Post Hoc Analysis of the PATRICIA Randomized Trial of the Efficacy of Human Papillomavirus Type 16 (HPV-16)/HPV-18 AS04-Adjuvanted Vaccine against Incident and Persistent Infection with Nonvaccine Oncogenic HPV Types Using an Alternative Multiplex Type-Specific PCR Assay for HPV DNA

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The efficacy of the human papillomavirus type 16 (HPV-16)/HPV-18 AS04-adjuvanted vaccine against cervical infections with HPV in the *Pa*pilloma *Tri*al against *C*ancer *in* Young *A*dults (PATRICIA) was evaluated using a combination of the broad-spectrum L1-based SPF$_{10}$ PCR-DNA enzyme immunoassay (DEIA)/line probe assay (LiPA$_{25}$) system with type-specific PCRs for HPV-16 and -18. Broad-spectrum PCR assays may underestimate the presence of HPV genotypes present at relatively low concentrations in multiple infections, due to competition between genotypes. Therefore, samples were retrospectively reanalyzed using a testing algorithm incorporating the SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ plus a novel E6-based multiplex type-specific PCR and reverse hybridization assay (MPTS12 RHA), which permits detection of a panel of nine oncogenic HPV genotypes (types 16, 18, 31, 33, 35, 45, 52, 58, and 59). For the vaccine against HPV types 16 and 18, there was no major impact on estimates of vaccine efficacy (VE) for incident or 6-month or 12-month persistent infections when the MPTS12 RHA was included in the testing algorithm versus estimates with the protocol-specified algorithm. However, the alternative testing algorithm showed greater sensitivity than the protocol-specified algorithm for detection of some nonvaccine oncogenic HPV types. More cases were gained in the control group than in the vaccine group, leading to higher point estimates of VE for 6-month and 12-month persistent infections for the nonvaccine oncogenic types included in the MPTS12 RHA assay (types 31, 33, 35, 45, 52, 58, and 59). This *post hoc* analysis indicates that the per-protocol testing algorithm used in PATRICIA underestimated the VE against some nonvaccine oncogenic HPV types and that the choice of the HPV DNA testing methodology is important for the evaluation of VE in clinical trials. (This study has been registered at ClinicalTrials.gov under registration no. NCT00122681.)

Persistent infection with oncogenic human papillomavirus (HPV) is a necessary prerequisite for the development of invasive cervical cancer (ICC) (1). HPV type 16 (HPV-16) and HPV-18 are found in approximately 70% of cases (2–5). Other common oncogenic HPV types causing ICC include types 31, 33, 35, 45, 52, and 58 (2–5). GlaxoSmithKline Vaccines has developed a prophylactic vaccine against HPV types 16 and 18, formulated with the AS04 adjuvant system. This vaccine has been shown to be

immunogenic and efficacious and to have a clinically acceptable safety profile (6–14).

In the large, randomized, double-blind, controlled *Pa*pilloma *Tri*al against *C*ancer *in* Young *A*dults (PATRICIA) (ClinicalTrials.gov registration no. NCT00122681), the HPV-16/18 AS04-adjuvanted vaccine prevented persistent infections and high-grade cervical lesions associated with HPV types 16 and/or 18 (11–13) and showed high efficacy against cervical intraepithelial

neoplasia grade 2 or greater (CIN2+) and CIN3+ irrespective of the HPV type [13] and cross-protection against some phylogenetically related nonvaccine oncogenic HPV types (i.e., types 31, 33, 45, and 51) [12, 14]. However, during both the event-triggered final analysis and the end-of-study analysis of PATRICIA, negative point estimates of vaccine efficacy (VE) were observed for other less common nonvaccine oncogenic HPV types [12, 14]. In particular, for 12-month persistent infections with HPV-58 in the end-of-study analysis, the associated upper and lower 95% confidence intervals (CIs) for VE were both less than zero [14], which might potentially indicate an increase in either the incidence or the duration of persistent infections with this genotype. On the other hand, estimates of VE against histopathological endpoints associated with HPV-58 were positive at the end-of-study analysis, which would not be expected if the vaccine had caused a true increase in the incidence or duration of persistent infection.

The present analysis investigated the hypothesis that the discrepant efficacy results for persistent infection versus lesional endpoints for some nonvaccine HPV types might be due to a technical issue in the PCR methodology used for HPV DNA detection [15]. The protocol-specified testing algorithm for PATRICIA included the broad-spectrum L1-based SPF_{10} PCR DNA immunoassay (DEIA) line probe assay ($LiPA_{25}$) system (here referred to as SPF_{10} PCR-DEIA/$LiPA_{25}$) and type-specific PCRs for HPV-16 and HPV-18 [16, 17] but did not include type-specific PCRs for nonvaccine HPV types. It is well recognized that broad-spectrum PCRs are affected by competition between different HPV genotypes present in the same sample and may underestimate the prevalence of genotypes present at low relative concentrations in multiple infections due to the differential efficiency of amplification [16], a phenomenon that has been described as masking [18]. In theory, due to the efficacy of the vaccine in preventing infection with HPV-16, HPV-18, and some nonvaccine oncogenic HPV types [14], such a scenario is more likely to arise in the control group than in the vaccine group, leading to a bias against the vaccine.

To increase the sensitivity of HPV DNA detection for a larger number of HPV genotypes, SPF_{10} PCR-DEIA-positive cervical

samples from PATRICIA were retrospectively reanalyzed using a testing algorithm that combined the results from the broad-spectrum SPF_{10} PCR-DEIA/$LiPA_{25}$ with a novel E6-based multiplex type-specific PCR and reverse hybridization assay (designated MPTS12 RHA), which permits detection of nine of the most common oncogenic HPV genotypes associated with ICC (HPV-16, -18, -31, -33, -35, -45, -52, -58, and -59) [19]. We report *post hoc* end-of-study (month 48) estimates of VE against incident and persistent infections using the alternative testing algorithm incorporating the MPTS12 RHA compared with those from the protocol-specified testing algorithm.

It is relevant to note that the purpose of this exploratory work was to investigate a potential technical issue in the protocol-specified testing algorithm used in PATRICIA, rather than to draw new conclusions regarding the efficacy of the HPV-16/18 AS04-adjuvanted vaccine in the prevention of ICC. The previously reported conclusions of PATRICIA, derived using the protocol-specified HPV DNA testing algorithm [11–14], are still valid.

MATERIALS AND METHODS

The trial methods of PATRICIA have been described in detail [11, 12], and the results of event-driven [12] and end-of-study [13, 14] analyses were reported previously. The data described here are from the end-of-study analysis after 48 months of follow-up. The protocol and other materials were approved by independent ethics committees or institutional review boards at each location. The study is registered with ClinicalTrials.gov, number NCT00122681.

Participants. Healthy women aged 15 to 25 years with no more than six lifetime sexual partners (this exclusion criterion was not applied in Finland, in accordance with local regulatory and ethical requirements) were included in the trial; the full inclusion and exclusion criteria, trial locations, and dates were described previously [11, 12]. Women were included regardless of their baseline HPV DNA status, HPV-16 or HPV-18 serostatus, or cytology. Written informed consent was obtained from all adult participants. For minors, written informed assent was obtained from the participants and from their parents.

Procedures. Women were randomized in a 1:1 ratio to receive either the HPV-16/18 AS04-adjuvanted vaccine (Cervarix; GlaxoSmithKline Vaccines) or control hepatitis A vaccine (GlaxoSmithKline Vaccines) at 0, 1, and 6 months in a double-blind manner. Cervical samples were obtained every 6 months for HPV DNA detection and typing. Specimens were stored in PreservCyt (Cytyc Corporation, Boxborough, MA) transport medium. HPV DNA was extracted using the MagNA Pure LC system (Roche Diagnostics, Almere, the Netherlands).

Protocol-specified algorithm for HPV DNA PCR testing. The protocol-specified HPV DNA testing algorithm combined the broad-spectrum SPF_{10} PCR-DEIA/$LiPA_{25}$ with the HPV-16 and HPV-18 type-specific (TS) PCR [16]. The SPF_{10} PCR-DEIA and $LiPA_{25}$ version 1 are produced by Labo Biomedical Products (Rijswijk, the Netherlands) and are based on licensed Innogenetics technology.

The SPF_{10} PCR primer set, which amplifies a 65-nucleotide region of the HPV L1 gene, was used to amplify a broad spectrum of HPV genotypes [20, 21]. After generic amplification, hybridization was done with a cocktail of nine conservative HPV probes using a DNA enzyme immunoassay (DEIA) recognizing at least 64 different HPV genotypes. SPF_{10} amplimers from HPV-positive clinical samples from PATRICIA were then genotyped using reverse hybridization on a line probe assay (LiPA) containing probes for 25 different HPV genotypes: 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74). If the SPF_{10} $LiPA_{25}$ detected no HPV-16, the specimen was tested by the HPV-16 TS PCR (a type-specific PCR which used primers that amplified a 92-nucleotide segment of the E6/E7 gene). Likewise, if the SPF_{10} $LiPA_{25}$ detected no HPV-18, the specimen was tested by the HPV-18 TS PCR (which used primers

that amplified a 126-nucleotide segment of the L1 gene). The cervical sample was considered positive for a particular HPV type if either the SPF_{10} PCR-DEIA/$LiPA_{25}$ or the HPV-16/18 TS PCR or both (the SPF_{10} PCR-DEIA/$LiPA_{25}$ plus HPV-16/18 TS-PCR) had a positive result for the HPV type.

Post hoc algorithm for HPV DNA PCR testing. Following completion of the PATRICIA end-of-study analysis, the cervical samples (but not the biopsy specimens) were reanalyzed using an HPV DNA PCR testing algorithm that included the validated MPTS12 RHA assay (19). The personnel involved in the retesting were blinded to the results of the protocol-specified HPV DNA PCR testing.

The first part of the *post hoc* algorithm was the same as that defined for the protocol-specified algorithm, i.e., SPF_{10} PCR-DEIA-positive samples were genotyped using a LiPA. In addition, the SPF_{10} PCR-DEIA-positive samples were genotyped using the MPTS12 PCR RHA system (Labo Biomedical Products, BV, Rijswijk, the Netherlands). The MPTS12 RHA detects and genotypes nine oncogenic HPV types (16, 18, 31, 33, 35, 45, 52, 58, and 59), even at very low copy numbers and in the presence of multiple infections in cervical liquid-based cytology samples, offering high sensitivity and specificity.

The MPTS12 RHA includes two sets of multiplex PCRs, one for HPV types 18, 31, 33, and 35 (MPTS1) and the other for types 16, 45, 52, 58, and 59 (MPTS2). In both the MPTS1 and MPTS2, each HPV type was specifically amplified by a pair of single reverse and single forward primers from the HPV E6 region. To analyze the PCR products generated by the two multiplex PCR mixes, the system used a strip-based reverse hybridization assay (RHA), hybridizing to 10 type-specific HPV probes which recognize the interprimer region of the MPTS1 and/or MPTS2 strand.

The cervical sample was considered positive for a particular HPV type if after SPF10 PCR-DEIA positivity either the SPF_{10} PCR-DEIA/$LiPA_{25}$ or the MPTS12 RHA or both (SPF_{10} PCR-DEIA/$LiPA_{25}$ plus MPTS12 RHA) had a positive result. For nonvaccine oncogenic HPV types, the testing algorithm, SPF10 PCR-DEIA/$LiPA_{25}$ plus MPTS12 RHA, has been shown to increase the sensitivity of detection for all SPF10 PCR-DEIA/$LiPA_{25}$ plus MPTS12 RHA-detectable genotypes, including HPV-58 and HPV-59, compared with that of either of the two tests alone (19).

Statistical analysis. To allow comparisons with previously published results from PATRICIA (12, 14), endpoints were evaluated in three cohorts: the according-to-protocol cohort for efficacy (ATP-E), the total vaccinated cohort (TVC), and the TVC-naive. Licensure of the vaccine was based on the analysis of the ATP-E to fully describe the vaccine's profile (12); therefore, the ATP-E is considered the primary cohort for the analyses described here. Supplementary analyses are presented for the TVC and TVC-naive for comparison with previous publications and because these cohorts are more relevant from a public health perspective. The ATP-E included women who were evaluable for efficacy (i.e., had a baseline PCR or cytology sample and one further sample available), met all of the eligibility criteria, complied with the protocol, received all three vaccine doses, and had negative or low-grade cytology at baseline. In the ATP-E, endpoints were assessed in women who were HPV DNA negative at months 0 and 6 for the HPV type analyzed. The TVC included all women who received at least one vaccine dose and were evaluable for efficacy. Endpoints were assessed in the TVC irrespective of a women's baseline HPV DNA, cytological status, or serostatus. The TVC-naive included women who had received at least one vaccine dose, who were evaluable for efficacy, and who at baseline were HPV DNA negative for the 14 oncogenic HPV types, were seronegative for HPV-16 and HPV-18, and had negative cytology.

We report VE against incident and 6-month and 12-month persistent infections for each of the nine oncogenic HPV types which can be genotyped using the MPTS12 RHA (i.e., HPV-16, -18, -31, -33, -35, -45, -52, -58, and -59). We also report VE for the composite endpoint of all 14 oncogenic HPV types which can be genotyped using the $LiPA_{25}$ (i.e., HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) and for the composite of nonvaccine oncogenic HPV types (i.e., HPV-31,

-33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68). Six-month persistent infection was defined as the detection of the same HPV type by PCR at two consecutive evaluations over approximately a 6-month interval. Twelve-month persistent infection was defined as the detection of the same HPV type by PCR at all available time points over approximately a 12-month interval. Results are reported for the protocol-specified HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ plus HPV-16/18 TS-PCR) and the alternative HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ plus MPTS12 RHA). It was not appropriate to statistically compare estimates of VE derived from the two testing algorithms, since the two estimates were based on data obtained from the same individuals and were not independent.

VE and 95% confidence intervals (CIs) were calculated using a conditional exact method (14). Results with the protocol-specified testing method were considered to confirm the statistically significant VE observed in the final event-driven analysis (12) if end-of-study estimates of VE and their 95% CIs were greater than zero. Event rates were calculated as the number of cases divided by the total follow-up in years and are expressed per 100 person-years. In the ATP-E, follow-up started the day after the third vaccine dose. In the TVC, follow-up started the day after the first vaccine dose. Follow-up for each outcome ended at the time the outcome occurred or at the last available sample (up to month 48).

The absolute change (Δ) in the number of cases of infection was calculated as the number of cases detected using the alternative testing algorithm minus the number detected using the protocol-specified testing algorithm. The relative difference in the number of cases (% Δ cases) was calculated as Δ divided by the number of cases detected using the protocol-specified testing algorithm × 100. The relative difference in terms of person-year rates is also reported (% Δ rates).

Statistical analyses were done with SAS version 9.2 and Proc StatXact-7 on Windows XP.

RESULTS

At the time of the PATRICIA end-of study analysis, a total of 18,644 women (vaccine $n = 9,319$, control $n = 9,325$) were included in the TVC, 16,114 women (vaccine $n = 8,067$, control $n = 8,047$) in the ATP-E, and 11,644 women (vaccine $n = 5,824$, control $n = 5,820$) in the TVC-naive (13, 14). The entire subset of SPF_{10} PCR-DEIA-positive samples (17,500 samples) from the end-of-study analysis were reanalyzed using the MPTS12 RHA.

Additional cases of 12-month persistent infection, 6-month persistent infection, and incident infection were detected in all cohorts when the MPTS12 RHA was included in the testing algorithm for the composite endpoints of any nonvaccine oncogenic HPV type and any oncogenic HPV type (Table 1). The absolute number of cases gained was largest in the TVC. For all cohorts, the relative difference between the two algorithms in the number of cases gained (% Δ cases) and in the rate of infection adjusted for duration of follow-up (% Δ rates) was larger for any nonvaccine oncogenic HPV type than for any oncogenic HPV type and was larger for persistent infections than for incident infections (Table 1).

In the ATP-E, more cases were gained in the control group than in the vaccine group for each of the composite endpoints, e.g., for any nonvaccine oncogenic HPV type, the relative increases in the numbers of cases (%Δ cases) in the control and vaccine groups, respectively, were 8.4 versus 4.5 for 12-month persistence, 4.4 versus 1.9 for 6-month persistence, and 2.2 versus 1.7 for incident infection (Table 1). In the TVC and TVC-naive, the differential between the control and vaccine groups in terms of the relative increase in the number of cases of any nonvaccine oncogenic HPV type was not as large as it was in the ATP-E, and for any oncogenic HPV type (including types 16 and 18), the rel-

TABLE 1 Vaccine efficacy against persistent and incident infections with any oncogenic HPV type and with any nonvaccine oncogenic HPV type (ATP-E, TVC and TVC-naive)[a]

Cohort and type of infection	Composite endpoint	Group	Results for protocol-specified HPV DNA testing algorithm (SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ + HPV-16/18 TS-PCR):					Results for alternative HPV DNA testing algorithm (SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ + MPTS12 RHA):					Results for additional cases (alternative algorithm − protocol-specified algorithm):		
			No.	Cases	Rate	VE (95% CI)	P value	No.	Cases	Rate	VE (95% CI)	P value	Δ cases	% Δ cases	% Δ rates
ATP-E[b]															
12-month PI	Nonvaccine HR-HPV	Vaccine	7,560	804	3.36	9.0 (−0.2 to 17.4)	0.0524	7,560	840	3.53	12.7 (4.1 to 20.5)	0.0045	+36	4.5	4.9
		Control	7,545	878	3.70			7,545	952	4.04			+74	8.4	9.2
	HR-HPV	Vaccine	7,560	824	3.45	27.8 (20.9 to 34.1)	<0.0001	7,560	858	3.61	28.2 (21.5 to 34.3)	<0.0001	+34	4.1	4.5
		Control	7,545	1,112	4.78			7,545	1,162	5.02			+50	4.5	5.1
6-month PI	Nonvaccine HR-HPV	Vaccine	7,672	1,399	6.13	11.2 (4.5 to 17.5)	0.0011	7,672	1,426	6.27	13.6 (7.2 to 19.6)	<0.0001	+27	1.9	2.2
		Control	7,656	1,556	6.91			7,656	1,624	7.26			+68	4.4	5.1
	HR-HPV	Vaccine	7,672	1,424	6.26	25.4 (20.0 to 30.4)	<0.0001	7,672	1,450	6.39	25.6 (20.3 to 30.6)	<0.0001	+26	1.8	2.1
		Control	7,656	1,837	8.39			7,656	1,874	8.59			+37	2.0	2.4
Incident	Nonvaccine HR-HPV	Vaccine	7,853	2,965	14.35	10.8 (6.2 to 15.1)	<0.0001	7,853	3,014	14.67	11.5 (7.0 to 15.8)	<0.0001	+49	1.7	2.2
		Control	7,852	3,262	16.09			7,852	3,334	16.58			+72	2.2	3.0
	HR-HPV	Vaccine	7,853	3,098	15.18	16.4 (12.2 to 20.3)	<0.0001	7,853	3,143	15.48	16.3 (12.1 to 20.2)	<0.0001	+45	1.5	1.9
		Control	7,852	3,560	18.16			7,852	3,608	18.48			+48	1.3	1.8
TVC[c]															
12-month PI	Nonvaccine HR-HPV	Vaccine	8,648	1,454	5.08	8.7 (1.9, 15.0)	0.0164	8,648	1,544	5.45	10.2 (3.7, 16.2)	0.0033	+90	6.2	7.4
		Control	8,671	1,579	5.56			8,671	1,700	6.07			+121	7.7	9.2
	HR-HPV	Vaccine	8,648	1,640	5.88	20.3 (14.8, 25.4)	<0.0001	8,648	1,720	6.22	19.6 (14.3, 24.7)	<0.0001	+80	4.9	5.9
		Control	8,671	2,001	7.37			8,671	2,081	7.74			+80	4.0	5.1
6-month PI	Nonvaccine HR-HPV	Vaccine	8,863	2,326	8.83	10.1 (4.9, 15.1)	0.0002	8,863	2,408	9.26	11.1 (6.1, 15.9)	<0.0001	+82	3.5	4.8
		Control	8,870	2,547	9.83			8,870	2,660	10.42			+113	4.4	6.0
	HR-HPV	Vaccine	8,863	2,532	9.93	18.7 (14.2, 22.9)	<0.0001	8,863	2,605	10.32	18.0 (13.5, 22.2)	<0.0001	+73	2.9	4.0
		Control	8,870	2,994	12.21			8,870	3,057	12.58			+63	2.1	3.0
Incident	Nonvaccine HR-HPV	Vaccine	9,316	4,299	19.40	8.3 (4.4, 12.0)	<0.0001	9,316	4,380	20.01	8.6 (4.7, 12.3)	<0.0001	+81	1.9	3.2
		Control	9,319	4,584	21.15			9,319	4,671	21.89			+87	1.9	3.5
	HR-HPV	Vaccine	9,316	4,559	21.53	12.2 (8.6, 15.6)	<0.0001	9,316	4,615	22.00	12.0 (8.4, 15.5)	<0.0001	+56	1.2	2.2
		Control	9,319	4,996	24.51			9,319	5,050	25.01			+54	1.1	2.0
TVC-naive[d]															
12-month PI	Nonvaccine HR-HPV	Vaccine	5,362	521	2.68	17.4 (7.1, 26.6)	0.0015	5,345	552	2.86	18.3 (8.4, 27.2)	0.0006	+31	6.0	6.7
		Control	5,329	619	3.24			5,299	660	3.49			+41	6.6	7.8
	HR-HPV	Vaccine	5,362	540	2.78	35.8 (28.3, 42.5)	<0.0001	5,345	568	2.95	35.1 (27.7, 41.8)	<0.0001	+28	5.2	5.9
		Control	5,329	809	4.33			5,299	839	4.54			+30	3.7	4.8

	Group	No.	Cases	Rate	VE (95% CI)	P value	No.	Cases	Rate	VE (95% CI)	P value	Δ cases	% Δ cases	% Δ rates
6-month PI														
Nonvaccine HR-HPV	Vaccine	5,427	907	4.84	19.0 (11.5, 25.9)	<0.0001	5,410	934	5.02	19.5 (12.2, 26.3)	<0.0001	+27	3.0	3.7
	Control	5,399	1,087	5.98			5,369	1,122	6.24			+35	3.2	4.4
HR-HPV	Vaccine	5,427	925	4.95	33.1 (27.1, 38.5)	<0.0001	5,410	951	5.13	31.8 (25.8, 37.3)	<0.0001	+26	2.8	3.5
	Control	5,399	1,307	7.40			5,369	1,317	7.52			+10	0.8	1.6
Incident														
Nonvaccine HR-HPV	Vaccine	5,568	1,940	11.30	13.4 (7.9, 18.6)	<0.0001	5,549	1,962	11.52	13.9 (8.4, 19.0)	<0.0001	+22	1.1	1.9
	Control	5,559	2,173	13.04			5,526	2,202	13.37			+29	1.3	2.5
HR-HPV	Vaccine	5,568	2,020	11.88	19.9 (15.0, 24.5)	<0.0001	5,549	2,038	12.08	19.8 (14.9, 24.4)	<0.0001	+18	0.9	1.6
	Control	5,559	2,396	14.83			5,526	2,409	15.05			+13	0.5	1.5

[a] No., number of evaluable women in each group; PI, persistent infection; Cases, number of women reporting at least one event in each group; Rate, number of cases divided by the sum of the follow-up period (per 100 woman years) (follow-up started on the day after the third vaccine dose for the ATP-E and on the day after the first vaccine dose for the TVC and TVC-naive); VE (%), vaccine efficacy (conditional exact method); Nonvaccine HR-HPV, any nonvaccine high-risk (oncogenic) HPV type (HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68); HR-HPV, any high-risk (oncogenic) HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68); Δ cases, absolute difference of number of cases = number of cases determined by the alternative algorithm − number of cases determined by the protocol-specified algorithm; % Δ cases, relative difference of number of cases = Δ × 100/(number of cases determined by the protocol-specified algorithm); % Δ rates, relative difference of rates = (rate determined by the alternative algorithm − rate determined by the protocol-specified algorithm) × 100/(rate determined by the protocol-specified algorithm).
[b] The ATP-E analysis was conducted for women who were HPV DNA negative for the corresponding HPV type at month 0.
[c] The TVC analysis was conducted for all women, irrespective of their baseline HPV DNA status.
[d] The TVC-naive analysis was conducted for women who were DNA negative for all 14 high-risk HPV types and seronegative for HPV-16 and HPV-18 and had negative cytology at month 0.

ative increase in the number of cases tended to be larger in the vaccine group than in the control group (Table 1).

In the ATP-E for vaccine HPV types, a few cases were gained (HPV-18) or lost (HPV-16) with inclusion of the MPTS12 RHA versus the HPV-16/18 TS PCR in the testing algorithm, without a major impact on the estimates of VE for 12-month persistent infection (Table 2), 6-month persistent infection (Table 3), or incident infection (Table 4). In the ATP-E for individual nonvaccine oncogenic HPV types included in the MPTS12 RHA, the relative difference in the number of cases (% Δ cases) of 12-month or 6-month persistent infections was higher in the control group than in the vaccine group for all genotypes, leading to higher point estimates of VE for these nonvaccine HPV types (Tables 2 and 3, respectively). The gain in cases of persistent infection detected by the alternative algorithm incorporating the MPTS12 RHA was particularly large for HPV-45, HPV-58, and HPV-59. For incident infection, the relative change in the number of cases detected was generally similar between the control and vaccine groups, except for HPV-45 and HPV-58; therefore, no major impact on the estimates of VE against incident infection was observed for most of the individual nonvaccine HPV types when the MPTS12 RHA was included in the HPV DNA testing algorithm (Table 4). Differentials between vaccine and control groups in terms of relative increases in the number of cases of individual nonvaccine HPV types tended to be less marked in the TVC (see Tables S1 to S3 in the supplemental material) and in the TVC-naive (see Tables S4 to S6 in the supplemental material) than in the ATP-E.

The reanalysis of samples from PATRICIA was driven by the negative estimate of VE observed for 12-month persistent infection with HPV-58 using the protocol-specified testing algorithm, for which the associated 95% CI did not include zero. On a comparison of the prespecified testing algorithm versus the alternative algorithm, the estimate for VE against 12-month persistent infection with HPV-58 in the ATP-E increased from −54.1% (−121.3 to −8.1) to −2.9% (−35.4 to 21.7), with an additional 28 and 54 cases detected in the vaccine and control groups, respectively (Table 2). The estimate for VE against 6-month persistent infection with HPV-58 increased from −18.3% (−51.8 to 7.7) to 8.2% (−12.9 to 25.4), with an additional 37 and 75 cases detected in the vaccine and control groups, respectively (Table 3).

Significant VE was already observed against persistent infections with HPV-31 and HPV-45 when the protocol-specified testing algorithm was used (Tables 2 and 3). For HPV-31, the relative differences in the number of cases in the ATP-E using the alternative testing algorithm in the control and vaccine groups were 11.0% versus 6.7% for 12-month persistent infection and 8.9% versus −6.9% for 6-month persistent infection, respectively, and there was no major impact on VE. For HPV-45, the relative difference in the number of cases was much larger in the control group than in the vaccine group (68.8% versus −7.7% for 12-month persistent infection; 38.9% versus 0% for 6-month persistent infection), resulting in an increase in VE.

DISCUSSION

Detection and identification of HPV genotypes in cervical samples and biopsy specimens are dependent on the accuracy and precision of the methods used. As vaccine trials use surrogate virological endpoints such as incident and persistent infection, in addition to histological endpoints, to predict vaccine efficacy, it is important that testing methods be reliable and robust. In this

TABLE 2 Vaccine efficacy against 12-month persistent infection in HPV DNA-negative subjects at months 0 and 6 (ATP-E)[a]

HPV type	Group	Results for protocol-specified HPV DNA testing algorithm (SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ + HPV-16/18 TS-PCR): No.	Cases	Rate	VE (95% CI)	P value	Results for alternative HPV DNA testing algorithm (SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ + MPTS12 RHA): No.	Cases	Rate	VE (95% CI)	P value	Results for additional cases (alternative algorithm − protocol-specified algorithm): Δ cases	% Δ cases	% Δ (rates)
HPV-16	Vaccine	7,103	20	0.08	93.6 (89.9 to 96.1)	<0.0001	7,106	18	0.08	93.9 (90.2 to 96.4)	<0.0001	−2	−10.0	−10.1
	Control	6,996	300	1.30			6,994	284	1.23			−16	−5.3	−5.4
HPV-18	Vaccine	7,356	8	0.03	92.4 (84.4 to 96.8)	<0.0001	7,346	12	0.05	89.1 (80.1 to 94.5)	<0.0001	+4	50.0	50.3
	Control	7,288	103	0.42			7,284	108	0.44			+5	4.9	4.9
HPV-31	Vaccine	7,295	30	0.12	78.1 (67.2 to 85.7)	<0.0001	7,277	32	0.13	79.0 (69.1 to 86.1)	<0.0001	+2	6.7	7.0
	Control	7,309	136	0.56			7,279	151	0.62			+15	11.0	11.7
HPV-33	Vaccine	7,426	38	0.15	35.8 (1.9 to 58.5)	0.0326	7,410	47	0.19	40.8 (14.0 to 59.6)	0.0041	+9	23.7	24.1
	Control	7,404	59	0.24			7,385	79	0.32			+20	33.9	34.5
HPV-35	Vaccine	7,468	37	0.15	−42.6 (−145.2 to 16.0)	0.2064	7,463	38	0.15	−41.0 (−140.2 to 16.1)	0.2135	+1	2.7	2.8
	Control	7,462	26	0.10			7,459	27	0.11			+1	3.8	3.9
HPV-45	Vaccine	7,485	13	0.05	59.6 (20.8 to 80.5)	0.0044	7,446	12	0.05	78.0 (58.3 to 89.3)	<0.0001	−1	−7.7	−7.2
	Control	7,445	32	0.13			7,400	54	0.22			+22	68.8	70.0
HPV-52	Vaccine	7,185	200	0.84	3.0 (−18.5 to 20.6)	0.7624	7,146	221	0.93	6.1 (−13.4 to 22.2)	0.5049	+21	10.5	11.3
	Control	7,134	205	0.87			7,102	234	0.99			+29	14.1	15.0
HPV-58	Vaccine	7,411	83	0.33	−54.1 (−121.3 to −8.1)	0.0159	7,335	111	0.45	−2.9 (−35.4 to 21.7)	0.8917	+28	33.7	35.5
	Control	7,403	54	0.22			7,335	108	0.44			+54	100.0	102.8
HPV-59	Vaccine	7,425	27	0.11	−35.2 (−154.3 to 26.9)	0.3809	7,338	58	0.24	−29.2 (−95.1 to 14.0)	0.2,353	+31	114.8	118.0
	Control	7,422	20	0.08			7,333	45	0.18			+25	125.0	128.2

[a] No., number of evaluable women in each group; Cases, number of women reporting at least one event in each group; Rate, number of cases divided by the sum of the follow-up period (per 100 woman years) (the follow-up period started on the day after the third vaccine dose); VE (%), vaccine efficacy (conditional exact method). Women had to be DNA negative for the corresponding HPV type at months 0 and 6. Δ cases, absolute difference of number of cases = number of cases determined by the alternative algorithm − number of cases determined by the protocol-specified algorithm; % Δ cases, relative difference of number of cases = Δ × 100/(number of cases determined by the protocol-specified algorithm); % Δ rates, relative difference of rates = (rate determined by the alternative algorithm − rate determined by the protocol-specified algorithm) × 100/(rate determined by the protocol-specified algorithm).

exploratory *post hoc* analysis, we retested cervical samples from PATRICIA, incorporating a recently developed E6-based multiplex type-specific PCR and reverse hybridization assay (designated MPTS12 RHA) in the HPV DNA testing algorithm (19). We showed that for some nonvaccine oncogenic HPV types, a large number of additional cases of persistent infection were detected when the MPTS12 RHA was utilized compared with the number detected with the protocol-specified testing algorithm. In the ATP-E, the relative increase in additional cases was larger in the control group than in the vaccine group, resulting in an increase in VE for persistent infection with some of these HPV types (e.g., HPV-45 and HPV-58 and to a lesser extent HPV-59 and HPV-33).

The reevaluation of virological endpoints in PATRICIA, using the recently developed MPTS12 RHA (19), was initiated in response to the finding of negative VE against persistent infection for some nonvaccine oncogenic HPV types, e.g., HPV-58 (14). One possible explanation was a potential bias against the vaccine due to technicalities in the PCR methodology used for HPV DNA testing (15). The broad-spectrum L1-based SPF$_{10}$ PCR-DEIA system has 10 primers that target relatively well-conserved genomic sequences, permitting the simultaneous amplification of at least 64 HPV genotypes in a single test. However, competition can oc-

cur between multiple HPV types present in the same sample, and genotypes present at low concentrations can remain undetected as they are outcompeted by genotypes present at high concentrations (16).

Mixed infections with more than one HPV type were commonly observed in PATRICIA and were more common in the control group than in the vaccine group because of the efficacy of the vaccine in preventing infections with HPV-16, HPV-18, and some other oncogenic HPV types (14). Genotyping of SPF$_{10}$ PCR-DEIA-positive samples using the E6-based MPTS12 RHA is known to be less prone to loss of sensitivity in the presence of multiple infections due to the use of individual primer sets specific for each of the nine genotypes (types 16, 18, 31, 33, 35, 45, 52, 58, and 59) included in the assay. In a previous evaluation, the combination of the SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ plus MPTS12 RHA systems resulted in a 14.3% increase in the detection of HPV genotypes compared with those detected by the SPF$_{10}$ PCR-DEIA/LiPA$_{25}$ system alone (19). Additionally, during progression from a low-grade lesion to a high-grade lesion or ICC, the HPV genome can integrate into the host DNA, and portions of the L1 and other HPV genes may be lost, whereas E6/E7 is retained (22). Thus, testing methods based on both early (E) and late (L) genes such

TABLE 3 Vaccine efficacy against 6-month persistent infection in HPV DNA-negative subjects at months 0 and 6 (ATP-E)[a]

HPV type	Group	No.	Cases	Rate	VE (95% CI)	P value	No.	Cases	Rate	VE (95% CI)	P value	Δ cases	% Δ cases	% Δ (rates)
		Results for protocol-specified HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ + HPV-16/18 TS-PCR):					Results for alternative HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ + MPTS12 RHA):					Results for additional cases (alternative algorithm − protocol-specified algorithm):		
PV-16	Vaccine	7,202	31	0.13	93.8 (91.0 to 95.8)	<0.0001	7,204	30	0.12	93.7 (90.9 to 95.8)	<0.0001	−1	−3.2	−3.3
	Control	7,099	473	2.07			7,097	456	1.99			−17	−3.6	−3.7
HPV-18	Vaccine	7,465	14	0.06	94.0 (89.7 to 96.8)	<0.0001	7,455	17	0.07	92.9 (88.4 to 95.9)	<0.0001	+3	21.4	21.7
	Control	7,394	227	0.93			7,390	234	0.96			+7	3.1	3.2
HPV-31	Vaccine	7,400	58	0.24	76.8 (69.0 to 82.9)	<0.0001	7,382	54	0.22	80.2 (73.4 to 85.5)	<0.0001	−4	−6.9	−6.7
	Control	7,414	247	1.01			7,383	269	1.11			+22	8.9	9.6
HPV-33	Vaccine	7,534	65	0.26	44.8 (24.6 to 59.9)	<0.0001	7,518	80	0.32	50.7 (35.2 to 62.8)	<0.0001	+15	23.1	23.5
	Control	7,513	117	0.47			7,494	161	0.65			+44	37.6	38.5
HPV-35	Vaccine	7,579	67	0.27	−19.8 (−74.1 to 17.2)	0.3654	7,573	68	0.27	−9.9 (−57.6 to 23.3)	0.6598	+1	1.5	1.6
	Control	7,569	56	0.22			7,565	62	0.25			+6	10.7	10.8
HPV-45	Vaccine	7,594	24	0.09	73.6 (58.1 to 83.9)	<0.0001	7,554	24	0.10	81.0 (70.5 to 88.3)	<0.0001	0	0.0	0.5
	Control	7,556	90	0.36			7,509	125	0.50			+35	38.9	40.1
HPV-52	Vaccine	7,289	346	1.46	8.3 (−6.5 to 21.0)	0.2515	7,250	364	1.55	12.9 (−0.6 to 24.5)	0.0553	+18	5.2	6.0
	Control	7,237	374	1.59			7,203	414	1.78			+40	10.7	11.6
HPV-58	Vaccine	7,518	144	0.58	−18.3 (−51.8 to 7.7)	0.1938	7,441	181	0.74	8.2 (−12.9 to 25.4)	0.4345	+37	25.7	27.4
	Control	7,511	122	0.49			7,442	197	0.80			+75	61.5	64.1
HPV-59	Vaccine	7,536	73	0.29	−7.5 (−51.8 to 23.8)	0.7352	7,446	135	0.55	2.1 (−25.1 to 23.3)	0.8548	+62	84.9	88.1
	Control	7,530	68	0.27			7,440	138	0.56			+70	102.9	106.5

[a] No., number of evaluable women in each group; Cases, number of women reporting at least one event in each group; Rate, number of cases divided by the sum of the follow-up period (per 100 woman years) (the follow-up period started on the day after the third vaccine dose); VE (%), vaccine efficacy (conditional exact method). Women had to be DNA negative for the corresponding HPV type at months 0 and 6. Δ cases, absolute difference of number of cases = number of cases determined by the alternative algorithm − number of cases determined by the protocol-specified algorithm; % Δ cases, relative difference of number of cases = Δ × 100/(number of cases determined by the protocol-specified algorithm); % Δ rates, relative difference of rates = (rate determined by the alternative algorithm − rate determined by the protocol-specified algorithm) × 100/(rate determined by the protocol-specified algorithm).

as the L1-based PCR-DEIA/$LiPA_{25}$ plus E6-based MPTS12 RHA can potentially detect HPV when deletions in the HPV genome have been introduced as a result of integration into cellular DNA (22).

When the alternative HPV DNA testing algorithm incorporating the MPTS12 RHA was used, there was no major impact on the estimates of VE for virological endpoints associated with HPV-16 and HPV-18. This was expected, as the MPTS12 RHA showed similar sensitivities to HPV-16 and HPV-18 TS PCRs during assay validation (19). Only a few cases of HPV-16 or HPV-18 were gained or lost using the alternative testing algorithm, and the high VE observed against vaccine HPV types using the protocol-specified algorithm was not affected by these subtle technical issues. It is not known if the cases which were gained or lost had low copy numbers, but aliquot effects alone might explain these small differences in detection rates. The nonvaccine oncogenic HPV types with lower observed estimates of VE might be expected to be affected to a greater extent by technical diagnostic issues. Indeed, using the alternative testing algorithm, the proportion of additional cases of persistent infection with nonvaccine oncogenic HPV types was higher in the control group than in the vaccine group, leading to higher estimates of VE for all nonvaccine geno-

types included in the MPTS12 RHA. It should be noted that no adjustment was made for multiple comparisons, and small increases in VE might arise by chance. However, the large differences observed for some genotypes (e.g., HPV-45 and HPV-58) are unlikely to be due to chance.

The differential detection of nonvaccine oncogenic HPV genotypes in the control group versus the vaccine group in the ATP-E using the alternative HPV DNA testing algorithm was greater for some genotypes (e.g., HPV-45 and HPV-58) than for others (e.g., HPV-31). This may reflect differences in viral concentration ("load") for genotypes such as HPV-45 or HPV-58, which may be present in lower concentrations than HPV-31. The HPV types at lower concentrations in a mixed infection would be less likely to be detected by the SPF_{10} PCR-DEIA/$LiPA_{25}$ (high proportion of false negatives) but would be detected using the more sensitive MPTS12 RHA. In contrast, the HPV types at higher concentrations in a mixed infection may still be detected by the SPF_{10} PCR-DEIA/$LiPA_{25}$ (low proportion of false negatives). Therefore, the addition of the MPTS12 RHA to the testing algorithm would have a lesser impact. We do not have viral load data and thus cannot test this hypothesis. Alternatively, the competition effects might be greater for some HPV types due to the extent of similarity between

TABLE 4 Vaccine efficacy against incident infection in HPV DNA-negative subjects at months 0 and 6 (ATP-E)[a]

HPV type	Group	\multicolumn{5}{l}{Results for protocol-specified HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ + HPV-16/18 TS-PCR):}					\multicolumn{5}{l}{Results for alternative HPV DNA testing algorithm (SPF_{10} PCR-DEIA/$LiPA_{25}$ + MPTS12 RHA):}					\multicolumn{3}{l}{Results for additional cases (alternative algorithm − protocol-specified algorithm):}		
		No.	Cases	Rate	VE (95% CI)	P value	No.	Cases	Rate	VE (95% CI)	P value	Δ cases	% Δ cases	% Δ (rates)
HPV-16	Vaccine	7,361	170	0.71	82.9 (79.9 to 85.6)	<0.0001	7,365	172	0.72	82.3 (79.2 to 85.1)	<0.0001	+2	1.2	1.1
	Control	7,273	936	4.17			7,272	915	4.07			−21	−2.2	−2.3
HPV-18	Vaccine	7,637	182	0.73	74.1 (69.4 to 78.1)	<0.0001	7,625	169	0.68	75.5 (70.9 to 79.4)	<0.0001	−13	−7.1	−7.1
	Control	7,583	676	2.83			7,579	664	2.78			−12	−1.8	−1.8
HPV-31	Vaccine	7,574	204	0.83	68.9 (63.6 to 73.6)	<0.0001	7,553	216	0.88	68.8 (63.5 to 73.3)	<0.0001	+12	5.9	6.2
	Control	7,598	642	2.67			7,567	674	2.82			+32	5.0	5.7
HPV-33	Vaccine	7,711	173	0.69	50.5 (40.4 to 59.0)	<0.0001	7,695	201	0.80	48.4 (38.7 to 56.7)	<0.0001	+28	16.2	16.6
	Control	7,706	346	1.39			7,687	385	1.56			+39	11.3	11.9
HPV-35	Vaccine	7,758	166	0.66	26.2 (9.4 to 40.0)	0.0029	7,752	179	0.71	23.1 (6.2 to 37.1)	0.0081	+13	7.8	8.0
	Control	7,763	225	0.89			7,758	233	0.93			+8	3.6	3.7
HPV-45	Vaccine	7,772	73	0.29	76.0 (68.9 to 81.7)	<0.0001	7,727	115	0.46	69.5 (62.3 to 75.5)	<0.0001	+42	57.5	58.8
	Control	7,744	300	1.20			7,692	370	1.49			+70	23.3	24.7
HPV-52	Vaccine	7,453	825	3.55	13.4 (4.9 to 21.2)	0.0017	7,414	874	3.80	13.5 (5.2 to 21.1)	0.0011	+49	5.9	7.0
	Control	7,415	945	4.10			7,381	1,002	4.39			+57	6.0	7.0
HPV-58	Vaccine	7,699	322	1.30	11.2 (−3.5 to 23.8)	0.1271	7,617	409	1.68	18.8 (7.3 to 28.9)	0.0017	+87	27.0	29.0
	Control	7,702	362	1.46			7,632	502	2.06			+140	38.7	41.1
HPV-59	Vaccine	7,711	338	1.36	7.4 (−7.7 to 20.4)	0.3156	7,618	491	2.02	9.2 (−2.8 to 19.8)	0.1143	+153	45.3	48.7
	Control	7,722	365	1.47			7,631	541	2.23			+176	48.2	51.6

[a] No., number of evaluable women in each group; Cases, number of women reporting at least one event in each group; Rate, number of cases divided by the sum of the follow-up period (per 100 woman years) (the follow-up period started on the day after the third vaccine dose); VE (%), vaccine efficacy (conditional exact method). Women had to be DNA negative for the corresponding HPV type at months 0 and 6. Δ cases, absolute difference of number of cases = number of cases determined by the alternative algorithm − number of cases determined by the protocol-specified algorithm; % Δ cases, relative difference of number of cases = Δ × 100/(number of cases determined by the protocol-specified algorithm); % Δ rates, relative difference of rates = (rate determined by the alternative algorithm − rate determined by the protocol-specified algorithm) × 100/(rate determined by the protocol-specified algorithm).

the DNA sequences of different HPV types detected by the SPF_{10} PCR primer set.

The increase in the absolute number of cases detected using the new testing algorithm was larger in the TVC than in the ATP-E because case counting started after dose 1 instead of dose 3 and also because there were fewer conditions that had to be fulfilled for a subject to be included in the TVC. The TVC includes all women for whom efficacy data were available, regardless of their baseline HPV or cytological status, while women in the ATP-E had to be HPV DNA negative for the corresponding HPV type at months 0 and 6 to have complied with the protocol and have normal or low-grade cytology at month 0. Thus, compared to the ATP-E, the TVC also includes cases resulting from prevalent infections at baseline. Since prevalent infections at baseline can be assumed to be roughly equally distributed between the two groups and the vaccine does not have a therapeutic effect, we expected to observe relatively more cases in the vaccine group than in the control group when comparing the TVC versus ATP-E, and this was indeed the case.

The addition of the MPTS12 RHA to the testing algorithm appears to have a greater impact on the rate of infection and associated VE for persistent infections than for incident infections.

This is because when multiple independent samples from the same subject are tested over time (with samples being collected at approximately 6-month intervals), there is an increasing chance that one of the samples will be a coinfection in which a vaccine-preventable HPV type is masked when tested using the protocol-specified algorithm. For example, if the HPV type was masked in one of two consecutive samples, the case would not meet the definition for persistent infection but would still meet the definition for incident infection. When the testing algorithm incorporating type-specific MPTS12 RHA is used, the HPV type would be detected in both samples and counted as an additional case of persistent infection.

We recognize that there are several other factors that may have contributed to the negative estimates of VE observed in PATRICIA, including a chance finding, an artifact of the colposcopy referral algorithm biasing against the vaccine, or HPV type replacement. We conducted additional exploratory statistical analyses to evaluate the likelihood of a chance finding (by adjusting for multiplicity) or bias due to the colposcopy referral algorithm (by conducting sensitivity analyses) and concluded that these factors were unlikely to explain the negative estimates of VE. While there is a theoretical concern that on-

cogenic HPV types not targeted by the vaccine may eventually take over the niche vacated by the eradication of vaccine types, type replacement has not been found in the sizeable PATRICIA trial or elsewhere, although only low-vaccine-coverage cohorts have been analyzed to date (15, 23). Thus, at present a diagnostic artifact is the most plausible explanation.

It is relevant to note that the HPV types detected by the broad-spectrum SPF_{10} PCR-DEIA/$LiPA_{25}$ system (14 oncogenic and 11 nononcogenic HPV types) but not covered by the highly sensitive MPTS12 RHA (9 oncogenic HPV types) would still be detected by the combination of assays included in the alternative testing algorithm if the copy numbers were high enough. As our aim was to interrogate differences between the protocol-specified and alternative algorithms, we have not reported results for the individual oncogenic HPV types not covered by the MPTS12 RHA (i.e., types 51, 56, 66, and 68), although data for these genotypes are included in composite endpoints for combined oncogenic HPV types.

In summary, we believe that the negative estimates of VE against persistent infection with some nonvaccine oncogenic HPV types in PATRICIA (e.g., HPV-58) were likely due to the shortcomings of the HPV DNA PCR testing methodology used in the original protocol-specified analyses. Inclusion of the MPTS12 RHA in the testing algorithm reveals a higher sensitivity of detection for some nonvaccine oncogenic HPV types, while the previous algorithm underestimated VE for virological endpoints with some of these HPV types. Overall, these exploratory results do not change the previously reported conclusions relating to the primary and secondary objectives of PATRICIA (11, 12, 14), which are still valid since the protocol-specified HPV DNA testing algorithm was used. However, our results may be useful to inform the choice of assays included in HPV DNA testing algorithms for the evaluation of virological endpoints in future studies. This is important in the evaluation of the efficacies of currently licensed and next-generation vaccines against oncogenic HPV types other than 16 and 18 (24, 25) and is particularly pertinent to geographic regions or ethnic groups that have an increased incidence of less common oncogenic HPV types.

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