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Article

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Burden of Human Papillomavirus (HPV)-Related Cancers Attributable to HPVs 6/11/16/18/31/33/45/52 and 58

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Abstract

Background: Many countries, mainly high- and upper-middle income, have implemented human papillomavirus (HPV) vaccination programs, with 47 million women receiving the full course of vaccine (three doses) in 2014. To evaluate the potential impact of HPV vaccines in the reduction of HPV-related disease, we aimed to estimate the HPV type distribution and burden of anogenital and head and neck cancers attributable to HPV types (HPVs 16/18/31/33/45/52/58/6/11) included in currently licensed HPV vaccines.
Methods: In all, 18 247 formalin-fixed paraffin-embedded specimens were retrieved from 50 countries. HPV DNA detection and typing were performed with the SPF-10 PCR/DEIA/LiPA25 system. With the exception of cervical cancer, HPV DNA-positive samples were additionally subjected to HPV E6*I mRNA detection and/or $p16^{INK4a}$ immunohistochemistry. For cervical cancer, estimates were based on HPV DNA, whereas for other sites, estimates were based on HPV DNA, E6*I mRNA, and $p16^{INK4a}$ biomarkers.
Results: The addition of HPVs 31/33/45/52/58 to HPVs 16/18/6/11 in the nonavalent HPV vaccine could prevent almost 90% of cervical cancer cases worldwide. For other sites, the nonavalent HPV vaccine could prevent 22.8% of vulvar, 24.5% of penile, 60.7% of vaginal, 79.0% of anal cancers, 21.3% of oropharyngeal, 4.0% of oral cavity, and 2.7% of laryngeal cancer cases.
Conclusions: Our estimations suggest a potential impact of the nonavalent HPV vaccine in reducing around 90% of cervical cancer cases and a global reduction of 50% of all the cases at HPV-related cancer sites.

Human papillomavirus (HPV)-related cancers remain a major cause of cancer both in men and women. Worldwide, HPV infection causes up to 4.5% (640 000 cases) of all new cancer cases worldwide (8.6% females; 0.9% males), representing 29.5% of all infection-related cancers (1,2).

The global burden of cervical cancer (530 000 cases) is substantially higher than that of HPV-related cancers other than cervix (113 000 cases) (1,2). However, an increase in the incidence of anal and HPV-related head and neck cancers (HNCs) has been noted in recent decades (3–6).

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HPV infection is unequivocally linked to almost all cervical cancer cases (≥95% of cases) and a large proportion of anal cancer cases (~88% of cases attributable to HPV) (2). HPV is also causally associated with a varying percentage of cancers of the vulva, vagina, penis, and a subset of HNCs, particularly tonsillar cancer (7,8). Within the spectrum of HPV oncogenic types, HPV 16 is the most prevalent in all HPV-related cancer sites. Based on previous analytical studies that demonstrated HPV to be a necessary cause of cervical cancer, HPV attribution has been based on the detection of HPV DNA alone. However, additional carcinogenic pathways other than HPV are suspected for anal and vaginal cancers, and validated for vulvar, penile, and HNCs. Thus, the identification of HPV DNA combined with E6/E7mRNA, a marker of viral transcriptional activity, or $p16^{INK4a}$, a cell surrogate marker of HPV carcinogenic transformation, provides a more accurate, although conservative, assessment of oncogenic activity of the virus in the specific sites (9–11).

Prophylactic HPV vaccines have been introduced worldwide since 2006, with a high efficacy and safety record in the prevention of vaccine-type HPV infection and disease. Further, the HPV vaccines show a high effectiveness profile on prevalent infections, precancerous lesions, and genital warts, as well as a robust herd-protection effect in nonvaccinated sexual partners with a high vaccine coverage rate (12,13). Currently, three HPV vaccines are commercially available (bivalent, tetravalent, and nonavalent). All three contain virus-like particles (VLPs) of HPVs 16/18. The tetravalent vaccine also contains VLPs from HPVs 6/11. The nonavalent vaccine additionally protects against HPVs 31/33/45/52/58.

The World Health Organization recognizes the importance of HPV-related diseases as global public health threats and has reiterated the recommendation to include HPV vaccines in national immunization programs (14). By October 2014, 80 countries (mainly high- and upper-middle income) had implemented HPV vaccination programs and 47 million women had received the full course of the vaccine. Even more, it has been estimated that approximately 379 000 cases of cervical cancer and 156 000 deaths could be averted in the 47 million fully vaccinated women by age 75 years, assuming lifelong protection (15).

The evidence suggests that the combination of high HPV vaccination coverage in adolescents together with high cervical screening coverage (followed by adequate treatment of the lesions detected by screening) can eliminate cervical cancer as a public health problem. As a result, it has been established that one of the greatest priorities and challenges in global health is to give access to HPV vaccination and cervical screening to the majority of women around the world. The availability of a wide range of cervical screening techniques (cytology, HPV-detection techniques, visual inspection with acetic acid) and HPV vaccination options (bivalent, tetravalent, and nonavalent vaccines with two- or three dose schedules) leads to an increase in the complexity of decision making regarding the combination of optimal prevention strategies in each region of the world.

To evaluate the potential impact of HPV vaccines in the reduction of HPV-related disease burden and to help formulate recommendations on HPV prevention, we estimated the HPV type distribution and burden of cancer cases attributable to the HPV types included in the nonavalent vaccine. For this we used new data derived from a large international study that provided information on HPV DNA, mRNA, and $p16^{INK4a}$ from formalin-fixed paraffin-embedded (FFPE) tissue of anogenital and HNC sites.

Materials and Methods

To estimate the prevalence and relative contribution (RC) of the types included in licensed HPV vaccines in HPV-related cancers, we used updated data from an international project designed and coordinated by the Catalan Institute of Oncology (ICO) (Barcelona, Spain) in collaboration with DDL Diagnostic Laboratory (Rijswijk, Netherlands) (5,16–20).

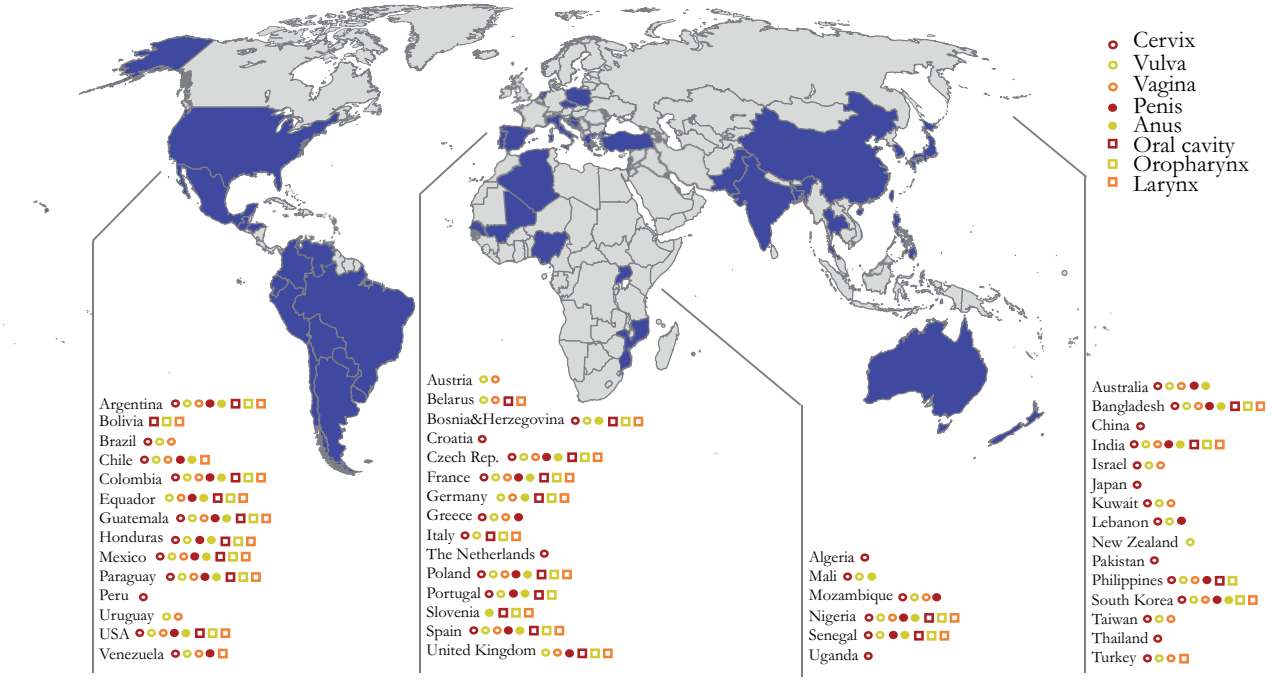

Figure 1. Anatomical sites included from each country. The number of cancer samples included at each anatomical site varies among countries.

Table 1. General characteristics of included cases*

General characteristics	Cervix	Vulva	Vagina	Anus	Penis	Oropharynx	Oral cavity	Larynx
Regions (%)	Eu (21%), Am (39%), Af (9%), AO (31%)	Eu (53%), Am (22%), Af (1%), AO (24%)	Eu (37%), Am (47%), Af (5%), AO (11%)	Eu (34%), Am (51%), Af (4%), AO (11%)	Eu (41%), Am (49%), Af (2%), AO (8%)	Eu (74%), Am (16%), Af (1%), AO (9%)	Eu (46%), Am (41%), Af (5%), AO (8%)	Eu (48%), Am (38%), Af (7%), AO (7%)
Period of diagnosis	1940–2010	1980–2011	1986–2011	1986–2011	1983–2011	1990–2012	1990–2012	1990–2012
Sex (%)				Fem (66.3%), Mal (31.7%)		Fem (16.3%), Mal (81.1%)	Fem (37.3%), Mal (61.8%)	Fem (10.5%), Mal (89.2%)
Mean age (SD), y	51.3 (13.3)	68.5 (15.3)	61.3 (15.3)	62.8 (14.7)	64.1 (15.0)	61.0 (11.2)	61.4 (14.0)	61.8 (10.9)
Histology, %	SCC—90% ADC—7% ADSC—2% Other—2%	SCC100WB—19% SCC100NonWB—72% SCC-Mixed—6% Other—3%	SCC100WB—31% SCC100NonWB—51% SCC-Mixed—4% Other—13%	SCC100WB—59% SCC100NonWB -33% SCC-Mixed—6% Other—2%	SCC100WB—23% SCC100NonWB—64% SCC-Mixed—7% Other—6%	SCC100WB—11% SCC100NonWB—88% SCC-Mixed—0% Other—1%	SCC100WB—3% SCC100NonWB—96% SCC-Mixed—0% Other—1%	SCC100WB—7% SCC100NonWB—92% SCCMixed—0% Other—1%
No. Total	11228	1709	408	496	1010	1090	1264	1042
HPV DNA+								
No. positive (%)	9516 (84.8%)	488 (28.6%)	303 (74.3%)	438 (88.3%)	334 (33.1%)	271 (24.9%)	93 (7.4%)	59 (5.7%)
Sin/mul/und %	93.0/6.3/0.7%	89.8/6.1/4.1%	95.7/4.0/0.3%	92.2/7.3/0.5%	89.2/9.0/1.8%	98.5/0.4/1.1%	87.1/5.4/7.5%	94.9/1.7/3.4%
HPV DNA+ and (E6*I mRNA/p16^{INK4a})								
No. positive (%)	NA	419 (24.6%)	289 (71.0%)	375 (82.6%)	280 (27.7%)	243 (22.4%)	55 (4.4%)	36 (3.5%)
Sin/mul/und %	NA	92.8/5.7/1.4%	96.2/3.8/0.0%	94.4/5.1/0.5%	90.0/8.9/1.1%	99.6/0.4/0.0%	96.4/3.6/0.0%	100.0/0.0/0.0%

*ADC = adenocarcinoma; ADSC = adenosquamous carcinoma; Af = Africa; AO = Asia and Oceania; Am = Americas; Eu = Europe; Fem = female; HPV = human papillomavirus; Mal = male; mul = multiple infections; N = number of cases; NA = not analyzed; Other = mainly ADC (for noncervical cancers, ADSC (for noncervical cancers, undifferentiated, and neuroendrocrine carcinomas; SCC = squamous cell carcinoma; sin = single infections; und = undetermined infections; WB = warty basaloid. HPV DNA+ & (E6*I mRNA/p16^{INK4a}) = cases that tested positive for HPV DNA and at least one of the following two markers: E6*I mRNA/p16^{INK4a}. E6*I mRNA was performed on HPV DNA-positive samples for types: 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/67/68/70/73/82. SCC100WB: SCC cases 100% with WB morphological features; SCC100NonWB: SCC cases 100% non-WB features; mixed histology includes SCC with variable percentages of WB and non-WB features. For more details, please see the methodological section from Castellsagué et al. 2016; de Sanjose et al. 2010; de Sanjosé et al. 2013; Alemany et al. 2014, Alemany et al. 2015, and Alemany et al. 2016 (5, 16–20).

Study Design

The project is a cross-sectional study. Case recruitment protocols were previously described (5,16–20). Briefly, consecutive (or randomly selected) FFPE specimens processed in pathology laboratories were obtained from hospital pathology archives in 50 countries (Figure 1). Other variables collected were sex, age at diagnosis, year at diagnosis, and original histological diagnosis. This article uses information from 11 228 cervical, 496 anal, 408 vaginal, 1709 vulvar, 1010 penile, 1090 oropharyngeal, 1264 oral cavity, and 1042 laryngeal cancer cases. Table 1 summarizes the main characteristics of the tumor series.

Pathology and Laboratory Procedures: FFPE Block Processing, Histological Evaluation, and HPV Detection and Genotyping

FFPE blocks were processed under strict conditions. At least five paraffin sections were systematically obtained for each block (sandwich method). First and last sections were used for histopathological evaluation after hematoxylin and eosin staining. The intermediate sections were used for HPV DNA and mRNA testing. HPV DNA detection was performed by polymerase chain reaction (PCR) with SPF-10 broad-spectrum primers. The amplified PCR products were tested for the presence of HPV DNA using a DNA enzyme immunoassay (DEIA) that recognized at least 54 mucosal HPV genotypes. DNA quality was evaluated by PCR testing for the human tubulin in all HPV DNA-negative vaginal, anal, and HNCs, and a subset of samples of cervical, vulvar, and penile cases (almost all HPV DNA-negative cervical cancer cases were due to technical artifacts, and 10% of the vulvar and penile cancers were due to poor DNA quality). Vaginal, anal, and HNCs that were negative both for HPV DNA and tubulin were excluded from the final analyses. Amplimers testing positive for viral DNA by DEIA were genotyped with a reverse hybridization line probe assay—LiPA25 that detects 25 HPV types (HPVs 6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/53/54/56/58/59/66/68/70/74) (21). Sequence analysis was performed to characterize HPV DEIA-positive samples with unknown types and to disentangle HPVs 68/73 because the LiPA system is unable to separate these types. If no HPV type could be attributed after DNA sequencing, the HPV type was labeled as undetermined.

With the exception of cervical cancer cases, all HPV DNA-positive samples, and a random selection of HPV DNA-negative cases for quality control, were further subjected to HPV E6*I mRNA detection, a biomarker of HPV viral transcriptional activity, and $p16^{INK4a}$ immunohistochemistry, a cellular surrogate marker for HPV carcinogenic transformation. The E6*I mRNA assay targets 20 HPV types (HPVs 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/67/68/70/73/82). For each case, type-specific E6*I mRNA PCR was performed for all types detected by the SPF-10 PCR/DEIA/LiPA25 system that had at least one of the mRNA targeted types, and additionally for HPV 16. $p16^{INK4a}$ was detected using the CINtec histology kit (clone E6H4, Roche mtm laboratories AG, Germany) following the manufacturer's protocol. A pattern of diffuse staining of more than 25% stained cells (nuclear and cytoplasmic) was considered positive.

Statistical Analysis

For cervical cancer, RC of the nine types included in the nonavalent vaccine was expressed as the proportion of women positive for a specific type among all HPV DNA-positive samples. HPV prevalence was calculated as those positive among all tested samples. For the other cancer sites, RC estimates were based on the combined positivity for HPV DNA and (E6*I mRNA or $p16^{INK4a}$). HPV prevalence indicates those positive for HPV DNA and (E6*I mRNA or $p16^{INK4a}$) among all those tested. For all sites, information on multiple infections was added to single types in accordance with a proportional weighting attribution (22,23). RCs by region, sex, and age at diagnosis were determined. To evaluate differences among proportions, we used the most suitable test (chi-squared, Fisher), adjusting for multiple comparisons (Bonferroni–Holms) when necessary. Trend test analysis for proportions was used to evaluate trends by age at diagnosis. The statistically significant *P* value was set at .05.

We further estimated the potential preventable number of cases per year by applying the estimated proportions attributed to HPV to the number of cases estimated to occur each year as reported by de Martel et al. (24).

Results

Squamous cell carcinomas (SCCs) accounted for more than 90% of cases reviewed, with the exception of vaginal cancer. Most cases were from Europe and the Americas (Table 1). Cervical cancer cases were diagnosed at younger ages (mean age = 51.1 years) compared to the other sites (mean ages ranging from 61.0 to 68.5 years).

Overall HPV and type-specific prevalence results are reported in Table 1 and Figures 2 and 3. The HPV prevalence for all types combined was 84.8% in cervical cancer cases (HPV DNA alone). For the other cancer sites, the HPV prevalence (HPV DNA

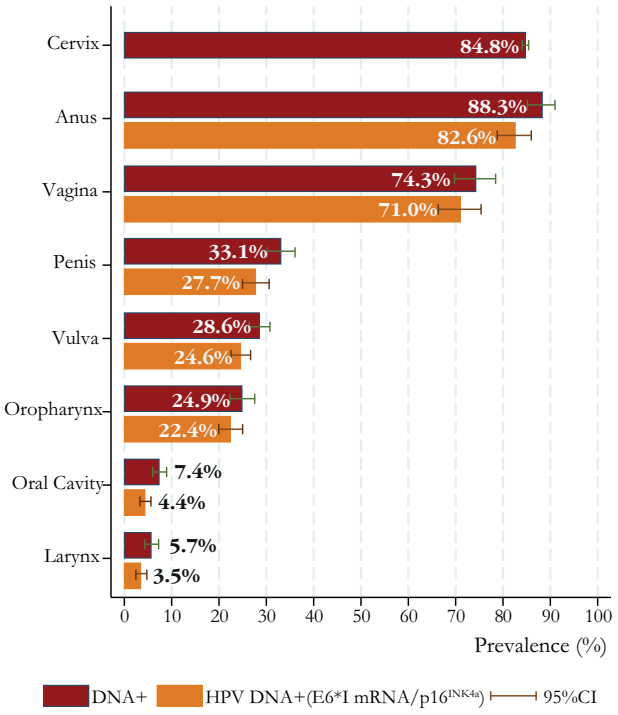

Figure 2. Overall HPV positivity for HPV DNA and HPV DNA + (E6*I mRNA or $p16^{INK4a}$) by anatomical site. 95% CI = 95% confidence interval (one-sided, 97.5% CI calculated when appropriate; HPV = human papillomavirus. For more details, please see the methodological section from Castellsagué et al. 2016; de Sanjose et al. 2010; de Sanjosé et al. 2013; Alemany et al. 2014, Alemany et al. 2015, and Alemany et al. 2016 (5,16–20).

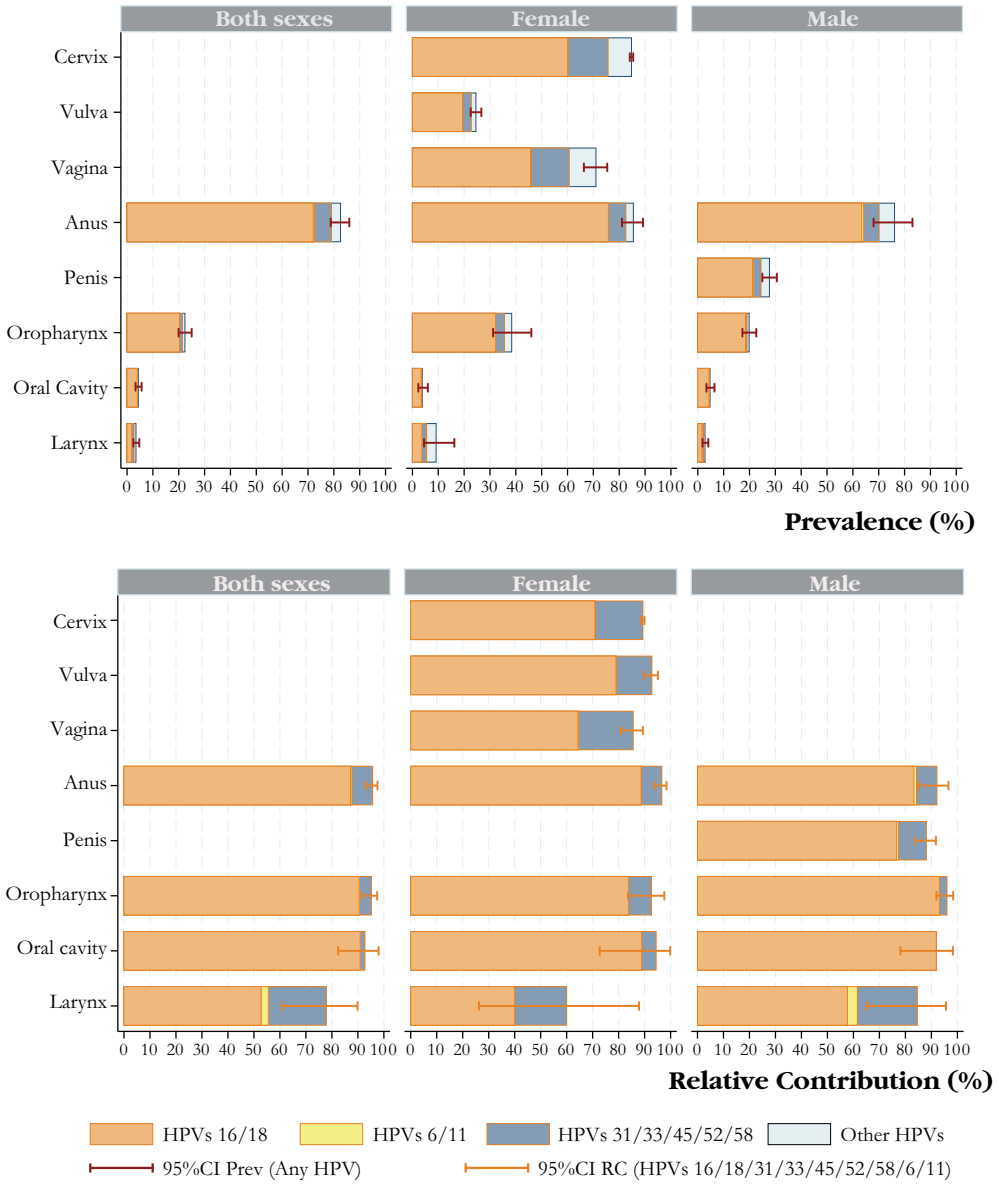

Figure 3. Worldwide HPV prevalence and relative contribution in HPV-related cancers, by sex. 95% CI = 95% confidence interval (one-sided, 97.5% CI calculated when appropriate); Cervix = based on HPV DNA; HPV = human papillomavirus; Other locations = based on information on three markers (HPV DNA + (E6*I mRNA or $p16^{INK4a}$)); Prev = prevalence and type-specific relative contribution estimations. For more details, please see the methodological section from Castellsagué et al. 2016; de Sanjose et al. 2010; de Sanjosé et al. 2013; Alemany et al. 2014, Alemany et al. 2015, and Alemany et al. 2016 (5, 16–20).

and "E6*I mRNA+ or $p16^{INK4a}$+") for all types combined was 82.6% anal, 71.0% vaginal, 27.7% penile, 24.6% vulvar, 22.4% oropharyngeal, 4.4% oral cavity, and 3.5% laryngeal cancers. When comparing HPV DNA detection estimates to the ones adding E6*I mRNA and $p16^{INK4a}$, a reduction of the proportion of cases attributed to HPV were higher for oral cavity and larynx (percentage reduction = 40.5% and 38.6%, respectively) than penis, vulva, oropharynx, anus, and vagina (percentage reduction = 16.3%, 14.0%, 10.0%, 6.4% and 4.4%, respectively), ($P < .05$ for oral cavity, larynx, penis, vulva, and anus).

Among HPV positives, RC of the nine types was 95.7% in the anus, followed by oropharynx (95.1%), vulva (92.8%), oral cavity (92.7%), cervix (89.3%), penis (88.1%), vagina (85.6%), and larynx (77.8%) (Tables 2 and 3 and Figure 2). RC of HPVs 16/18 was especially prominent in oral cavity, oropharyngeal, and anal cancer sites (90.9%, 90.5%, and 87.2%, respectively), mostly attributable to HPV 16 (90.9%, 88.5%, and 83.7%, respectively). RCs of HPVs 16/18 in other anogenital and HNCs was 79.0% in the vulva, followed by penis (76.6%), cervix (70.9%), vagina (64.3%), and larynx (52.8%). The additional contribution of HPVs 31/33/45/52/58 was higher in the larynx (22.2%), vagina (21.0%), and cervix (18.3%) compared to other cancer sites (Tables 2 and 3). HPV 16 was by far the most frequently detected genotype across all anatomical sites. RCs by individually tested types are detailed in Supplementary Material Table 1 (available online). RC of the nine HPV types was significantly higher when taking into account the combined detection of HPV DNA, and E6*I mRNA or $p16^{INK4a}$ for vulvar and oropharyngeal cancers as compared to HPV DNA detection alone (Tables 2 and 3).

HPV RCs by region are provided in Figure 4 and Supplementary Material Tables 2 and 3 (available online). HPV 16 was again the dominant type in HPV-related cancers in

Table 2. Worldwide relative contribution of HPVs 16/18/31/33/45/52/58/6 and 11 in anogenital HPV-related cancers

	Cervix	Vulva		Vagina		Anus		Penis	
HPV type	DNA+(n = 9516) RC% (95% CI)	DNA+(n = 488) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 419) RC% (95% CI)	DNA+(n = 303) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 289) RC% (95% CI)	DNA+(n = 438) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 375) RC% (95% CI)	DNA+(n = 334) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 280) RC% (95% CI)
Combined nine HPV types†	89.3 (88.7 to 89.9)	87.1 (83.8 to 89.9)	92.8 (89.9 to 95.1)	85.3 (80.6 to 89)	85.6 (80.9 to 89.3)	95.3 (92.8 to 97.0)	95.7 (93.2 to 97.5)	84.4 (80.1 to 88.1)	88.1 (83.8 to 91.7)
HPVs 16/18	70.9 (69.9 to 71.8)	72.6 (68.4 to 76.5)	79.0 (74.8 to 82.8)	63.7 (58.0 to 69.1)	64.3 (58.5 to 69.9)	84.3 (80.5 to 87.5)	87.2 (83.4 to 90.4)	70.2 (64.8 to 74.9)	76.6 (71.4 to 81.6)
HPVs 31/33/45/52/58	18.3 (17.6 to 19.1)	13.0 (10.2 to 16.4)	13.6 (10.5 to 17.3)	20.1 (15.8 to 25.1)	21.0 (16.5 to 26.3)	8.0 (5.6 to 10.9)	8.0 (5.5 to 11.2)	9.1 (6.2 to 12.7)	10.7 (7.3 to 14.9)
Specific HPV type:									
HPV 16	60.3 (59.4 to 61.3)	68.0 (63.7 to 72.2)	74.0 (69.5 to 78.1)	58.7 (53.0 to 64.3)	59.4 (53.6 to 65.2)	80.7 (76.8 to 84.4)	83.7 (79.6 to 87.3)	68.7 (63.3 to 73.5)	75.3 (69.9 to 80.3)
HPV 18	10.5 (9.9 to 11.2)	4.6 (2.8 to 6.7)	5.0 (3.1 to 7.6)	5.0 (2.8 to 8.0)	4.9 (2.7 to 8.0)	3.6 (2.1 to 5.9)	3.5 (1.9 to 5.9)	1.5 (0.5 to 3.5)	1.4 (0.4 to 3.6)
HPV 31	3.6 (3.2 to 4.0)	1.3 (0.5 to 2.7)	1.0 (0.3 to 2.4)	5.3 (3.0 to 8.4)	5.5 (3.2 to 8.8)	1.9 (0.8 to 3.6)	1.1 (0.3 to 2.7)	0.8 (0.2 to 2.6)	0.9 (0.2 to 3.1)
HPV 33	3.7 (3.4 to 4.1)	5.9 (4.0 to 8.4)	6.5 (4.3 to 9.2)	4.8 (2.8 to 8.0)	4.7 (2.7 to 8.0)	2.7 (1.4 to 4.7)	3.1 (1.7 to 5.5)	2.9 (1.4 to 5.4)	3.2 (1.5 to 6.0)
HPV 45	6.1 (5.6 to 6.6)	2.9 (1.6 to 4.8)	3.4 (1.8 to 5.5)	3.6 (1.8 to 6.4)	3.8 (1.9 to 6.7)	0.9 (0.2 to 2.3)	1.1 (0.3 to 2.7)	2.7 (1.2 to 5.1)	3.2 (1.5 to 6.0)
HPV 52	2.7 (2.4 to 3.1)	1.8 (0.8 to 3.5)	1.7 (0.7 to 3.4)	2.9 (1.4 to 5.6)	3.1 (1.4 to 5.8)	0.7 (0.1 to 2.0)	0.5 (0.1 to 1.9)	1.5 (0.5 to 3.5)	1.9 (0.6 to 4.1)
HPV 58	2.2 (1.9 to 2.5)	1.1 (0.3 to 2.4)	1.0 (0.3 to 2.4)	3.6 (1.8 to 6.4)	3.8 (1.9 to 6.7)	1.8 (0.8 to 3.6)	2.1 (0.9 to 4.2)	1.3 (0.3 to 3.0)	1.6 (0.4 to 3.6)
HPV 6	0.1 (0.1 to 0.2)	1.1 (0.3 to 2.4)	0.3 (0.0 to 1.3)	1.0 (0.2 to 2.9)	0.0 (0.0 to 1.3)	1.8 (0.8 to 3.6)	0.5 (0.1 to 1.9)	3.7 (1.9 to 6.2)	0.7 (0.1 to 2.6)
HPV 11	0.0 (0.0 to 0.1)	0.5 (0.0 to 1.5)	0.0 (0.0 to 0.9)	0.3 (0.0 to 1.8)	0.3 (0.0 to 1.9)	1.1 (0.4 to 2.6)	0.0 (0.0 to 1.0)	1.5 (0.5 to 3.6)	0.0 (0.0 to 1.3)

†Nine HPV types includes the ones in the nonavalent HPV vaccine: HPVs 16/18/31/33/45/52/58/6/11. 95% CI = 95% confidence interval (one-sided, 97.5% CI calculated when appropriate; DNA+ = cases that tested positive for HPV DNA; E6*I mRNA/$p16^{INK4a}$ = cases that tested positive for HPV DNA+ (E6*I mRNA positive or $p16^{INK4a}$ positive; HPV = human papillomavirus; N = number of cases; RC = relative contribution. E6*I mRNA was performed in HPV-DNA-positive samples for types: HPVs 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/67/68/70/73/82. Type-specific RC estimations are estimated among HPV DNA positive and among HPV DNA positive + (E6*I mRNA positive or $p16^{INK4a}$ positive). For cervix, RC estimations are calculated only among DNA positive. For more details, please see methodological section from de Sanjose et al. 2010; de Sanjosé et al. 2013; Alemany et al. 2014, Alemany et al. 2015, and Alemany et al. 2016 (16–20).

Table 3. Worldwide relative contribution of HPVs 16/18/31/33/45/52/58/6 and 11 in head and neck HPV-related cancers

	Oropharynx		Oral cavity		Larynx	
HPV type	DNA+(n = 271) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 243) RC% (95% CI)	DNA+(n = 93) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 55) RC% (95% CI)	DNA+(n = 59) RC% (95% CI)	and (E6*I mRNA/ $p16^{INK4a}$) (n = 36) RC% (95% CI)
Combined nine HPV types†	90.0 (85.8 to 93.3)	95.1 (91.5 to 97.4)	81.7 (72.4 to 89.0)	92.7 (82.4 to 98.0)	83.1 (71.0 to 91.6)	77.8 (60.8 to 89.9)
HPVs 16/18	85.2 (80.4 to 89.2)	90.5 (86.1 to 93.9)	72.0 (61.8 to 80.9)	90.9 (80.0 to 97.0)	57.6 (44.1 to 70.4)	52.8 (35.5 to 69.6)
HPVs 31/33/45/52/58	8.6 (3.8 to 16.2)	4.5 (2.3 to 8.0)	4.4 (2.3 to 7.6)	1.8 (0.0 to 9.7)	16.9 (8.4 to 29.0)	22.2 (10.1 to 39.2)
Specific HPV type						
HPV 16	83.4 (78.4 to 87.6)	88.5 (83.8 to 92.2)	70.9 (60.6 to 79.9)	90.9 (80.0 to 97.0)	50.8 (37.5 to 64.1)	44.4 (27.9 to 61.9)
HPV 18	1.8 (0.6 to 4.3)	2.1 (0.7 to 4.7)	1.1 (0.0 to 5.8)	0.0 (0.0 to 6.5)	6.8 (1.9 to 16.5)	8.3 (1.8 to 22.5)
HPV 31	0.0 (0.0 to 1.4)	0.0 (0.0 to 1.5)	0.0 (0.0 to 3.9)	0.0 (0.0 to 6.5)	3.4 (0.4 to 11.7)	2.8 (0.1 to 14.5)
HPV 33	3.3 (1.5 to 6.2)	3.3 (1.4 to 6.4)	0.0 (0.0 to 3.9)	0.0 (0.0 to 6.5)	3.4 (0.4 to 11.7)	2.8 (0.1 to 14.5)
HPV 45	0.4 (0.0 to 2.0)	0.4 (0.0 to 2.3)	0.0 (0.0 to 3.9)	0.0 (0.0 to 6.5)	8.5 (2.8 to 18.7)	13.9 (4.7 to 29.5)
HPV 52	0.0 (0.0 to 1.4)	0.0 (0.0 to 1.5)	7.6 (3.1 to 14.9)	0.0 (0.0 to 6.5)	0.0 (0.0 to 6.1)	0.0 (0.0 to 9.7)
HPV 58	0.7 (0.1 to 2.6)	0.8 (0.1 to 2.9)	1.1 (0.0 to 5.8)	1.8 (0.0 to 9.7)	1.7 (0.0 to 9.1)	2.8 (0.1 to 14.5)
HPV 6	0.4 (0.0 to 2.0)	0.0 (0.0 to 1.5)	0.0 (0.0 to 3.9)	0.0 (0.0 to 6.5)	6.8 (1.9 to 16.5)	2.8 (0.1 to 14.5)
HPV 11	0.0 (0.0 to 1.4)	0.0 (0.0 to 1.5)	1.1 (0.0 to 5.8)	0.0 (0.0 to 6.5)	1.7 (0.0 to 9.1)	0.0 (0.0 to 9.7)

†Nine HPV types includes the ones in nonavalent HPV vaccine: HPVs 16/18/31/33/45/52/58/6/11. 95% CI = 95% confidence interval (one-sided, 97.5% CI calculated when appropriate); DNA+ = cases that tested positive for HPV DNA; E6*I mRNA/$p16^{INK4a}$ = cases that tested positive for HPV DNA+ (E6*I mRNA positive or $p16^{INK4a}$ positive; HPV = human papillomavirus; N = number of cases; RC = relative contribution. E6*I mRNA was performed in HPV-DNA–positive samples for types: HPVs 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/67/68/70/73/82. Type-specific RC estimations are estimated among HPV DNA positive and among HPV DNA positive + (E6*I mRNA positive or $p16^{INK4a}$ positive). For more details, please see the methodological section from Castellsagué et al. 2016 (5).

all regions. Within cervical cancer, HPV 16 RC ranged from 47.3% (Africa) to 65.5% (Europe); in vulvar cancer from 66.7% (Americas) to 87.5% (Africa); in vaginal cancer from 46.2% (Africa) to 66.9% (Europe); in anal cancer from 66.7% (Africa) to 87.3% (Europe), in penile cancer from 71.4% (Africa) to 78.9% (Europe), in oropharyngeal cancer from 78.1% (Americas) to 94.7% (Asia and Oceania), in oral cavity cancer 90.9% (Americas and Europe), and in larynx cancer from 0.0% (Africa based on small number of samples) to 58.3% (Europe). Although cumulative RC of the nine types varied slightly by region, differences were not statistically significant when comparing regions with world data. The only exception was observed in cervical cancer in Africa and Asia and Oceania (World—89.3%, Africa—86.3%, Asia and Oceania—91.2%; $P < .05$).

Differences by sex were not detected in the combined RC of HPVs 16/18, HPVs 31/33/45/52/58, or the nine HPVs for anal and HNCs, except for a higher RC of HPVs 16/18 in male oropharynx ($P < .05$) (Figure 3).

No specific nine HPV RC age trends were observed by cancer site, except in the cervix (Supplementary Material Table 4, available online), where the combined RC of the nine types decreased with age ($P < .001$); mainly explained by the decrease of HPVs 16/18/45 in older ages (more details in Serrano et al. 2012) (25).

The potentially preventable fraction of the nonavalent HPV vaccine would be around 50% worldwide, taking into account our type-specific prevalence estimates and the estimated number of incident cases at HPV-related cancer sites provided by de Martel et al. (24). These would include most cervical and anal cancers and a substantial fraction of vaginal, penile, vulvar, and HNCs (Table 4).

Discussion

This comprehensive analysis of the contribution of HPV vaccine types in selected anogenital and HNCs provides robust estimates to be used to monitor the vaccine impact worldwide. We present data from all HPV-related sites, updating the number of the cervical series, and adding the evaluation of molecular and immunohistochemistry markers of viral activity beyond HPV DNA detection. In the absence of a unique carcinogenic pathway and of detection of specific infections leading to cancer, the addition of these markers increases our ability to attribute causality (8,11). Our data confirm the biological role of HPV in the great majority of HPV DNA-positive anogenital and oropharyngeal cancers, and to a much lesser extent in oral cavity and laryngeal cancers.

We have estimated that the combined detection of HPV DNA and (E6*I mRNA or $p16^{INK4a}$) confirms that the addition of HPVs 31/33/45/52/58 to the current vaccine types (HPVs 16/18/6/11), could prevent almost 96% of the HPV-positive cancers of the anus, vulva (93%), cervix (90%), penis (88%), vagina (86%), oropharynx (95%), oral cavity (93%), and larynx (78%). A marked heterogeneity in the incremental HPV-related preventable fraction added by the nonavalent vaccine compared to bivalent and tetravalent vaccines was observed, ranging from 8.0% (anal) to 21.0% (vaginal) in anogenital cancer sites, and from 1.8% (oral cavity) to 22.2% (larynx) in HNC sites.

Similar to previous data, overall HPV prevalence was lower for vulvar (24.6%), penile (27.0%), oral cavity (4.4%), laryngeal (5.7%), and oropharyngeal (22.4%) cancers, compared with vaginal (71.0%), anal (82.6%), and cervical (84.8%) cancers (2). Therefore, overall the nonavalent vaccine could prevent 22.8% of vulvar, 24.5% of penile, 60.7% of vaginal, 79.0% of anal, and 90% of cervical cancer cases, 21.3% of oropharynx, 4.0% of oral cavity, and 2.7% of larynx cancers cases. Some of our prevalence estimates are lower than those published in previous meta-analyses (6,26) or in series using similar protocols (27–30). These differences were expected, as we used a more restrictive protocol in terms of HPV attribution by adding additional markers. This study included cases from numerous countries, some of which had scarce data published. This is particularly relevant in

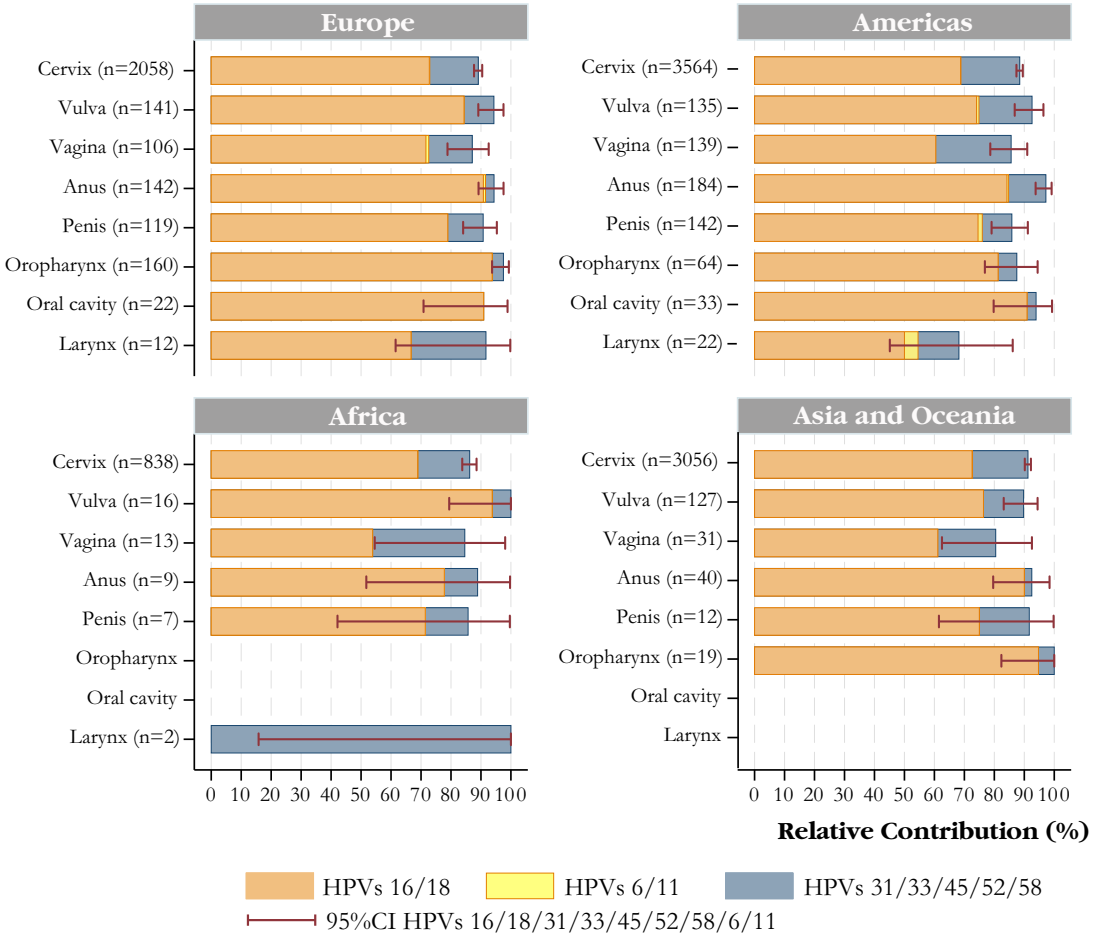

Figure 4. HPV relative contribution of types included in licensed HPV vaccines in HPV-related cancers, by region. 95% CI = 95% confidence interval (one-sided, 97.5% CI calculated when appropriate); HPV = human papillomavirus; n = number of cancer cases represented in the region. Type-specific relative contribution estimations: Cervix = based on HPV DNA positive; Other locations = based on information on three markers (HPV DNA positive + (E6*I mRNA or $p16^{INK4a}$)). For more details, please see the methodological section from Castellsagué et al. 2016; de Sanjose et al. 2010; de Sanjosé et al. 2013; Alemany et al. 2014, Alemany et al. 2015, and Alemany et al. 2016 (5, 16–20).

Table 4. Preventable fraction of human papillomavirus (HPV)-related cancer cases per year

HPV-related cancer site	Incident cases, No.*	Cases attributable to HPV, No.	Nine HPV types, %	Cases attributable to HPV, No.	Nine HPV types, %
Cervix uteri	530 000	405 450†	76.5†	477 000‡	90.0‡
Anus	40 000	31 600	79.0	31 600	79.0
Vulva	34 000	7752	22.8	7752	22.8
Vagina	15 000	9105	60.7	9105	60.7
Penis	26 000	6370	24.5	6370	24.5
Oropharynx	96 000	20 448	21.3	20 448	21.3
Oral cavity	200 000	8000	4.0	8000	4.0
Larynx	160 000	4320	2.7	4320	2.7
Total HPV-related sites	1 101 000	493 045		564 595	
Potentially preventable cases, %		44.8%		51.3%	

*Source: de Martel, 2017
†Assumption: 85% HPV DNA-positivity and 90% relative contribution (RC) of nine HPV types.
‡Assumption: HPV is a necessary cause of invasive cervical cancer; and 90% RC of nine HPV types.

HNC sites where a substantial variability in the HPV-related attribution has been observed across regions (2). Contrary to this, our series have a distinctive limitation on samples from areas reported to have an increased trend of HPV-driven HNCs, such as the United States and Northern Europe, where higher attributable fractions in HNCs have been recently reported (30). Although the exclusive use of HPV DNA results in higher attribution to HPV (30), when adding other biomarkers the attribution was reduced to 67.2% in oropharynx SCC, 5.9% in oral cavity SCC, and 1.7% in larynx SCC (27–29).

Worldwide incidence rates at HPV-related cancer sites other than the cervix are much lower than that observed for cervical cancer. However, incidence rates in anal, vulvar, and HPV-related HNCs are increasing, probably linked to changes in sexual behavior, tobacco and alcohol consumption, and in cervical cancer screening policies (2–4).The large contribution of HPV infection, mainly of HPV 16, the absence of well-established screening instruments, and algorithms for the detection of HPV-related cancer cases other than the cervix, and the current evidence on HPV vaccine efficacy, reinforces the role that HPV vaccines can play in primary prevention of HPV-related lesions. Moreover, even if a higher impact of HPV vaccination would be expected in cervical and anal cancer, the confirmed increasing trends of HNCs attributable to HPV could be translated to a high number of cases prevented by vaccination despite the low proportion of HPV-related cases estimated for these sites (2).

HPV 16 was the most important oncogenic type (≥60% in anogenital cancer sites, and 44% to 91% in HNCs). The inclusion of additional markers increased the HPV 16 RC in vulvar, penile, oral cavity, and oropharyngeal cancers, and confirmed the causative role for other, less frequently occurring mucosal HPV types. Some non-HPV 16 types, usually involved in cervical cancer, are also related to other cancers of the genital tract and HNCs, but to a lesser extent. Although HPV18 is reported to be the second most common type in cervical cancer, variations were observed in other cancer sites. HPV 33 follows HPV 16 in vulvar and oropharyngeal cancers, HPV 31 in vaginal cancer, HPVs 31/45 in penile cancer, HPVs 18/33 in anal cancer, HPV 58 in oral cavity cancer, and HPVs 18/45 in laryngeal cancers. However, results in oral cavity and larynx should be interpreted with caution because of the limited number of cases included. HPVs 6/11, contribution is minimal in the overall burden of HPV-related cancers, although they still rank relatively high in penile and anal cancer.

We found little heterogeneity by geographical region, sex, and age at diagnosis. Regional differences in the HPV RCs were significant only for cervical and oropharyngeal cancers. For cervical cancer a significantly higher contribution was observed in Asia and Oceania, and for oropharyngeal cancer a significantly higher contribution was observed in Europe compared to the Americas. However, further research will be necessary to provide robust estimates by region for noncervical lesions. Overall, HPV RC estimates were higher in women than in men, although differences were significant only for oropharyngeal cancer. The magnitude of RCs decreased with increasing age for all cancer sites. RC age trends were evaluated for the nine types combined. A similar analysis for individual HPV types was not possible because of the limited number of cases in cancer sites other than the cervix. Assessment of multiple infections was possible by means of a proportional weighting attribution (22,23). A decrease in the proportion of cases with multiple infections was observed in oropharyngeal cancers. The proportion of cases with multiple infections (0.4%) was 15 times lower than in cervical cancer (6.3%). It is unknown why there is this difference in the proportion of multiple infections, but it could be explained by differences in cell susceptibility to HPV infection in different tissues.

Despite the large sample size and the strong design, this study is not free of limitations. The principal one is that while the presence of HPV DNA was assessed in all tested samples, the two additional biomarkers E6*I mRNA and $p16^{INK4a}$ were assessed only in HPV DNA-positive samples as well as in a small fraction of HPV DNA-negative cases (of which 10% were mRNA positive), and not for all HPV genotypes. Therefore, we cannot completely rule out that we are not excluding some truly HPV-driven cases because of imperfect detection of the HPV DNA test, although we used the most sensitive test available to date. Moreover, although HPV E6*I mRNA and $p16^{INK4a}$ together with HPV DNA are well established markers in research and even in clinical settings for specifically attributing one case to be etiologically related to the virus, we are missing other potential approaches such as laser capture microdissection that can specifically analyze the markers in tumoral cells instead of the whole paraffin-embedded tissue block section that may be even more specific. However, we have applied the most suitable markers analyses taking into account the design of the study, a large epidemiological biorepository. Another limitation of the study was the small number of samples included from some regions (such as Africa) and ages, thus restricting the validity of our results for certain regions and ages. Finally, the use of fresh tissue samples would have been preferred but unrealistic to obtain when including such a large number of countries. Additionally, the studies reflect the epidemiology of each country and the impact of potential variation in screening or other environmental modifications in HPV attribution is difficult to fully evaluate in this study. Finally, for the estimated preventable fraction we make several vaccine effects assumptions: efficacy for all HPV-related cancers, long-term duration of the protection, among others.

Our estimations suggest that if HPV vaccination programs with the nonavalent HPV vaccine are effectively implemented, around 90% of cervical cancers and 50% of all HPV cancer sites related to HPV could be prevented.

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Authors and Contributions

All authors made substantial contribution to the conception and design of the work. Beatriz Serrano, Laia Alemany, and Sara Tous were responsible for the data analysis and preparations of the tables and figures. All authors contributed to the writing of the article and Beatriz Serrano, Laia Alemany, and Silvia de Sanjosé were responsible for the preparation of the article for submission. Laia Alemany had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, and had the final responsibility to submit the article for publication. All authors read and approved the final article. No financial support for the project from commercial partners was provided to Massimo Tommasino.

Conflicts of Interest

Silvia de Sanjosé: received institutional research funding from Merck, GSK and Seegene.

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All other authors declare no potential conflicts of interest.

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