

Immunogenicity of 2 Doses of HPV Vaccine in Younger Adolescents vs 3 Doses in Young Women

A Randomized Clinical Trial

Simon R. M. Dobson, MD

Shelly McNeil, MD

Marc Dionne, MD

Meena Dawar, MD

Gina Ogilvie, MD

Mel Krajden, MD, PhD

Chantal Sauvageau, MD

David W. Scheifele, MD

Tobias R. Kollmann, MD, PhD

Scott A. Halperin, MD

Joanne M. Langley, MD

Julie A. Bettinger, PhD

Joel Singer, PhD

Deborah Money, MD

Dianne Miller, MD

Monika Naus, MD

Fawziah Marra, PharmD

Eric Young, MD

GLOBALLY, CERVICAL CANCER IS the second most common cause of cancer morbidity and mortality in women.¹ Human papillomavirus (HPV) infection has been identified as a necessary cause for the development of cervical cancer, with HPV genotypes 16 and 18 accounting for approximately 70% of cervical cancer cases. Prevention of cervical cancer using either the bivalent

For editorial comment see p 1832.

Author Video Interview available at www.jama.com.

Importance Global use of human papillomavirus (HPV) vaccines to prevent cervical cancer is impeded by cost. A 2-dose schedule for girls may be possible.

Objective To determine whether mean antibody levels to HPV-16 and HPV-18 among girls receiving 2 doses was noninferior to women receiving 3 doses.

Design, Setting, and Patients Randomized, phase 3, postlicensure, multicenter, age-stratified, noninferiority immunogenicity study of 830 Canadian females from August 2007 through February 2011. Follow-up blood samples were provided by 675 participants (81%).

Intervention Girls (9-13 years) were randomized 1:1 to receive 3 doses of quadrivalent HPV vaccine at 0, 2, and 6 months (n=261) or 2 doses at 0 and 6 months (n=259). Young women (16-26 years) received 3 doses at 0, 2, and 6 months (n=310). Antibody levels were measured at 0, 7, 18, 24, and 36 months.

Main Outcomes and Measures Primary outcome was noninferiority (95% CI, lower bound >0.5) of geometric mean titer (GMT) ratios for HPV-16 and HPV-18 for girls (2 doses) compared with young women (3 doses) 1 month after last dose. Secondary outcomes were noninferiority of GMT ratios of girls receiving 2 vs 3 doses of vaccine; and durability of noninferiority to 36 months.

Results The GMT ratios were noninferior for girls (2 doses) to women (3 doses): 2.07 (95% CI, 1.62-2.65) for HPV-16 and 1.76 (95% CI, 1.41-2.19) for HPV-18. Girls (3 doses) had GMT responses 1 month after last vaccination for HPV-16 of 7736 milli-Merck units per mL (mMU/mL) (95% CI, 6651-8999) and HPV-18 of 1730 mMU/mL (95% CI, 1512-1980). The GMT ratios were noninferior for girls (2 doses) to girls (3 doses): 0.95 (95% CI, 0.73-1.23) for HPV-16 and 0.68 (95% CI, 0.54-0.85) for HPV-18. The GMT ratios for girls (2 doses) to women (3 doses) remained noninferior for all genotypes to 36 months. Antibody responses in girls were noninferior after 2 doses vs 3 doses for all 4 vaccine genotypes at month 7, but not for HPV-18 by month 24 or HPV-6 by month 36.

Conclusions and Relevance Among girls who received 2 doses of HPV vaccine 6 months apart, responses to HPV-16 and HPV-18 one month after the last dose were noninferior to those among young women who received 3 doses of the vaccine within 6 months. Because of the loss of noninferiority to some genotypes at 24 to 36 months in girls given 2 doses vs 3 doses, more data on the duration of protection are needed before reduced-dose schedules can be recommended.

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(HPV-16 and HPV-18) or quadrivalent (HPV-6, HPV-11, HPV-16, and HPV-18) vaccine is the goal of immu-

Author Affiliations are listed at the end of this article.
Corresponding Author: Simon R. M. Dobson, MD, Vaccine Evaluation Center, University of British Columbia, British Columbia Children's Hospital, 950 W 28th Ave, Vancouver, BC V5Z 4H4, Canada (sdobson@cw.bc.ca).

nization programs in many countries. Both vaccines are safe, highly immunogenic, and effective at protecting against persistent infection and disease. The HPV vaccines, which are designed to prevent cervical cancer outcomes in adults, need to be administered before persons become sexually active.

The quadrivalent HPV vaccine was approved for use in young adolescents based on immunogenicity-bridging studies rather than efficacy studies.² More than 99% of male and female adolescent participants seroconverted following a 3-dose schedule with antibody levels 1.7- to 2.0-fold higher among adolescents than in adults, with participants 9 through 13 years of age having the highest antibody levels.^{3,4}

School-based HPV vaccine programs were introduced in Canada in 2007 using the quadrivalent HPV vaccine. Given the high cost of the vaccines, their strong immunogenicity profile, and high efficacy, interest existed in alternate dose schedules. Canadian experts and policy makers identified alternate dose schedules as being among the top research priorities in 2005.⁵ A posttrial, nonrandomized analysis of girls who received fewer than 3 doses of the bivalent vaccine in a clinical trial in Costa Rica using efficacy end points showed that fewer doses were as protective as 3 doses.⁶

In this study, we examined whether 2 doses of quadrivalent HPV vaccine given 6 months apart to girls aged 9 through 13 years produced an immune response noninferior to 3 doses in young women aged 16 through 26 years in whom efficacy against disease has been demonstrated. As a secondary outcome, we also examined the incremental benefit in antibody titers of a third dose given to girls and durability of antibody to 36 months after vaccination.

METHODS

Study Design

The study was approved by Health Canada and ethics review boards at each of the 3 provincial centers. An external advisory panel and data and safety

monitoring board were created by the Michael Smith Foundation for Health Research to oversee the study conduct and participant safety. Cervical HPV detection and genotyping assays were conducted by the Provincial Health Services Authority Laboratory. This was a phase 3, postlicensure, age-stratified, noninferiority immunogenicity study conducted at 3 provincial centers in Canada with 3 parallel groups in 2 age groups receiving open-label quadrivalent HPV vaccine. Enrollment was conducted from August 1, 2007, through February 29, 2008, and was limited to healthy participants 9 through 13 years of age (girls) or 16 through 26 years of age (young women), with 4 or fewer lifetime sexual partners. Study exclusions were pregnancy at enrollment or at vaccine visit, history of genital warts or cervical intraepithelial neoplasia, or prior receipt of any HPV vaccine. Presence of HPV-16, HPV-18, HPV-6, and HPV-11 antibodies (all participants) or virus infection (among sexually active women participants) at study enrollment was an exclusion criterion from per-protocol study participant analysis for that genotype-specific outcome.

At study entry girls were randomized (1:1) in balanced, stratified blocks of 6 to receive either 2 doses (at 0 and 6 months) or 3 doses (at 0, 2, and 6 months).⁷ The coordinating center used SAS, version 9.2 (SAS Institute Inc) to generate randomization lists for each site. Each site was treated as a stratum. Women were not randomized and received the standard 0-, 2-, and 6-month vaccine schedule (FIGURE 1 and FIGURE 2).

Immunogenicity was assessed at 7 months (1 month after the last dose). Participants were eligible for follow-up blood samples to 36 months if they completed all immunizations and met criteria for subsequent blood sampling. All eligible participants provided a blood sample at 24 months after the first dose. To enhance study retention, participants within each cohort were randomized (1:1) into blocks of 6 for a blood sample to be taken either at 18 or 36 months after the first

dose. Participants received no compensation.

Study Procedures

Participants were recruited by advertising in newspapers, at local colleges, and by approved established recruitment procedures at each site. Consent for girls required written consent from parents or a legal guardian and written assent of study participants, and women followed the appropriate consent procedures for each province. Participants provided written consent a second time for blood samples drawn after 7 months.

We purchased the licensed, commercially available quadrivalent vaccine,² which was administered using pre-filled syringes with 25-gauge, 2.54-cm needles, into the deltoid muscle. Serum samples were taken from all participants at months 0, 7, and 24, and an additional serum sample was taken at either month 18 or 36.

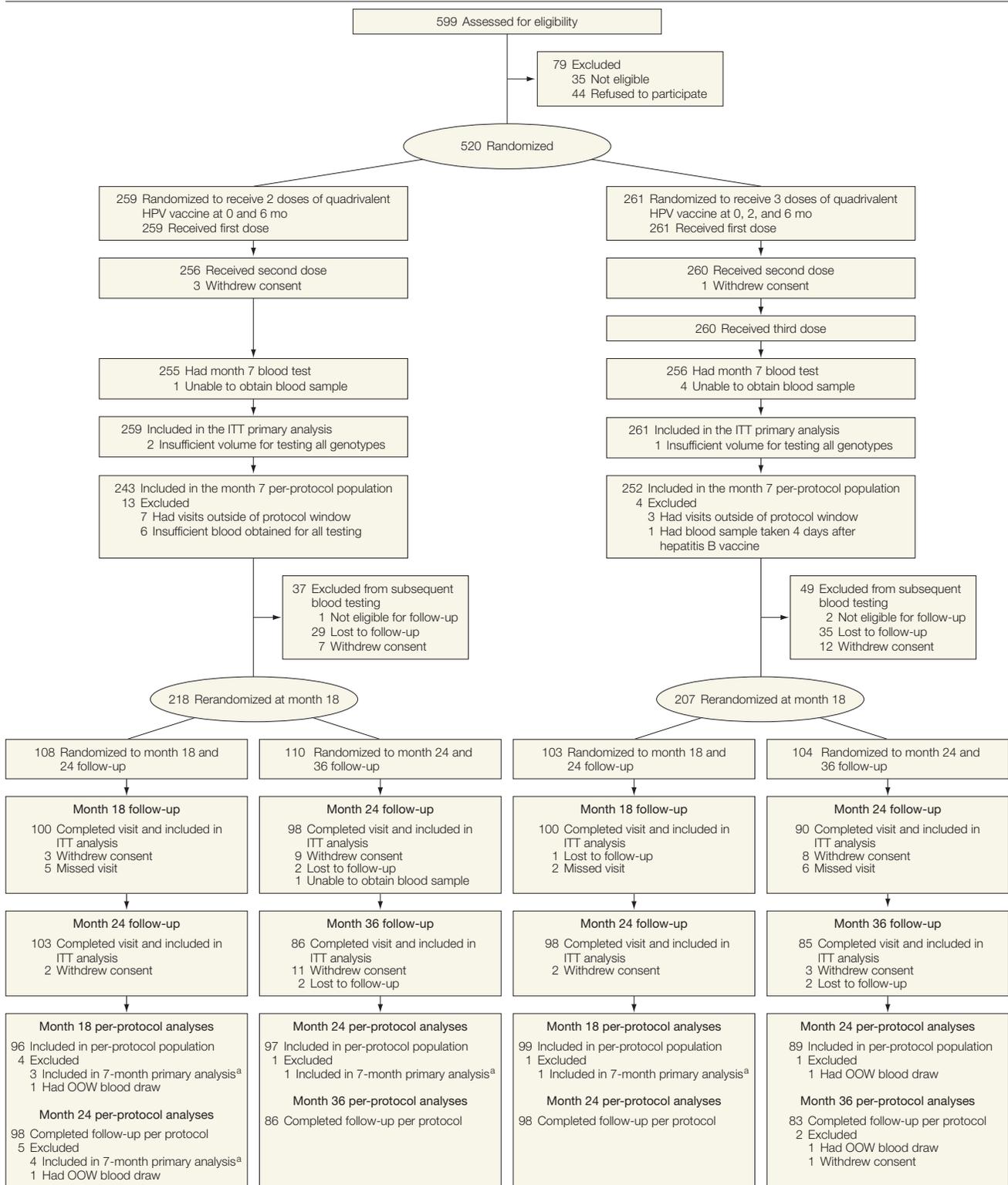
Health assessment, including sexual history, was obtained at study entry. Self-identified ethnicity was used to establish demographic similarities between groups. Vaccine was administered at 0, 2, and 6 months to girls and women receiving 3 doses, and at 0 and 6 months to girls receiving 2 doses. Sexually active women provided a vaginal swab at study entry for HPV detection and genotyping. Because this was a postlicensure study, data were only collected on serious adverse events occurring within 30 days of each vaccination. This information was collected at the next visit or if the participant called with concerns.

Laboratory Analysis

For HPV detection and genotyping, vaginal swabs were placed into specimen transport media, stored frozen, and transported to the Provincial Health Services Authority Laboratory. Vaginal swabs were screened for the presence of 37 HPV genotypes, including the 13 high-risk genotypes, using a commercial reverse line-blot assay.

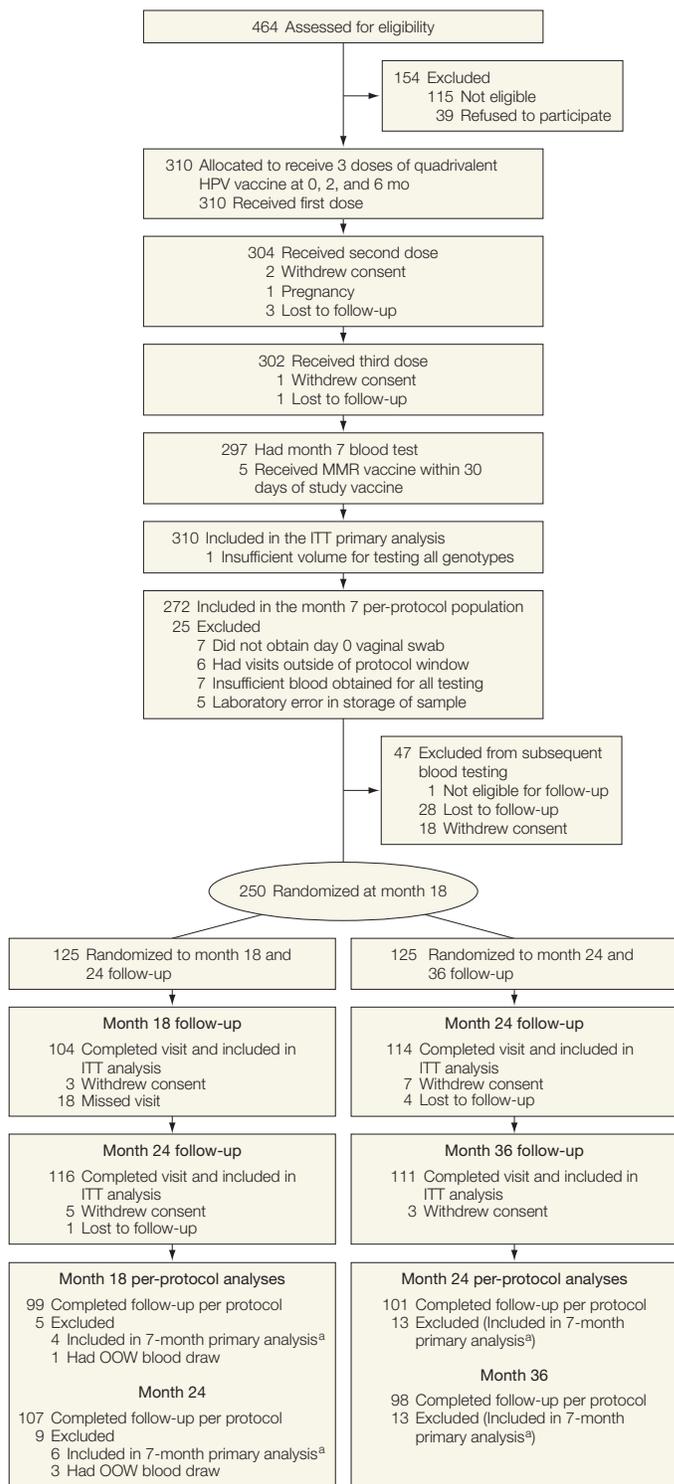
Merck Laboratory staff, blinded to group assignment, conducted the HPV

Figure 1. Flowchart of Participants Through the Study, Girls Aged 9 Through 13 Years



HPV indicates human papillomavirus; ITT, intention to treat; OOW, out of window.
^aParticipants included in the ITT analysis at month 7 could not be reintroduced into the per-protocol analysis.

Figure 2. Flowchart of Participants Through the Study, Women Aged 16 Through 26 Years



HPV indicates human papillomavirus; ITT, indicates intention to treat; MMR, measles-mumps-rubella; OOW, out of window.

^a Participants included in the ITT analysis at month 7 could not be reintroduced into the per-protocol analysis.

antibody assays using a competitive Luminex immunoassay to detect HPV-16, HPV-18, HPV-6, and HPV-11 antibodies. The immunoassay measures genotype-specific neutralizing antibodies in human serum, which displace labeled detection monoclonal antibodies targeting neutralizing epitopes of the respective HPV types. Serostatus cutoff values were those determined in validation studies for use in both patients who were previously infected and those vaccinated (ie, ≥ 20 milli-Merck units per mL [mMU/mL] for HPV-16, ≥ 24 mMU/mL for HPV-18, ≥ 20 mMU/mL for HPV-6, and ≥ 16 mMU/mL for HPV-11).^{8,9}

Statistical Analysis

The primary objective of this study was to determine whether geometric mean titer (GMT) antibody levels at 7 months (1 month after the last dose) among girls receiving 2 doses was noninferior to GMT antibody levels among young women receiving 3 doses for HPV-16 and HPV-18. Secondary objectives included comparisons of GMT antibody levels and seropositivity between girls receiving 2 doses and young women receiving 3 doses for HPV-6 and HPV-11, and between girls receiving 2 doses vs 3 doses for HPV-16, HPV-18, HPV-6, and HPV-11 at month 7. An important secondary objective was to examine durability of antibody response at 18, 24, and 36 months after the first dose by examining seropositivity and GMTs in the 3 study groups for antibodies to the 4 vaccine antigens.

Sample size was calculated using a 1-sided α equals .025 of noninferiority among the young women group and the 2 treatment groups, equal allocation in the 3 groups, with a power of 99%.¹⁰ An estimate of assay variance was inferred from the published immunogenicity trial data.^{2,11} The clinically relevant difference in GMT was computed as the exponential of the difference of the mean of 2 groups in the log scale. A P value of .05 was implicitly used to declare statistical significance, but the focus was on 95% CIs for the between-group comparisons. Cri-

teria for declaring noninferiority of a treatment group were defined as the lower bounds of the multiplicity-adjusted 95% CI for a GMT ratio (girls or women) greater than 0.5. This noninferiority margin was based on benchmarks set by Merck for other bridging studies leading to licensure, according to regulatory guidance.^{2-4,12} We needed 235 evaluable participants per group for a total of 705 participants. Sample size was further inflated by 10% in the girls cohort and 30% in the women cohort to allow for loss to follow-up and higher baseline HPV antibody positivity in the women for an anticipated recruitment of 825 participants.

Analyses Population

The primary interest was in the per-protocol population; however, the results presented are the intention-to-treat population because these results can be more readily generalizable. The per-protocol population included individuals who were seronegative (all participants) and had a negative result for a HPV genotype at enrollment (assessed in women only), received all assigned doses of the vaccine, and adhered to all study procedures. Participants who did not follow protocol and/or were seropositive or polymerase chain reaction–positive for HPV-16, HPV-18, HPV-6, or HPV-11 at enrollment were excluded from the per-protocol population analysis but retained for the intention-to-treat population analysis. Participants were eligible to continue with the 18- and 36-month follow-up if they had all of their doses of vaccine and a 7-month blood sample collected. If participants were excluded from the per-protocol population analysis at 7 months, they remained excluded for the remainder of the study but were retained for intention-to-treat analysis.

Geometric mean titer ratios and corresponding 95% CIs were calculated using general linear models (SAS, version 9.2). The main intention-to-treat analysis was performed excluding missing values, but a sensitivity analysis

Table 1. Baseline Characteristics of Female Study Participants at Enrollment

	No. (%) of Participants		
	Girls, 9-13 y		Women, 16-26 y
	2 Doses (n = 259)	3 Doses (n = 261)	3 Doses (n = 310)
Age, mean (SD), y	12.3 (1.4)	12.3 (1.4)	19.3 (2.8)
Weight, mean (SD), kg	46.8 (10.8)	47.8 (12.3)	62.3 (12.1)
BMI, mean (SD)	19.6 (3.5)	19.8 (3.9)	22.9 (3.8)
Race/ethnicity			
White	242 (93.4)	239 (91.6)	276 (89.0)
Chinese	2 (0.8)	8 (3.1)	7 (2.3)
Other	15 (5.8)	14 (5.4)	27 (8.7)
Sexual history			
Postmenarchal	116 (44.8)	118 (46.2)	310 (100)
Sexually active	0	0	204 (65.8)
Age of sexual debut, mean (range), y ^a			16.7 (13-24)
No. of sexual partners, mean (range)			1.3 (0-4)
Baseline HPV results			
Seropositivity			
HPV-16	0	1 (0.4)	12 (3.9)
HPV-18	0	0	7 (2.3)
HPV-6	2 (0.8)	2 (0.8)	14 (4.5)
HPV-11	0	1 (0.4)	3 (1.0)
Missing blood samples			
DNA detection by polymerase chain reaction	3 (1.2)	0	6 (1.9)
HPV-16			21 (6.8)
HPV-18			6 (1.9)
HPV-6			3 (1.0)
HPV-11			0
Missing vaginal swabs			7 (2.3)

Abbreviations: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; HPV, human papillomavirus.

^aBlank cells reflect an absence of data.

using multiple imputation to generate values for missing data was done for results at 7 months. Seroconversion rates and 95% CIs among groups were calculated using the Wilson risk sum score method.¹⁰

RESULTS

A total of 830 participants were enrolled from August 2007 through February 2008 with 767 participants (92.4%) evaluable for the per-protocol population analysis at month 7 (Figure 1 and Figure 2). Missing baseline blood samples and participant withdrawal of consent were the most frequent reasons for exclusion from the per-protocol population analysis. For months 18, 24, and 36, 675 participants were evaluable for the intention-to-treat population analysis. Characteristics of study

participants in the intention-to-treat population are presented in TABLE 1. Within each enrollment site girls receiving 2 or 3 doses were balanced for demographics (eAppendix 1, available at <http://www.jama.com>). The aggregated data for both girls groups were comparable regarding age, weight, body mass index, and ethnicity, whereas the women receiving 3 doses were older (mean age, 19 years), had higher weight, and were more ethnically diverse (11% non-white). Scheduled vaccine doses were received by 98.6% of study participants with no serious adverse events reported.

Results were consistent between the intention-to-treat (TABLE 2) and per-protocol (TABLE 3) populations with the 95% CI overlapping in all cases. Only the intention-to-treat results are dis-

cussed below. The multiple imputation of the intention-to-treat population's 7-month data (eAppendix 2) are also consistent (Table 2 and Table 3).

For the primary outcome, at 7 months, all but 2 participants (>99%) seroconverted; 1 from the girls group receiving 2 doses and 1 from the women group receiving 3 doses did not seroconvert to HPV-6. The GMT antibody levels in girls receiving 2 doses were 7344 mMU/mL (95% CI, 6310-8547) for HPV-16 and 1169 mMU/mL (95% CI, 1021-1338) for HPV-18; and in women receiving 3 doses, 3545 mMU/mL (95% CI, 3083-4076) for HPV-16 and 664 mMU/mL (95% CI, 586-752) for HPV-18. The levels in girls receiving 2 doses were noninferior to the respective GMTs in women receiving 3 doses, with GMT ratios of 2.07 (95% CI, 1.62-2.65) for HPV-16 and 1.76 (95% CI, 1.41-2.19) for HPV-18 (Table 2). The GMTs in girls receiving 2 doses were 2117 mMU/mL (95% CI, 1787-2508) for HPV-6 and

2339 mMU/mL (95% CI, 2088-2619) for HPV-11; and for women receiving 3 doses, 943 mMU/mL (95% CI, 807-1101) for HPV-6 and 1268 mMU/mL (95% CI, 1143-1408) for HPV-11. The levels in girls receiving 2 doses were noninferior to the GMTs in women with ratios of 2.25 (95% CI, 1.71-2.96) for HPV-6 and 1.84 (95% CI, 1.53-2.22) for HPV-11.

Girls receiving 3 doses had GMT levels of 7736 (95% CI, 6651-8999) for HPV-16, 1730 (95% CI, 1512-1980) for HPV-18, 1876 (95% CI, 1585-2221) for HPV-6, and 2117 (95% CI, 1891-2370) for HPV-11. Girls given 2 doses vs 3 doses had a noninferior antibody response for all 4 vaccine genotypes, with GMT ratios of 0.95 (95% CI, 0.73-1.23) for HPV-16; 0.68 (95% CI, 0.54-0.85) for HPV-18; 1.13 (95% CI, 0.85-1.50) for HPV-6; and 1.10 (95% CI, 0.91-1.34) for HPV-11 (Table 2).

The majority of participants (>99%) remained seropositive for HPV-16,

HPV-6, and HPV-11 for the duration of follow-up out to month 36. At 24 months, HPV-18 seropositivity was 89% (95% CI, 83%-92%) among girls receiving 2 doses, 94% (95% CI, 89%-96%) among girls receiving 3 doses, and 83% (95% CI, 77%-87%) among women receiving 3 doses. At 36 months, HPV-18 seropositivity was 86% (95% CI, 77%-92%) for girls receiving 2 doses, 95% (95% CI, 89%-98%) for girls receiving 3 doses and 79% (95% CI, 70%-86%) for women receiving 3 doses. The antibody levels for all 4 vaccine genotypes declined between months 7 and 18 to a plateau level that was maintained out to 36 months. Both girls groups continued to maintain higher plateau levels of antibody at 36 months than women. When comparing girls receiving 2 doses with girls receiving 3 doses, evidence for noninferiority was lost for HPV-18 by month 24 and for HPV-6 by month 36 (Table 2).

Table 2. Summary of Month 7, 18, 24, and 36 Anti-Human Papillomavirus Competitive Immunoassay Geometric Mean Titers in the Intention-to-Treat Population^a

Antibodies	Girls, 9-13 y				Women, 16-26 y		GMT Ratio (95% CI), mMU/mL		
	2 Doses		3 Doses		3 Doses		Girls (2 Doses)/ Women (3 Doses)	Girls (2 Doses)/ Girls (3 Doses)	Girls (3 Doses)/ Women (3 Doses)
	No. of Patients	GMT (95% CI), mMU/mL	No. of Patients	GMT (95% CI), mMU/mL	No. of Patients	GMT (95% CI), mMU/mL			
Month 7									
HPV-16	254	7344 (6310-8547)	256	7736 (6651-8999)	300	3545 (3083-4076)	2.07 (1.62-2.65) ^b	0.95 (0.73-1.23)	2.18 (1.71-2.79)
HPV-18	254	1169 (1021-1338)	256	1730 (1512-1980)	300	664 (586-752)	1.76 (1.41-2.19) ^b	0.68 (0.54-0.85)	2.61 (2.09-3.25)
HPV-6	253	2117 (1787-2508)	254	1876 (1585-2221)	300	943 (807-1101)	2.25 (1.71-2.96)	1.13 (0.85-1.50)	1.99 (1.51-2.62)
HPV-11	254	2339 (2088-2619)	256	2117 (1891-2370)	300	1268 (1143-1408)	1.84 (1.53-2.22)	1.10 (0.91-1.34)	1.67 (1.39-2.01)
Month 18									
HPV-16	100	1579 (1322-1885)	100	1806 (1512-2156)	104	840 (706-999)	1.88 (1.40-2.53)	0.87 (0.65-1.18)	2.15 (1.60-2.89)
HPV-18	100	137 (107-176)	100	238 (186-305)	104	77 (61-98)	1.78 (1.17-2.69)	0.58 (0.38-0.88)	3.08 (2.03-4.66)
HPV-6	100	346 (291-411)	100	351 (295-417)	104	203 (171-241)	1.70 (1.27-2.28)	0.99 (0.74-1.32)	1.73 (1.29-2.31)
HPV-11	100	451 (381-532)	100	429 (363-507)	104	286 (242-336)	1.58 (1.19-2.09)	1.05 (0.79-1.39)	1.50 (1.14-1.99)
Month 24									
HPV-16	201	1407 (1234-1606)	188	1726 (1506-1978)	230	844 (746-954)	1.67 (1.34-2.07)	0.82 (0.65-1.02)	2.05 (1.64-2.55)
HPV-18	201	131 (108-158)	188	264 (218-321)	230	96 (81-114)	1.36 (1.00-1.85)	0.49 (0.36-0.68)	2.75 (2.01-3.77)
HPV-6	201	278 (244-315)	188	357 (313-407)	230	217 (193-244)	1.28 (1.04-1.58)	0.78 (0.62-0.97)	1.65 (1.33-2.04)
HPV-11	201	370 (326-420)	188	423 (371-482)	230	272 (242-306)	1.36 (1.11-1.67)	0.87 (0.70-1.09)	1.56 (1.26-1.92)
Month 36									
HPV-16	86	1151 (919-1441)	85	1407 (1122-1764)	111	719 (590-876)	1.60 (1.12-2.29)	0.82 (0.56-1.20)	1.96 (1.37-2.80)
HPV-18	86	104 (76-141)	85	237 (174-322)	111	74 (57-97)	1.40 (0.86-2.29)	0.44 (0.26-0.74)	3.19 (1.95-5.21)
HPV-6	86	243 (199-296)	85	376 (308-460)	111	189 (159-225)	1.28 (0.94-1.76)	0.65 (0.46-0.90)	1.99 (1.45-2.74)
HPV-11	86	298 (245-363)	85	404 (332-493)	111	215 (181-255)	1.39 (1.01-1.90)	0.74 (0.53-1.03)	1.88 (1.37-2.58)

Abbreviations: GMT, geometric mean titer; HPV, human papillomavirus; mMU/mL, milli-Merck units per milliliter.

^aThis table excludes missing values from the recipients of the 2-dose and 3-dose vaccine.

^bResults corresponding to the primary objective.

DISCUSSION

Although effective and safe HPV vaccines to prevent cervical cancer are available, several key questions remain unanswered before global implementation of vaccine programs occur. In particular, more information is needed on the immunogenicity and efficacy of reduced-dose schedules, and the duration of immune responses after completion of a full or reduced-dose series. We have established that the immunogenicity of a 2-dose schedule at 0 and 6 months in girls 9 through 13 years of age is statistically noninferior for HPV-16 and HPV-18 to the immunogenicity in women receiving 3 doses, assessed 1 month after the final dose. The GMTs in girls receiving a 2-dose schedule were between 1.77- to 2.24-fold higher than those in women receiving a 3-dose schedule, assessed 1 month after the final dose, which is consistent with the bridging studies that led to the licensing of the 3-dose

vaccine for use in children as young as 9 years of age. We have determined that the majority of girls receiving 2 doses seroconvert by month 7, and although they decline, GMTs plateau at month 18, and remain detectable to month 36 and noninferior to women for the same time frame. These are the first data, to our knowledge, on the duration of the immune response of young adolescent girls to a reduced-dose schedule of quadrivalent HPV vaccine out to 3 years. These data will help to inform public health program planning.

Licensure of quadrivalent HPV vaccine for preadolescent and adolescent girls was based on immunogenicity-bridging studies that established better immune responses in girls than in women who participated in the efficacy trials.³ Bridging studies were required because there is no available correlate of protection, and efficacy studies requiring cervical cancer

screening in young girls are not feasible. The setting of the noninferior criterion relies on regulatory guidance and opinions about what is a clinically meaningful difference. The lower confidence bound of the 95% CI for the GMT ratios, girls receiving 2 doses and women receiving 3 doses of greater than 0.5 for all HPV types, is consistent with other prelicensure trials of quadrivalent HPV²⁻⁴ vaccine and with other vaccines for which no correlate of protection was identified.¹³ Our study shows that the noninferior immune response previously found in girls receiving 3 doses³ compared with women is also present with 2 doses of quadrivalent HPV vaccine given at 0 and 6 months. A study of a 2-dose schedule using the bivalent HPV vaccine showed noninferiority of immune responses in girls up to 24 months compared with women aged 15 to 25 years receiving 3 doses.¹⁴ The antibody responses of a

Table 3. Summary of Month 7,18, 24, and 36 Anti-Human Papillomavirus Competitive Immunoassay Geometric Mean Titers in the Per-Protocol Population

Antibodies	Girls, 9-13 y				Women, 16-26 y		GMT Ratio (95% CI), mMU/mL		
	2 Doses		3 Doses		3 Doses		Girls (2-Dose)/Women (3-Dose)	Girls (2-Dose)/Girls (3-Dose)	Girls (3-Dose)/Women (3-Dose)
	No. of Patients ^a	GMT (95% CI), mMU/mL	No. of Patients ^a	GMT (95% CI), mMU/mL	No. of Patients ^a	GMT (95% CI), mMU/mL			
Month 7									
HPV-16	243	7457 (6388-8704)	251	7640 (6561-8896)	246	3574 (3065-4169)	2.09 (1.61-2.71) ^b	0.98 (0.75-1.27)	2.14 (1.65-2.77)
HPV-18	243	1207 (1054-1384)	252	1703 (1489-1946)	264	661 (580-754)	1.83 (1.46-2.29) ^b	0.71 (0.56-0.89)	2.57 (2.06-3.22)
HPV-6	241	2186 (1846-2588)	248	1856 (1571-2192)	256	938 (796-1105)	2.33 (1.76-3.09)	1.18 (0.89-1.56)	1.98 (1.50-2.62)
HPV-11	243	2348 (2090-2638)	251	2096 (1869-2350)	269	1277 (1144-1427)	1.84 (1.52-2.23)	1.12 (0.92-1.36)	1.64 (1.36-1.98)
Month 18									
HPV-16	96	1598 (1333-1916)	98	1804 (1508-2160)	92	837 (695-1008)	1.91 (1.40-2.60)	0.89 (0.65-1.20)	2.16 (1.58-2.94)
HPV-18	96	137 (106-177)	99	236 (184-304)	95	74 (57-95)	1.86 (1.21-2.87)	0.58 (0.38-0.89)	3.21 (2.09-4.93)
HPV-6	96	347 (291-414)	97	351 (294-418)	93	200 (168-240)	1.73 (1.28-2.34)	0.99 (0.74-1.33)	1.75 (1.30-2.36)
HPV-11	96	451 (380-535)	99	424 (359-502)	98	281 (238-333)	1.60 (1.20-2.14)	1.06 (0.80-1.42)	1.51 (1.13-2.01)
Month 24									
HPV-16	195	1414 (1235-1618)	186	1739 (1514-1998)	189	813 (709-933)	1.74 (1.38-2.19)	0.81 (0.64-1.02)	2.14 (1.69-2.70)
HPV-18	195	132 (109-160)	187	267 (220-324)	202	91 (76-110)	1.44 (1.05-1.99)	0.49 (0.36-0.68)	2.92 (2.11-4.03)
HPV-6	193	276 (243-313)	186	359 (315-409)	195	197 (173-224)	1.40 (1.13-1.74)	0.77 (0.62-0.96)	1.82 (1.47-2.27)
HPV-11	195	368 (324-420)	186	422 (369-482)	206	267 (235-303)	1.38 (1.11-1.72)	0.87 (0.70-1.09)	1.58 (1.27-1.97)
Month 36									
HPV-16	86	1151 (918-1444)	83	1413 (1122-1780)	86	678 (540-850)	1.70 (1.16-2.49)	0.81 (0.55-1.20)	2.09 (1.42-3.07)
HPV-18	86	104 (77-141)	83	239 (175-327)	96	71 (53-95)	1.46 (0.88-2.41)	0.43 (0.26-0.73)	3.35 (2.02-5.58)
HPV-6	84	239 (195-292)	83	372 (304-456)	92	176 (145-213)	1.36 (0.97-1.90)	0.64 (0.46-0.90)	2.12 (1.51-2.96)
HPV-11	86	298 (244-364)	82	410 (335-503)	97	208 (172-251)	1.43 (1.03-1.99)	0.73 (0.52-1.02)	1.97 (1.42-2.75)

Abbreviations: GMT, geometric mean titer; HPV, human papillomavirus; mMU/mL, milli-Merck units per milliliter.
^aNumber of negative samples available for a specific HPV genotype at baseline. Per-protocol population criteria also required a negative HPV DNA vaginal swab result at baseline for the specific HPV genotype.
^bResults corresponding to the primary objective.

2-dose vaccine series shown in both the study of bivalent vaccine and our study of quadrivalent HPV vaccine are high. However, the noninferiority definition used for the prelicensure immunogenicity-bridging studies and for our study does not answer the question of efficacy because there were no clinical outcomes and no literature to guide interpretation of the titers. In addition, the noninferiority definition does not answer the question of the durability of these antibody responses, which can only be answered through long-term studies of effectiveness for regimens using either 2 or 3 doses of the HPV vaccine.

Though it was not the primary objective of our study, as part of a comprehensive evaluation comparing the response of girls receiving 2 doses with women receiving 3 doses, we also compared the incremental value of a third dose in girls. Although GMTs were higher in girls receiving 3 doses compared with girls receiving 2 doses, noninferiority was demonstrated for 2 of the genotypes, HPV-16 and HPV-11, out to 3 years. However, HPV-18 responses at month 24 and HPV-6 responses at month 36 were no longer noninferior after a 2-dose schedule compared with a 3-dose schedule in girls. For immunization program decision makers, deciding what constitutes a clinically meaningful difference in the immunogenicity between the girls receiving 2 or 3 doses is important in considering reduced dose schedules. So far, vaccine efficacy has been demonstrated out to 60 months in women aged 16 to 23 years, even when antibody level has waned, especially with respect to HPV-18.¹⁵ The vaccine is thought to provide protection through the production of serum neutralizing anti-HPV IgG antibodies to the basal stem cells of epithelial mucosa where they bind to viral particles^{3,14} and only small amounts of antibody need to be present.^{16,17} So few events have occurred in follow-up of the efficacy trials' participants that it has not been

possible to determine the antibody threshold associated with protection. The clinically meaningful difference between the 2- and 3-dose schedules for girls cannot yet be determined.

Three-dose schedules have been implemented across the world, mainly for preadolescent girls because maximal benefit is obtained if immunization is completed before the onset of sexual activity. The need for additional doses of the vaccine later in adult life is unknown. The results of our study suggest that advantage can be taken of the better immunogenicity afforded girls compared with young women by receiving at least an initial 2-dose schedule and leaving open the possibility of receiving a third dose later in adolescence. Smolen and coauthors¹⁸ explored B-cell memory responses in a subset of our cohort. They found no difference between the recipients of 2 and 3 doses but did find a significantly lower response in older recipients than with younger recipients. The impact of this age-dependent difference in B-cell memory formation, as well as the unknown effect on affinity maturation, on long-term protection is currently unknown and will require careful follow up. A fourth dose given at 60 months after the first dose in a 3-dose schedule resulted in significantly increased antibody, implying an anamnestic response.¹⁹ Protecting through early adolescence with 2 doses would allow for boosting in late adolescence to provide a high level of antibody through early adulthood. This is a cautious approach until effectiveness of reduced schedules can be demonstrated. Such a program has been introduced in the Canadian provinces of British Columbia²⁰ and Quebec,²¹ with program evaluation underway.

The immunogenicity outcome in our study is only an interim measure that allows continued exploration of the effectiveness of reduced schedules. In the absence of an immunological correlate of protection, an ideal study comparing 2- vs 3-dose

schedules would examine protection against disease as the primary outcome. The efficacy of this vaccine means that the sample size required to detect a clinically significant difference between recipients of 2 and 3 doses would need to consist of several thousand participants. The length of time from vaccination of girls to ascertainment of disease outcomes, given the natural history of HPV disease, is at least 5 to 10 years, with the important ethical constraints of conducting gynecologic assessments in young adolescents. A careful evaluation of the effectiveness of a reduced-dose schedule would be required, with persistent infection outcomes perhaps being more realistically obtainable, in the continuing absence of an immunological correlate of protection.²²

One limitation of our study is the potential differences in sensitivity of serological assays that test for HPV antibodies.²³ In a recent study, involving women who received quadrivalent HPV vaccine and did not have evidence of antibodies to HPV-18 at 48 months after the first dose as measured by competitive immunoassay, more than 95% demonstrated HPV-18 antibodies using a total IgG immunoassay, which targets a broader range of HPV genotype-specific antibodies and does not discriminate neutralizing and nonneutralizing epitopes.²⁴ The total IgG immunoassay may also detect neutralizing epitopes, which can be missed by the existing array of monoclonal antibodies in the competitive immunoassay. This helps to explain the continuing efficacy noted for HPV-18, even as HPV-18 seropositivity declines with time.¹⁵ Because we used the competitive immunoassay, it is possible that both seroconversion and titers of antibodies to both HPV-6 and HPV-18 were underestimated.²⁵ Further testing of the sera from our study using the total IgG immunoassay is warranted and underway. Other limitations are the definition of noninferiority and its unknown correlation with clinical relevance in public programs.

The number of doses and cost of HPV vaccines are barriers to global implementation, in both developed and developing nations. Reducing the number of doses affects vaccine and administration costs as well as potentially improving uptake rates.^{26,27} Evidence-based decision making in public health has led to reduced-dose schedules for hepatitis B, pneumococcal, and meningococcal serogroup C vaccine programs.²⁸⁻³⁰ There is a balance to be found between the incremental value of an additional dose on population effectiveness and the opportunity costs of using the resources required for the extra dose in other public health programs. This is especially the case for HPV vaccines at their present cost.

Author Affiliations: Department of Pediatrics (Drs Dobson, Scheifele, Kollman, and Bettinger), School of Population and Public Health (Drs Dawar, Ogilvie, Singer, and Naus), Department of Pathology and Laboratory Medicine (Dr Kraiden), Department of Obstetrics and Gynaecology (Drs Money and Miller), and Faculty of Pharmaceutical Sciences (Dr Marra), University of British Columbia, Vancouver, Canada; Vaccine Evaluation Center (Drs Dobson, Scheifele, Kollman, and Bettinger), Vancouver, Canada; Departments of Medicine (Dr McNeil) and Pediatrics (Drs Halperin and Langley), Canadian Center for Vaccinology, Dalhousie University, IWK Health Centre and Capital Health, Nova Scotia, Canada; Centre hospitalier universitaire de Québec, Canada (Dr Dionne and Dr Sauvageau); Vancouver Coastal Health Authority (Dr Dawar); British Columbia Center for Disease Control (Drs Ogilvie, Kraiden, Naus, and Marra); British Columbia Cancer Agency (Dr Miller); and British Columbia Ministry of Health, Vancouver, Canada (Dr Young).

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Study concept and design: Dobson, McNeil, Dionne, Dawar, Ogilvie, Sauvageau, Scheifele, Singer, Money, Miller, Naus, Marra, Young.

Acquisition of data: Dobson, McNeil, Dionne, Sauvageau, Scheifele, Kollman, Halperin, Langley. **Analysis and interpretation of data:** Dobson, McNeil, Dionne, Dawar, Ogilvie, Kraiden, Sauvageau, Scheifele, Langley, Bettinger, Singer, Money, Marra.

Drafting of the manuscript: Dobson, Dionne, Dawar, Ogilvie, Marra.

Critical revision of the manuscript for important intellectual content: All authors.

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Children learn at their own pace, and it is a mistake to try to force them. The great incentive to effort, all through life, is experience of success after initial difficulties. The difficulties must not be so great as to cause discouragement, or so small as not to stimulate effort. From birth to death, this is a fundamental principle. It is by what we do ourselves that we learn.

—Bertrand Russell (1872-1970)