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Second-Generation Prophylactic HPV Vaccines: Successes and Challenges

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Abstract

The role of human papillomavirus (HPV) as the causative factor in cervical cancer has led to the development of the HPV vaccines Gardasil and Cervarix. These vaccines effectively protect against two HPV types associated with 70% of cervical cancer cases. Despite this success, researchers continue to develop second generation HPV vaccines to protect against more HPV types and allow increased uptake in developing countries. While a reformulated vaccine based on the current technology is currently in clinical trials, another strategy consists of targeting highly conserved epitopes in the minor capsid protein of HPV, L2. Vaccines targeting L2 induce broadly neutralizing antibodies, capable of blocking infection by a wide range of HPV types. Several vaccine designs have been developed to optimize the display of L2 epitopes to the immune system and to reduce the cost of manufacture and distribution. L2-based vaccines show considerable promise as a potential next-generation HPV vaccine.

Keywords

HPV; Cervical Cancer; Vaccines; L2; Virus-like Particles

Introduction

Human papillomavirus (HPV) is one of most common sexually transmitted pathogens in the world, with a reported 11% world-wide prevalence in women with normal cytology [1]. In the United States, over 6 million new HPV infections are reported each year and greater than 20 million people are currently infected. Over 100 different HPV types have been identified, but the most common HPV-associated cancer, cervical cancer, is associated with infection by one of a subset of 14–20 HPVs termed “high-risk” types (reviewed in [2]). Two high-risk HPV types, HPV 16 and HPV 18, are found in approximately 70% of all cervical cancer cases [3]. Cervical Cancer is the second most common and fifth deadliest cancer in women worldwide, with over 500,000 new cases and 275,000 deaths each year [4] Approximately 85% of cervical cancer cases occur in developing countries [5]. High-risk HPV infection is also associated with other anogenital cancers (of the vulva, vagina, penis, and anus) as well as growing percentage of oropharyngeal cancers (reviewed in [6–10]). In total, HPV infection is estimated to be responsible for about 5% of human cancers worldwide [11].

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Cervical cancer is one of the few cancers that can be prevented using a prophylactic vaccine. The current HPV vaccines (Gardasil and Cervarix) are comprised of a mixture of virus-like particles (VLPs) derived from the HPV major capsid protein, L1 of two high-risk HPV types (HPV16 and 18). Gardasil also contains VLPs derived from two low-risk HPV types associated with genital warts (HPV6 and 11). Both Gardasil and Cervarix have excellent safety profiles and strongly protect immunized individuals against infection with the HPV types included in the vaccines [12–16]. However, these vaccines provide modest cross-protection against other high-risk HPV types, leaving vaccinated individuals at a decreased risk, but still vulnerable to the development of cancer. This review will discuss efforts to develop second generation HPV vaccines that will provide broader protection against the HPV types associated with cancer. In particular, we will focus on the considerable progress that has been made in developing vaccines targeting the minor capsid protein of HPV, L2. Vaccines targeting L2 may provide a relatively simple and effective way to generate cross-neutralizing immunity against diverse high-risk HPV types.

Biology

HPV is a non-enveloped double-stranded DNA virus from the family *Papillomaviridae* (HPV biology reviewed in [17]). Its circular, covalently-closed genome is approximately 8kb in length and encodes 8 genes, divided into early (E) and late (L) proteins. Papillomaviruses have a strict tropism for cells of the squamous epithelium and are peculiar in that their life cycle is dependent upon differentiation of the host cell. In short, upon entering the basal cells, transcription of the viral genome is regulated by E2. Proteins E6 and E7 interact with p53 and retinoblastoma protein, respectively, to deregulate the cell cycle and promote division. As the keratinocytes continue to differentiate and migrate to the surface, the late structural proteins, L1 and L2 are produced to encapsidate the viral genome and virions are eventually sloughed off from the dead cells.

HPV virions consist of two viral structural proteins, L1 and L2. L1, the major viral structural protein, assembles into pentamers, 72 of which form an icosahedral capsid with T-7 symmetry. The minor capsid protein, L2, is present in much lower amounts than L1, with a maximum of 72 copies per virion at the vertices [18]. Although both viral capsid proteins are present in virions, natural HPV infection typically results in the induction of low-titer antibody responses directed towards L1 only, demonstrating the immunodominance of L1 epitopes as well as the occlusion of L2. Structural studies have indicated that L2 is poorly displayed on the surface of mature virions, and is only revealed later in the complex infection process, presumably after binding of the virion to the basement membrane, which exposes the amino terminus of L2 [19–21]. Once exposed, 12 or so amino acids at the N-terminus of L2 are cleaved by a furin, a cellular proprotein convertase, leading to surface exposure of one or more domains of L2 on the virion surface [21–23]. Although HPV virus-like particles (VLPs, described below) can be formed by L1 protein alone, L2 is required for productive infection. L2 is required for both HPV endosomal escape and also plays a role in facilitating trafficking of the viral genome to the nucleus [23–26]. L2 also plays a critical role in the encapsidation of viral DNA prior to virion release [27].

HPV cancer epidemiology

While HPV infection is common, infections rarely progress to cancer. It is thought that most HPV infections are cleared by the immune system. Nevertheless, persistent infection can occur in a subset of individuals, and this persistent infection with high-risk HPV types has been shown to be necessary for the development of cervical cancer (reviewed in [2,28]). Of the high-risk HPV types, HPV16 and HPV18 stand out. These two HPV types are found in approximately 70% of all cervical cancer cases, and HPV16 infection is associated with 90% of HPV-related oropharyngeal cancers [3,8], reflecting the enhanced oncogenic potential of these HPV types relative to other high-risk HPVs [29,30]. Although there are geographic differences in HPV genotype distribution in cancers [29,31–33], there is strong evidence that about eight HPV types (namely HPV16, HPV18, HPV31, HPV 33, HPV35, HPV45, HPV52, and HPV58) are responsible for at least 90% of the global burden of cervical cancer [34]. Nevertheless, the abundance of high-risk HPV types that cause a small percentage of cancer cases, and regional differences in these types, complicate efforts to protect against all oncogenic types and represent a significant hurdle in efforts to develop a vaccine that provides 100% protection against HPV infection.

Current HPV Vaccines

There are currently two prophylactic HPV vaccines on the market: Gardasil and Cervarix. Both vaccines contain virus-like particles (VLPs) composed of the HPV L1 protein. The development of these vaccines was made possible by the observation that recombinant L1, when overexpressed, spontaneously self-assembles into VLPs that structurally resemble infectious virus but lack genomic material [35–37]. Randomized clinical trials of HPV VLP-based vaccines have established that Gardasil and Cervarix are safe and induce high-titer antibody responses. Importantly, vaccination largely protects women from HPV16 and 18 DNA acquisition, and the vaccines are remarkably effective (nearly 100%) at preventing HPV 16- and HPV 18-associated cervical intraepithelial neoplasia grade III (CIN III), the precursor lesion for cervical cancer [13–16,38,39]. Notably, studies in the US and Australia have begun to show a drop in the prevalence of vaccine HPV types both in vaccinated and non-vaccinated populations, indicating that the vaccines may be establishing herd immunity [38,39].

Although the precise immunological mechanism of protection by the HPV vaccines has not been definitely established, it is likely that the protection provided upon vaccination with HPV VLPs is mediated by neutralizing antibodies. Both Gardasil and Cervarix elicit high titers of neutralizing antibodies in vaccinated individuals after intramuscular immunization [40]. HPV neutralizing antibodies in vaccinated individuals can be measured using sensitive *in vitro* neutralization assays that assess the ability of HPV pseudovirus (PsV; HPV VLPs which encapsidate a reporter plasmid, described in detail in Roberts *et al.* [41]) to infect cell lines [42]. In addition, animal studies have shown that passively transferred sera from Gardasil-vaccinated mice can protect naïve mice from cervico-vaginal challenge by HPV PsV [43]. Antibody responses to HPV VLPs are also quite durable. Although antibody titers drop about 10-fold in the first year after vaccination, levels are stable thereafter (after 8 years of follow-up, in one study), indicating that the HPV vaccines provide long-lasting

protection [12,44]. These desirable characteristics, the induction of high-titer and long-lasting antibody responses, appear to be general characteristics of VLP-based vaccines. The dense, highly ordered presentation of L1 on VLPs strongly activates B cells through B cell receptor cross-linking. Also, VLPs are readily taken up and presented by professional antigen presenting cells due to their particulate nature (VLPs reviewed in [45,46]), further enhancing their immunogenicity. It should be noted that Gardasil and Cervarix contain an aluminum salt adjuvant that may contribute to their ability to elicit high titer antibody responses; Cervarix is additionally adjuvanted with monophosphoryl lipid A (MPL), a Toll-like receptor 4 (TLR4) agonist. However, clinical trials have demonstrated that even unadjuvanted HPV VLPs elicit high-titer antibody responses [47], highlighting the innate immunogenicity of VLP-based immunogens.

One major limitation of the current vaccines is that antibodies elicited by L1 VLPs are type restricted, in that they largely protect against infection by the HPV types included in the vaccine and provide suboptimal protection against other high-risk HPV types (reviewed in [48]). Thus, vaccinated individuals are still at risk for cancer. The type-restricted nature of neutralizing antibodies against L1 has been borne out by epidemiological studies as well as in in vitro studies using the HPV PsV neutralization assay. Although there is evidence that the titer and breadth of cross-reactive antibodies are greater after vaccination with Cervarix than Gardasil [49,50], in either case the titer of cross-reactive antibodies is quite low compared to those elicited against HPV16 and 18. There are also several aspects of the current vaccines that are barriers to worldwide implementation. Both Gardasil and Cervarix are quite costly, at over US$100 dollars for each of the three immunizations, though recent agreements brokered by the GAVI Alliance have lowered the price to a little less than US$5 in as many as 40 developing countries. Also, the vaccine requires a cold-chain which increases the cost of transportation and storage. Finally, both Cervarix and Gardasil are given as a recommended three-dose series over an extended six-month period (although recent data has shown that two doses of Cervarix are as protective as three doses [51]; several Canadian provinces are now recommending a two shot vaccine regimen). Taken together, these factors (price, cold-chain, and requirement for multiple doses) reduce the uptake of the HPV vaccine in developing countries, where it is most needed.

Next Generation HPV Vaccines

The next HPV vaccine will need to address many, if not all, of the issues associated with current vaccines while retaining their effectiveness against HPV 16 and 18. Foremost among them is increasing the number of HPV high-risk types for which immunization confers protection. One way to address this issue using the current vaccine technology is to include additional high-risk HPV VLPs in the vaccine formulation. This strategy has been adopted by Merck, which has developed a nonavalent HPV vaccine (V503) that is currently in Phase III trials (http://clinicaltrials.gov/ct2/show/NCT00543543). In addition to VLPs of HPV types 16/18/6/11, high-risk types 31, 33, 45, 52, and 58 have been added to the vaccine. Assuming the vaccine is equally effective against all nine HPV types, the immunization with V503 will theoretically prevent over 90% of cervical cancer cases. One study, modeling the increase in protection between current vaccines and the new nonavalent vaccine, estimates that the decrease in cervical cancer cases due to the uptake of the new vaccine could range

from 9% to 30%, depending on the region and the amount of cross-neutralization seen after vaccination [52]. While it is somewhat premature to speculate about this vaccine since the results of the trial have yet to be published, our experience in animals is that we can immunize a single animal with a mixture of eight VLPs and still obtain high titer antibody responses to each of the individual components of the vaccine [53]. Thus, inducing high-titer antibody responses against the HPV types included in the formulation is highly possible (although it is also possible that the levels of neutralizing antibodies against the individual components of the vaccine may vary). It is likely, however, that V503 will face many of the same barriers to worldwide implementation as the current vaccines (i.e. high-cost and requirement of a cold-chain). It is also unlikely that the nonavalent vaccine will be universally effective against high-risk HPV infection, so Pap screening will continue, although potentially at reduced frequency.

L2 as the target of Second Generation HPV Vaccines

Another strategy to increase the breadth of protection conferred by vaccination is to target the immune response against epitopes that are more broadly conserved between HPV types. One target that has generated considerable interest is the HPV minor capsid protein, L2. In studies beginning in the early 1990s, several labs showed that vaccination with recombinant L2 protein could provide protection from infection with animal papillomavirus [54–56]. Subsequent studies showed that antibodies targeting L2 could not only mediate homologous neutralization, but could also neutralize diverse papillomavirus types [57]. Mapping studies have shown that broadly neutralizing epitopes within L2 are located in the N-terminal domain of the protein (roughly amino acids 13–120), which is consistent with both the sequence conservation of this region and also the fact that this region of the protein appears to be transiently exposed on the surface of virions. For example, antibodies raised against the N-terminal 88 amino acids of bovine papillomavirus type 1 (BPV-1) are broadly cross-neutralizing against several HPV types, whereas other domains of BPV-1 L2 do not induce cross-neutralizing antibodies [42]. Subsequent studies have identified peptide domains within N-terminus that appear particularly promising targets for vaccines (described in more detail below).

Although vaccines targeting L2 have the potential to cross-neutralization diverse HPV types, they also have challenges to overcome before they can be a viable option as a next-generation HPV vaccine. Most prominently, the antibody titers elicited upon vaccination with recombinant L2 alone are much lower than those elicited by the L1 VLPs. Furthermore, despite the fact that HPV L2 is relatively conserved amongst types, it still displays sequence heterogeneity. Thus, a broadly effective L2 vaccine will need to protect against as many HPV types as possible. Of course, this must be done while keeping anti-HPV 16 and 18 neutralizing antibody titers high. Finally, the vaccine needs to be cost-effective for uptake in developing countries.

Strategies for Targeting HPV L2

Most of the efforts to target HPV L2 have focused on vaccines targeting specific epitopes or domains within the N-terminus of the protein and the use of different techniques to increase

the immunogenicity of L2-derived peptides (Table 1). Many of these efforts have focused on targeting the L2 domain encompassing amino acids 17–36. This domain is referred to as the RG-1 epitope because it is targeted by a neutralizing monoclonal antibody (RG-1) that binds to this region, strongly neutralizes HPV16 and HPV18, and, upon passive immunotherapy, protects mice from challenge with HPV16 PsV [58]. This region of L2 contains two cysteine residues (which form a disulfide bond in mature virions) that are present in all known papillomavirus types [59]. Although this is a linear epitope, there is evidence that both the oxidation state and structural context of the RG-1 epitope contributes to its immunogenicity [60,61]. The RG-1 epitope is not the only potential vaccine target; it has been shown that other domains within L2 (for example amino acids 108–120 and 69–81) can also elicit cross-neutralizing antibodies [62–64].

Because L2 displays some sequence heterogeneity, it is possible that no single L2 epitope will be capable of inducing antibodies that can cross-neutralize all high-risk HPV types. To account for this possibility, and also to increase the immunogenicity of a recombinant protein-based vaccine, one strategy has been to covalent fuse the N-terminal regions of L2 from diverse HPV types together and express this construct as a concatemeric peptide in *E. coli* [65]. Used with a variety of adjuvants, these multimeric recombinant vaccines elicit broadly neutralizing antibodies that were protective against *in vivo* HPV PsV challenge in a mouse genital challenge model of HPV infection. For example, a fusion peptide of HPV L2 amino acids 11–88 from HPV types 1, 5, 6, 16, and 18 (11–88×5), injected five times with Freund's adjuvant, induced high *in vitro* neutralization titers against all of the HPV types included in the polypeptide, as well as types 45, 31, and 58. In another study, the same 11–88×5 peptide was mixed with type HPV 16 L1 capsomeres, a structural component of L1 VLPs comprised of 5 L1 proteins [66]. Capsomeres are less expensive to produce than the full VLP, but elicited type-specific neutralizing antibodies when used in vaccination [67]. Coimmunization elicited antibodies that strongly neutralize HPV16 and also cross-neutralize other HPV types. Finally, in a recent study attempting to optimize their multimeric peptide by determining essential regions needed for neutralization, Jagu *et al.* showed that vaccination with a fusion peptide displaying the 11–88 region from eight different HPV types to be more immunogenic than vaccinating with multimeric peptides representing smaller portions of the same region of L2, implying that the domain contains multiple neutralizing epitopes and suggesting that immunization with a larger portion of the N-terminus may increase antibody titers [68].

Another strategy for presenting L2 epitopes to the immune system is the use of HPV L1 VLPs as a display scaffold [63,69,70]. The rationale for these studies is that multivalent display of L2 on HPV16 VLPs will enhance its immunogenicity without sacrificing a strong anti-HPV16 response. The Kirnbauer group, in particular, has had considerable success inserting L2 peptides into exposed loops of BPV and HPV16 L1 VLPs [71]. Schellenbacher *et al.* genetically inserted overlapping sequences derived from the N-terminus representing amino acids 2–200 from L2 into L1 proteins and attempted to generate chimeric VLPs. Although some of the chimeric proteins failed to assemble into intact VLPs, they found that L1/L2 chimeric VLPs displaying L2 aa 17–36 in combination with adjuvants, provoked the greatest amount of cross-neutralizing antibodies in *in vitro* neutralization assays.

Specifically, neutralizing antibodies were detected against HPV high-risk types 16, 18, 45, 52, and 58, as well as types 11 and 5. This degree of cross-neutralization was seen when using Freund's adjuvant or the more physiologically relevant Aluminum hydroxide-monophosphoryl lipid A (Alum-MPL), though at lower titers with Alum-MPL. Importantly, the insertion of L2 epitopes did not reduce the observed titer of antibodies directed against the VLP vehicle itself, whether BPV or HPV 16. Schellenbacher *et al.* expanded on this study in a recent paper, rigorously investigating the breadth of cross-neutralization induced by vaccination with HPV 16 L1 VLPs displaying the L2 17–35 (RG-1 VLP) epitope with Alum-MPL [72]. Variable *in vitro* cross-neutralization titers were observed against all relevant high-risk types as well as common low-risk and cutaneous types as well. Further, immune rabbit sera was passively transferred into mice which were then challenged with a comprehensive panel of high- and low-risk HPV PsVs. Protection was seen against all the 21 tested PsVs, even against types for which the *in vitro* neutralization titers were quite low. Crucially, this cross-protection was observed to be long lasting; passively transferred sera drawn 52 weeks after the initial vaccination was still protective against a heterologous PsV challenge.

A similar technique to increase the immunogenicity of L2 epitopes is to display them on non-HPV VLPs. Heterologous vaccine targets can be genetically inserted or conjugated to the surface of VLPs, creating the same dense, ordered display that strongly activates B cells and leads to high-titer antibody production against the displayed epitope. This has been accomplished on a number of different VLPs, ranging from plant viruses to bacteriophages [73–76]. For example, the Palmer group conjugated the streptavidin bound N-terminus of L2 from canine oral papillomavirus (COPV) to biotinylated Tobacco Mosaic Virus (TMV) VLPs, showing an increase in anti-L2 antibodies when compared to immunizing with the L2 peptide alone [73]. Similarly, the Kleinschmidt group made use of Adeno-associated Virus VLPs (AAVLPs), genetically displaying the RG-1 epitope (17–36) from HPV 16 and 31 on the same particle [75]. Immunization with montanide ISA 51 as an adjuvant induced high-titer anti-L2 antibodies that were able to neutralize HPV PsVs 16, 31, 18, 45, 58, and 52. Importantly, Nieto *et al.* demonstrated that lyophilized and re-constituted AAVLPs were also immunogenic, provoking anti-L2 antibody production. This finding could be advantageous in lowering the cost of storage and distribution of the vaccine for developing countries.

Our lab has focused on the use of bacteriophage VLPs for the display of the RG-1 epitope. Bacteriophage VLPs can be produced in bacterial expression systems, such as *E. coli*, which lowers manufacturing difficulty and cost while generating a high yield of recombinant VLPs. Genetically inserting the L2 epitope into an exposed loop on the surface of PP7 bacteriophage coat protein, we observed induction of high-titer neutralizing anti-L2 antibodies [53]. Notably, when the RG-1 epitope was inserted in a unconstrained format at the N-terminus of MS2 bacteriophage coat protein, we observed a marked increase in the cross-protection against diverse HPV types when compared to display in other regions of the VLP[61]. Using the PsV *in vivo* challenge model, we observed significant protection against HPV types 5, 6, 16, 18, 31, 33, 35, 39, 45, 51, 53, and 58 in mice vaccinated with our L2 displaying VLPs. More recent studies have examined the longevity and potency of immune

responses to bacteriophage vaccination ([77] and unpublished data). Mice immunized with PP7 bacteriophage VLPs displaying the RG-1 epitope were found to have high anti-L2 titers for at least 18 months after vaccination. These antibodies were also shown to be protective against PsV challenge after the same time period. We also measured the immunogenicity of PP7 and MS2 bacteriophages with or without adjuvants and found the immune response to be only mildly boosted when mixed with alum, demonstrating the high innate immunogenicity of bacteriophage VLPs. In another study, we have had some success conjugating L2 peptides to the surface of Qβ VLPs, another bacteriophage. We created a consensus sequence of region aa 65–85 of L2 from the high-risk HPV sequences to increase the cross-neutralization of this region and tested the cross-neutralization in an *in vitro* L2 PsV neutralization assay (assay described in [78]). Sera from mice immunized with VLPs displaying the consensus sequence showed considerably higher titers of neutralizing antibodies against heterologous PsV types than those immunized with non-consensus sequences from the same region (data not published).

Finally, L2-derived peptides have been fused to a variety of immune-activating substances. Richard Roden's group fused the RG-1 epitope to p25, a T helper epitope, and P2C, a Toll-like receptor 2 activating lipopeptide [79]. Immunization with this fusion peptide, either subcutaneously or intra-nasally, induced a strong anti-L2 response that was cross-neutralizing in both *in vitro* and *in vivo* PsV neutralization assays. Of note is their observation of a high-titer response to the intra-nasal immunization, suggesting that this vaccine could be delivered needle-free, possibly easing its uptake. The Müller group used bacterial thioredoxin as an adjuvant for L2 peptides, displaying one or more L2 peptides into a surface-exposed loop of the protein [80]. Inserting a number of small, overlapping peptides derived from the N-terminus of HPV 16 L2, Rubio *et al.* found that immunizing with these thioredoxin constructs with CFA and IFA adjuvants did induce a strong anti-L2 response. Of the N-terminal regions tested, they found vaccines that displayed aa 20–38, a peptide overlapping the RG-1 epitope, to induce the greatest breadth of cross-neutralization in *in vitro* PsV neutralization assays. Finally, in a rather unique approach, Yoon *et al.* genetically inserted a large region of the N-terminus of L2, aa 1–240, into a surface protein of *Lactobacillus casei* (*L. casei*) [81]. The lyophilized, recombinant bacteria were given to the mice enterically via intra-gastric gavage, after which anti-L2 antibodies were found in both sera and vaginal washes. Immune sera and vaginal washes also cross-neutralized HPV types 18, 45, 58, and BPV1 in *in vitro* PsV neutralization assays. Immunized mice were modestly protected upon *in vivo* PsV vaginal challenges.

Expert Commentary and Five-year view

Despite the effectiveness of the current HPV vaccines, there remains a need to provide broader protection against rarer high-risk HPV types and to make it more affordable for developing countries. While the nonavalent L1-based vaccine that is in clinical trials may be a potential solution, several groups have aimed to develop cross-protective vaccines based on L2. Although many labs have developed strategies to elicit high-titer broadly cross-neutralizing antibodies against L2, our bias is that multivalent display on VLPs (or some other particulate carrier) is the most promising and potent technique for eliciting the high titer and long-lasting antibody responses that may be required for sustained cross-protection.

L2-displaying VLP vaccines confer remarkable *in vivo* cross-protection in HPV PsV challenges against a large variety of HPV types, both high-risk and otherwise. These studies exhibit the versatility and effectiveness of VLP-based vaccines in displaying heterologous targets to the immune system. In all cases, targeting the RG-1 epitope of L2 provokes the broadest cross-neutralizing responses. Nevertheless, there remain obstacles that must be overcome before any next-generation HPV can be deemed successful, some of which have been examined in the context of non-VLP-based L2 vaccines. For example, several groups have looked for ways to reduce the necessity of a cold-chain in the delivery of the vaccine. The lipopeptide-L2 construct that was shown to be effective when delivered intra-nasally is one example; others have lyophilized their vaccines and shown continued effectiveness. Also, techniques that are needle-free could possibly ease uptake of the virus. It is possible that the VLP-based vaccines that have shown so much promise [61,72,75] in cross-protection will also need to incorporate these features in order to be globally successful. Indeed, our lab has begun to investigate formulation and long-term storage issues, including whether bacteriophage VLPs can be stored in a lyophilized format under environmental conditions.

In the long-run, the greatest hurdle may be showing an increase in clinical effectiveness compared to the upcoming nonavalent L1 VLP vaccine. Clinical trials of L2-based vaccines will require the establishment of high-throughput and standardized assays to measure anti-L2 antibody responses and the ability of patient sera to neutralize diverse HPV types. The recent development of an in vitro neutralization assay that is optimized to sensitively detect neutralizing L2 antibodies should prove useful for these evaluations [78]. Although L2-based vaccines have shown effectiveness in preventing infection with cutaneous animal papillomaviruses [82], it remains to be seen whether these successes will translate to protection from genital infection by HPV. Finally, the clinical effectiveness of Gardasil and Cervarix will set a high bar an L2-based vaccine and an effective nonavalent vaccine could establish even broader protection for a second-generation vaccine that would be difficult to surpass. In clinical trials L2-based vaccines will need to directly compared to the established HPV vaccines and the trials will need to powered sufficiently to demonstrate protection against rare high-risk HPV types. Although these barriers are not insurmountable, they are substantial.

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Key Issues

- A subset of HPV types have been categorized as high-risk due to their involvement in the development in cervical cancer
- Current HPV vaccines target HPV types 16 and 18; the two types responsible for 70% of cervical cancer cases
- Current HPV vaccines largely do not protect against infection by other high-risk HPV types
- A nonavalent vaccine targeting 7 high-risk HPV types is in clinical trials
- It is unlikely that vaccines targeting type-specific epitopes in the viral major capsid protein will be able to protect against all high-risk types
- The minor capsid protein of HPV, L2, has been shown to contain epitopes that elicit cross-neutralizing antibodies when used as a recombinant protein vaccine
- A number of approaches have been applied to increase the immunogenicity and cross-protective activity of L2-based vaccines.

Table 1

Vaccine Strategy	L2 displayed as a:	Notable Findings	References
Recombinant L2 Proteins	Concatameric polypeptide	L2 sequences from multiple HPV types may broaden cross-neutralizing potential and also increase immunogenicity by providing a degree of multivalent display	[65,66,68]
	Lipopeptide fusion	Use of P25, a TLR2 agonist, may enhance immunogenicity. Effective as an intranasal vaccine	[79]
	Thioredoxin fusion	Bacterial fusion protein; potentially low cost.	[60,80]
Mulivalent display on Virus-Like Particles	Papillomavirus VLPs	Also provokes strong anti-L1 neutralizing antibody titers against the HPV16 platform	[69–72]
	Bacteriophage VLPs	Vaccine effective without requiring exogenous adjuvants Long-lived immunity; mice were protected from HPV pseudovirus challenge 18 months after vaccination Compatible with genetic display and chemical conjugation	[53,61,77]
	Adeno-associated VLPs	Particles maintain immunogenicity upon lyophilization	[75]
	Tobacco Mosaic Virus and Potato Virus X	Production in plants	[73,74]
Recombinant Bacteria	*Lactobacteria casei*	Compatible with oral delivery	[81]