WHITE PAPER

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

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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 10 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 approved. Since that time, evidence from observational studies suggests that a single dose of HPV vaccine may also provide protection against HPV and its sequelae.

The primary objective of this review is to summarize and assess the current evidence that could support a change to a single-dose schedule of HPV vaccine. The review 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 incredible 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), the Wits Reproductive Health and HIV Institute (Wits RHI) at the University of Witwatersrand, and the World Health Organization (WHO).

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 delivery, effectiveness, and efficacy. 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 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]. In 2012, prior to widespread HPV vaccine introduction, there were estimated to be 528,000 new cases and 266,000 cervical cancer-related deaths per annum globally [2], with over 80% of ICC cases occurring in low- and middle-income countries (LMICs) [2, 3]. In settings where effective cervical screening programs are available, the incidence of cervical cancer has markedly decreased [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 HPV and licensed vaccines

Primary prevention for cervical cancer is now possible through vaccination with one of three licensed vaccines: the bivalent vaccine (2vHPV) that targets HPV 16 and 18 infection, the quadrivalent vaccine (4vHPV) that targets HPV 6, 11, 16, and 18 infections, and the nonavalent vaccine (9vHPV) that targets HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 infections. 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].

Vaccine composition

All three vaccines contain synthetically manufactured virus-like particles (VLPs) of the L1 epitope 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. 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 HPV vaccine contains more than twice the adjuvant content of the 4vHPV HPV 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 concentration of adjuvant as the 9vHPV HPV vaccine. None of the vaccines contains a preservative.

Table 1. Summary of available HPV vaccines targets and composition

	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)
Substrate	*Trichoplusia ni* (Hi 5) insect cell line infected with L1 recombinant baculovirus	*Saccharomyces cervisiae* (bakers yeast) expressing L1	*Saccharomyces cervisiae* (bakers 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)	0, 6-12 months	0, 6-12 months	0, 6-12 months
Injection Schedule (3 doses)	0, 1 months, 6 months	0, 2 months, 6 months	0, 2 months, 6 months

Adapted from [5] and updated for dosing schedule licensure modifications and global vaccination recommendations [8].

2.3 HPV vaccine schedules and vaccine introduction

Uptake of HPV vaccines since 2006 has been highly variable and based primarily on country income levels. Uptake was 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 for the 2vHPV and 4vHPV vaccines. This regimen, based on evidence of non-inferior immunogenicity compared with women for whom efficacy was demonstrated in clinical trials with a three-dose schedule [9-11], included administering the two doses at an interval of six months for girls aged 9-14 years old [8]. WHO guidelines allow for dosing flexibility for the second dose of the two-dose schedule, as early as five months and up to 15 months after the first dose. According to the recommendations, girls aged 15 years or older and girls who are immune-compromised, including those HIV-infected, should continue to receive three doses as per original dosage recommendations [8].

Despite the fact that LMICs bear the greatest burden of cervical cancer and the highest mortality rates due to the disease [2], uptake of HPV vaccine has been substantially faster in HIC than LIC. This, combined with a wider age range target in developed countries and compared to single or more restricted-year cohorts in LMICs (such as nine-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) [12].

A number of factors have influenced the slower introduction of HPV vaccines in LMICs. 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, Gavi requirements that demonstration projects be conducted if the country had no prior experience of HPV vaccine delivery or adolescent multidose schedules, had not prioritized cervical cancer as a public health problem [13], and perceptions that the vaccine is difficult and expensive to deliver.

A recent study collating evidence and lessons learned from HPV vaccine delivery in 37 LMICs 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 LMICs 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 LMICs 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 may not be functioning effectively [14].

A single-dose vaccine 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 are now of interest for a number of reasons, following scientific examination of the virus and the immune response, data from observational and registry studies of females that have received a single dose, and modeling data. 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 LMICs. 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 for/against the hypothesis.

This white paper aims to assess (i) the current evidence on single-dose schedules of HPV vaccine, (ii) the strength of that evidence, and (iii) the gaps in the evidence. It also 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 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.

Sections of the white paper include 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 study designs or approaches to use (and the ethical considerations therein) to fill such gaps; acknowledgment of any known studies or datasets that might be ongoing or available that could contribute to these evidence gaps; and an overall conclusion for the strategic direction needed to inform decisions about HPV single-dose or alternative schedules.

3 Sources of evidence

Sources of evidence covered in this review 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 information;
- Observational data from partially vaccinated participants during clinical trials of HPV vaccine efficacy and immunogenicity; data from post-licensure vaccine effectiveness evaluations; and other observational data;
- Modeling analyses.

3.1 Biological plausibility for protection with single-dose HPV vaccine

Plausible biological explanations for the unexpected potency of HPV sub-unit vaccines were examined and recently reviewed, following observational data from several clinical studies that suggested a single dose of HPV vaccine could provide protection against HPV infection [15].

3.2 Observational data from partially vaccinated participants in RCT and post-licensure effectiveness evaluations

Observational data on single-dose HPV vaccination come from both three-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 have been used as the primary immunogenicity endpoint. Secondary immunological endpoints included antibody avidity and B- or T-cell responses.

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 the lack of a reference serum for establishing cut-off values [16].

Immune parameters other than antibody levels, which might correlate with protection, have not been defined and data on antibody avidity are scarce [17]. 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 [17,18].

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

For each of the outcomes listed above, published data (and data in press for (i)) was compiled from any geographical location that compared at least one of the outcomes of interest after one and two or three doses of HPV vaccine (in any schedule). A review was undertaken with the aid of key stakeholders in order to identify potentially relevant published literature.

3.3 Modeling data

While limited, the published studies on modeling of reduced dose strategies for the 2vHPV, 4vHPV and the 9vHPV vaccines were examined in order to identify key factors related to the impact of reduced dosages and their cost-effectiveness.

4 Results

4.1 Biological plausibility for single-dose protection

Below, the paper provides a summary of a recently published comprehensive review [15].

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 [19]. 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 vaccines 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" [20].

4.1.2 The immunogenicity of a single 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 [21]. In healthy young women, seroconversion rates are virtually 100%, peak in vitro neutralizing titers of 1000-10,000 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 [22]. Responses in pre-adolescent girls and boys are even stronger [10, 23]. The stability of antibody responses, now observed for almost 10 years post-vaccination [24, 25], 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 [26, 27]. 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 [28]. 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 [29]. 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 [30]. 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 [31]. 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 [32]. 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 [33]. 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 [34]. Epitope spacing at this distance is found on the surface of most viruses (HIV being a notable exception [35]) 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 [36]. 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 [37].

The high-density display on a VLP surface can efficiently break B-cell peripheral tolerance and even reactivate anergic self-reactive B cells [38, 39]. The BCRs on a majority of newly produced B cells are thought to bind self-antigens, which renders them functionally anergic [40, 41]. 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 [42]. 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) [43]. 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 [44]. 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 [45].

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 [46] 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 [47]. 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 [48] (**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 [49].

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 [50]. 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) [51] 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 [48]. 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 [52]. 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 [49]. 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 [53].

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 [52]. 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.

4.2 Clinical trial data - evidence from randomized controlled trials

As of April 2018, there were no data on the immunogenicity, efficacy, or effectiveness of one-dose HPV vaccination schedule compared to two- or three-dose schedules that originated from specifically designed randomized studies comparing one-dose to two- or three- dose groups.

4.3 Non-randomized observational data from clinical trials

Observational data from RCTs where participants failed to complete their schedule of two or three doses provide some evidence that one dose of HPV vaccine may provide protection against persistent HPV infection with vaccine genotypes and protective immune responses. The methods of these studies are described below in order to highlight different designs and laboratory and statistical analyses.

The results presented below are for the 2vHPV and 4vHPV vaccines. There are currently no data available on immune responses or efficacy against HPV infection of one dose compared to two or three doses of the 9vHPV vaccine.

4.3.1 The Costa Rica HPV Vaccine Trial

This section 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]. The content was edited for this paper.

4.3.1.1 SUMMARY AND STUDY DESIGN

The US National Cancer Institute (NCI) and the Agencia Costarricense de Investigaciones Biomédicas (ACIB) conducted a publicly funded, four-year, community-based, randomized phase III clinical trial prior to licensure of the HPV vaccines (the Costa Rica HPV Vaccine Trial or CVT- registered with Clinicaltrials.gov NCT00128661) [55]. From 2004 to 2005, 7,466 women were consented and randomized to receive either the 2vHPV or a control hepatitis A vaccine in a 1:1 ratio on a three-dose schedule at 0, 1, and 6 months. Participants were followed at least annually for four years. Protocols were approved by the Institutional Review Boards (IRB) of the U.S. National Cancer Institute, the Costa Rican INCIENSA (for

the CVT) and the National University Review Board (for the long-term follow-up [LTFU] component); all participants signed informed consent.

During the vaccination phase, time windows for each vaccine dose were pre-defined based on the first vaccination date. Women who became pregnant during the vaccination phase or who were referred to colposcopy were deferred and missed that dose if the vaccination window was closed; this occurred in roughly 20% of women in the CVT. Reasons for missing vaccine doses are discussed in the results section.

At enrollment and follow-up visits, participants provided a serum sample, and for sexually experienced women, a pelvic exam was performed at which time cervical cells were collected for cytology and HPV DNA testing. At the end of the four-year trial, participants were offered the vaccine they had not received at enrollment (cross-over vaccination) and were invited to stay in a long-term follow-up study [56]. During this study, HPV-vaccinated participants were followed biennially for six additional years with a pelvic examination and collection of a cervical sample and a serum sample as long as they had normal cytology. If they had minor HPV-related cytological abnormalities, they were followed every six months. To replace the original control group, 2,836 unvaccinated women from the same birth cohorts and geographic regions as the original trial participants were recruited into an Unvaccinated Control Group (UCG) and were also followed every two years. The new control group had similar characteristics to the trial participants, particularly with regard to risk of HPV acquisition [56].

4.3.1.2 LABORATORY METHODS

HPV DNA detection and genotyping from cervical specimens were performed at DDL Diagnostic Laboratory in the Netherlands [57-59]. Extracted DNA was used for PCR amplification with the SPF10 primer sets. The same SPF10 amplimers were used on SPF10-DEIA–positive samples to identify HPV genotype by reverse hybridization on a line probe assay (LiPA; SPF10-DEIA/HPVLiPA25, version 1; Labo Bio-Medical Products, Rijswijk, the Netherlands), which detects 25 HPV genotypes.

HPV 16 and HPV 18 serum antibody levels were measured by ELISA using HPV 16 and HPV 18 VLP at the NCI HPV Immunology Laboratory, USA [27]. The laboratory-determined seropositivity cut-offs for HPV 16 and HPV 18 were 8 EU/mL and 7 EU/mL, respectively. Laboratory-blinded replicates were included in each batch and the inter-plate coefficient of variation (CV) was ≤10%.

HPV 16 avidity was measured in serum by coating plates with HPV 16 L1 VLP. Each serum sample was tested at a dilution that yielded an absorbance reading of 1.0 ± 0.5 as previously

determined in an HPV 16 VLP ELISA. Guanidine-HCl (GuHCl) was added to the samples at various concentrations (0.5 to 3.5 M); the concentration of GuHCl that reduced the optical density by 50%, compared with sample wells without GuHCl treatment, defined the Avidity Index.

For specimens collected at the last (48-month) clinic visit, HPV 16 and HPV 31 neutralization titers were determined using a previously described pseudovirion-based secreted alkaline phosphatase neutralization (SEAP) assay [60].

4.3.1.3 STATISTICAL ANALYSIS

For analyses of the efficacy of fewer than three doses during the randomized, blinded phase (first four years of study), the primary endpoint was newly detected HPV 16 or 18 infection that persisted for at least six months (i.e. detection of the same genotype consecutively at least four months apart with no intervening negatives). Event counting started at the 12-month study visit or later to exclude prevalent infections at enrollment and bias due to differential infection assessment by missed visits during the vaccination phase (i.e. possible bias from assessing outcomes differentially for women who missed or received the six-month vaccination). Secondary endpoints were 12-month persistent HPV 16 and 18 infections and HPV 31, 33, and 45 infections (after excluding women with prevalent HPV 31, 33 and 45 infections detected at enrollment).

The analytic cohort excluded women who were both HPV 16 and 18 DNA positive at enrollment, as well as women with no follow-up visits post-enrollment (for analyses of cross-protection, the analytic cohort was restricted to women who were HPV DNA negative for types 31, 33, or 45 at enrollment, instead of restricting to those who were DNA negative for HPV 16 or 18 at enrollment). Within each dose group, vaccine efficacy was calculated using the complement of the ratios of the attack rates for the HPV arm and the control arm, instead of conducting a direct comparison by number of doses within the HPV arm only. This at least partially controlled for confounding by risk behavior - i.e. the fact that women who missed dose(s) may have lower rates of exposure to genital HPV infection.

For analyses occurring at the seven-year study visit, multiple endpoints were assessed, including year seven incident and prevalent HPV infections. This focus on the year seven results enabled assessment of the longevity of the protection against HPV infections, instead of allowing the early-term protection to potentially drive the longer-term findings, as a cumulative endpoint may have done. Comparisons of endpoints are made between the HPV dose groups and the new, non-randomized, unvaccinated control group, since the original control group was exited by this time. P-values comparing rates in the two-dose (0/6 month),

two-dose (0/1 months), and one-dose groups with the rate in the three-dose group were produced using the Fisher's test. A comparison of HPV prevalence by group was conducted in lieu of vaccine efficacy (VE).

4.3.1.4 RESULTS

Enrollment characteristics

*Comparing participants randomized to the HPV arm versus the control arm within each dose group, there were no differences between arms in those that received a single dose by age at vaccination, number of clinic visits attended (a metric for increased opportunities for endpoint assessment), or HPV 16 and 18 DNA / serologic status at enrollment. Sexual risk-taking behavior using presence or absence of Chlamydia trachomatis (CT) was also investigated. The prevalence of CT was balanced within the single-dose group by arm (**Table 2**). However, between HPV-vaccinated groups, HPV 16 and 18 DNA positivity at enrollment was higher in the one-dose compared to three-dose HPV vaccine recipients; HPV 16 and 18 seropositivity did not differ across dose groups.*

Reasons for receiving fewer doses

*Among vaccinated women, reasons for not receiving all doses were similar in both HPV and HAV arms, conditional on the number of doses received (**Table 3**) [61]. The most common reasons for not receiving all three doses were involuntary, including pregnancy and colposcopy referral (~35% of instances). It was less common for participants to refuse the vaccine.*

Antibody levels when all dose groups received only a single dose

The antibody levels measured at one month following the initial dose, when all women received the same initial number of doses irrespective of the total number of doses they finally received, were not significantly different [27]. Specifically, the one month HPV 16 Geometric Mean Titers (GMTs) were 419.7 (95% Confidence Interval [CI] 251.0 to 701.7) for one dose, 646.2 (95% CI 478.2 to 873.4) for two doses, and 597.0 (95% CI 454.1 to 784.8) for three doses (p=0.4); the respective data for HPV 18 were 207.0 (95% CI 114.9 to 372.8), 244.1 (95% CI 184.2 to 323.4), and 207.9 (95% CI 163.3 to 264.5) (p=0.7). These findings allayed concerns that the single-dose recipients may have had a more robust intrinsic ability to respond to the vaccine.

HPV infections during the follow-up.

*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 [61]. Since balance in enrollment characteristics (**Table 2**) 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 [61, 62]. At the four-year analysis [61], 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 [62], 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.*

Evidence of protection

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 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 [62]. 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 (**Figure 2**). 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.

Evidence of protection from a combined analysis of CVT and the PATRICIA trial

Data from another trial, the PATRICIA trial, found that women who received one dose had the same VE as two and three doses [63]. 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 [64]. 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 [64].

*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 (**Figure 3**) 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.*

Evidence for cross-protection with a single dose of 2vHPV

The analysis of CVT data seven years following initial HPV vaccination found that the prevalence of HPV 31, 33, and 45 was similar between the three-dose (2.3%; 95% CI: 1.8 to 3.1%), two-dose (0/6 months; 0.0%; 95%CI: 0.0 to 3.7%; p=0.26 compared to three doses) and one-dose groups (1.5%; 95% CI: 0.3 to 4.8%; p=0.77 compared to three doses), with a seven-year prevalence of the same genotypes in the control group of 5.5% (95% CI: 4.7 to 6.5%). In the combined analysis of the CVT and PATRICIA trials, VE against one-time detection of incident HPV 31, 33, and 45 infections at four years of follow-up was 59.7% (95% CI: 56.0 to 63.0%) for three doses, 37.7% (12.4 to 55.9%) for two doses, and 36.6% (–5.4 to 62.2%) for one dose [63]. VE was 0% in women who received a second dose one month after the first dose, whereas women who received a second dose six months after the first dose had a higher efficacy estimate (68.1%). In the four-year work, underlying group differences may have contributed to observed differences in the presence of cross-protection for a single dose. Thus, the question of whether reduced dosage regimens of the HPV 16 and 18 vaccine retain partial cross-protection against HPV 31, 33, and 45 deserves continued investigation.

Serum Antibody Patterns (CVT trial)

In the CVT trial, among women who received one dose, 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 [27]. Titers remained stably elevated at seven years post-vaccination at four- to five-fold lower levels than for three doses [62] (**Figure 4**).*

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- compared to the three-dose group [27]. 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 [61, 62]).

4.3.1.5 INTERPRETATION

Dose-specific data were obtained in the context of a randomized clinical trial but are observational in nature. After extensive analyses, a single dose of 2vHPV provided strong and lasting protection against HPV 16 and 18 to the CVT and PATRICIA trial participants up to seven years post-vaccination. There may be the additional benefit of cross-protection against phylogenetically-related HPV types. Trial data have been extensively interrogated to rule out much of the potential for bias and confounding by dose group.

4.3.2 The India HPV Vaccine Trial

Portions of this section were excerpted from a review of evidence of single-dose HPV vaccine protection from the India HPV Vaccine Trial [66] and edited for this paper.

4.3.2.1 STUDY DESIGN

The International Agency for Research on Cancer (IARC), Lyon, France, initiated a multi-center cluster randomized trial in India in September 2009 to evaluate the comparative efficacy of two versus three doses of 4vHPV in preventing persistent HPV infection and cervical neoplasia [26]. The study planned to recruit 20,000 unmarried girls aged 10 to 18 years and the statistician at IARC randomly assigned them to receive either two doses on days 1 and 180 (n = 10,000) or three doses on days 1, 60, and 180 (n = 10,000) [26].

At each project site, the identified clusters were randomly assigned either to the two-dose group or the three-dose group. All eligible girls in the randomizead clusters were enumerated into the study. Vaccinations occurred from study start until April 8, 2010, when the Indian authorities suspended all further recruitment and vaccination of girls in all HPV vaccination trials in India because of events unrelated to the HPV vaccine study. The suspension meant that the study included four groups of girls who were vaccinated, including those vaccinated on days 1, 60, and 180 or later (original three-dose group); those vaccinated on days 1 and 180 or later (original two-dose group), those vaccinated on days 1 and 60 by default (two-dose default group), and those with one dose only by default (one-dose default group). At the time of suspension, 17,729 girls (89% of the target) had already been randomized to the two study arms, but many could not complete their allocated vaccine schedules.

The study participants who received more than a single dose of vaccine had the immunogenicity (antibody levels both total and neutralizing and their avidity) and safety assessed at one month after the last dose of the vaccine. Single-dose recipients had the immunogenicity and safety assessment at 12 months. All were then followed up to monitor safety and immunogenicity at different time points over a 48-month period from the beginning of vaccination. Frequency of incident infection and persistence of both the vaccine-included HPV types (HPV 16 and 18) and other HPV types was assessed. Cervical samples for HPV genotyping could be obtained only from the married participants, and this was done 18 months after marriage or six months after first child-birth, whichever was earlier.

An age and site-matched cohort of unvaccinated married women was recruited from different study sites in 2011 to serve as an unvaccinated control group of women [66]. Cervical samples were collected at recruitment and yearly thereafter for HPV genotyping.

Both the vaccinated and the unvaccinated cohorts are being followed up with yearly to collect cervical samples for HPV genotyping from the eligible participants. The study aims to collect four consecutive yearly samples from each eligible woman. The married participants are screened for cervical cancer using the Hybrid Capture II HPV detection test as they reach 25 years of age. The screen positive women are being further investigated with colposcopy and biopsy to diagnose/rule out cervical neoplasias.

4.3.2.2 LABORATORY ANALYSIS

Blood samples to determine immunogenicity were obtained by nurses during the vaccination session at a clinic or during household visits. They were collected on day 1 and at 7, 12, 18, 24, 36, 48, and 60 months from a cohort of a convenience sample of participants,

which represented all included ages (10 to 18 years of age at study entry) of the vaccinated study population. The concentration of binding antibodies against L1-glutathione s-transferase fusion proteins of vaccine types HPV 16, 18, 6, and 11 as a geometric mean of median fluorescence intensity (MFI) was assessed using Luminex-based (Austin, TX, USA) multiplex serology assay [67, 68] at the European Molecular Biology Laboratory, Heidelberg, Germany. "A seropositivity cut-off for each HPV type was estimated to determine the rate of seroconversion after vaccination. The cut-off was based on the MFI values of serum samples obtained prior to vaccination after allowing for an arbitrary 5% seropositivity in the total baseline samples "[66]. Antibody avidity, which reflects the degree of antibody affinity maturation, was measured by analyzing a subset of 18-month plasma samples in a modification of the HPV-L1 VLP genotype specific binding antibody assay.

> *A subset of plasma samples collected at 18 months were analyzed for the neutralizing antibodies specific for the neutralizing epitopes situated on the HPV L1 protein. The high-throughput pseudovirion-based neutralization assay (PBNA) was used with bovine papillomavirus (BPV) pseudovirion assays as controls. The PBNA titer was considered sero-positive if it was ≥ 50 and ≥ 2 times the BPV titer. A PBNA titre > 50 and < 2 times the BPV titre was considered indeterminate.*

HPV type-specific E7 PCR bead-based multiplex genotyping [69, 70] was performed on the cervical samples to detect 19 high-risk or probable high-risk types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73, and 82), and two low-risk HPV types (6 and 11). The method was validated at the Rajiv Gandhi Centre for Biotechnology (RGCB; Thiruvananthapuram, India) under the supervision of scientists from IARC and the cervical cell samples were tested at RGCB. Persistent infection was defined as the detection of the same HPV type in two consecutive cervical samples collected at least at an interval of 12 months.

4.3.2.3 STATISTICAL ANALYSIS

MFI values were used as a measure of antibody concentration and quantified by multiplex serology. Non-inferiority of antibody concentrations in different dose groups were quantified using log-transformed mean MFIs in linear regression models to obtain MFI ratios and their corresponding 95% CIs. Non-inferiority of a vaccination group was considered if the lower bound of the 95% CI for its MFI ratio was greater than 0·5.

> *The antibody avidity index was calculated as the following: MFI values of urea-treated samples/ the MFI values of the untreated samples x 100. To compare the index in the one- or two-dose regimens with the three-dose regimen, the log-transformed avidity index values*

were used in linear regression models to obtain avidity index ratios (along with 95% CIs). The lower bound of the 95% CI of the avidity index ratio exceeding 0.5 indicated non-inferiority of the avidity of less than three doses to that of three doses.

Type-specific seroprevalence for neutralization titers was reported as a proportion. The neutralization GMTs and their 95% CIs were compared by use of the log-transformed titer values in linear regression models. Ratios of the type-specific GMTs were measured at the last (48 month) visit for each of the two dose and one dose group compared with the three-dose group. Non-inferiority of the GMT was concluded if the 95% CI lower bound of the neutralization GMT ratio was greater than 0·5.

4.3.2.4 RESULTS

At the time of suspension of vaccination on April 8, 2010; 21,258 girls were enumerated and 17,729 had received at least one dose of vaccine. Of these, 4,348 (25%) girls received three doses on days 1, 60, and 180 or later; 4,979 (28%) received two doses on days 1 and 180 or later; 3,452 (19%) received two doses on days 1 and 60 by default; and 4,950 (28%) received single dose by default (**Table 4**). The baseline characteristics of participants in the four groups show more or less similar age distribution, with some differences in average monthly household income and education [26].

Incident and persistent HPV infections

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 (**Table 5**).

Immune responses

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.

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 single 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 [26] and continued to be inferior by month 48 (**Figure 5, Figure 6**). 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 (**Figure 5**).

The values for geometric mean avidity index for HPV types 16 and 18 for the one-dose group at 18 months was non-inferior to the value after the three-dose regimen at 18 months [28]: 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) (**Figure 7**). 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 006 (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.

4.3.3 Strengths and weaknesses of observational data from clinical trials

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 paper.

4.3.3.1 STRENGTHS OF OBSERVATIONAL DATA FROM CLINICAL TRIALS

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 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;*

- *Reasons for missed 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 analysis were performed in a blinded manner. Although this was originally a randomized trial, the original dose randomization could not be maintained. The study became an observational one following the stoppage of vaccination due to extraneous reasons. The creation of the single dose cohort was therefore unintentional and not controlled by the investigators.

4.3.3.2 WEAKNESSES OF OBSERVATIONAL DATA FROM CLINICAL TRIALS

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 enrollment, such as monthly household income, religion, and education [66]. The frequency of detection of HPV vaccine genotypes not targeted by the 4vHPV, were however, similar across the vaccinated and unvaccinated women [71]. 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.

4.3.4 Summary of observational data from clinical trials

In the CVT, the initial strong protection observed among women who received a single dose of the 2vHPV vaccine [62] indicates no evidence of diminishing at seven years of follow-up. Single-time point infection rates by types targeted by the vaccine remain remarkably low. Incident HPV infections in the PATRICIA study were also similar by vaccine dose. In the India HPV vaccine trial of 4vHPV, although antibody concentrations (measured by MFI) for both anti-HPV 16 and anti-HPV 18 were lower among girls receiving one dose compared to three doses at 48 months, the frequency of incident HPV 16, 18, 6, and 11 infections were similar, regardless of the number of vaccine doses received, and no persistent HPV 16 or 18 infections were detected in any dose group over a follow-up period of seven years.

4.4 Immunogenicity studies of partially vaccinated populations

Two observational (non-trial related) studies—one in Uganda after 2vHPV [74] and one in Fiji after 4vHPV [73]—have evaluated antibody response after one, two, and three doses of HPV vaccine. In both studies, previously vaccinated girls were recruited and enrolled in studies to determine seropositivity and antibody titers to HPV vaccine types. In the Uganda study, antibody was determined at about three years post vaccination, and in the Fiji study, at six years post vaccination.

4.4.1 The Uganda study

4.4.1.1 STUDY DESIGN

The Uganda study was a cross-sectional study among 376 adolescent girls (age 10 to 11-years) 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 [72, 74]. HPV vaccine was administered by immunization program vaccinators in a three-dose schedule (0, 1, and 6 months). Three-dose completion among girls aged 10 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 desired sample size was

reached. Participants were recruited based on data in vaccine registries, but final vaccine status was based on information in the vaccination card (provided by parent).

4.4.1.2 LABORATORY METHODS

Participants provided 10 mL of blood at enrollment. Serum was stored at −70◦C, sent to the HPV Immunology Laboratory of the NCI (Fredrick, Maryland, USA), and tested by ELISA for HPV 16 and HPV 18 antibody as described under the CVT. Seropositivity cut-offs for HPV 16 and HPV 18 were 8 EU/mL and 7 EU/mL, respectively, which mirrored the seropositivity cut-offs of the CVT.

4.4.1.3 STATISTICAL ANALYSIS

Analyses included comparison of GMTs in girls who received one or two doses compared to those who received three doses. Antibody in the one- and two-dose groups was also compared with the lowest antibody in the three-dose group, and in an exploratory analysis with GMTs in the CVT [27, 61]. 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.

4.4.1.4 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. 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 has been demonstrated.

4.4.2 The Fiji study

4.4.2.1 STUDY DESIGN

The Fiji study [73] 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. In 2015, girls were recruited into a study designed to compare antibody responses in one- or two-dose vaccine recipients with three-dose recipients. 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 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.

4.4.2.2 LABORATORY METHODS

Blood was drawn on enrollment to the study and 28 days after the challenge dose of 2vHPV. The serum samples 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 [75]. 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.

4.4.2.3 STATISTICAL ANALYSIS

The primary analysis was a comparison of the GMTs of NAb (and 95% confidence intervals) 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 ***P*** value <.05 was considered statistically significant for all analyses. A sample-size calculation determined that

for 80% power to detect a 30% difference in HPV antibodies with a 2-sided 5% significance level, the number needed in each group was 26 and 47.

4.4.2.4 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 (**Table 6**). 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:

- HPV 6 (three-dose: 2216 [95% CI, 1695–2896] vs. two-dose: 1476 [1019–2137]; P =.07);
- HPV 11 (three-dose: 4431 [3396–5783] vs. two-dose: 2951 [1984–4390]; P =.09);
- HPV 16 (three-dose: 3373 [2511–4530] vs. two-dose: 3275 [2452–4373]; P =.89); and
- HPV 18 (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 5- 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 following 2vHPV and were not significantly different from the two-dose and three-dose groups (**Figure 8**).

4.4.3 Strengths and weaknesses of immunogicity studies of partially vaccinated girls

There are several strengths of these immunogenicity studies to be noted. Both studies used the same laboratory assay to assess immune responses, which allowed for comparison to antibody levels reported from clinical trials of adult women in which efficacy had been demonstrated. Both studies had long follow-up time to accommodate an immunogenicity plateau observed 24 months after initial vaccination. Both studies reported antibody levels for single-dose recipients that were higher than those reported in women naturally infected.

These observational studies also have a number of limitations. Neither the Uganda nor the Fiji study was an RCT and, therefore, girls 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. However, data to evaluate comparability across groups were more limited from the Uganda study. While neither 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.

Sample sizes were not large enough in either study, especially among those who received only a single dose. 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.

4.4.4 Summary of immunogenicity studies of partially vaccinated girls

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 [27, 61]. Furthermore, in Uganda, even though immune responses were inferior in the single-dose group, they were still four-fold higher than natural infection.

The same ELISA and calibrated standards as used in the CVT were used in Uganda to measure immunogenicity. 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 (HPV 6, $P = .074$; HPV 11, $P = .086$; HPV 16, $P = .887$; HPV 18, $P = .885$). 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 anamnestic response after a challenge dose of 2vHPV, was evident in all one-, two- and three-dose vaccine recipients.

4.5 Non-trial observational studies, registry linkages and other studies

This section summarizes and includes excerpts from a recent systematic review of the literature [76] on evidence of the effectiveness of HPV vaccination by the number of doses, as measured in post-licensure studies.

4.5.1 Method for systematic review

4.5.1.1 STUDY SELECTION

Studies were eligible if they fulfilled the following inclusion criteria: 1) reported effectiveness of HPV vaccination (2vHPV or 4vHPV) on 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, 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 authors MD and NP on title and abstract first, and secondly on the full-text article (full authorship in Acknowledgments section).

4.5.1.2 DATA EXTRACTION

Two authors (NP and LM) independently extracted the main study characteristics and outcomes using standardized forms. MD 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.

4.5.2 Current evidence from non-trial observational studies of HPV vaccine effectiveness

The literature search identified 3,787 articles, from which 26 full articles were assessed. After reading full texts, 12 articles were excluded, leaving 14 [77-90] (**Figure 9**). These publications were published between 2013 and 2017 and included studies from Australia (three), Scotland (three), United States (two), Sweden (two), and one each from Belgium, Canada, Denmark, and Spain (**Table 7**). All evaluations were conducted within the context of a recommended three-dose schedule of either 2vHPV vaccine (three) or 4vHPV vaccine (eleven). Articles included analyses of effectiveness for prevention of HPV infection (two), anogenital warts (six), and cervical cytological or histological abnormalities (six) (**Table 8**). 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 most studies

(**Table 7**). Four studies also evaluated the impact of buffer periods and four evaluated different intervals between doses for two-dose vaccine recipients.

4.5.2.1 HPV PREVALENCE

The 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 that had achieved high coverage in the routine and catch-up target age groups. Kavanagh et al. found statistically significant effectiveness for three doses but not for two doses or one dose [77]. In the second study, Cuschieri et al. over selected women who were partially vaccinated [78]. Statistically significant effectiveness was found for three doses, two doses, and one dose. There was no formal comparison of effectiveness of three doses vs. fewer doses in either study; confidence intervals for the effectiveness estimates of three, two, and one dose(s) overlapped.

4.5.2.2 ANOGENITAL WARTS

The six evaluations of anogenital wart outcomes were retrospective cohort studies from five countries that had introduced 4vHPV vaccination [79-84]. All studies adjusted analyses for age at vaccination and some were able to adjust for educational level or markers of socioeconomic status (**Table 7**). Most two-dose vaccine recipients received doses separated by two months. Two studies also included assessment of different buffer periods [81, 84] and three included assessment of different intervals between doses in two-dose vaccine recipients [80, 82, 84].

Four of the six studies included a comparison of three, two, and one dose(s) with no dose. All found highest effectiveness with three doses, and lower but significant effectiveness with two doses. Three of the four studies found significant effectiveness with one dose [79, 80, 83].Four 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 [79, 80, 82, 84]. Two studies examined different buffer periods; a longer buffer period decreased differences in effectiveness between three and two doses in one study [81]. In the three studies that explored intervals between doses in two-dose vaccine recipients [80, 82, 84], one found no difference between three doses and two doses with an interval longer than four months [80].

4.5.2.3 CERVICAL CYTOLOGICAL HISTOLOGICAL ABNORMALITIES

Six studies evaluated vaccine effectiveness for prevention of cervical cytological or histological abnormalities, including five for 4vHPV vaccine and one for 2vHPV vaccine [85-

90]. Characteristics of women differed by number of doses in most studies, particularly in the age at first vaccine dose. (**Table 7**). Among the six studies, all found effectiveness for three doses. Four studies found some effectiveness for prevention of high-grade histological abnormalities with two doses, and two studies found effectiveness with one dose among some age groups in analyses with longer buffer periods [86, 87]. Most two-dose vaccine recipients received two doses at a one- or two-month interval. One study examined intervals between doses and found no impact on the effectiveness estimate [87].

4.5.3 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. The following include important weakness of the available post-licensure studies and caveats that should be considered when interpreting the findings:

1) 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.

2) In 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 [91]. 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.

3) 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 [10, 92, 93]. 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, 94].

   Although the number of girls vaccinated with two doses separated by at least six months was small in the studies identified in the review, four studies evaluated the interval between doses [80, 82, 84, 87]. Blomberg et al. found that, as the time between dose 1 and 2 increased from two to six months, the difference in effectiveness between two and three doses decreased; with an interval of more than four months, there was no difference [80].However, two studies did not find that varying intervals between two doses had that same effect [82, 87]. 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.

4) 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 [86, 87]. 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.

4.5.4 Summary of non-trial observational studies

In this systematic review of HPV vaccine effectiveness by number of doses, most of the 14 studies, including studies of the bivalent and quadrivalent vaccines, found the highest point estimate of effectiveness with three doses, followed by two doses, and then one dose. 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. All found statistically significant effectiveness for three doses and 11 studies found effectiveness for two doses [78-88]. In six studies (including studies for both vaccines), significant effectiveness was observed for one dose in some analyses [78-80, 83, 85, 87].

Across all endpoints (prevalence, AGW, and cervical abnormalities), variation in effectiveness by number of doses was observed. Not all studies evaluted buffer periods, but there were generally consistent findings among studies that used buffer periods. With longer buffer periods, three of four studies found higher effectiveness estimates for one and two doses and a decrease in the differences by number of doses. 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. Few studies evaluated varying time intervals between two doses. Two studies of anogenital warts found higher two-dose effectiveness with increasing interval through six or seven months [79, 84]; however, the one study of cervical abnormalities that evaluated interval between two doses did not find a difference [87].

Most post-licensure studies examining HPV vaccine effectiveness by number of doses report highest effectiveness with three doses, but some found no statistically significant difference between two and three doses. Additionally, almost half of the studies found some effectiveness for one dose. There are several biases in currently available data that impacted the estimates, with most biasing the one- or two-dose results towards lower vaccine effectiveness.

4.6 Mathematical modeling studies evaluating reduced dosage immunization schedules

4.6.1 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 [95-99]. 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 [95, 100, 101]. Standardization of model reporting to increase transparency and interpretability of model assumptions, inputs, and outputs is also critical [102].

In contrast to the large body of model-based evidence on the impact and cost-effectiveness of three-dose HPV vaccination [103-107], 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 one-dose vaccination is anticipated, corresponding with the growing empirical data summarized in **sections 4.2-4.5**.

4.6.2 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 [108-111]. 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 [108, 109]. 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 10 years, the added incidence reduction was 17% (5-23%) [108].

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 10 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 1-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) [109]. 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 10 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 10 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 [110], routine vaccination was targeted to 9-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 10 or 20 years and the third dose would extend protection by 10 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 [110].

One United States (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) [111]. 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 (10 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).

4.6.2.1 MODELS OF ONE-DOSE HPV VACCINATION

Two analyses–one in the UK and one in the US–have evaluated single-dose HPV 16 and 18 vaccination, both in the context of routine girls-only vaccination in HICs [112, 113]. An analysis forthcoming 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 [114].

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 (10 or 20 years) and cross-protection against HPV 31, 33, and 45 [112]. 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 10 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 10 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 (10 years, 15 years and lifetime) and achievable vaccination coverage (70%, 90%) [113]. 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 projections of one-dose and two-dose vaccination effects were applied to the burden of HPV and cervical cancer in the setting of Uganda [114], 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.

4.6.3 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.

4.6.4 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:

i. 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 10 years;
ii. The impact and cost-effectiveness of adding a third vaccine dose hinges on the relative duration of protection for two versus three doses and will be minimal if two-dose protection is 20-30 years, assuming no initial waning in the first 10 years for either two or three doses;
iii. If two-dose protection is only 10 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:

i. Compared to no vaccination, single-dose HPV vaccination yields substantial health benefits and is good value for money, even at lower vaccine efficacy (80%) and when duration of reduced-dose protection is only 10 years;
ii. 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.

5 Summary of all 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, at least for the 2vHPV vaccine), may still provide protection against HPV [15].

Non-randomized observational study data comparing one dose with two or more doses of the 2vHPV and the 4vHPV vaccines show consistency in terms of protection against HPV persistent infection, and this appears to be sustained into the medium term. With the exception of the CVT trial [56] and the Uganda study [72], where samples were tested in the same laboratory using the same assays, it is difficult to compare the immunological data on

a single dose for the two vaccines because different laboratories and assays were used in the CVT/Uganda, India, and Fiji studies.

Most post-licensure studies examining HPV vaccine effectiveness by number of doses report highest effectiveness with three doses, but some found no statistically significant difference between two and three doses [76]. Almost half of the studies found some effectiveness after one dose. Several biases in currently available data impact estimates, with most biasing two- and one-dose results away from showing effectiveness. Future studies of real-world HPV vaccination effectiveness, which examine persons vaccinated prior to sexual activity and use methods to reduce potential sources of bias, are warranted.

There are limited modeling analyses evaluating single-dose HPV vaccination and these studies have used data from HIC settings. However, 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 to be good value for money. This applies even of the vaccine has a lower vaccine efficacy than two or more doses, as long as one-dose protection lasts at least 10 years. 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

Data from several non-randomized studies, such as the CVT, PATRICIA, and India studies, 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. The results from these studies 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 is 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 the 2vHPV or 4vHPV vaccines. It is also important to note that there are no current data from prospective randomized controlled studies that are specifically designed to answer the question of single-dose protection or immune responses.

Other studies 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 women who received one dose of HPV vaccine, for which no breakthrough cases have been detected four years after vaccination [72, 74]. 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. The results can be compared with studies using ELISAs with data based on the EU/mL standards, although inter-laboratory differences could potentially still affect results. A unique aspect of the Fiji study [73] 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.

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. Several limitations also existed, including selection bias; 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 second limitation is information bias--for example, misclassification of vaccination status due to recall, misclassification of outcome due to diagnostic bias, interviewer bias, or tools used (**Table 9**).

In addition, modeling studies have only utilized data from high-income countries and are reliant on assumptions about the duration of one- and two-dose vaccine protection. Ultimately, modeling results will only be confirmed by long-term follow-up of post-vaccination cohorts.

7 Identification of gaps in evidence & research priorities

7.1 Gaps in efficacy and immunogenicity data and research priorities for randomized controlled trials

A number of clinical studies have examined single-dose regimens and have demonstrated results that challenge the prevailing dogma that protein-based subunit vaccines require a prime-boost 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.

There are a number of evidence gaps that are being addressed or will need to be addressed in the coming years.

It is not known if a single dose of 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, analysis of efficacy is published out to seven years and a subset of participants will be followed out to 15 years for immunogenicity outcomes.

Additional data on incident persistent infections in the India study will be obtained from at least an additional 1000 women who are initiating sexual activity over the next few years, including women in the single-dose arm. 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 generate data on the efficacy of a single dose to protect against cervical sequelae of HPV infection, by comparing rates of CIN2+ in one-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, 249 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.

The India study will increase 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, 1300 women have been recruited and an additional 3,000 women will be recruited over the next two years, with the aim of maintaining the age-matching. The analysis plan is currently being drafted.

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 trials without efficacy endpoints. A large-scale randomized controlled trial is now 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) [115] aims to find out if one dose of either the 2vHPV or 9vHPV HPV vaccines is as effective as two doses of these vaccines to 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 enrollment in 2019 with four-year follow-up data available in 2023 (**Figure 10**).

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 studies across numerous countries is challenging, but current studies (CVT, India and ESCUDDO) are already being conducted on two continents. Immunobridging studies will be important to allow conclusions to be drawn about the potential efficacy of a single dose across different populations and age groups.

Several immunogenicity studies are underway or planned that will help address these gaps. The DoRIS trial (Dose Reduction Immunobridging and Safety Study of Two HPV Vaccines in Tanzanian Girls; ClinicalTrials.gov Identifier: NCT02834637) [116] is an ongoing (enrollment start: February 2017) 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 is randomizing 900 girls to six groups and following them for 36 months. Girls will receive the 2vHPV or the 9vHPV HPV vaccine as one, two, or three doses. Girls receiving one or two doses will be compared with those receiving three doses of the same vaccine in order to ensure that the reduced-dose regimen produces an immune response that is not inferior to the standard three doses. Results from the DoRIS trial will be used to immunobridge to historical cohorts, such as the CVT and India studies, where a single dose has been shown to be protective, as well as to the ESCUDDO trial. 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 will complete the first year of follow-up by the end of 2018 and final data will be available in 2021 (**Figure 10**).

A second immunogenicity trial in younger children in The Gambia is under discussion. At the time of this writing, there were no prospective studies on single-dose HPV vaccine from Europe, Central Asia, Australia, or Southeast/East Asia.

Data on immune responses following single-dose vaccination will continue to accrue from the CVT and India studies. A delayed second-dose trial in the United States, where subjects are given a second dose at 24 months, will also provide some limited information on immune responses to a single dose up to two years post-first dose [clinicaltrials.gov registration: NCT02568566] [117].

The current prospective studies are working across a wide age range from 9 to 18 years and are covering study populations on four continents. The CVT trial participants were aged 18 to 25 years at enrollment, the India trial has enrolled participants aged 10 to18 years, and ESCUDDO and DoRIS are enrolling 12 to 16-year-olds and 9 to 14-year-olds respectively.

Durability of efficacy and immunogenicity is a further gap that will be addressed in the non-randomized and randomized prospective studies. Efficacy data on immune responses will be measured to at least four years in ESCUDDO and durability of immune responses will be measured to three years in DoRIS; they will be measured to 15 and 20 years in the non-randomized CVT and India studies, respectively.

The inability to compare immune responses to a single-dose HPV vaccine across studies 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. Antibody avidity indicates the degree of antibody affinity maturation and generally increases over time following encounter with an antigen. Antibody avidity could also be explored as a surrogate of induction of immunological memory. 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.

Current research on single-dose vaccination is ongoing, including studies on the 2vHPV, 4vHPV, and 9vHPV vaccines.

7.2 Gaps in effectiveness data and research priorities for non-trial observational 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 work will be done to update the systematic review as new studies are published, as well as to conduct a meta-analysis 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).

7.3 Gaps in data and research priorities for modeling studies

7.3.1 Factors influencing modeling results

These 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, these 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 assumed 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 [110, 111]. 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 4.3**); however, ongoing clinical trials (summarized in **Section 7.2**) 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 [111, 113].

7.3.2 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 long-term follow-up 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 (Mark Jit and Marc Brisson in The Gambia, and Jane Kim in Costa Rica). The close involvement of the modelers in the ongoing 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 also be used to explore the 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.

8 Forthcoming evidence

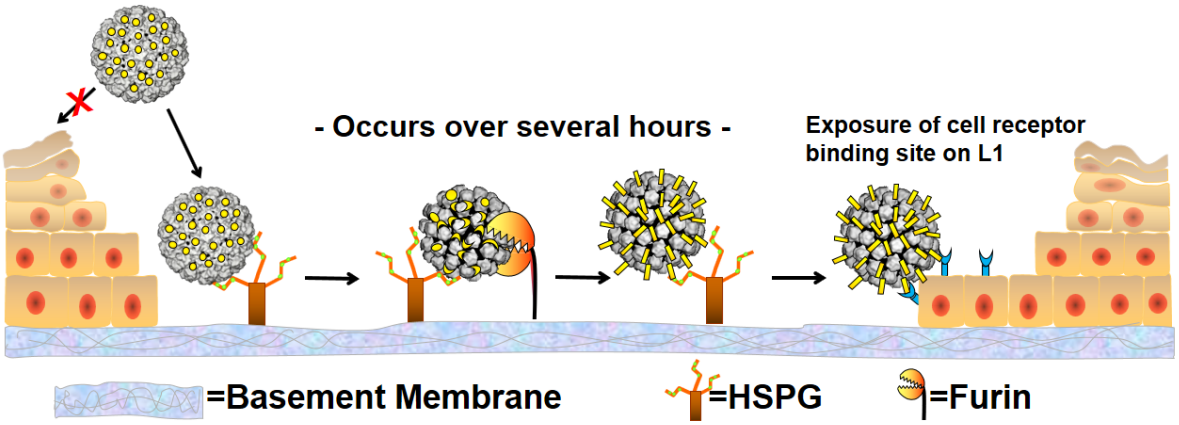
As of April 2018, there were no data on the immunogenicity, efficacy, or effectiveness of a one-dose HPV vaccination schedule compared to a two- or three-dose schedules that originated from a randomized comparison of vaccination groups. Two trials are ongoing that will either investigate efficacy and/or immune responses and safety of a single dose of HPV vaccine compared to recommended dose regimens (**Figure 10)**.

While not being conducted as part of this Consortium, the data generated from these studies will be reviewed in the future. Further prospective data generated in the IARC India and CVT trials will also be available (section 7.1). Estimates of effectiveness of HPV vaccines against HPV infection and related disease may increase with time, since vaccination, as most infections are due to prevalent infection, and will have cleared in the years following enrollment. As single-dose recipients in the current trials appear to be at greater risk of prevalent infection, the effect may be more acute in this group.

A study will be undertaken in 2018 with the aim of systematically reviewing the literature on both the immunogenicity of one dose compared to two or three doses of HPV vaccines, and the efficacy of one-dose compared to two- or three-dose HPV vaccine regimens against HPV infection, anogenital warts, and HPV-associated disease endpoints. Databases including Medline, Embase, and Global Health will be searched for publications and conference abstracts using search terms for the following: human papillomavirus (HPV), vaccine, immunogenicity, efficacy, anogenital warts (AGW), cervical intraepithelial lesions (CIN), anal intraepithelial lesions (AIN). The search will be carried out until May 31, 2018. A protocol for the review is being finalized. The systematic review of non-trial effectiveness will be updated in 2018 and a meta-analysis conducted, if possible. These reviews will add to the next edition of this white paper.

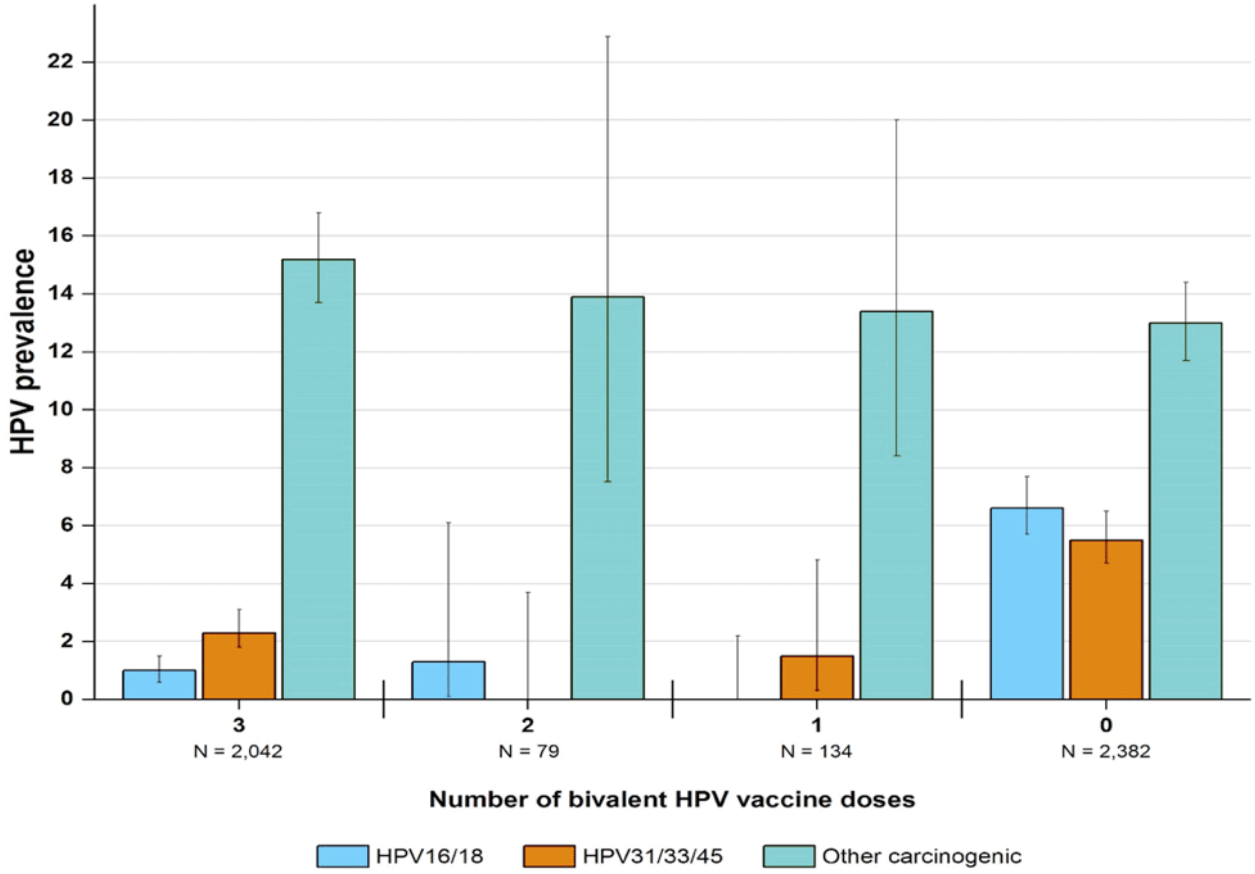
Modeling work will focus on integrating new trial, non-trial, and effectiveness data into existing models, as well as conducting model-based analyses in LMICs with different sexual behavior and epidemiological profiles.

Figure 1. In Vivo Murine Model of Vaginal HPV Infection



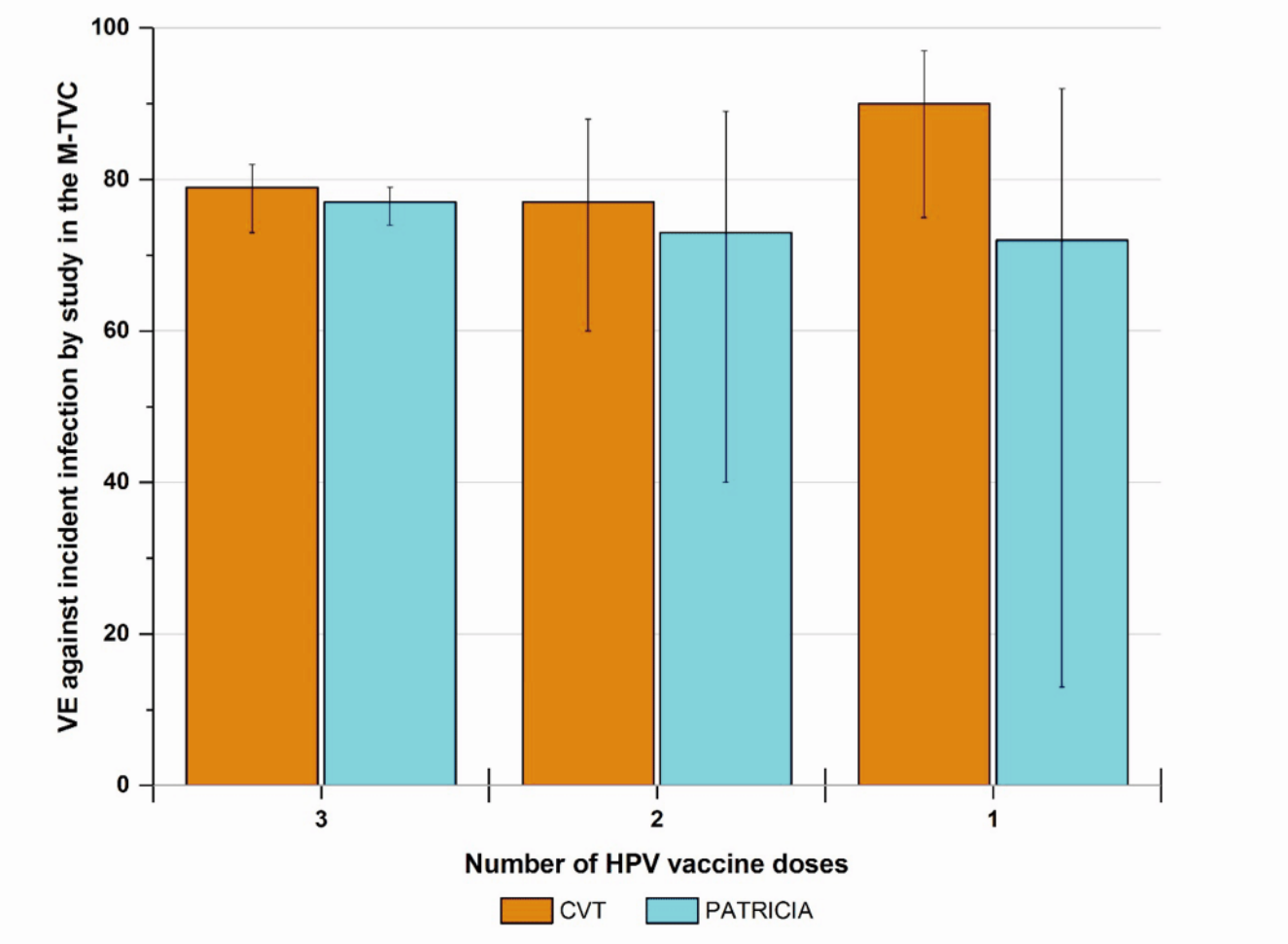
In Vivo Murine Model of Vaginal HPV Infection. A disrupted cerviovaginal epithelium is depicted. "X" indicates the inability of virions to bind the apical surface of intact epithelium. HSPG = heparan sulfate proteoglycan. The L2 minor capsid protein, cleaved by furin after a HSPG binding-induced conformational change in the capsid, is shown in yellow. Adapted from [15]

Figure 2. HPV prevalence measured seven years after initial vaccination among women who received 3, 2, 1, and 0 doses in the Costa Rica HPV Vaccine Trial.



Legend. The endpoint was HPV 16 or 18 infections detected seven years following enrollment among the HPV vaccine groups and the contemporaneous visit among the unvaccinated control group. This was assessed among the total vaccinated cohort and the unvaccinated control group.

Figure 3. Four-year efficacy against incident HPV 16 and 18 infections, by dose group, in the CVT and PATRICIA trials.



Legend. The endpoint assessed was cumulative HPV 16 or 18 infections in an analytical cohort of women who were HPV 16 and 18 DNA negative at the enrollment visit. VE Vaccine efficacy M-TVC Modified total vaccinated cohort

It should be noted that in comparisons of VE by dose group, the authors benchmarked the one dose VE against that of three doses (the historical gold standard) instead of interpreting the absolute VE, which is influenced by the cohort and endpoint chosen for the analysis.

The endpoint is not persistent infection, which is why the point estimate decreases.

Figure 4. Human Papillomavirus (HPV) type 16 (panel A) and type 18 (panel B) antibody levels up to seven years following initial HPV vaccination in the Costa Rica HPV Vaccine Trial, by number of doses received.

Panel A.

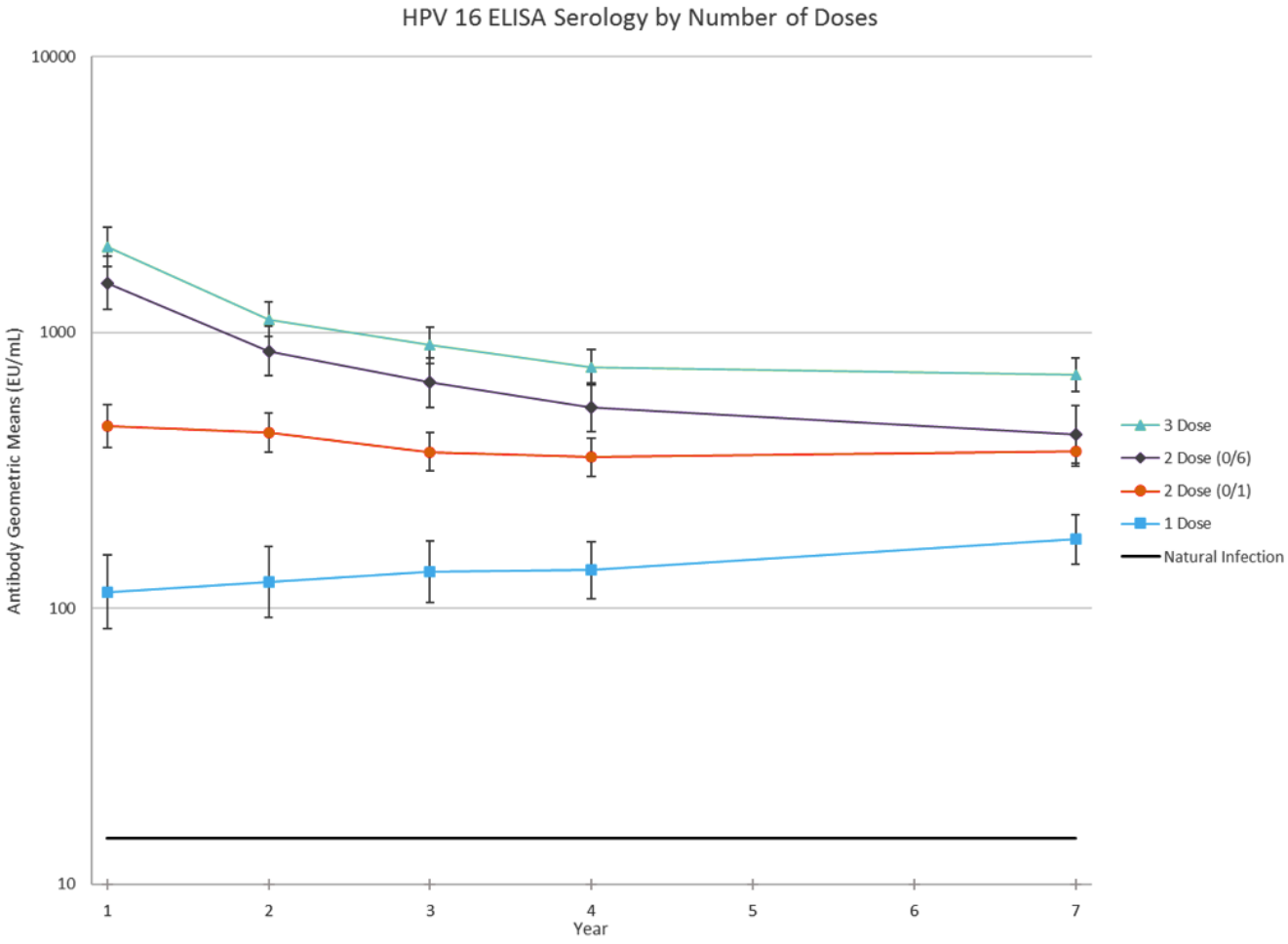


Figure 4, Panel B.

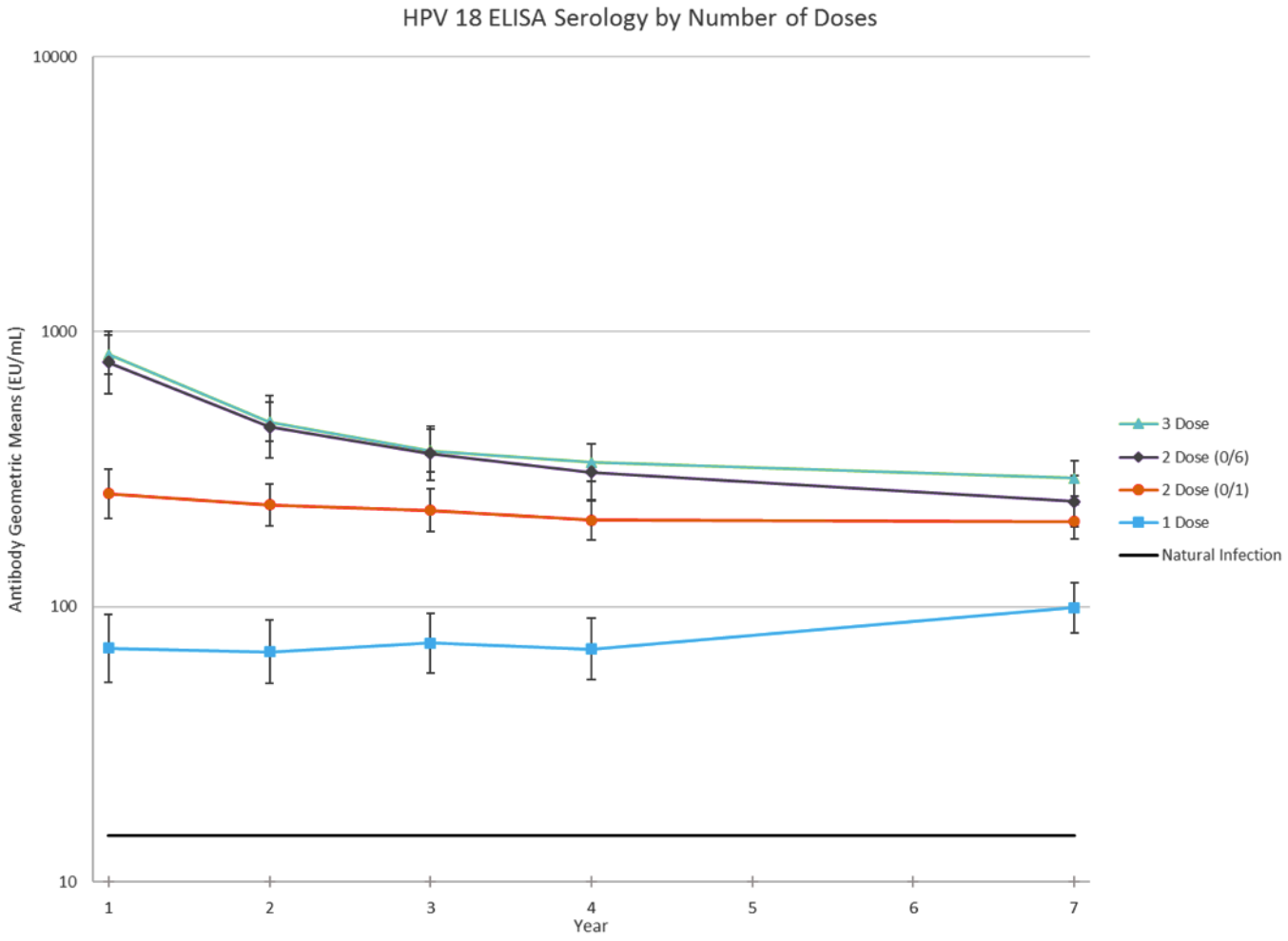
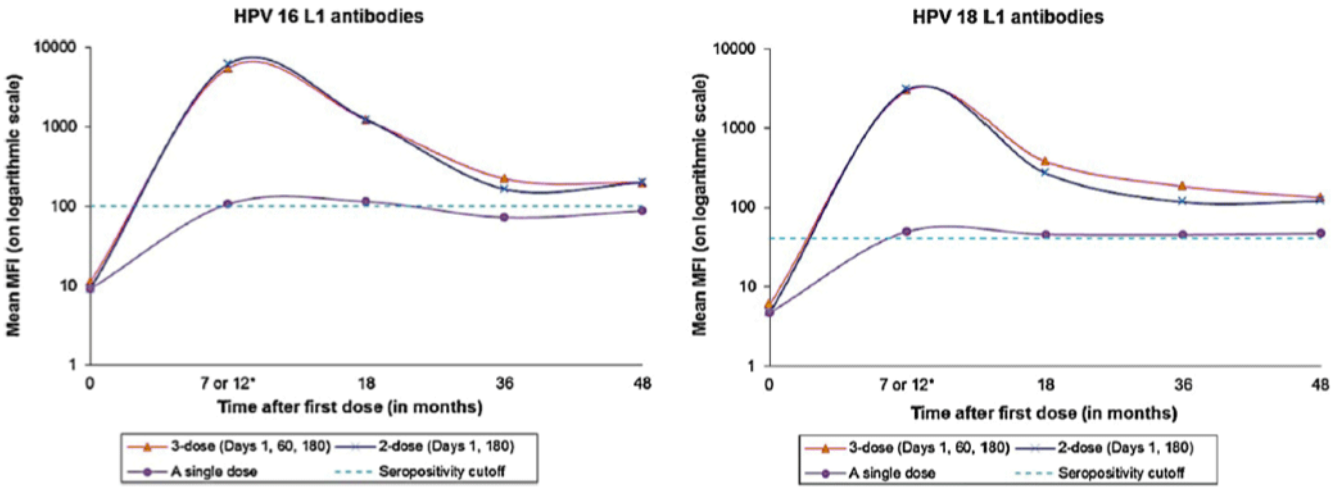


Figure 5. Mean MFI values for HPV types 16, 18, 6, and 11 L1 antibodies in the India HPV Vaccine Trial.

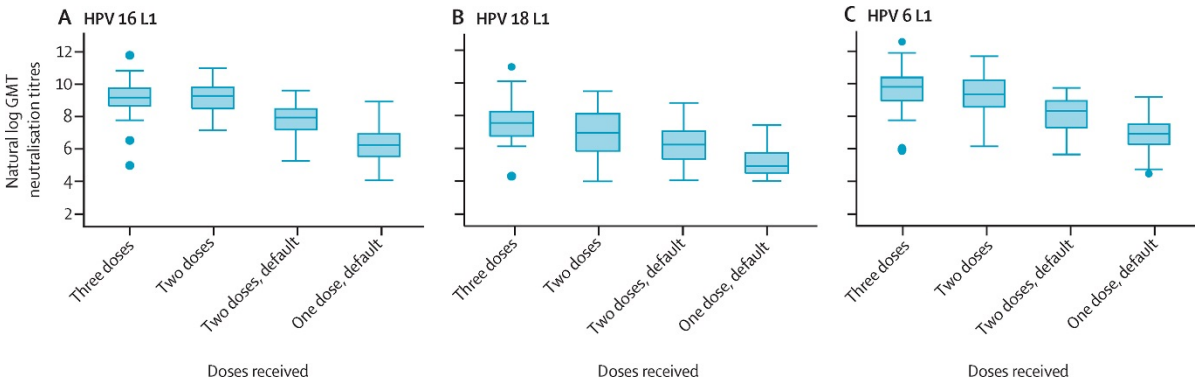


Mean median fluorescent intensity (MFI) values for HPV 16 and 18 L1 antibodies at different time points among girls who completed vaccination as per protocol (vaccination days 1, 60 and 180 (3-dose group) or days 1 and 180 (2-dose group)), and those who received only a single dose of vaccination by default.

(Key: * For the 3-dose (Day 1, 60, 180 or later), 2-dose (Day 1, 180 or later) one month after last dose MFI values are used, while for single dose group, month 12 MFI values are used.) [From Sankaranarayanan et al., Single-dose efficacy using the quadrivalent HPV vaccine: early results from an Indian study. HPV World. Newsletter on Human Papillomavirus. September 2017 – year 1 No. 17–26.]

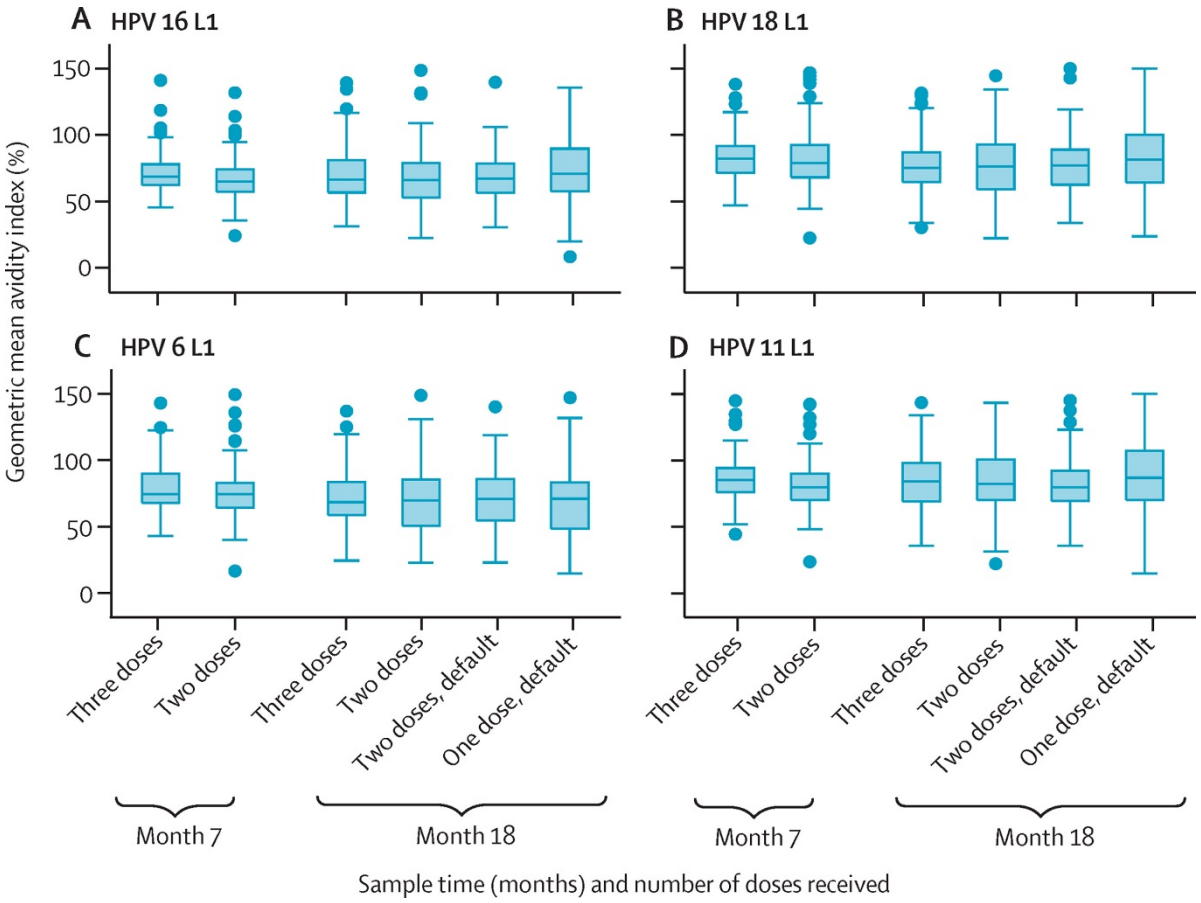
Dashed lines show the threshold (cut-off) values for seroconversion. MFI=median fluorescence intensity. *MFI values for month 7 were used for the three-dose and two-dose vaccine groups, whereas MFI values for month 12 were used for the two-dose default and one-dose default groups. Adapted from [66] .

Figure 6. Box plots of neutralization titers of HPV types 16 (A), 18 (B), and 6 (C) L1 antibodies at 18 months after the first dose in the India HPV Vaccine Trial.



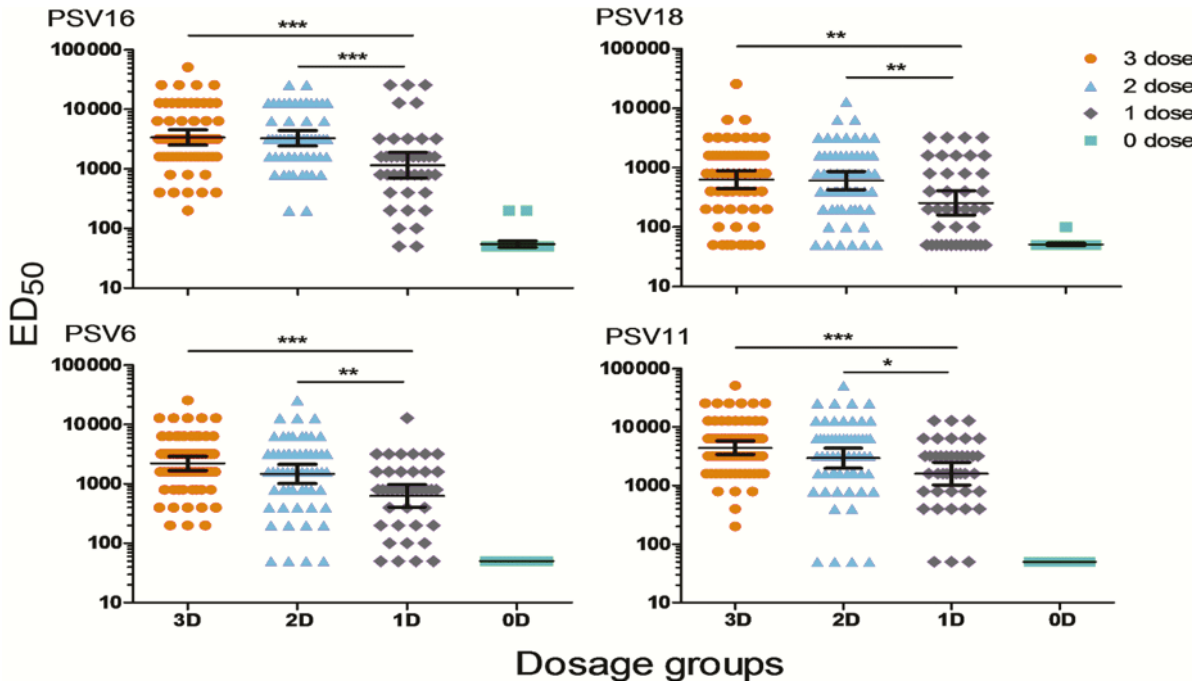
Samples without neutralizing activity were not included in the GMT analyses. GMT=geometric mean neutralization titer. Adapted from [26].

Figure 7. Box plots of the avidity index of MFI for HPV types 16 (A), 18 (B), 6 (C), and 11 (D) L1 antibodies at 7 months and 18 months after the first dose in the India HPV Vaccine Trial.



Adapted from [26].

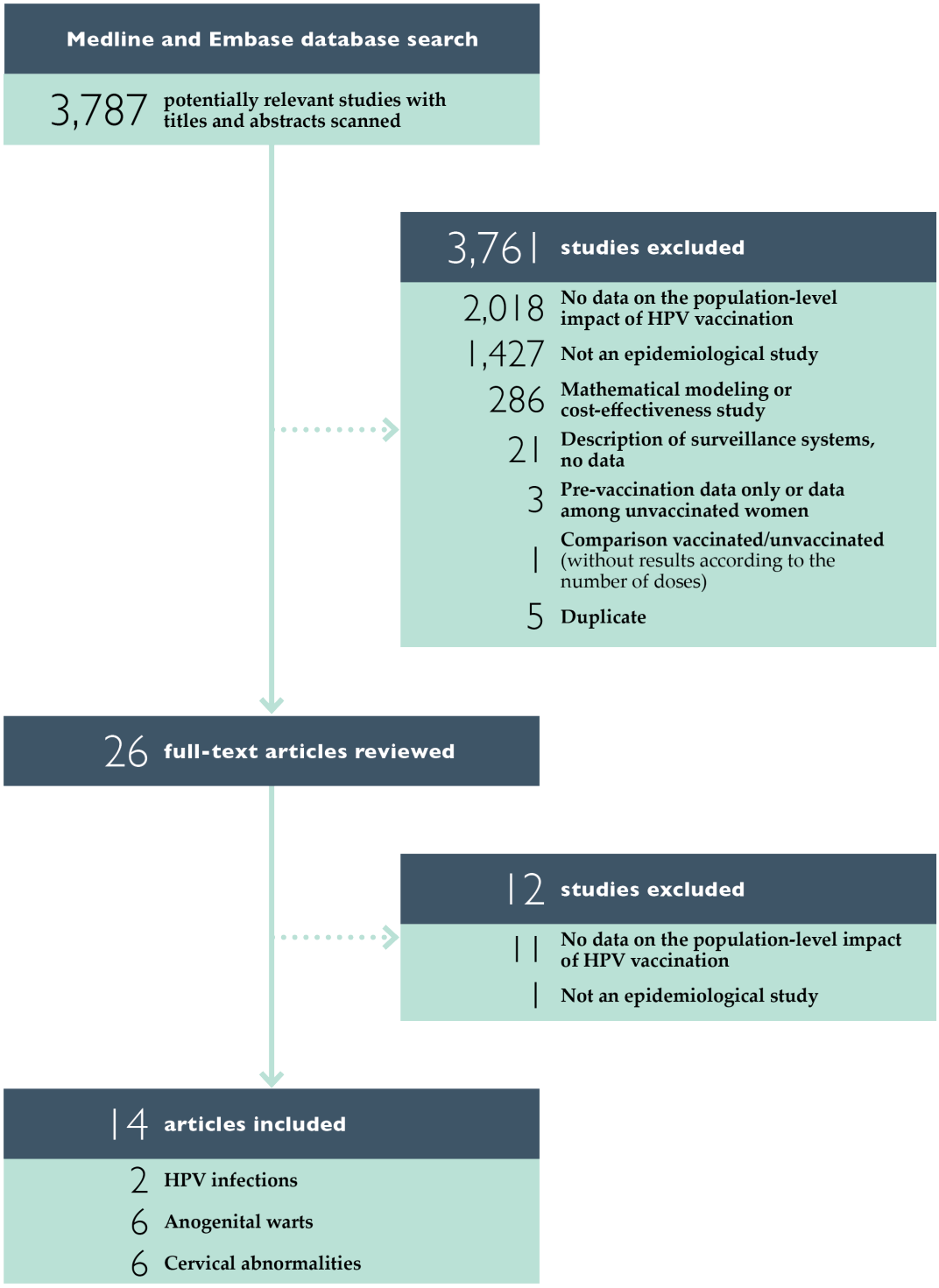
Figure 8. Neutralizing antibody (NAb) titers against HPV types 16, 18, 6, and 11, six years after last dose of quadrivalent HPV vaccine (4vHPV) in the Fiji study.



Dose-response was seen across all dosage groups for all 4 HPV types. There was no significant difference between the 2- and three-dose groups. The one-dose group had significantly lower NAb titers than the 2- and three-dose groups, but significantly higher than the zero-dose group. Error bars represent geometric mean titer ± 95% confidence interval. *P < .05; **P < .01; ***P < .001. Abbreviations: ED₅₀, effective dose 50; PSV, pseudovirion.

Adapted from [73].

Figure 9. Flow diagram of study selection process as part of a systematic review of the evidence on the effectiveness of HPV vaccination by the number of doses.



Adapted from [76].

Figure 10. Timing of data from studies evaluating a single-dose HPV vaccine.

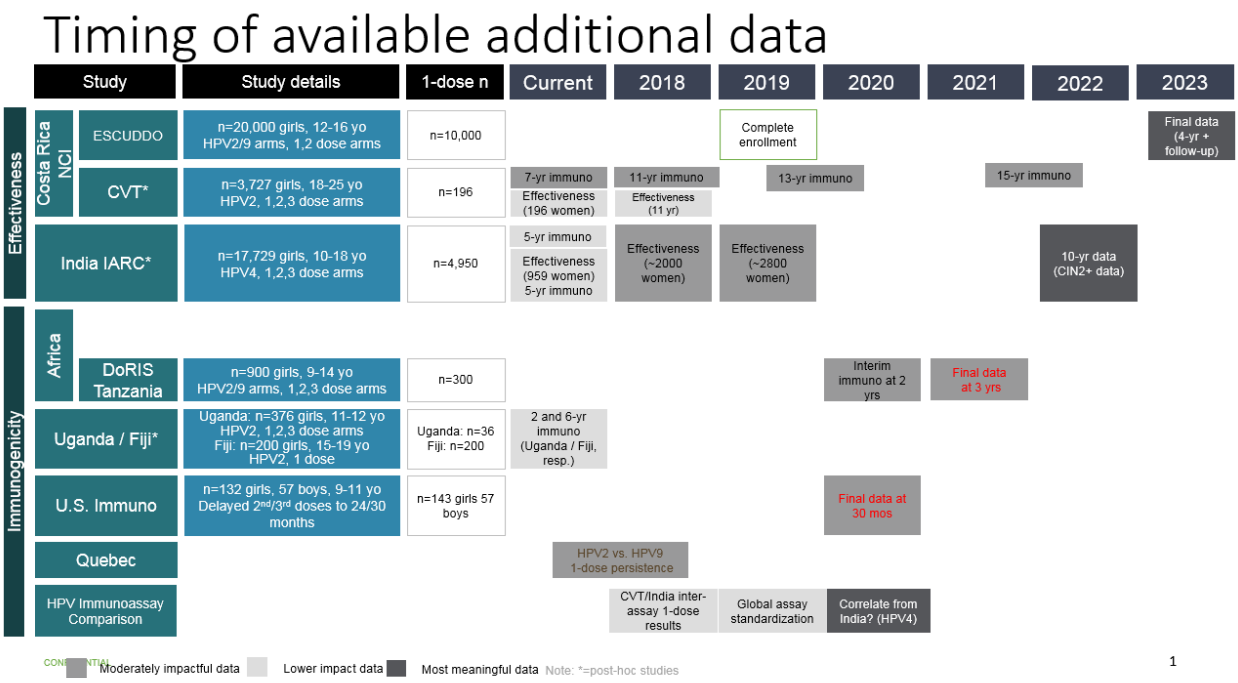


Table 2. Balance in enrollment characteristics by vaccine arm and number of vaccine doses received in the Costa Rica Vaccine Trial.

	One dose			Two doses (0/1)			Two doses (0/6)			Three doses		
Group (N)	**HPV (277)**	**Control (274)**	**p**	**HPV (382)**	**Control (364)**	**p**	**HPV (106)**	**Control (77)**	**p**	**HPV (2965)**	**Control (3021)**	**p**
Age												
≤ 20 or less	163 (58.8%)	163 (59.5%)	0.9	233 (61.0%)	224 (61.5%)	0.9	64 (60.4%)	45 (58.4%)	0.8	1679 (56.6%)	1718 (56.9%)	0.9
≥ 21 or older	114 (41.2%)	111 (40.5%)		149 (39.0%)	140 (38.5%)		42 (39.6%)	32 (41.6%)		1286 (43.4%)	1303 (43.1%)	
P										0.3	0.3	
Visits												
0	87 (31.4%)	94 (34.3%)	0.9	65 (17.0%)	60 (16.5%)	0.8	4 (3.8%)	4 (5.2%)	0.03	49 (1.7%)	38 (1.3%)	0.3
1 to 3	46 (16.6%)	42 (15.3%)		66 (17.3%)	60 (16.5%)		15 (14.2%)	18 (23.4%)		366 (12.3%)	401 (13.3%)	
4	44 (15.9%)	47 (17.2%)		79 (20.7%)	66 (18.1%)		23 (21.7%)	14 (18.2%)		640 (21.6%)	615 (20.4%)	
5	68 (24.5%)	63 (23.0%)		117 (30.6%)	126 (34.6%)		50 (47.2%)	21 (27.3%)		1354 (45.7%)	1361 (45.1%)	
6+	32 (11.6%)	28 (10.2%)		55 (14.4%)	52 (14.3%)		14 (13.2%)	20 (26.0%)		556 (18.8%)	606 (20.1%)	
P										<0.0001	<0.0001	
HPV16/18 DNA positivity												
Negative	244 (88.4%)	238 (87.8%)	0.8	339 (88.7%)	332 (91.5%)	0.2	99 (93.4%)	70 (90.9%)	0.5	2737 (92.5%)	2749 (91.1%)	0.05
Positive	32 (11.6%)	33 (12.2%)		43 (11.3%)	31 (8.5%)		7 (6.6%)	7 (9.1%)		223 (7.5%)	270 (8.9%)	
P										0.01	0.3	
HPV16/18 Seropositivity												
Negative	166 (61.5%)	154 (58.6%)	0.5	222 (59.5%)	214 (60.3%)	0.8	60 (58.3%)	43 (58.1%)	1.0	1834 (63.2%)	1829 (62.0%)	0.3
Positive	104 (38.5%)	109 (41.4%)		151 (40.5%)	141 (39.7%)		43 (41.7%)	31 (41.9%)		1066 (36.8%)	1122 (38.0%)	
P										0.4	0.6	
Chlamydia												
Negative	245 (91.4%)	242 (90.6%)	0.8	321 (85.4%)	318 (88.1%)	0.3	92 (86.8%)	64 (83.1%)	0.5	2646 (89.7%)	2657 (88.3%)	0.09
Positive	23 (8.6%)	25 (9.4%)		55 (14.6%)	43 (11.9%)		14 (13.2%)	13 (16.9%)		303 (10.3%)	351 (11.7%)	
P										0.04	0.3	
Lifetime # of sex partners												
0-1	149 (53.8%)	131 (48.3%)	0.4	165 (43.3%)	187 (51.8%)	0.03	62 (58.5%)	42 (54.5%)	0.9	1631 (55.0%)	1702 (56.5%)	0.5
2	59 (21.3%)	63 (23.2%)		91 (23.9%)	85 (23.5%)		17 (16.0%)	14 (18.2%)		611 (20.6%)	591 (19.6%)	
3+	69 (24.9%)	77 (28.4%)		125 (32.8%)	89 (24.7%)		27 (25.5%)	21 (27.3%)		721 (24.3%)	72 (23.9%)	
P										0.001	0.1	

	One dose			Two doses (0/1)			Two doses (0/6)			Three doses		
Group (N)	**HPV (277)**	**Control (274)**	**p**	**HPV (382)**	**Control (364)**	**p**	**HPV (106)**	**Control (77)**	**p**	**HPV (2965)**	**Control (3021)**	**p**
Lifetime # of pregnancies												
0	152 (54.9%)	149 (54.4%)	0.7	197 (51.6%)	202 (55.5%)	0.4	55 (51.9%)	48 (62.3%)	0.4	1541 (52.0%)	1563 (51.7%)	0.3
1	67 (24.2%)	74 (27.0%)		112 (29.3%)	106 (29.1%)		32 (30.2%)	19 (24.7%)		861 (29.0%)	922 (30.5%)	
2+	58 (20.9%)	51 (18.6%)		73 (19.1%)	56 (15.4%)		19 (17.9%)	10 (13.0%)		563 (19.0%)	536 (17.7%)	
P										0.8	0.4	
Oral contraceptive use												
Never	117 (42.2%)	99 (36.7%)	0.2	139 (36.4%)	136 (37.5%)	0.8	49 (46.2%)	32 (41.6%)	0.5	1169 (39.5%)	1202 (39.9%)	0.8
Yes	160 (57.8%)	171 (63.3%)		243 (63.6%)	227 (62.5%)		57 (53.8%)	45 (58.4%)		1789 (60.5%)	1809 (60.1%)	
P										0.2	0.6	
Smoking												
Never	246 (88.8%)	232 (85.0%)	0.4	303 (79.3%)	305 (83.8%)	0.1	93 (87.7%)	65 (84.4%)	0.1	2569 (86.7%)	2628 (87.2%)	0.5
Former	15 (5.4%)	20 (7.3%)		34 (8.9%)	19 (5.2%)		7 (6.6%)	2 (2.6%)		160 (5.4%)	170 (5.6%)	
Current	16 (5.8%)	21 (7.7%)		45 (11.8%)	40 (11.0%)		6 (5.7%)	10 (13.0%)		235 (7.9%)	217 (7.2%)	
P										0.004	0.06	

Legend. Three sets of p values are provided: one is a test for differences by arm within dose, one is a test across dose in the HPV arm, and one is a test across dose in the HAV arm. The p-values in the separate columns are for the HPV arm vs Control arm comparisons within a dose group and p-values in the three-dose column are for the across dose group comparisons within an arm.

Adapted from [54].

Table 3. Reasons for missed dosing at one month and six months, among women who received one of two doses of the vaccine, by arm, in the Costa Rica Vaccine Trial.

	Missed dose at 1 month		Missed dose at 6 months	
	HPV arm N (%)	**HAV arm N (%)**	**HPV arm N (%)**	**HAV arm N (%)**
Pregnancy	35 (9.1)	35 (10.0)	205 (31.1)	202 (31.7)
Colposcopy referral	58 (15.1)	46 (13.1)	69 (10.5)	53 (8.3)
Medical condition	61 (15.9)	67 (19.1)	110 (16.7)	116 (18.2)
Vaccine refusal	42 (11.0)	38 (10.8)	150 (22.8)	142 (22.3)
Missed Visit	122 (31.9)	98 (27.9)	54 (8.2)	76 (11.9)
Other	65 (17.0)	67 (19.1)	71 (10.8)	49 (7.7)

The three most common 'other' reasons included: woman could not get time off work, personal reasons, woman not using an acceptable form of birth control.

Adapted from [54].

Table 4. Participant baseline characteristics in the India HPV Vaccine Trial, by dose received

Characteristics	3-dose (Day 1, 60, 180+) n (%)		2-dose (Day 1, 180+) n (%)		2 doses (Day 1, 60) n (%)		A single dose n (%)		Overall vaccinated group n (%)		Unvaccinated group n (%)	
Number recruited	4348		4979		3452		4950		17,729		1574	
Site												
Ambillikai	1446	(33.3)	1532	(30.8)	111	(3.2)	211	(4.3)	3300	(18.6)	200	(12.7)
Barshi	744	(17.1)	824	(16.5)	2699	(78.2)	2825	(57.1)	7092	(40.0)	189	(12.0)
Delhi	416	(9.6)	480	(9.6)	62	(1.8)	42	(0.8)	1000	(5.6)	200	(12.7)
Ahmedabad	0	(0.0)	0	(0.0)	0	(0.0)	1011	(20.4)	1011	(5.7)	50	(3.2)
Hyderabad	0	(0.0)	0	(0.0)	315	(9.1)	479	(9.7)	794	(4.5)	300	(19.1)
Mumbai	0	(0.0)	490	(9.8)	0	(0.0)	24	(0.5)	514	(2.9)	0	(0.0)
Pune	1266	(29.1)	1182	(23.7)	246	(7.1)	324	(6.5)	3018	(17.0)	400	(25.4)
Sikkim	233	(5.4)	230	(4.6)	13	(0.4)	24	(0.5)	500	(2.8)	135	(8.6)
Mizoram	243	(5.6)	241	(4.8)	6	(0.2)	10	(0.2)	500	(2.8)	100	(6.4)
Age at recruitment												
10–14	2833	(65.2)	3184	(63.9)	2081	(60.3)	2970	(60.0)	11,068	(62.4)	0	(0.0)
15–18	1515	(34.8)	1795	(36.1)	1371	(39.7)	1980	(40.0)	6661	(37.6)	119	(7.6)
19–23	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1455	(92.4)
Type of house												
Thatched	399	(9.2)	361	(7.3)	329	(9.5)	668	(13.5)	1757	(9.9)	359	(22.8)
Tiled	2844	(65.4)	3299	(66.3)	2657	(77.0)	3411	(68.9)	12,211	(68.9)	786	(49.9)
Concrete	1018	(23.4)	1129	(22.7)	453	(13.1)	839	(16.9)	3439	(19.4)	428	(27.2)
Unknown	87	(2.0)	190	(3.8)	13	(0.4)	32	(0.6)	322	(1.8)	1	(0.1)
Average monthly household income (in Rupees)												
<2000	1451	(33.4)	1454	(29.2)	588	(17.0)	952	(19.2)	4445	(25.1)	100	(6.4)
2000–4999	1808	(41.6)	2103	(42.2)	2291	(66.4)	3184	(64.3)	9386	(52.9)	696	(44.2)
5000–9999	776	(17.8)	1009	(20.3)	421	(12.2)	673	(13.6)	2879	(16.2)	447	(28.4)
10000+	312	(7.2)	412	(8.3)	149	(4.3)	127	(2.6)	1000	(5.6)	330	(21.0)
Unknown	1	(0.0)	1	(0.0)	3	(0.1)	14	(0.3)	19	(0.1)	1	(0.1)
Religion												
Hindu	3925	(90.3)	4344	(87.2)	2961	(85.8)	4769	(96.3)	15,999	(90.2)	1393	(88.5)
Muslim	70	(1.6)	161	(3.2)	462	(13.4)	119	(2.4)	812	(4.6)	38	(2.4)
Christian	308	(7.1)	392	(7.9)	28	(0.8)	56	(1.1)	784	(4.4)	121	(7.7)
Other	45	(1.0)	82	(1.6)	1	(0.0)	6	(0.1)	134	(0.8)	22	(1.4)
Participant's education												
Nil	46	(1.1)	35	(0.7)	26	(0.8)	47	(0.9)	154	(0.9)	106	(6.7)
Primary	364	(8.4)	443	(8.9)	182	(5.3)	629	(12.7)	1618	(9.1)	176	(11.2)
Middle	2018	(46.4)	2383	(47.9)	1174	(34.0)	1773	(35.8)	7348	(41.4)	395	(25.1)
High	1464	(33.7)	1556	(31.3)	1442	(41.8)	1746	(35.3)	6208	(35.0)	634	(40.3)
College	456	(10.5)	561	(11.3)	626	(18.1)	755	(15.3)	2398	(13.5)	263	(16.7)
Unknown	0	(0.0)	1	(0.0)	2	(0.1)	0	(0.0)	3	(0.0)	0	(0.0)

*All characteristics had non-significant Pearson chi-square p-value from hierarchical log-linear modelling using iterative proportional fitting (IPF) with conditional dependency of characteristics on randomization cluster.

Adapted from [66]

Table 5. Persistent HPV infection in women vaccinated with 4vHPV vaccine over a 7-year period (2009-2017) and in the unvaccinated women.

Group	Persistent HPV 16/18 infection	Persistent HPV 31/33/45 infection	Persistent non-vaccine targeted HPV infection excluding 31, 33, and 45
Three doses (Days 1, 60 and 180+)			
N positive/Total	1/604	1/604	16/604
%	0.2	0.2	2.6
95% CI	(0.0–0.9)	(0.0–0.9)	(1.5–4.3)
Two doses (Days 1 and 180+)			
N positive/Total	0/608	1/608	6/608
%		0.2	1.0
95% CI		(0.0–0.9)	(0.4–2.1)
Two doses (Days 1 and 60)			
N positive/Total	3/818	2/818	9/818
%	0.4	0.2	1.1
95% CI	(0.1–1.1)	(0.0–0.9)	(0.5–2.1)
One dose (Day 1)			
N positive/Total	0/959	7/959	16/959
%		0.7	1.7
95% CI		(0.3–1.5)	(1.1–2.7)
All vaccinated			
N positive/Total	4/2989	11/2989	47/2989
%	0.1	0.4	1.6
95% CI	(0.0–0.3)	(0.2–0.7)	(1.2–2.1)
Unvaccinated			
N positive/Total	14/1141	6/1141	27/1141
%	1.2	0.5	2.3
95% CI	(0.7–2.1)	(0.2–1.1)	(1.5–3.3)

* Persistent infections defined as incident infections that persisted for 12+ months without an HPV negative test (for HPV type in question) between positive tests.

Adapted from [66]

Table 6. Baseline characteristics of participants enrolled in the Fiji study.

Characteristic	Dosage Group				P Value
	3 Doses (n = 66)	two doses (n = 60)	one dose (n = 40)	0 Doses (n = 32)	
Age at recruitment, y, median (IQR)	17.0 (16.0–18.0)	17.0 (16.0–18.0)	17.0 (15.8–17.3)	18.0 (16.8–19.0)	.045
Age at first dose of 4vHPV, median (IQR)	11.0 (10.0–12.0)	10.0 (10.0–11.0)	10.5 (9.0–11.0)	NA	0.14
Time in months between:					
[a] Doses 1 and 2, median (IQR)	1.0 (1.0–5.0)	6.0 (1.0–11.0)	NA	NA	<.0001
[b] Doses 1 and 3, median (IQR)	6.0 (6.0–8.0)	NA	NA	NA	NA
Time, y, since last dose of 4vHPV, median (IQR)	5.8 (5.7–5.8)	5.8 (5.4–6.3)	6.3 (6.3–6.3)	NA	<.0001
Height, cm, median (IQR)	162.0 (156.0–167.3)	161.0 (154.5–166.8)	160.0 (154.3–165.8)	151.0 (130.0–169.3)	.087
BMI, kg/m2, median (IQR)	24.6 (20.7–27.7)	24.6 (21.3–26.9)	23.9 (20.0–25.9)	23.6 (21.7–26.5)	.880
Ethnicity					.611
[l] Taukei (Indigenous Fijian)	36 (55)	32 (53)	17 (42.5)	15 (47)	
Fijian of Indian descent	30 (45)	28 (47)	23 (57.5)	17 (53)	
Participant education at time of enrollment					.0009
No schooling	7 (11)	9 (15)	2 (5)	4 (12.5)	
Secondary school	47 (71)	48 (80)	33 (82.5)	15 (47)	
University	12 (18)	3 (5)	5 (12.5)	13 (40.5)	
Partner status at enrollment					
Boyfriend/married	8 (12)	5 (8.3)	7 (17.5)	1 (3)	.223
No. of children	0	0	0	1	.157
Consumption of alcohol					.062
Never consumed	44 (67)	41 (68)	26 (65)	20 (62.5)	
Currently <1 alcoholic drink per week	18 (27)	18 (30)	8 (20)	12 (37.5)	
Currently ≥1 alcoholic drink per week	4 (6)	1 (2)	6 (15)	0 (0)	
Consumption of kava[c]					.045
Never consumed kava	38 (57.5)	27 (45)	27 (67.5)	17 (53)	
<1 kava drink per week	21 (32)	26 (43)	7 (17.5)	15 (47)	
≥1 kava drink per week	7 (10.5)	7 (12)	6 (15)	0 (0)	
Cigarette smoking					
Smoked ≥100 cigarettes in a lifetime	2 (3)	2 (3)	2 (5)	2 (6)	.862

Characteristic	Dosage Group				P Value
	3 Doses (n = 66)	two doses (n = 60)	one dose (n = 40)	0 Doses (n = 32)	
Parental education					
Mother					.786
Primary school or lower	9 (14)	8 (13)	8 (20)	5 (16)	
Secondary school or higher	56 (85)	50 (83)	31 (77.5)	24 (75)	
Missing data	1	2	1	3	
Father					.609
Primary school or lower	9 (14)	11 (18)	8 (20)	4 (13)	
Secondary school or higher	52 (79)	46 (77)	25 (62.5)	26 (81)	
Missing data	5	3	7	2	
Parental employment					
Mother					.607
Not employed	46 (70)	37 (62)	29 (72.5)	18 (56)	
Employed	20 (30)	23 (38)	11 (27.5)	11 (34)	
Missing data	0	0	0	3	
Father					.131
Not employed	25 (38)	15 (25)	8 (20)	5 (16)	
Self-employed (own business)	40 (61)	42 (70)	25 (62.5)	25 (78)	
Missing data	1	3	7	2	
Socioeconomic status[d]					.881
Poorer	33 (50)	32 (53)	18 (45)	16 (50)	
Richer	33 (50)	28 (47)	22 (55)	16 (50)	
Family and household occupants					
No. of people living at home, median (IQR)	5.0 (4.0–7.0)	5.0 (4.0–6.0)	5.0 (4.0–6.0)	5.0 (4.0–6.8)	.968
No. of cigarette smokers	22 (33)	22 (37)	7 (18)	6 (19)	.088
No. of alcohol drinkers	22 (33)	22 (37)	12 (30)	12 (38)	.885
No. of kava drinkers	30 (45)	36 (60)	15 (38)	17 (53)	.136
Seropositivity to HPV					
HPV6	66 (100)	56 (93)	36 (90)	0 (0)	
HPV11	66 (100)	56 (93)	37 (93)	0 (0)	
HPV-16	66 (100)	60 (100)	38 (95)	2 (6)	
HPV-18	58 (88)	54 (90)	27 (68)	1 (3)	

Data are presented as No. (%) unless otherwise indicated. Abbreviations: 4vHPV, quadrivalent human papillomavirus vaccine; BMI, body mass index; HPV, human papillomavirus; y, years; IQR, interquartile range; NA, not applicable.

[a] Data missing for 1.

[b] Data missing for 3.

[c] Kava is the root of a plant that is made into a sedative drink that is commonly consumed in Fiji.

[d] Socio-economic status was derived using principal component analysis of household assets (derived from local inquiry) as described in [17]. Based on the weighted scores from the 3 most correlated assets, a single numeric score was derived; the score was divided into upper and lower median scores, which represent the richer and poorer groups, respectively. Adapted from [73].

Table 7. Characteristics of studies that evaluated HPV vaccine effectiveness by number of doses.

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									
Bivalent vaccine									
Kavanagh 2014[9]	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[10]	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
Anogenital Warts									
Quadrivalent vaccine									
Herweijer 2014[11]	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[12]	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[13]	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[14]	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[15]	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[16]	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
Cervical Abnormalities									
Quadrivalent vaccine									
Gertig 201317	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[18]	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[19]	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[20]	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[21]	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
Bivalent vaccine												
Pollock 2014[22]	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

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; 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.

Adapted from [76].

Table 8. Studies that evaluated HPV vaccine effectiveness by number of doses: analyses and main findings.

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						
Bivalent vaccine						
Kavanagh 20149	15-17	20-21	0	Yes/No/No	No	• Statistically 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 201610	15-17	20-21	0	No/No/No	No	• Statistically 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
Anogenital Warts						
Quadrivalent vaccine						
Herweijer 2014[11]	10-19	10-24	3	Yes/Yes/No	Yes	• Statistically 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[12]	12-27	12-27	1	Yes/No/Yes	Yes	• Statistically significant effectiveness for 1 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[13]	10-23 16-23	1	No/No/No	No	• Statistically significant effectiveness for 3 and two doses, but not 1 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[14]	9-25 9-25	0	No/Yes/Yes	Yes	• Statistically 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 doses, 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[15]	14 14-19	0	No/No/No	No	• Statistically 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[16]	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
Cervical Abnormalities[c]					
Quadrivalent vaccine					
Gertig 201317	12-19 12-21	0	No/No/No	No	Outcome summarized: CIN3/AIS • Statistically significant effectiveness for 3, but not 2 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 201418	12-26 11-31	0	Yes/Yes/No	No	Outcome summarized: High grade histological lesions • Statistically significant effectiveness for 3 and two doses, but not 1 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

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
Brotherton 2015[19]	12-26 12-30	0	Yes/Yes/Yes	No	Outcome summarized: CIN3/AIS • Statistically 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[20]	11-20 11-27	1	Yes/No/No	No	Outcome summarized: Any abnormal cytology • Statistically 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[21]	10-15 18-21	0	No/No/No	No	Outcome summarized: High grade cytology • Statistically 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
Bivalent vaccine					
Pollock 2014[22]	15-17 20-21	0	No/No/No	No	Outcome summarized: CIN3 • Statistically 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

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

[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.

Adapted from [76].

Table 9. Threats to validity of single-dose HPV protection, and evaluations of bias and confounding within these rubrics.

Threat to validity	Evaluation of bias and confounding
Are 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 arm, 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 Chlamydiatrachomatis.
Did single-dose women receive less than a complete schedule for reasons related to HPV vaccination?	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.
Are women who received a single dose of the HPV vaccine immunologically different from women who received multiple doses of the HPV vaccine?	Compared to the two- and three-dose groups, women in the one-dose HPV group had similar HPV antibody titers 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 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 arm 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.

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Single-Dose HPV Vaccine
EVALUATION CONSORTIUM

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

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