WHITE PAPER

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Review of the current published evidence on single-dose HPV vaccination

2nd Edition

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Single-Dose HPV Vaccine
EVALUATION CONSORTIUM

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Abbreviations

2vHPV	Bivalent HPV vaccine
4vHPV	Quadrivalent HPV vaccine
9vHPV	Nonavalent HPV vaccine
AAHS	Amorphous aluminium hydroxyphosphate sulfate
ACIB	Agencia Costarricense de Investigaciones Biomédicas
AGW	Anogenital warts
AIN	Anal intraepithelial lesions
AIS	Adenocarcinoma in situ
aOR	Adjusted odds ratio
aRR	Adjusted relative risk
AS04	Adjuvant system 04
BCR	B-cell receptors
BMI	Body mass index
BPV	Bovine papillomavirus
CDC	US Centers for Disease Control and Prevention
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN2+	CIN grade 2 or worse
CIN3	CIN grade 3
CT	*Chlamydia trachomatis*
CV	Coefficient of variation
CVT	Costa Rica HPV vaccine trial
DoRIS	A dose reduction immunobridging and safety study of two HPV vaccines in Tanzanian girls
ELISA	Enzyme-linked immunosorbent assay
ESCUDDO	Scientific evaluation of one or two doses of the bivalent or nonavalent prophylactic HPV vaccines
EU	ELISA unit
EPI	Expanded programs on immunization
GDP	Gross domestic product
GM	Geometric mean
GMT	Geometric mean neutralization titer
GuHCl	Guanidine hydrochloride
HAV	Hepatitis A vaccine
HIC	High income countries
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
HSPG	Heparan sulfate proteoglycan
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
ICD-9	International classification of disease, ninth revision
IRB	Institutional review board
IVIR-AC	Immunization and vaccines implementation research advisory committee
LLOQ	Lower limit of quantitation

LLPCs	Long lived plasma cells
LMIC	Low- and middle-income countries
LSHTM	London School of Hygiene and Tropical Medicine
LTFU	Long-term follow-up
LSIL	Low-grade squamous intraepithelial lesions
MeSH	Medical subject headings
MFI	Median fluorescence intensity
MHC	Major histocompatability complex
MHMS	Ministry of Health and Medical Services
MSM	Men who have sex with men
M-TVC	Modified total vaccinated cohort
Nabs	Neutralizing antibody
NCI	US National Cancer Institute
OR	Odds ratio
PATRICIA	PApilloma TRIal against Cancer In young Adults
PBNA	Pseudovirion-based neutralization assay
PSV	Pseudovirion
NITAG	National immunization technical advisory group
NNV	Number needed to vaccinate
QALY	Quality-adjusted life year
RCT	Randomized controlled trial
RGCB	Rajiv Gandhi Centre for Biotechnology
RITAG	Regional immunization technical advisory group
RR	Relative risk
SAGE	Strategic advisory group of experts
SEAP	Secreted alkaline phosphatase neutralization assay
SES	Socioeconomic status
UBC	University of British Columbia
U Laval	Université Laval
UCG	Unvaccinated control group
UK	United Kingdom
UMIC	Upper middle-income country
Wits RHI	Wits Reproductive Health and HIV Institute
WHO	World Health Organization
US	United States
VE	Vaccine efficacy
VLP	Virus-like particle

1 Introduction

Prophylactic human papillomavirus (HPV) vaccines have been licensed for over ten years. They were initially administered as a three-dose regimen over a six-month period. In 2014, following a review of the evidence for dose reduction by the World Health Organization (WHO) Strategic Advisory Group of Experts (SAGE) on Immunization, a two-dose regimen for individuals less than 15 years of age was recommended. Since that time, evidence from observational studies suggests that a single dose of HPV vaccine may also provide protection against HPV infection and its sequelae.

The primary objective of this White Paper is to summarize and assess the current evidence that could support a change to a single-dose schedule of HPV vaccine. The White Paper also aims to identify gaps that remain in determining whether a single dose could be sufficiently protective to have a major impact against HPV infection and its sequelae within the context of immunization programs.

This White Paper has been compiled by a working group of the Single-Dose HPV Vaccine Evaluation Consortium, whose members represent technical depth, a wide global reach, and extensive expertise in immunization programs, HPV vaccine introductions, and vaccine policy. Coordinated by PATH, the Consortium includes the London School of Hygiene & Tropical Medicine (LSHTM), US Centers for Disease Control and Prevention (CDC), Harvard University (Harvard), US National Cancer Institute (NCI), Université Laval (U Laval), University of British Columbia (UBC), and the Wits Reproductive Health and HIV Institute (Wits RHI) at the University of Witwatersrand.

The Consortium leverages the experience of expert groups working in HPV vaccine and other vaccine introductions. Members represent groups that have actively generated evidence for HPV vaccine safety and efficacy, as well as post-licensure effectiveness and delivery. They have implemented HPV vaccine delivery programs in numerous countries, comprehensively evaluated the delivery and impact of HPV vaccines, and contributed to both WHO- and Gavi-led global vaccine policy processes.

The agencies also complement each other at both the global and country level through their existing work with WHO, SAGE, Gavi, ministries of health, regional immunization technical advisory groups (RITAG), national immunization technical advisory groups (NITAG), and national expanded programs on immunization (EPI programs). Specific contributors are listed in **Appendix table 1**.

2 Background

2.1 Cervical cancer burden

Invasive cervical cancer (ICC), caused by persistent infection with HPV, is a major public health problem, especially in developing countries (1). **As of 2018, the International Agency for Research on Cancer (IARC) estimates that there are nearly 570,000 new cases of cervical cancer and over 311,000 cervical cancer-related deaths per annum globally, with over 85% of ICC cases occurring in low- and middle-income countries (LMIC) (2, 3).** In settings where effective cervical screening programs are available, the incidence of cervical cancer markedly decreased after their introduction (3, 4). However, in many developing countries, screening programs are not in place or are only available on a limited scale. This means that women frequently present late with the disease, leading to high associated morbidity and mortality rates.

2.2 Licensed HPV vaccines

Primary prevention for cervical cancer is now possible through vaccination with one of three licensed vaccines: the bivalent vaccine (2vHPV) contains L1 antigens from HPV 16 and 18, the quadrivalent vaccine (4vHPV) contains L1 antigens from HPV 6, 11, 16, and 18, and the nonavalent vaccine (9vHPV) contains L1 antigens from HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. These vaccines are highly efficacious against persistent infection with vaccine genotypes, a necessary prerequisite for the development of cervical cancer and related cervical lesions (5).

2.2.1 Vaccine composition

All three vaccines contain virus-like particles (VLPs) of the L1 protein produced in cultured cells and are formulated with adjuvants to increase their immunogenicity. The vaccines differ in several aspects, including HPV types targeted, valency, dose, substrate, and adjuvant (summarized in Table 1).

Although the 4vHPV and 9vHPV vaccines are produced by the same manufacturer with similar substrate and adjuvant, there are several differences between the two. In addition to the five additional VLPs, the 9vHPV has an increased amount of VLPs for HPV 6, 16 and 18 compared to the 4vHPV (6). While the 4vHPV and 9vHPV vaccines contain the same

adjuvant (Amorphous Aluminium Hydroxyphosphate Sulfate [AAHS]), the 9vHPV vaccine contains more than twice the adjuvant content of the 4vHPV vaccine (500 μg vs. 225 μg).

The 2vHPV vaccine has the lowest VLP dose of the three vaccines (Table 1). It contains a novel adjuvant for enhanced immunogenicity called the Adjuvant System 04 (AS04). AS04 is a combination of the Toll-like receptor 4 agonist monophosphoryl lipid A (MPL) and aluminium hydroxide, which provides direct stimulation of antigen-presenting cells, pronounced cellular and humoral immune responses, and long-lasting antibody responses (7). The 2vHPV vaccine contains a similar amount of aluminium salt as the 9vHPV vaccine. None of the vaccines contain a preservative.

2.3 HPV vaccine schedules and introduction

Uptake of HPV vaccines since their introduction in 2006 has been highly variable and broadly correlated with country income levels. Programs were initially predominated by high income countries (HIC) in Europe, the Americas, and Australia. Tiered pricing later facilitated introduction in middle-income countries but, for several years, introduction in low-income countries (LIC) was largely dependent on external support for limited-scale demonstration projects. In 2012, Gavi initiated support for HPV vaccination to encourage introduction in LIC.

In 2014, the WHO SAGE on Immunization revised its recommendations from a schedule of three doses to two doses, administered at an interval of at least six months, for the 2vHPV and 4vHPV vaccines for girls aged 9 to 14 years old (8). This revised recommendation was based on evidence of non-inferior VLP antibody responses in female adolescents aged 9 to 14 years compared with women for whom efficacy was demonstrated in clinical trials with a three-dose schedule (9-11). WHO guidelines allow for dosing flexibility for the second dose of the two-dose schedule as early as five months. According to the recommendations, persons aged ≥15 years or immunocompromised, including those who are HIV-infected, should continue to receive three doses as per original dosage recommendations (8).

Despite the fact that LMIC bear the greatest burden of cervical cancer and the highest mortality rates due to the disease (2), introduction of HPV vaccine has been substantially more widespread among HIC than LIC. This, combined with a wider age range target in developed countries (compared to single or more restricted-year cohorts in LMIC, such as 9-year-olds or 12- to 13-year-olds), has meant that the proportion of vaccinated 10- to 25-year-old females is substantially higher in HIC and upper middle-income countries (UMIC) than in LIC (12).

A number of factors have influenced the slower introduction of HPV vaccines in LMIC. These include the initial cost of the vaccines and a delay in provision of financial mechanisms to support countries in obtaining the vaccine, which was partly due to the financial climate when HPV vaccines became available. Other challenges have included absence of a mechanism for rapid vaccine introduction, previous Gavi requirements that demonstration projects be conducted if the country had no prior experience of HPV vaccine delivery or adolescent multidose schedules, low prioritisation of cervical cancer as a public health problem, and perceptions that the vaccine is difficult and expensive to deliver (13).

A recent study collating evidence and lessons learned from HPV vaccine delivery in 37 LMIC found that the countries that did introduce HPV vaccine, either through demonstration projects or national programs, achieved high coverage, especially if their programs or demonstration projects incorporated school-based delivery strategies (14).

However, key informants from LMIC reported that the sustained financial commitment for the cost of vaccine procurement and vaccine delivery has been a key factor in their governments' hesitancy to commit to national HPV vaccine introduction (14). Various approaches to making the HPV vaccine more affordable for LMIC have been suggested, including integrating vaccination into existing adolescent or school-health programs. Integration has proved challenging in many settings since these programs may be vertically funded, only operating in selected districts of a country or not functioning effectively (14).

A single-dose regimen for HPV vaccines could be another way to reduce costs and simplify delivery. A dose-reduction recommendation to a single-dose regimen could potentially reduce the costs of vaccine supply and delivery, since different delivery strategies might be available for a single-dose schedule (e.g. integration with measles campaigns). This could, in turn, increase accessibility and sustainability of the vaccination programs in both Gavi-eligible and non-eligible countries. Single-dose delivery of HPV vaccines is now of interest for a number of reasons following accumulating evidence along several lines: biologic plausibility based on understanding of host-virus interactions at the mucosal level; data from randomized, observational and registry studies; and vaccine impact modeling assessments. These topics are reviewed below.

2.4 Rationale for this White Paper

As discussed above, the cost of the HPV vaccine and its delivery in a multi-dose schedule have created barriers to HPV vaccine introduction and program sustainability in LMIC. Some observational data and biologically plausible mechanisms exist to suggest that a single dose of HPV vaccine may be sufficient to elicit a protective immune response against

incident and persistent HPV infection, which are the necessary prerequisites to further development of cervical lesions and, in the longer term, cervical cancer. Randomized controlled trials (RCT) are underway to provide high-quality evidence to assess this hypothesis (15-18).

This document is an updated version (2nd Edition) of a previously published White Paper (1st Edition, 30 April 2018) (19), aiming to assess (i) the current evidence on efficacy, effectiveness, immunogenicity and modeling of single-dose schedules of HPV vaccine, (ii) the strength of that evidence, and (iii) the gaps in the evidence. The Second Edition of the White Paper builds on the first by including further evidence published up to the end of March 2019. Significant updates made in the Second Edition of the White Paper compared to the first are highlighted in bold. It presents the current evidence base together in one document in order to facilitate access to and understanding of the myriad of individually published scientific studies that comprise the evidence base as a whole.

It is envisaged that this White Paper could be used in early policy conversations with key global stakeholders, such as the WHO Immunization and Vaccines Implementation Research Advisory Committee (IVIR-AC) and SAGE. It may help to highlight what information is needed for policy deliberations and help clarify a timeline for when new evidence addressing critical unanswered questions will become available for use in these discussions.

The White Paper includes a detailed summary of published evidence; interpretation of the implications of the results relevant to single-dose HPV vaccine immunogenicity, efficacy, or effectiveness; identification of gaps in the evidence; discussion of possible approaches (and the ethical considerations therein) to fill such gaps; description of any known studies or datasets that might be ongoing or available that could address evidence gaps; and an overall conclusion for the strategic direction needed to inform decisions about HPV single-dose or alternative schedules.

Table 1. Summary of available HPV vaccines

	Bivalent HPV vaccine (2vHPV)	Quadrivalent HPV vaccine (4vHPV)	Nonavalent HPV vaccine (9vHPV)
Manufacturer	GlaxoSmithKline	Merck & Co, Inc.	Merck & Co, Inc.
Trade name	Cervarix®	GARDASIL®	GARDASIL9®
HPV VLPs included	16, 18	6, 11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58
L1 protein dose	20 μg (HPV16) 20 μg (HPV18)	20 μg (HPV6) 40 μg (HPV11) 40 μg (HPV16) 20 μg (HPV18)	30 μg (HPV6) 40 μg (HPV11) 60 μg (HPV16) 40 μg (HPV18) 20 μg (HPV31) 20 μg (HPV33) 20 μg (HPV45) 20 μg (HPV52) 20 μg (HPV58)
Substrate	*Trichoplusia ni* (Hi 5) insect cell line infected with L1 recombinant baculovirus	*Saccharomyces cervisiae* (baker's yeast) expressing L1	*Saccharomyces cervisiae* (baker's yeast) expressing L1
Adjuvant	500 μg of aluminium hydroxide and 50 μg of 3-O-desacyl-4'-monophosphory lipid A (GSK AS04 adjuvant)	225 μg of Amorphous Aluminium Hydroxyphosphate Sulfate (AAHS) (Merck aluminium adjuvant)	500 μg of Amorphous Aluminium Hydroxyphosphate Sulfate (AAHS) (Merck aluminium adjuvant)
Injection Schedule (2 doses)[a, b]	0, 6-12 months	0, 6-12 months	0, 6-12 months
Injection Schedule (3 doses)[b, c]	0, 1, 6 months	0, 2, 6 months	0, 2, 6 months

[a] A two-dose schedule is recommended for girls aged 9 to 14y (for 2vHPV or 9vHPV) or 9 to 13y (for 4vHPV). SAGE recommends that the second dose should be administered between months 5 and 13 for 2vHPV and 9vHPV, and at month 6 for 4vHPV. If the second dose is administered earlier than recommended, a third dose should be given (8, 20).

[b] In some countries, the vaccines are also licensed and recommended for boys in the same dosing schedules as for girls.

[c] A three-dose schedule is recommended for girls aged ≥15y (for 2vHPV or 9vHPV) or ≥14y (for 4vHPV). For 2vHPV, SAGE recommend that the second and third doses are administered between months 1 and 2.5, and months 5 and 12, respectively. For 4vHPV and 9vHPV, the second dose should be given at least one month after the first, and the third dose should be given at least three months after the second (8).

Table adapted from (5) and updated for dosing schedule licensure modifications and global vaccination recommendations (8).

3 Sources of evidence

Sources of evidence covered in this White Paper include publicly available peer-reviewed scientific publications on:

- The biological plausibility for protection with single-dose HPV vaccine, based on vaccine immune response and virological data;
- Nonrandomised data from partially-vaccinated participants in clinical trials and immunogenicity studies;
- Data from post-licensure vaccine effectiveness evaluations and other observational data;
- Mathematical modeling of the impact of reduced dosing schedules for HPV vaccines.

3.1 Biological plausibility for protection with single-dose HPV vaccine

Plausible biological explanations for the unexpected potency of HPV subunit vaccines were examined and recently reviewed after observational data from several clinical studies suggested that a single dose of HPV vaccine could provide protection against HPV infection (21).

3.2 Nonrandomised data from partially-vaccinated participants in clinical trials, immunogenicity studies, and post-licensure effectiveness evaluations

Observational data on single-dose HPV vaccination come from both multi-dose vaccine clinical trials (where some participants did not receive a full course of vaccination) and from non-trial observational, phase IV, registry linkages, and other studies.

Specific outcomes of interest examined in the observational clinical studies include:

i. **Efficacy:** Efficacy against HPV infection (genotype-specific prevalence, incidence and/or persistence) or clinical outcomes (e.g. anogenital warts [AGW], cervical intraepithelial neoplasia [CIN]);

ii. **Immunogenicity:** HPV vaccine-type antibody titers or concentrations (used as the primary immunogenicity endpoint), antibody avidity, and B- or T-cell responses (used as secondary immunological endpoints);

Currently, there is no immune correlate, antibody concentration, or other immune measurement that has been defined, which correlates with protection. The

pseudovirion-based neutralization assay (PBNA) is the "gold standard" for detection of HPV antibodies, although comparisons between sero-epidemiological studies are difficult due to the use of different serological assays and lack of a reference serum for establishing cut-off values (22). **The search for an immune correlate of protection has been hampered because there are very few clearly documented "vaccine failures" among vaccine recipients where prior infection could be conclusively excluded and where relevant blood samples were also collected for immunological assessments.**

Immune parameters other than functional (neutralizing) and binding antibody levels, which might correlate with protection, have not been defined; and data on antibody avidity are scarce (23). Antibody avidity indicates the degree of antibody affinity maturation and generally increases over time following an encounter with an antigen. Memory responses are characterized by the production of high-avidity antibodies. Vaccine-derived neutralizing antibody levels correlate with antibody avidity at both six months and one year after HPV vaccination (23, 24).

iii. **Effectiveness and impact**: Effectiveness against HPV infection (e.g., genotype-specific incidence, persistence) or other clinical outcomes (e.g. AGW, CIN).

Published data were compiled from any geographical location that compared at least one of the outcomes of interest after one versus two or three doses of HPV vaccine (in any schedule), or versus no HPV vaccination. **Previously, a comprehensive review of the published literature was undertaken in order to identify potentially relevant studies and articles, which were collated and summarized in the First Edition of the White Paper. In the Second Edition, we report on a number of key updates to the published literature:**

- **A new systematic review of the literature on the efficacy, effectiveness and immunogenicity of a single HPV vaccine dose compared to multi-dose schedules (and compared to no HPV vaccination) from HPV vaccine clinical trials, published between January 1, 1999 and August 14, 2018. The review includes seven articles describing four studies; one conducted in India, one in Costa Rica, one multi-nationally and one in the United States. A narrative quality assessment was conducted for the included trials. An updated literature search for articles published between August 2018 and March 2019 for the purpose of this White Paper (not using systematic review methodology) did not yield any further data from RCTs.**
- **An updated literature search for non-RCT immunogenicity studies published up to March 2019. Five further studies since the First Edition of the White Paper were identified. One was a further evaluation of the previously described Fijian cohort, two were from Canada, and two were from the United States.**
- **An updated comprehensive systematic literature review conducted to include non-trial vaccine effectiveness studies published from June 2017 through March 2019. Nine new papers are included in this review, including two studies from Scotland, four from the United States, one from Canada, and two from Denmark. Therefore, the Second Edition of the White Paper includes a total of 23 studies (summarized below) that examined HPV vaccine effectiveness by number of doses from January 1, 2017 to March 20, 2019.**

Each of these updates is described in detail below (Section 4).

3.3 Mathematical modeling of the impact of reduced dosing schedules for HPV vaccines

In the First Edition of the White Paper, we examined and summarized the published studies of reduced-dose strategies for the 2vHPV, 4vHPV and the 9vHPV vaccines to identify key factors related to the impact of reduced dosages and their cost-effectiveness. A comprehensive literature search conducted since that edition of the White Paper identified only one further analysis evaluating reduced dosage HPV vaccination. The analysis, published as part of a special-issue supplement in the journal *Vaccine* (25), focuses on estimating the impact of single-dose 9-valent HPV (9vHPV) vaccination in HIV-positive and HIV-negative women in South Africa.

4 Results

4.1 Biological plausibility for single-dose protection

Below, we provide a summary of a recently published comprehensive review (21).

4.1.1 Mechanism of vaccine-induced protection

All three available vaccines are produced using recombinant, genotype-specific, viral outer coat L1 proteins. During a natural infection, the L1 protein is only 'visible' to the immune system prior to cell invasion; once a cell is invaded by the virus, the L1 protein locates in the nucleus and is not displayed on the cell surface. Vaccine-induced antibodies to the L1 protein are therefore likely to elicit protection against infection by preventing initial cell invasion events. This mechanism of protection would also explain why already established infections are unaffected by vaccination. The principal mediator of HPV vaccine-induced protection seems to be humoral; however, given the high immunogenicity of the vaccine and the rarity of "breakthrough" infections, the minimum systemic or mucosal antibody level required for protection has not yet been established.

Additionally, it is unknown whether persistent levels of antibodies need to be maintained long term or whether an anamnestic response, mediated by memory B cells, can elicit protection from persistent infection and subsequent disease. It is likely that neutralizing antibodies need to be present at the time of exposure for the HPV vaccines to be most effective (26). Therefore, "long lived plasma cells (LLPCs) that continuously produce antigen-specific antibodies are likely to be the key immune effectors that underlie the strong type-restricted protection induced by the HPV vaccines. It is possible that even the few vaccine recipients with undetectable levels of anti-HPV antibody four years after vaccination remain protected by circulating antibodies, because very low levels of VLP antibodies appear to be sufficient for protection against infection of cervicovaginal tissue" (27).

4.1.2 The immunogenicity of a single vaccine dose

This section was excerpted from a review of evidence on the immunologic considerations of HPV vaccination [15] and edited for this paper.

The exceptionally strong, consistent, and durable antibody responses to the three HPV vaccines is well documented (28). In healthy young women, seroconversion rates are virtually 100%, peak in vitro neutralizing titers of 1,000-10,000 are generally obtained and, after a relatively steep 10-fold drop in titer over the first two years, IgG titers plateau or decline very slowly, stabilizing at levels that are substantially higher than the antibody titers induced by natural infection (29). Responses in pre-adolescent girls and boys are even stronger (9, 30). The stability of antibody responses, now observed for almost 10 years post vaccination (31, 32), is unprecedented for a subunit vaccine.

Surprisingly this pattern of antibody response is observed even after a single-dose of vaccine, with stable geometric mean IgG binding and in vitro neutralizing titers that are about four-fold lower than the plateau titers measured after the standard three doses (33, 34). Avidity, as measured in a VLP-based chaotrope enzyme-linked immunosorbent assay (ELISA), similarly rose over the first four years after immunization with one or three doses of 2vHPV, and then stabilized for both dose regimens (35). The long-term antibody levels, regardless of dose number, are almost certainly due to efficient induction of LLPC, which primarily reside in the bone marrow and continuously produce antibodies, probably independent of additional antigen exposure (36). It is unlikely that successive rounds of memory B-cell activation from putative secondary exposure to virion antigens are primarily responsible for the durable levels, as intermittent increases and decreases in antibody levels would be expected if repeated episodic antigen exposure were involved, while the antibody levels in individuals generally remain constant or decrease at a slow rate. In addition, essentially all vaccinees maintain a stable level of antibodies against the VLP types in the vaccine, and it is doubtful that virtually all the women would have experienced immunizing levels of environmental exposure to each of the multiple genital HPV types targeted by the vaccines. Therefore, the central immunological question is why the HPV vaccines are such potent inducers of LLPCs. The specific structure of the VLPs that comprise the HPV vaccine may be key to their ability to efficiently induce LLPCs.

HPV VLPs are composed of 360 ordered protein subunits that form a particulate 55nm structure displaying a repetitive array of epitopes on their surface. Particles of this size efficiently enter the lymphatic system and traffic to lymph nodes, where they induce primary antibody responses (37). The closely spaced arrangement of determinants on the VLP surface can lead to the stable binding of natural low avidity IgM and complement, thereby promoting acquisition of the VLPs by follicular dendritic cells, which present antigens for the induction of B-cell responses in the lymph node (38). Particles in this size range are also efficiently taken up and processed by phagocytic antigen-presenting cells for Major Histocompatibility Complex (MHC) Class II presentation, leading to the induction of potent T-helper responses (39). Polyvalent binding of the HPV VLPs to human monocytes,

macrophages, and dendritic cells induces the release of a variety of cytokines that may promote antibody induction (40). The ordered display of epitopes at intervals of 50-100Å on the VLP surface is a pathogen-specific danger signal to the humoral immune system (41). Epitope spacing at this distance is found on the surface of most viruses (HIV being a notable exception (42)) and on other microbial structures, such as bacterial pili. Binding and subsequent cross-linking of the B cell receptors (BCR) on the surface of naïve B cells by these ordered repetitive antigens transmit exceptionally strong activation and survival signals (43). Naïve B cells generally express both IgM and IgD BCRs. While both monomeric and repetitive antigens can activate IgM BCRs, signaling through IgD is preferentially activated by repetitive antigens, raising the possibility that IgD BCR crosslinking is an important component in the efficient induction of LLPCs by HPV VLPs (44).

The high-density display on a VLP surface can efficiently break B-cell peripheral tolerance and even reactivate anergic self-reactive B cells (45, 46). The BCRs on a majority of newly produced B cells are thought to bind self-antigens, which renders them functionally anergic (47, 48). The polyvalent interaction of repetitive VLP epitopes might also lead to stable engagement and subsequent B-cell activation through BCRs whose affinity, if they were engaged by a monomeric antigen, would be too low to be activating. These conjectures that identify potential mechanisms for activating a large variety of distinct naïve B-cell clones can provide a mechanistic explanation for the remarkable consistency of VLP antibody responses across individuals.

The above considerations may also help to explain the patterns of antibody responses observed for other classes of vaccines compared to the HPV VLPs. Other subunit vaccines composed of monomer or low valency antigens, such as bacterial toxoids and polysaccharide/protein conjugates, only induce protective antibody responses after several doses and require periodic boosting, as the antibody titers continue to wane over time. This is presumably because these antigens do not deliver the strong signals induced by BCR oligomerization that promote differentiation into LLPCs. Hepatitis B vaccines are multivalent particulate antigens; however, they often do not induce seroconversion after a single dose and generally fail to induce stable antibody responses (49). Induction of LLPCs may be limited because the HBV particles are only 22nm in diameter, the surface antigen in the HBV particles float in a lipid membrane, and there are a relatively small number of repetitive elements (24 knuckle-like protrusions of the surface antigen for HBV compared to 360 L1 molecules arranged into 72 pentameters for HPV) (50). Each of these factors could limit the potentially critical oligomerization and downstream signaling through the BCRs.

Inactivated virus vaccines are particulate and have a dense array of repetitive surface elements, and yet are administered in multiple doses and generally fail to induce stabilizing antibody responses. However, it is likely that the inactivation process (e.g. protein

crosslinking with formalin) disrupts the dense repetitive array of their surface epitopes to ablate their "virus-like" character (51). An exception may be the Hepatitis A inactivated virus vaccine (HAV), which appears to induce durable protective antibody responses after a single dose and therefore may retain a sufficient number of repetitive surface epitopes after inactivation to retain its virus-like character (52).

The observation that live attenuated vaccines, such as yellow fever and vaccinia, induce potent, durable antibody responses and immunity to infection after the primary inoculation in most vaccinees (53) has previously been attributed to the infectious nature of the inoculum. In light of the findings with the HPV vaccines, the alternative explanation—that they are highly immunogenic primarily because they contain authentic virion surface structures—should now be considered.

4.1.3 Virologic considerations

This section was excerpted from a review of evidence on the virologic considerations of HPV vaccination [15] and edited for this paper.

Papillomaviruses have a unique life cycle in which production of virions occurs only in the terminally differentiated layer of a stratified squamous epithelium. However, completion of its productive life cycle depends upon establishing infection in the cells of the basal layer of the epithelium (54). To ensure that initial infection occurs only in basal epithelial cells, the virus cloaks its cell surface receptor binding domain until after it has undergone a series of conformational changes. These changes are induced by binding specifically modified forms of heparan sulfate proteoglycans specific to the basement membrane that separates the dermis from the epithelium (55) (Figure 1).

This unusual strategy of initiating infection on an acellular surface may substantially increase the susceptibility of the virus to serum-derived neutralizing antibodies for a number of reasons (56).

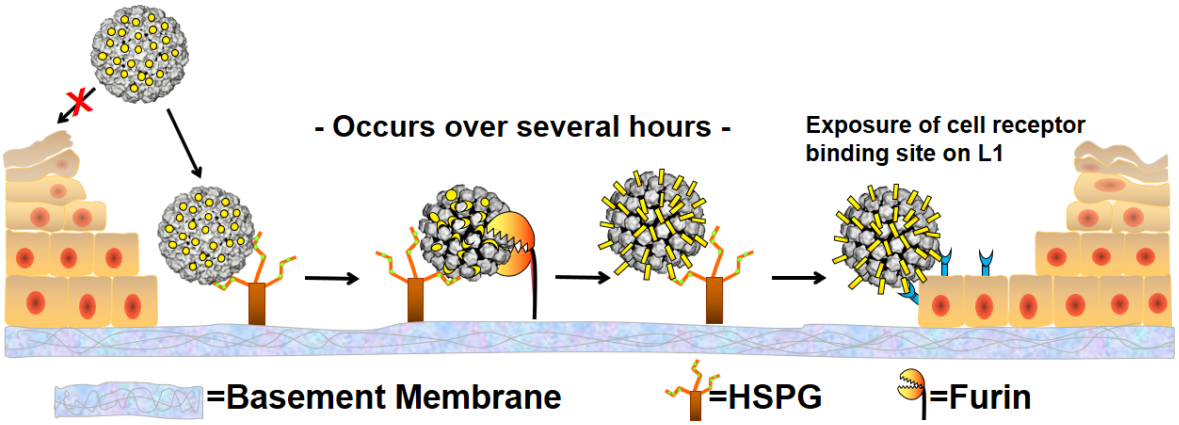
First, exposure of the basement membrane to the virus requires disruption of the epithelial barrier, which results in direct exudation of capillary and interstitial antibodies at these sites. A consequence of this event is that HPV encounters systemic antibodies at potential sites of infection. This mechanism can explain why induction of systemic antibodies via intramuscular vaccination can be so effective in preventing a mucosal infection. There is also significant transudation of systemic antibodies via the neonatal Fc receptor in the female genital tract (57). However, this latter mechanism may play a secondary role in protection, because levels of transudated VLP-specific antibodies in cervical mucus of vaccinated women are 10 to 100-fold lower than serum levels (depending on the stage of the

menstrual cycle) (58) and because the vaccines are highly protective against infections of cutaneous epithelia (e.g. external genital warts), which are not routinely bathed in mucus.

Secondly, the factor that contributes to increased susceptibility of the virus to neutralizing antibodies is the exceptional slowness of the initial stages of the papillomavirus life cycle. In a mouse cervicovaginal challenge model, HPV virions remain on the exposed basement membrane for hours before they attach to the epithelial cells that migrate in to close the disrupted tissue; internalization of the cell-bound virus takes a further several hours (55). Thus, the virions are exposed to neutralizing antibodies for an exceptionally long time. High concentrations of passively transferred VLP antisera can prevent infection by inhibiting basement membrane binding; lower doses that permit basement membrane binding are nonetheless effective at preventing infection (59). The long exposure of antibody-bound virions on the basement membrane and cell surface may make the complexes highly susceptible to opsinization by phagocytes which would also be attracted to the sites of trauma (56). The observation that antibody levels that are more than 100-fold lower than the minimum level detected in the in vitro neutralizing assay are able to prevent in vivo infection are consistent with the idea that there are potent antibody-mediated mechanisms relevant to in vivo inhibition that are not detected in vitro (60).

Thirdly, remarkably low levels of VLP antibodies are protective in vivo. For example, in the mouse cervicovaginal model, circulating antibody levels in recipient mice that were 10,000-fold lower than in the donor HPV16 VLP-vaccinated rabbit potently inhibited infection from high-dose HPV16 cervicovaginal pseudovirus challenge (59). Although the titers of in vitro neutralizing antibodies induced by HPV VLP vaccination are approximately 10-fold lower in humans than in rabbits, it is plausible that the levels of VLPs antibodies in human vaccinees considerably exceed the minimum level required for prevention of genital infection and that protective levels are lower than those that can be reproducibly detected in current in vitro antibody binding and neutralizing assays. Therefore, the four-fold lower, but readily detectable, plateau titers induced by one-dose compared with three-dose vaccine regimens discussed below might not substantially reduce the long-term protection induced by the HPV VLP vaccines.

Figure 1. In vivo murine model of vaginal HPV infection



In Vivo Murine Model of Vaginal HPV Infection. A disrupted cerviovaginal epithelium is depicted. "X" indicates the inability of virions to bind the apical surface of intact epithelium. HSPG = heparan sulfate proteoglycan. The L2 minor capsid protein, cleaved by furin after a HSPG binding-induced conformational change in the capsid, is shown in yellow.

Figure adapted from (21).

4.2 Clinical trials of HPV vaccines

This section summarizes evidence on the efficacy, effectiveness and immunogenicity of a single HPV vaccine dose compared to multi-dose schedules (and compared to no HPV vaccination) from clinical trials of HPV vaccines. Evidence is primarily derived from a recent systematic review aimed at evaluating the literature on single-dose HPV vaccination from clinical trials (61). When the database search for the systematic review was conducted (August 2018), there were no data on the immunogenicity, efficacy, or effectiveness of single-dose HPV vaccination compared to two- or three-dose schedules that originated from specifically designed randomized controlled trials comparing one-dose to two- or three-dose groups. Only one small randomized, unblinded pilot intervention study in ten individuals compared immunological responses in HPV16-seropositive women after a single dose with no vaccination (62). Thus, most evidence comes from comparisons made between clinical trial participants who completed and failed to complete standard two- or three-dose schedules.

This systematic review will be updated regularly. However, an updated search of the literature up to March 31, 2019 (not using the systematic review methodology), conducted for the Second Edition of the White Paper, yielded no further RCT data on one dose of HPV vaccine versus two or three doses, or versus no HPV vaccination. One new publication describes the protocol for a new trial of single-dose HPV vaccination (63), and this is described further in Section 7.1.

Additional information is also presented in the Second Edition of the White Paper from a 2018 Cochrane review on the efficacy of HPV vaccines, which presents data on 'at least one' dose of HPV vaccine compared to non-HPV vaccinated controls (64).

The trials-based evidence obtained from systematic reviews provided in the Second Edition of the White Paper has largely replaced the individual descriptions of two vaccine trials (the Costa Rica Vaccine Trial [CVT] and the IARC India HPV Vaccine Trial) that were presented in the First Edition. However, given that these studies still form the majority of the trials-based evidence base on single-dose HPV vaccination, key excerpts from the First Edition of the White Paper are retained here.

4.2.1 Systematic review of evidence on single-dose HPV vaccination from clinical trials

The available literature from RCTs on the immunogenicity and efficacy of single-dose HPV vaccination compared to either no vaccination or to multi-dose schedules was systematically reviewed in a recent study (61). The research questions were:

- **Does a single dose of HPV vaccine provide equivalent efficacy against HPV infection and associated clinical outcomes, and produce non-inferior immune responses, compared to a two-dose or three-dose HPV vaccination schedule?**
- **Does a single dose of HPV vaccine provide efficacy against HPV infection and associated clinical outcomes compared to no HPV vaccination?**

The systematic review was specifically designed to identify clinical trials that randomized participants to receive one dose of HPV vaccine versus no dose or multiple doses, as well as trials in which some participants received only one dose due to non-completion of a multi-dose schedule.

The following sections include excerpts from the trials systematic review (61). The content was edited for the Second Edition of the White Paper.

4.2.1.1 SEARCH STRATEGY

Medline, EMBASE, Global Health Database and Cochrane Central Register of Controlled Trials were searched systematically for publications and conference abstracts using medical subject headings (MeSH) and non-MeSH terms under the following themes: human papillomavirus AND vaccines AND (immunogenicity OR efficacy OR effectiveness) AND dosage. MeSH terms and operators were adapted as required for each database searched. Searches were limited to articles published between January 1, 1999 and August 14, 2018, and (where allowed by the database) studies conducted in humans. No language restrictions were applied.

Reference lists of relevant review articles and all full-text articles identified for inclusion through the database searches were additionally hand-searched.

4.2.1.2 ELIGIBILITY SCREENING

Search results were screened using pre-defined eligibility criteria, based on the population, intervention, comparison, outcome, or PICO format. Titles and abstracts of all search results were double-screened for eligibility based on a limited number of eligibility criteria; articles were excluded if they did not describe a research study of human participants who had received 2vHPV, 4vHPV, or 9vHPV, and/or did not generate data on immunogenicity, infection, and/or disease outcomes. Full texts of all remaining and potentially relevant publications were subsequently double-screened against full eligibility criteria.

4.2.1.3 DATA EXTRACTION, QUALITY ASSESSMENT, DATA SYNTHESIS AND ANALYSIS

Data were extracted using a standardized extraction form. Extracted data included: publication details; target population and setting; study design; study population; intended and actual intervention and comparators; evaluated outcomes; results and findings; and authors' conclusions.

Included studies were assessed for selection bias (i.e. the selection of participants in each dose group), confounding, retention and survival bias, misclassification of exposure and outcome, and statistical analysis approach. Study populations were evaluated for generalizability. Where articles described a sub- or post-hoc analysis of a clinical trial cohort, the 'parent' clinical trial population was additionally assessed for generalizability. Biases were specifically assessed for the probability that they would artificially increase the vaccine efficacy in the one-dose group, or artificially decrease the vaccine efficacy in the three-dose group.

A narrative synthesis of the data was conducted using three elements: (i) development of a preliminary synthesis of findings of included studies; (ii) exploration of relationships within and between studies; and (iii) assessment of the robustness of the synthesis.

Infection endpoints evaluated in this review were as reported in included studies. To standardize statistical reporting of incidence risk, persistence and prevalence, event and denominator data extracted from each article were used to calculate proportions (expressed as percentages (%)) and 95% confidence intervals (95%CI), using the exact (Clopper-Pearson) method for calculating CIs for proportions, assuming a binomial distribution. Unadjusted infection risk ratios (RRs) and

prevalence ratios (PRs) were calculated for one- versus two- or three-dose HPV vaccine arms, and for one-dose HPV vaccine versus control (no HPV vaccine) arms. The Haldane-Anscombe correction was used for calculation of RRs and PRs where no events were detected in one or both comparison arms. Fisher's exact test (2-sided) was used to assess for statistical significance between the groups and compute p values. RRs and PRs calculated for one versus two or three doses must be interpreted with caution because of potential for selection bias due to differences in follow-up between the groups.

In the absence of a known correlate of protection for HPV vaccination, data capture for this systematic review were not limited to a specified humoral immunogenicity endpoint and instead included any data on binding and/or neutralising antibody seropositivity, titres and/or avidity. To standardize statistical reporting of seropositivity results, extracted data on numbers of participants seropositive for HPV16/18 antibodies and denominator data were used to calculate seropositivity proportions (%) and 95%CIs, as above.

Pooling and meta-analysis of data from multiple studies was not considered appropriate due to heterogeneity in study designs and methods.

4.2.1.4 SEARCH RESULTS

Of 6,523 unique records identified from the database and hand searches, seven articles were included in this review (33, 34, 62, 65-68) (Figure 2; Table 2). Of these, six were considered as observational studies because allocation to the dosing schedule arms (i.e. one dose versus alternative schedules or no vaccination) was according to what participants actually received rather than participants being prospectively allocated to a specific dosing schedule (33, 34, 62, 65-68). One small randomized study prospectively allocated participants to one HPV vaccine dose versus no vaccination (62).

4.2.1.5 NESTED OBSERVATIONAL STUDIES OF SINGLE-DOSE HPV VACCINATION

All six observational studies were based on data from three clinical trials. Two studies (33, 68) were based on the International Agency for Research on Cancer (IARC) Trial of Two Versus Three Doses of HPV Vaccine in India (33). Three studies (34, 65, 67) were based on the Costa Rica Vaccine Trial (CVT) (69), and one (66) was based on combined data from CVT and the PApilloma TRIal against Cancer In young Adults (PATRICIA) Trial (70).

IARC India HPV Vaccine Trial

This study was designed as an open-label cluster-randomized trial aiming to compare two versus three doses of 4vHPV among healthy unmarried females aged 10 to 18 years in India (33, 71). Participants were recruited from 188 geographical clusters across nine locations from September 2009 and randomized to either two- or three-dose arms. However, in April 2010, the Indian government suspended all HPV vaccine trials for reasons not related to the IARC India HPV Vaccine Trial, and enrolment into the trial therefore stopped early. At the point of suspension, 17,729 participants had been recruited (88.6% of the targeted recruitment of 20,000 girls), but many had not yet completed their full dose schedules. Thus, the clinical trial of two versus three HPV vaccine doses became a prospective observational cohort study of one versus two versus three vaccine doses.

Of the two identified publications arising from the IARC India HPV Vaccine Trial, the first presents HPV infection and immunogenicity data up to 48 months following the first vaccine dose for participants who received one dose (at day 0), two doses (at day 0 and either month 2 or month 6), and three doses (at day 0, month 2 and month 6) (33). The second presents immunogenicity data up to 48 months, and HPV infection data up to seven years, following the first vaccine dose for the same dosing schedules (68). A supplementary cohort of married, unvaccinated females aged 18-23 years (corresponding to the age of the married vaccinated females at the time of follow up) was recruited from different study sites in India during 2012 to 2015, allowing comparison of HPV infection data between participants vaccinated with one, two, or three doses and those who had not received any vaccine doses.

CVT

This was a community-based double-blind RCT aimed at evaluating the efficacy of a three-dose regimen of 2vHPV against persistent vaccine type-specific HPV infection and subsequent development of HPV-associated pre-cancerous lesions among healthy women aged 18-25 years in two regions of Costa Rica (69, 72). A total of 7,466 women were recruited from seven study clinics between June 2004 and December 2005, all of whom were randomized to receive three doses of either HPV vaccine or Hepatitis A vaccine (HAV; control). Some women did not complete their full vaccination schedule for reasons including pregnancy, colposcopy referral, other medical conditions, vaccine refusal or missed study visits.

The first identified one-dose study arising from CVT describes a post-hoc analysis of HPV infection data up to 48 months following first vaccine dose in participants

who received one dose (at day 0), two doses (at day 0 and either month 1 or month 6), and three doses (at day 0, month 1 and month 6) (65). The second study describes a post-hoc analysis of HPV vaccine-induced immunogenicity up to month 48 for the same dosing schedules (34). A subsequent manuscript extends the HPV infection and immunogenicity data from this study to seven years following first vaccine dose. At the completion of the randomized, blinded phase of CVT, control participants were offered the HPV vaccine (67). Thus, for the most recent (2018) study, a new cohort of 2,836 unvaccinated women, age-matched to the trial participants, were recruited to replace the original control group.

PATRICIA Trial

This was a large-scale, phase III, double-blind RCT among healthy women aged 15-25 years from 14 countries in Asia Pacific, Europe, Latin America and North America, also aiming to evaluate the efficacy of a three-dose regimen of 2vHPV (70). The PATRICIA trial enrolled 18,729 women between May 2004 and June 2005, all of whom were randomized to receive three doses of HPV or HAV (control). 18,644 women received at least one vaccine dose; some participants did not receive all scheduled doses for similar reasons as in CVT.

One study identified for inclusion in the systematic review reports a post-hoc analysis of combined CVT and PATRICIA trial data (66). This publication describes HPV infection data up to 48 months following first vaccine dose in participants who received one dose (at day 0), two doses (at day 0 and either month 1 or month 6), and three doses (at day 0, month 1 and month 6).

4.2.1.6 RANDOMISED INTERVENTION STUDY OF SINGLE-DOSE HPV VACCINATION

The only randomized intervention study was a small pilot study conducted in the USA aimed at evaluating antibody and memory B-cell responses following one dose of HPV vaccine compared with no vaccine in participants with prior HPV16 infection (62). The study randomized ten healthy HPV16-seropositive women aged 27-45 years at day 0 to receive a single dose of 4vHPV or no intervention. Humoral and cellular immunogenicity results for the two arms are presented up to month 6.

4.2.1.7 HPV16 AND HPV18 INFECTION RESULTS

HPV16/18 infection results for participants who received one HPV vaccine dose compared to any comparator group are reported in five of the included studies (33, 65-68). HPV infection-related outcome measures most commonly reported include one-time or cumulative incident infection, and six or 12-month persistent

infection. Three studies report results up to four years post vaccination (65, 66), and two studies report results up to seven years (67, 68). Methods used for detection of infection and definitions of endpoints reported by each of the five studies are summarized in Table 3.

Table 4 ***summarizes efficacy results for each of the five studies. In brief, incident, persistent and prevalent infection with HPV16/18 were extremely low in all participants who received any HPV vaccine, and significantly lower than participants who were either unvaccinated or received HAV. All studies reported comparable efficacy against HPV16/18 infection in one-dose and two- or three-dose arms.***

Further detail on efficacy results from CVT, PATRICIA and the IARC India HPV Vaccine Trial is provided below.

HPV infection and vaccine protection data from CVT and PATRICIA

This section, included in the First Edition of the White Paper, was excerpted from a review of evidence of single-dose HPV vaccine protection from the Costa Rica HPV vaccine trial and future research studies [54]. As in the First Edition, the content was edited for the Second Edition of the White Paper.

After four years of follow-up, in the HAV (control) arm the attack rates of incident HPV 16 or HPV 18 infections that persisted for at least six months were similar among women who received three doses (7.6%; 95% CI: 6.7 to 8.6%), two doses (6.3%; 95% CI: 4.2 to 9.1%), or one dose (8.0%; 95% CI: 4.7 to 12.5%), indicating that they were at similar risk for acquiring HPV infections regardless of the number of HAV doses they received (65). Since balance in enrollment characteristics was observed between the HPV and HAV arms, indicating successful randomization, it could be inferred that there is likely balance in HPV 16/18 exposure by dose group among the HPV-vaccinated arms. Assessment of HPV genotypes not protected by the 2vHPV vaccine showed balance across dose groups at both years four and seven, indicating continued equality in HPV exposure (65, 67). At the four-year analysis (65), the cumulative detection of carcinogenic HPV types, excluding HPV 16/18/31/33/45, was 14.9% (95% CI: 13.6 to 16.2%) for women who received three doses, 14.1% (95% CI: 11.0 to 17.6%) for women who received two doses, and 12.7% (95% CI: 8.6 to 17.9%) among women who received one dose. At year seven (67), the point prevalence for the same group of HPV types was 15.2% (95% CI: 13.7 to 16.8%) for women who received three doses, 14.3% (95% CI: 10.5 to 18.9%) for women who received two doses (at 0/6), and 13.4% (95% CI: 8.4 to 20.0%) for women who received one dose.

Single-dose efficacy of 2vHPV was assessed at two time points: first, during the initial four-year randomized blinded phase that included the randomized control arm (although not randomized by dose) to assess background rates of HPV infection, and then at seven years in the long-term follow-up (LTFU) study that included a new control arm. At four years, cumulative HPV infections over the four-year follow-up were assessed. At the seven-year data point, point prevalence of HPV was assessed in order to determine continued duration of protection.

Four years after initial vaccination, one dose of the 2vHPV vaccine had comparable efficacy to three doses of the vaccine using an endpoint of cumulative persistent HPV infection (67). The four-year efficacy against HPV 16 or 18 infections that persisted for at least six months among women who were HPV DNA negative for these types at first vaccination was the following: for three doses = 84% (95% CI=77 to 89%; 37 and 229 events in the HPV [n=2957] and control [n=3010] arms, respectively); for two doses = 81% (95% CI: 53 to 94%; 5 and 24 events among HPV [n=422] and control [n=380] arms, respectively); and one dose = 100% (95% CI: 79 to 100%; 0 and 15 events among HPV [n=196] and control [n=188] arms, respectively).

The CVT trial has published data following up to seven years. Among the participants who received one dose, no HPV 16/18 cervical infections were detectable at year seven. This was similar to women who received the three-dose regimen, where there were 20 (1.0%) HPV 16/18 infections. For comparison, there was a 6.6% HPV 16/18 prevalence among the unvaccinated women at year seven, suggesting that a single dose continued to provide protection against HPV 16/18 infection. Again, carcinogenic HPV types not protected against by the HPV vaccine were detected with similar frequency among vaccinated (15.0%) and unvaccinated (13.0%) women, indicating similar exposure to HPV infections.

Data from another trial, the PATRICIA trial, found that women who received one dose had the same VE as two and three doses (66). The PATRICIA trial was a phase III, randomized, double-blind placebo-controlled trial of 2vHPV, conducted in 18,644 women aged 15 to 25-years who were enrolled between May 2004 and June 2005 (73). VE for one-time detection of incident HPV 16 and 18 infection in the PATRICIA trial was 76.8% (95% CI 74.2–79.2) for three doses, 73.3% (40.4–89.2) for two doses, and 72.2% (13.6–92.4) for one dose (73).

The four-year efficacy against an endpoint of cumulative incident HPV 16/18 infection hovers around 80% for all dose groups in the PATRICIA and CVT trials and demonstrates that one dose of HPV VE is not inferior to three-dose VE among the same analytic population and utilizing the same endpoint for analyses.

HPV infection and vaccine protection data from IARC India vaccine trial

This section describes infection data from the IARC India vaccine trial, as presented in the First Edition of the White Paper.

> *The frequencies of cumulative incident HPV 16 and 18 infections over seven years from vaccination were similar and uniformly low in all the study groups. The frequencies of HPV 16 and 18 infections were higher in 1,481 unvaccinated women (6.2%) than among the vaccine recipients (0.9% in 1,180 three dose recipients, 0.9% in 1,179 two dose recipients, 1.7% in 1,473 two dose (default) recipients and 1.6% among 1,823 single dose recipients).*
>
> *Findings from the India study, based on the comparison between the rate of persistent infection in 2,989 vaccinated women who provided at least two cervical samples, and the rate in 1,141 unvaccinated women providing at least two samples suggest high vaccine efficacy in preventing persistent HPV 16 and 18 infections, regardless of the number of doses received. There were a total of four (0.1%) persistent HPV 18 infections and no persistent HPV 16 infection among the 2,989 vaccine recipients compared to 14 (1.2%) persistent infections with HPV 16 or 18 among 1,141 unvaccinated control women. No persistent HPV 16/18 infection was detected in 959 women in the single-dose arm.*

4.2.1.8 IMMUNOGENICITY RESULTS

The following text is excepted from the trials systematic review (61). The content was edited for this Second Edition of the White Paper.

> ***HPV16/18 humoral immunogenicity results for participants who received one HPV vaccine dose compared to any comparator group are reported in five of the included studies (33, 34, 62, 67, 68). HPV16/18 immunogenicity-related outcome measures most commonly reported include: seropositivity, geometric mean (GM) antibody levels (titers or MFI) and antibody stability. Some studies additionally reported on antibody avidity or neutralizing antibody seropositivity/titers. Methods used for measurement of immune responses and, where applicable, definitions of endpoints reported by each of the five studies are summarized in Table 5.***
>
> ***Table 6 summarizes seropositivity and antibody level results for the four studies comparing one dose versus other vaccine dosage schedules. In brief, the proportions of participants reportedly seroconverting to HPV16/18 antibody-positive were generally high in all HPV vaccine arms, reaching 100% in some studies. However, the definition of seroconversion differs between studies (Table 5). Antibody levels were lower with one dose than for two or three doses. However,***

whilst levels for two and three-dose arms declined following an initial increase, plateauing thereafter, this trend was typically less pronounced in the one-dose arms, in which levels remained more stable throughout follow-up. Furthermore, antibody levels were significantly higher in participants vaccinated with one dose of HPV vaccine compared to pre-vaccination levels in participants with natural infection (Table 6).

In CVT, post-vaccination HPV16/18 antibody avidity was lower in the reduced dosage groups; however, avidity remained stable between the 4 and 7-year time points within each of the dosage groups. Proportions of CVT participants who were seropositive for HPV16/18 neutralising antibodies at month 48 were similar across the HPV vaccine dosage arms. In the IARC India HPV vaccine trial, HPV16/18 antibody avidity was comparable across the dosage groups at 18 months post vaccination, but neutralising antibody levels were lower in reduced dose schedules.

Further detail on immunogenicity results from CVT and the IARC India HPV Vaccine Trial is provided below.

Immunogenicity data from CVT

This section, from the First Edition of the White Paper, was excerpted from a review of evidence of single-dose HPV vaccine protection from the Costa Rica HPV vaccine trial and future research studies [54]. As in the First Edition, the content was edited for the Second Edition of the White Paper.

Among women who received one dose in CVT, 100% seroconverted, and HPV 16 and 18 antibody titers (assessed by ELISA) were substantially higher than those among naturally-infected unvaccinated women (approximately nine-fold higher for HPV 16 and five-fold higher for HPV 18) four years after initial vaccination (34). Titers remained stably elevated at seven years post vaccination at four to five-fold lower levels than for three doses (67).

Neutralizing antibodies measured at year four were highly correlated with levels measured by ELISA. Spearman correlations were high for three- (0.87), two- (0/1; 0.72), two- (0/6; 0.80), and one- (0.79) dose groups, although decreased correlation was noted for the one-dose group compared to the three-dose group (34). By the SEAP assay, HPV 16 seropositivity was greater than 95% for all HPV-dose groups and was no different by dose group (p=0.6).

In the CVT trial, HPV 16 VLP antibody avidity, a measure of the quality of the antibody response, was measured at years four and seven. The data for three doses showed that avidity increases considerably over the first four years and then stabilizes to year seven.

Since the avidity for one dose was similar to three doses at year four, we assume that avidity similarly increased during this period after one dose. These results suggest that HPV 16 antibody quality is not substantially increased by boosting (65, 67)).

Immunogenicity data from IARC India vaccine trial

This section describes immunogenicity data from the IARC India vaccine trial, as presented in the First Edition of the White Paper.

Follow-up data are available up to 48 months. All vaccinated girls in the study groups seroconverted against HPV 16 and 18 after vaccination, and all remained seropositive at 48 months regardless of the number of doses received.

The immune response in the two-dose HPV vaccine group was non-inferior to the three-dose group at seven months (MFI ratio for HPV 16 was 1.12 [95% CI 1.02-1.23] and for HPV 18 was 1.04 [0.92-1.19]), but was inferior in the two-dose default (0.33 [0.29-0.38] for HPV 16 and 0.51 [0.43-0.59] for HPV 18) and one-dose default (0.09 [0.08-0.11] for HPV 16 and 0.12 [0.10-0.14] for HPV 18) groups at 18 months (33) and continued to be inferior by month 48. Although the MFI values for HPV 16 and 18 L1 antibodies for the single-dose group had values equivalent to or lower than the seropositivity cut-off, they are several times higher than the baseline values.

The values for geometric mean avidity index for HPV types 16 and 18 for the one-dose group at 18 months were non-inferior to the values after the three-dose regimen at 18 months (33): the avidity index ratio of the one-dose default group compared with the three-dose group for HPV 16 L1 was 1·10 (95% CI 1·01–1·19). One dose induced detectable concentrations of neutralizing antibodies to HPV 16 and 18, but at lower concentration than two or three doses. The GMT ratio of HPV 16 L1 neutralization titers was 0.06 (0.04-0.08) for the one-dose default group compared with the three-dose group at 18 months; 0·08 (0.05-0.13) for HPV 18 L1 and 0·06 (0.04-0.09) for HPV 6 L1.

Immunogenicity data from US randomized pilot intervention study in previously HPV infected women

The following text has been excepted from the trials systematic review (61). As in the First Edition, the content was edited for the Second Edition of the White Paper.

In the small randomized study (62), four of the five HPV16-seropositive women receiving a single dose of the 9-valent HPV vaccine exhibited increases in HPV16 and HPV 18 binding antibody levels and neutralization against HPV16 by one month following vaccination, and responses remained increased compared to baseline at month 6 (62). Two women had observed increases in HPV16/18 antibody binding levels at one week post vaccination. Increases in memory B cells

numbers were also observed. Conversely, non-neutralizing antibodies were observed in women with natural HPV infection and no changes in antibody responses or memory B cell numbers were seen among the five infected women who did not receive any HPV vaccine dose.

4.2.1.9 RESULTS OF QUALITY ASSESSMENT

The quality of evidence from all seven studies was assessed, and a descriptive synthesis is presented in Table 7 for the CVT, PATRICIA and IARC India trials. The presence of enrolled comparator groups of young women who did not receive HPV vaccine in these trials allowed authors to assess the risk of bias and the presence of a number of confounders that could have artificially inflated the vaccine efficacy in the one-dose group or deflated the vaccine efficacy in the three-dose group. Sociodemographic characteristics (e.g. age, household income, education level), HPV seropositivity at baseline, and the incidence of non-vaccine type HPV infections during follow up (proxy measures for participants' risk of HPV16/18 exposure during follow-up) were very similar across comparator groups (dose groups and control groups). Participants' reasons for non-completion of the vaccination schedule and rates of loss-to-follow up (indicators of survival bias) were also very similar across all comparator groups and were controlled for in some analyses conducted by the authors of the included studies. The risk of exposure or outcome misclassification was low and the included analyses were appropriate.

The intervention study by Scherer et al was a very small (n=5 per arm) pilot study among HPV16 seropositive women, limiting the precision of estimates and generalizability of results. Allocation to one-dose HPV vaccine versus no intervention was randomized but not blinded; however, the latter point likely has little implication as the study endpoints were immunological.

4.2.2 Data on 'one or more' HPV vaccine doses from Cochrane review

A recently published Cochrane review of clinical trial data on the efficacy and safety of HPV vaccines (monovalent, 2vHPV, 4vHPV or 9vHPV) compares 'at least one' dose of HPV vaccine (bivalent or quadrivalent) to placebo (vaccine adjuvants or another control vaccine) (64). The specific objective of the Cochrane review was '*To evaluate the harms and protection of prophylactic human papillomaviruses (HPV) vaccines against cervical precancer and HPV16/18 infection in adolescent girls and women.*' The review included

phase II and III randomized controlled trials enrolling female participants of any age receiving HPV vaccine or placebo published up to June 2017.

The review included trials of three vaccine doses. Therefore, women who received only one or two doses were those who did not complete their allocated three-dose schedule. Efficacy outcomes evaluated by the review included high-grade cervical intraepithelial neoplasia or worse, invasive cervical cancer, and incident and persistent infection with vaccine HPV types. Whilst primarily presenting data for 'at least one' dose, the review also stratified results by actual number of doses received, as follows: one dose, two doses, three doses, two or three doses (calculated as the difference between three-dose and 'at-least-one' dose participants in a post-hoc analysis).

4.2.2.1 SINGLE-DOSE VERSUS COMPARATOR GROUPS

The Cochrane review included 56 references describing 26 randomized trials of a three-dose HPV vaccination regimen, including a total of 73,428 women. Of these, only three articles report efficacy data for single-dose HPV vaccination compared to comparator groups (65-67). These three articles were derived from the CVT and PATRICIA trial, and are included in the results of the systematic review described above (61) (Section 4.2.1). The IARC India HPV Vaccine Trial (33) was not included in the Cochrane review presumably because, due to suspension of the trial mid-way through randomization, it could no longer be reported as an RCT and/or because no placebo group was included.

Since the three articles identified by the Cochrane review were also identified by the systematic review of evidence on single-dose HPV vaccination from clinical trials described above, and the corresponding studies are already presented in Section 4.2.1, the results are not repeated here.

4.2.2.2 'ONE OR MORE' DOSE VERSUS PLACEBO GROUPS

The main comparison in the Cochrane review was 'at least one' HPV vaccine dose versus placebo (vaccine adjuvants or another control vaccine, such as HAV). The usefulness of these data for evaluating a single-dose regimen is limited because the vast majority of participants received three doses (i.e. completed their allocated schedule). However, in a post-hoc analysis, the review authors determined measures of effect and association for participants who received one or two vaccine doses (combined) by calculating the difference between three-dose and 'at least one' dose groups (where reported) (65, 70, 73-92). Among one- or two-dose recipients, significant protection was seen against HPV16/18-associated CIN2+ and CIN3+ (bivalent and quadrivalent vaccine, women aged 16-25 years) (70, 73-75, 80-92), incident HPV16/18 infection (bivalent vaccine, women aged 15-26 years) (74, 75, 83-85, 88-90), and six-month persistent HPV16/18 infection (bivalent

and quadrivalent vaccine, women aged 15-45 years) (74, 75, 80, 81, 83-85, 91, 92) compared to women receiving placebo. Again, these data are limited in terms of evaluating efficacy of single-dose HPV vaccination as some (presumably most) participants included in the post-hoc 'one or two' dose groups received two doses of vaccine.

4.2.3 Strengths and weaknesses of evidence from clinical trials

4.2.3.1 STRENGTHS AND WEAKNESSES OF SYSTEMATIC REVIEW OF EVIDENCE ON SINGLE-DOSE HPV VACCINATION FROM CLINICAL TRIALS

The systematic review of trials data described in Section 4.2.1 provides rigorous search and evaluation of the published literature on single-dose HPV vaccination compared to no vaccination or standard dosing regimens among clinical trial participants. The study's strengths include: a robust and comprehensive search strategy; searches of multiple scientific databases as well as clinical trials registers; duplicate screening of all abstracts and full-text articles by two authors; independent verification of extracted data and STATA calculations by a separate author; and a quality assessment of included studies, specifically evaluating biases that might lead to increased efficacy in the single-dose arms or reduced efficacy in the standard dose arms.

The systematic review of evidence on single-dose HPV vaccination from clinical trials also has several limitations. The following text contains excerpts from the trials systematic review (61). The content was edited for the Second Edition of the White Paper.

> ***This systematic review is limited by the small number of studies reporting clinical trial-based evaluations of single-dose HPV vaccination, and in some studies, limited sample size of the one-dose group. The review identified only seven publications describing studies of single-dose HPV vaccination compared to either no vaccination or two- or three-dose schedules. Six were observational studies arising from three randomised clinical trials (that were investigating efficacy and immune responses in three doses versus control, or two versus three doses), with participant allocation to one-dose or comparator arms occurring retrospectively (due to non-completion of originally-allocated schedules). Only one very small pilot study allocated participants to one-dose versus no-dose arms prospectively.***
>
> ***Furthermore, the systematic review was not able to evaluate the effects of gender, age or HIV status, as proposed in the study protocol, as all studies conducted to date have been in young, healthy females. This highlights a paucity of evidence in potential alternative target populations. Additionally, all trial-based data of single-dose HPV vaccination published to date come from Cervarix® and***

Gardasil® recipients; no studies have evaluated Gardasil-9®. Whilst 12 national programme-based studies included in the published review by Markowitz et al report on vaccine efficacy against AGW and cervical abnormalities, the trial-based efficacy studies in the trials-based review reported only on HPV infection endpoints.

Studying CVT, PATRICIA and IARC India HPV Vaccine Trial-derived cohorts for evaluation of single- versus multi-dose vaccination schedules minimizes many of the biases that confound the national program-based studies, despite the retrospective allocation to exposure versus comparator arms. However, retrospective allocation is still sub-optimal, so this approach does not preclude the requirement for gold-standard, purpose-designed, prospectively randomized controlled trials. Also, although the point estimates of vaccine effectiveness in the trial-based observational studies are high, the confidence intervals around the estimates are very wide, which limits any strong conclusions from these data on whether one dose is sufficient for protection. It was not possible to combine results of the included studies and perform a meta-analysis in this review due to considerable heterogeneity between the studies.

Whilst a quality assessment of included studies was conducted, this did not utilise a standardised risk of bias tool due to the lack of availability of a suitable tool. Co-authors of the systematic review have developed an adapted ROBINS-I tool to take into account the characteristics of reduced-dose observational studies (e.g., different types of study design, use of buffer periods to control for prevalent infection at 1st dose) to formally assess the quality of these studies. These data will be included in an updated systematic review of evidence on single-dose HPV vaccination from clinical trials that is scheduled to be conducted within the next year.

Specific quality considerations for CVT, the IARC India HPV vaccine trial, and PATRICIA are provided below in Section 4.2.3.3.

4.2.3.2 STRENGTHS AND WEAKNESSES OF THE COCHRANE REVIEW ON 'ONE OR MORE' HPV VACCINE DOSES

The Cochrane review has several strengths, including a high-quality review of the trials-based evidence on the safety and efficacy of HPV vaccines and inclusion of data from a large number of studies. However, the review did not specifically aim to evaluate single-dose HPV vaccination, so has a number of limitations in relation to this question. First, the main comparison, 'one or more' doses versus placebo, only includes trials randomizing participants to receive three doses of vaccine or placebo, so the majority of participants included in the analyses received three doses. Only a proportion received

one dose, and we do not know who these participants are. The post-hoc analyses the authors conducted enabled evaluation of vaccine efficacy among participants who received one or two doses (combined) compared with placebo, but did not examine efficacy for single-dose participants. The authors did present data by number of doses received where provided in included studies (CVT, PATRICIA). However, the review was limited to phase II and III RCTs of three-dose HPV vaccine versus placebo or other control vaccine, so would not capture trials of other designs that could provide informative data on efficacy on single-dose HPV vaccination. Whilst an assessment of risk of bias for studies was included in the Cochrane review, this did not include an evaluation of the risk of bias due to differences in reduced-dose and placebo/control participants. Finally, the review did not present any immunogenicity data from the included RCTs.

4.2.3.3 STRENGTHS AND WEAKNESSES OF CVT, IARC INDIA HPV VACCINE TRIAL, AND PATRICIA STUDIES

The strengths and weaknesses of the CVT, IARC India HPV vaccine trial, and PATRICIA studies are summarized in Section 4.2.1.9 and Table 7, both of which are extracted from the systematic review of evidence on single-dose HPV vaccination from clinical trials. Given that, at present, the majority of trials-based evidence for single dose HPV vaccination is derived from the CVT, PATRICIA, and IARC India trials, their strengths and weaknesses are described here in more detail. Portions of this section were excerpted from a review of evidence of single-dose HPV vaccine protection from the Costa Rica HPV vaccine trial, as well as future research studies [54]. The content was edited for this White Paper.

Strengths of studies

For the CVT trial, a concurrent control group was enrolled, and extensive analyses were conducted to rule out much of the potential bias and confounding that could relate to an underlying characteristic shared by women who received only a single dose. The findings on the protection conferred by single-dose vaccination were consistent in the PATRICIA study before the combined analysis with CVT was done.

Several metrics were used to evaluate potential biases and confounding in the CVT and PATRICIA data, including by dose assessment of the following:

- *Demographic and HPV-related differences at enrollment, including sexual behavior and presence or absence of Chlamydia trachomatis by dose group;*
- *Follow up time and reasons for missed visits and doses;*

- *Vaccine antibody response elicited one month after the first dose, when all women received the same number of doses irrespective of the total number of doses they received; and,*
- *Prevalence of HPV genotypes not protected by the vaccine, as an indicator of genital HPV exposure, accumulated over the four years of follow-up.*

For the India HPV vaccine trial, strengths of the study include a large sample size across all arms (including the single-dose arm), high cohort retention (>80%) at seven years after recruitment, the frequency of the immunogenicity and efficacy measures, and the fact that laboratory analyses were performed in a blinded manner. **The original allocation to two versus three doses was cluster randomised. Although, the halt to enrolment resulted in formation of new study groups (one versus two versus three dose arms) and this 're-allocation' was determined by time of enrolment (and not controlled by the investigators). Thus, it is unlikely to be linked to any pre-existing HPV risk status.**

In all three studies, the incidence of infection with HPV vaccine genotypes not targeted by the 4vHPV was similar across vaccinated participants, regardless of the number of doses received. This provides some reassurance against potential bias and confounding relating to underlying characteristics of participants not completing their allocated vaccine schedule.

Weaknesses of studies

For the CVT and PATRICIA trials, the group of women receiving one dose of the 2vHPV vaccine was relatively small, and they were not randomized to a reduced-dose schedule. The combined analysis of the CVT and PATRICIA trials used one-time detection of HPV incident infection, rather than persistent infection. This measurement could also include virus deposition from an infected partner, short-term infections that clear spontaneously or intermittently activated latent infections that were not detected at vaccination.

Although the India HPV Vaccine trial was originally a randomized trial, the original dose randomization could not be maintained. The different vaccine dose cohorts were comparable for age but there were differences in several socio-demographic factors at enrolment, such as monthly household income, religion, and education (68). However, as described above, the frequency of detection of HPV vaccine genotypes not targeted by the 4vHPV were similar across the vaccinated and unvaccinated women (93). Clinical outcomes were only measured in married women for cultural reasons and this reduced the sample size for analysis. The unvaccinated cohort was created post-hoc in 2011 by selecting married women matched to married participants on age, study site, and time of follow-up. Biases in selection of this cohort cannot be ruled out.

4.2.4 Summary of observational data from clinical trials

The following text contains excerpts from the systematic review of evidence on single-dose HPV vaccination from clinical trials (61). The content was edited for the Second Edition of the White Paper.

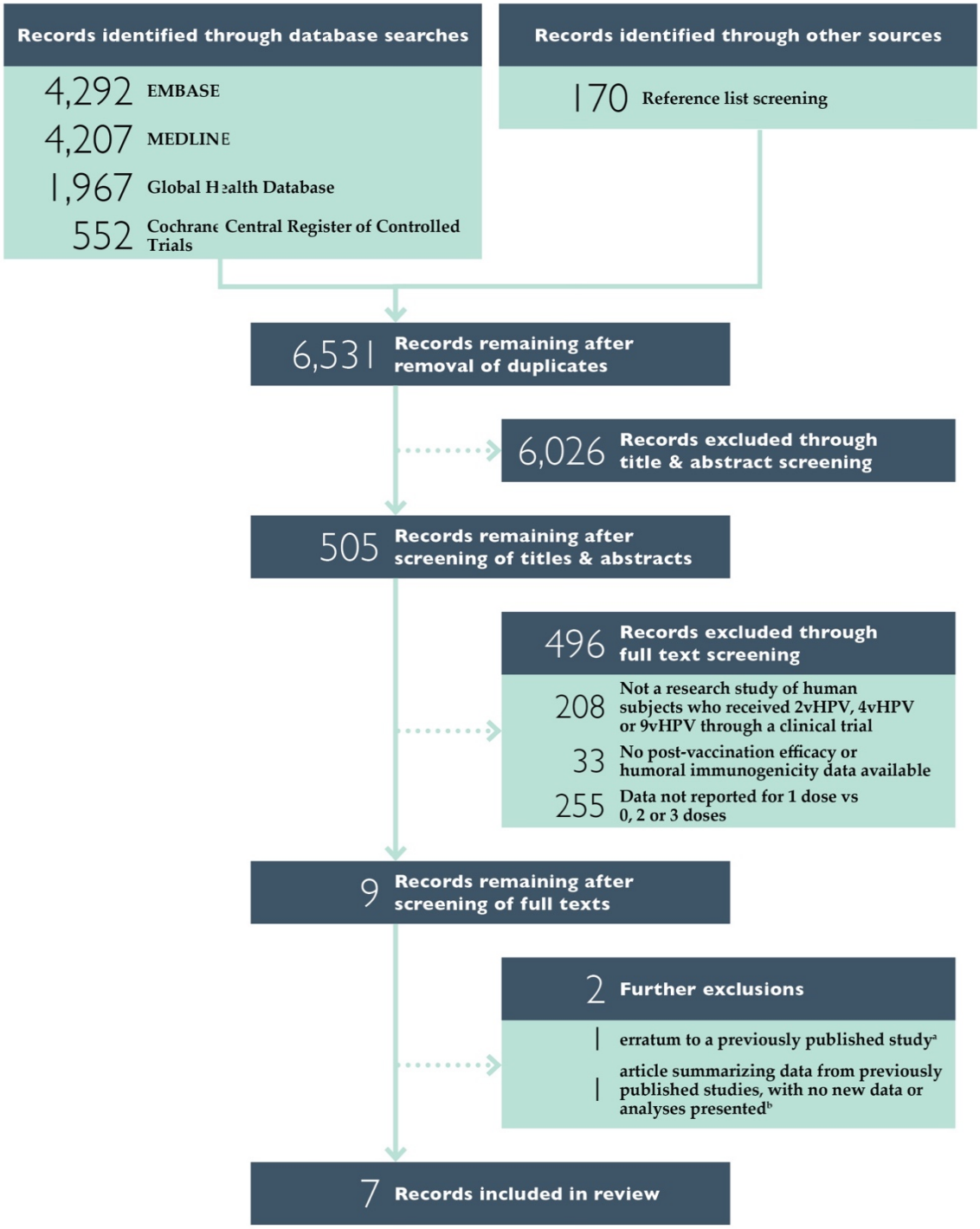
> *The systematic review of the literature on single-dose HPV vaccination from clinical trials supports the premise that one dose may be as effective in preventing HPV infection as two or three doses in healthy young females up to seven years post vaccination. Incident, persistent and prevalent infection with HPV16/18 were extremely low in all efficacy trial participants who received any HPV vaccine, and significantly lower than participants who were either unvaccinated or received a control vaccine such HAV. All included efficacy studies reported comparable efficacy against HPV16/18 infection in one-dose and two- or three-dose arms.*

The Cochrane review (64) did not identify any studies contributing evidence specifically on efficacy of single-dose HPV vaccination further to those captured by the systematic review of trials on single-dose HPV vaccination. However, the authors' post-hoc analysis demonstrated high efficacy of one or two doses of HPV (combined analysis) vaccine compared to control using data from eight studies. As described above, these data must be interpreted with caution, as one- and two-dose participants cannot be disaggregated, and there is already strong evidence for efficacy of two doses.

> *Across studies reporting immunogenicity outcomes, the proportions of participants reportedly seroconverting to HPV16/18 antibody-positive were generally high in all HPV vaccine dosage arms, reaching 100% in some studies. However, the definition of seroconversion differs between studies (Table 5), so caution must be applied in interpreting these results. Antibody levels were lower with one dose than for two or three doses, but levels in single-dose arms remained stable throughout follow-up. Furthermore, antibody levels were significantly higher in participants vaccinated with one dose of HPV vaccine compared to pre-vaccination levels in participants with natural infection (61).*
>
> *Whilst producing promising results, the systematic review also highlighted the existing paucity of available evidence appropriate for informing policies and guidelines on HPV vaccination strategies. Ongoing clinical trials assessing the efficacy and immunogenicity of single-dose HPV vaccination compared to currently recommended schedules will go a long way towards addressing this knowledge gap for the target populations in those trials. However, research on efficacy of, and immune responses to, single-dose HPV vaccination may need to be expanded to other target groups, such as boys, alternative age groups and HIV-*

positive individuals, and should evaluate all licensed HPV vaccines, as well as new vaccines currently in development.

Figure 2. Clinical trials systematic review flow diagram



[a] Corrected results presented in the erratum (94) were incorporated into data extraction for the corresponding article (34).
[b] Article (95) presents previously published data from CVT (34, 65-67).

Figure adapted from (61).

Table 2. Summary of studies selected for inclusion in the trials systematic review

Reference, location	Study design	HPV-vaccinated population (healthy females in all studies)								Control group
		No. in efficacy cohort	No. in immuno. Cohort	Age at vaccination (years)	Baseline HPV16/18 DNA status[a]	Baseline HPV16/18 Serology[a]	Vaccine administered	Vaccination schedule(s)	Follow-up duration	
Kreimer 2011, Costa Rica[b] *(65)*	Post-hoc analysis of RCT (CVT)	3,575	NA	18-25	HPV16 *and* 18 positive excluded; Unstated proportion HPV16 *or* 18 positive	Unstated proportion positive *(method not stated)*	2vHPV	3d (M0,1,6), 2d (M0,1 / 0,6) 1d (M0)	Efficacy: 4 years	3,578 healthy females receiving HAV in CVT
Safaeian 2013, Costa Rica[c] *(34)*	Post-hoc analysis of RCT (CVT)	NA	390	18-25	5% HPV16 *or* 18 positive	15% HPV16 positive *(by IgG ELISA)*	2vHPV	3d (M0,1,6), 2d (M0,1 / 0,6) 1d (M0)	Immuno: 4 years	115 healthy HPV16/18 seropositive females in CVT, pre-vaccination
Kreimer 2015, multiple LMIC & HIC worldwide[d] *(66)*	Combined retrospective analysis of CVT and PATRICIA data	12,159	NA	15-25	HPV16 *and* 18 positive excluded; Unstated proportion HPV16 *or* 18 positive	Unstated proportion positive *(method not stated)*	2vHPV	3d (M0,1,6), 2d (M0,1 / 0,6) 1d (M0)	Efficacy: 4 years	12,194 healthy females receiving HAV in CVT or PATRICIA
Sankaranarayanan 2016, India[e] *(33)*	Prospective observational cohort study	2,649	1,552 – 1,937	10-18	Not measured; unmarried	5% of immuno. cohort HPV16 positive, 5% HPV18 positive; Not reported for efficacy cohort *(by Luminex)*	4vHPV	3d (M0,2,6), 2d (M0,2 / 0,6) 1d (M0)	Efficacy: 4 years Immuno: 3 years	None
Scherer 2016, USA[f] *(62)*	Randomised unblinded pilot intervention study.	NA	5	27-45	Not measured	HPV16 positive *(by IgG binding assay)*	4vHPV	1d (M0)	Immuno: 6 months	5 healthy HPV16-seropositive unvaccinated females
Sankaranarayanan 2018, India[e] *(68)*	Prospective observational cohort study	5,655	879 – 1937	10-18	Not measured; unmarried	Not reported	4vHPV	3d (M0,2,6), 2d (M0,2 / 0,6) 1d (M0)	Efficacy: 7 years Immuno: 4 years	1,481 age-matched healthy unvaccinated females
Safaeian 2018, Costa Rica[g] *(67)*	Prospective observational cohort study of prior CVT participants	2,449	486	18-25	8% HPV16/18 positive	38% HPV16/18 positive *(by IgG ELISA)*	2vHPV	3d (M0,1,6), 2d (M0,1 / 0,6) 1d (M0)	Efficacy & immuno: 7 years	2,386 age-matched healthy unvaccinated females

CVT: Costa Rica Vaccine Trial; D: Dose; HAV: Hepatitis A vaccine; HIC: High income county; HPV: Human papillomavirus; Immuno.: Immunogenicity; LMIC: Low and middle-income country; M: Month; No.: Number; PATRICIA: PApilloma TRIal against Cancer In young Adults Trial; RCT: Randomized controlled trial; USA: United States of America.

[a] HPV16/18 DNA status refers to PCR/genotyping results in cervical samples; HPV16/18 serology refers to antibody seropositivity results in serum or plasma. Baseline refers to pre-vaccination.

[b] Analytic cohort included all 7,153 CVT participants who were seen each year during four years of follow-up, and who were not HPV16 and 18 DNA positive at baseline. At enrolment, participants were randomized to receive HPV vaccine (n = 3,575) or HAV (3,578). HAV control arms received vaccine and were followed up according to the same schedule as HPV vaccine arms.

[c] Included all 270 CVT participants who received one or two HPV vaccine doses, and a random selection of 120 participants who received three HPV vaccine doses, all with sera available for each study visit. Pre-vaccination samples from 115 HPV16/18-seropositive CVT participants (DNA status not reported) were used as single timepoint controls.

[d] Analytic cohort included all 25,055 CVT and PATRICIA participants who had adequate follow up and available HPV DNA results at baseline, and who were not HPV16 and 18 DNA positive at baseline. Inadequate follow up was defined as no M12 or later visit, or <300 days between the M12 (or later) visit and the last study visit. At enrolment, participants were randomized to receive HPV vaccine (n = 21.013) or HAV (12,042). HAV control arms received vaccine and were followed up according to the same schedule as HPV vaccine arms. Results were additionally reported in the study for a 'naïve' cohort excluding women who were HPV DNA positive for any of 14 high-risk HPV types, HPV16/18 seropositive, and cytology positive at enrolment. Results from the 'naïve' cohort are not included in the systematic review.

[e] Efficacy cohort included all IARC India HPV Vaccine Trial participants (all unmarried at enrolment) who received one or more dose of HPV vaccine and had at least one cervical sample collected during follow up (2,649 up to Y4; 5,655 up to Y7). Collection of cervical samples commenced six months after delivery of a baby or 12 months after marriage, whichever was earlier. Participants for the immunogenicity cohort were selected by convenience sampling; numbers of samples vary at each time point. 1,481 age-matched healthy married and HPV-unvaccinated control participants were enrolled two years after the start of enrolment into the IARC India HPV Vaccine Trial and followed up for four years.

[f] Included 10 HPV16-positive females with ≤5 heterosexual lifetime partners. Five were randomized to receive one dose of 4vHPV and five to receive no vaccine. Both arms were enrolled together and followed up at the same timepoints.

[g] Efficacy cohort included all 2,449 HPV-vaccinated CVT participants who agreed to enter the long-term follow up study at the end of the four-year trial. The immunogenicity cohort included a subset of 321 one- or two-dose participants who were tested previously and had sufficient available sera, and a random subset of 165 three-dose participants. 2,836 age-matched healthy and HPV-unvaccinated women were enrolled at the start of the long-term follow up study and followed up for three years.

Table adapted from (61).

Table 3. Sampling, laboratory methods and definitions used and reported by each study in the trials systematic review for HPV16/18 infection-associated endpoints

Study	Sampling	Methods	Endpoints reported (measure / unit)[a]	Endpoint definitions
Kreimer 2011 **(65)** and Safaeian 2018 **(67)**	Vaccinated cohort: Cervical cell samples collected from sexually experienced women at enrolment, M6, and then annually (from day 0) for 4y. Thereafter, samples collected biennially up to Y7 from all women in follow-up study. Unvaccinated cohort: Cervical cell samples collected biennially.	SPF_{10} PCR DEIA[b] and $LiPA_{25}$ for 25 HPV types[c]	6m persistent infection (% risk, 95%CI)	New infection detected at M6 or later and persisting for ≥4m, confirmed by 2 samples collected ≥4 months apart testing positive for the same HPV type, with no intervening negative tests
			12m persistent infection (% risk, 95%CI)	New infection detected at M6 or later and persisting for ≥10m (as above, with samples collected ≥10m apart)
			One-time incident infection (% risk, 95%CI)	All infections detected at Y7 that were not detected at Y4
			Cumulative incident infection (% risk, 95%CI)	All detectable infection between M12 and Y7 among women type-specific negative at enrolment
			One-time prevalent infection (%, 95%CI)	All infections detected at Y7
Kreimer 2015 **(66)**	CVT vaccinated cohort: as above, up to Y4 timepoint. PATRICIA vaccinated cohort: Cervical samples collected from sexually experienced women at enrolment and biennially thereafter for 4y.	SPF_{10} PCR DEIA[b] and $LiPA_{25}$ for 25 HPV types[c]	One-time incident infection (% rate, 95% CI)	All first detectable infections occurring from M12, accumulated up to Y4
			6m persistent infection (% rate, 95% CI)	New infection detected at M12 or later and persisting for ≥6m, confirmed by 2 samples collected ≥150d apart testing positive for the same HPV type, with no intervening negative tests
			12m persistent infection (% rate, 95% CI)	New infection detected at M12 or later and persisting for ≥12m (as above, with samples collected ≥300d apart)
Sankaranarayanan 2016 **(33)** and 2018 **(68)**	Vaccinated cohort: Cervical samples collected 18m after marriage or 6m after first child-birth[d], and annually thereafter until 4 consecutive yearly samples obtained. Unvaccinated cohort: Cervical samples collected at enrolment and annually thereafter for up to 4 collections.	HPV type-specific E7 PCR bead-based multiplex genotyping[e]	Cumulative first incident infection (% risk, 95%CI)	All first detectable infections accumulated during follow up
			12m persistent infection (% risk, 95%CI)	Presence of type-specific HPV DNA on repeated cervical samples over ≥12 month interval (in women with ≥2 samples tested)
			Cumulative incident infection (% risk, 95%CI)	All detectable infections at any visit up to Y7

CI: Confidence interval; CVT: Costa Rica Vaccine Trial; D: days; DNA: Deoxyribonucleic acid; HPV: Human papillomavirus; M: Month; PATRICIA: PApilloma TRIal against Cancer In young Adults; PCR: Polymerase chain reaction; Y: Year.

[a] Incidence risk denotes the number of new cases occurring per population at risk (i.e. using the number of women in the analytical population as the denominator). Incidence rate denotes the number of new cases per population at risk in a given time period (i.e. using person-years as the denominator).

[b] SPF_{10} PCR DEIA: SPF_{10} PCR primer system and DNA enzyme immunoassay detection of amplimers (DDL Diagnostic Laboratory, Voorburg, the Netherlands).

[c] $LiPA^{25}$: HPV line probe assay containing probes for 25 HPV genotypes (Labo Biomedical Products, Rijswijk, the Netherlands).

[d] Whichever occurred earlier.

[e] For 19 high-risk and two low-risk HPV types.

Table adapted from (61).

Table 4. Summarized HPV16/18 infection results from studies in the trials systematic review

Reference	Follow up duration	Infection endpoint[a]	3 dose HPV arm		2 dose HPV arm[b]		1 dose HPV arm		Control arm[c]		RR or PR (95%CI), *p value*[d]		
			# events / participants	% (95%CI)[d]	# events / participants	% (95%CI)[d]	# events / participants	% (95%CI)[e]	# events / participants	% (95%CI)[d]	1 dose / 3 doses[e]	1 dose / 2 doses[e]	1 dose / control
CERVARIX®													
One-time incident and cumulative incident infections													
Kreimer 2015 *(66)*	Mean: 4.0y SD: 0.7y	One-time incident	529 / 11,110	4.8 (4.4-5.2)	22 / 611	3.6 (2.3-5.4)	8 / 292	2.7 (1.2-5.3)	45 / 251	17.9 (13.4-23.2)	0.6 (0.3-1.1) ***0.12***	0.8 (0.3-1.7) ***0.56***	0.2 (0.1-0.3) ***<0.01***
Safaeian 2018 *(67)*	Median: 6.9y IQR: 6.5-7.3y	One-time incident	9 / 2,042	0.4 (0.2-0.8)	0 / 78	0.0 (0.0-4.6)	0 / 134	0.0 (0.0-2.7)	-	-	0.8 (0.0-13.6) ***1.0***	0.6 (0.0-29.2) ***UTC[i]***	-
		Cumulative incident	88 / 2,036	4.3 (3.5-5.3)	3 / 78	3.8 (0.8-10.8)	2 / 133	1.5 (0.2-5.3)	-	-	0.3 (0.1-1.4) ***0.17***	0.4 (0.1-2.3) ***0.36***	-
One-time prevalent infections													
Safaeian 2018 *(67)*	Median: 6.9y IQR: 6.5-7.3y	One-time prevalent	20 / 2,043	1.0 (0.6-1.5)	1 / 79	1.3 (0.0-6.9)	0 / 134	0.0 (0.0-2.7)	158 / 2,382	6.6 (5.7-7.7)	0.4 (0.0-6.1) ***0.63***	0.2 (0.0-4.8) ***0.37***	0.1 (0.0-0.9) ***<0.01***
Persistent infections[h]													
Kreimer 2011 *(65)*	Median: 4.2y[g]	6m persistent	37 / 2957	1.3 (0.9-1.7)	5 / 422	1.2 (0.4-2.7)	0 / 196	0.0 (0.0-1.9)	15 / 188	8.0 (4.5-12.8)	0.2 (0.0-3.2) ***0.17***	0.2 (0.0-3.5) ***0.18***	0.0 (0.0-0.5) ***<0.01***
		12m persistent	25 / 2957	0.9 (0.6-1.2)	3 / 422	0.7 (0.1-2.1)	0 / 196	0 .0 (0.0-1.9)	10 / 188	5.3 (2.6-9.6)	0.3 (0.0-4.8) ***0.40***	0.3 (0.0-5.9) ***0.56***	0.0 (0.0-0.8) ***<0.01***
Kreimer 2015 *(66)*	Mean: 4.0y SD: 0.7y	6m persistent	114 / 11,104	1.0 (0.8-1.2)	4 / 611	0.7 (0.2-1.7)	1 / 292	0.3 (0.0-1.9)	24 / 250	9.6 (6.2-13.9)	0.3 (0.0-2.4) ***0.37***	0.5 (0.1-4.7) ***1.00***	0.0 (0.0-0.3) ***<0.01***
		12 persistent	84 / 11,104	0.8 (0.6-0.9)	3 / 611	0.5 (0.1-1.4)	1 / 292	0.3 (0.0-1.9)	17 / 249	6.8 (4.0-10.7)	0.5 (0.1-3.2) ***0.72***	0.7 (0.1-6.7) ***1.00***	0.1 (0.0-0.4) ***<0.01***
GARDASIL®													
One-time incident and cumulative incident infections													
Sankaranarayanan 2016 *(33)*	Median: 4.7y IQR: 4.2-5.1y	Cumulative first incident	2 / 536	0.4 (0.0-1.3)	4 / 526	0.8 (0.2-1.9)	10 / 870	1.1 (0.6-2.1)	-	-	3.1 (0.7-14.0) ***0.17***	1.5 (0.5-4.8) ***0.059***	-
Sankaranarayanan 2018 *(68)*	Up to 7y[f]	Cumulative incident	11 / 1,180	0.9 (0.5-1.7)	11 / 1,179	0.9 (0.5-1.7)	30 / 1,823	1.6 (1.1-2.3)	92 / 1,481	6.2 (5.0-7.6)	1.8 (0.9-3.5) ***0.1***	1.8 (0.9-3.5) ***0.1***	0.3 (0.2-0.4) ***<0.01***
Persistent infections[h]													
Sankaranarayanan 2018 *(68)*	Up to 7y[f]	12m persistent	1 / 604	0.2 (0.0-0.9)	0 / 608	0.0 (0.0-0.6)	0 / 959	0.0 (0.0-0.4)	14 / 1,141	1.2 (0.7-2.1)	0.2 (0.0-5.1) ***0.39***	0.6 (0.0-31.9) ***UTC[i]***	0.0 (0.0-0.7) ***<0.01***

CI: confidence interval; HPV: Human papillomavirus; IQR: Inter-quartile range; M; Month; PR: Prevalence ratio; RR: Risk ratio; SD: Standard deviation; UTC: Unable to compute; Y: Year.

[a] Definitions of infection endpoints used in each study are provided in Table 3.

[b] Results are shown only for two-dose arms where participants received dose one at day 0 and dose two at day 180.

[c] Results are shown for one-dose control vaccine (HAV) arms for Kreimer et al (2011) and Kreimer et al (2015), and unvaccinated control arms for Sankaranarayanan et al (2018) and Safaeian et al (2018; persistent infection only). Comparison of the single-dose HPV vaccine arm with the single-dose HAV (rather than multi-dose HAV) arm in the Costa Rica trial minimizes the potential for selection bias due to differences in follow up. No control arm was reported in Sankaranarayanan et al (2016).

[d] Proportions (%), unadjusted RRs and PRs, 95%CIs and 2-sided Fisher's exact p values were calculated by the authors of the systematic review using data provided in the included articles. Haldane-Anscombe correction was used for calculation of RRs and PRs where no events were detected in one or both comparison arms. In most cases, the 95%CIs for proportions calculated by the authors of this review matched those reported in the included studies. Where they do differ, the 95%CIs calculated in this review are wider than those reported in the articles.

[e] Risk and prevalence ratios calculated for one versus two or three doses must be interpreted with caution because of potential for selection bias due to differences in follow up between the groups.

[f] Mean, median, IQR or SD were not reported for this study.

[g] IQR or SD were not reported for this study.

[h] Sankaranarayanan et al (2016) detected no persistent infections in any arm up to the median follow up of 4.7y among 838 women with two or more samples available for analysis.

[I] STATA does not compute a p value using Fisher's exact test where both numerators are 0.

Table adapted from (61).

Table 5. Sampling, laboratory methods and definitions used and reported by each study in the trials systematic review for HPV16/18 immunogenicity-associated endpoints

Study	Sampling	Methods	Endpoints reported (measure / unit)[a] with definitions where applicable
Safaeian 2013 **(34)** and 2018 **(67)**	Vaccinated cohort: Serum collected at enrolment and at M1, 6, 12, 24, 36 and 48. Serum additionally collected at Y4 and Y7. Naturally infected cohort: Serum collected at baseline, pre-vaccination.	HPV16/18 L1 VLP ELISA	- Antibody titres (GM EU/ml, 10th, 25th, 75th and 90th percentiles, 95%CI) - HPV16/18 seropositivity (% of analytical population seroconverting) *Laboratory-determined seropositivity cut-offs (8 EU/ml for HPV16, 7 EU/ml for HPV18)* - Antibody stability (% of analytical population with stable GMTs; Safaeian 2013 only) *Stability defined as titres not declining by ≥2-fold between two specified timepoints*
		PsV-based SEAP neutralisation assay	- HPV16 neutralising antibody seropositivity (% of analytical population seroconverting) *Laboratory-determined seropositivity cut-off (25.1 TU/ml)*
		GuHCl-modified HPV L1 VLP avidity ELISA	- Antibody avidity levels (GM avidity level, 95% CI, IQR)
Sankaranarayanan 2016 **(33)** and 2018 **(68)**	Plasma collected from convenience sample at enrolment and M7, 12, 18, 24, 36, 48 and 60.	Luminex-based multiplex binding assay	- Antibody levels (GM MFI, 95% CI) - HPV16/18 seropositivity (% of analytical population seroconverting) *Seropositivity cut-offs (100 for HPV16, 41 for HPV18) calculated based on MFI values of plasma samples from study participants at baseline after allowing for 5% seropositivity*
		Modified HPV-L1 genotype-specific binding antibody assay	- Antibody avidity index (GM avidity index (%), 95%CI)
		Automated PsV-based neutralisation assay	- Antibody titres (GMT, 95% CI) - HPV16/18 neutralising antibody seropositivity (% of analytical population with neutralisation titres) *Seropositivity defined as sample titre ≥50 and ≥2x control (BPV) titre*
Scherer 2016 **(62)**	PBMCs and plasma collected 6m prior to vaccination, on day of vaccination and at 1w, 1m and 6m post vaccination.	Anti-L1 binding assay using GST-HPV L1 fusion proteins on BioPlex with magnetic beads	- Antibody levels (MFI converted to U/ml) - HPV16 seropositivity *Seropositivity cut-off (3 U/ml) based on 3x SD above mean for sera from sexually-unexperienced controls*
		293TT PsV-based SEAP neutralisation assay	- HPV16 neutralising antibody levels (IC50 plasma dilution[a], SD)
		Flow cytometry	- HPV16-specific memory B cell responses (frequency)

BPV: Bovine papillomavirus; CI: Confidence interval; ELISA: Enzyme-linked immunosorbent assay; GM: Geometric mean; GST: Glutathione-S-transferase; GuHCl: Guanidine hydrochloride; HPV: Human papillomavirus; MFI: Mean fluorescent intensity; M: Month; Psv: Pseudovirus; SD: Standard deviation; SEAP: Secreted alkaline phosphatase; VLP: Virus-like particle; W: Week; Y: Year.

[a] Plasma dilution at which half-maximal inhibition occurred.

Table adapted from (61).

Table 6. Summarized HPV16/18 seropositivity and GM antibody level results from studies in the trials systematic review

Reference	Time point	# seropositive[b] / participants (% Seropositive, 95%CI[c])			GM titers / MFI (95%CI)			
		3 doses	2 doses[a]	1 dose	3 doses	2 doses[a]	1 dose	Naturally infected
CERVARIX®								
HPV16								
Safaeian 2013[d] *(34)*	D0	18 / 120 (15.0, 9.1-22.7)	-	6 / 78 (7.7)	<LOD	<LOD	<LOD	-
	M6	-	-	-	724 EU/ml	102 EU/ml	145 EU/ml	-
	M12	-	-	-	2,034 EU/ml	1,484 EU/ml	115 EU/ml	-
	M24	-	-	-	1,115 EU/ml	837 EU/ml	124 EU/ml	-
	M36	-	-	-	899 EU/ml	642 EU/ml	136 EU/ml	-
	M48	78 / 79 (98.7, 93.1-100.0)	52 / 52 (100.0, 93.2-100.0)	120 / 120 (100, 97.0-100.0)	748 EU/ml (648-865)	520 EU/ml (422-641)	137 EU/ml (106-178)	15 EU/ml (11-19)
Safaeian 2018 *(67)*	M48	2,043 / 2,043 (100.0, 99.8-100.0)	79 / 79 (100.0, 95.4-100.0)	134 / 134 (100.0, 97.3-100.0)	803 EU/ml (708-909)	555 EU/ml (447-690)	205 EU/ml (165-255)	
	M84	2,043 / 2,043 (100.0, 99.8-100.0)	79 / 79 (100.0, 95.4-100.0)	134 / 134 (100.0, 97.3-100.0)	716 EU/ml (630-814)	460 EU/ml (367-576)	194 EU/ml (158-237)	
HPV18								
Safaeian 2013[d] *(34)*	D0	-	-	-	<LOD	<LOD	<LOD	-
	M6	-	-	-	408 EU/ml	53 EU/ml	76 EU/ml	-
	M12	-	-	-	827 EU/ml	763 EU/ml	71 EU/ml	-
	M24	-	-	-	471 EU/ml	446 EU/ml	69 EU/ml	-
	M36	-	-	-	369 EU/ml	358 EU/ml	74 EU/ml	-
	M48	-	-	-	335 EU/ml (285-392)	305 EU/ml (238-391)	70 EU/ml (54-91)	15 EU/ml (12-19)
Safaeian 2018 *(67)*	M48	2,043 / 2,043 (100.0, 99.8-100.0)	79 / 79 (100.0, 95.4-100.0)	134 / 134 (100.0, 97.3-100.0)	360 EU/ml (313-414)	296 EU/ml (240-366)	112 EU/ml (93-134)	
	M84	2,043 / 2,043 (100.0, 99.8-100.0)	79 / 79 (100.0, 95.4-100.0)	134 / 134 (100.0, 97.3-100.0)	322 EU/ml (281-369)	270 EU/ml (221-330)	125 EU/ml (105-150)	
GARDASIL®								
HPV16								
Sankaranarayanan 2016[e] *(33)*	D0	46 / 1,000 (4.6, 3.4-6.1)	52 / 937 (5.5, 4.2-7.2)	-	MFI 11 (10-12)	MFI 9 (8-10)	-	-
	M7	308 / 308 (100.0, 98.8-100.0)	316 / 317 (99.7, 98.3-100.0)	-	MFI 5,460 (5,195-5.738)	MFI 6,125 (5,785-6,485)	-	-
	M12	-	-	260 / 528 (49.2, 44.9-53.6)	-	-	MFI 106 (96-116)	-
	M18	311 / 313 (99.4, 97.7-99.9)	312 / 314 (99.4, 97.7-99.9)	255 / 476 (53.6, 49.0 – 58.1)	MFI 1,209 (1,105-1,323)	MFI 1,222 (1,116-1,338)	MFI 113 (102-126)	-
	M36	225 / 271 (83.0, 78.0-87.3)	197 / 278 (70.9, 65.1-76.1)	166 / 510 (32.5, 28.5-36.8)	MFI 221 (197-247)	MFI 163 (147-181)	MFI 72 (66-78)	-
Sankaranarayanan 2018 *(68)*	M36	271 / 271 (100.0, 98.6-100.0)	278 / 278 (100.0, 98.7-100.0)	510 / 510 (100.0, 99.3-100.0)	MFI 221 (197-247)	MFI 163 (147-181)	MFI 72 (66-78)	-
	M48	239 / 239 (100.0, 98.5-100.0)	243 / 243 (100.0, 98.5-100.0)	397 / 397 (100.0, 99.1-100.0)	MFI 196 (170-226)	MFI 197 (172-225)	MFI 86 (75-99)	-
HPV18								
Sankaranarayanan 2016[e] *(33)*	D0	41 / 1,000 (4.1, 3.0-5.5)	63 / 937 (6.7, 5.2-8.5)	-	MFI 6 (5-7)	MFI 5 (4-5)	-	-
	M7	308 / 308 (100.0, 98.8-100.0)	317 / 317 (100.0, 98.8-100.0)	-	MFI 2,942 (2,733-3,167)	MFI 3,068 (2,812-3,347)	-	-

Reference	Time point	# seropositive[b] / participants (% Seropositive, 95%CI[c])			GM titers / MFI (95%CI)			
		3 doses	2 doses[a]	1 dose	3 doses	2 doses[a]	1 dose	Naturally infected
	M12	-	-	304 / 528 (57.6, 53.2-61.8)	-	-	MFI 50 (45-55)	-
	M18	307 / 313 (98.1, 85.9-99.3)	305 / 314 (97.1, 94.6-98.7)	259 / 476 (54.4, 49.8-59.0)	MFI 377 (337-422)	MFI 269 (241-299)	MFI 46 (40-51)	-
	M36	249 / 271 (91.9, 88.0-94.8)	238 / 278 (85.6, 80.9-89.5)	271 / 510 (53.1, 48.7-57.5)	MFI 184 (162-208)	MFI 117 (104-132)	MFI 45 (41-49)	-
Sankaranarayanan 2018 *(68)*	M36	271 / 271 (100.0, 98.6-100.0)	278 / 278 (100.0, 98.7-100.0)	510 / 510 (100.0, 99.3-100.0)	MFI 184 (162-208)	MFI 117 (104-132)	MFI 45 (41-49)	-
	M48	239 / 239 (100.0, 98.5-100.0)	243 / 243 (100.0, 98.5-100.0)	397 / 397 (100.0, 99.1-100.0)	MFI 133 (115-154)	MFI 120 (105-136)	MFI 47 (41-53)	-

CI: confidence interval; HPV: Human papillomavirus; M Month; RR: Risk ratio.

[a] Results are shown only for two-dose arms where participants received dose one at day 0 and dose day at day 180.

[b] Definitions of seropositivity used in each study are provided in Table 5.

[c] Seropositivity proportions (%) and 95%CIs, and percentage change in GM levels, were calculated by the authors of the systematic review using data provided in the included articles.

[d] HPV GMTs (95%CI) among 113 unvaccinated but naturally infected controls were 15 (11-19) for HPV16 and 15 (12-19) for HPV18.[22] This article did not report rates of seropositivity for M6, 12, 24 or 36 for HPV16, or at any time point for HPV18. It also did not report 95%CIs for HPV16/18 antibody titers prior to M48; 10th, 25th, 75% and 90th percentiles were reported in the article but not presented in the systematic review.

[e] Month 48 results not shown as reported only for two- and three-dose arms, not for the one-dose arm.

Table adapted from (61).

Table 7. Quality assessment of studies in the trials systematic review

Studies	Parameter	Summary (including adjustment or consideration by study authors)
Kreimer 2011 (65) Kreimer 2015 (66) Safaeian 2013 (34) Safaeian 2018 (67)	Selection bias	CVT and PATRICIA were individually randomised trials of 3d HPV vaccination compared to control HAV. Participants were blinded to vaccine allocation. The '1d' HPV vaccine group were non-completers of the 3d schedule (due to pregnancy, referral to colposcopy, medical conditions, refusal of subsequent vaccinations or missed study visits). Confounding factors could differentially affect whether a participant completed the schedule and their risk of HPV infection during FU; e.g. pregnancy and colposcopy may indicate higher levels of sexual activity and greater exposure to HPV. However, the prevalence of pregnancy and colposcopy was balanced between the HPV 1d group and the HAV 1d control group, against which 1d HPV efficacy was estimated; therefore, pregnancy and colposcopy did not appear to be associated with higher rates of HPV infection during FU. Analyses also assessed whether groups were comparable with respect to sexual activity by looking at HPV DNA or antibody positivity at enrolment. The 1d group had slightly higher HPV DNA detection at enrolment but similar rates of HPV seropositivity as the 3d group. I.e. the 1d group may have been more sexually active on average and, in theory, this would lead to lower VE in the 1d group, yet the data appears to suggest very high VE in the 1d group despite these differences at baseline.
	Retention / survival bias	Kreimer *et al* 2011 set the primary endpoint as newly detected HPV16/18 at the 6m visit or later. The 6 month visit was the time of third dose administration so it is likely that those who missed their third dose, in the 1d or 2d groups, missed this study visit and therefore had a lower probability of detection of incident HPV detection than the 3d group. However, the vaccine efficacy calculated for the 1d group may still be unbiased as it was calculated against a sub-set of the HAV control group that attended/missed the same study visits. The later analysis of the same data combined with the PATRICIA trial data (Kreimer *et al* 2015), addressed this limitation by assessing HPV outcomes at the 12m visit or later, the first visit at which women in the different dose groups may have had an equal chance of attending. The limitation of this later analysis was that LTFU at 12m was higher in the 1d group than in the 1d or 3d groups. This could have again introduced bias; however, the VE was calculated within each dose group compared to the HAV group, controlling for the differential likelihood of HPV detection due to visit attendance. The dose groups and their control groups had very similar prevalence of the different reasons for non-completion and study visit attendance, and were balanced with respect to other confounders measured, leading us to believe the VEs of each dose group are unbiased. When we compare the VEs of the different dose groups we may be comparing slightly different populations. I.e. the 1d VE was calculated in a group of trial enrolees who did not attend every visit and may, on average, have lower health seeking behaviour and be less healthy than the population who attended all study visits. Conversely, the 3d VE was calculated in a group of trial participants who attended all study visits, and could be healthier on average than the 1d group (the 'healthy vaccinee' effect). If these imbalances between the trial groups were borne in reality we would expect a lower VE in the 1d arm; however, even in the presence of this potential bias, the VE of 1d is still high.
	Misclassification	Misclassification of the exposure (the number of vaccine doses received) is unlikely across all analyses as the vaccine was not freely available to trial participants outside of the studies. However, none of the texts mention whether there was any verification of vaccination status at FU visits. All studies used highly sensitive HPV assays and standardised assays for the assessment of IgG. Misclassification of HPV incident or persistent infection is possible if HPV is simply undetectable within the cervix at the time of sampling yet latently infecting the epithelial cells. This is an unavoidable problem given the limitations of HPV sampling techniques and would likely be non-differential across comparison groups.
	Statistical analysis	Appropriate comparisons were made among CVT and PATRICIA trial participants, using the HAV control group. It is legitimate to restrict analysis to those who are HPV negative at enrolment given that is the population targeted for vaccination.
	Generalisability	The trial recruited generally healthy, HIV-negative young women with few exclusion criteria and were therefore relatively pragmatic and representative of the general population. However, trial participants are in general healthier and less heterogenous than the general population.
Sankaranarayanan 2016 (33) Sankaranarayanan 2018 (68)	Selection bias	In the Indian vaccine trial the number of doses a participant received was dependent on their time of enrolment onto the study. It is unlikely that time of enrolment would have significantly affected the distribution of relevant confounders between the groups; e.g. their risk of HPV exposure. The 3d group was, on average, slightly poorer; potentially predisposing them to poorer HPV infection outcomes and poorer immunogenicity. However, both the 3d and 1d groups had similar rates of non-vaccine type infection over the full period of FU (excluding types 31, 33, 45).

Studies	Parameter	Summary (including adjustment or consideration by study authors)
	Retention / survival bias	The lack of a control group in the early analyses of the Indian Vaccine Trial makes differential rates of LTFU across comparison groups a problem. At m36, 75% of the 1d group remained in FU, compared to 88% of the 3d group. No analysis of whether those LTFU were different with respect to baseline characteristics is available in the published texts. Differential LTFU could decrease the rate of HPV detection in the 1d arm simply because the cervical sample wasn't available and therefore biases the vaccine efficacy estimate higher than the true value. However, in the later analysis with follow up to 48m, retention rates had become more similar (75% in the 1d group vs. 78% in the 3d group), reducing the risk of survival bias when comparing VE across groups.
	Misclassification	Misclassification of the exposure (the number of vaccine doses received) is unlikely across all analyses as the vaccine was not freely available to trial participants outside of the studies. However, none of the texts mention whether there was any verification of vaccination status at FU visits. All studies used highly sensitive HPV assays and standardised assays for the assessment of IgG. Misclassification of HPV incident or persistent infection is possible if HPV is simply undetectable within the cervix at the time of sampling yet latently infecting the epithelial cells. This is an unavoidable problem given the limitations of HPV sampling techniques and would be non-differential across comparison groups.
	Statistical analysis	The later analysis of the India Vaccine trial was improved with the enrolment of an unvaccinated control group, allowing comparison of HPV infection outcomes and controlling for visit attendance. Marriage and sexual activity may have influenced both the sampling timepoints for HPV infection (6m after first delivery or 18m after marriage) and risk of HPV acquisition (due to exposure), so the control group of unvaccinated married women is necessary to control for confounding by sexual activity.
	Generalisability	The trial recruited generally healthy, HIV-negative young women with few exclusion criteria and were therefore relatively pragmatic and representative of the general population. However, trial participants are in general healthier and less heterogenous than the general population.

CVT: Costa Rica Vaccine Trial; PATRICIA: PApilloma TRIal against Cancer In young Adults Trial; HAV: Hepatitis A vaccine; DNA: ; VE: Vaccine efficacy; IgG: Immunoglobulin G; HIV: Human Immunodeficiency Virus; M: month; D: Dose; FU: Follow up; LTFU: Loss to follow up.

Table adapted from (61).

4.3 Immunogenicity studies of partially vaccinated populations

In the first edition of the White Paper, we reported on two observational (non-trial) studies that evaluated immune responses after one, two, and three doses of HPV vaccine. One study of 2vHPV was conducted in Uganda (96) and the other of 4vHPV was conducted in Fiji (97). In both studies, seropositivity and antibody titers to HPV vaccine types were evaluated among previously vaccinated individuals. In the Uganda study, antibody responses were evaluated at approximately three years post vaccination, and in the Fiji study, at six years post vaccination.

An updated literature search performed subsequent to the First Edition of the White Paper found an additional five articles published up to the end of March 2019 that provide non-trials immunological data for single-dose HPV vaccination. One paper reported on an additional analysis of the Fijian cohort described above, in which cellular immunological responses were compared in one, two and three-dose participants (98). Another paper describes a retrospective cohort study, utilizing routine data, of women who received one, two or three doses of 4vHPV through the US Department of Defense (DoD) vaccination program (99). Routine serum samples, collected pre and post vaccination, were used for measurement of seropositivity to the 4vHPV types. Two other papers were published by the same research group in Quebec, Canada. The first of these reported on a study in which participants received a dose of 9vHPV between three and eight years following an initial dose of 4vHPV (100). Measurement of antibody seropositivity and titers prior to administration of the second dose allowed assessment of durability of immune responses following a single dose of 4vHPV. The other study from Quebec compared HPV antibody responses between the delayed-second-dose cohort (from the first study) and an independent cohort of girls and boys receiving two doses of 9vHPV with an interval of six months (101). Finally, the most recently published paper described a prospective observational cohort study that compared HPV antibody responses among HIV-positive and negative adolescents who received one, two and three doses of 4vHPV in the United States (102).

All seven studies are described in detail below and summarized in Table 8. Summarized humoral immunogenicity results from these studies are provided in Table 9.

4.3.1 The Uganda study

4.3.1.1 STUDY DESIGN

The Uganda study was a cross-sectional non-inferiority immunogenicity study among 376 adolescent girls (age 10 to 11 years at the time of vaccination) who had been vaccinated as part of a government-run HPV vaccination demonstration program implemented between October 2008 and October 2009 in one district of the country (96, 103). HPV vaccine was administered by immunization program vaccinators in a three-dose schedule (months 0, 1 and 6). Three-dose completion among girls aged ten years was 52-60%. The cross-sectional immunogenicity study recruited girls who had received one, two, or three doses; recruitment was district-wide but started in specific sub-districts. Enrollment closed in each group as soon as the desired sample size was reached. Participants were recruited based on data in vaccine registries, but final vaccine status was based on information in vaccination cards (provided by parents).

4.3.1.2 LABORATORY METHODS

Participants provided 10 mL of blood at enrollment. Serum was stored at −70^{O}C, sent to the HPV Immunology Laboratory of the NCI (Fredrick, Maryland, USA), and tested by ELISA for HPV 16 and HPV 18 antibodies. Seropositivity cut-offs for HPV 16 and HPV 18 were 8 EU/mL and 7 EU/mL, respectively. The laboratory, assay, and seropositivity cut-offs were the same as those used in the Costa Rica Vaccine Trial (CVT), and subsequent studies of the trial cohorts.

4.3.1.3 STATISTICAL ANALYSIS

Analyses included comparison of GMTs in girls who received one or two doses compared to those who received three doses. Antibody levels in the one- and two-dose groups were also compared with the lowest antibody levels in the three-dose group, and in an exploratory analysis with GMTs in the CVT (34, 65). To test non-inferiority of one and two vaccine doses relative to three doses, GMT ratios (one:three dose and two:three dose) with multiplicity-adjusted 97.5% CI were determined. Non-inferiority was defined as the lower bound of the CI of the GMT ratio greater than 0.50.

4.3.1.4 RESULTS

Overall, vaccine registries indicated that 3,785 girls had received three doses, 1,044 received two doses, and 291 received one dose. Study enrollment and blood draws were completed for 195 three-dose recipients, 145 two-dose recipients, and 36 one-dose vaccine recipients. Enrollment of one-dose vaccine recipients was lower than expected, due to persistent follow-up by government vaccination nurses to ensure girls received missed doses of HPV

vaccine. However, the recording of follow-up doses was not re-entered into the original vaccination register for the demonstration project.

Study participant demographic characteristics were comparable across dose groups. The mean time between last dose and blood collection was 33, 39, and 33 months, respectively, for three-, two- and one-dose groups. Overall, 99% were HPV 16 and HPV 18 seropositive. GMTs of anti-HPV 16 were the following: 1607 EU/mL in three-dose recipients, 808 EU/mL in two-dose recipients, and 230 EU/mL in single-dose recipients. Anti-HPV 18 GMTs were the following: 296 EU/mL for three-dose recipients, 270 EU/mL in two-dose recipients, and 87 EU/mL in one-dose recipients. The GMT ratios for two:three doses and one:two doses did not meet the non-inferiority criteria for HPV 16 (0.50) or HPV 18 (0.68). However, in the cross-study comparison, GMTs for one dose recipients were not lower in the Ugandan girls than in adult women who received one dose in the CVT (HPV16=124 EU/mL, HPV18=69 EU/mL) in whom efficacy had been demonstrated (69).

4.3.2 The Fiji study

4.3.2.1 STUDY DESIGN

The Fiji study (97) was a follow-up study of 200 girls 15 to 19 years of age who had been vaccinated in 2008–2009 when the Fiji Ministry of Health and Medical Services (MHMS) received a donation of 4vHPV. At that time, all girls aged 9 to 12 years were eligible to receive the recommended three-dose schedule (0, 2, 6 months); however, some received only one or two doses due to non-completion of the vaccine schedule. In 2015, girls were recruited into a study designed to compare neutralizing antibody responses of vaccinees who received one or two doses of HPV vaccine compared to those who received three doses. Girls were enrolled into vaccine dose groups based on immunization lists obtained from MHMS. A group of unvaccinated girls was also recruited. A secondary aim of the study was to assess whether vaccination had elicited immune memory and if there were differences by dose group. In order to do this, a challenge dose of 2vHPV was administered to girls in the one-, two-, and three-dose groups and neutralizing antibody responses measured in samples collected post 2vHPV vaccination.

In a sub-study of 59 girls randomly selected from the main cohort study, cellular immune responses were additionally measured (98).

4.3.2.2 LABORATORY METHODS

Blood was drawn on enrollment into the study (six years after vaccination with 4vHPV) and 28 days after the challenge dose of 2vHPV. Serum samples for humoral immunogenicity evaluations were frozen at –80°C and shipped on dry ice to Murdoch Children's Research

Institute in Melbourne, Australia for analysis. Neutralizing antibody (NAbs) against HPV types 6, 11, 16, and 18 were measured using the pseudovirion-based neutralization assay (104). The neutralizing titer (ED50) was defined as the highest serum dilution that reduces the secreted alkaline phosphatase activity by at least 50% in comparison to a control (pseudovirions without serum). A sample with an ED50 value of ≥100 was considered HPV seropositive; seronegative samples were given a value of 50.

Peripheral blood mononuclear cells (PBMCs) were additionally isolated from whole blood and cryopreserved. For cellular immunogenicity evaluations, PBMCs were thawed and stimulated with pooled peptides of HPV 16 and 18 L1 proteins. Numbers of HPV16/18-specific IFNy-producing cells were quantified by ELISpot, and a panel of Th1 and Th2 cytokines were measured in culture supernatants by multiplex bead array. Flow cytometry was performed in order to enumerate memory CD4+ and CD8+ cell populations.

4.3.2.3 STATISTICAL ANALYSIS

For the humoral immunogenicity analyses, the primary analysis was a comparison of the GMTs of NAb (and 95%CIs) against HPV 6, 11, 16, and 18 in girls who previously received one or two doses of 4vHPV compared to girls who had received three doses. Within the two-dose group, the investigators also stratified girls into those who received two doses at an interval of <6 or ≥6 months. The secondary analyses included comparisons of NAb GMTs at one month after a dose of 2vHPV between girls who had received one, two or three 4vHPV doses. NAb titers were compared using the Student *t* test or Mann-Whitney test. A sample-size calculation determined that for 80% power to detect a 30% difference in HPV antibodies with a two-sided 5% significance level, the number needed in each group was 26 and 47.

As for the humoral responses, the primary analysis for the cellular immunogenicity responses compared the number of HPV-specific IFNy-producing cells (from ELISpot) in girls receiving one or two doses of 4vHPV versus those who received three doses at six years post vaccination. In secondary analyses, the number of IFNy-producing cells was compared between the same groups one month after a boost dose of 2vHPV was administered. The same comparisons were made for the multiplex and flow cytometric data. A sample-size calculation determined that a sample size of 10 and 16 per group for HPV16 and 18, respectively, would give 66% power to detect a 20% difference in number of HPV16/18-specific IFNy-producing cells with a two-sided 5% significance level.

4.3.2.4 RESULTS

Humoral immunogenicity results

A total of 200 girls were enrolled: 66 in the three-dose group; 60 in the two-dose group, 40 in the one-dose group and 34 in the unvaccinated group. The baseline characteristics of participants did not differ by vaccine group except for small differences by time since last vaccine dose and differences in timing of doses one and two in the three- and two-dose groups. Compared with the other groups, age at enrollment was higher in the unvaccinated group and a larger percentage attended university. At enrollment, six years after initial vaccination, 90-100% of girls were seropositive for HPV 6, 93-100% for HPV 11, 95-100% for HPV 16 and 68-88% for HPV 18. GMTs for all 4vHPV types were not statistically different between three- and two-dose recipients:

- **HPV6** (three-dose: 2216 [95% CI, 1695–2896] vs. two-dose: 1476 [1019–2137]; P =.07);
- **HPV11** (three-dose: 4431 [3396–5783] vs. two-dose: 2951 [1984–4390]; P =.09);
- **HPV16** (three-dose: 3373 [2511–4530] vs. two-dose: 3275 [2452–4373]; P =.89); and
- **HPV18** (three-dose: 628 [445–888] vs. two-dose: 606 [462–862]; P =.89).

One-dose recipients had significantly lower NAb titers than two- or three-dose recipients; among all groups, titers were five to 30-fold higher than unvaccinated girls. There were no differences in titers in two-dose girls who received dose one and dose two more or less than six months apart.

After a dose of 2vHPV, NAb titers for HPV 16 and 18 in the one-dose group increased 46- and 84-fold and were not significantly different from the two-dose and three-dose groups, suggesting that a single dose of 4vHPV may be sufficient to prime for immunologic memory to HPV16 and HPV18.

Cellular immunogenicity results

Fifty-nine girls were included in the cellular immunogenicity sub-study. At the time of enrollment, 15 girls had received three doses of 4vHPV, 14 had received two doses, 15 had received one dose, and 15 were unvaccinated. Flow cytometry was performed for fewer participants (≤7 per group) due to limited availability of cells. The baseline characteristics were similar in the sub-study cohort compared to the full Fijian cohort, except that the three-dose participants in the sub-study cohort were older at the time of first vaccination with 4vHPV and at enrolment into the study.

At six years post 4vHPV vaccination (and pre 2vHPV vaccination), numbers of HPV16-specific IFNy-producing cells were similar among one, two and three-dose participants. Numbers of HPV-18-specific IFNy-producing cells were lower among two-dose

participants (but not one-dose participants) compared to three-dose participants (p = 0.004). Post boost vaccination with 2vHPV, HPV16- and HPV18-specific IFNy-producing cells were similar among participants previously receiving one, two and three doses of 4vHPV.

In flow cytometry, no significant differences in HPV16- and HPV18-specific memory CD4+ cells were observed between the different dosage groups either pre or post 2vHPV administration. Low levels of HPV16- and HPV18-specific memory CD8+ cells were observed across all groups at both timepoints. In multiplex array, levels of cytokines released in response to HPV16 and HPV18 stimulation were largely similar in the one-dose and three-dose 4vHPV recipients, both pre and post 2vHPV administration, though levels of a few cytokines were lower in the one-dose group (pre-2vHPV: IL2 for HPV16 and 18, and IL10 for HPV18 only; post-2vHPV: IFNy for HPV16 and 18, and IL-10 for HPV16 only).

4.3.3 The US DoD study

4.3.3.1 STUDY DESIGN

The US DoD study was a retrospective cohort analysis of women vaccinated at age 17-26 years with one, two, or three doses of 4vHPV (66, 99). HPV vaccine was provided through a routine US DoD vaccination program, which administers a three-dose HPV vaccination schedule. Thus, one- and two-dose recipients were non-completers of the intended three-dose schedule. The study obtained records of vaccinated women using routine data from the Defense Medical Surveillance System (DMSS), which maintains medical records, immunization records, and demographic data for US military personnel. Women were included if routine serum samples collected within one year pre-vaccination and four to six years post vaccination were available in the DoD Serum Repository (DoDSR). The samples were used to test for seropositivity to each of the 4vHPV types, and seropositivity rates were compared across vaccine dosage arms.

4.3.3.2 LABORATORY METHODS

Serum samples collected pre- and post-HPV vaccination were retrieved from the DoDSR and shipped to the John Hopkins University. Samples were tested for seropositivity to HPV6, 11, 16 and 18 by VLP ELISA. Seropositivity cut-offs were not provided in the published article.

4.3.3.3 STATISTICAL ANALYSIS

The authors first calculated the percentage of women who were seropositive to HPV6, 11, 16 or 18 pre-vaccination. Post-vaccination seropositivity rates for each HPV type were then calculated, by number of doses received, among women who were sero-negative for the corresponding HPV type(s). Additionally, binomial proportions of women seroconverting were calculated by number of doses received, stratified by demographic category. 95% CIs were calculated using the Agresti-Coull method, and statistical significance was assessed using Fisher's Exact test.

4.3.3.4 RESULTS

The authors obtained records of 2,091 women who had received 4vHPV through the US DoD vaccination program, and who had pre- and post-vaccination serum samples available. 1,260 women completed the intended three-dose schedule, 420 received two doses, and 411 received only one dose. Pre-vaccination, 62.1% of women (61.9% of three-dose recipients, 60.5% of two-dose recipients and 64.5% of one-dose recipients) tested positive for at least one HPV type (of HPV6, 11, 16 and 18). There was no statistical difference in pre-vaccination seropositivity rates between vaccine dosage arms (p=0.4777).

99.8% of three-dose recipients, 100% of two-dose recipients and 100% of single-dose recipients who were HPV6, 11, 16 *and* 18 sero-negative pre-vaccination seroconverted to all four HPV types post vaccination. There was no statistical difference in the proportion of sero-negative women seroconverting to all four HPV types post vaccination between vaccine dosage arms (p=1.0). Equivalent results for individual HPV types were as follows:

- **HPV6 – Seroconversion in 98.1% of three-dose recipients, 96.8% of two-dose recipients and 92% of one-dose recipients;**
- **HPV11 – Seroconversion in 99.4% of three-dose recipients, 99.7% of two-dose recipients and 97.6% of one-dose recipients;**
- **HPV16– Seroconversion in 98.8% of three-dose recipients, 97.0% of two-dose recipients and 89.8% of one-dose recipients;**
- **HPV18 – Seroconversion in 79.6% of three-dose recipients, 81.1% of two-dose recipients and 82.7% of one-dose recipients.**

The difference in seropositivity rates between vaccine dosage arms was statistically significant for HPV6 ($p<0.0001$) and HPV16 ($p<0.0001$), but not HPV11 ($p = 0.0258$) or HPV18 ($p = 0.4473$).

4.3.4 The Quebec studies

4.3.4.1 STUDY DESIGN

The first Quebec study (ClinicalTrials.gov Identifier: NCT03431246) was a small single-group study of 31 girls aged 13 to 18 years who received a single dose of 4vHPV between three and eight years prior to enrolment (100). At the time of entry into the study, the girls were given a boost dose of 9vHPV. The prior 4vHPV vaccine was provided to the girls through a school-based national vaccination program; the reason for only receiving a single dose was non-completion of the intended three-dose schedule. Immunization status was determined from regional vaccination registry data and vaccination cards, and confirmed with participants and their parents. The objectives of the study were two-fold: to assess persistence of HPV-specific antibodies after a single dose of 4vHPV (using blood samples collected prior to the boost dose of 9vHPV) and to assess the effect of a dose of 9vHPV given several years later (using blood samples collected one month following the boost dose of 9vHPV).

The second Quebec study was a post-hoc analysis comparing antibody responses among the 31 girls included in the study above with those from an independent cohort of 88 girls and 85 boys aged nine to ten years old who received two doses of 9vHPV six months apart (range 5.7 to 6.9 months) (101). This independent cohort of boys and girls were from a clinical trial of a two-dose 9vHPV schedule conducted by the same authors (ClinicalTrials.gov Identifier: NCT02567955) (105). Clinical trial participants were eligible for inclusion in the post hoc comparison if they had blood samples available before and one month following their second vaccine dose.

4.3.4.2 LABORATORY METHODS

Blood collection and antibody testing were harmonized in the two studies. Blood was collected before and one month following vaccination with the second vaccine dose (9vHPV), and serum samples were shipped to the CDC in Atlanta (USA) for analyses. 9vHPV vaccine-type antibody titers were measured using multiplex direct IgG ELISA to HPV L1 and L2 virus-like particles on MSD platform. Antibody titers were measured in international units (IU/ml) for HPV16 and 18, and in arbitrary units (AU/ml) for other types. Samples were considered positive for HPV antibodies if they passed parallel line method conditions and were above the median plus two standard deviations of the PLL/titer generated from control samples. Cut-off values for seropositivity were applied individually for each of the nine HPV types tested.

4.3.4.3 STATISTICAL ANALYSIS

Proportions of participants with detectable HPV vaccine specific antibodies and GMTs with 95%CIs were determined. In the post-hoc analysis, proportions of participants seropositive for each HPV type were compared between the two cohorts using two-tailed Fisher's Exact Test. Titer distributions and GMTs were compared between the two cohorts using two-tailed Wilcoxon's test.

4.3.4.4 RESULTS

Thirty-one girls were enrolled into the first cohort between three and eight years (mean 5.4 years) after their single dose of 4vHPV; all participants were administered a dose of 9vHPV. In the second study, 173 girls and boys were administered two doses of 9vHPV six months apart.

All participants in both studies were seropositive to HPV6, 11, 16 and 18 (i.e. all four 4vHPV types) prior to receiving their second dose. GMTs for the first cohort (n = 31) were as follows: HPV6 - 6.1 AU/ml; HPV11 - 7.7 AU/ml; HPV16 - 20.1 IU/ml; HPV18 - 6.3 IU/ml. GMTs for the second cohort (n = 173) were as follows: HPV6 – 5.3 AU/ml; HPV11 – 5.8 AU/ml; HPV16 – 29.7 IU/ml; HPV18 – 11.0 IU/ml. Titers were significantly higher among the second cohort compared to the first for HPV18 (p=0.005), but not for the other three types (HPV6, 11 and 16). Of note, between 58% and 87% of participants in the first cohort were also seropositive to non-4vHPV types prior to administration with 9vHPV, with GMTs ranging from 2.0 to 5.2 AU/ml.

Following vaccination with the second vaccine dose, all participants in both cohorts were seropositive for the nine 9vHPV types. Among the first cohort, GMTs increased 60- to 82-fold for the four types included in both vaccines, indicating that long term memory is induced after a single dose of 4vHPV.

4.3.5 The US PHACS study

4.3.5.1 STUDY DESIGN

This was a prospective observational cohort study of children who received one, two or three doses of 4vHPV at an average age of 13 years (interquartile range (IQR) 11-15 years) through a national vaccination program (102). The reason for some participants receiving one or two doses was non-completion of the intended three-dose schedule. The study was conducted within the Pediatric HIV/AIDS Cohort Study (PHACS) Adolescent Master Protocol and included children who were either perinatally HIV-infected (PHIV) or perinatally HIV-exposed but not infected (PHEU). The study evaluated 4vHPV-type

antibody seropositivity and titers approximately three years after the last vaccine dose (IQR 1.8-4.1 years). Sexually active but non-HPV vaccinated children of the same age as the vaccinated children at the time of enrolment were additionally included as a control group to allow evaluation of natural seroconversion. Comparisons were made across PHIV and PHEU, and between different dosing groups.

Of note, whilst incidence rates of cervical abnormalities and genital warts were also evaluated for PHIV versus PHEU within the study, the numbers of participants and events were small, and the data were not stratified by number of doses received. These results are therefore not presented in this White Paper.

4.3.5.2 LABORATORY METHODS

Sera were collected from vaccinated participants at least 20 days after their most recent HPV vaccine dose. For control, non-vaccinated participants, sera were collected after sexual debut. Samples were tested for neutralizing IgG to the four 4vHPV types using a competitive Luminex Immunoassay (cLIA). HPV18 antibody titers were additionally measured using an anti-HPV IgG enzyme immunoassay (EIA). Sample testing was conducted by Merck laboratories. Cut-off values for seropositivity were applied individually for each of the four HPV types tested in cLIA, and for HPV18 in EIA.

4.3.5.3 STATISTICAL ANALYSIS

The study reports proportions of participants within each cohort and dosage group who were seropositive for each 4vHPV type with binomial confidence intervals. Groups were compared using Fisher's Exact Test. Additionally least squares mean regression was performed using log-transformed titers, adjusted for time between last vaccine dose and sample collection, to predict GMTs as a function of cohort and number of doses received.

4.3.5.4 IMMUNOGENICITY RESULTS

The authors reported antibody seropositivity and titer data for 310 PHIV and 148 PHEU. Among PHIV, 90 received three doses, 34 received two doses, 154 received one dose, and 32 were unvaccinated. Among PHEU, 11 received three doses, 13 received two doses, 91 received one dose, and 33 were unvaccinated.

Overall seropositivity rates (measured by cLIA) for HPV6, 11, 16 and 18 among PHIV who received at least one does of 4vHPV were 83%, 84%, 90% and 62%, respectively. Among PHEU, corresponding proportions were 94%, 96%, 99% and 87%. Seropositivity rates did not vary considerably by number of doses received within either PHIV or PHEU, but were significantly higher among vaccine recipients (regardless of the number of doses

received) compared to unvaccinated participants. Among PHIV, proportions seropositive for the four 4vHPV types, by number of doses received, were as follows:

- **HPV6 – three doses: 82.2%, two doses: 82.4%, one dose: 84.4%, no dose: 12.5%**
- **HPV11 – three doses: 84.4%, two doses: 88.2%, one dose: 83.1%, no dose: 12.5%**
- **HPV16 – three doses: 92.2%, two doses: 91.2%, one dose: 87.7%, no dose: 12.5%**
- **HPV18 – three doses: 61.1%, two doses: 61.8%, one dose: 62.3%, no dose: 12.5%**

Among PHEU, proportions seropositive for the four 4vHPV types, by number of doses received, were as follows:

- **HPV6 – three doses: 100%, two doses: 100%, one dose: 92.3%, no dose: 27.3%**
- **HPV11 – three doses: 100%, two doses: 100%, one dose: 94.5%, no dose: 30.3%**
- **HPV16 – three doses: 100%, two doses: 100%, one dose: 98.9%, no dose: 30.3%**
- **HPV18 – three doses: 81.8%, two doses: 92.3%, one dose: 86.8%, no dose: 21.2%**

Whilst seropositivity rates appeared higher among two- and three-dose recipients compared to one-dose recipients among PHEU, numbers of participants in the two- and three-dose arms were very small (13 and 11, respectively), and thus 95% CIs were wide and overlapped with those for the single-dose group.

GMTs for the four 4vHPV types also did not differ considerably between three, dose and one dose recipients, and GMTs were significantly higher for all vaccine recipients (regardless of number of doses received) than in unvaccinated participants. Among PHIV, GMTs by number of doses received were as follows:

- **HPV6 – three doses: 109 mMU/ml, two doses: 125 mMU/ml, one dose: 118 mMU/ml, no dose: 16 mMU/ml**
- **HPV11 – three doses: 121 mMU/ml, two doses: 146 mMU/ml, one dose: 129 mMU/ml, no dose: 12 mMU/ml**
- **HPV16 – three doses: 430 mMU/ml, two doses: 497 mMU/ml, one dose: 519 mMU/ml, no dose: 19 mMU/ml**
- **HPV18 – three doses: 57 mMU/ml, two doses: 71 mMU/ml, one dose: 67 mMU/ml, no dose: 16 mMU/ml**

Among PHEU, GMTs by number of doses received were as follows:

- **HPV6 – three doses: 236 mMU/ml, two doses: 252 mMU/ml, one dose: 164 mMU/ml, no dose: 24 mMU/ml**
- **HPV11 – three doses: 314 mMU/ml, two doses: 421 mMU/ml, one dose: 287 mMU/ml, no dose: 22 mMU/ml**
- **HPV16 – three doses: 1367 mMU/ml, two doses: 2129 mMU/ml, one dose: 1464 mMU/ml, no dose: 39 mMU/ml**
- **HPV18 – three doses: 142 mMU/ml, two doses: 245 mMU/ml, one dose: 165 mMU/ml, no dose: 23 mMU/ml**

Again, numbers of participants in the two and three-dose PHEU arms were small, so 95% CIs for GMTs were wide and overlapped with those for the single-dose PHEU arm for each HPV type.

4.3.6 Strengths and weaknesses of immunogenicity studies of partially vaccinated individuals

There are several strengths of these immunogenicity studies. Some of the studies used the same laboratory assay to assess immune responses as previous clinical HPV vaccine trials, which allowed for comparison to antibody titres reported from clinical trials of adult women receiving single-dose schedules among whom efficacy had been demonstrated. **The lack of WHO international standards for HPV types 16 and 18 assays until recently meant that earlier immunogenicity studies could not use these standard assays.** Some studies had long follow-up time to accommodate an immunogenicity plateau observed 24 months after initial vaccination. **The Quebec study evaluated persistence of HPV-specific antibodies between three and eight years after vaccination with a single dose of 4vHPV.**

Where included (e.g., in the US PHACS study), non-HPV vaccinated participants had lower antibody titres than single-dose recipients. Furthermore, single-dose recipients from these immunogenicity studies had higher antibody titres than naturally infected women from prior trials of HPV vaccine. The US PHACS study provides data for a cohort of HIV-positive adolescents, a sub-group for whom data has been lacking; whilst the US DoD study provides data for women vaccinated at an older age compared to other immunogenicity studies. A major strength of the US DoD study was the availability of pre-vaccination serum samples for all study participants, enabling the authors to determine HPV seropositivity status and thus numbers of sero-negative women who seroconverted after vaccination, according to the number of vaccine doses received.

These observational studies also have a number of limitations. None of the studies was an RCT and, therefore, participants might have differed by dose group. The results could suffer from selection bias and confounding. The Fiji study had data on participants six years after their initial vaccination, including body mass index (BMI), ethnicity, and some socioeconomic and behavioral characteristics. **Many of these data were also available for the US PHACS cohort; however, they were not stratified by number of doses received (only by PHEU versus PHIV). Data to evaluate comparability across groups were more limited from the Uganda study. While neither the Uganda or the Fiji study reported data on sexual behavior, all girls in the Uganda study were aged 10 or 11 years at the time of vaccination, and prevalent infections prior to vaccination are highly unlikely in this context. The US PHACS study did report data on sexual activity and age at sexual debut but, again, data were not stratified by number of doses received. The US DoD study used routine data obtained from the Defense Medical Surveillance System, so available data on potential confounders, or data that could be used to assess for biases due to differing characteristics between dosage arms, were limited.**

The first Quebec study included only a single group of participants, all of whom received one dose of 4vHPV and were boosted with a dose of 9vHPV. Therefore, no comparisons in immune response can be made with either unvaccinated individuals or multi-dose recipients within the study. Participants were non-completers of a three-dose national HPV national programme. In the second Quebec study, results from the single-dose 4vHPV cohort receiving a delayed second dose of 9vHPV were compared with those from a cohort of adolescents receiving two doses of 9vHPV vaccine. Whilst laboratory methods were harmonised between the two studies, there may be differences in the two cohorts that could lead to bias or confounding.

Sample sizes were relatively small in all the studies except the US DoD study, especially among single-dose groups, thus limiting the statistical precision of estimates. In the Uganda study, the sample size was too small to test the primary hypothesis of non-inferiority of one dose compared with three doses with sufficient power. Nevertheless, in a cross-study comparison among girls who received only a single dose in Uganda, GMTs were not lower than those in women who received a single HPV vaccine dose in the CVT, among whom no breakthrough cases have been detected four years after vaccination. **Whilst the US PHACS study followed up participants to obtain incidence rates of cervical abnormalities and genital warts, the authors were not able to compare these between dosage arms due to the small numbers of participants in each group.**

Finally, several studies measured immune responses at only one time point following vaccination, and thus the kinetics of the response over time cannot be evaluated.

4.3.7 Summary of immunogenicity studies of partially vaccinated individuals

Together, the immunogenicity studies demonstrate that single-dose HPV vaccination results in high rates of seroconversion and sustained seropositivity to vaccine-type HPV over time. In both the Uganda and Fiji studies, GMTs after one dose of HPV vaccine were lower than after two or three doses. In the Uganda study, GMTs after one or two doses of 2vHPVvaccine (measured about three years after the last dose) did not meet the threshold to be declared non-inferior to three doses. However, GMTs of antibody in adolescents who received only one dose in Uganda were still higher than women who received one dose of 2vHPV vaccine in the CVT, among whom there have been no breakthrough cases of persistent infection up to four years after vaccination (34, 65). Furthermore, in Uganda, even though immune responses were inferior in the single-dose group, they were still four-fold higher than natural infection.

In the Fiji study, no significant differences in the GMTs across all four HPV types were found between girls who previously received two or three doses of 4vHPV. Antibody was detected among one-dose recipients six years after vaccination, but GMTs were significantly lower than among two- or three-dose recipients. Immune memory, as measured by the humoral anamnestic response after a challenge dose of 2vHPV, was evident in all one-, two- and three-dose vaccine recipients.

Cellular immune responses were detectable among 4vHPV recipients in a sub-cohort of the Fiji study six years after vaccination, regardless of number of doses received. HPV16-specific responses were generally similar between the dosage groups, but some HPV18-specific responses were lower among one- or two-dose groups compared to the three-dose groups. Cellular responses (both HPV16 and HPV18-specific) were mostly similar between dosage groups after a dose of 2vHPV was administered.

The US DoD study found that, among women aged 17 to 26 years who were sero-naïve to all four 4vHPV types prior to vaccination, seroconversion to the four HPV types post vaccination was very high (approaching 100%), and did not differ by number of vaccine doses received.

In the Quebec studies, all girls who received a single dose of 4vHPV between three and eight years previously were seropositive for the four vaccine-types. Antibody titers prior to the second dose were not significantly different in the two cohorts for three of the four quadrivalent vaccine HPV types: HPV6, 11 and 16. Only titers of antibodies to HPV18 were higher in the second cohort (two dose 9vHPV) compared to the first (delayed second dose 9vHPV). In both groups, boosting with 9vHPV induced a memory response to all four types.

In the US PHACS study, seropositivity rates to all 4vHPV vaccine HPV types were high, but lower in PHIV (ranging from 62 to 99%) compared to PHEU (ranging from 87 to 99%). Stratified by HIV status, seropositivity rates and antibody titers did not differ significantly for participants who received one, two, or three vaccine doses. Furthermore, all vaccinated participants had significantly higher seropositivity rates and antibody titers than unvaccinated control participants, regardless of number of doses received.

Although most of these studies have shown that GMTs after a single dose of HPV vaccine are lower than after multi-dose regimens, a minimal immunogenicity level sufficient for protection has not been identified so the clinical relevance of these differences is unclear.

Table 8. Summary of non-trial immunogenicity studies

Reference, location	Study design	Study population	Vaccination setting	Actual vaccination schedule(s)	Age at vaccination	Sampling	Immunogenicity endpoint(s)	Method(s)
LaMontagne 2014; Uganda (96)	Cross-sectional study of girls with prior HPV vaccination	376 girls aged 13-15y	Government demonstration program of 3d 2vHPV	3d 2vHPV (n=195) 2d 2vHPV (n=145) 1d 2vHPV (n=36)	10y	Serum collected at enrolment	HPV16/18 seropositivity & titres	ELISA; Cut-offs for seropositivity – HPV16: 8 EU/mL; HPV18: 7 EU/mL
Hurt 2016; USA (99)	Retrospective cohort routine data study of women with prior HPV vaccination	2,091 women aged 17-26y	U.S Department of Defence vaccination program of 3d 4vHPV	3d 4vHPV (n=1,260) 2d 4vHPV (n=420) 1d 4vHPV (n=411)	17-26y	Serum collected within 1y prior to first dose and 4-6y post last dose	HPV6/11/16/18 seropositivity	ELISA; Cut-offs for seropositivity not stated
Toh 2017; Fiji (97)	Intervention study of girls with prior HPV vaccination administered a challenge dose	200 girls aged 15-19y	Prior vaccine: National vaccination campaign of 3d 4vHPV Challenge vaccine: Study intervention	Prior to study: 3d 4vHPV (n=66) 2d 4vHPV (n=60) 1d 4vHPV (n=40) 0d HPV vaccine (n=32) Challenge vaccine: 1d 2vHPV (all subjects)	Previous vaccine: 9-12y Challenge vaccine: 15-19y	Serum collected at enrolment & 28d after challenge dose of 2vHPV	HPV6/11/16/18 neutralizing seropositivity & titres	PBNA; Cut-off for seropositivity – ED50 ≥100
Toh 2018; Fiji (98)		59 girls aged 15-19y		As above – 3d (n=15); 2d (n=14); 1d (n =15); 0d (n=15)		PBMCs collected at enrolment & 28d after challenge dose of 2vHPV	HPV16/18-specific IFNy-producing cells (& memory CD4+/ CD8+ cells)	ELISpot; Flow cytometry; Multiplex bead array
Gilca 2019 (1); Canada (100)	Intervention study of girls with prior HPV vaccination administered a boost dose	31 girls aged 13-18y	Prior vaccine: School-based national vaccination program of 3d 4vHPV Challenge vaccine: Study intervention	Prior to study: 1d 4vHPV (n=31) Challenge vaccine: 1d 9vHPV (all subjects)	Previous vaccine: 9-14y Challenge vaccine: 13-18y	Serum collected before & one month post 2nd vaccine dose	HPV6/11/16/18/31/33/45/52/58 seropositivity & titres	Multiplex direct IgG ELISA on MSD platform; Cut-offs for seropositivity – HPV6: 0.1 AU/mL ; HPV11: 0.1 AU/mL HPV16: 0.5 AU/mL; HPV18: 0.4 AU/mL
Gilca 2019 (2); Canada (101)	Post-hoc comparison of two HPV-vaccinated cohorts	Cohort 1: Described above Cohort 2: 173 girls & boys aged 9-10y	Cohort 1: Described above Cohort 2: Prior intervention study of 2d 9vHPV	Cohort 1: 1d 4vHPV & 1d 9vHPV 3-8y later Cohort 2: 2d 9vHPV	Cohort 1: Described above Cohort 2: 9-10y			
Mosckicki 2019; USA (102)	Prospective cohort study of adolescents with prior HPV vaccination, embedded in PHACS cohort	310 PHIV & 148 PHEU girls & boys aged 7-16y at time of entry into PHACS cohort	National vaccination program of 3d 4vHPV	3d 4vHPV (n=101) 2d 4vHPV (n=47) 1d 4vHPV (n=245) 0d HPV vaccine (n=65; sexually active)	Mean: 13y IQR: 11-15y	Serum collected ≥20d after last vaccine dose Age at sampling – Mean: 16y; IQR: 13-18y	HPV6/11/16/18 binding & neutralizing seropositivity & titres	Direct IgG EIA; Cut-offs for seropositivity – HPV6: 15 mMU/mL; HPV11: 15 mMU/mL HPV16: 7 mMU/mL; HPV18: 10 mMU/mL cLIA; Cut-offs for seropositivity – HPV6: 20 mMU/mL; HPV11: 16 mMU/mL HPV16: 20 mMU/mL; HPV18: 24 mMU/mL

HPV: Human papillomavirus; Y: Years; D: Dose; M: Month; N: Number; ELISA: Enzyme-linked immunosorbent assay; PBMCs: Peripheral blood mononuclear cells; IFNy: Interferon-gamma; PBNA: pseudovirion-based neutralization assay; ELISpot: Enzyme-linked immunosorbent spot; IgG: Immunoglobulin G; MSD: Meso-scale discovery; USA: United States of America; PHACS: Pediatric HIV/AIDS Cohort Study; PHIV: perinatally HIV-infected; PHEU: perinatally HIV-exposed, uninfected; IQR: Inter-quartile range; EIA: Enzyme immunoassay; cLIA: Competitive Luminex immunoassay

Table 9. Summarized HPV16/18 seropositivity and GM antibody level results from non-trial immunogenicity studies

Reference	Antibody response measured	Time since last vaccine dose	HPV type	# Seropositive / total (%)				GM titers (95%CI)			
				3 doses	2 doses	1 dose	0 dose	3 doses	2 doses	1 dose	0 dose
2vHPV											
LaMontagne 2014 *(96)*	Binding	Mean (IQR) – 3d group: 38m (29-43m) 2d group: 39m (29-49m) 1d group: 33m (17-48m)	HPV16	Individual results not provided; 99.25% of all participants seroconverted				1607.92 EU/mL (1381.78–1871.07)	808.38 EU/mL (631.86–1034.22)	229.86 EU/mL (139.27–379.38)	NA
			HPV18					395.51 EU/mL (331.15–472.37)	270.21 EU/mL (213.15–342.55)	86.87 EU/mL (54.98–137.23)	NA
4vHPV											
Hurt 2016[a] *(99)*	Binding	4-6y	HPV16	917 / 928 (99%)	294 / 303 (97%)	237 / 264 (90%)	596 / 2,091 (29%)	NA	NA	NA	NA
			HPV18	839 / 1054 (80%)	287 / 354 (81%)	291 / 352 (83%)	331 / 2,091 (16%)	NA	NA	NA	NA
Gilca 2019[b] *(100)*	Binding	Mean (IQR) – 65.3m (36-96m)	HPV16	NA	NA	31 / 31 (100%)	NA	NA	NA	20.1 AU/mL (12.0-33.7)	NA
			HPV18	NA	NA	31 / 31 (100%)	NA	NA	NA	6.3 AU/mL (3.8-10.2)	NA
Toh 2017[c] *(97)*	Neutralizing	Median (IQR) – 3d group: 5.8y (5.7-5.8y) 2d group: 5.8y (5.4-6.3y) 1d group: 6.3y (6.3-6.3y)	HPV16	66 / 66 (100%)	60 / 60 (100%)	38 / 40 (95%)	2 / 32 (6%)	F: 2095 (1461-3004) I: 5971 (3942-9046)	F: 2030 (1405-2934) I: 5655 (3865-8273)	F: 1359 (536-3447) I: 1018 (572.4-1811)	F: 54.84 (44-98-66.87) I: 54.25 (45.64-64.49)
			HPV18	58 / 66 (88%)	54 / 60 (90%)	27 / 40 (68%)	1 / 32 (3%)	F: 392.4 (248.3-620) I: 1106 (687.9-1777)	F: 358.9 (223.1-577.5) I: 1104 (701.1-1738)	F: 384 (174-847.5) I: 188.3 (102.3-345.1)	F: 52.36 (47.42-57.82) I: 50 (50-50)
Moscicki 2019[d] *(102)*	Neutralizing	Mean (IQR) – 2.9y (18.-4.1y)	HPV16	94 / 101 (93%)	44 / 47 (94%)	225 / 245 (92%)	14 / 65 (22%)	PHIV+: 430 mMU/mL PHEU: 1367 mMU/mL	PHIV+: 497 mMU/mL PHEU: 2129 mMU/mL	PHIV+: 519 mMU/mL PHEU: 1464 mMU/mL	PHIV+: 19 mMU/mL PHEU: 39 mMU/mL
			HPV18	64 / 101 (63%)	33 / 47 (70%)	175 / 245 (71%)	11 / 65 (17%)	PHIV+: 57 mMU/mL PHEU: 142 mMU/mL	PHIV+: 71 mMU/mL PHEU: 245 mMU/mL	PHIV+: 67 mMU/mL PHEU: 165 mMU/mL	PHIV+: 16 mMU/mL PHEU: 23 mMU/mL

HPV: Human papillomavirus; GM: Geometric mean; CI: Confidence intervals; IQR: Interquartile range; M: Months; Y: Years; NA: Not applicable; F: Indigenous Fijians; I: Fijians of Indian descent; PHIV+: perinatally HIV-infected; PHEU: perinatally HIV-exposed, uninfected.

[a] Seropositivity results shown for '0 dose' are pre-vaccination results for the vaccinated cohort in Hurt et al's study (99). Seropositivity results for 1, 2 and 3 dose recipients are shown for participants who were seronegative to the corresponding HPV type pre-vaccination.

[b] Results are shown for the intervention study of 31 girls with prior single-dose HPV vaccination (100). Results shown are those measured prior to the boost dose of 9vHPV.

[c] Results are shown only for Toh et al 2017, which provides humoral immunogenicity results (97). Humoral immunogenicity results shown are those measured prior to the challenge dose of 2vHPV. Neutralizing titers (ED_{50}) are shown for two ethnicity groups: indigenous Fijians (F) and Fijians of Indian descent (I). Results are not shown for Toh et al 2018, which provides cellular immunogenicity results (98).

[d] Antibody titer data are shown separately for PHIV+ and PHEU. 95%CIs are not provided in the publication (102).

4.4 Post-licensure vaccine effectiveness evaluations and other observational data

This section summarizes and includes excerpts from a previously published systematic review of the literature (106), combined with a recent update, on evidence of the effectiveness of HPV vaccination by the number of doses, as measured in post-licensure studies.

4.4.1 Systematic review of evidence on single-dose HPV vaccination from non-trial observational studies

4.4.1.1 STUDY SELECTION

Studies were eligible if they fulfilled the following inclusion criteria: 1) reported effectiveness of HPV vaccination (2vHPV or 4vHPV) on vaccine-type HPV infections, anogenital warts, or cervical abnormalities (based on cytological or histopathological results) or 2) assessed effectiveness of HPV vaccination by the number of doses received (one, two, or three). Studies were excluded if vaccine was administered as part of an RCT (e.g. post-hoc evaluations of clinical trials).

Medline and EMBASE databases were searched for studies published between January 1, 2007 to June 15, 2017, **and again for studies published from June 16, 2017 through March 20, 2019,** *using a combination of Medical Subject Headings (MeSH) terms, title or abstract words, without restriction on the language of publications. These included the following:*

- *"papillomavirus vaccines", "HPV vaccine", "HPV vaccination", "papillomavirus vaccine", or "papillomavirus vaccination," and;*
- *"program evaluation", "immunization programs", "population surveillance", "sentinel surveillance", "incidence", "prevalence", "rate", "rates", "effectiveness", "doses," and;*
- *"papillomavirus infections", "HPV", "uterine cervical neoplasms", "cervical intraepithelial neoplasia", "HPV related diseases", "condylomata acuminata", "genital warts".*

The selection of eligible articles was performed independently by two authors on title and abstract first, and secondly on the full-text article (full authorship in Acknowledgments section).

4.4.1.2 DATA EXTRACTION

Two authors independently extracted the main study characteristics and outcomes using standardized forms. One author resolved any discrepancy between extractions. The main study characteristics were the country, study design, age of study population at vaccination and outcome assessment, sample size according to the number of doses received, case definition, and statistical analyses (procedure used to assign the number of doses and adjustment for potential confounders). Information was also collected on use of buffer periods (lag time between vaccination and counting of outcomes). Buffer periods delay the case counting to try to exclude conditions caused by a prevalent infection at the time of vaccination.

Sources of bias in post-licensure studies examining the effectiveness by number of doses include the following: 1) differences in the characteristics and age at vaccination between groups vaccinated with different number of doses; 2) likelihood of prevalent infection at vaccination; and 3) interval between the first and second dose of the HPV vaccine among two-dose vaccine recipients. Since one of the aims of the systematic review was to discuss the limitations of these studies, no studies were excluded on the basis of the methodological quality.

The main outcome of the review was effectiveness of HPV vaccination, comparing the incidence or prevalence of HPV-related endpoints between individuals vaccinated with different numbers of doses (three vs none, two vs none, one vs none, three vs two, three vs one, and two vs one) of 4vHPV or 2vHPV vaccine. Because eligible studies used different buffer periods or age groups at vaccination and at outcome assessment, it was not possible to pool results from the studies.

4.4.1.3 RESULTS

The first literature search identified 3,787 articles, from which 26 full articles were assessed. After reading full texts, 12 articles were excluded, leaving 14 (20, 107-119) (**Figure 3**). These publications were published between January 2013 and June 2017 and included studies from Australia (three), Scotland (three), United States (two), Sweden (two), and one each from Belgium, Canada, Denmark, and Spain (**Table 10**). **The second literature search identified an additional 1,626 articles, from which 50 full articles were assessed. After reading full texts, 41 articles were excluded, leaving nine new papers) (120-128). These included studies from Scotland (two), United States (four), Canada (one), Denmark/Sweden (one), and Denmark (one).** All evaluations were conducted within the context of a recommended three-dose schedule of either 2vHPV vaccine or 4vHPV vaccine (18).

Overall, the articles included analyses of effectiveness for prevention of HPV infection (four), anogenital warts (nine), or cervical cytological or histological abnormalities (ten) (Table 11). All investigators attempted to control for or stratify by potentially important variables, such as age at vaccination. However, there were few other variables available in many studies (Table 10). **Five studies also evaluated the impact of buffer periods for case counting and seven studies evaluated different intervals between doses for two-dose vaccine recipients.**

HPV prevalence

In the original review, two studies that reported vaccine effectiveness for reduction of prevalent vaccine-type infection (HPV 16 or 18) were both from Scotland, conducted in the context of a three-dose 2vHPV vaccination program. In the updated review, two additional studies were identified, one from Scotland (120), using the same monitoring system as original two studies identified and one from the United States (121), evaluating vaccine effectiveness among males.

The first study from Scotland found statistically significant effectiveness for three doses but not for two doses or one dose (107). The analysis was also stratified by age at vaccination; results were similar with effectiveness significant only for three doses. In the second study, the authors over selected women who were partially vaccinated (108). Statistically significant effectiveness was found for three doses, two doses, and one dose. There was no formal comparison of effectiveness of three doses versus fewer doses in either study; confidence intervals for the effectiveness estimates of three, two, and one dose(s) overlapped. The additional study identified from Scotland used the same surveillance as the first two but included data through 2015. Statistically significant effectiveness was found for three and two doses but not one dose. One small study from the United States was conducted among men (121); there was no statistically significant effectiveness for at least one dose and no difference in effectiveness by number of doses.

Anogenital warts

In the original review, the six evaluations of anogenital wart outcomes were retrospective cohort studies among women from countries that had introduced 4vHPV vaccination (20, 109-113). In the updated review, three additional studies were identified, including one of men and women (122-124). Overall, the nine studies of anogenital warts were from six different countries. All studies adjusted or stratified analyses for age at vaccination and some were able to adjust for educational level or markers of socioeconomic status (Table 10). The more recent studies adjusted for more characteristics and several attempted to adjust for sexual behavior by various composite measures. Most two-dose vaccine

recipients received doses separated by two months. Three of the nine studies also included assessment of different buffer periods (109, 111, 122) and five included assessment of different intervals between doses in two-dose vaccine recipients (20, 111, 113, 122, 123).

Of the nine studies, seven included a comparison of three, two, and one dose(s) with no dose. All seven found highest point estimate of effectiveness with three doses, and six found lower point estimates but significant effectiveness with two doses. Five of the seven studies found significant effectiveness with one dose (20, 109, 112, 122, 123). Six studies also formally compared three and two doses, finding either no significant difference in the primary analysis or in analyses with different buffer periods or two-dose intervals (20, 109, 111, 113, 122, 123). Three studies examined different buffer periods (109, 111, 113); a longer buffer period decreased differences in effectiveness between three and two doses in one study (109). In the five studies that explored the interval between doses in two-dose vaccine recipients (20, 111, 113, 122, 123), two found that a longer interval changed effectiveness estimates or resulted in no difference between three and two doses (20, 122).

All five studies that stratified by age at vaccination found higher vaccine effectiveness point estimates with younger age at vaccination, although the differences were not all formally tested (20, 109, 113, 123, 124). One study was limited to those vaccinated at age 14 years, due to the structure of the national vaccination program, and found similar effectiveness estimates by number of doses (112). One study found similar point estimates of effectiveness with one, two, and three doses among those vaccinated at age 15 to 19 years and no significant difference in effectiveness between one and three doses (123).

Cervical cytological histological abnormalities

In the original review, six studies evaluated vaccine effectiveness for prevention of cervical cytological or histological abnormalities, including five for 4vHPV vaccine and one for 2vHPV vaccine (114-119). In the updated review, four additional studies were included (three for 4vHPV and one for 2vHPV) (125-128) (Table 10). Overall, the ten studies were from five different countries. Characteristics of women differed by number of doses in most studies, including for age at first vaccine dose.

Among the ten studies, all found effectiveness for three doses. Five studies found some effectiveness for prevention of high-grade histological abnormalities with two doses (115-117, 127, 128), and three studies found effectiveness with one dose among some age groups or in analyses with longer buffer periods (115, 116, 128). Most two-dose vaccine recipients received two doses at a one- or two-month interval. Two studies examined

intervals between two doses; one found no impact on the effectiveness estimate (116); the other found that longer intervals decreased the difference between two and three doses in those vaccinated at age 20 years or younger (127).

Five studies that stratified by age at vaccination found higher vaccine effectiveness point estimate with younger age at vaccination although the differences were not all formally tested (115-117, 125, 127). In four studies that evaluated effectiveness by number of doses stratified by age at vaccination, differences by number of doses remained in three (115-117). One study found similar point estimates by number of doses when stratifying by age at vaccination, but significant effectiveness only for three doses (127). However, in a large study that was limited to those vaccinated at age 16 or younger, effectiveness was found for one, two and three doses and there was no difference between number of doses (128).

4.4.2 Strengths and weaknesses of data from non-trial observational studies

Strengths of the data from the observational studies included the size of the studies, data on buffer periods for some studies, and some information on intervals between doses. **Some studies stratified by age at vaccination or limited analyses to those vaccinated at younger ages.** The following include important weaknesses of the available post-licensure studies and caveats that should be considered when interpreting the findings:

- The post-licensure studies were all conducted in settings of a national three-dose recommendation, and girls who received one or two doses differed from those completing the recommended schedule. Most studies included girls who were vaccinated beyond the routine target age as part of catch-up vaccination programs. In several studies, fewer-than-three-dose vaccine recipients were older than three-dose vaccine recipients at the time of vaccination, had lower socioeconomic status, and/or had indicators of earlier sexual exposure. Because of these differences, girls who received fewer doses were likely to be at higher risk of incident HPV infection, presence, or history of prevalent HPV infection, which biases results towards a greater effectiveness of three doses compared to one or two doses. Most studies adjusted analyses for some risk factors; however, it is highly likely that residual confounding remained.
- In most retrospective studies, it is impossible to identify individuals who were already infected with HPV at the time of vaccination. Since girls vaccinated with one or two doses in the studies were often older when vaccinated, prevalent infections at the time of vaccination could have biased results towards a lower vaccine effectiveness of less than three doses. Some researchers used buffer periods in the analyses, which delay case counting to exclude conditions caused by a prevalent infection. The importance of buffer periods might differ by the condition evaluated. Longer buffer periods might be more helpful for evaluation of vaccine effectiveness

against cervical high-grade histological abnormalities than anogenital warts, since the former takes more time to develop after infection (129). In addition, buffer periods could be of greater importance at an older age at vaccination compared to those of a younger age who are more likely to be HPV negative at vaccination. A disadvantage of buffer periods in effectiveness studies is that they reduce the number of person-years with one or two doses, resulting in low statistical power.

- Since all post-licensure studies published to date were conducted in settings of a national three-dose recommendation, most individuals vaccinated with two doses had received doses at a 0,1 month or 0,2 month interval. However, immunogenicity studies have found non-inferior results with two doses compared to three doses when the two doses were separated by about six months (9, 130, 131). The longer interval is thought to allow maturation of B cells and the second vaccination to act as a booster dose. Results of the immunogenicity studies led to the recommendation for a two-dose schedule administered at 0 and 6–12 months for females aged 9 through 14 years old at the time of their first dose (8, 132).

  Although the number of girls vaccinated with two doses separated by at least six months was small in the studies identified in the review, seven studies evaluated the interval between doses (20, 111, 113, 116, 122, 123, 127). Three of five studies evaluating anogenital wart outcomes (109, 111, 122) and one of two studies evaluating cervical outcomes (127) found that varying the interval increased effectiveness estimates. It is possible that the finding of higher effectiveness with a longer interval between two doses in these observational studies is the result of the longer interval acting as a buffer period and not related to the spacing between doses. If so, the inconsistent findings by interval between doses could be due to differing importance of buffer periods for the endpoints and age groups evaluated.

- The accuracy of vaccine history is important for vaccine effectiveness studies. Most studies included in this review were conducted in countries with national vaccine registries. However, underreporting of vaccinations to registries can occur (115, 116). In studies using claims or insurance data, vaccination history could be incomplete if girls moved or changed insurers during the vaccination series. Incomplete vaccination histories could lead to overestimating effectiveness of fewer than three doses.

4.4.3 Summary of non-trial observational studies

In this systematic review of HPV vaccine effectiveness by number of doses, most of the 23 studies found the highest point estimate of effectiveness with three doses, followed by two doses, and then one dose. However, more recent studies with younger vaccine recipients have found small or no differences by number of doses.

All studies, except one small study among males (121), found statistically significant effectiveness for three doses and 15 studies found effectiveness for two doses (20, 108-117, 120, 122, 123, 128). In nine studies, significant effectiveness was observed for one dose in some or all analyses (20, 108, 109, 112, 114, 116, 120, 122, 123, 128). Few studies directly

compared three, two, and one dose(s) and some effectiveness estimates had wide confidence intervals due to the small number of outcomes in one- and two-dose vaccine recipients.

Across all endpoints (prevalence, AGW, and cervical abnormalities), variation in effectiveness by number of doses was observed in most studies. There were generally consistent findings among studies that used buffer periods; with longer buffer periods, four of five studies found higher effectiveness estimates for one and two doses and a decrease in the differences by number of doses. Three of five studies of anogenital warts that evaluated interval between two doses found higher two-dose effectiveness with increasing interval (109, 113, 122). Among the studies of cervical abnormalities that evaluated interval between two doses, one did not find a difference (116) and another found that a longer interval decreased difference between two and three doses but only for those vaccinated at age 20 years or younger (127).

Among studies presenting results stratified by age group, there were higher effectiveness estimates with younger age at vaccination, although the differences were not formally tested. Important findings emerged from some of the recent studies identified. These either stratified by age at vaccination or were limited to those vaccinated at younger ages (123, 128). Along with a study which was limited to persons vaccinated in a younger age group in the first review (112), the studies found similar effectiveness for one, two, and three doses. These studies overcome some of the limitations of earlier studies, which likely included more women who had prevalent infection at the time of vaccination. Continued review of future published reports will be done to determine vaccine effectiveness by number of doses as studies include more persons vaccinated in early adolescence.

Figure 3. Non-trial observational studies systematic review flow diagram

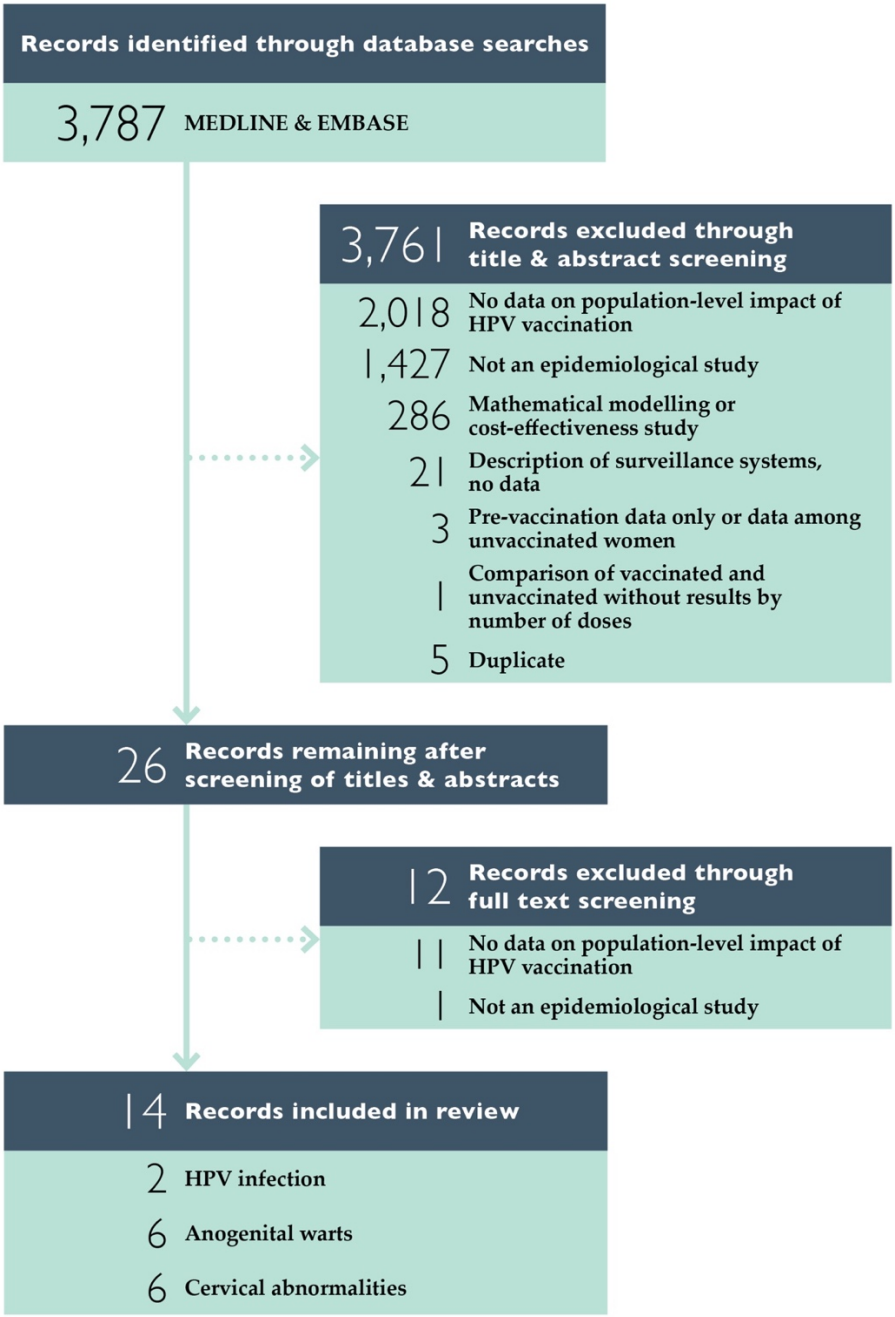


Figure adapted from (106).

Figure 4. Non-trial observational studies systematic review flow diagram - update

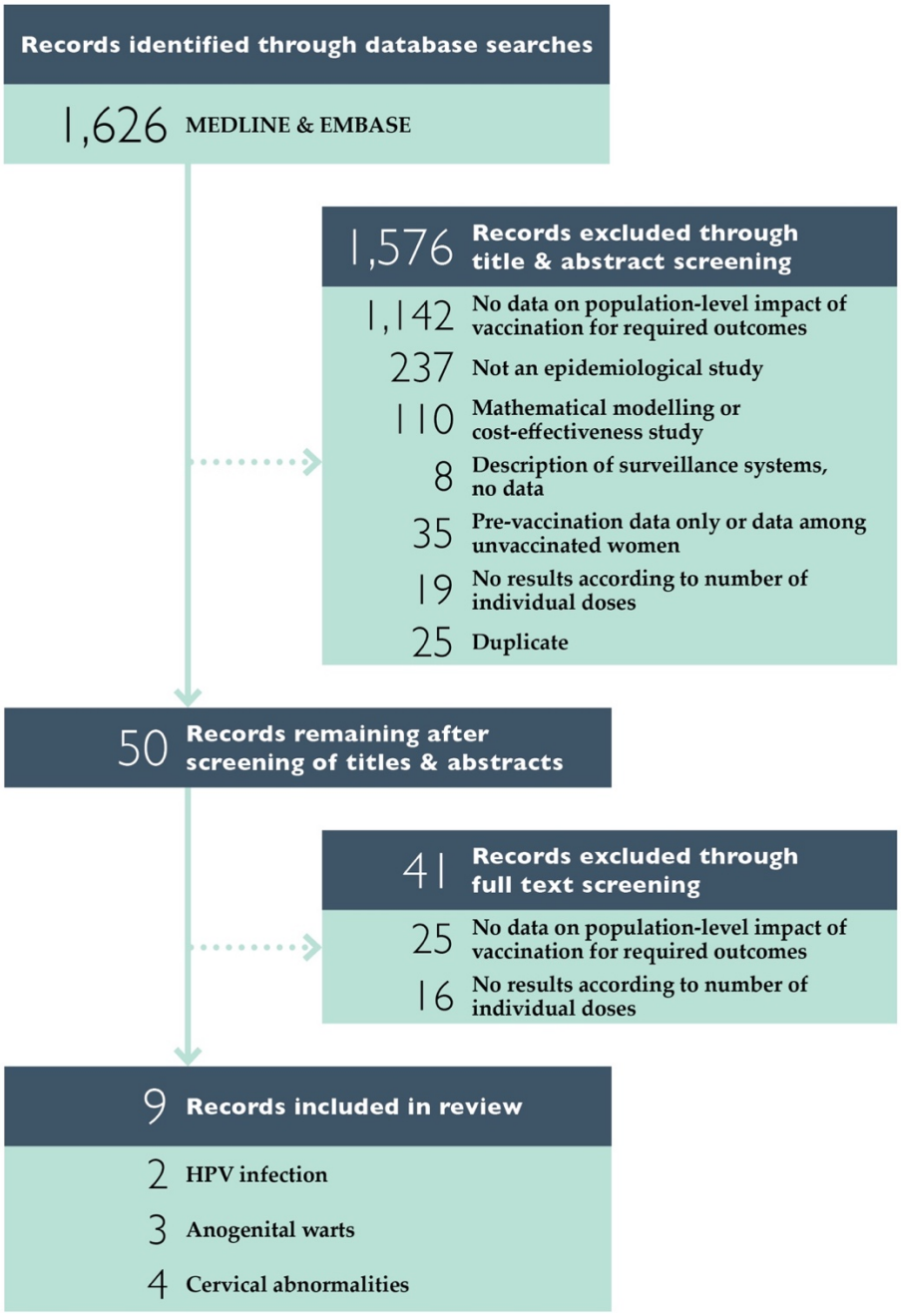


Table 10. Summary of studies selected for inclusion in the non-trial observational studies systematic review

Endpoint/ Vaccine/ Authors	Country	Study Design	Study population		Vaccination,	Case definition	Statistical analyses		
			Age (years) at Vaccination Outcome		N by dose number		Assignment of dose number	Buffer periods[a] (months)	Adjustment or stratification
Vaccine Type HPV Prevalence									
2vHPV vaccine									
Kavanagh 2014 (107)	Scotland	Cross-sectional study using screening registry data	15-17	20-21	0: 3,418 1: 55 2: 106 3: 1,100	HPV 16 or 18 DNA positivity in liquid-based cytology samples [b]	Final status	0	Birth year cohort, deprivation score
Cuschieri 2016 (108)	Scotland	Cross-sectional study using screening registry data with additional sampling of those with <3 doses	15-17	20-21	0: 3,619 1: 177 2: 300 3: 1,853	HPV 16 or 18 DNA positivity in liquid-based cytology samples [c]	Final status	0	Birth year cohort, deprivation score, age at first dose
Kavanagh 2017 (120)	Scotland	Cross-sectional study using screening registry data	12-18	20-21	0: 4008 1: 223 2: 391 3: 3962	HPV 16 or 18 DNA positivity in liquid-based cytology samples [c]	Final status	0	Birth year cohort, deprivation score
4vHPV vaccine									
Chandler 2018 (121)	United States	Cross-sectional study using self-reported data from men	NA	13-26	0: 82 1: NA 2: NA 3: NA	HPV 6,11,16, or 18 DNA positivity in self collected penile and perianal/anal swabs	Final status	0	None
Anogenital Warts									
4vHPV vaccine									
Herweijer 2014 (109)	Sweden	Retrospective cohort study using population-based health registries	10-19	10-24	0: 926,119 1: 115,197 2: 107,338 3: 89,836	First observed diagnosis: ICD-10 code A63.0 or podophyllotoxin/ imiquimod prescription	Time-dependent Final status	0 to 12	Age at first vaccination, age at outcome, parental education
Blomberg 2015 (20)	Denmark	Retrospective cohort study using population-based health national registries	12-27	12-27	0: 188,956 1: 55,666 2: 93,519 3: 212,549	First diagnosis: ICD-10 code A63.0 or podophyllotoxin prescription	Time-dependent	1	Age at vaccination, maternal education disposable income, calendar year
Dominiak-Felden 2015 (110)	Belgium	Retrospective cohort study using sick-fund / insurance data	10-23	16-23	0: 63,180 1: 4,020 2: 3,587 3: 35,792	First prescription of imiquimod and reimbursement	Time-dependent	1	Age at first dose
Perkins 2017 (111)	United States	Retrospective cohort study using commercial claims database	9-25	9-25	0: 201,933 1: 30,438 2: 36,583 3: 118,962	ICD-9 codes [d]	Final status	0, 12	Age, regions, SES indicators, calendar year, differential observation periods

Navarro-Illana 2017 (112)	Spain	Retrospective cohort study using national registries	14	14-19	0: NA [e] 1: NA 2: NA 3: NA	First diagnosis of ICD-9-CM code 078.11	Time-dependent	0	Age, calendar year, health department
Lamb 2017 (113)	Sweden	Retrospective cohort study using national registries	10-19	10-27	2: 79,042 3: 185,456	First diagnosis of ICD-10 code A63.0 or podophyllotoxin/ imiquimod prescription	Time-dependent	0	Age at outcome, time between doses
Hariri 2017 (122)	United States	Retrospective cohort study in integrated health-care delivery systems	16-17 mean	11-22	0: 31,563 1: 5,864 2: 5,459 3: 21,631	ICD-9 code 078.10, 078.11, 078.19), specialty of diagnosing provider, and STI tests ordered	Final status	6 from last dose 12 from first dose	Race/ethnicity, health plan, age at enrollment in the health plan, age at beginning of study period, age at first evidence of sexual activity (as defined by a composite measure), age at first dose, continuous enrollment indicator, months enrolled in health plan, Medicaid enrollment
Zeybek 2018 (123)	United States	Matched retrospective cohort study using health insurance claims databases (males and females)	9-26	10-31	0: 286,963 1: 54,280 2: 55,632 3: 177,051	ICD-9-CM or 10 code 078.11 or A63.0	Final status	3	Age group, sex, region of residence, and history of STDs.
Willows 2018 (124)	Canada	Matched retrospective cohort study using linked vaccine registry and claims and population-based databases	9-26	10-33	0: 94,327 1: 3,521 2: 6,666 3: 21,277	ICD-9-CM or 10 code 078.11 or A63.0 and related procedure code	Final status	0	Age at vaccination, area-level income, birth date, previous hospitalizations and previous physician visits, and for girls >19 years old, sexual activity (based on evidence using a composite measure)
Cervical Abnormalities									
4vHPV vaccine									
Gertig 2013 (114)	Australia	Retrospective cohort study using linked data from registries	12-19	12-21	0: 14,085 1: 1,422 2: 2,268 3: 21,151	Histology: CIN3/AIS, CIN2, CIN1, any high grade Cytology: low grade and high grade	Time-dependent Final status	0	Age at first screen, remoteness area, SES
Crowe 2014 (115)	Australia	Case control study using linked data from registries	12-26	11-31	0: 53,761 1: 9,649 2: 10,950 3: 23,106	Histology: CIN2+/AIS	Final status	0, 1, 6, 12	Year of birth, remoteness area, SES, follow-up time
Brotherton 2015 (116)	Australia	Retrospective cohort study using linked regional data registries	12-26	12-30	0: 133,055 1: 20,659 2: 27,500 3: 108,264	Histology: CIN3/AIS, CIN2, any high-grade Cytology: low grade and high grade	Final status	0, 1, 6, 12, 24	Age, remoteness, SES, screening start (before or after vaccination)
Hofstetter 2016 (117)	United States	Retrospective cohort study using medical center records	11-20	11-27	0: 1,632 1: 695 2: 604 3: 1,196	Cytology: low grade and high-grade [f]	Final status	1	Age, insurance, language, clinic type, CT screening, and baseline cytology

Kim 2016 (118)	Canada	Nested case-control study using linked data from registries	10-15	18-21	0: 5,712 1: 327 2: 490 3: 3,675	Cytology: low grade and high-grade [g]	Final status	0	Age, urban/rural, neighborhood income
Silverberg 2018 (125)	United States	Nested case-control study of women enrolled in an integrated health-care delivery system	14- 21	18-26	0: 23,293 1: 756 2: 554 3: 1,527	Histology: CIN2+/AIS	Final status	6	Smoking, parity, recent outpatient visits, race/ethnicity. sexually transmitted infections, hormonal contraceptives, immunosuppression
Dehlendorff 2018 (127)	Denmark/ Sweden	Retrospective cohort study using linked national registry data	13-30	13-30	0: 2,091,579 1: NA 2: NA 3: NA	Histology: CIN2+/AIS	Time-dependent	0	Attained age, age at vaccination, maternal education
Verdoodt 2019 (128)	Denmark	Retrospective cohort study using linked national registry data	12-16	17-25	0: 374,327 1: 10,480 2: 30,259 3: 174,532	Histology: CIN2+ CIN3+	Time-dependent (final status for the comparison between doses)	0 6 in secondary analysis	Attained age, maternal education
2vHPV vaccine									
Pollock 2014 (119)	Scotland	Retrospective cohort study using linked national registry data	15-17	20-21	0: 75,113 1: 1,315 2: 2,725 3: 25,898	Histology: CIN1, CIN2, CIN3	Final status	0	Age, birth year cohort year, deprivation score
Cameron 2017 (126)	Scotland	Retrospective cohort study using linked national registry data	14-17	20-21	0: 75,683 1: 2,258 2: 4,462 3: 55,303	Histology: CIN1, CIN2, CIN3	Final status	0	Deprivation score, birth year cohort

Abbreviations: CT, chlamydia trachomatis; SES, socioeconomic status, CIN, cervical intraepithelial neoplasia; CIN2+, CIN grade 2 or worse; AIS, adenocarcinoma in situ; ICD-9, International Classification of Disease, ninth revision; ICD-10, International Classification of Disease, tenth revision; NA, not available

[a] Buffer period is the lag time between vaccination and counting of outcomes;

[b] By multimetrix HPV assay detecting 24 types including all established high risk types;

[c] By Optiplex HPV assay detecting 24 types including all established high risk types;

[d] Three possible scenarios: a) ≥ 1 diagnosis of ICD-9 code 078.1; b) ≥ 1 diagnosis of ICD-9 code 078.1, 078.10, 078.19 plus destruction/excision procedure or ICD-9 code 211.4, 216.5, 221.8, 222.9; c) ≥ 1 prescription for anogenital warts plus destruction/excision procedure or ICD-9 code 211.4, 216.5, 221.8, 222.9.

[e] Presented as person-years in this article.

[f] Low-grade cytology defined as atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. High-grade cytology defined as atypical squamous cells, cannot rule out a high-grade lesion, or high-grade squamous intraepithelial lesion.

[g] High-grade cytology defined as possible high-grade squamous intraepithelial lesion (HSIL), HSIL, HSIL with possible microinvasion/invasion, squamous cell carcinoma, possible high-grade endocervical glandular lesion, AIS, AIS with possible microinvasion/invasion and adenocarcinoma. Low-grade cytology defined as possible low-grade squamous intraepithelial lesions (LSIL), LSIL and atypical endocervical cells of uncertain significance.

Table adapted from (106).

Table 11. Analyses and main findings from studies in the non-trial observational studies systematic review

Endpoint/ Vaccine/ Authors	Study Population Age (years) at Vaccination	Outcome	Buffer[a] (months)	Sensitivity analyses by age group/ buffer/ dose interval[b]	Formal comparison of 3 vs 2 or one doses	Main Findings
HPV Prevalence						
2vHPV vaccine						
Kavanagh 2014 (107)	15-17	20-21	0	Yes/No/No	No	• Significant effectiveness for 3, but not 2 or one doses compared to 0 3: aOR = .43 (CI .34, .55); 2: aOR = .68 (CI .42, 1.12); 1: aOR = .95 (CI .51, 1.76) • Effectiveness CI overlap for 3, 2 and one doses • Similar results when stratified by age at vaccination
Cuschieri 2016 (108)	15-17	20-21	0	No/No/No	No	• Significant effectiveness for 3, 2 and one doses compared to 0 3: aOR = .27 (CI .20, .37); 2: aOR = .45 (CI .29, .69), 1: aOR = .52 (CI .31, .83) • Effectiveness CI overlap for 3, 2 and one doses
Kavanagh 2017 (120)	12-18	20-21	0	No/No/No	No	• Significant effectiveness for 3 and 2 but not one doses compared to 0 3: aOR = .40 (CI .33, .48); 2: aOR = .75 (CI .57, .99); 1: aOR = .89 (CI .63, 1.25) • Effectiveness CI do not overlap for 3 vs 2 and one doses
4vHPV vaccine						
Chandler 2018 (121)	NA	13-26	0	No/No/No	Yes	• No significant effectiveness for at least one dose compared to 0 • No significant differences for effectiveness of 3 vs 1, or 3 vs 2 doses
Anogenital Warts						
4vHPV vaccine						
Herweijer 2014 (109)	10-19	10-24	3	Yes/Yes/No	Yes	• Significant effectiveness for 3, 2 and one doses compared to 0 3: aRR = .20 (CI .17, .23), 2: aRR = .32 (CI .26, .40), 1: aRR = .54 (CI .43, .68) • Significantly higher effectiveness of 3 compared to 2 and one doses • With buffer periods >4 months, no significant difference between 3 and two doses • Similar results for age groups 10-16 and 17-19, except effectiveness for one dose without buffer period statistically significant for 10-16 yr olds
Blomberg 2015 (20)	12-2	12-27	1	Yes/No/Yes	Yes	• Significant effectiveness for one compared to 0 dose, RR = .51 (CI .46, .56) • Effectiveness not reported for 3 and two doses compared to 0 • Effectiveness significantly increased with each dose: RR 2 vs one dose = .44 (CI .37, .51); RR 3 vs two doses = .46 (CI .39, .54) • With dose interval >4 months, no significant difference between 3 and two doses • Similar results when stratified by age at vaccination

Endpoint/ Vaccine/ Authors	Study Population Age (years) at Vaccination	Outcome	Buffer[a] (months)	Sensitivity analyses by age group/ buffer/ dose interval[b]	Formal comparison of 3 vs 2 or one doses	Main Findings
Dominiak-Felden 2015 (110)	10-23	16-23	1	No/No/No	No	• Significant effectiveness for 3 and two doses, but not one compared to 0 3: aRR = .12 (CI .07, .21); 2: aRR = .34 (CI .14, .83); 1: aRR = .63 (CI .35, 1.16) • Effectiveness CI overlap for 3 and two doses; no overlap for 3 and one doses
Perkins 2017 (111)	9-25	9-25	0	No/Yes/Yes	Yes	• Significant effectiveness for 3 doses compared to 0, aRR =.52 (CI .46,.60) • Effectiveness not reported for 2 and one doses compared to 0 • Higher effectiveness for 3 compared with one dose, aRR = .82 (CI .71, .95); but no significant difference between 3 and two doses, aRR = .89 (CI .78, 1.03) • With buffer period of 1 year, no change in findings (data not shown) • Similar results with dose interval >5 months for two doses
Navarro-Illana 2017 (112)	14	14-19	0	No/No/No	No	• Significant effectiveness for 3, 2, and one doses compared to 0 3: aRR = .24 (CI .15, .34); 2: aRR = .36 (CI .14, .68); 1: aRR = .39 (CI .13, .80) • Effectiveness CI overlap for 3, 2 and one doses
Lamb 2017 (113)	10-19	10-27	0	Yes/No/Yes	Yes	• Effectiveness not reported for 3, 2 and one doses compared to 0 • Higher effectiveness of 3 doses compared to two doses, when two doses administered either 0-3 months or >8 months apart; whereas no significant difference between 3 and two doses when the two doses administered within 4-7 months • Similar results when stratified by age at vaccination
Hariri 2017 (122)	16-17 (mean)	15-22	6 from last dose 12 from first dose	No/Yes/Yes	Yes	6-month buffer from last dose • Significant effectiveness for 3 and 2[d], but not for one dose compared to 0 3: aHR = .23 (CI .17, .31); 2: aHR = .32 (CI .17, .59); 1: aHR = .81 (CI .60, 1.08) • No significant difference for effectiveness of 3 vs 2 doses, aHR = .74 (CI .38, 1.43) when 2 doses ≥ 6-month interval • Significantly greater effectiveness of 3 vs 1 dose, aHR = .29 (CI .20, .42) 12-month bufffer from first dose • Significant effectiveness for 3, 2[d], and one doses compared to 0 3: aHR = .20 (CI .15, .27); 2: aHR = .24 (0.13, .44); 1: aHR = .32 (CI .20, .52) • No significant difference for effectiveness of 3 vs 1 doses, aHR = .63 (CI .37, 1.09)
Zeybek 2018 (123)	9-26	10-31	3 from last dose	Yes/No/Yes	Yes	Results for those vaccinated at age 15-19 years below; no significant effectiveness in older or younger age groups. • Significant effectiveness for 3, 2, and one doses compared to 0 3: aRR = . 58 (CI .49, .70); 2: aRR = .67 (CI .51, .89); 1: aRR = .65 (CI .49, .85) • Similar results with dose interval <6 or ≥6 months for two doses • No significant differences for effectiveness of 3 vs 1, 3 vs 2, or 2 vs 1 doses

Endpoint/ Vaccine/ Authors	Study Population Age (years) at Vaccination	Outcome	Buffer[a] (months)	Sensitivity analyses by age group/ buffer/ dose interval[b]	Formal comparison of 3 vs 2 or one doses	Main Findings
Willows 2018 (124)	9-18	10-32	0	Yes/No/No	No	Results for those vaccinated at age 9-18 years below; no significant effectiveness for those vaccinated at older ages. • Significant effectiveness for 3, but not 2 or one doses compared to 0 3: aHR = .4 (CI .3, .7); 2: aHR = 1.4 (CI .6, 3.3); 1: aHR = .6 (CI .2, 1.8). • Effectiveness CI overlap for 3, 2 and one doses
Cervical Abnormalities[c]						
4vHPV vaccine						
Gertig 2013 (114)	12-19	12-21	0	No/No/No	No	Outcome summarized: CIN3/AIS • Significant effectiveness for 3, but not two and one doses compared to 0 3: aRR = .53 (CI .36, .77); 2: aRR = .87 (CI .46, 1.67); 1: aRR = 1.40 (CI .75, 2.61) • Effectiveness CI overlap for 3, 2 and one doses
Crowe 2014 (115)	12-26	11-31	0	Yes/Yes/No	No	Outcome summarized: High grade histological lesions • Significant effectiveness for 3 and 2doses, but not one compared to 0 3: aOR = .54 (CI .43, .67); 2: aOR = .79 (CI .64, .98); 1: aOR = .95 (CI .77, 1.16) • Effectiveness CI overlap for 3 and two doses, no overlap for 3 and one doses • Buffer periods from 1 to 12 months - no consistent impact on 3, 2 and one dose effectiveness estimates • Similar results when stratified by age at vaccination
Brotherton 2015 (116)	12-26	12-30	0	Yes/Yes/Yes	No	Outcome summarized: CIN3/AIS • Significant effectiveness for 3, but not 2 and one doses compared to 0 3: aRR = .69 (CI .58, .81); 2: aRR = 1.17 (CI .92, 1.48); 1: aRR = 1.41 (CI 1.12, 1.77) • Effectiveness CI for 3, 2 and one doses do not overlap • With increasing buffer periods, some effectiveness for 2 and one doses in several age groups • No difference in effectiveness by interval between two doses • Similar results when stratified by age at vaccination
Hofstetter 2016 (117)	11-20	11-27	1	Yes/No/No	No	Outcome summarized: Any abnormal cytology • Significant effectiveness for 3 and 2, but not one dose compared to 0 3: aRR = .58 (CI .48, .69); 2: aRR = .81 (CI .66, .99); 1: aRR = 1.05 (CI .88, 1.26) • Effectiveness CI overlap for 3, 2 and one doses • Similar results when stratified by age at vaccination, although effectiveness of two doses compared to 0 not always significant
Kim 2016 (118)	10-15	18-21	0	No/No/No	No	Outcome summarized: High grade cytology • Significant effectiveness for 3, but not 2 and one doses compared with 0 3: aOR = .48 (CI .28, .81); 2: aOR = .17 (CI .02, 1.20); 1: aOR = .45 (CI .11, 1.83) • Effectiveness CI overlap for 3, 2 and one doses

Endpoint/ Vaccine/ Authors	Study Population Age (years) at Vaccination	Outcome	Buffer[a] (months)	Sensitivity analyses by age group/ buffer/ dose interval[b]	Formal comparison of 3 vs 2 or one doses	Main Findings
Silverberg 2018 (125)	14 - 26	18-26	6	Yes/No/No	No	Outcome summarized: CIN2+/AIS • Significant effectiveness for 3, but not 2 or one doses compared with 0 3: aRR = .76 (CI .64, .89); 2: aRR = .98 (CI .78, 1.24); 1: aRR = .84 (CI .68, 1.03) • Effectiveness CI overlap for 3, 2 and one doses
Dehlendorff 2018 (127)	13-30	13-30	0	Yes/No/Yes	Yes	Outcome summarized: CIN2+/AIS (age <16 years) • Significant effectiveness for 3, but not 2 or one doses compared with 0, but point estimates similar for 3 and 1 doses 3: aRR = .23 (CI .11, .49); 2: aRR = .44 (CI .10, 2.03); 1: aRR = .23 (CI .01, 5.24) • No significant difference when delay between dose 1 and 2 > 5 months and age at vaccination < 20 years
Verdoodt 2019 (128)	12-16	17-25	0 (6 months for the comparison between doses)	No/No/No	Yes	Outcome summarized: CIN2+/AIS • Significant effectiveness for 3, 2, and one doses compared with 0 3: aIRR = . 43 (CI .36, .51); 2: aIRR = . 49 (CI .32 .76); 1: aIRR = .34 (CI .13, .87) • No significant differences for effectiveness of 3 vs 1 or 2 vs 1 doses
2vHPV vaccine						
Pollock 2014 (119)	15-17	20-21	0	No/No/No	No	Outcome summarized: CIN3 • Significant effectiveness for 3, but not 2 and one doses compared with 0 3: aRR = .45 (CI .35, .58); 2: aRR = .77 (CI .49, 1.21); 1: aRR = 1.42 (CI .89, 2.28) • Effectiveness CI overlap for 3 and two doses, no overlap for 3 and one doses
Cameron 2017 (126)	14-17	20-21	0	No/No/No	No	Outcome summarized: CIN 2/CIN 3 Significant effectiveness with 3 doses, in all deprivation categories, compared with unvaccinated in most deprived; no significant effectiveness with 1 or 2 doses

Abbreviations: RR, relative risk; aRR, adjusted RR; aOR, adjusted odds ratio; CI, 95% confidence intervals; CIN3, cervical intraepithelial neoplasia grade 3; AIS, adenocarcinoma in situ
Significant; 95% CI does not include 1.

[a] Buffer period is the lag time between vaccination and counting of outcomes. This column shows buffer period in main analysis.
[b] Interval between doses for two-dose vaccine recipients.
[c] Several outcomes were presented in some articles for cervical cytological or histological abnormalities. We summarized results for the outcome most proximal to cervical cancer.
[d] Data presented for 2 doses are those with an interval ≥6 months between doses

Table adapted from (106).

4.5 Mathematical modeling of the impact of reduced dosing schedules for HPV vaccines

4.5.1 Overview

Given the long natural history process of HPV and cervical carcinogenesis, empirical studies have relied on intermediate endpoints as measures of efficacy and effectiveness of HPV vaccination, such as the incidence of persistent HPV infection and CIN. Mathematical models that simulate the disease burden of HPV in populations can be used to complement these data by projecting longer-term outcomes of most interest to decision-makers (e.g. cancer cases and deaths averted, or life expectancy gained) and generating evidence under conditions of uncertainty or where data do not exist. Such models have been used extensively to evaluate the health and epidemiologic impacts, budget impacts, and cost-effectiveness of strategies to prevent HPV-related diseases globally.

Important features of different model types, attributes, functionalities, and structures have been covered extensively elsewhere (133-137). The best suited models for questions related to HPV vaccination are "dynamic" transmission models that explicitly simulate the acquisition of HPV infections through sexual behavior in the population and can therefore capture both direct and indirect (i.e., herd protection) effects. Given the increased use of mathematical models to inform decisions globally, ensuring appropriate model adaptation to different populations (i.e., model calibration), assessing the quality of predictions (i.e., model validation), and comparing predictions across independent models (i.e., comparative modeling) are important to enhance credibility of findings (133, 138, 139). Standardization of model reporting to increase transparency and interpretability of model assumptions, inputs, and outputs is also critical (140).

In contrast to the large body of model-based evidence on the impact and cost-effectiveness of three-dose HPV vaccination (141-145), analyses evaluating reduced-dose vaccination schedules are limited. To date, most have focused on two-dose vaccination; however, an increasing number of analyses on the impact and value of single-dose vaccination is anticipated, corresponding with the growing empirical data summarized in Sections 4.2, 4.3, and 4.4.

4.5.2 Models of two-dose HPV vaccination

Four published analyses have addressed the question of reducing vaccination from three to two doses in the context of high-income settings; three with either the 2vHPV or 4vHPV vaccines and one with the 9vHPV vaccine (146-149). These analyses explored the impact of duration of protection, with equivalent or shorter duration for two doses compared to three doses. Consistent with observed data, they assumed equivalent vaccine efficacy between the dose regimens (95-100% efficacy) in base-case scenarios but explored differential vaccine efficacy in sensitivity analyses.

Comparative analyses of two-dose 2v/4vHPV vaccination using independent dynamic transmission models fitted to the United Kingdom (UK; Public Health England model) and Canada (HPV-ADVISE model) found that the health benefits, in terms of cancer incidence reduction and quality-adjusted life years (QALYs) gained, were substantial with two-dose HPV vaccination, even when vaccine protection waned at 30, 20, or 10 years (146, 147). However, the incremental benefit of adding a third dose varied greatly dependent on duration of two-dose protection. For example, in the UK model, at 80% vaccination coverage with two-dose protection lasting 30 years, the added cervical cancer incidence reduction from the third dose (assuming lifelong protection) at 70 years post vaccination was only 1% (90% range, 0-6%) of pre-vaccination incidence; however, when two-dose protection was only ten years, the added incidence reduction was 17% (5-23%) (146).

The Canadian model projected similar cancer incidence reductions as the UK model, except it estimated a lower benefit from two-dose vaccination when protection lasted only ten years, which made the incremental benefit associated with the third dose greater than in the UK model (49% in the Canada model versus 17% in the UK model). These trends were similar when vaccination coverage was 40% (although with lower absolute benefit) and when results were reported in terms of the number needed to vaccinate (NNV) to prevent an additional cancer.

Despite different cost inputs and willingness to pay thresholds in the two countries, the cost-effectiveness results of two-dose (2vHPV or 4vHPV) HPV vaccination in the UK and Canada were also qualitatively similar. The UK analysis evaluated routine vaccination of 12-year-old girls plus a one-year catch-up campaign to age 18 and included health benefits and costs related to all HPV-related diseases (i.e., cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancers, AGW and respiratory papillomatoses) (147). The model estimated that two-dose HPV vaccination was cost-effective compared to no vaccination at the UK willingness-to-pay threshold (£30,000 per QALY gained), even when the duration of protection was only ten years and at a vaccine cost up to £300 per dose (much higher than list price at the time of £86.50 per dose). Similar to the health benefits, the cost-effectiveness

of adding a third dose depended heavily on the assumption of duration of two-dose protection; for example, three-dose vaccination (assuming lifelong protection) was not cost-effective when two-dose vaccination provided at least 20 years of protection. However, if two-dose protection was only ten years, three-dose vaccination was cost-effective, provided the vaccine cost was less than £147 per dose. These results were robust irrespective of vaccine type (2vHPV versus 4vHPV) and assumptions on cross-protection against non-vaccine types; they were replicated when using HPV-ADVISE and adapted to include UK cost and cancer inputs.

In the Canadian analysis using the HPV-ADVISE model (148), routine vaccination was targeted to nine-year-olds and included a five-year, three-dose catch-up campaign; strategies of two- and three-dose vaccination were also evaluated for girls only or with girls and boys, and included outcomes related to all HPV diseases. As in the UK analysis, two-dose vaccination was found to be cost-effective (versus no vaccination) at a willingness-to-pay threshold of Gross Domestic Product (GDP) per capita in Canada (i.e., $40,000 per QALY gained). Adding a third dose for girls was not cost-effective unless protection of two-dose vaccination was ten or 20 years and the third dose would extend protection by ten years; if two-dose vaccine protection was 30 years, the third vaccine dose was not cost-effective unless the cost for the third dose was drastically reduced below the base case cost per dose ($85).

Extending vaccination to girls and boys at either two or three doses was uniformly cost-ineffective, unless the cost for vaccinating boys was substantially reduced (10-40% of the cost for vaccinating girls) or under other extreme conditions, including high prevalence of men who have sex with men (MSM), much higher relative risk of disease among MSM (versus heterosexual men), and no effect of girl-only vaccination on MSM disease risk. Interestingly, vaccinating both girls and boys with two doses was found to be dominated by vaccinating girls only with three doses, given the similar health gains but higher cost of extending two doses to all boys versus adding one more dose to all girls (148).

One US-based analysis using the HPV-ADVISE model (calibrated to US HPV epidemiology and sexual behavior) evaluated reduced doses in the context of the 9vHPV vaccine for girls only, assuming comparable vaccine efficacy (95%) between two and three doses, vaccine cost of $158 per dose, and variable duration of two-dose protection (10 years to lifelong) (149). Despite a greater absolute benefit from the 9vHPV vaccine on all HPV-related diseases, the findings regarding two-dose vaccination were qualitatively similar to the previous analyses assuming the 2vHPV or 4vHPV vaccines in the UK and Canada. Compared to no vaccination, two-dose HPV vaccination was found to be cost-saving or cost-effective, even when duration of protection from two doses was short (ten years). As in the other analyses, adding a third dose was unlikely to be cost-effective if duration of two-dose

protection was at least 20 years. Unlike previous studies, this analysis explored modest increases in vaccination coverage with a two-dose regimen and found that an increased uptake of 5-15% of two-dose vaccination could compensate for the loss in not administering the third dose. Given the higher cost, three-dose vaccination was therefore found to be dominated (i.e., costlier and less effective).

4.5.3 Models of single-dose HPV vaccination

Two analyses, one in the UK and one in the US, have evaluated single-dose HPV 16 and 18 vaccination in the context of routine girls-only vaccination in HICs (150, 151). An analysis published in the *Vaccine* theme issue on single-dose HPV vaccination extends the findings from the US-based analysis to evaluate the impact and cost-effectiveness of single-dose HPV 16 and 18 vaccination in the setting of Uganda (152).

The UK analysis involved comparative modeling using the Public Health England (UK) and the Canadian HPV-ADVISE models, in which one dose was assumed to have equivalent efficacy against HPV 16 and 18 as two doses, but varied in terms of duration of protection (ten or 20 years) and cross-protection against HPV 31, 33, and 45 (150). Results for single-dose vaccination were qualitatively consistent with findings regarding two-dose vaccination. Compared to no vaccination, single-dose vaccination resulted in substantial reductions in cervical cancer incidence (range 18-74%) and was highly cost-effective, even when protection was only ten years and did not include cross-protection. Adding a second dose resulted in additional cancer reductions ranging from 4-44% and was cost-effective if single-dose protection was only ten years and the second dose extended protection to 20 years, irrespective of cross-protection. In contrast, adding a second dose was not cost-effective if single-dose vaccination protected for 20 years, even if the second dose extended protection over the lifetime. The large uncertainty intervals in predictions are driven, at least partly, by uncertainty around sexual behavior, and suggest that information about these parameters will be key to comparing the impact of different vaccine schedules.

The US analysis explored the epidemiologic impact of single-dose vaccination under varied assumptions of duration of single-dose protection (ten years, 15 years, and lifetime) and achievable vaccination coverage (70%, 90%) (151). This analysis also assumed lower vaccine efficacy for one dose (80% against HPV 16 and 18 infections) than for two doses (100%). The analysis projected that both one-dose and two-dose vaccination provide substantial reductions in population HPV 16 prevalence over time, even when protection with one dose is not lifelong. When no waning of protection after one-dose vaccination was assumed, HPV 16 prevalence reductions over time were lower for one-dose vaccination than two-dose

vaccination, as expected with the lower efficacy; however, this loss in benefit was almost completely offset when there was an increase in one-dose vaccination coverage from 70% to 90%. The ability for increased coverage to compensate for decreased efficacy was diminished under assumptions of waning protection.

When these model assumptions and projections of one-dose and two-dose vaccination effects were applied to the burden of HPV and cervical cancer in the setting of Uganda (152), one-dose vaccination was found to be cost-saving or very cost-effective compared to no vaccination, consistent with prior analyses. Adding a second dose was found to be cost-effective unless one-dose vaccination was accompanied by higher coverage and had equivalent (i.e., lifelong) protection.

One published modeling study evaluated the population-level impact of single-dose 9vHPV vaccination on reducing cervical cancer (CC) incidence and mortality in South Africa, taking into consideration HIV status, CD4 count, and ART coverage (25). The analysis utilized a dynamic HIV transmission model calibrated and validated to data from KwaZulu-Natal, South Africa. This model was adapted to including not only sexual transmission of HIV but also high-risk HPV and the natural history of cervical precancerous lesions (i.e., CIN1, CIN2, CIN3) and invasive cancer. HIV infection impacted HPV transmission, as well as progression and regression of HPV and precancer, as a function of CD4 count.

Unlike previous analyses of single-dose vaccination (1st Edition, White Paper, section 4.6.2.1), this analysis did not compare the comparative effectiveness (or cost-effectiveness) of two doses versus one dose; rather it was used to project the long-term effects of single-dose 9vHPV vaccination of nine-year-old girls on CC incidence and mortality by age and over time, varying important vaccine characteristics and programmatic assumptions. In the base case, vaccination coverage of 90% for nine-year-old girls was assumed starting in year 2018, with 80% protection over the lifetime against 90% (i.e., approximate type distribution of 9vHPV) of cervical cancer cases. Sensitivity analysis examined the impacts of vaccination coverage (50%, 70%) and duration of vaccine protection (waning at ten, 15, 20 years of full protection followed by linear decline to no protection over 20 years).

Assuming 80% lifetime protection and 90% coverage, CC incidence for all women irrespective of HIV status was reduced by 74% (CC mortality reduced by 71%) after 70 years of the start of 9vHPV vaccination in South Africa. As expected, lower vaccination coverage resulted in lower incidence and mortality reductions; with 50% coverage and lifelong protection, reductions in CC incidence and mortality decreased to 48% and 45%, respectively. Waning protection at ten to 20 years also reduced benefits, ranging from 72% CC incidence reduction among all women when full protection lasted only 20 years down

to 67% CC incidence reduction when full protection lasted only ten years (decreases in CC mortality reductions were also similar). Interestingly, the impact of HIV status (and CD4 count among HIV-positive women) on relative reductions in incidence and mortality was minimal – roughly 2-3% for CC incidence, and 2-5% for CC mortality – at all included levels of coverage and vaccine waning.

The study did not evaluate costs and did not vary CC screening, but identified cost-effectiveness analysis of single-dose HPV vaccination, including threshold analysis for the cost of 9vHPV in an HIV-endemic setting, as a priority for future work. The authors concluded that single-dose 9vHPV vaccination has the potential to achieve high reduction in CC burden, even with lower efficacy (80%) and possible waning protection (ten to 20 years), and despite a high prevalence of HIV among women in South Africa.

4.5.4 Strengths and weaknesses of model-based evidence

It is important to highlight that the model-based evidence on reduced-dose HPV vaccination to date relies on findings from three independent models that have been developed using data from high-income settings with similar HPV epidemiologic profiles. The emerging evidence on vaccine efficacy and durability from the ongoing studies – and the extension of these analyses into settings with more variable epidemiological, demographic, and behavioral profiles – will be critical to fill important evidence gaps regarding the impact and value of reduced-dose HPV vaccination.

The latest analysis (25) makes several contributions to the limited literature on reduced-dose HPV vaccination. First and foremost, the study is the first of its kind to take into consideration the comorbidity of HPV and HIV when evaluating the impact of single-dose 9vHPV vaccination. The explicit modeling of the interactive effects of HPV and HIV is critical to understand the mediating or exacerbating effects of CC prevention strategies in many low- and middle-income countries where HIV is highly prevalent. Second, the model was adapted to the setting of South Africa, leveraging rich data on sexual behaviours, the natural history of HIV and HPV, and longstanding programs in both HIV and cervical cancer prevention and control. Third, the study was led by a modeling group that was independent from the model-based studies summarized in the first edition of the White Paper, adding to the number of different research groups assessing the impacts of single-dose HPV vaccination. Continued model-based work evaluating the relative trade-offs of multiple doses (at recommended or delayed schedules) and integrating emerging evidence on the efficacy, costs, and acceptability of single-dose HPV vaccination can inform various stakeholders and decision-makers on the value of HPV vaccination in different settings.

4.5.5 Summary of model-based evidence

These initial studies suggest that the duration of protection afforded by reduced dosages is a critical factor in determining impact and cost-effectiveness. Several findings were consistent across analyses evaluating two-dose HPV vaccination, including:

- Compared to no vaccination, two-dose HPV vaccination yields substantial health benefits and is good value for money, even when duration of reduced-dose protection is only ten years;
- The health impact and cost-effectiveness of adding a third vaccine dose hinges on the relative duration of protection for two versus three doses
- The relative gain in health impact by adding a third vaccine dose will be minimal if two-dose protection is 20-30 years, assuming no initial waning in the first ten years for either two or three doses;
- If two-dose protection is less than ten years, adding a third vaccine dose will have greater health impact and is likely to be cost-effective.

Similar themes emerged in the limited analyses evaluating single-dose HPV vaccination:

- Compared to no vaccination, single-dose HPV vaccination yields substantial health benefits and is good value for money, even at a lower vaccine efficacy (level of 80%) and a lower duration of protection of only ten years;
- The impact and cost-effectiveness of adding a second dose is driven by the duration of single-dose vaccine protection and, possibly, the ability to achieve higher coverage with a single dose versus multiple doses.
- **Single-dose 9vHPV vaccination in a high HIV prevalence setting can yield high reductions in cervical cancer incidence and mortality, and these relative reductions are similar irrespective of HIV status, CD4 count, or ART coverage.**

5 Summary of results

A recent review on the virological and immunological properties of HPV infections and HPV vaccines provides a plausible theoretical mechanism to explain why a single dose of HPV vaccine should be able to elicit a robust immune response and why lower antibody titers observed for one dose, compared with two or more doses (which are higher than those following natural infection), may still provide protection against HPV.

A systematic review of data on single-dose HPV vaccination from participants vaccinated through clinical trials supports the premise that a single HPV vaccine dose may be as effective in preventing HPV infection as multi-dose schedules in healthy young females. The review identified seven articles describing six nested observational studies from three clinical trials (CVT, PATRICIA and the IARC India HPV Vaccine Trial) and one small pilot intervention study. Participants receiving HPV vaccine through the clinical trials had very low rates of HPV16/18 infection up to seven years post vaccination, regardless of the number of doses received. Furthermore, participants receiving only one HPV vaccine dose had significantly lower infection rates than control participants who did not receive any HPV vaccine. Rates of HPV16/18 antibody seropositivity were very high among participants receiving one, two, or three HPV vaccine doses. However, seropositivity data must be interpreted with caution due to differences in methodologies and definitions between studies. HPV16/18 antibody titers were consistently lower for single-dose arms compared to multi-dose arms, though this may have limited clinical significance if the titers induced by a single dose are sufficient to confer long-term protection against infection, as the evidence suggests. Even in single-dose arms, the data indicate that HPV16/18 antibodies are sustained to at least 84 months post vaccination.

An updated literature search for articles on single-dose HPV vaccination through clinical trials published up to March 2019, as well as a Cochrane review on the efficacy and safety of one or more doses of HPV vaccine versus placebo within the context of clinical trials, did not identify any additional articles on single-dose HPV vaccination besides those presented here.

A number of non-randomized observational studies have recently been published that compare immune responses among adolescents receiving three, two, or one HPV vaccine doses through national vaccination campaigns or programs. Most of these evaluate humoral immune responses to the vaccines, though one also presents cellular immunogenicity data. The published studies demonstrate high rates of seroconversion for vaccine-type HPV antibodies in all dosage groups, albeit with the same caveat as trial-

derived data, whereby methodologies used and definitions of seropositivity are variable. Again, antibody titers are mostly lower for single-dose recipients compared to multi-dose recipients. However, where immunogenicity studies have used the same laboratory methods as the clinical trials described above, they have been able to demonstrate higher antibody titers among adolescents receiving a single dose of HPV vaccine through national campaigns or programmes than the titers associated with protection in previous clinical trial participants (of older age). Furthermore, the immunogenicity studies present evidence of a sustained immune response to single-dose HPV vaccination into the mid- to long-term, with one study presenting data up to eight years post vaccination.

Most post-licensure studies examining HPV vaccine effectiveness by number of doses report highest effectiveness with three doses, though some found no statistically significant difference between two and three doses. Almost half of the studies found some effectiveness after one dose. **Importantly, more recent studies with younger vaccine recipients, have found minimal or no differences in effectiveness by number of doses.** Several biases in available data impact estimates, with most biasing two-dose and one-dose results away from showing effectiveness. Future studies of real-world HPV vaccination effectiveness, which examine people vaccinated prior to sexual activity and use methods to reduce **potential sources of bias, are warranted.**

Modeling analyses have evaluated single-dose HPV vaccination in the US, UK, South Africa, and Uganda. Initial analyses indicate that, if the choice is between no vaccination and a single dose, a single dose is likely to provide health benefits and be good value for money. This applies even if the vaccine has a lower vaccine efficacy than two or more doses, as long as single-dose protection lasts at least ten years. **Single-dose 9vHPV vaccination in a high HIV prevalence setting can yield high reductions in cervical cancer incidence and mortality, and these relative reductions are similar irrespective of HIV status, CD4 count, or ART coverage.** If the choice is between one-dose and two-dose vaccination, then the second dose becomes the most cost-effective option if it can extend protection up to at least 20 years. Extension of these analyses into settings with more variable epidemiological, demographic, and behavioral profiles will be critical to fill important evidence gaps regarding the impact and value of reduced-dose HPV vaccination.

6 Strengths and weaknesses of the evidence

A major strength of this Second Edition of the White Paper is the inclusion of two high-quality and purpose-designed systematic reviews of the evidence on single-dose HPV vaccination compared to either no vaccination or to multi-dose schedules. One systematic review presented evidence on efficacy and immunogenicity derived from clinical trials, and the other from post-licensure observational (surveillance and ecological) studies of national HPV vaccination programmes. Both reviews utilised a robust and comprehensive search strategy and encompassed data from multiple sources. A limitation of the reviews was that, while the authors have evaluated the quality of the included studies, they have not utilised a formal quality assessment tool due to the previous lack of availability of a suitable tool. Recently, members of this Consortium have adapted the Risk of Bias 2.0 framework to allow a formal quality assessment of the studies included in the two reviews. Thus, a formal quality assessment utilising a standardised framework will be included in future updates to the evidence base.

To date, there has been no systematic review of the evidence derived from observational immunogenicity studies of participants who received different dosing schedules of HPV vaccine through national programmes or campaigns. The evidence presented in this edition of White Paper comes from a literature search (not using systematic review methodology) conducted by Consortium members.

Data from the non-randomized studies included in the trials-based systematic review (derived from CVT, PATRICIA and the IARC India HPV Vaccine Trial), have provided encouraging indications that a single dose of the HPV VLP vaccine may provide protection from HPV infections over several years. These are well-conducted, prospective studies implemented in the context of clinical trial protocols with rigorous enrollment, clinical procedures, and laboratory protocols and good retention to follow-up. Their results have provided the strongest evidence to date to support further investigations on the efficacy and immunogenicity of single-dose HPV vaccine strategies; analyses from some of these studies are ongoing. These published studies are, however, heterogeneous in design and outcome assessment. Immune response data are difficult to compare across these studies because of the different assays and laboratories used for these trials, although

clinical data on protection against HPV infection provide consistent results for a single dose of either 2vHPV or 4vHPV vaccines. It is also important to note that no data are yet available from prospective randomized controlled studies that are specifically designed to answer the question of single-dose protection or immune responses.

The immunogenicity studies identified through literature searches have also provided useful data. In Uganda, among adolescents who received only one dose, the GMTs measured nearly three years after vaccination were no different compared to those observed in CVT women who received one dose of HPV vaccine, for which no breakthrough cases have been detected four years after vaccination. Furthermore, the Uganda study has shown the importance of consistency in laboratory methods for the outcome measurements in using the same ELISA and calibrated standards to measure immunogenicity as those used in the CVT trial. A unique aspect of the Fiji study was the ability to examine the immunogenicity of mixed HPV vaccine schedules comprising both 4vHPV and 2vHPV; the study reported that a single dose of 4vHPV elicits antibodies that persist for at least six years and also induced immune memory. **A strength of the first Quebec study was the availability of seropositivity results pre-vaccination, but the study suffered from the limitations of using routine data. The second study from Quebec demonstrated sustained antibody responses to a single dose of 4vHPV between three and eight years post vaccination in a small cohort of 31 girls, but was unable to compare results for a single dose versus either no dose or multiple doses. The US PHACS study presented immunogenicity data following one, two, or three doses of 4vHPV (as well as no vaccination) for HIV-infected adolescents, an important population who is at particularly high risk of HPV infection and related clinic sequalae, yet for whom there is currently little evidence base in regards to HPV vaccine dosing schedules.**

Strengths of the data included in the systematic review of evidence from the post-licensure observational studies included the overall size of the studies, data on buffer periods for some studies, and some information on intervals between doses. Several limitations were noted: post-licensure studies were all conducted in settings of a national three-dose recommendation, and girls who received one or two doses differed from those completing the recommended schedule. These studies also included girls who were vaccinated beyond the routine target age group in the early years of the vaccination programs when catch-up programs had been implemented, who were older than three-dose vaccine recipients at the time of vaccination, who had lower socioeconomic status, and/or who had indicators of earlier sexual exposure. A third limitation was information bias–for example, misclassification of vaccination status due to recall, misclassification of outcome due to diagnostic bias, interviewer bias, or tools used.

In the majority of studies comparing participants who received single versus multi-dose HPV vaccination schedules, whether through trials or through national campaigns/programs, a major limitation was sample size, particularly for the single-dose groups, limiting statistical precision of estimates.

Three of the five identified modeling studies have only used data from high-income countries and are reliant on assumptions about the duration of one-dose and two-dose vaccine protection. The South Africa modeling study is the first to consider HPV and HIV comorbidity when evaluating single-dose 9vHPV vaccination impact, which is critical to understanding the effects of HIV infection on cervical cancer prevention strategies in many LMIC where HIV is highly prevalent. Ultimately, modeling results will only be confirmed by LTFU of post-vaccination cohorts.

Table 12. Threats to validity of single-dose HPV protection from previous clinical trials, and evaluations of bias and confounding within these rubrics

Threat to validity	Evaluation of bias and confounding
Are girls/women who received a single dose of the HPV vaccine different from women who received a single dose of the control vaccine?	Within the single-dose arms of CVT and PATRICIA, women who were in the HPV and control arms were similar with regard to age, number of clinic visits, HPV16/18 DNA- and sero-status, and prevalence of other surrogates of infection risk such as Chlamydia trachomatis.
Did single-dose girls/women receive less than a complete schedule for reasons related to HPV vaccination?	In CVT and PATRICIA, assessment of reasons for missed doses revealed that most reasons were involuntary and unrelated to randomization arm, such as pregnancy and colposcopy referral. It was less common for participants to refuse the vaccine or have a medical condition that was contraindicated to vaccination. For the IARC India study, subjects received only a single dose due to a government-requested halt to enrolment (for reasons unrelated to the study itself).
Are girls/women who received a single dose of the HPV vaccine immunologically different from girls/women who received multiple doses of the HPV vaccine?	In CVT, women in the one-dose HPV group had similar HPV antibody titers compared to the two- and three-dose groups following the initial HPV vaccine dose, when all women received the same number of doses.
Is HPV exposure during the follow-up phase similar among girls/women who received a single dose of the HPV vaccine compared to the control HPV vaccine or other dose groups?	Cumulatively over the first four years of follow-up, women in the active control arms of CVT and PATRICIA had the same HPV attack rate regardless of the number of doses received. Seven years after initial vaccination, women in the HPV arm had similar prevalence of non-vaccine HPV genotypes, a metric of HPV exposure, independent of dose group. Similarly, girls who received HPV vaccine in the IARC India study had similar rates of cumulative incident infections with non-vaccine HPV types over seven years of follow up.

Table adapted from (95).

7 Gaps in the evidence, research priorities & forthcoming evidence

7.1 Efficacy and immunogenicity data from RCTs and observational studies

Several clinical studies have examined single-dose regimens and demonstrated results that challenge the prevailing dogma that protein-based subunit vaccines require a multi-dose regimen. These observations and the potential public health impact of an effective single-dose strategy suggest that further studies on single-dose efficacy of the HPV vaccines, including cross-protective efficacy and duration of protection, and data from different study populations, are warranted. Several evidence gaps are being addressed or will need to be addressed in the coming years. **These are discussed below, and new and ongoing studies are summarized in Table 13.**

Durability of protection

Currently, it is not known if a single dose of HPV vaccine will provide a sufficient and durable enough level of efficacy against persistent HPV infection to support a recommendation for a policy change to a single-dose vaccination strategy. This question is being addressed through the CVT trial and continued follow-up of the India study cohort.

In the CVT trial **[ClinicalTrials.gov Identifier: NCT00128661; NCT00867464; NCT03309033]**, analysis of efficacy is published out to seven years and a subset of participants will be followed out to 15 years for immunogenicity outcomes in a study called CVT EXTEND (72, 153, 154). **11-year immunogenicity and efficacy by dose will soon be available.**

Additional data on incident persistent infections in the IARC India HPV vaccine study **[ClinicalTrials.gov Identifier: NCT00923702]** will be obtained from at least 1,000 additional women who are initiating sexual activity over the next few years, including women in the single-dose arm (71). Data from these women will be used to compare the efficacy of one dose of 4vHPV against persistent infection, compared to the two- and three-dose vaccine recipients and unvaccinated women.

The India study will also generate data on the efficacy of a single dose to protect against cervical sequelae of HPV infection by comparing rates of CIN2+ in single-dose recipients (compared to unvaccinated women and women receiving two or three doses) who initiate cervical cancer screening within the next few years. **To date, 792 women in the single-dose group have initiated cervical cancer screening (using Hybrid Capture II HPV assay). A further 500 women per year will be screened up to 2021.**

An additional future benefit from the India study will be the increase in the number of age-matched unvaccinated, married women above 25 years of age. This will allow a more robust comparison of the efficacy of a single dose compared to age-matched unvaccinated women to prevent CIN 2+ disease. To ensure age- and site-matching of the new unvaccinated cohort, unvaccinated women between 25 and 27 years of age will be recruited at each site. **To date, over 3,100 women of the 3,500 planned have been recruited, with the aim of maintaining the age-matching.** The analysis plan is currently being drafted.

Durability of efficacy and immunogenicity will also be addressed through new randomized and non-randomized prospective intervention studies, which are described below.

Evidence from purpose-designed intervention studies of single-dose HPV vaccine versus no vaccination or multi-dose schedules

The systematic and Cochrane reviews of trials data highlighted a paucity of evidence from RCTs that prospectively randomized participants to receive one HPV vaccine dose versus either no HPV vaccine dose or multi-dose schedules. Prospective, randomized trials will be able to provide more definitive data on whether single-dose HPV vaccination can protect against HPV-persistent infection and provide immunobridging data to other trials without efficacy endpoints. **Several ongoing trials are investigating efficacy and/or immune responses and safety of a single dose of HPV vaccine compared to recommended dose regimens or controls (Table 13, Figure 5).**

A large-scale randomized controlled trial is underway in Costa Rica. The ESCUDDO trial (Scientific Evaluation of One or Two Doses of the Bivalent or Nonavalent Prophylactic HPV Vaccines; ClinicalTrials.gov Identifier: NCT03180034) (16) aims to find out if one dose of either the 2vHPV or 9vHPV vaccines is as effective as two doses of these vaccines among young women aged 12 to 20 years in Costa Rica. The study is a four-arm trial of 20,000 12 to 16-year-old girls to formally evaluate the non-inferiority of one versus two doses of each of 9vHPV and 2vHPV vaccines. The participants have been randomized in two stages to receive one or two doses of the vaccines and to be followed initially for four years. As a primary endpoint, the trial will focus on the prevention of new, persistent infection by HPV

types 16 and 18. The trial will also evaluate protection against the other cancer- and genital wart-causing HPV types, while documenting infection by non-vaccine HPV types to verify continued exposure among trial participants. In addition to the evaluation of efficacy against HPV infection, the immunological response to vaccination will be monitored in order to demonstrate robust, stable, and durable antibody responses following one- and two-dose vaccination, and to enable studies to compare immune responses induced by the two vaccines, which contain different adjuvants. The ESCUDDO trial should complete enrolment in 2020 with four-year follow-up data available in 2025 (Figure 5).

A second efficacy RCT commenced in Kenya in December 2018. The Kenya Single-dose HPV vaccine Efficacy (KEN-SHE) study [Clinicaltrials.gov Identifier: NCT03675256], is enrolling 2,250 sexually-active females aged 15 to 20 years and randomizing participants to receive either immediate single-dose HPV vaccination (2vHPV or 9vHPV) and delayed second dose of meningococcal vaccine or immediate meningococcal vaccine and delayed HPV vaccine (9vHPV) (17). Study participants will be followed until month 36 to assess vaccine efficacy against HPV infection and measure humoral immune responses. The delayed vaccine will be administered at the end of follow up.

Whilst not randomized, three further intervention studies evaluating the efficacy or effectiveness of single-dose HPV vaccination are also underway: the PRIMAVERA study in Costa Rica, the IVIHPV1 study in Thailand, and the HOPE study in South Africa.

PRIMAVERA [ClinicalTrials.gov Identifier: NCT03728881] is a clinical trial in Costa Rica comparing immune responses following one dose of 2vHPV vaccine among 520 girls aged 9 to 14 years (the intervention arm) versus three doses of 4vHPV vaccine in 520 women aged 18 to 25 years (the control arm) (155). The primary aim is to demonstrate that HPV16 and 18 antibody responses among 9 to 14-year-old, single-dose 2vHPV recipients are non-inferior to those of 18 to 25-year-old, three-dose 4vHPV recipients at 24 and 36 months after first vaccine dose. Efficacy of three doses of 4vHPV has already been demonstrated among women of this age group, and thus non-inferior immune responses among the younger age group would imply protection against HPV16/18 and associated precancerous lesions following a single dose of 2vHPV. This study started in March 2019.

The Effectiveness of Single Dose or Two Doses of Bivalent HPV Vaccine in Thailand (IVIHPV1) study [ClinicalTrials.gov Identifier: NCT03747770] (17) is a community intervention study of female students in Thailand, which started in December 2018. The study involves vaccination of grade 8 female students from two provinces with either one or two doses of HPV vaccine (2vHPV) and a series of cross-sectional surveys (at baseline, year 2 and year 4) among grade 10 and 12 female students to measure the population-level

impact on HPV prevalence, with DNA being measured in, and genotyped from, urine. Immune responses will be measured in a subset of vaccinated participants, as well as a subset of survey participants.

The HPV One/Two Dose Population Effectiveness (HOPE) study also aims to assess the population-level effectiveness of one versus two HPV vaccine doses, and is embedded within the South African national HPV vaccination program, which has been administering two doses of 2vHPV to girls aged nine years since 2014 (156). In 2019, HOPE performed a one-year catch-up demonstration project among girls aged 17 and 18 years in one South African district, administering a single dose of 2vHPV to approximately 7,000 girls. Cross-sectional surveys of at least 3,260 girls aged 17-18 years across districts offered the national program alone and the district offered single-dose catch-up vaccination will be used to determine HPV prevalence at baseline and follow-up time points, enabling measurement of population effectiveness of the two-dose national program and the single-dose demonstration project. The impact of HIV infection on the protective effectiveness of HPV vaccination will additionally be determined.

Randomized, controlled immunogenicity trials are also underway. The Dose Reduction Immunobridging and Safety Study of Two HPV Vaccines in Tanzanian Girls (DoRIS) [ClinicalTrials.gov Identifier: NCT02834637] (15) is an ongoing RCT among Tanzanian girls aged 9 to 14 years, intended to establish whether a single dose of HPV vaccine (2vHPV and 9vHPV) produces immune responses that are likely to be effective in preventing cervical cancer. **The trial has randomized 930 girls to six groups, which are being followed for 36 months. Girls received the 2vHPV or the 9vHPV vaccine in one, two, or three dose schedules. Immune responses of girls receiving one or two doses will be compared with those receiving three doses of the same vaccine. Results from the DoRIS trial will be used to immunobridge to historical cohorts, such as the CVT and the IARC India HPV vaccine trial, where a single dose has been shown to be protective, as well as to the new RCTs—ESCUDDO and KEN-SHE.** The intent of the immunobridging analyses would be to support efficacy claims across different geographies (among an African population) and age groups (among girls as young as nine years). This study will be one of the first randomized trials of one and two doses of any HPV vaccine in Africa. **The DoRIS trial cohort completed the first year of follow-up in January 2019 and final data will be available in 2021.**

The HPV Vaccination in Africa – New Delivery Schedules or HANDS trial [ClinicalTrial.gov Identifier: NCT03832049] (18) is a second immunogenicity trial in The Gambia which will compare one and two doses of 9vHPV in four to eight-year-olds and 9 to 14-year-olds with three doses in 15 to 26-year-olds. This trial will begin later in 2019. This randomized, open-label, single-centre, phase III non-inferiority trial will recruit 1,720 female participants. The primary and secondary immunogenicity objectives will be

analysed based on serological samples taken four to six weeks after the last dose of vaccine received according to group. A sub-study will be undertaken within the main trial to compare early immunological events.

Finally, a non-randomized delayed second-dose immunogenicity trial in the US, where 200 male and female subjects aged 9 to 12 years receive a second dose of 9vHPV at 24 months, determine the persistence and stability of serologic geometric mean titer of HPV 16/18 between six, 12, 18, and 24 months after the prime dose and prior to the administration of the second dose, thus also providing some limited information on immune responses to a single dose up to two years after the first dose [Clinicaltrials.gov registration: NCT02568566] (157).

Evidence from different populations and using different vaccines

It is important that research on a single dose of HPV vaccine is carried out across a wide range of age groups and populations. **Undertaking multiple, large-scale efficacy and effectiveness studies across numerous countries is challenging, but current studies (including CVT, India, ESCUDDO, KEN-SHE, IVIHPV1, HOPE) are already being conducted across multiple continents.** Immunobridging studies will be important to allow conclusions to be drawn about the potential efficacy of a single dose across further populations and age groups. The current prospective studies are working across a wide age range from 4 to 26 years and are covering study populations on five continents (Table 13).

While the evidence base to date is largely derived from studies of the bivalent and quadrivalent HPV vaccines, new and ongoing research on single-dose vaccination spans the three commercially available vaccines (2vHPV, 4vHPV, and 9vHPV).

Standardized measurement and reporting of immunogenicity outcomes

The inability to compare immune responses of a single-dose HPV vaccine across studies due to heterogeneity in laboratory methods and cutoff thresholds for seropositivity creates a significant gap in evidence. Efforts are now underway to standardize the immunological testing for antibody levels so that the results of the CVT and India trials can be compared directly as well as for future trials **(including ESCUDDO, DoRIS, KEN-SHE)**. Antibody avidity indicates the degree of antibody affinity maturation and generally increases over time following encounter with an antigen. Avidity data are available from the CVT and India studies and will be collected in the ESCUDDO and DoRIS trials. Studies are also underway in the DoRIS trial to compare cellular immune responses following one, two, and three doses of HPV vaccines.

To date, there has been no systematic review of immunogenicity data from observational studies of participants receiving a single dose of HPV vaccine versus either no vaccination or versus multi-dose schedules through national programs or campaigns. However, a systematic review of immunogenicity data among vaccine recipients, stratified by number of doses received, is currently underway. This review is being conducted by the Strategic Analysis, Research and Training (START) Center at the University of Washington. Once results are available, these will enhance the evidence-base regarding the immunogenicity of single-dose HPV vaccination.

7.2 Efficacy data from post-licensure surveillance and ecological studies

The systematic review of the literature conducted to date identified studies that: 1) reported the effectiveness of HPV vaccination (2vHPV or 4vHPV vaccine) on HPV infections, anogenital warts, or cervical lesions abnormalities; and 2) assessed the effectiveness of HPV vaccination by the number of doses received (one, two, and three). However, because eligible studies used different outcomes, buffer periods, and/or age groups at vaccination and at outcome assessment, it was not possible to pool the results from the different studies.

Further surveillance and ecological studies evaluating the effectiveness of single-dose HPV vaccination are expected to be published over the year ahead, including studies from the US, Canada, and Australia. The systematic review of effectiveness studies will be updated regularly, allowing inclusion of these and other newly published studies, and it is anticipated that future updates will include meta-analyses of the population-level effectiveness of HPV vaccination (2vHPV or 4vHPV vaccine) with reduced doses. This work will include contacting authors of eligible studies to request supplementary data extractions in order to standardize data stratifications between studies for comparison and pooling (e.g. same age at first vaccination, buffer periods, and outcomes).

Until recently, there has not been a suitable tool for assessing the quality of evidence and risk of bias derived from post-licensure surveillance and ecological studies comparing single-dose HPV vaccination to either no vaccination or multi-dose schedules. There is an ongoing study to adapt the ROBINS-I framework (158) to take into account the characteristics of reduced dose observational studies (e.g. different types of study design, use of buffer periods to control for prevalent infection at 1st dose) to formally assess the quality of these studies. This quality assessment will be presented in future updates of this White Paper.

7.3 Modeling studies

Factors influencing modeling results

The early studies on reduced-dose vaccination have revealed several key issues and areas of uncertainty that the models can continue to explore as data emerge. Collectively, the analyses demonstrate that the duration of vaccine protection with reduced-dose regimens is a key determinant of impact and value and that the function of waning protection is important. Most analyses assume fixed duration with or without a gradual decline, based on sustained efficacy from over 10 years of trials of three-dose regimens and three years of trials of two-dose regimens.

Efficacy of single-dose vaccination will also have a key influence on overall effectiveness, although preliminary results suggest that it could be less important than duration of protection. Small changes in efficacy (5-10%) had little impact on results in the context of two versus three doses (148, 149). Likewise, cross-protection, which in previous analyses has been shown to be potentially influential in the choice of vaccine (2vHPV versus 4vHPV vaccine, and incremental value of 9vHPV), thus far has not been shown to have much effect in analyses of reduced doses. However, that could change as evidence regarding the efficacy and duration of cross-protection associated with reduced doses emerges. It currently remains unclear whether the difference in the plateauing of GMTs will influence long-term efficacy (see Section 514.3); however, ongoing clinical trials (summarized in Section 7.1) are expected to provide stronger evidence on the magnitude of efficacy.

The impact of duration of protection and efficacy will also undoubtedly be influenced by the level of vaccination coverage achievable and possible increase in coverage with reduced-dose schedules. Preliminary analyses showed that modest increases in coverage with reduced doses can compensate for waning protection and/or lower efficacy (149, 151).

In the South African modeling study, the authors found that changes in vaccination coverage was influential in reductions in CC incidence and mortality, whereas the duration of vaccine protection ranging from 10 to 20 years (followed by a linear decline over 20 years) did not degrade the level of health benefits as much as in previous studies evaluating reduced-dose HPV vaccination.

Future modeling priorities

Given the ongoing activities related to evaluating single-dose vaccination, several important priorities exist for future modeling work. First, it will be critical for the models to continue to synthesize and integrate new data as they emerge from the ongoing studies and trials. Results from the LTFU of the CVT and Indian trials will continue to refine the plausible lower limits of duration of protection. Model-based impact and cost-effectiveness analyses are already included as part of the existing single-dose HPV vaccine trials, being led by the three modeling groups in this Consortium. The close involvement of the modelers in the ongoing efficacy and immunogenicity trials will enable timely and relevant model updates and analyses. The consortium will provide a venue for the modelers to share assumptions and explorations, and–under agreed-upon circumstances–perform comparative modeling exercises to unveil important similarities and differences in results.

Given the limited clinical trial settings, it will also be important to conduct modeling extrapolations and analyses in different countries with varied epidemiological profiles, population demographics, and sexual behaviors in order to continue to identify important factors and uncertainties that could inform decision-making in a particular setting. Likewise, it will be essential to explore single-dose vaccination in the context of both settings that have already initiated multi-dose HPV vaccination programs (the one- versus two/three-dose scenario), as well as settings in which HPV vaccination has not yet been adopted (the single-dose versus no-vaccine scenario). Moreover, the models can be used to explore opportunities for, and design of, innovative strategies for vaccine delivery given the unconventional target age group of adolescents and the requirement for multiple doses over multiple contacts.

The South African study found that the relative reductions in CC incidence and mortality did not vary substantially across HIV-negative and HIV-positive women (irrespective of CD4 count or ART coverage). However, the analysis assumed the same efficacy across all vaccinated girls. Given current recommendations for HPV vaccination with a full three-dose series for HIV-positive individuals, it will be critical to generate more evidence on the health and economic impacts of reduced-dose HPV vaccination in this population. Model-based analyses that are in the context of settings with high HIV prevalence will need to revisit assumptions regarding vaccine characteristics as data become available from clinical trials on vaccine efficacy and durability in HIV-positive women.

Table 13. Ongoing and forthcoming efficacy, effectiveness and immunogenicity studies of single-dose HPV vaccination

Study	Country	Study population	Vaccine(s)	Study design	Key endpoint(s)	Start date & FU / duration
CVT EXTEND (153, 154)	Costa Rica	1000 females vaccinated aged 18-25y	2vHPV	Long-term follow up study of participants previously vaccinated with 1 v 2 v 3 doses through an RCT	Humoral immunogenicity	Start: July 2018 Follow-up: To 15 years post first vaccination
DoRIS (15)	Tanzania	930 females aged 9-14y	2vHPV & 9vHPV	RCT of 1 v 2 v 3 doses	Humoral & cellular immunogenicity; Cost-effectiveness; Acceptability	Start: Feb 2017 Follow-up: 36 months
ESCUDDO (16)	Costa Rica	20,000 females aged 12-16y (RCT) & 4,000 females aged 17-20y (epi study)	2vHPV & 9vHPV	RCT of 1 v 2 doses, & epidemiological study of 1 dose v no vaccination	Vaccine efficacy against HPV infection; Humoral immunogenicity	Start: Nov 2017 Follow-up: 48 months
HANDS (18)	Gambia	1,720 females aged 4-26y	9vHPV	RCT of 1 v 2 v 3 doses	Humoral immunogenicity; Safety; Tolerability	Start : Jul 2019 Follow-up: 36 months
HOPE (156)	South Africa	~7,000 girls aged 15-16y (1-dose catch up) & ≥3,260 sexually active girls aged 17-18y per surveys	2vHPV	Intervention study of 1 dose catch up v 2 dose national program, using repeat cross-sectional surveys	Population effectiveness against HPV infection; Cross-protection; Herd protection; Sociodemographic & behavioural correlates of uptake & impact	Start: Feb 2018 Duration: 48 months
IARC India HPV-vaccine efficacy study (71)	India	17,729 vaccinated females aged 10-18y & 1,540 age-matched unvaccinated females	4vHPV	Observational cohort study of 1 v 2 v 3 doses, and v no vaccination (extended follow up)	Vaccine efficacy against HPV infection; Humoral immunogenicity	Start: Sep 2009 Follow-up: To 11 years post first vaccination
IVIHPV1 (17)	Thailand	~18,000 female students (intervention), & between ~4,000 and 9,200 female students per survey	2vHPV	Intervention study of 1 v 2 doses, using repeat cross-sectional surveys	Population effectiveness against HPV infection; Humoral immunogenicity	Start: Dec 2018 Duration: 48 months
KEN SHE (17)	Kenya	2,250 sexually-active females aged 15-20y	2vHPV & 9vHPV	RCT of 1 dose v delayed vaccination	Vaccine efficacy against HPV infection; Humoral & cellular immunogenicity; Cost-effectiveness	Start: Dec 2018 Follow-up: 36 months
PRIMAVERA (155)	Costa Rica	520 girls aged 9-14y & 520 women aged 18-25y	2vHPV & 4vHPV	Non-inferiority trial of 1 dose 2vHPV in girls v 3 doses 4vHPV in women	Immunogenicity	Start: Mar 2019 Follow-up: 36 months
US study (72)	US	200 males and females aged 9-11y	9vHPV	Intervention study of 1 dose v deferred-booster dosing schedule	Immunogenicity	Start: Mar 2016 Follow-up: 48 months

US: United States of America; HPV: Human papillomavirus; FU: Follow up; Y: Years; RCT: Randomized controlled trial.

Figure 5. Timing of data from new and ongoing studies evaluating single-dose HPV vaccination

Study name *(country)*	Evidence type	Vaccine(s)	Brief description	2019	2020				2021				2022				2023				2024	2025
				Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4		
ESCUDDO *Costa Rica*	Efficacy	2vHPV and 9vHPV	Girls 12-16 yo randomized to 1 or 2 doses of 2vHPV or 9vHPV; n=5000 each arm																			4y f/u
India IARC *India*	Efficacy	4vHPV	Girls 10-18 yo received 1, 2, 3 doses of 4vHPV; n=17586, 1-dose n=4980	10y f/u						n=3000 1-dose			12y f/u									
KEN-SHE *Kenya*	Efficacy	2vHPV v 9vHPV v MenACWY (delay HPV)	Girls 15-20 yo randomized to 1 dose of 2vHPV, 9vHPV, or MenACWY; n=750 each arm; delayed dose 2 planned							18m f/u										3y f/u		
Primavera *Costa Rica*	Immunogenicity	2vHPV and 4vHPV	Girls 10-13 yo 1-dose 2vHPV immunobridge to women 18-25 yo 3-doses 4vHPV; n=520 each											2y f/u						3y f/u		
DoRIS *Tanzania*	Immunogenicity	2vHPV and 9vHPV	Girls 9-14 yo randomized to 1, 2, or 3 doses of 2vHPV or 9vHPV; n=155 each arm					2y f/u				3y f/u										
HANDS *The Gambia*	Immunogenicity	9vHPV	Girls 4-8 yo and 9-14 yo randomized to 1 or 2 doses; girls 15-26 yo given 3 doses; n=344 each arm									2y f/u				3y f/u						
Thailand impact study *Thailand*	Effectiveness	4vHPV	Girls in grade 8 given 1 or 2 doses; n=~8000 each arm | prevalence surveys of girls grades 10, 12; n=2,400 each grade x 2 provinces								Year 2								Year 4			
HOPE *South Africa*	Effectiveness	2vHPV	Girls 17-18 yo serial prevalence surveys: unvaccinated (17-18 yo), 1-dose catch up (15-16 yo), and 2-dose routine (9 yo) cohorts; n≥3260									1-dose									Year 4	
CVT *Costa Rica*	Efficacy / Immunogenicity	2vHPV v control	Women 18-25 yo received 1, 2, or 3 doses of 2vHPV; n=3727, 1-dose n=196	13y f/u							15y f/u											

Interim results Final results

HPV: Human papillomavirus; V: versus; yo: years-old; N: Number; Q: Quarter.
The information provided in this schematic is correct at 30th June 2019 but may be subject to change.

8 References

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Appendix 1: Contributors and acknowledgments

Appendix table 1. Individuals that contributed to the White Paper (in alphabetical order)

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Appendix 2: Summary of updates

Significant updates made in the Second Edition of the White Paper compared to the first are highlighted in bold and summarized below. Minor amendments or clarifications have not been highlighted.

Appendix table 2. Summary of changes made in the 2nd Edition of the White Paper compared to the 1st Edition

SECTION	SUMMARY OF UPDATES
Contents	The list of contents has been updated to reflect changes to the White Paper content.
List of figures	The list of figures has been updated to reflect changes to the White Paper content.
List of tables	The list of tables has been updated to reflect changes to the White Paper content.
2 – BACKGROUND	
2.1. Cervical cancer burden	Text has been updated with 2018 statistics and the corresponding reference has been updated accordingly.
2.4. Rationale for the White Paper	Text has been updated to inform the reader that this is an updated version (2nd Edition) of the previously published White Paper (1st Edition), now presenting evidence published up to March 2019.
Table 1. Summary of available HPV vaccines	Amendments have been made to the table from the 1st Edition of the White Paper to include further detail on the composition and dosing schedules of the vaccines.
3 - SOURCES OF EVIDENCE	
3.2. Observational data from partially vaccinated participants in RCTs and post-licensure effectiveness evaluations	Text has been added regarding challenges in research on immune correlates of protection. Additionally, a summary of updates included in the 2nd Edition of the White Paper has been added.
3.3. Modeling data	Text has been updated to reflect the additional evidence presented the 2nd Edition of the White Paper version compared to Edition 1.
4 – RESULTS	
4.2. Clinical HPV vaccine trials	Text has been added on a systematic review of the clinical trials-based literature on the efficacy and immunogenicity of single-dose HPV vaccination compared to no vaccination or multiple doses (including a narrative quality assessment of the included studies). Additionally, data is presented from an updated Cochrane review of clinical trials on HPV vaccines, which includes data on the efficacy of 'one or more' HPV vaccine doses.
Figure 2. Clinical trials systematic review flow diagram	A flow chart included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.
Table 14. Summary of studies selected for inclusion in the trials systematic review	A summary table of studies included in the clinical trials-based systematic review been edited and reproduced in this White Paper edition.
Table 15. Sampling, laboratory methods and definitions used and reported by each study in the trials systematic review for HPV16/18 infection-associated endpoints	A table of the laboratory methods and definitions for HPV infection endpoints used by studies included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.
Table 16. Summarized HPV16/18 infection results from studies in the trials systematic review	A table summarizing HPV infection results from studies included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.

Table 17. Sampling, laboratory methods and definitions used and reported by each study in the trials systematic review for HPV16/18 immunogenicity-associated endpoints	A table of the laboratory methods and definitions for immunogenicity endpoints used by studies included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.
Table 18. Summarized HPV16/18 seropositivity and GM antibody level results from studies in the trials systematic review	A table summarizing HPV immunogenicity results from studies included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.
Table 19. Quality assessment of studies in the trials systematic review	A table presenting a narrative qualitive assessment of studies included in the clinical trials-based systematic review has been edited and reproduced in this White Paper edition.
4.3. Immunogenicity studies of partially vaccinated populations	Text has been added on five non-trial immunogenicity studies presenting results for single-dose HPV vaccination versus no vaccination or other vaccine schedules that have been published since the 1st Edition of the White Paper.
Table 8. Summary of non-trial immunogenicity studies	A summary table of the non-trial immunogenicity studies has been added.
Table 9. Summarized HPV16/18 seropositivity and GM antibody level results from non-trial immunogenicity studies	A table summarizing the results of the non-trial immunogenicity studies has been added.
4.4. Non-trial observational studies, registry linkages and other studies	Text has been updated to incorporate results of an updated systematic review on the efficacy or effectiveness of single dose HPV vaccination compared to no vaccination or multiple doses.
Figure 3. Non-trial observational studies systematic review flow diagram	A flow chart included in the non-trials systematic review published paper (and presented in the 1st Edition of the White Paper (previously as Figure 9)) has been edited and reproduced in this White Paper edition.
Figure 4. Non-trial observational studies systematic review flow diagram - update	A flow chart of the update to the non-trials systematic review has been edited and reproduced in this White Paper edition.
Table 10. Summary of studies selected for inclusion in the non-trial observational studies systematic review	A table summarizing studies included in the non-trials systematic review (and presented in the 1st Edition of the White Paper (previously as Table 7)) has been updated, edited and reproduced in this White Paper edition.
Table 11. Analyses and main findings from studies in the non-trial observational studies systematic review	A table summarizing HPV infection results from studies included in the non-trials systematic review (and presented in the 1st Edition of the White Paper (previously as Table 8)) has been updated, edited and reproduced in this White Paper edition.
4.5. Mathematical modeling studies evaluating reduced dosage immunization schedules	Text has been added on one modeling study of single-dose HPV vaccination published since the 1st Edition of the White Paper.
5 – SUMMARY OF RESULTS	
-	Text has been updated to include a summary of the new information and data included in Section 4 of this White Paper edition.
6 – STRENGTHS AND WEAKNESSES OF THE EVIDENCE	
-	Text has been updated to include a summary of the strengths and weaknesses of studies that generated the new data included in Section 4 of this White Paper edition.
Table 12. Threats to validity of single-dose HPV protection from previous clinical trials, and evaluations of bias and confounding within these rubrics	Amendments have been made to the table from the 1st Edition of the White Paper (previously Table 9) to include information on additional trials-based studies of single-dose HPV vaccination.
7 – GAPS IN THE EVIDENCE, RESEARCH PRIORITIES & FORTHCOMING EVIDENCE (Previously presented across two sections (Section 7 and Section 8) in the 1st Edition of the White Paper)	
7.1 Efficacy and immunogenicity data from RCTs and observational studies	Text has been updated to reflect the evidence gaps existing at the time of production of the 2nd Edition of the White Paper. New clinical trials and observational studies that will address these evidence gaps are additionally described.
7.2 Efficacy data from post-licensure surveillance and ecological studies	Text has been added regarding an ongoing study that is developing a framework for standardized quality assessment of studies included in the systematic review of data on single-dose HPV vaccination.
7.3 Modeling studies	Text has been updated to reflect the evidence gaps from modeling studies existing at the time of production of the 2nd Edition of the White Paper.

Table 13. Ongoing and forthcoming efficacy, effectiveness and immunogenicity studies of single-dose HPV vaccination	A table summarizing ongoing and forthcoming clinical trials and observational studies evaluating the efficacy, effectiveness and/or immunogenicity of single-dose HPV vaccination has been added.
Figure 5. Timing of data from new and ongoing studies evaluating single-dose HPV vaccination	Amendments have been made to the figure from the 1st Edition of the White Paper (previously Figure 10) to include new studies and provide updated information on timelines.
8 – REFERENCES (Previously Section 10 of the 1st Edition of the White Paper)	
-	The list of references has been updated to reflect changes to the White Paper content.
APPENDIX 1: CONTRIBUTORS AND ACKNOWLEDGMENTS (Previously Section 9 of the 1st Edition of the White Paper)	
Appendix table 1. Individuals that contributed to the White Paper (in alphabetical order)	New contributors involved in the production of the 2nd Edition of the White Paper have been added to the table.

Single-Dose HPV Vaccine
EVALUATION CONSORTIUM

Disclaimer: The content, findings, and conclusions of this report are those of the authors and do not necessarily represent the official position of their agencies or institutions of employ.

For information about the Single-Dose HPV Vaccine Evaluation Consortium, visit path.org/singledosehpv.

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