

Carcinogenic human papillomavirus infection.

[Schiffman M](#)¹, [Doorbar J](#)², [Wentzensen N](#)¹, [de Sanjosé S](#)³, [Fakhry C](#)⁴, [Monk BJ](#)⁵, [Stanley MA](#)², [Franceschi S](#)⁶.

[Author information](#)

Abstract

Infections with human papillomavirus (HPV) are common and transmitted by direct contact. Although the great majority of infections resolve within 2 years, 13 phylogenetically related, sexually transmitted HPV genotypes, notably HPV16, cause - if not controlled immunologically or by screening - virtually all cervical cancers worldwide, a large fraction of other anogenital cancers and an increasing proportion of oropharyngeal cancers. The carcinogenicity of these HPV types results primarily from the activity of the oncoproteins E6 and E7, which impair growth regulatory pathways. Persistent high-risk HPVs can transition from a productive (virion-producing) to an abortive or transforming infection, after which cancer can result after typically slow accumulation of host genetic mutations. However, which precancerous lesions progress and which do not is unclear; the majority of screening-detected precancers are treated, leading to overtreatment. The discovery of HPV as a carcinogen led to the development of effective preventive vaccines and sensitive HPV DNA and RNA tests. Together, vaccination programmes (the ultimate long-term preventive strategy) and screening using HPV tests could dramatically alter the landscape of HPV-related cancers. HPV testing will probably replace cytology-based cervical screening owing to greater reassurance when the test is negative. However, the effective implementation of HPV vaccination and screening globally remains a challenge.

PMID:

27905473

DOI:

[10.1038/nrdp.2016.86](https://doi.org/10.1038/nrdp.2016.86)

[PubMed - in process]

3 comments

[How to join PubMed Commons](#)

- [Sin Hang Lee](#) 2017 Feb 19 01:15 a.m. (yesterday)

Dr. Schiffman's response to my initial comment on the Primer needs rebuttal to point out its misleading and obfuscating statements.

The statement in the response "virus like particles (VLPs)...do not contain full length HPV DNA genomes" is a red herring. Neither the Primer nor my comment on it mentioned full length HPV DNA genomes at all. The concern is about recombinant HPV L1 gene DNA fragments bound to AAHS adjuvant, transfected into the host's immune cells. Viral DNA does not need full length genomes to induce an innate immune response. The pathogen-recognition receptors, or PRRs, in the macrophages can distinguish viral DNA from the host DNA by its nucleotide sequence and elicit a

strong antiviral pro-inflammatory response [1]. The proinflammatory cytokine TNF- α and IL-1 β are toxic to the neurons and myocardium.

The statement “VLPs....are intrinsically immunogenic eliciting high antibody titres with or without adjuvant.” created a false dilemma. The fact that VLPs can elicit high antibody titres does not negate the need for adjuvantation of HPV VLPs in formulating a useful vaccine product, as demonstrated in the official formulation of Gardasil and Cervarix. Although HPV L1 VLPs alone can activate several innate immune pathways via dendritic cells and B cells, immunization with VLPs formulated with a special aluminum adjuvant elicits a significantly stronger immune response with higher peak antibody titers both at four weeks post vaccination (12.7 to 41.9-fold higher) as well as in the persistent phase at week 52 (4.3 to 26.7-fold higher) than that with VLPs alone. In addition, the aluminum adjuvant formulated HPV VLP vaccine elicits a predominantly T helper type 2 response [2].

Instead of addressing the extremely high death rate in an FDA report based on analysis of the clinical trial data on women aged 24 to 45 years and the sudden unexpected deaths of adolescents after HPV vaccinations, Dr. Schiffman depends on an appeal to authority, such as the March 12, 2014 Statement of the Global Advisory Committee on Vaccine Safety of the World Health Organization (GACVS) to support his declaration of HPV vaccination safety. Perhaps, Dr. Schiffman would like to respond on behalf of the WHO to the Open Letter I sent to Dr. Margaret Chan questioning the bias of the GACVS members in reviewing the safety issues of the HPV vaccination programs worldwide <http://sanevax.org/wp-content/uploads/2016/01/Allegations-of-Scientific-Misconduct-by-GACVS.pdf> Citing publications in pediatric journals does not help explain away the deaths in the HPV vaccine clinical trial on these women documented in the FDA report.

I disagree with the statement “there is no scientific evidence that aluminum-containing vaccines cause harm...”. I was the expert pathologist on the case (Court of Federal Claims No: 15-0160V) involving sudden unexpected death of a healthy 14-year boy who died with a recent myocardial infarction without coronary diseases in sleep in the night after the second injection of Gardasil. Free viral DNA fragments bound to certain aluminum salts may elicit a very strong innate immune response, causing tissue damages in certain genetically and physically predisposed individuals. Activated macrophages laden with viral DNA/AAHS nanoparticles can travel throughout the body, including the myocardium.

Dr. Schiffman’s endorsement in using high grade intraepithelial lesions which often include CIN2 and CIN3 as surrogate endpoint for efficacy evaluation in clinical trials for cervical cancer vaccine prevention is open to question. The CIN3 self-regressing rate is 19-31% [3] and CIN2 is not a true biologic entity, but rather an equivocal diagnosis of precancer, representing an admixture of HPV infection and precancer, according to a report co-authored by Dr. Schiffman himself [4]. The FDA independent analysis report stated correctly that efficacy in the prevention of high grade cervical disease has not been established among women aged 24 to 45 years, let alone among the 9 to 13-year old females currently targeted for mass HPV vaccination. Dr. Schiffman stated that cervical cancer cannot be used for ethical reasons as an endpoint in clinical trial. However, he feels quite comfortable in promoting mass vaccination of young females aged 9-13 without proven efficacy for cancer prevention perhaps in 30 years. In the United States, cervical cancer which is 100% treatable with early detection is a disease of the grandmothers who have already accomplished what they wanted to do in life. However, there are now 304 deaths [5] with thousands of permanent disabilities, most of them teenagers, after HPV vaccination. This latter group of people might have lost the dream of their lives prematurely and unnecessarily. Based on the FDA documents, Gardasil9 will generate 2,300 serious adverse events (2.3%) per 100,000 vaccinees.

Are the American families willing to interrupt the normal education of 2,300 out of every 100,000 granddaughters for the possibility of preventing 1.7 grandmothers from dying of cervical cancer 30 years from now, at the cost of \$50 million?

Dr. Schiffman’s claim “The figure he quotes of 58% sensitivity is the result of a controversial applicationin detection of CIN2 or worse, in a single trial.” is incorrect.

The original statement is “the CIN3+ detection sensitivity of screening algorithm with commercial HPV test kits is only 58.26%”.

Dr. Schiffman changed the word “CIN3+” to “CIN2 or worse” to create a strawman.

In surgical pathology, a “CIN3+” diagnosis calls for immediate intervention.

A “CIN2” lesion can wait as it may self-regress to normalcy. The most important value of a screening test, virology or cytology, is to detect the CIN3+ lesions. As cellular carcinogenesis progresses while

the cytoplasmic: nuclear ratio decreases in the epithelium in the course of persistent HPV infection, the copies of episomal HPV DNA per cell may go down to single digits in number, or even to 1 copy of HPV DNA per cervical cancer cell as in the SiHa cell line. It needs a more sensitive HPV test to screen for invasive or in-situ cervical cancer (CIN3+) than for CIN2 lesions in which there are many low-grade koilocytes containing thousands of copies of episomal HPV DNA per cell. The HPV screen for the detection of cervical cancer is based on the classic work of "Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer" in which the HPV DNA detection was by a nested PCR amplification of a segment of the L1 gene followed by direct automated DNA sequencing for accurate genotyping [6]. The statement in the Primer "The choice between well-validated HPV tests is not crucial; HPV DNA tests approved by the US FDA for co-testing and/or primary screening show very similar performance." is questionable and potentially detrimental to good patient care because the current FDA test kits are not sensitive enough to increase the rate of 58.26% detection for CIN3+ lesions or genotype-specific enough for follow-up of persistent HPV infections. Compared to the technology of nested PCR followed by DNA sequencing performed on split samples, an FDA-approved test kit detected only 388 (57.6%) of the 674 high-risk HPV isolates in clinical patient specimens [7], missing >40% of the high risk HPV-positive cases.

The promotional Primer by Dr. Schiffman and his colleagues for the HPV industry should be further discussed before it can be used for health care policy decision making. References: 1.Orzalli MH, Knipe DM. Annu Rev Microbiol. 2014;68:477-92. 2.Ruiz W, et al. J Immune Based Ther Vaccines. 2005;3:2. 3.Trimble CL, et al. Clin Cancer Res. 2005;11:4717-23. 4.Castle PE, et al. Am J Clin Pathol. 2007;127:805-15.

5.[http://www.medalerts.org/vaersdb/findfield.php?TABLE=ON&GROUP1=AGE&EVENTS=ON&VAX\[\]=HPV2&VAX\[\]=HPV4&VAX\[\]=HPV9&VAX\[\]=HPVX&DIED=Yes](http://www.medalerts.org/vaersdb/findfield.php?TABLE=ON&GROUP1=AGE&EVENTS=ON&VAX[]=HPV2&VAX[]=HPV4&VAX[]=HPV9&VAX[]=HPVX&DIED=Yes) 6.Wallin KL, et al. N Engl J Med. 1999;341:1633-8. 7. Ge S, et al. Cancer Med. 2012;1:59-67.

[PermalinkShare](#)

- [Sin Hang Lee](#) 2017 Feb 18 11:55 p.m. (yesterday)
Comment deleted by user.
- [Mark Schiffman](#) 2017 Feb 14 10:44 a.m. (6 days ago)
Prophylactic HPV vaccines consist of the major coat protein L1 assembled into macromolecular structures – virus like particles (VLPs) that mimic the geometry and morphology of the wild type virus coat or capsid but do not contain full length HPV DNA genomes. VLPs, with their repeat crystalline array of L1 pentamers as in the wild type virus, are intrinsically immunogenic(1) eliciting high antibody titres with or without adjuvant(2). The safety profile of the licensed vaccines was assessed extensively in randomized clinical trials (RCTs)(3-5). In the 10 years since the first 2 commercial vaccines Gardasil and Cervarix were licensed, the safety profile has been intensively monitored in the post-licensure setting by robust pharmacovigilance using both passive and active surveillance (3, 6). These studies, which collectively have included millions of subjects, provide no evidence whatsoever to support the speculation that HPV vaccines by virtue of their protein content, adjuvants or any other element within the formulation –could induce, trigger or exacerbate auto-immune disorders, thromboembolic events, demyelinating diseases or other chronic conditions.
The Global Advisory Committee on Vaccine Safety of the World Health Organisation has reviewed the safety data for HPV vaccines on several occasions http://www.who.int/vaccine_safety/committee/topics/hpv/en/ GACVS stated in 2014: "In summary, the GACVS continues to closely monitor the safety of HPV vaccines and, based on a careful examination of the available evidence, continues to affirm that its benefit-risk profile remains favorable. The Committee is concerned, however, by the claims of harm that are being raised on the basis of anecdotal observations and reports in the absence of biological or epidemiological substantiation. While the reporting of adverse events following immunization by the public and health care providers should be encouraged and remains the cornerstone of safety surveillance, their interpretation requires due diligence and great care. As stated before, allegations of harm from vaccination based on weak evidence can lead to real harm when, as a result, safe and effective vaccines cease to be used. To date, there is no scientific evidence that aluminium-containing vaccines cause harm, that the presence of aluminium at the injection site (the MMF "tattoo") is related to any autoimmune syndrome, and that HPV DNA fragments are responsible for inflammation, cerebral vasculitis or other immune-mediated phenomena."http://www.who.int/vaccine_safety/committee/topics/hpv/GACVS_Statement_HP_V_12_Mar_2014.pdf Efficacy against vaccine type high grade cervical intraepithelial neoplasia CIN3 (the

well-established precursor of cervical cancer(7)) has been demonstrated for the vaccines in the relevant RCTs (8-10). Cervical cancer cannot be used for ethical reasons as an end point in clinical trials (7). With regard to screening, contrary to the misleading comments by Dr. Lee, there is a large and authoritative body of evidence (including many RCTs) showing that any of the approved HPV tests is substantially more sensitive for detection of CIN2, CIN3, or cancer than cytology (11). The figure he quotes of 58% sensitivity is the result of a controversial application of “verification bias adjustment” in detection of CIN2 or worse, in a single trial. Large systematic reviews have consistently reported much higher sensitivity of HPV testing compared to cytology (12). The sensitivity of HPV testing is not at issue; rather specificity is a concern. As we emphasized in the article, HPV testing does require a secondary triage method to identify persistent infection and cancer precursors that require treatment, because HPV is very common and most infections “clear”. There are several choice of triage strategy prior to treatment; HPV typing and cytology or its analogues are most often proposed. Automated methods will soon be available. Carcinogenic human papillomavirus infections are a global public health problem, >80 % of the annual ≥530,000 cervical cancer cases occur in resource poor countries in which the disease is often incurable (13). Whatever preventive measures are adopted, evaluating the impact of interventions to control infection and disease requires a global perspective; from this perspective the promise of HPV vaccination and HPV testing are overwhelmingly supported by highly credible data. • Mark Schiffman • , John Doorbar • , Nicolas Wentzensen • , Silvia de Sanjosé • , Carole Fakhry • , Bradley J. Monk • , Margaret A. Stanley • & Silvia Franceschi

1. Bachmann MF, Zinkernagel RM. The influence of virus structure on antibody responses and virus serotype formation. *Immunology today*. 1996;17(12):553-8.
2. Harro CD, Pang YY, Roden RB, Hildesheim A, Wang Z, Reynolds MJ, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst*. 2001;93(Feb 21. 4): 284-492.
3. Vichnin M, Bonanni P, Klein NP, Garland SM, Block SL, Kjaer SK, et al. An Overview of Quadrivalent Human Papillomavirus Vaccine Safety: 2006 to 2015. *Pediatr Infect Dis J*. 2015;34(9):983-91.
4. Moreira ED, Jr., Block SL, Ferris D, Giuliano AR, Iversen OE, Joura EA, et al. Safety Profile of the 9-Valent HPV Vaccine: A Combined Analysis of 7 Phase III Clinical Trials. *Pediatrics*. 2016.
5. Descamps D, Hardt K, Spiessens B, Izurieta P, Verstraeten T, Breuer T, et al. Safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for cervical cancer prevention: A pooled analysis of 11 clinical trials. *Hum Vaccin*. 2009;5(5).
6. Angelo MG, Zima J, Tavares Da Silva F, Baril L, Arellano F. Post-licensure safety surveillance for human papillomavirus-16/18-AS04-adjuvanted vaccine: more than 4 years of experience. *Pharmacoepidemiol Drug Saf*. 2014;23(5):456-65.
7. Pagliusi SR, Teresa Aguado M. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine*. 2004;23(Dec 16. 5):569-78.
8. Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *The New England journal of medicine*. 2007;356(19):1915-27.
9. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsague X, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol*. 2012;13(1):89-99.
10. Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *The New England journal of medicine*. 2015;372(8):711-23.
11. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJ; International HPV screening working group.. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*. 2014 Feb 8;383(9916):524-32. Erratum in: *Lancet*. 2015 Oct 10;386(10002):1446.
12. Arbyn M, Ronco G, Anttila A, Meijer CJLM, Poljak M, Ogilvie G et al. Evidence Regarding Human Papillomavirus Testing in Secondary Prevention of Cervical Cancer. *Vaccine*. 2012; 30 Suppl 5:F88-99.
13. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob Health*. 2016 Sep;4(9):e609-16. doi: 10.1016/S2214-109X(16)30143-7.

- [Sin Hang Lee](#) 2017 Jan 19 12:27 p.m.

Schiffman and colleagues finally admitted in the end of the abstract that implementation of HPV vaccination and screening globally remains a challenge. However, the authors did not present the whole truth for a balanced analysis.

1. The statement “Currently available HPV vaccines consist of VLPs comprising the major HPV coat protein L1. VLPs have the geometry of the native virus particle but lack DNA and are non-infectious” was ambiguously constructed to hide the fact that the HPV vaccine Gardasil does contain recombinant HPV L1 gene DNA fragments [1, 2]. Viral DNA fragments are potent pathogen-associated molecular patterns (PAMPs) [3] for the receptors of the innate immune system in stimulating antiviral gene transcription to generate interferons and pro-inflammatory cytokines [4], including the myocardial depressant tumor necrosis factor (TNF)- α and IL-1 β [5].
2. The authors highlighted the efficacy of HPV VLP vaccines in generating very high serum antibody concentrations, but avoided mentioning that this can be achieved only with a special aluminum-based adjuvant, for example, the patented amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant for Gardasil [6] or another proprietary adjuvant as mentioned for Cervarix.
3. The authors avoided acknowledging the role of free DNA molecules released from the dying host cells at the site of vaccine injection [7, 8], commonly referred to as damage-associated molecular patterns (DAMPs) [9] serving as the mediator in enhancing immunogenicity of the antigens in vaccinology [7, 8]. Free DNA molecules bind the aluminum adjuvant particles through a ligand exchange process between the phosphate groups of the DNA and the hydroxyl groups on the aluminum adjuvant surface, and can be transfected into macrophages (APCs) and transported throughout the body [10]. The APCs can recognize the HPV DNA as PAMPs and release antiviral interferons and pro-inflammatory cytokines [3, 4, 9]. The antiviral innate immune response to PAMPs may have triggered generation of the extremely high levels of antibodies against the VLPs in HPV vaccination [3, 4, 9]. Highly augmented immune responses may be harmful to some genetically or physically predisposed persons, causing unexpected adverse reactions.
4. The statement “All vaccines are highly efficacious and are without major adverse effects, conferring virtually complete protection ...” is not supported by solid scientific data. HPV vaccines were designed to prevent a viral infection which is self-regressing in >90% of the cases at the cost of >\$50 million for 100,000 vaccinated young people in the United States. The claimed potential life-saving three-decades down the road at a yet-to-be-proven 70% successful rate is 1 in 100,000 vaccinated females because statistically only 1.7 women among 100,000 die of cervical cancer each year [11]. Even this single cervical cancer death can be prevented by making the cervical screening program more effective and more available to the general population. Cervical cancer is primarily a disease among unscreened or rarely screened women [12].
5. The authors presented no evidence that HPV vaccination has prevented a single cervical cancer, using survival or irreversible morbidity as the endpoint required by drug law for efficacy evaluation. The primary endpoints used for clinical trials were persistent infection (PCR positive for one HPV type on two consecutive visits at least 6 months apart) or low grade cervical disease. Efficacy in the prevention of high grade cervical disease has not been established [13]. In one study conducted in ~3800 subjects (women aged 24 to 45 years), who were randomized 1:1 to receive Gardasil or AAHS control, there were 7 deaths among the 1911 Gardasil-vaccinated and one death among the control subjects [13]. Such high death rate in any vaccine clinical trials is disturbing. There are no efficacy studies on adolescent girls 9-13 years of age now targeted for mass HPV vaccination.
6. The authors avoided the facts that serious adverse event was reported in 2.3% of the Gardasil9 vaccinees and in 2.5% of the Gardasil vaccinees in clinical studies [14]. Among 12,424 reported adverse events following Gardasil vaccination there were 32 deaths with a mean age of 18 years old, 6 confirmed to be cardiac-related [15]. The case of a 14-year old healthy boy who died of a recent myocardial infarct after Gardasil vaccinations was filed in the vaccine court [16]. These sudden unexpected cardiac deaths of healthy teenagers after HPV

vaccination raise concerns about the potential link between excess pro-inflammatory cytokines and myocardial damage.

7. The authors proposed replacing the traditional cervical cytology screen with HPV screen. However, they avoided acknowledging that the CIN3+ detection sensitivity of screening algorithm with commercial HPV test kits is only 58.26% [17] and that these test kits cannot distinguish persistent high-risk HPV infection, the real virologic indication of cancer risk, from consecutive transient infections by different HPV strains through exposures to multiple sources because these test kits may generate 40% errors in HPV detection or genotyping in testing real patient samples [18].

The misrepresentation and omission of facts should be considered if this Primer is used for health care policy decision making.

Sin Hang Lee Milford Molecular Diagnostics Laboratory Shlee01@snet.net

References

1. <http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm276859.htm>
2. Lee SH. J Inorg Biochem. 2012;117:85.
3. Orzalli MH, Knipe DM. Annu Rev Microbiol. 2014;68:477.
4. Schiffman, et al. Nat Rev Dis Primers. 2016;2:16086.
5. Fernandes CJ Jr, de Assuncao MS. Crit Care Res Pract. 2012;2012:896430.
6. US Patent 7,709,010 B2
7. Marichal T, et al. Nature Medicine 2011;17: 996.
8. McKee AS, et al. Proc Natl Acad Sci U S A. 2013;110:E1122.
9. Paludan SR, Bowie AG. Immunity 2013;38:870.
10. Mold M, et al. Sci Rep. 2016: 6: 31578.
11. <https://healthcaredelivery.cancer.gov/icsn/cervical/mortality.html>
12. Janerich DT, et al. Am J Public Health 1995; 85: 791.
13. <http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM251763.pdf>
14. <http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM426457.pdf>
15. Slade BA, et al. JAMA 2009; 302:750.
16. Court of Federal Claims No: 15-0160V <http://www.gpo.gov/fdsys/pkg/FR-2015-03-19/html/2015-06279.htm>
17. Austin RM, Zhao C. Cytojournal 2014;11:14.
18. Lee SH, et al. Int J Mol Sci. 2016; 17:229.