2020. HPV vaccination was not provided by the Costa Rican government to girls in this age range at any point during the trial period (the National Immunization Program started vaccination of 10-year-old girls in 2019). To prevent inducing herd protection as a consequence of the trial, we restricted enrollment to 35% or less of the girls in any district by recruiting from randomly selected minimal geostatistical units.

Participants had to be in good health and could not have received any previous HPV vaccination (full eligibility criteria are provided in the protocol, available with the full text of this article at NEJM.org).[9] After enrollment, participants were randomly assigned to receive a bivalent (HPV16 and HPV18) AS04-adjuvanted vaccine (Cervarix, GlaxoSmithKline Biologicals) or a nonavalent (HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58) aluminum-adjuvanted vaccine (Gardasil 9, Merck Sharp and Dohme). Six months later, the participants underwent randomization again to receive either a second dose of the assigned vaccine or a tetanus, diphtheria, and pertussis vaccine (Adacel, Sanofi Pasteur) as a control to maintain blinding (details regarding randomization and blinding are provided in the Supplementary Methods section in the Supplementary Appendix, available at NEJM.org). Participants were followed every 6 months for 5 years; girls younger than 15 years of age were followed annually until their 15th birthday and were then followed every 6 months. The trial-group assignments were concealed until the database lock on April 2, 2025.

For the nonrandomized survey, we enrolled girls and women 16 to 21 years of age from the geostatistical units that were not randomly se-

lected for the enrollment of trial participants. Survey enrollment coincided with the 4.5-year visit for the trial participants. The inclusion criteria were generally the same as those for the trial participants. The survey participants attended an enrollment visit as well as a second clinic visit approximately 6 months later. On the basis of the premise that HPV vaccination does not alter the outcome of an established infection, the survey participants were offered two doses of the HPV vaccine as a benefit to participation.

PROCEDURES

At each visit, a participant-collected cervicovaginal specimen was obtained from trial participants who were 15 years of age or older and from all survey participants, regardless of whether they reported that they had become sexually active. Participants used a Dacron swab for collection, which was immediately placed in 2 ml of PreservCyt. The trial participants and survey participants completed questionnaires that addressed schooling, cigarette smoking, pubertal development, and (among participants ≥15 years of age) sexual history. Adverse events, both serious and nonserious, were coded and reported according to the *International Classification of Diseases, 10th Revision*, and were monitored until resolution, regardless of whether they were considered to be related to vaccination.

HPV TESTING

TypeSeq2, a targeted sequencing assay that has been shown to detect 46 HPV types with high positive agreement in repeated testing and against established assays for most carcinogenic and noncarcinogenic genotypes, was used for outcome determination.[11] Additional details are provided in the Supplementary Methods section in the Supplementary Appendix.

END POINTS AND ANALYSES

The primary end point for the noninferiority analysis was incident, persistent HPV16 or HPV18 infection (HPV16 or HPV18 infection that occurred during the period from month 12 to month 60 and persisted for at least 6 months). Incident infection was defined as infection that occurred after negative HPV results had been shown at both enrollment and at month 6 (on the basis of cervicovaginal specimens [among participants ≥15 years of age] or initiation of sexual activity as reported by the participants who did not have HPV results from a cervicovaginal specimen). Persistent infection was defined as a positive test result of the same HPV genotype at two consecutive trial visits. The noninferiority analysis was performed in the per-protocol population, which included all the participants who had received both assigned doses (the two assigned HPV vaccine doses or one HPV vaccine dose and one dose of the control vaccine) and had no major protocol deviations.

Vaccine effectiveness was assessed in the per-protocol population and among survey participants who had no major protocol deviations. For the analysis of vaccine effectiveness, HPV16 or HPV18 infection was assessed at month 54 and month 60 among trial participants and at month 0 (the enrollment visit) and month 6 (the second visit) among survey participants. The definition of the end point for the survey is described below, in the Statistical Analysis section. Trial participants with missing data at month 54 and month 60 and survey participants with missing data at month 0 and month 6 were excluded because they would have contributed no data for the estimation of vaccine effectiveness. Details regarding secondary end points, which addressed other HPV types, are provided in the Supplementary Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

The noninferiority of one dose to two doses was assessed on the basis of the difference in the rate of incident, persistent HPV16 or HPV18 infection between one dose and two doses of each vaccine.[10] The prespecified noninferiority margin was 1.25 infections per 100 participants. This margin was selected to provide convincing evidence that the vaccine efficacy of one dose is more than 80%. We believe an efficacy exceeding 80% would provide substantial public health utility, especially given the additional benefits of indirect protection conferred by high vaccine coverage. Given that the expected efficacy of two doses was 93.6%, and on the basis of infection rates observed in the original Costa Rica HPV Vaccine Trial,[10] a one-dose efficacy of more than 80% would be equivalent to a difference in the infection event rate between one and two doses that is smaller than 1.25 infections per 100 participants ($0.092 \times [0.936 - 0.8] = 0.0125$; i.e., if we

followed an unvaccinated population for 5 years, the event rate of HPV infection would be 9.2%[10]). The 95% confidence interval was calculated with the use of the Farrington–Manning approach.[12] If the upper bound of the 95% confidence interval was less than or equal to 1.25 infections per 100 participants, the null hypothesis of inferiority would be rejected at a one-sided significance level of 0.025. In the secondary analyses, one additional noninferiority margin was prespecified for the comparison of one dose with two doses for protection against the carcinogenic HPV types included in the nonavalent vaccine formulation: a noninferiority margin of 2.55 infections per 100 participants was considered to be equivalent to the difference in the event rate with a one-dose efficacy of 80% and a two-dose efficacy of 93.6%, under the assumption of an event rate of 18.8% in an unvaccinated population. Additional details, including details regarding the assessment of all secondary end points, are provided in the Supplementary Methods section in the Supplementary Appendix.

The effectiveness of one dose or two doses of each vaccine was estimated by comparing the rates of incident, persistent HPV16 or HPV18 infection among the survey participants (at month 0 and month 6) with the rates in each trial group (at month 54 and month 60).[10] Two adjustments were made when we calculated the vaccine effectiveness. First, to align the end point among the trial participants (incident, persistent HPV infection) with that among the survey participants (persistent HPV infection), we estimated the proportion of prevalent, persistent infections that occurred in the trial and subtracted this value from the infection rates estimated for the survey (a prevalent infection was defined by a positive HPV result at either enrollment or month 6, and a persistent infection was defined by a positive HPV result of the same genotype at both month 54 and month 60). Second, because the survey participants did not undergo randomization, we used propensity-score adjustment to account for possible differences in covariate distributions (i.e., age, geographic region, and sexual activity) between the trial participants and survey participants. The 95% confidence interval for vaccine effectiveness was calculated with the use of the nonparametric bootstrap method with 500 replicates. We then tested the null hypothesis: if the lower bound of the 95% confidence interval of the vaccine effectiveness was greater than 0.80, the null hypothesis of low vaccine effectiveness would be rejected at a one-sided significance level of 0.025.

Both the noninferiority analysis and the analysis of vaccine effectiveness account for missing HPV data to more accurately estimate the event rate. The full details regarding the methods for handling missing data are provided in the Supplementary Appendix[10] and in the prespecified statistical analysis plan (available with the protocol). In brief, for each HPV type, if there was a data gap for a participant such that definitive incident, persistent infection (or the absence of infection) could not be determined, a "reference group" of similar girls and women with complete HPV data within the gap was used; therefore, the probability of any infection patterns during the gap could be calculated for that participant. With this method, we calculated the expected number of events (observed plus estimated) in each trial group. Among all the trial groups, 73.2% of the events were observed (either there was no missing data or missing data did not affect the identification of events), and the remaining 26.8% were estimated on the basis of the probability calculation for the gaps. We performed a sensitivity analysis that was restricted to the observed events. The analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PARTICIPANTS

A total of 20,330 participants were enrolled and underwent randomization. After the exclusion of 868 participants, 4880 were assigned to receive one dose of the bivalent vaccine, 4880 to receive two doses of the bivalent vaccine, 4851 to receive one dose of the nonavalent vaccine, and 4851 to receive two doses of the nonavalent vaccine (Fig. 1). Two participants had major protocol deviations and were excluded from the noninferiority analysis. Vaccine efficacy was assessed in 4068 participants in the one-dose bivalent vaccine group, 4040 in the two-dose bivalent vaccine group, 4109 in the one-dose nonavalent vaccine group, and 4083 in the two-dose nonavalent vaccine group. A total of 3005 unvaccinated girls and women were enrolled in the survey. After the exclusion of 15 participants who

had missing HPV test results, 2990 were included in the analysis of vaccine effectiveness. Enrolled participants attended 91.6% of the trial visits and 95.6% of survey visits. Adherence to collection of cervicovaginal specimens exceeded 94% (Table S4 in the Supplementary Appendix).

Randomization variables and other covariates were balanced across the trial groups (Tables S1 and S2). The characteristics of the survey participants were similar to those of the trial participants, including the time of initiation of sexual activity (Table S3), as well as the risk of HPV infection, as evidenced by the similar prevalence and distribution of noncarcinogenic HPV genotypes among the trial participants and the survey participants (Fig. S1).

NONINFERIORITY ANALYSIS

In the analysis of the primary end point (incident, persistent HPV16 or HPV18 infection), one dose was noninferior to two doses for both vaccines. The rate difference between one and two doses of the bivalent vaccine was −0.13 infections per 100 participants (95% confidence interval [CI], −0.45 to 0.15; P<0.001 for noninferiority), which indicates that every 100 participants who received one dose of the vaccine had 0.13 fewer infections within 5 years after vaccination than those who received two doses (Table 1). The rate difference between one and two doses of the nonavalent vaccine was 0.21 infections per 100 participants (95% CI, −0.09 to 0.51; P<0.001 for noninferiority), which indicates that every 100 participants who received one dose had 0.21 additional infections within 5 years after vaccination than those who received two doses (Table 1). The numbers of observed events were evenly distributed from month 24 to the end of follow-up and therefore did not show evidence of waning protection (Table S11). The differences in event rates of HPV16 and HPV18 infection individually between one and two doses were 0.14 or fewer infections per 100 participants for both vaccines (Table S6). As a sensitivity analysis, the primary noninferiority analysis was conducted with the use of observed events only (52 events) and yielded similar results to those of the main analysis (Table S12).

We also assessed noninferiority for the prevention of any of the seven carcinogenic HPV types included in the nonavalent vaccine formulation: the observed rate difference was 0.56 infections per 100 participants (95% CI, 0.01 to 1.11; P<0.001 [noninferiority margin, 2.55 infections per 100 participants]), a finding that shows that one dose was noninferior to two doses (Table 1). Among the participants who received the bivalent vaccine, the rate differences for infection with HPV31 (which is not included in the bivalent vaccine formulation) that were observed between one and two doses suggested that protection against this HPV type might be greater with two doses (Table S6).

ANALYSIS OF VACCINE EFFECTIVENESS

With respect to the effectiveness of the vaccines in preventing HPV16 or HPV18 infection that persisted for at least 6 months, the effectiveness of one dose of the bivalent vaccine was 98.2% (95% CI, 96.1 to 99.6), of two doses of the bivalent vaccine was 97.8% (95% CI, 95.6 to 99.3), of one dose of the nonavalent vaccine was 97.0% (95% CI, 94.3 to 99.1), and of two doses of the nonavalent vaccine was 98.5% (95% CI, 96.7 to 99.7) (Table 2 and Fig. S2). In secondary analyses, vaccine effectiveness against HPV16 and HPV18 infection individually was at least 97.1% in each of the four groups (Table 3). The effectiveness of the nonavalent vaccine against the secondary end point of incident, persistent HPV infection with any of the seven carcinogenic HPV types included in the nonavalent vaccine formulation was 94.5% (95% CI, 92.3 to 96.6) for one dose and 95.8% (95% CI, 93.8 to 97.6) for two doses (Table 2). The effectiveness of one and two doses against all the individual HPV types included in the nonavalent vaccine formulation was at least 90%, with the exception of HPV11 (the prevalence of infection with this type in the two-dose group was too low to give a precise estimate of effectiveness) (Table 3). The effectiveness of the bivalent vaccine against HPV31 was 38.3% (95% CI, 18.1 to 54.1) with one dose and 82.6% (95% CI, 73.9 to 88.8) with two doses; the effectiveness against HPV45 was 58.8% (95% CI, 28.4 to 78.5) with one dose and 72.1% (95% CI, 46.0 to 87.1) with two doses (Table 3). The results for effectiveness with respect to the secondary end points are reported in Table S8. The results of the sensitivity analysis, which used observed outcomes only, were similar to those of the primary analysis (Table S13). In the intention-to-treat analysis, the rate differences were similar to those in the per-protocol analyses (Table S9), and the

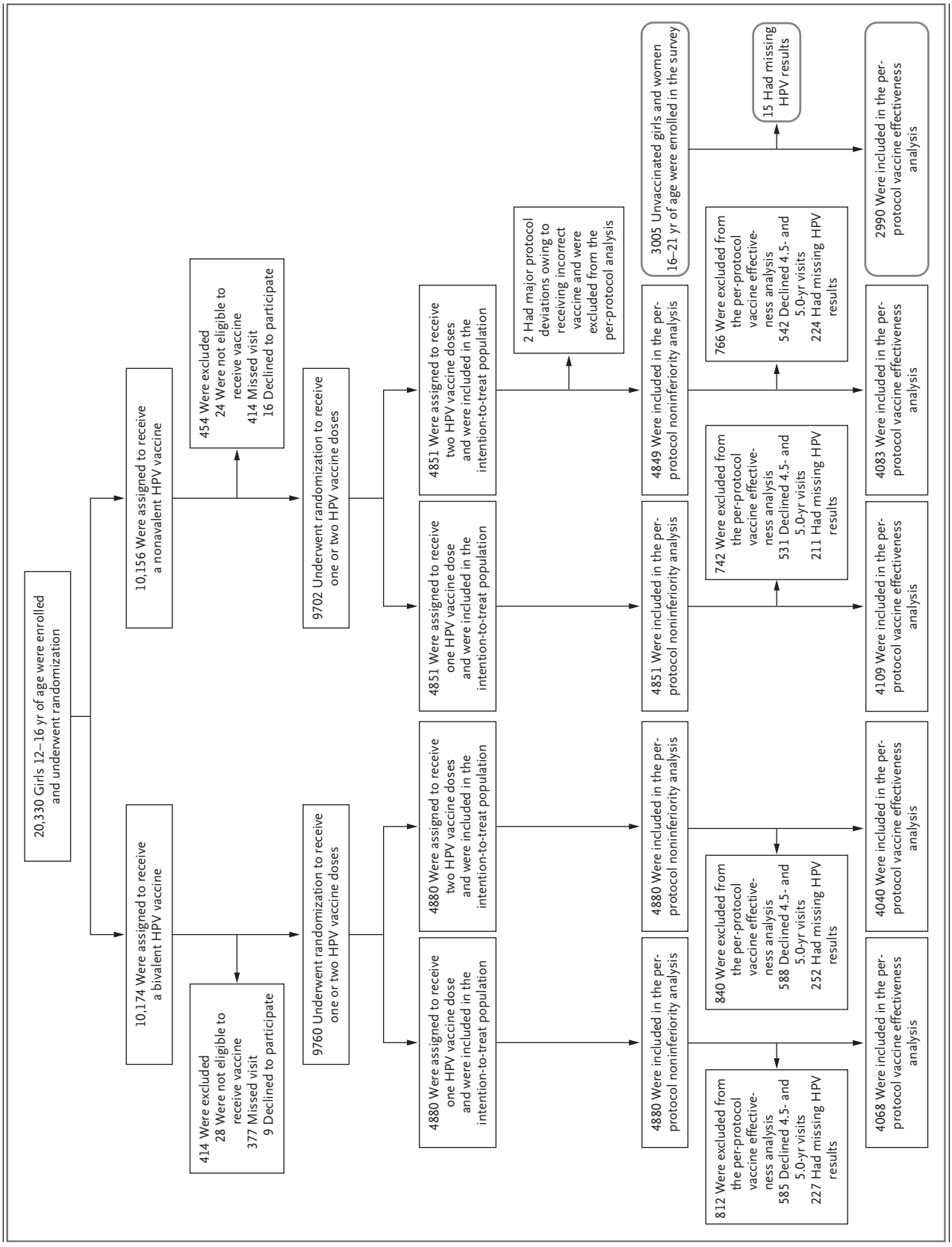

Figure 1 (facing page). Enrollment, Randomization, and Follow-up.

Trial participants were excluded from the analysis of vaccine effectiveness if they had missing results for human papillomavirus (HPV) infection at both month 54 and month 60 and survey participants were excluded if they had missing results at month 0 (the enrollment visit) and month 6 (the second visit) because they would have contributed no data for the estimation of vaccine effectiveness. The survey participants did not undergo randomization.

effectiveness against HPV16 or HPV18 infection was at least 92% in each of the four trial groups (Table S10).

Serious adverse events that were considered by the investigators to be "possibly, probably, or definitely related to HPV vaccination" occurred in 7 of 20,330 trial participants (0.03%) over a period of 5 years (Table S14). Nonserious adverse events are shown in Table S15. No pattern was observed in safety data.

DISCUSSION

After 5 years of follow-up, a single dose of either a bivalent or nonavalent HPV vaccine provided similar protection to that of two doses, which is the standard regimen for adolescents. Assessment of the timing of the events over the 5-year trial period indicated that protection persisted through at least 5 years. The primary end point assessed infection with HPV16 or HPV18, the two HPV genotypes that account for more than 77% of cervical cancers worldwide. We observed that one dose of the nonavalent vaccine was noninferior to two doses with respect to protection against the seven carcinogenic HPV types in the vaccine formulation, which account for approximately 95% of cervical cancers.[1] A single dose of the bivalent vaccine provided substantial protection against HPV45 infection, and two doses of the bivalent vaccine conferred greater protection against HPV31 than one dose. These single-dose results are consistent with previous estimates from observational studies in Costa Rica[4,5] and India[6] and from the KEN SHE randomized trial.[7]

Effectiveness against HPV16 or HPV18 infection was at least 97% in the one-dose groups, with narrow confidence intervals, a finding that supports projections[13] that a single dose will prevent most new infections and subsequent disease associated with these types. Single-dose HPV vaccine effectiveness was high with respect to all HPV types included in the nonavalent vaccine formulation, a result that emphasizes the eventual usefulness of increased valency in single-dose vaccines to better control cervical cancer.

HPV vaccines comprise recombinant L1 major capsid proteins that assemble into viruslike particles with a densely ordered repetitive array of B-cell epitopes on their surface. These viruslike particles are strong B-cell immunogens that can induce sterilizing immunity in most vaccine recipients and consistently induce high and durable titers of infection-inhibiting antibodies, even after a single dose. In addition, HPVs are very susceptible to antibody inhibition.[14] The vaccines induce the production of long-lived plasma cells that consistently produce antigen-specific antibodies,[15] independent of additional antigen exposure, even after a single dose. Because consistent, long-term stabilization of antibodies after a single dose had not been observed in subunit vaccines before the development of HPV vaccines, the current trial advances the science suggesting that viruslike particles should be considered for future vaccines.

Our trial had many strengths, including its population-based design, well-powered sample size for noninferiority and effectiveness assessments, high participant adherence, excellent balance among groups, and the use of a comparator group. We ensured the assessment of individual-level effectiveness by enrolling no more than 35% of the girls 12 to 16 years of age in any district in the trial area so that the results would be generalizable to settings where HPV vaccination has yet to be introduced (i.e., areas that do not have herd protection). The trial focused on the population at greatest risk for HPV-driven cancer — adolescent girls — and generated data that are applicable to girls in other regions of the world (Table S16).

The trial had limitations. Our estimates of vaccine effectiveness were based on a control group that consisted of participants who did not undergo randomization but were similar to the trial participants in relevant aspects, notably in the almost identical distribution of low-risk HPV types not included in the vaccine formulations. For the analysis of vaccine effectiveness, we did not have information about previous HPV infection rates among participants in the survey, which limited our ability to create a per-protocol

Table 1. Noninferiority Analysis.*

End Point	Bivalent HPV Vaccine				Nonavalent HPV Vaccine			
	No. of Participants	No. of Events	Cumulative Event Rate/100 Participants (95% CI)	Rate Difference (95% CI)†	No. of Participants	No. of Events	Cumulative Event Rate/100 Participants (95% CI)	Rate Difference (95% CI)†
Primary end point: infection with HPV type 16 or 18								
One dose	4880	14	0.29 (0.15 to 0.52)		4851	23	0.48 (0.28 to 0.75)	
Two doses	4880	21	0.42 (0.23 to 0.71)	−0.13 (−0.45 to 0.15)	4849	13	0.27 (0.12 to 0.51)	0.21 (−0.09 to 0.51)
P value‡				<0.001				<0.001
Secondary end point: infection with HPV type 16, 18, 31, 33, 45, 52, or 58								
One dose	4880	824	16.88 (15.71 to 18.11)		4851	79	1.64 (1.25 to 2.10)	
Two doses	4880	721	14.77 (13.63 to 15.96)	2.12 (0.46 to 3.76)	4849	52	1.08 (0.75 to 1.50)	0.56 (0.01 to 1.11)
P value‡				Not calculated				<0.001

* The noninferiority analysis was performed in the per-protocol population, which included all the participants who had received both assigned doses (the two assigned human papillomavirus [HPV] vaccine doses or one HPV vaccine dose and one dose of the control vaccine [tetanus, diphtheria, and pertussis vaccine]). The primary end point was new HPV16 or HPV18 infection that occurred during the period from month 12 to month 60 and persisted for at least 6 months. The secondary end point was new infection with the HPV types shown that occurred during the period from month 12 to month 60 and persisted for at least 6 months. Missing data have been imputed. The event numbers have been rounded to the nearest integer. Details regarding the methods for handling missing data are provided in the Supplementary Methods section in the Supplementary Appendix.

† The rate difference is the event rate in the one-dose group minus that in the two-dose group.

‡ The P value is for the noninferiority of one dose to two doses. A one-sided P value of less than 0.025 was considered to indicate statistical significance (i.e., the observed rate difference was significantly lower than the prespecified noninferiority margin). The prespecified noninferiority margin was 1.25 infections per 100 participants for the primary end point. The noninferiority test for the secondary end point was performed only for the nonavalent vaccine (prespecified noninferiority margin, 2.55 infections per 100 participants) and was not performed for the bivalent vaccine because the HPV types included in the secondary end point are not in the bivalent vaccine formulation.

Table 2. Analysis of Vaccine Effectiveness.*

End Point	Bivalent HPV Vaccine				Nonavalent HPV Vaccine			
	No. of Participants	No. of Events	Event Rate/100 Participants (95% CI)	Vaccine Effectiveness (95% CI)†	No. of Participants	No. of Events	Event Rate/100 Participants (95% CI)	Vaccine Effectiveness (95% CI)†
Primary end point: infection with HPV type 16 or 18								
Survey	2990	160	5.37 (4.55–6.17)		2990	159	5.32 (4.49–6.17)	
One dose	4068	4	0.10 (0.02–0.21)	98.2 (96.1–99.6)	4109	7	0.16 (0.05–0.30)	97.0 (94.3–99.1)
P value‡				<0.001				<0.001
Survey	2990	162	5.43 (4.56–6.24)		2990	160	5.35 (4.54–6.22)	
Two doses	4040	5	0.12 (0.03–0.23)	97.8 (95.6–99.3)	4083	3	0.08 (0.01–0.16)	98.5 (96.7–99.7)
P value‡				<0.001				<0.001
Secondary end point: infection with HPV type 16, 18, 31, 33, 45, 52, or 58								
Survey	2990	390	13.03 (11.88–14.24)		2990	389	13.01 (11.61–14.29)	
One dose	4068	363	8.93 (8.01–9.79)	31.5 (21.5–40.1)	4109	29	0.72 (0.45–0.99)	94.5 (92.3–96.6)
Survey	2990	385	12.89 (11.59–14.18)		2990	393	13.16 (11.91–14.50)	
Two doses	4040	311	7.69 (6.93–8.56)	40.3 (31.3 to 48.8)	4083	22	0.55 (0.31–0.81)	95.8 (93.8–97.6)

* Vaccine effectiveness was assessed in the per-protocol population. Shown are infections that were observed at the visits at month 54 and month 60 among the trial participants and at month 0 (the enrollment visit) and month 6 (the second visit) among the survey participants. Missing data have been imputed. The estimated numbers of events among the survey participants have been adjusted for prevalent infections, and propensity-score adjustment was used to adjust for different distributions in age, geographic region, and sexual activity between the trial participants and the survey participants (the adjusted number of events in the survey population is considered to be the standardized number of events in the same population as the trial group in the comparison). The event numbers have been rounded to the nearest integer. Details regarding the methods for estimating the vaccine effectiveness are provided in the Supplementary Methods section in the Supplementary Appendix.

† The vaccine effectiveness values are expressed as percentages.

‡ A one-sided P value of less than 0.025 was considered to indicate statistical significance (i.e., the vaccine effectiveness was higher than 80%).

Table 3. Analysis of Vaccine Effectiveness According to HPV Type.*

HPV Infection	Bivalent HPV Vaccine				Nonavalent HPV Vaccine			
	No. of Participants	No. of Events	Event Rate/100 Participants (95% CI)	Vaccine Effectiveness (95% CI)	No. of Participants	No. of Events	Event Rate/100 Participants (95% CI)	Vaccine Effectiveness (95% CI)
HPV16								
Survey	2990	109	3.66 (2.91 to 4.33)		2990	109	3.64 (2.97 to 4.33)	
One dose	4068	3	0.08 (0.01 to 0.17)	97.7 (95.1 to 99.7)	4109	4	0.10 (0.02 to 0.20)	97.2 (94.1 to 99.4)
Survey	2990	112	3.73 (3.01 to 4.43)		2990	110	3.67 (2.99 to 4.40)	
Two doses	4040	2	0.06 (0.01 to 0.14)	98.4 (96.1 to 99.8)	4083	1	0.03 (0.00 to 0.08)	99.2 (97.8 to 100)
HPV18								
Survey	2990	61	2.05 (1.56 to 2.64)		2990	61	2.03 (1.54 to 2.56)	
One dose	4068	1	0.02 (0.00 to 0.06)	99.3 (97.0 to 100.0)	4109	2	0.06 (0.00 to 0.14)	97.1 (92.5 to 100)
Survey	2990	61	2.05 (1.52 to 2.57)		2990	61	2.03 (1.51 to 2.59)	
Two doses	4040	2	0.06 (0.00 to 0.15)	97.1 (92.5 to 100.0)	4083	2	0.05 (0.00 to 0.12)	97.6 (93.7 to 100)
HPV31								
Survey	2990	119	3.99 (3.25 to 4.80)		2990	121	4.05 (3.29 to 4.86)	
One dose	4068	100	2.46 (1.92 to 3.03)	38.3 (18.1 to 54.1)	4109	3	0.08 (0.01 to 0.17)	98.0 (95.4 to 99.7)
Survey	2990	119	3.97 (3.19 to 4.77)		2990	123	4.11 (3.36 to 4.83)	
Two doses	4040	28	0.69 (0.46 to 0.99)	82.6 (73.9 to 88.8)	4083	6	0.14 (0.04 to 0.26)	96.6 (93.5 to 99.1)
HPV33								
Survey	2990	30	1.00 (0.67 to 1.39)		2990	29	0.96 (0.62 to 1.33)	
One dose	4068	23	0.57 (0.35 to 0.81)	42.6 (2.1 to 68.4)	4109	1	0.02 (0.00 to 0.07)	97.5 (91.2 to 100)
Survey	2990	29	0.97 (0.63 to 1.32)		2990	29	0.96 (0.60 to 1.31)	
Two doses	4040	30	0.73 (0.44 to 0.99)	24.8 (−26.2 to 58.7)	4083	3	0.07 (0.01 to 0.17)	93.0 (78.6 to 100)
HPV45								
Survey	2990	38	1.28 (0.89 to 1.68)		2990	38	1.28 (0.86 to 1.70)	
One dose	4068	21	0.53 (0.30 to 0.78)	58.8 (28.4 to 78.5)	4109	5	0.12 (0.02 to 0.22)	90.5 (79.4 to 98.2)
Survey	2990	38	1.27 (0.87 to 1.74)		2990	39	1.32 (0.90 to 1.80)	
Two doses	4040	14	0.36 (0.19 to 0.57)	72.1 (46.0 to 87.1)	4083	3	0.08 (0.01 to 0.19)	93.6 (84.7 to 100)

HPV52								
Survey	2990	95	3.16 (2.61 to 3.76)		2990	94	3.13 (2.48 to 3.76)	
One dose	4068	128	3.16 (2.65 to 3.75)	0.3 (−31.5 to 23.7)	4109	3	0.08 (0.00 to 0.17)	97.3 (93.9 to 100)
Survey	2990	94	3.14 (2.50 to 3.84)		2990	95	3.18 (2.48 to 3.84)	
Two doses	4040	143	3.55 (2.99 to 4.14)	−13.2 (−49.6 to 13.5)	4083	2	0.05 (0.00 to 0.12)	98.5 (96.1 to 100)
HPV58								
Survey	2990	88	2.95 (2.35 to 3.60)		2990	89	2.98 (2.35 to 3.57)	
One dose	4068	143	3.51 (2.92 to 4.09)	−19.3 (−53.8 to 7.5)	4109	12	0.29 (0.13 to 0.47)	90.2 (83.4 to 95.6)
Survey	2990	84	2.81 (2.22 to 3.48)		2990	89	2.99 (2.44 to 3.62)	
Two doses	4040	127	3.15 (2.60 to 3.76)	−12.0 (−48.2 to 16.6)	4083	5	0.13 (0.05 to 0.26)	95.5 (90.7 to 98.6)
HPV6								
Survey	2990	61	2.03 (1.53 to 2.62)		2990	61	2.05 (1.49 to 2.55)	
One dose	4068	63	1.55 (1.21 to 1.96)	23.6 (−7.9 to 45.4)	4109	8	0.20 (0.08 to 0.37)	90.0 (81.5 to 96.3)
Survey	2990	61	2.05 (1.54 to 2.59)		2990	62	2.09 (1.61 to 2.62)	
Two doses	4040	74	1.82 (1.40 to 2.23)	11.4 (−27.0 to 36.5)	4083	1	0.03 (0.00 to 0.10)	98.7 (95.0 to 100)
HPV11								
Survey	2990	12	0.42 (0.21 to 0.66)		2990	12	0.41 (0.19 to 0.64)	
One dose	4068	5	0.13 (0.03 to 0.26)	69.2 (23.0 to 94.5)	4109	0	0.00†	100†
Survey	2990	12	0.39 (0.15 to 0.63)		2990	12	0.41 (0.19 to 0.62)	
Two doses	4040	11	0.28 (0.13 to 0.47)	27.1 (−86.7 to 68.7)	4083	4	0.10 (0.01 to 0.22)	76.3 (27.5 to 97.1)

* Vaccine effectiveness was assessed in the per-protocol population. Shown are infections that were observed at the visits at month 54 and month 60 among the trial participants and at month 0 (the enrollment visit) and month 6 (the second visit) among the survey participants. Missing data have been imputed. The estimated numbers of events among the survey participants have been adjusted for prevalent infections, and propensity-score adjustment was used to adjust for different distributions in age, geographic region, and sexual activity between the trial participants and the survey participants (the adjusted number of events in the survey population is considered to be the standardized number of events in the same population as the trial group in the comparison). The event numbers have been rounded to the nearest integer. HPV16 is responsible for 61.7% of the global burden of cervical cancer, HPV18 for 15.3%, HPV31 for 2.8%, HPV33 for 3.8%, HPV45 for 4.8%, HPV52 for 2.8%, and HPV58 for 3.5%.

† The confidence interval was not calculated because no HPV11 infections were observed among the participants who had received one dose of the nonavalent vaccine.

population; data obtained from age-matched trial participants at enrollment facilitated the necessary statistical adjustments to ensure comparison with the trial participants. We followed the participants for 5 years, so longer-term durability of response between one and two doses would require additional monitoring. The prespecified noninferiority margin that considered 80% effectiveness to be noninferior to 93.6% may be considered to be too broad, but the sample-size requirements for a smaller margin would have been infeasibly large. Yet, the observed confidence intervals for the estimates of rate differences indicate that the data are compatible with a true difference in vaccine effectiveness between one and two doses of no more than 5.5 percentage points (0.51÷9.2, where 0.51 infections per 100 participants is the upper bound of the 95% confidence interval for the rate difference between one and two doses of the nonavalent vaccine, and 9.2 infections per 100 participants is the expected infection rate in an unvaccinated population), which is much smaller than the prespecified margin of 13.6 percentage points for the difference in vaccine effectiveness.

The trial was not designed to evaluate safety because all the trial participants received at least one HPV vaccine dose and participants in the one-dose groups received a control vaccine. No safety concerns were identified, a finding that is consistent with that in our phase 3 Costa Rica HPV Vaccine Trial.[16] The safety profiles of these commercial HPV vaccines have been evaluated extensively in hundreds of millions of persons.

High-coverage HPV vaccination is a mainstay of cervical cancer control efforts, but to date not even one third of eligible adolescent girls worldwide have received the vaccine, which has been licensed for almost 20 years. The evidence from this trial supports the WHO alternative recommendation for single-dose HPV vaccination[17] to achieve higher coverage while maintaining sufficiently high efficacy.

The views expressed in this article are those of the authors alone and do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer or the World Health Organization.

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We dedicate this article to the memory of our beloved colleague and friend Paula González, extraordinary scientist and fighter for women's health, who led the initial phase of the trial with inspiration and motivation.

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REFERENCES

1. Wei F, Georges D, Man I, Baussano I, Clifford GM. Causal attribution of human papillomavirus genotypes to invasive cervical cancer worldwide: a systematic analysis of the global literature. Lancet 2024;404:435-44.
2. Bruni L, Saura-Lázaro A, Montoliu A, et al. HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010-2019. Prev Med 2021;144:106399.
3. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-63.
4. Kreimer AR, Rodriguez AC, Hildesheim A, et al. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst 2011;103:1444-51.
5. Kreimer AR, Sampson JN, Porras C, et al. Evaluation of durability of a single dose of the bivalent HPV vaccine: the CVT Trial. J Natl Cancer Inst 2020;112:1038-46.
6. Basu P, Malvi SG, Joshi S, et al. Vaccine efficacy against persistent human papillomavirus (HPV) 16/18 infection at 10 years after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre, prospective, cohort study. Lancet Oncol 2021;22:1518-29.
7. Barnabas RV, Brown ER, Onono MA, et al. Durability of single-dose HPV vaccination in young Kenyan women: randomized controlled trial 3-year results. Nat Med 2023;29:3224-32.
8. Watson-Jones D, Changalucha J, Whit-

worth H, et al. Immunogenicity and safety of one-dose human papillomavirus vaccine compared with two or three doses in Tanzanian girls (DoRIS): an open-label, randomised, non-inferiority trial. Lancet Glob Health 2022;10(10):e1473-e1484.
9. Porras C, Sampson JN, Herrero R, et al. Rationale and design of a double-blind randomized non-inferiority clinical trial to evaluate one or two doses of vaccine against human papillomavirus including an epidemiologic survey to estimate vaccine efficacy: the Costa Rica ESCUDDO trial. Vaccine 2022;40:76-88.
10. Sampson JN, Hildesheim A, Herrero R, Gonzalez P, Kreimer AR, Gail MH. Design and statistical considerations for studies evaluating the efficacy of a single dose of the human papillomavirus (HPV) vaccine. Contemp Clin Trials 2018;68:35-44.
11. Sierra MS, Coto C, Porras C, et al. Validation of TypeSeq2, a next-generation-based sequencing assay for the detection of 46 human papillomavirus genotypes, at the US National Cancer Institute and Costa Rica laboratories. J Infect Dis 2025 August 12 (Epub ahead of print).
12. Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med 1990;9:1447-54.
13. Bénard É, Drolet M, Laprise J-F, et al. Potential population-level effectiveness of one-dose HPV vaccination in low-income and middle-income countries: a mathematical modelling analysis. Lancet Public Health 2023;8(10):e788-e799.
14. Schiller J, Lowy D. Explanations for the high potency of HPV prophylactic vaccines. Vaccine 2018;36:4768-73.
15. Porras C, Romero B, Kemp T, et al. HPV16/18 antibodies 16-years after single dose of bivalent HPV vaccination: Costa Rica HPV vaccine trial. J Natl Cancer Inst Monogr 2024;67:329-36.
16. Hildesheim A, Wacholder S, Catteau G, Struyf F, Dubin G, Herrero R. Efficacy of the HPV-16/18 vaccine: final according to protocol results from the blinded phase of the randomized Costa Rica HPV-16/18 vaccine trial. Vaccine 2014;32:5087-97.
17. World Health Organization. Human papillomavirus vaccines: WHO position paper (2022 update). December 2022 (https://www.who.int/publications-detail-redirect/who-wer9750-645-672).

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