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Review Article

# HPV vaccine: Current status and future directions

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ABSTRACT

HPV Vaccine was introduced to prevent cervical cancer known to be caused by infection with one or more of the high risk subtypes of the Human papilloma virus (HPV). Since introduction, trials have proven its efficacy in preventing Cervical intraepithelial neoplasia (CIN) beyond doubt and its effectiveness in preventing cervical cancer though presumptive is reasonably certain as per mathematical modelling. It also prevents other HPV related anogenital and oropharyngeal malignancies in both sexes. HPV vaccines have courted many controversies related to its efficacy, safety, ideal age of vaccination, use in HPV infected individuals and use in males. The currently available vaccines are based on L1 Viral like particles (VLP) and hence highly species specific, thermolabile, costly and are purely prophylactic. The quest for a cheaper, thermostable and broad spectrum vaccine has led to many newer prophylactic vaccines. Therapeutic vaccines were born out of the inescapable necessity considering high HPV related morbidity projected in the non HPV naïve population. Therapeutic vaccines would immediately reduce this burden and also help in the management of HPV related cancers alone or as part of combination strategies. Ongoing research is aimed at a total control over HPV related malignancies in the near future.



## Introduction

HPV (Human Papilloma Virus) has its presence in about 5.2% of cancers in the world, and is proven to cause cervical, anogenital and head and neck cancers.[1] HPV was recognized as a cause of cervical cancer as early as 1992 and almost all cervical malignancies demonstrated oncogenic strains of HPV DNA.[2] While the organized screening programs has brought down the incidence of cancer cervix and associated mortality in the developed world, the lack of the same has increased the burden of the disease in countries like India. Lack of adequate resources and infrastructure are likely to keep cervical screening programs a distant dream in resource starved nations, alternate strategies are needed to reduce the cervical cancer burden in these nations. The recent prophylactic HPV vaccines aimed at preventing cervical cancer and its precursors has thrown in an opportunity for countries like India

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to control this epidemic.[3] Human papilloma Virus (HPV) is a non – enveloped double stranded DNA virus with a genome of 8000 base pairs encoding two protein types – 'Late proteins' $L_1$ and $L_2$ which are the structural components of viral capsid and are involved in packaging of the virus, and the 'Early proteins' $E_{1,2,4,5,6,7}$which regulate the replication of viral DNA. While the early proteins are expressed throughout the life cycle of the virus, the late proteins are expressed only during the initial stages of infection fading away later.[4] There are more than 170 different HPV types identified of which 40 are purely mucosal subtypes that includes the 15 high risk oncogenic types[5] (Table 1). The maximum number of HPV infections occur during the early years. Most HPV infections, however are transient and are spontaneously cleared by the immune system of the host except in susceptible individuals and in immunocompromised, when they persist and lead to preinvasive and invasive lesions of the genital tract.[6] The two currently available vaccines against HPV are both prophylactic vaccines meant for HPV naïve individuals – The bivalent vaccine (Cervarix) against HPV 16 & 18 and the quadrivalent vaccine (Gardasil) effective against HPV 16, 18, 6 and 11. The quest for newer vaccines continues with the aim of making it more affordable, more thermostable, more coverage towards larger number of strains and for therapeutic use too.

## The vaccine

These vaccines are produced by recombinant DNA technology by incorporating $L_1$ capsid gene of HPV 16 and 18 into a host cell (Baculovirus/Yeast) which replicates the $L_1$ proteins which then self-assemble into viral like particles (VLP) or empty viral shells similar in size and shape to HPV viron but non infective and non-oncogenic. The VLP's are mixed with a suitable adjuvant to promote immunogenicity.[3,4] Though the vaccine is species specific and the protection against HPV is limited to the two high risk oncogenetic strains interestingly there is some augmented protection exhibited by the vaccine on account of cross reactivity and the specific adjuvant used. HPV 16 (A9 Species) is phylogenetically related to HPV strains 31, 33, 52, and 58 and HPV 18 (A-7 species) to HPV strain 45 thus providing some protection against these non-vaccine strains too and increasing protection.[7]

## Immunogenicity of HPV vaccines

Though HPV infections are very common, they are cleared through an immune response mounted by the body. However natural infections produce only transient local immunity at the level of basal keratinocytes. As the viral capsid and proteins do not reach beyond the basement membrane and do not incite systemic humoral immunity they fail to prevent reinfection with the same species or infection with a different HPV species.[4,8] Prophylactic $L_1$ based VLP HPV vaccines induce much stronger immunogenicity with a long lasting and effective humoral immunity thus offering prolonged and effective protection from HPV.[8] Unfortunately the vaccines are highly type specific thus narrowing the protection only to the target species.[9]

## Drawbacks of current vaccines

### *Limited species coverage*

The $L_1$ protein being highly type specific, the protection against HPV infection is only against the vaccine strains (HPV 16, 18 in case of the bivalent and HPV 16, 18, 6, 11 in case of the quadrivalent vaccine) with some happenstance protection against a few related species as discussed earlier. Though the vaccine protects against a vast majority of HPV infections (70–80%) the remaining strains still pose the danger of HPV related disease even after vaccination.[7,9]

### *Absence of therapeutic role*

Both the presently available vaccines are $L_1$ based VLP vaccines which induce only humoral immunity, has only a prophylactic effect against HPV infections and hence effective only in HPV naïve individuals. $L_1$ proteins being structural capsid proteins involved in packaging of the virus are not expressed once infection is established in the mucosa or after infection becomes systemic and hence $L_1$ based vaccines have no therapeutic effect. A therapeutic vaccine should target oncogenetic proteins that are expressed throughout the life-cycle of the virus (E6, 7 proteins) and should be capable of inciting cell mediated immunity.[10]

### *Cost and affordability*

$L_1$ protein based vaccines are highly species specific and the only way to increase the coverage is to produce vaccine separately against each oncogenic species thus increasing the cost of production especially when one relies on the assembly of viral like particles for vaccine production. The addition of suitable adjuvant to enhance the immunogenicity not only increases the cost of production but also makes the vaccine thermolabile thus necessitating an efficient cold chain further adding storage and delivery costs making the vaccine even more expensive and unaffordable to the very population who need it most. Cost reducing strategies tried out include use of a two dose regimen in place of the currently recommended three dose schedule, bacteria based vaccines and $L_2$ protein based vaccines.

**Table 1 – Pathogenic HPV viruses.**

| Low risk | High risk | Non classified |
|---|---|---|
| 6, 11, 42, 43, 44, 55 | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 | 2a, 3, 7, 13, 26, 27, 28, 29, 30, 34, 40, 53, 54, 57, 61, 67, 70, 72, 73, 74, 81, 82, 83, 84, 87, 89, 90, 91 |

## Current controversies

### *Immunogenicity, duration of protection and requirement of booster doses*

One of the foremost concerns regarding the HPV vaccines has been the clinical efficacy and immunogenicity, its expected duration of protection and, the possible requirement of booster doses. The clinical efficacy of the vaccine has been evaluated in two phase III trials each for the bivalent (PATRICIA and Costa Rica trials) and Quadrivalent (FUTURE I and II Trials) vaccines.[11–13] High efficacy of upto 96–100% has been demonstrated by both the vaccines in HPV naïve population of fresh HPV infections in preventing CIN2 and 3 related changes. Both vaccines have exhibited very high immunogenicity with antibody titres several folds higher than after natural infections. The titres remain high enough to prevent infections for a duration of 5 years for the quadrivalent vaccine (with 98.8% seropositivity) and 8.4 years for the bivalent vaccines (With 100% seropositivity) and trials are continuing to study the protective effectiveness beyond this time period. Mathematical models have predicted the duration of clinical protection to exceed 30 years which would mean life time protection against infections. A strong anamnestic response has also been demonstrated after administration of a fourth dose of vaccine after 7 years and 5 years for the bivalent and quadrivalent vaccines respectively thus confirming enhanced response after reinfections in the immunized.[14] The present evidence confirm adequate immunogenicity, duration of protection and does not recommend booster doses.

### *Efficacy in preventing Ca cervix*

Though it has been demonstrated that all the cells of cancer cervix express HPV antigens of high risk oncogenic viruses and a cause-effect relationship has been established, the capability of HPV vaccination to prevent cancer cervix is still presumptive because the end points of all the clinical trials have been prevention of CIN 2/3 disease and not cancer cervix because the HPV vaccinated population till date has been followed up only for a period of 13 years and due to the long latent period and the prolonged pre-invasive phase after HPV infections we would have to wait for the results of the ongoing clinical trials for evidence to declare its efficacy against Ca cervix.[15] Till then what we know for sure is that the vaccine does prevent fresh HPV infections, and HPV related CIN 2/3 disease and hence is likely to prevent development of HPV related Ca cervix. However presently available vaccines do not protect against all high risk oncogenic types of HPV and hence is not going to be 100% effective in preventing Ca cervix. This also means that the screening for Ca cervix needs to continue even in the vaccinated individuals.

### *Age of vaccination*

Prophylactic vaccines are effective only in HPV naïve individuals and hence are less effective after sexarche when there is maximum chance of acquiring HPV infections. Hence the vaccine has been recommended to be initiated at a very early age of 9 years which has generated a debate whether vaccination is indeed recommended at such an early age when sexual activity is unlikely for many more years. Since it has been proven beyond doubt that the dynamic squamocolumnar junction which eventually forms the transformation zone is highly immature and susceptible to HPV infection and HPV related oncogenic damage, maximum benefit of vaccination would be only if vaccination is completed before sexarche. Moreover the maximum immunogenicity of the vaccine is between 9 and 14 years compared to later – another reason to administer the vaccine at an early age.[16] Another argument against early vaccination is that the peak age of HPV infection is only after 20 years of age. But the duration of protection now has been proven to be definitely for more than 10 years and possibly life long and with a very strong anamnestic response being demonstrated at reinfection with HPV later, this argument does not hold good.

### *Vaccination beyond 26 years and in HPV infected population*

There are women at all ages who will benefit from prophylactic HPV vaccination, but the proportion of women who will benefit decreases with age. The benefits of vaccination with either the bivalent or quadrivalent HPV vaccine is greater in younger women as compared to that in older women (Younger than 25 years of age vs. 25 years or more).[13] Rationally, to extend HPV vaccination to older women, cost-effectiveness must be taken into account. Two novel strategies that integrate vaccination and screening in older women have been proposed to reduce the need for screening, but need validation. First, a vaccinate and screen strategy, in which women would be vaccinated and then screened 1 year after vaccination for the presence of high-risk HPV in the cervix.[17] Women who tests positive for high-risk HPV at follow-up will very likely have persistent high-risk HPV from a pre-existing HPV infection present at the time of vaccination and be at high risk of having or developing CIN2+, and could therefore be managed aggressively. Second, a screen and vaccinate strategy, in which women would first be screened for high-risk HPV, with those who test positive given follow-up management or treatment and not being vaccinated, and those who test negative being vaccinated. In both scenarios, vaccinated, low-risk women are protected against acquisition of new infections by the highest risk HPV genotypes, and might need screening either never again or at a much lower frequency than if not vaccinated. Both approaches, if validated, are promising, especially for low-resource settings in which several rounds of screening might not be financially sustainable. Skinner and colleagues affirm that prophylactic HPV vaccination is safe and prevents the acquisition of target HPV genotypes at any age, and that woman of any age could gain some potential benefit from HPV vaccination.[18]

### *Vaccine resistance*

It has been proven that HPV vaccine would reduce the rate of infections and related disease due to vaccine types, but there is a genuine concern regarding the possibility of an increase in proportion of infections with other non-vaccine oncogenic types, development of escape mutations of vaccine strains

and evolution of new oncogenic strains thus making vaccines less effective.[16] However, HPV viruses are considered genetically stable viruses and are highly unlikely to undergo type replacements nor are likely to develop escape mutants and hence vaccine resistance is an unlikely possibility even after increasing vaccine coverage.[19]

### *Vaccination of males*

Though Ca cervix is the first HPV related malignancy identified; now it is known that HPV infection in males leads to anal, penile and oral cancers and anogenital warts. Males also account for the disease load by increasing the transmission to females and thus an indirect role in causation of cervical cancer.[20] Quadrivalent HPV vaccines have been propagated as a means of not only reducing the incidence of HPV related malignancies and anogenital warts in males but also to protect women against HPV related infection by promoting herd immunity. Though routine vaccination of males is recommended as part of vaccination schedules of some countries (Australia, New Zealand) cost – benefit analysis has shown that increasing vaccine coverage in girls would be more effective in reducing HPV infection and HPV related disease.[21]

### *Vaccine safety*

With increasing popularity of this vaccine and its introduction in many national immunization programs allegations have continued to surface in the media and elsewhere about the safety of the vaccine with unsubstantiated reports of some neurological sequelae like Multiple sclerosis and mortality. With >175 million doses distributed worldwide WHO and CDC have investigated in detail the safety profile of this vaccine and have reassured the users of its safety but has proposed continuous surveillance for adverse events. Surveillance of outcomes among women inadvertently vaccinated during pregnancy has not detected any adverse outcomes. However vaccination during pregnancy is not recommended.[22]

## The quest for newer vaccines

Both the currently available vaccines are $L_1$ VLP based and hence highly species specific, purely prophylactic, but thermolabile and costly. The quest for a cheaper, thermostable vaccine with wider coverage against all the oncogenic viruses has resulted in the development of the new generation of prophylactic vaccines. These include a nine-valent vaccine, an L2 based vaccine, a Capsomere vaccine and a Chimeric L1, L2 vaccine.

### *Nonavalent HPV vaccine*

The currently available vaccines (HPV 16 and 18) protect against only 70% of squamous cancers. To increase the protection a nonavalent vaccine is undergoing trials. This vaccine code named V 503 in addition to the four HPV oncotypes of quadrivalent vaccine (HPV 16, 18, 6, 11) also covers for five more strains of HPV (HPV 31, 33, 45, 52 and 58) thus increasing vaccine coverage to infection by nine oncotypes thus making it more efficacious and cost effective.[23]

### *Prophylactic $L_2$ vaccines*

The type specificity of $L_1$ based vaccine reduces the spectrum of protection and increases vaccine cost. The research for a pan HPV vaccine that would augment vaccine efficacy and reduce cost, led to the discovery of the $L_2$ based vaccine based on the finding that small proportion of the $L_2$ protein between amino acids 20 and 38 are highly preserved in most high risk oncotypes of HPV and antigens against this region generate neutralizing antibodies against a wide spectrum of HPV species including most oncogenic types. However the problem of poor immunogenicity prompted search for strategies to enhance immunogenicity. These include Bacteriophage PP7 derived $L_2$ VLP and oral immunization using Lactobacillus caseii based HPV 16 $L_2$. The possibility of mass production of $L_2$ based vaccine in the bacteria *Escherichia* coli as opposed to Yeast (Gardasil) and insect host cells (cervarix) would add to further reduction in cost.[24]

### *$L_1$ capsomere vaccines*

Currently available prophylactic vaccines are VLP based and require 360 copies of $L_1$ protein to constitute one VLP and this increases cost. A cheaper, equally effective and thermostable alternative would be the capsomere based vaccine. A capsomere is the basic structural component of viral capsids and only five $L_1$ monomers are required to assemble a thermostable pentavalent capsomere of HPV with comparable immunogenicity. This can be produced in bacteria (Salmonella typhimurium and *E coli*) further reducing the cost. A phase II clinical trial of an *E coli* derived HPV 16/18 $L_1$ capsomere vaccine is currently being conducted (NCT 01355823).[25]

### *Chimeric VLP vaccines*

$L_1$ vaccines have good immunogenicity but are limited by specificity of protection and $L_2$ based vaccines though have wider protection have poor immunogenicity. Genetically fusing and chemically conjugating $L_1$ and $L_2$ proteins would combine the advantages of both resulting in a highly immunogenic vaccine with wider coverage. $L_2$ proteins would thus have a vaccinogenic effect when combined with an $L_1$ vaccine due to the innate cross-reactivity of $L_2$ proteins thus increasing the efficacy of the vaccine.[26]

## Therapeutic vaccines

Current HPV vaccines are purely prophylactic and is effective only in HPV naïve individuals. These vaccines lack therapeutic effect because of two main reasons. (a) They are based on $L_1$ viral capsid proteins whose expression fades away after primary HPV infection and hence undetectable in HPV associated disease and malignancy. (b) They induce only humoral immunity and not cell mediated immunity that is essential for a therapeutic effect. A successful therapeutic vaccine would be

one where the antigen is presented to T cells for inciting adequate cell mediated response. This requires the vaccine be based on target antigens expressed actively throughout life-cycle of HPV. HPV $E_6$ and $E_7$ proteins are strongly expressed on tumour cells and has been the target of research for development of therapeutic vaccine. The challenge however is to present these antigens to the immune system to induce cell mediated immunity against HPV infected cells.[27]

## Do we actually need a therapeutic vaccine?

Prophylactic HPV vaccines would definitely bring down the incidence of HPV infections. But even after the vaccine coverage reaches 50% or more it would take at least 20 more years for it to translate into significant reduction of HPV related morbidity. Hence without an effective means to control natural progression of the HPV infections, pre invasive and invasive cervical disease would continue even in vaccinated population. This necessitates a simultaneous therapeutic vaccination that can be administered to Non HPV naïve individuals and individuals with proven HPV related disease to bring down the incidence of HPV related disease in recent years.

This led to the search for therapeutic vaccines that would incite a strong cell mediated immune response against HPV to control infection and kill tumour. The strategies being tried include use of bacterial and viral vectors for introduction of HPV antigen into host, direct introduction of viral peptides, proteins or even DNA into the host and a dendritic cell based strategy for antigen presentation and T cell activation.[27]

### *Live vector based HPV vaccine*

A live vector which may be bacteria or virus is used to deliver $E_6$ and $E_7$ antigens to the antigen presenting cells of the host to induce the CD8+ cytotoxic cells and CD4+ T helper cells that attack the specific HPV target antigen. An unique property of the vectors to replicate within the body initiates the active spread of the antigen thus potentiating the vaccine. However the intrinsic pathogenic potential of the vector poses safety hazard especially in immunocompromised. The possibility of vaccine resistance by development of vector specific antibodies or by a pre-existing vector-specific immunity thus making the vaccine potentially ineffective needs to be addressed.[28,29]

### *Bacterial vector based vaccine*

Many bacteria have been studied as vectors for HPV immunization. One of which, the Listeria monocytogenes, invades macrophages and evades phagocytosis within the phagosome by using Listeriolysin O (LLO) a pore forming toxin, can successfully deliver the antigen into cytoplasm and in turn activate CD8+ cytotoxic cells and CD4+ T helper cells finally creating cell mediated immunity and is being tried as ADXS II-001, a live attenuated Listeria based HPV 16 E7 vaccine on patients with HPV associated oropharyngeal cancers, cervical cancers and CIN 2/3.[28] Lactobacillus casei and Lactobacillus lactis also has been tried as bacterial vectors.

### *Viral vector based vaccines*

An enveloped double stranded DNA – 'Vaccinia virus' has been developed as a viral vector when encoded with E7 fused to Calreticulum or Listriolysin O (LLO) due to its large genome and high infectivity. A recombinant vaccinia vector based therapeutic vaccine expressing HPV 16 & 18 E6/E7 antigen (TA-HPV) has been evaluated in Phase I and II clinical trials in cervical cancers, VIN and VAIN. MVA-E2, MVA-HPV – IL2 and TG 4001/R3484 are some other vector based vaccines being tried out in clinical trials.

### *Peptide and protein based vaccines*

Peptides derived from HPV antigens when directly administered leads to direct uptake of peptides by dendritic cells for antigen processing and presentation thus activating antigen specific T cell immunity. These vaccines are likely to be safe, stable and easy to produce but are of low immunogenicity and hence require adjuvants to be effective. As part of the research to develop a well-tolerated formulation that is effective in generating a strong immune response, HspE7 a chimeric protein of BCG heat shock protein (Hsp65) and HPV 16E7 is being tried in phase II trials in HSIL and CIN2/3.[29]

### *DNA vaccines*

DNA based vaccines employ direct injection of plasmid DNA encoding HPV antigen into host cells thus promoting expression and presentation of encoded antigen by the transfected cells thus stimulating appropriate cell mediated immunity with or without associated humoral response. Naked DNA is easy to manufacture, safe from inherent risks associated with bacteria and virus and do not develop antibodies and hence vaccine resistance. However poor immunogenicity is the main problem. Novel vaccine delivery methods to increase potency that are being tried include Gene gun, microencapsulation, electroporation etc. pnGVL4a-CRT/E7 (detox) is a therapeutic DNA vaccine undergoing phase II trial for treating CIN2/3 lesions.[26,28,29]

### *Dendritic cell based vaccines*

Dendritic cells are the dedicated antigen presenting cells in the body which induce T cell response through MHC –I and II pathways. 'Pulsing' dendritic cells with HPV antigens or peptides or DNA encoding the antigens in vivo enables loading of the MHC I and II molecules with HPV epitopes. On readministration of these dendritic cells they elicit a strong and specific immune response against these HPV antigens. Preliminary phase I clinical trials of HPV 16 and 18 E7 based vaccines have proven safety and immunogenicity and is awaiting phase II trials.[30]

## Combination strategies

Treatment of frank invasive cancer related to HPV uses in addition to surgery, radiotherapy and chemotherapy. For

developing a more effective control of HPV related disease a combination strategy involving other modalities used along with therapeutic vaccines is expected to increase disease control. The combination of radiation, chemotherapy and immunotherapy has been tried and has shown cumulative antitumour effects in mice models. Chemotherapeutic agents in combination with DNA based vaccine is also being tried. The chemotherapeutic agent Apigenin being used along with a DNA-encoding heat shock protein 70 (HSP70) and HPV 16 E7 DNA has shown high frequency of effector CD8+ T cells and memory CD8+ T cells and an increased tumour susceptibility to E7 specific cytotoxic immune responses and maximum antitumour effect.[26]

## Conclusion

The development of HPV Vaccine has been a landmark, not only among the discoveries of 'Vaccines', but in the prevention of HPV related infections and on towards prevention of cervical cancer. The vaccine is safe and is also efficacious. The real efficacy would be proven approximately 7 years from now when it would complete a total period of 20 years follow up. Many more lives would be lost if mankind waits for another decade for the final results. For this reason the Indian Academy of Paediatricians, too have already included the HPV vaccine in its schedule as done already by more than 120 countries around the globe. However more robust evidence needs to unfurl before it is included in the National Immunisation Programme. The benefits of such a programme would be maximal in a nation like ours where the incidence of cervical cancer is not only high but the implementation of the screening program is less than optimal.

These vaccines also need further modifications so that future generations of vaccines would not only be more effective, with wider coverage but also cheap and affordable to all. The next generation vaccines also need to have a therapeutic effect so that they can be used not only in HPV infected individuals for preventing future infections but also in the treatment of HPV related disease including frank cancer alone or in combination with other modalities of cancer therapy as a synergistic agent in prime boost regimens. Continuing progress and active research in this field would eventually allow us total control over HPV associated malignancies and especially over cervical cancer in the foreseeable future.

## Conflicts of interest

All authors have none to declare.

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