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Alternative dosage schedules with HPV VLP vaccines

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Summary

Human papillomavirus (HPV) vaccines can prevent multiple cancers in women and men. Difficulties in the cost and completion of the three-dose vaccine series have led to considerations of alternative dose schedules. In clinical trials, three doses given within a 12-month period versus the standard six-month period yielded comparable results, and immunogenicity appears comparable with two doses in adolescent females compared to the three-dose series in adult females. While the data are generally supportive of moving to a two-dose vaccine schedule among young female adolescents, the adoption of a two-dose vaccine schedule still poses a potential risk to the strength and longevity of the immune response. Public health authorities implementing a two-dose vaccine schedule should devise risk management strategies to minimize the potential impact on cancer prevention.

Keywords

HPV vaccination; dosing; virus-like particle; dose schedules; immunogenicity

Introduction

Human papillomaviruses (HPV) cause multiple cancers in women (cervical, vaginal, vulvar, anal, and oropharyngeal) and men (oropharyngeal, anal, and penile), as well as benign conditions such as genital warts [1-3]. Prophylactic vaccination against these viral infections should prevent the vast majority of HPV-associated cancers and related benign conditions, and thus have a major public health impact [4-6].

HPV vaccines are subunit vaccines consisting of virus-like particles (VLPs) made of only one protein: the major HPV coat or capsid protein, L1. HPV VLPs are made using recombinant technology in which the L1 gene is expressed in recombinant yeast or baculovirus vectors. Expressed L1 proteins spontaneously assemble into VLPs that are

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morphologically and immunologically similar to the native virus but lack DNA and are therefore non-infectious.

Two commercial HPV VLP prophylactic vaccines are licensed: Cervarix®, a bivalent HPV16/18 product (bHPV) from GlaxoSmithKline Biologicals (Rixensart, Belgium), and Gardasil® (also known as Silgard), a quadrivalent HPV6/11/16/18 product (qHPV) from Merck (Whitehouse Station, New Jersey, USA), each designed to be delivered in a three-dose series over a six-month period.

There is a compelling public health case for considering alternatives to the standard three-dose regimen delivered on day 1, month 1 or 2, and month 6. The vast burden of HPV-associated malignant and benign disease is in developing countries without effective screening programs and with poor access to medical services [7]. There are significant logistical challenges in administering three doses over six months to young adolescents and young adults, as most countries have no infrastructure for adolescent vaccination. High rates of completion of a 3-dose regimen have been achieved in certain countries such as the United Kingdom and Australia, primarily by delivering the vaccine in school programs [8]. However, in many countries, including low- and high-resource countries, immunization programs struggle to deliver the full three-dose regimen, leading to wide variability in vaccination coverage rates [9]. Many factors contribute to poor vaccine completion rates, including logistical factors such as difficulties in attending three clinical visits in a 6-month period, lack of provider reminder systems, and misunderstanding by families regarding the need for three doses [10]. If new infections and subsequent cases of disease are to be effectively prevented, high vaccination coverage with a target of at least 70% of the population vaccinated with the necessary number of doses is needed [11].

HPV vaccines, even at discounted cost, are beyond the health budget for a large number of low-resource countries. The funds available via agencies such as the GAVI Alliance are finite and, in the current economic climate, uncertain. Middle-resource countries have limited or no access to external funding for vaccines, which can result in a lag in vaccine dissemination. A case could be made that implementing a two-dose HPV vaccination regimen in low-resource countries would be programmatically easier and achieve higher coverage. The reduction in costs achieved by implementing a two-dose schedule in terms of administration and vaccine price is an attractive option to public health authorities. However, the key question concerns the robustness of the evidence base for a change from the three-dose schedule.

To increase vaccine dissemination while potentially reducing costs and maintaining population protection, two potential modifications to the HPV vaccine dosing schedule have generated interest: 1) altering (extending) the schedule for delivering three doses of HPV vaccine, and 2) delivery of less than three doses. Prior to presenting clinical evidence, it is informative to review the basics of vaccine immunology, the rationale for the number of doses currently provided, and the results of the hepatitis B vaccine clinical trials, another subunit vaccine on which the HPV vaccine clinical program was based and which also evaluated a two-versus three-dose schedule.

Immunization schedules and the generation of immune memory

Successful immunization depends upon immune memory and vaccine immunization schedules (i.e. the number of doses and the time elapsed between doses) that are designed to generate optimal immune memory. For most, but not all, of the currently licensed antiviral vaccines, the main effectors of the vaccine-induced response are antibodies produced after the differentiation of antigen-primed B lymphocytes into antibody-secreting cells or plasma cells, a process that depends upon cognate help from CD4+ T lymphocytes [12]. Memory for antibody has two components [13]:

1. serological memory: the long-term persistence of antibody generated by long-lived antibody-secreting plasma cells residing in niches in the bone marrow and secondary lymphoid organs; and
2. recall memory (memory B cells): antigen-specific B lymphocytes persisting in a resting or quiescent state in lymphoid organs and circulating throughout the body that can be reactivated rapidly upon encountering antigen to generate large numbers of antibody-secreting cells.

Memory is evoked as a consequence of initial or primary immunization, which elicits a primary immune response. This “priming” enables the immunized individual to mount a more potent and rapid response to subsequent challenges with the same antigen. The response to each subsequent immunization (secondary, tertiary, etc.) thus increases in intensity.

About one month after primary immunization, both antibody concentration and memory B cell populations peak. Upon re-exposure to the same antigen, this pool of memory B cells is reactivated, and the secondary immune response is evoked. This response differs from the primary immune response in several ways [14]. In a secondary response, antibody, predominantly of the IgG class, appears quickly (within 2-5 days), and peak antibody concentrations are usually orders of magnitude higher than in a primary response. Furthermore, the binding affinity of antibody for antigen is significantly increased. The increase in affinity (affinity maturation) is a consequence of somatic hypermutation of B cell receptor (BCR) gene sequences as the mature B cells proliferate. This produces a heterogeneous population of B cells with a range of BCR affinities. These B cells compete for binding to antigen displayed by follicular dendritic cells present in the germinal center of the lymph node. Low-affinity BCRs bind weakly and undergo death by apoptosis. Cells with high-affinity BCRs bind strongly, receive a survival signal, proliferate, and differentiate into antigen-secreting cells, some of which continue as long-lived plasma and memory B cells. Affinity maturation increases with each immunization and, importantly, continues for several months. Consequently, maximum antibody affinity and avidity (the sum of epitope-specific affinities) is reached only when sufficient time has elapsed after priming. This is the basis for the classic prime boost schedule of 0, 1/2, and 6 months used for most protein vaccines, allowing at least 4-6 months between prime and boost. Binding strength or affinity is important, as the higher the affinity of antibody for antigen, the less antibody is needed to eliminate the antigen. Antibodies with high affinity bind at lower antigen concentrations and are therefore more effective.

The magnitude of the vaccine immune response depends upon several factors, including the nature of the antigen (i.e. live, killed, or protein), the route of immunization, and the dose [12]. Most vaccines are delivered by injection into tissue – subcutaneous, intra-dermal, or intramuscular – and the optimal route and dose are usually determined empirically. Live viral vaccines activate robust innate immune responses, undergo limited replication with persistence of antigen, and therefore elicit potent antibody- and cell-mediated immune responses and long term memory for these responses. Unlike live viral vaccines, which elicit innate immune responses, protein vaccines require adjuvants in their formulation for immunogenicity. Adjuvants are substances that enhance immunogenicity by targeting innate immunity thus focusing and biasing the adaptive immune response.

Hepatitis B vaccine – model for the development of HPV vaccines

The hepatitis B vaccine dosing schedule was developed on the basis of the above immunological principles. With this successful program as a precedent, the HPV vaccine clinical development program proceeded with a three-dose vaccine regimen within a six-month time frame [15,16]. Similar to the HPV vaccine, even after public health recommendations were issued for routine vaccination, completion of the three-dose hepatitis B vaccine series was lacking in adolescents. Therefore, there was interest in altering the dosing schedule and/or reducing the number of doses delivered while maintaining robust protection against infection and disease. Various vaccine dosing schedules were explored, including altering the three-dose time schedule, accelerating the schedule for high-risk populations, and reducing the number of doses from three to two.

Fewer than three hepatitis B vaccine doses—Immunogenicity and non-inferiority of the two-dose *adult* formulation (higher antigen dose) compared to the three-dose *pediatric* formulation of the hepatitis B vaccine in adolescents aged 11-15 years [17-20] was assessed in several trials. Non-inferiority of the two-dose schedule compared to the three-dose schedule has consistently been observed in adolescent populations. In addition, when antibody persistence was evaluated five years post-vaccination, the two-dose schedule met the protective antibody cutoff level, with 100% of participants responding to a challenge vaccine dose, indicating immune memory [17].

Since these early studies, hepatitis B vaccine alternative dose schedules using a two-dose regimen have continued to be studied in adolescent populations. A study of alternative dose intervals in adolescents demonstrated that anti-hepatitis B geometric mean titers (GMTs) were significantly higher with a 6-11 month interval between the first and second doses, compared to an interval of 1-2 months [21]. Three additional studies examined administration of hepatitis B vaccine to adolescents following a two-dose schedule of varying intervals (0 and 4 months, 0 and 6 months, or 0 and 12 months) assessing seroprotection one month after the second dose [19,20,22]. Seroprotection rates were similar across dosing schedule groups (93.4%-97.9%), with anti-hepatitis B GMTs ranging from 1386-4155 IU/L. Based on these data, the US Centers for Disease Control and Prevention (CDC) currently recommends catch-up vaccination for adolescent children aged 11-15 years old to receive a two-dose series (at 0 and 6 months) of the adult formulation of the hepatitis

B vaccine. However, most hepatitis B vaccinations are delivered worldwide as a three-dose schedule, beginning at birth [23].

Long-term duration of efficacy for hepatitis B—Antibody concentrations over time are not a clear marker of protection, in contrast to immune memory [24]. Long-term follow-up of children from Alaska and Taiwan enrolled in hepatitis B vaccine clinical trials demonstrate that 50% of adolescents studied have no measureable antibodies 15 years post-vaccination, yet there was no breakthrough infection or disease [25,26].

In most countries where hepatitis B infection and related disease is endemic (e.g., Asia and South Africa), hepatitis B is acquired at young ages through perinatal or early childhood transmission [27]. Therefore, the duration of protection following hepatitis B vaccination is essential through young childhood when exposure and acquisition are greatest. In contrast, regardless of world region, HPV prevalence is highest in young women but remains constant across the lifespan of males (beyond age 70) [28]. As such, duration of protection needs to be long-lived, with decades of protection for females and perhaps even longer for males. Therefore, the discussion regarding whether potential alternative dosing schedules, or two- vs. three doses of HPV vaccine, are highly effective should consider the duration of efficacy and factors that influence long-term duration.

HPV vaccine immunogenicity: licensed three-dose regimen

At present, the assumption is that the major basis for the protection afforded by VLP HPV vaccines is neutralizing antibody, although other mechanisms cannot be ruled out. This assumption is supported by animal models that demonstrate protection against viral challenge in animals immunized by passive transfer of antibody from VLP-immunized individuals [29-31]. The licensed administration schedules for the two vaccines include three doses delivered by intramuscular injection at months 0, 2, and 6 for the qHPV vaccine and at months 0, 1, and 6 for the bHPV vaccine. In the pivotal randomized control trials FUTURE 1 and 2 for the qHPV vaccine[5] and PATRICIA for the bHPV vaccine [6], virtually all subjects (women 15-26 years of age) seroconverted. This is in contrast to natural infection, in which seroconversion is observed in only 50-70% of women with an incident HPV infection [32], and 2-51% of males [33]. GMTs of HPV genotype-specific antibody at one month after the third vaccine dose (month 7) were 2-4 orders of magnitude greater than those measured in natural infection. Furthermore, after 18 months, GMTs remained tenfold greater than for those recorded from individuals with natural infection, and these levels appear to be preserved over time with the exception of HPV 18, whose titers drop to the level of natural infection [34-36]. While both vaccines generate higher titers than natural infection, and both are efficacious at preventing cervical lesions, in a randomized control trial comparing immunogenicity between the two vaccines, the GMTs for anti-HPV-16 and -18 serum neutralizing antibodies were significantly higher for the bHPV compared to the qHPV vaccine after 24 months of follow-up [37,38].

Both the qHPV and bHPV vaccines have undergone large, Phase III, double blind, placebo-controlled, randomized trials that have demonstrated remarkable efficacy in individuals naïve for the HPV types in the relevant vaccines at trial entry and at the completion of the

three-dose immunization regimen delivered at day 1, month 1 or 2, and at month 6 [39-43]. Follow-up studies of qHPV- and bHPV-vaccinated cohorts are ongoing and have extended up to 9 years thus far, with no breakthrough cases of cervical intraepithelial neoplasia (CIN2/3) caused by qHPV or bHPV vaccine types [44,45].

Non-inferiority immunogenicity bridging studies have been conducted for both vaccines. For the qHPV vaccine, these studies were conducted in 9-15-year-old males and females with the objective of bridging the efficacy findings in young women to pre-adolescents and adolescents. HPV-specific antibody GMTs were non-inferior in adolescents and 1.7-2.7-fold higher than in the clinical efficacy group of 16-23-year-old females [46,47]. In fact, antibody responses to vaccine were highest among the youngest adolescents, with males demonstrating a higher antibody response than females at ages younger than 15 years [46-48]. Preliminary data from the adolescent extension study of these cohorts (P018) at six years shows no breakthrough cases of infection or disease related to the qHPV vaccine types in the per-protocol population of girls. Likewise, there were no breakthrough cases of infection related to qHPV types reported in boys. Disease endpoints are not yet available in boys for this interim analysis [49]. In an immunobridging study, the bHPV vaccine induced GMTs in 10-14-year-old girls that were 2.1-2.5-fold higher than those induced in 15-25-year-old women [50].

The robust antibody response after HPV VLP immunization is attributed to the route of immunization. Natural HPV infections are exclusively intraepithelial, with virus shed sporadically from mucosal surfaces. There is no known viremia; therefore, virus particles and capsid protein have limited access to lymphoid organs (draining lymph nodes and spleen), and systemic antibody and cellular immune responses are consequently weak. In contrast, VLPs are delivered intramuscularly, allowing rapid access of antigen to vascular channels and lymphatics and thus to lymph nodes and spleen [51]. To date, there is no immune correlate for vaccine-induced protection against infection or disease [52,53]. The minimal level of antibody needed for such protection, the long-term durability of neutralizing antibody, and the role of memory B cell if antibody wanes have yet to be established in HPV vaccinated subjects [54].

Alternative dosing schedules

HPV vaccination programs have been introduced in many countries. These programs primarily target adolescent girls 11-15 years of age with or without catch-up vaccination for older adolescents and young women through age 26 years. In a few countries (i.e., US and Australia), gender-neutral vaccination programs are in place that include both females and males. The robust immunogenicity of VLP vaccines, the higher responses in young adolescents, and the positive experience of testing two versus three doses of the hepatitis B vaccine have evoked intense interest in testing alternative HPV vaccine schedules, including reducing the number of vaccine doses.

Three doses with longer time intervals—Several clinical trials have examined adjusting the HPV vaccine dosing schedule to better fit school calendars in hopes of increasing completion of the three-dose series. In one trial (Table 1), young women were

randomized to receive the bHPV vaccine at the standard dosing schedule versus an alternative schedule at 0, 1, and 12 months. Non-inferiority criteria between the two groups were confirmed, with similar rates of seroconversion and comparable antibody GMTs between groups [55]. A similar study assessed an alternative schedule of the qHPV vaccine at 0, 2, and 12 months in young women. Similar to the results with bHPV vaccine, non-inferiority of the alternate dose schedule was confirmed for the qHPV vaccine [56]. In an extension of these trials, an on-going study is assessing alternative dose schedules with the qHPV vaccine in college-aged men (NCT01184079). A trial among Vietnamese girls (ages 11 to 13) with the qHPV vaccine assessed altered dosing schedules of 0, 3, and 9 months; 0, 6, and 12 months; and 0, 12, and 24 months vs. the standard schedule [57]. Similar to the above trials, the alternative dosing schedules at 0, 3, and 9 months and 0, 6, and 12 months met non-inferiority criteria for HPV 6, 11, 16, and 18 compared to the standard dose schedule group. However, the alternative schedule group at 0, 12, and 24 months met non-inferiority criteria for HPV 11 and 18 but not for HPV 6 and 16 compared to the standard dose schedule group. These studies [55,56] demonstrate that alternative dose schedules are effective in producing adequate antibody titers when three doses are delivered within a 12-month period. Therefore, the vaccination schedule could be more liberal in order to complete the vaccine series in females that missed their originally scheduled third dose.

Two versus three doses—The concept of reducing the number of HPV doses relies partially on the argument that, in addition to vaccination occurring in a population, the population continues to be naturally exposed to HPV, thus promoting “booster dose” immune responses and sustaining antibody titers. Depending on rates of vaccine dissemination and migration of unvaccinated cohorts, it is unclear whether this natural boosting will be sufficient to provide long-term duration of protection, should a reduced number of vaccine doses be implemented.

The highest risk for HPV infection occurs 5-10 years after sexual debut for women [47]. This high-risk period of infection solidifies the need for vaccination to occur prior to sexual debut where vaccine will have the greatest impact on preventing future infection upon exposure. While HPV infection prevalence and incidence tends to decrease with age in women, it remains fairly constant in males across the lifespan [58]. Worldwide, approximately 90% of anal canal cancers, 50% of penile cancers, and 33-72% of oropharyngeal cancers (OPCs), depending on world region, are attributable to HPV and occur several years or decades after the initial infection [59]. For example, the median age of diagnosis is 49 years for cervical cancer, 60 years for anal cancer, 62 years for oropharyngeal cancer, and 68 years for penile cancer [60]. While administration of three doses of the HPV vaccine demonstrates continued efficacy after eight years of follow-up [61,62], true reduction in cancer cannot be assessed for several more years or decades. Reducing the number of vaccine doses may have a large impact on the duration of vaccine efficacy, but true indicators of this will not be known for more than a decade.

The following studies demonstrate that, in the short term, two doses of HPV VLP-based vaccines may be immunologically equivalent to three doses, especially if delivered to younger adolescents whose immune responses are more robust than those of young adults (Table 2). However, there are few studies of avidity or immune memory comparing reduced

vaccine doses to the standard three dose series in order to derive conclusive statements regarding long-term duration of effectiveness with reduced vaccine dose schedules for the prevention of cancer.

Bivalent HPV (bHPV) vaccine—Immunogenicity and safety of the bHPV vaccine administered as a two-dose schedule compared with the licensed three-dose schedule has been assessed in two separate studies [63,64] sponsored by the manufacturer. In the first study [63], in addition to the licensed formulation of 20 μg of each antigen (20/20F), an alternative formulation of 40 μg of each antigen (40/40F) was delivered, similar to the approach utilized in reducing the number of hepatitis B vaccine doses delivered to young adolescents by formulating a higher dose vaccine. Females, stratified into three age groups (9-14, 15-19, and 20-25 years) were randomized to four groups: licensed vaccine 20/20F at 0 and 6 months, alternative formulation 40/40F at 0 and 6 months, alternative formulation 40/40F at 0 and 2 months, or licensed vaccine 20/20F at 0, 1, and 6 months. For both HPV 16 and 18 at months 7 and 24, the two-dose schedule in girls ages 9-14 was non-inferior to the three-dose schedule in women ages 15-25 in whom clinical efficacy had been demonstrated.

In a trial conducted in Mexico [64], non-inferiority between two vs. three doses of the bivalent HPV vaccine was assessed among females ages 9-10 years receiving two doses at months 0 and 6 or three doses at the standard dose schedule compared to 18-24-year-olds receiving vaccine at the standard dose schedule. After 21 months of follow-up, the immunogenicity of the two-dose regimen in young girls was statistically non-inferior compared to the immunogenicity of the three-dose regimen in both the young girls and adult women. A third trial, Evaluation of Immunogenicity and Safety of Two-dose Human Papillomavirus (HPV) Vaccine Schedules in 9-14-year-old Girls (NCT01381575) is currently on-going; currently there are no data from this trial in the public domain.

Quadrivalent (qHPV) vaccine—At present, there are two randomized controlled trials assessing immunogenicity and/or effectiveness of two vs. three doses of the qHPV vaccine in adolescent cohorts [65,66]. In the first of these studies, the British Columbia GOV01 trial among Canadian adolescents (NCT00501137), two age groups of females were randomized as follows: 9-13-year-old girls to receive two doses of vaccine at 0 and 6 months; 9-13-year-old girls to receive three doses of vaccine at 0, 2, and 6 months; and 16-26-year-old women to receive three doses of vaccine 0, 2, and 6 months.[65] At 7, 18, and 24 months following a two-dose regimen, antibody responses to HPV 6, 11, and 16 were non-inferior in 9-13-year-old girls compared to the three-dose regimen in the same age group and in young adult women. At 36 months, HPV 6, 11, 16, and 18 antibody responses in both two- and three-dose regimens among 9-13-year-old girls remained non-inferior to the three-dose regimen in young adult women. However, among 9-13-year-old girls, the two-dose regimen was inferior for HPV 18 by month 18 and for HPV 6 at month 36 when compared to the three-dose regimen.

B cell and T cell memory responses, measured by modified ELISPOT assays at 0 and 7 months, have been reported from the British Columbia GOV01 trial [67]. Data from this trial indicate that for all three groups, a statistically significant increase was observed for

memory B cells (MBC) specific for HPV 6, 11, and 16 at month 7 compared to month 0. However, among 9-13-year-old girls receiving two doses, HPV 18-related MBC was not significantly higher at month 7 compared to month 0, while significant increases were observed among those that received three vaccine doses. Significantly higher MBC responses were observed in 9-13-year-olds receiving three doses compared to the three-dose adult group. In addition, T cell memory responses were dose-related. Both adult and adolescent three-dose groups had similar T memory responses to each HPV type, but 9-13-year-old females receiving two vaccine doses had significantly lower responses to HPV 6, 16, and 18 compared to the three-dose vaccine group.

In mice and humans, there are several subsets of activated MBCs defined by surface markers whose contribution to long-term memory remains to be defined [68]. Furthermore, there is considerable heterogeneity in the lifespan of circulating MBCs, with only a subpopulation destined to be long-lived [69]. Studies that have quantified HPV-specific MBCs after VLP vaccination have not provided any information on the longevity of these cells, the key determinant for long term protection. Only the work of Dauner and colleagues [70] showing avidity maturation suggests selection over time for high-affinity clones.

The second randomized controlled trial assessing immunogenicity and effectiveness of two versus three doses of the qHPV vaccine is on-going in India among girls aged 10-18 years, randomized to either two doses at 0 and 6 months or three doses at 0, 2, and 6 months [66]. All girls are to be followed during the five years of the project to document outcomes, with follow-up visits at months 12, 24, 36, and 48. The trial was suspended while the Indian authorities investigated reports of deaths of girls participating in a separate trial sponsored by the nongovernmental organization PATH. After consideration, these deaths were determined to be coincidental to, and not caused by, vaccination. Despite no causal link being identified, the authorities remained cautious; thus, vaccination series in many subjects will not be completed. There is no serological or effectiveness data available yet, but participants will be followed for several years to enable future analyses.

Efficacy of two- versus three-dose schedules – Long-term duration of protection?—At present, there are no data on long-term vaccine effectiveness, either for infection or disease, in the adolescent two- and three-dose cohorts in either the qHPV or bHPV vaccine trials, which are being followed prospectively. The only evaluation of clinical efficacy for less than three doses has been reported in adult women in the Costa Rica Vaccine Trial using the bHPV vaccine [71]. In this trial, women (18-25 years) were randomly assigned to receive three doses of a control vaccine or bHPV vaccine at 0, 1, and 6 months. However, 20% received less than three doses of the bHPV vaccine due to a variety of reasons, primarily pregnancy. Analysis of clinical efficacy data at 48 months provided suggestive evidence that two doses or even one dose is efficacious in the prevention of incident HPV 16 and 18 infections that persist for 12 months or more. Unexpectedly, protection against infection decreased from 100% with one dose, to 84% with two doses, and 81% with three doses. These data are intriguing; however, these are efficacy point estimates with overlapping confidence intervals from a study that was not designed to evaluate clinical efficacy of less than three doses. Furthermore, the study included women who received less than three doses, primarily due to pregnancy, therefore representing a

select population. Interestingly, although cross-protection against HPV 31, 33, or 45 incident infection over one year was observed in the three-dose group, this was not observed in the two-dose group, suggesting differences in the antibody species generated after two, compared to three, doses. However, the numbers are small, and the results of this study should not be over-interpreted.

The Costa Rica Vaccine Trial was further evaluated for durability of antibody response for less than three doses [72]. In this analysis, the magnitude and durability of antibodies to the vaccine was assessed by measuring HPV 16- and 18-specific antibodies from serum at enrollment through four years of follow-up in four groups: one dose (n=78), two doses separated by one month (n=140), two doses separated by six months (n=52), and three doses per standard schedule (n=120). In addition, antibody titers among naturally infected women (n=113) were monitored. At four years, 100% of vaccinated women remained HPV16/18 seropositive. Women receiving one dose of vaccine had nine-fold and five-fold higher GMTs compared to those with natural infections for HPV 16 and 18, respectively. Two vaccine doses led to 24-fold and 14-fold higher GMTs, and three vaccine doses resulted in 51-fold and 23-fold higher GMTs, compared to titers in response to natural infection with HPV 16 and 18, respectively. The durability of the antibody response and the robustness of the MBC response following one or two vaccine doses remains unknown.

As immune memory is central to the long-term protection afforded by vaccines, data regarding circulating HPV-specific MBC populations after differing dosage schedules could be informative. In an early study using the bHPV vaccine, Giannini and colleagues [73] showed a significantly higher HPV 16 MBC population at month 7 (after the third dose of vaccine) compared to that after the second dose. HPV 18-specific MBCs increased after the third dose, but this difference did not reach statistical significance ($p=0.05$). In a study using an unadjuvanted HPV 16 vaccine, antigen-specific MBCs measured at 7 months correlated with antibody concentration, but avidity was unrelated to either [70].

Gender, sexuality, and age differences in immune response

A randomized controlled trial comparing immunogenicity and reactogenicity of the qHPV vaccine in adolescent males and females (ages 9 to 15) compared to young adult females (ages 16 to 26) confirmed that younger age groups have higher GMTs for all four types compared to young adult females [46]. This same trend for higher GMTs in adolescent populations compared to young adult males also holds true for the quadrivalent vaccine [46,48]. In addition to differences in antibody titers based on age, gender also plays a role. Among young adolescents (ages 9-15 years), males have higher antibody titer response to vaccine than females, with greater than 91% remaining seropositive after one year [46,47]. However, following sexual maturation (around 16 years old in most countries), vaccine antibody response declines, and higher titers are observed in females compared to males in the 16-26 year age group [46-48].

The qHPV vaccine trial in adult men (16-26 years old) assessed immunogenicity of the vaccine among men who have sex with men (MSM) and men who have sex with women (MSW). This trial demonstrated that MSM have lower antibody titers than MSW [48] in response to three doses of vaccine. However, despite the lower antibody titers, efficacy of

the vaccine to prevent genital and anal HPV infections and lesions related to these infections was demonstrated among MSM and MSW [48,74], with an examination of durability of this protection for more than five years currently underway [74]. Alternative dosing schedules, including an evaluation of immunogenicity with less than the three recommended vaccine doses, has not been evaluated in males or in populations at high risk of disease, such as MSM and HIV-positive individuals.

Unresolved issues

Information central to an argument for reducing the number of HPV vaccine doses is whether the duration of protection provided by two doses of the vaccine is equivalent to that provided by three doses in adolescents, the primary vaccination cohort. Protection will need to be maintained for decades among adolescents who are immunized at 12-13 years of age. Duration of protection is a key issue for public health officials, as cost-effectiveness analyses upon which policy decisions have been based have used estimates of the duration of protection based on the clinical trials and long-term follow-up studies completed or currently in progress, with cancer as the endpoint. Currently, the duration of protection against disease (CIN2/3) provided by the three-dose schedule is up to 8.4 years for the bHPV vaccine [45] and up to 7 years and 9.5 years, respectively, for the qHPV in 16-23-year-old women and for the HPV 16 L1 VLP of the qHPV vaccine [75]. However, there is no immune correlate - no antibody concentration or other measurable immune marker that correlates with protection against clinical disease - that can be utilized as a surrogate marker of protection lasting decades.

Evidence from experimental studies demonstrates that very low serum antibody concentrations are protective, but the importance of antibody affinity and avidity in relation to this with respect to HPV vaccines is not known [76]. Recent studies have shown that vaccine-induced HPV antibody avidity is uniformly high, whereas in natural infections, there is a wide range of avidities [77]. Importantly, in a recent study by Boxus et al., bHPV vaccine-induced antibody avidity after a two-dose schedule in 10-14 year old girls was non-inferior to that in 15-23-year-old females [78]. However, the available serological assays provide only a partial characterization of the immune status in vaccinated individuals, and current understanding of this response is superficial. The kinetics of the response and specific details, such as avidity, affinity, and epitope specificity, remain to be elucidated.

Utilizing data from non-inferiority immunogenicity trials, the European Medicines Agency (EMA) approved administration of the bHPV vaccine as a two-dose schedule (0, 6 months) for females aged 9-14 years old in December 2013[79]. As of February 2014, EMA approved use of the qHPV vaccine as a two-dose schedule (0, 6 months) for individuals 9 to 13 years old [79,80]. Currently, in addition to the European Union (EU), 12 countries (Panama, Guatemala, Honduras, El Salvador, Haiti, Suriname, Chile, Guyana, Nigeria, Ghana, Pakistan, and Bangladesh) have licensed the bHPV vaccine for use as a two-dose series in females ages 9-14 years. Similarly, use of two doses of the qHPV vaccine has been approved by regulatory authorities in Chile, the Philippines, Brazil, Colombia, Guatemala, Honduras, the Dominican Republic, and South Africa. In April 2014, taking into consideration these approvals by EMA and other countries, the World Health Organization

Strategic Advisory Group of Experts (SAGE) on Immunization recommended a two-dose schedule (0, 6 months) for girls under 15 years of age and a three-dose schedule for girls 15 years of age and older, as well as for immunocompromised populations (e.g., HIV positive) [80]. In the SAGE document, WHO recommended that decision makers assess the degree of risk and benefits of various schedules and their ability to implement effective surveillance post immunization and devise risk management strategies in the event of a worst case scenario after two vaccine doses.

Conclusion

The current understanding of the immune response engendered after HPV VLP immunization is rudimentary. Antibody concentration is the only consistently measured immune parameter, a marker that is relatively insensitive and non-specific to measure long-term duration of vaccine efficacy. There is no immune correlate of protection. The clinical development programs for the currently licensed HPV vaccines were based on clinical efficacy data for a three-dose regimen of prime, prime, boost. This has led to the current indications for these vaccines approved by regulatory authorities around the world. The evidence from clinical trials for two-dose schedules in 9-14-year-old adolescents is restricted to immunogenicity. At present, there is no evidence from these trials demonstrating duration of protection. There is no robust evidence from any randomized trial in 15-26-year-old women that shows clinical efficacy against disease for a two-dose schedule.

The adoption of an alternative to the licensed three-dose regimen in the absence of robust data regarding duration of protection is a potential risk for breakthrough infection and disease years or decades after the immunization series has been completed. As stated by WHO, public health and regulatory authorities adopting alternatives to the licensed three-dose regimens, will need to make in-depth assessments of the risks and devise risk management strategies for worst case scenarios to minimize any impact on cancer prevention strategies and other immunization programs. Disease surveillance data from countries adopting a two-dose vaccine schedule will be instructional on the potential need for a third booster dose. Low-resource countries that do not have screening programs will be reliant on high-resource countries for this information. In addition to the opportunity to provide a third dose of vaccine if necesary, adoption of new lower cost technologies for cervical cancer screening should be considered for the future.

The current three-dose HPV vaccination practices in Australia and other countries highlight the success of this schedule for reduction of cervical pre-cancerous lesions. Although immunogenicity data support reduced/alternative schedules in 9-13 year old females, in the absence of data regarding efficacy, changes in schedule are a risk. The extent of risk and its significance are critical questions when considering policy changes, and risk assessment to inform decision makers is standard practice. Individual governments and public health authorities will need to make such risk assessments in the context of their specific circumstances. Risk management strategies can then be developed to meet worst case scenarios such as failure to achieve two doses, no increase in or poor coverage in adolescents, and/or a decrease in strength or duration of protection.

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Expert Commentary

Alternative dose schedules to HPV vaccination may be efficient strategies to ensuring broad population dissemination of vaccines. However, clinical efficacy and long-term duration of a reduced dosing schedule has not yet been established. As such, countries adopting a two- vs. three-dose vaccine schedule should devise backup cancer prevention strategies should this regimen be shown to be less effective than a three-dose vaccine schedule.

Five-year review Five years from now, we will have data from long-term follow-up of variably vaccinated cohorts with which to draw firm conclusions regarding optimal HPV vaccine schedules to prevent cancer.

Key Issues

1. The cost of HPV vaccines and the difficulty in reaching adolescents with a three-dose series have led to strong interest in modifying the dosing schedule of HPV vaccines by allowing a more liberal schedule over 12 months vs. 6 months and/or by reducing the number of doses.
2. Alternative dose schedules of HPV vaccines are comparable to the standard three-dose series over six months.
3. Several clinical trials have shown that a two-dose vaccine schedule delivered at 0 and 6 months among young adolescents is equivalent to a three-dose schedule delivered at 0, 1/2, and 6 months among 16-26-year-old females with respect to immunogenicity.
4. Long-term duration of immunogenicity following a two-dose versus a three-dose vaccine schedule has not been assessed beyond three years post-dose two.
5. Clinical efficacy of a two-dose versus a three-dose HPV vaccine schedule has not been evaluated.
6. A two-dose HPV vaccine schedule has not been evaluated in males or among those at high risk of HPV-related disease (e.g., men who have sex with men, HIV-positive individuals)
7. Some countries have licensed a two-dose HPV vaccine regimen among girls ages 9-14 years. WHO SAGE provides guidance for a two-dose vaccine schedule in females under age 15.
8. Countries need to make in-depth assessments of the risks and devise risk management strategies for worst case scenarios to minimize impact on cancer prevention strategies and other immunization programs if a two-dose HPV vaccine schedule is implemented.

Table 1

Clinical trials examining alternative schedules for three doses of licensed HPV vaccines in males and females.

Trial Number	Study Location	Vaccine	Gender	Age	Dose Timeline	Results
NCT00552279	Romania, Slovakia, and Italy	bHPV	female	15-25 years	(0, 1, 12 months) vs (0, 1, 6 months)	similar seroconversion rates and non-inferiority of antibody GMTs
NCT00572832	United States	qHPV	female	18-23 years	(0, 2, 12 months) vs (0, 2, 6 months)	non-inferiority of antibody GMTs
NCT01184079	United States	qHPV	males	18-24 years	(0, 2, 12 months) vs (0, 2, 6 months)	ongoing
NCT00524745	Vietnamese	qHPV	female	11-13 years	(0,3,9 months) or (0,6,12 months) or (0,12,24 months) vs (0,2,6 months)	Dose schedules at 0, 3, 9 months and 0, 6, 12 months met non-inferiority for all four HPV types compared to standard dosing schedule; however, the group vaccinated at 0, 12, 24 months did not met non-inferiority criteria for all four types compared to standard

*Note: bHPV=bivalent HPV vaccine (HPV genotypes 16/18). qHPV= quadrivalent HPV vaccine (HPV genotypes 6/11/16/18). GMTs=geometric mean titers.

Table 2

Clinical trials comparing immunogenicity of two versus three doses of HPV vaccines in females.

Trial Number	Study Location	Vaccine	Age	Formulation	Dose Timeline	Results
NCT00541970	Canada and Germany	bHPV	9-14, 15-19, and 20-25 years	licensed vs increased antigen content	(0, 6 months) or (0, 2 months) vs (0, 2, 6 months)	Two-dose schedule in girls 9-14 years was non-inferior to three-dose schedule in women 15-25 years
NCT01717118	Mexico	bHPV	9-10 years and 18-24 years	licensed	(0, 6, 60 months) vs (0, 1, 6 months)	Two-dose schedule in girls 9-10 years was non-inferior to three-dose schedule in women 18-24 years
NCT01381575	Germany, Canada, Italy, Taiwan, and Thailand	bHPV	9-14 years vs 15-25 years	licensed	(0, 6 months or 0, 12 months) vs (0, 1, 6 months)	ongoing
NCT00501137	Canada	qHPV	9-13 years and 16-26 years	licensed	(0, 6 months) vs (0, 2, 6 months)	At 36 months, antibody responses to all four HPV types were non-inferior for both two- and three-dose regimens among 9-13-year-old girls compared to three-dose regimen in women ages 16-26.
NCT00923702	India	qHPV	10-18 years	licensed	(0, 6 months) vs (0, 2, 6 months)	Study stopped

*Note: bHPV=bivalent HPV vaccine (HPV genotypes 16/18). qHPV= quadrivalent HPV vaccine (HPV genotypes 6/11/16/18).