

Prophylactic HPV vaccines

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SUMMARY

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Human papillomavirus (HPV) is one of the most common sexually transmitted diseases worldwide. Cervical and other anogenital cancers, cervical and anal intraepithelial neoplasia, genital warts, and recurrent respiratory papillomatosis are HPV-associated diseases. Prophylactic HPV vaccines are composed of HPV L1 capsid protein that self-assemble into virus-like particles (VLPs) when expressed in recombinant systems. Two types of prophylactic vaccines are designed as a bivalent vaccine to protect against high-risk HPV types 16 and 18 and a quadrivalent vaccine designed to protect against HPV 16 and 18, and low-risk, genital wart-causing HPV 6 and 11. Proof-of-principle trials have suggested that intramuscular injections of VLPs result in strong adaptive immune responses that are capable of neutralizing subsequent natural infections. Recent research on the safety and efficacy of candidate prophylactic vaccines against HPV have shown very promising results with nearly 100% efficacy in preventing the development of persistent infections and cervical precancerous lesions in vaccinated individuals.

Key words: Papillomavirus Vaccines; Papillomavirus Infections; Cervical Intraepithelial Neoplasia

INTRODUCTION

Cervical cancer is a major cause of death in women of reproductive age in parts of the developing world (1). In developed countries, incidence and mortality rates for cervical cancer have declined dramatically, due to the effectiveness of screening programs that assess cervical cytology by Papanicolaou smear (2,3).

HPV

Papillomaviruses are small DNA viruses that infect epithelial tissues. Whether cutaneous or mucosal, more than 100 types of HPV described have a circular DNA genome of about 8000 base pairs in common. These small genomes are organized into an early, a late, and a long control region. The products of 2 genes from the early control region, genes *E6* and *E7*, are essential in the HPV-induced processes of cellular transformation and immortalization, and 2 genes from the late control region, genes *L1* and *L2*, encode the viral capsid proteins (Figure 1).

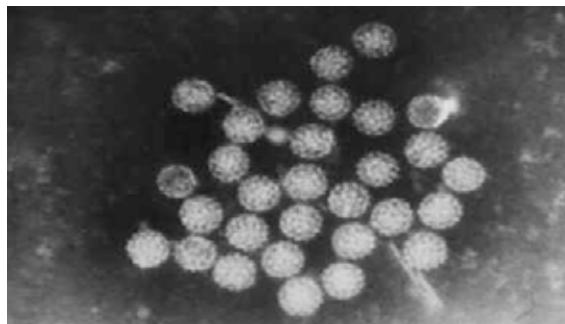


Figure 1. The general organization of a papillomavirus genome and HPV by electrophotomicrography (thanks to Prof. Dr. M. Janicek)

There are many papers showing an importance of persistent HPV infections as a major risk for development of a cervical intraepithelial neoplasia (CIN), characterized by dysplastic changes showing varying degrees of disordered maturation. CIN is classified as either:

- CIN I or low-grade squamous intraepithelial lesions (LSIL)
- CIN II/III or high-grade squamous intraepithelial lesions (HSIL)

These precursor lesions may last continually for several years until some of these HSIL lesions progress in invasive form (4-6).

Human papillomavirus (HPV) is one of the most common sexually transmitted diseases worldwide. Clinical manifestations of HPV infection are exceedingly common and subclinical infection is widespread. More than 100 types of HPV were identified until now and more than 40 types affected anogenital region divided in two groups (Table 1).

Table 1. Main low- and high-risk types of HPV

High-risk types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Low-risk types: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
Potentially high-risk types: 26, 53, 66

Four HPV types implicated in the majority of HPV-related diseases. These four types have been the focus of vaccine development efforts. HPV 6 and 11 are low-risk types associated with the majority of cases of genital warts, and HPV 16 and 18 are high-risk types implicated in approximately 50% of cases of high-grade cervical intraepithelial neoplasia (CIN), invasive cancer at a variety of anogenital sites, and 60–72% of cervical cancers (7,8).

VACCINE: A NEW WAY OF PRIMARY PREVENTION

Today, vaccines are available for many serious human pathogens such as bacteria and viruses, and for about half of all human parasites. Traditionally, attenuated vaccines were made by repeated passaging of the infectious agent in tissue culture or animal hosts until its virulence was greatly decreased but its immunogenicity was retained. Alternatively, chemicals such as formalin were used to destroy infectivity. More recently, parts of an infectious agent, usually a surface antigen, have been used as a subunit vaccine. The current vaccines against hepatitis B virus and Lyme disease rely on recombinant DNA technology (9).

It was therefore reasonable to assume that a vaccine that prevents HPV infection will reduce the incidence of precancerous or cancer lesions on cervix by preventing the major risk factor such as a persistent HPV infection. The L1 capsid protein has been targeted for neutralizing antibody formation (10).

Empty viral capsids, termed virus-like particles (VLPs) were represented as leading candidate for prophylactic vaccine. VLPs was found to bind very

well to human and mouse immune cells that expressed markers of antigen-presenting cells (APCs) such as MHC class II, CD80 and CD86, including dendritic cells, macrophages and B cells.

Purified VLPs are morphologically identical to natural HPV virions (11,12). Because VLPs do not contain viral genetic material, there is no risk of oncogenic progression or productive infection associated with vaccination (13,14). Two pharmaceutical companies, GlaxoSmithKline (GSK) and Merck&Co., Inc. have been the major forces in research and development of prophylactic HPV vaccines today.

During the last five years, both companies put in a great effort to obtain and publish the results about prophylactic effects of two HPV vaccines, bivalent by GSK and quadrivalent by Merck&Co.

The main goals of these studies around the world were to get the information about efficacy, immunogenicity, and safety of these vaccines.

At the beginning of the first decade of the 21st century two randomized controlled trials had been published with highly promising proof-of-principle results (15,16). Vaccine efficacy was 100% in preventing acquisition of persistent HPV infection (of the target types) in both studies. Both studies also showed encouraging results concerning prevention of CIN but the precision of the estimates of efficacy were much lower given that these trials had not been designed with sufficient power to detect reductions in CIN incidence (17).

During the next few years, several studies around the world were continued to prove the benefits of both prophylactic vaccines in general populations, Cervarix™, a bivalent HPV16/18 VLP vaccine from GlaxoSmithKline; the Gardasil™, also known as Silgard, a quadrivalent HPV16/18/6/11 VLP vaccine from Merck Vaccines (18-20).

The efficacy, immunogenicity, and safety of these vaccines were the major endpoints. High-grade CIN (CIN2/3) is accepted as the immediate precursor of invasive

cervical cancer and for vaccine licensing; the endpoint of CIN 2/3 or worse has been accepted widely as an ethically acceptable proxy for cervical cancer (21).

The efficacy

In women who have no evidence of exposure or infection to the HPV genotypes in the vaccine, both vaccines show high efficacy, with more than 90% reduction in persistent infection (HPV DNA of the same type detected on two successive occasions 6–12 months apart in a woman previously HPV DNA-negative) and 100% reduction in high-grade cervical lesions (19, 20). In the according-to-protocol (ATP) groups in the phase II trial of the bivalent vaccine, there was 100% efficacy against the development of HPV16/18-associated high-grade CIN2/3, despite the small numbers (19).

For both the bivalent and quadrivalent vaccines, results of different trials allow for the examination of broad trends in efficacy in preventing HPV 6/11/16/18-related disease in several groups of patients classified according to their HPV status at baseline. The quadrivalent vaccine was 100% effective in reducing the incidence of HPV 6/11/16/18-related disease in women who were serologically and DNA PCR negative at baseline to the relevant HPV type, as well as in women who had been previously exposed to at least 1 HPV type vaccine at enrollment, but had no ongoing HPV infection (22,23). Furthermore, the vaccine was shown to reduce the risk of developing disease by 27-28% in those individuals who were post-vaccination PCR positive and seronegative to the same HPV type for an average follow-up of 3 years. However, there was no clear evidence of protection from disease caused by HPV types for subjects that were HPV DNA positive by PCR and/or seropositive at baseline (24,25). Similar results were obtained for the bivalent vaccine (19). Vaccination of HPV16/18 DNA positive women does not enhance clearance of the viral infection (26). In a recent publication of a phase III trial, this bivalent vaccine showed 90% prophylactic efficacy against CIN2+ associated with HPV 16 or HPV 18 (27) (Table 2).

Table 2. The efficacy of Bivalent and Qadriivalent HPV vaccine (19-27)

Study feature	GSK study (Phase II)	Merck study (Phase II)
Vaccine type	Bivalent HPV-16 and -18 L1 VLPs	Quadrivalent HPV-6, -11, -16, -18 L1 VLPs
Expression system	Insect cells (baculovirus)	Yeast
Concentration	20 µg HPV-16, 20 µg HPV-18	20 µg HPV-6, 40 µg HPV-11, 40 µg HPV-16, 20 µg HPV-18
Adjuvant	AS04 (proprietary)	Aluminum hydroxyphosphate sulfate
Dose, administration	0.5 ml, intramuscular	0.5 ml, intramuscular
Schedule	0, 1 and 6 months	0, 2 and 6 months
Trial size	560 vaccinees, 553 placebo	277 vaccinees, 275 placebo
Age range of participants	15-25 years	16-23 years
Key eligibility requirements	No history of cervical lesions, few sexual partners	No history of cervical lesions, few sexual partners
Duration	Up to 54 months	Up to 60 months
Efficacy for incident/transient infections	97% (89-100)	96% (83-100)
Efficacy for persistent infections	100% (77-100)	100% (16-100)
Efficacy for cytological abnormalities	97% (84-100)	
Efficacy for pre-invasive lesions	100% (42-100)	100% (32-100)
Acceptable rate of adverse events	Yes	Yes
Serious adverse events	No	No
Sustained seroconversion	100%	100%
Specific titers compared with natural infection	50-80-times greater	10-20-times greater

HPV = Human papillomavirus; VLP = Virus-like particle.; GSK = GlaxoSmithKline

Immunogenicity

The measurement of specific serum immunoglobulin G (IgG) anti-L1 VLP antibodies by immunoassays in vaccinated and unvaccinated individuals is the main parameter used in the current vaccine trials to monitor vaccine-induced immune responses. VLPs are highly immunogenic and, in VLP-immunized individuals, the peak anti-VLP antibody responses are substantially greater than those made at seroconversion in natural infections (15, 28). The serum antibody levels falling from the peak levels achieved after the third immunization to a lower concentration that persists at the same level (at least 10–20 times that of natural infection) for at least 60 months post-vaccination (19, 20). The long-term duration of protection depends on immune memory and there is evidence that both vaccines induce good immune memory. Increased numbers of circulating memory cells are generated after immunization with the bivalent vaccine and this is attributed to the novel adjuvant ASO₄ (29). Early results from a challenge study of 241 women, in which vaccinated women were given a booster 5 years after enrolment, showed rapid and enhanced antibody responses after the fourth immunization characteristic of an anamnestic response (20).

Adverse events

The bivalent vaccine was very safe, and the adverse events were both mild and transient. The vaccine group had significantly more injection-site reactions than did the placebo group, but these symptoms were observed to be transient and mild. General symptoms such as fatigue, gastrointestinal complaints, headache, itching, and rash, were equally distributed between the placebo and vaccine groups. Discontinuations were not attributed to adverse events related to the vaccine. The quadrivalent vaccine was well tolerated. Injection site reactions were more common in women receiving active vaccine injection, with injection-site pain being the most common adverse event. Headache was the most frequent systemic adverse event. The vast majority of adverse events were mild or moderate (94%), and there were no vaccine-related serious adverse events. Only one patient discontinued treatment due to an adverse event, and this patient was in the placebo group (20,30,31).

Cross-neutralization

There is preliminary evidence that the vaccines may offer some degree of cross protection against types phylogenetically related to the target types, such as HPV-45 (related to HPV-18) and HPV-31 (related to HPV-16), although at antibody concentrations that are 1–2 logs lower than the dominant type-specific neutralizing antibodies (19).

In the second-generation vaccine, some researchers are considering modifying the L1 molecules that make up the VLPs in such a way that the particle surface induces more broadly neutralizing antibodies. Until now, there have been two ways to try to get such a vaccine by use of pools of randomly mutagenized L1 genes for the direct ("genetic") immunization of mice and by use of the minor structural protein L2. The isolated protein, L2 or specific L2-derived epitopes (e.g., of HPV-16) induce antibodies that neutralize infection by other HPV types but the titers induced by L2 are at least 1000-fold lower than when L1 VLPs are used. Multimerization of immunogenic epitopes or their engineering into the surface loops of L1 VLPs have improved the L2-specific immunogenicity, although not yet to a satisfactory level. The introduction of rationally designed and highly efficient adjuvants then the

antibody titers can be further increased to the point where they may become relevant (32).

CONCLUSION

Two HPV L1 VLP vaccines have been developed: a quadrivalent HPV6/11/16/18 and a bivalent HPV16/18 highly immunogenic and well tolerated. The vaccines have been shown in the various trials to be effective at preventing infection and diseases related to the vaccine HPV genotypes in women who were HPV DNA-PCR-negative at baseline. The protection generated by the vaccines persists for at least 5 years and, the antibody levels remain high even after 5 years. The primary target group for immunization with the HPV vaccines is likely to be pre-adolescent girls, but there could be benefit in vaccinating other groups (men, sexually active women of all ages).

Incorporation of HPV vaccination in the public health sector is still to be seen in the developing world, mostly due to vaccine cost.

Despite good efficacy of the vaccine, the secondary screening with Pap tests (or HPV DNA testing) will still be required to detect cervical cancers and pre-cancers caused by non-vaccine HPV types. The vaccines do not protect against all high-risk types of HPV.

The prophylactic vaccine has no therapeutic effects.

The durability of these vaccines has been evaluated only for up to 5 years. The monitoring of antibody levels and high grade disease caused by the HPV vaccine types in sentinel groups of immunized individuals will be required over the next decades.

Vaccinated populations should be followed-up for long-term safety, sustained immune responses, and vaccine disease efficacy.

Education of physicians, policy makers, parents, and adolescents will be crucial for delivering HPV vaccines, which ultimately will result in the reduction of cervical cancer rates and other HPV-related diseases worldwide.

Conflict of interest

We declare no conflicts of interest.

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