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Immunogenicity of HPV prophylactic vaccines: Serology assays and their use in HPV vaccine evaluation and development

Ligia A. Pinto [a,*], Joakim Dillner [b], Simon Beddows [c], Elizabeth R. Unger [d]

[a] Vaccine, Cancer and Immunity Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD, USA
[b] Department of Laboratory Medicine, Karolinska Institutet, 141 86 Stockholm, Sweden
[c] Virus Reference Department, Public Health England, London, UK
[d] Chronic Viral Diseases Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

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ABSTRACT

When administered as standard three-dose schedules, the licensed HPV prophylactic vaccines have demonstrated extraordinary immunogenicity and efficacy. We summarize the immunogenicity of these licensed vaccines and the most commonly used serology assays, with a focus on key considerations for one-dose vaccine schedules.

Although immune correlates of protection against infection are not entirely clear, both preclinical and clinical evidence point to neutralizing antibodies as the principal mechanism of protection. Thus, immunogenicity assessments in vaccine trials have focused on measurements of antibody responses to the vaccine. Non-inferiority of antibody responses after two doses of HPV vaccines separated by 6 months has been demonstrated and this evidence supported the recent WHO recommendations for two-dose vaccination schedules in both boys and girls 9–14 years of age. There is also some evidence suggesting that one dose of HPV vaccines may provide protection similar to the currently recommended two-dose regimens but robust data on efficacy and immunogenicity of one-dose vaccine schedules are lacking. In addition, immunogenicity has been assessed and reported using different methods, precluding direct comparison of results between different studies and vaccines. New head-to-head vaccine trials evaluating one-dose immunogenicity and efficacy have been initiated and an increase in the number of trials relying on immunobridging is anticipated. Therefore, standardized measurement and reporting of immunogenicity for the up to nine HPV types targeted by the current vaccines is now critical. Building on previous HPV serology assay standardization and harmonization efforts initiated by the WHO HPV LabNet in 2006, new secondary standards, critical reference reagents and testing guidelines will be generated as part of a new partnership to facilitate harmonization of the immunogenicity testing in new HPV vaccine trials.

1. Current licensed HPV prophylactic vaccines

There are currently three licensed HPV prophylactic vaccines: Cervarix®, a bivalent HPV-16/18 product from GlaxoSmithKline; Gardasil®, a quadrivalent HPV-6/11/16/18 product and Gardasil®9, a nonavalent HPV-6/11/16/18/31/33/45/52/58 vaccine, both from Merck & Co., Inc. [1–4]. These products were licensed following highly encouraging efficacy data from large phase III vaccine trials and have the potential (nonavalent vaccine) to prevent up to 90% of cervical cancer cases. The first two products to be licensed, Cervarix® and Gardasil®, used a placebo arm and relied on cervical disease as the primary endpoints [5–7]. The Gardasil®9 trial used the previously licensed Gardasil® vaccine as the control arm [8]. For the 4 HPV types targeted by both vaccines, the primary endpoint was non-inferiority in antibody response, while cervical disease endpoints were used for the 5 additional types in Gardasil®9. All the vaccine trials have demonstrated outstanding long-term efficacy. This remarkable vaccine efficacy is now starting to be seen as effectiveness at the population level following introduction of HPV vaccines into national immunization programs [9].

All three vaccines are based on non-infectious recombinant type-specific L1 capsid proteins assembled into viral-like particles (VLPs) as immunogens. The expressed recombinant L1 capsids self-assemble in arrays of 72 pentamers that present an exterior surface closely mimicking HPV virions and it is this multiplicity of L1 domains that bestows the VLP antigen with significant immuno-

* Corresponding author.
E-mail addresses: pintol@mail.nih.gov (L.A. Pinto), Joakim.Dillner@ki.se (J. Dillner), Simon.Beddows@phe.gov.uk (S. Beddows), eru0@cdc.gov (E.R. Unger).

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genicity, even in the absence of concomitant immune modulators (adjuvants) [10,11].

The vaccines differ in the antigen expression system used, antigen composition and adjuvants included (Table 1) [12–14]. Both Gardasil® and Gardasil®9 products are produced in yeast and formulated with amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant, which has an increased capacity to bind to L1 VLPs compared with other aluminum salts [15]. In contrast, Cervarix® is produced in insect cells using a baculovirus expression vector system and adjuvanted with AS04 which contains aluminum hydroxide plus an additional immunostimulant, the toll-like receptor 4 agonist monophosphoryl lipid A [16]. AS04 has been shown to enhance innate as well as humoral and cell-mediated immune responses [17], and may be responsible for differences in the overall immunogenicity described in head-to-head studies of the two vaccines [18–21]. Other differences between the vaccines include the concentration of each of the L1 VLPs, and the ratio of antigen to adjuvant. Gardasil® has two-fold higher concentrations of HPV-16 L1 VLP and an equal concentration of HPV-18 L1 VLP compared with Cervarix®. Gardasil®9 contains twice the amount of HPV-18 L1 VLP, 50% more HPV-16 antigen and more than twice the level of adjuvant contained in Gardasil®.

The vaccines were originally licensed for use as a three-dose regimen, administered intramuscularly. The licensed HPV vaccines have demonstrated remarkable efficacy in phase III clinical trials in HPV-naïve young women, providing nearly complete protection against incident infection and cervical disease caused by the HPV types that they specifically target [5–8,22]. This is coincident with the induction of a high level, high affinity polyclonal anti-L1 IgG antibody response to the HPV types included in the vaccine, and essentially 100% seroconversion to all targeted HPV types [23–28]. The robust immunogenicity of the HPV vaccines contrasts with the immune responses observed after natural infection, in which seroconversion is found in only a proportion of individuals following incident infection [29]. Vaccine antibodies persist for several years after vaccination at levels that are orders of magnitude higher than those observed in natural infection [24,30–32]. Antibodies generated during natural infection do not appear to consistently protect against subsequent infection. In addition, some studies suggest that protection may be limited to individuals with relatively high levels of naturally-acquired antibody [33–34]. These findings are consistent with a recent study indicating that antibodies cloned from naturally elicited memory B cells were generally non-neutralizing while those isolated after vaccination had strong neutralization activity [35].

A degree of efficacy against some non-vaccine types (i.e. cross-protection) has been demonstrated in vaccine trials, particularly for HPV-31 and HPV-45 [36,37]. Cervarix® appears to confer greater cross-protection than Gardasil® and this difference is reflected in antibody levels against these non-vaccine types [38]. For the purposes of this review, however, only antibody responses against vaccine-targeted types are considered.

Correlates of protection against infection afforded by HPV vaccines have not been formally identified and are difficult to study because of the exceptional efficacy of the vaccines. There have been very few cases of breakthrough infection or disease with the current vaccines to identify the threshold of antibody level that confers immunity among vaccinated subjects. Most studies attempting to address this question have analyzed the protective levels of naturally acquired antibodies in the placebo arm of vaccine trials [33,34] or in cohort studies of infection [29]. Evaluation of different immunogenicity measures in reduced dose schedule trials, in which virological failures may be more likely to occur, could make it possible to establish immune correlates of protection.

Immunogenicity testing of prophylactic HPV vaccines in clinical trials has focused primarily on antibody responses because neutralizing antibodies are thought to be the principal effectors of protection against HPV infection. This is primarily based on experimental evidence in animal models demonstrating that protection against papillomavirus infection can be passively transferred in serum or purified immunoglobulin (Ig) G from VLP-vaccinated animals [39]. It has also been shown that very low levels of HPV antibodies are able to neutralize HPV-16; the 50% inhibitory concentration being estimated to range from 1.9 picomolar to 5.4 nM for three monoclonal antibodies [40]. Vaccine-induced antibodies against vaccine types are detectable not only in serum but also at mucosal sites of infection, such as the cervix and oral cavity [41–44]. These antibodies are believed to reach the site of infection through transudation from serum and by exudation at sites of potential trauma that expose the basement membrane to infection [43,45].

2. Review of immunogenicity of licensed HPV vaccines and duration of responses

2.1. Immunogenicity of three-dose schedules

A number of recent publications have thoroughly reviewed the immunogenicity and efficacy of licensed HPV vaccines in the context of the standard three-dose and reduced dose regimens [46,47]. While all three licensed vaccines have similar efficacy against HPV infection and precancer lesions in clinical trials, the products do differ in immunogenicity, as demonstrated in a variety of assays. Head-to-head trials of three doses of Cervarix® and Gardasil® in 18–45 year-old women and in 12–15 year-old girls have found that HPV-16 antibody levels were significantly lower for Gardasil® when compared with Cervarix® [19–21,48], although they had similar patterns of peak and decay over time. HPV-18 antibody levels and seropositivity were significantly lower for Gardasil®

Table 1
Main characteristics of the licensed HPV prophylactic vaccines.

	Cervarix®	Gardasil®	Gardasil9®
Manufacturer	GlaxoSmithKline	Merck and Co, Inc.	Merck and Co, Inc.
VLP Types Included	HPV-16/18	HPV-6/11/16/18	HPV-6/11/16/18/31/33/45/52/58
Dose of L1 VLP (μg)	20/20	20/40/40/20	30/40/60/40/20/20/20/20/20
Expression system	*Trichoplusia ni* (Hi 5) insect cell line infected with L1 recombinant baculovirus	*Saccharomyces cerevisiae* expressing L1	*Saccharomyces cerevisiae* expressing L1
Adjuvant	500 μg aluminum hydroxide salt and 50 μg 3-O-Desacyl-4′-monophosphoryl lipid (MPL) A	225 μg amorphous aluminum hydroxyphosphate sulfate	500 μg amorphous aluminum hydroxyphosphate sulfate
Initially approved injection schedule	0, 1 and 6 months	0, 2, and 6 months	0, 2, and 6 months
Manufacturing components	4.4 mg NaCl, 0.624 mg sodium dihydrogen phosphate dihydrate	9.56 mg NaCl, 0.78 mg L-Histidine, 50 μg Polysorbate 80, 35 μg Sodium borate	9.56 mg NaCl, 0.78 mg L-Histidine, 50 μg Polysorbate 80, 35 μg Sodium borate
Route of administration	Intramuscular	Intramuscular	Intramuscular

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