

## Human Papilloma Virus (HPV) Vaccines: Scope and Implications

Roshan Kumar Singh<sup>1</sup>, Sandeep Satapathy<sup>2\*</sup> and Abhilasha Singh<sup>1</sup>

<sup>1</sup>Department of Zoology, University of Delhi, Delhi, India

<sup>2</sup>Indian Institute of Science Education and Research, Bhopal

\*Corresponding author: Sandeep Satapathy, Indian Institute of Science Education and Research, Bhopal,

Tel: +91 9437543777; E-mail: [satapathys@live.in](mailto:satapathys@live.in)

Received date: June 08, 2016; Accepted date: June 23, 2016; Published date: July 12, 2016

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### Abstract

High risk human papilloma virus has often been associated with cancer onset and progression by the virtue of the viral oncogenes. Genital cancers and Oropharyngeal cancers are the two leading carcinomas often induced by HPV infection. These viral encoded oncogenes (e.g. E6/E7/E8) are known for their ability to integrate, recombine and interfere with the host cellular genes. Although significant work has been undertaken and several more in progress in understanding the disease biology, yet attaining an effective and long term protection from HPV associated cancers remains unachieved. Lack of any drug based therapy or post-infection treatment strategy along with limited commercially available vaccines (e.g. Gardasil and Cervarix) have crippled our efforts to curb the disease manifestation cancers, remains. Therefore, in this review we aim to address the contemporary rational of HPV vaccine designing and development. We have also analyzed the scope and future perspectives of the commercially available vaccine regimes.

**Keywords:** Human papilloma virus; Vaccines; Gardasil; Cervarix; Viral oncogenes; Papilloma

### Introduction

The Human Papillomavirus (HPV) belongs to the ancient taxonomic group of Papillomaviridae comprising the non-enveloped DNA viruses. These viruses are known to pathologically infect mammals [1] and certain reptiles like snakes and tortoise [2-4]. Even though several sporadic reports confirm the detection of these viruses in some non-mammalian representatives, mammals especially humans serve as their principal hosts [3]. The double stranded and circular genomic DNA of the HPV spans approximately 8Kb [2]. HPVs have a skewed preference for infection site [1] and several reports have established that the basal layer of the stratified epithelium is the primary site of infection of these viruses [1]. On the basis of such a tissue tropism, HPVs have been characterized into 200 subtypes [1,5]. A broad classification of these subtypes into two groups has been described by Mistry et.al. As such this classification denotes HPVs as either cutaneous HPVs [5] (infecting the skin) or mucosal HPVs (infecting the mucosal epithelium) [6].

### Knowing the HPVs: Life and life cycle

The HPV targets the highly dividing lower basal membrane of the stratified epithelium through any micro lesions or micro abrasions that exposes it [7]. The uppermost layer of epithelial cells infected with viruses is sloughed with cell division of the lower layers. This transmits the infection either directly to the next host (mucosal types) or by finding a new epithelial layer for infection (cutaneous type) [7].

The mode of internalisation is specific to different subtypes of HPVs [7]. Often these modes of internalisation include either clathrin-mediated or caveolar-endocytosis and subsequently transported to the nucleus [7]. Upon nuclear entry, these viruses substantially

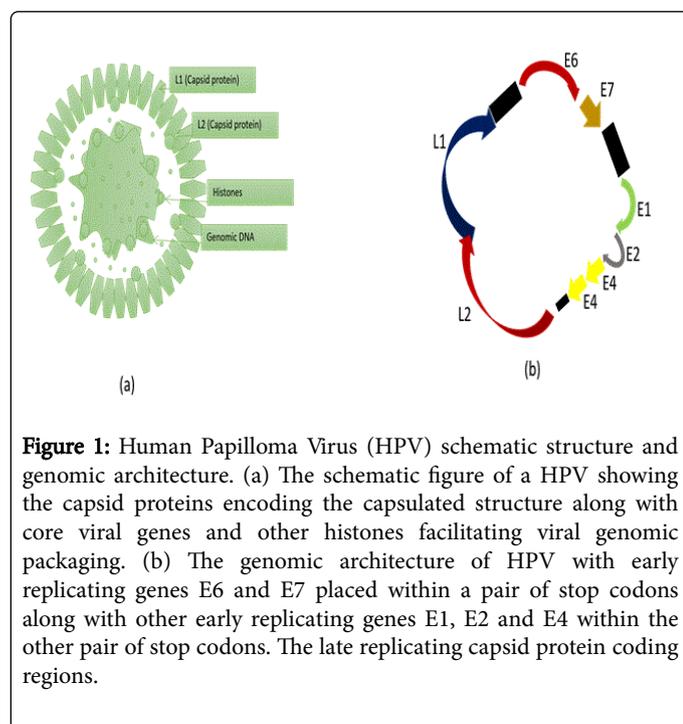
replicate their genome using the host cellular machinery [7]. These genetic elements of virus integrated into host genome encodes for virus specific and modified or recombinant host proteins that modulate and interfere with several biological processes [7,8]. Newly synthesized viral capsid proteins are assembled into new virus particles in the layers of the partially differentiated upper epithelium with around 50-100 copies of virus per cell [8]. Therefore, a successful integration into host genome leads to expression of the viral encoded genes (core genes and the accessory genes e.g. capsid protein coding genes).

The genome of the HPV virus is broadly organised into six early replicating genes (E1, E2, E4, E5, E6 and E7), two late replicating genes (L1 and L2) (Figure 1) and a long control region (LCR) [9]. Among these E6 and E7 encoded (viral oncogenes) protein products are decisive in tumour severity, progression and the onset of malignancy [9] (Figure 1). The E7 protein of high risk HPVs binds and degrades the tumour suppressor Retinoblastoma (Rb) leading to free activation of genes involved in cell cycle progression mediated by elongation factor E2F (the typical binding transcription factor of Rb) [10] (Figure 1). In addition, E6 viral oncogene binds to tumour suppressor protein, p53 and leads to its proteosomal degradation by ubiquitination [9]. Consequently, the G1 to S cell cycle arrest is inhibited. E6 also promotes the expression of telomerase (TERT) that repairs the chromosomal telomeres and is involved in cell division [9,10]. Collectively, these E7/E6 mediated effects drive the cell towards proliferation, which, due to the loss of restraining effects of Rb and p53 results in cancers of varied kinds [10].

### HPV and diseases

The most common mode of transmission of HPVs has been via sexual transmission [11]. In addition it has been reported that transmission of viral infection from the pregnant mothers to the neonates and under the condition of immunosuppression also exist [11]. Though the majority of the HPV infections remain dormant,

asymptomatic and clear off on their own within 1-2 years of initiation, some of them bear the capacity to become potentially pathogenic to the hosts [10,11]. The cutaneous (beta-type) HPV infections predominantly result in benign papillomas and pre-malignant lesions, for instance skin and genital warts [11]. However, in contrast, the chronic infections are often caused by alpha-type mucosal HPVs with a high oncogenic transformation potential of benign tumours [11]. Interestingly mucosal HPVs are usually associated with chronic viral infections and often result in carcinogenic transformation of host cell and further complications due to malignancies [11]. In addition, these mucosal HPVs have been categorized into low risk and high-risk types depending on the extent of malignancies inflicted. The latter group comprising HPVs16 /18/ 31/ 33/ 45/ 51/ 52/ 58 etc. is associated with more than 90% of the mucosal cancers including cervical, anal, oropharyngeal, penile etc [12]. Several studies have been conducted in the past and many more are ongoing to explore this virus mediated oncogenic transformation and mechanism [13-16]. However, the further analysis of the same is beyond the scope of this paper.



**Figure 1:** Human Papilloma Virus (HPV) schematic structure and genomic architecture. (a) The schematic figure of a HPV showing the capsid proteins encoding the capsulated structure along with core viral genes and other histones facilitating viral genomic packaging. (b) The genomic architecture of HPV with early replicating genes E6 and E7 placed within a pair of stop codons along with other early replicating genes E1, E2 and E4 within the other pair of stop codons. The late replicating capsid protein coding regions.

## HPV associated cancers

**Genital cancers:** The cancer of the genitals comprises the most prevalent group of cancers caused by the high risk HPV types [14]. In such cases sexual transmission remains the primary mode of transmission in both, men and women [15]. Most cases of HPV mediated genital cancers are that of cervical cancers of which, over 65% are caused by HPV 16 and 18 [17-19]; vulvar cancers [20,21]; penile cancers [22-24] and the anal cancers [25,26].

**Oropharyngeal cancers:** HPV infection of the oral and respiratory mucosa leads to oropharyngeal cancers including that of head and neck cancers [27]. The oropharyngeal cancers usually initiate near the base of the tongue or tonsils and progress into other areas [27,28]. While the prevalence of oral HPV infections is comparatively much lesser than the genital ones, still almost 95% of the head and neck cancers are caused by HPV16 infection [29,30]. Several other studies

have indicated the pathogenic role of HPVs in case of non-small cell lung carcinomas [31], although further experimental validation is essential for a better understanding of the HPV pathogenicity and carcinogenesis [31].

Another aspect of HPV infection is its involvement in a rare pathological condition known as Recurrent Respiratory Papillomatosis (RRP) [32]. This disorder is characterized by the development and growth of wart-like lesions in the oropharyngeal pathways by HPV 6 and 11 infections [32]. HPV 6 and 11 are known to cause more than 90% of the RRP cases [32]. Certain other HPV sub-types such as 16 and 18 have also been reported to be responsible for RRP albeit to a much lesser extent [33]. Furthermore RRP may also prove to be life threatening despite its benign nature in cases where these wart-like lesions become obstructive in the respiratory tract [32,33].

**HPV immune response:** The prevalence of HPV infection and the association of high risk HPVs with carcinogenesis involve about 5% of all human cancers [32]. The low risk and high risk HPVs are different in terms of their resident time within the host and the mechanism of elimination of this virus. Infections with the low risk HPVs are usually cleared spontaneously (i.e., viral DNA undetectable) within 4-8 months while the majority of high risk HPVs take almost 12-18 months to clear off from the human physiological system [33]. The most common association of chronic HPV infection has been in promoting the tumour progression and onset of malignancy [33]. It is important to note that these viruses are able to evade the immune system response while being able to establish itself in a chronic manner. In order to accomplish such an effective evasion of host immune response HPVs utilise several strategies discussed here. Firstly, during early HPV infection the target of infection is the basal epithelium wherein the virus replicates with that of the host dividing cells [34]. As a result, these cells are exposed to a minimal viral load that is within the tolerance capacity of host immune system [34]. This suppresses immune response in the local environment at the site of infection otherwise known as host cells that are immunologically unresponsive to the presence of the virus [34]. Secondly, the virus proliferates itself in the differentiated keratinocytes comprising the upper layer of epithelium farther from vasculature. This distance from the vasculature keeps the virus away from immune surveillance and thus enabling its propagation [35]. Also, the release of viral load from the keratinocytes that are committed to natural cell-death, does not result in usual cytotoxic/cytolytic effects [35]. This limits any subsequent inflammatory or apoptotic response and the associated activation of the immune system. Thirdly, the virus inhibits the synthesis and signaling of virus-targeting immune molecule IFN- $\gamma$  (Interferon- $\gamma$ ) and hence downregulates antiviral genes induced by it [36]. Also the viral oncoproteins E6 and E7 hinder the interferon signaling pathways [36]. In addition to these immune evasion strategies, the virus is successfully able to escape the activation of TLR-9 (Toll-like receptor -9) [35], chemokines [35], NK cells [36] and the Langerhans Cells (LC) [36] facilitating its prolonged survival in the epithelium [34-36].

## HPV Vaccines: rationale and development

With a worldwide prevalence of HPVs and the growing clinical and scientific evidence of its association with cancer onset and progression, the biology of vaccine designing against HPVs has become an urgent scientific need and concern. In addition, the unavailability of any drug based therapy or other post-infection treatment strategy for the cure of HPV infection substantiates for further critical analysis and

development of HPV vaccines. Following the idea, that the prevention of HPV infections could in turn prevent HPV associated cancers, research accomplished in the last decades set in motion the development of vaccines targeting HPVs. The present scenario of use of such vaccines through various government and institutionally aided vaccination programs for teenaged/young males and females could play a key role in subsiding the incidences of HPV related cancers especially those that are transmitted through sexually contact.

Due to skewed species-restricted infection ability, HPVs are unable to induce carcinomas in animals other than humans [37]. Therefore, this has enabled the use of various animal models or closely related species for their respective animal papillomaviruses (PVs) have led to the development of prophylactic vaccines against HPVs. Principally, a prophylactic viral vaccine is designed to generate adequate amounts of virion antibodies in the host. These antiviral antibodies can effectively neutralise the viral load in the infected cells and thus effectively restricting the infection [37]. An enormous breakthrough in the development anti-HPV vaccine was the work done by Kirnbauer et al. [38]. Their work demonstrated that the overexpression of major capsid protein L1 of HPVs could self-assemble into virus like particles (VLPs) even in the absence of viral genome [38]. Moreover, the co-expression of L1 with the minor capsid protein L2 of HPVs produced VLPs that induction of neutralising antisera with high titers [39,40]. Presently using effective bioengineering and pharmaceutical skills these key findings have resulted in the development of two prophylactic HPV vaccines. Gardasil and Cervarix are these FDA approved vaccines developed by Merck & Co. (USA) and GlaxoSmithKline (Europe) respectively [41]. These commercially available vaccines utilize the major capsid protein L1 overexpressed in yeast/insects and self-assembled into VLPs as antigens [41]. The clinical trials for both the vaccines exhibited their 100% efficacy against chronic infections of HPV types 16 and 18 [41]. Specifically, Cervarix responds to 70% of all cervical cancers and Gardasil for most cases of HPVs 6/11/16 or 18 infections [41]. A more detailed overview about the clinical trial results, efficacy data and the cost estimation data for Gardasil and Cervarix in women with cervical cancers has been reported elsewhere [42].

Recently Merck & Co. (USA) have developed another nonavalent vaccine V503 or Gardasil9 directed against the seven most frequently detected HPV types in cervical carcinomas and HPV6 and 11 in genital warts. The preliminary results of advanced phase 3 clinical trials of this vaccine indicate its efficacy to be at least comparable to Gardasil with additional protection against five more cervical cancer-inducing HPV types apart from 16 and 18 [43,44]. The additional HPV types targeted by Gardasil9 are HPV 31/33/45/52/58 [45]. The cost effectiveness for its mass usage needs further evaluations in the coming years.

### Collectively, the novel therapeutic vaccines can be briefed as under

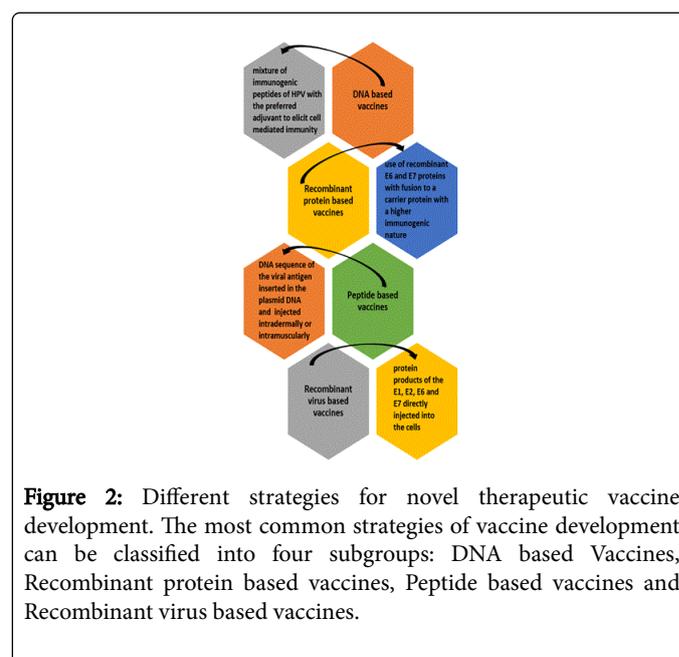
**Peptide based vaccines:** These vaccines utilise a mixture of immunogenic peptides of HPV with the preferred adjuvant to elicit cell mediated immunity [46] (Figure 2). The antigen presenting capability of the peptide is carefully taken care of while designing the vaccines [46].

**Recombinant protein based vaccines:** The use of recombinant E6 and E7 proteins comprises another strategy for the production of therapeutic HPV vaccine (Figure 2). Such a long length of recombinant

viral protein enhances the epitope content even after processing of the proteins by antigen presenting cells [47]. However, this has also been seen to reduce the immunogenicity [47]. The work done by Frazer et al. [47] and Kaufmann et al. [48] have attempted to compensate the reduced immunogenicity by fusing the viral protein to a carrier protein with a higher immunogenic nature (Figure 2) and mixing with appropriate adjuvants [47,48].

**DNA based vaccines:** These vaccines are based on a comparatively newer approach for the designing of HPV vaccines. The DNA sequence of the viral antigen is inserted in the plasmid DNA, which is then injected intradermally or intramuscularly (Figure 2) [49]. The antigenic viral proteins encoded by the plasmid DNA aid in stimulating immune response by both, humoral and cell based ways [50].

**Recombinant virus based vaccines:** The protein products of the early replicating genes (E1, E2, E6 and E7) of the virus are directly injected into the cells that cause a stronger immune response due to the number of antigens involved and superior antigen presentation than other approaches (Figure 2) [51]. These are easy to manufacture and highly immunogenic vaccines [52].



**Figure 2:** Different strategies for novel therapeutic vaccine development. The most common strategies of vaccine development can be classified into four subgroups: DNA based Vaccines, Recombinant protein based vaccines, Peptide based vaccines and Recombinant virus based vaccines.

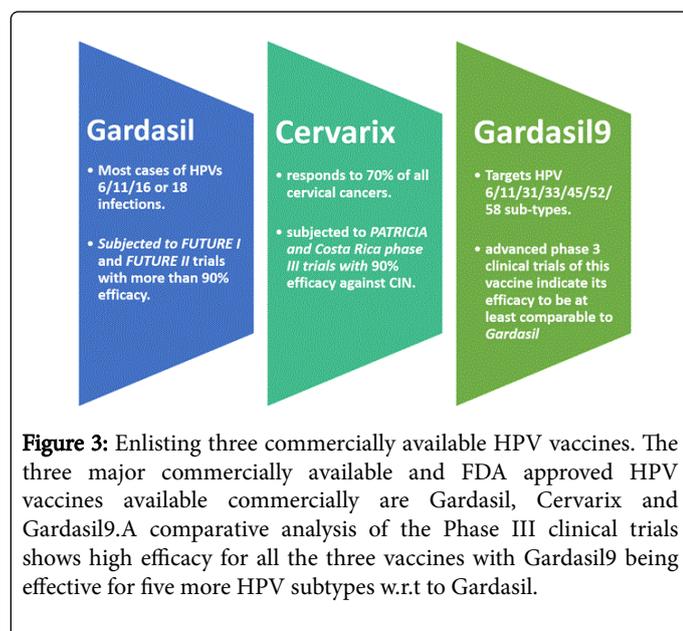
Even though the above novel strategies have yielded variable results, the recombinant virus based strategy has generated some interesting outcomes with respect to treatment of the HPV-induced lesions [53].

### Implications and future perspectives

The two commercially available HPV prophylactic vaccines, Cervarix and Gardasil have been introduced in many countries. Majority of the vaccination programs prescribe a regular scheduled use in teenaged girls. Certain programs offer vaccination even in older females who did not have access to these vaccines previously. Lately, routine programs of immunization with quadrivalent HPV vaccines for adolescent males have also been initiated in several countries [46]. Due to the time lag between infection and cancer onset, estimation of the efficacy of both the vaccines as preventive measures against HPV induced carcinomas in real-world conditions becomes challenging

[47]. In addition further examinations are in progress to assess the impact of these vaccines for future programs and policies [54,55].

The efficacies of both Cervarix and Gardasil in clinical trials are positive for prophylactic action. The bivalent vaccine Cervarix was subject to two phase III trials namely, PATRICIA and Costa Rica phase where it exhibited an efficacy of over 90% against Cervical Intraepithelial Neoplasia (CIN) (Figure 3). Cervarix is effective against CIN 2+ and CIN3+ irrespective of the type of HPV causing the infection [48]. Similarly, the According to Protocol (AIP) outcomes of the FUTURE I and FUTURE II trials of Gardasil too revealed more than 90% efficacy in CIN2+ and CIN3+ cases [48] (Figure 3). However, the Intention to Treat (ITT) analysis depicted 40-50% of efficacy in the same vaccine trials [56]. Apart from the CINs, a study originating based on cases from several many nations reflected that the vaccine administration provided a significant protection in the cases of genital warts with an efficacy of almost 83% in women and men [57]. When analyzed with respect to the age of the client, a higher efficacy was observed in younger girls ( $\leq 14$  years of age) with most efficient response at 22 years and a decline thereafter [58].



**Figure 3:** Enlisting three commercially available HPV vaccines. The three major commercially available and FDA approved HPV vaccines available commercially are Gardasil, Cervarix and Gardasil9. A comparative analysis of the Phase III clinical trials shows high efficacy for all the three vaccines with Gardasil9 being effective for five more HPV subtypes w.r.t to Gardasil.

The evaluation of the immunogenicity of these vaccines reflects a highly immunogenic nature of both vaccines with differential titers of antibodies in each case. The neutralising antibody titers against HPV 16 and 18 stood 3.7 times for Cervarix and 7.3 times for Gardasil [59], 7 months post- vaccination in women belonging to 18-26 years of age [60]. These levels were observed to be more or less sustained even after 48 months after vaccination suggesting that the immunogenic responses to these vaccines were long term. Lastly, the prophylactic effects of the bivalent and the quadrivalent vaccines are known to target HPVs other than sub-types 6, 11, 16 and 18 [53]. In addition to primary HPV targets the bivalent vaccine offers protection to HPV 31/33/45 and the quadrivalent vaccine exhibited to additionally cross-protect only against HPV 31 [52,53]. The efficacies of the cross-protection are considerable (~50-60%) [62] While no such additional protection was observed against HPV 52/58 with either of the vaccines [63-65].

## Conclusion

The contemporary HPV vaccines are largely based on developing immunity for blocking the oncogenic transformations due to HPV infection. However, these vaccines need to be administered prior to HPV induced carcinogenesis and thus presents a major technical limitation. In addition, such a stage specific administration of vaccine hints a key role of immune cells and immune memory development prior to the actual infection. This can be explained by the fact that remedying the existing HPV linked disease would require activation of cell-mediated immune responses especially that of helper CD4+ and cytotoxic CD8+ T cells. Moreover, such a process requires the expression of viral antigen/s to effectively identify and eliminate the viral load in the affected cells. This fact has led to the emergence of newer studies for the development of novel HPV Therapeutic Vaccines as a new line of treatment. Since therapeutic vaccines require the viral antigens for evoking T-cell immune response, they essentially involve the use of various viral proteins. While the viral capsid proteins L1 (major) and L2 (minor) are poor antigens owing to their weak expression in the keratinocytes, the protein products of early genes E1, E2, E6 and E7 of HPV bear the potential to serve as superior immune-stimulators due to their constitutive expression in multiple stages of carcinomas. Both E1 and E2 are DNA binding proteins and are known to bind to the origin of replication and various promoter elements of the virus genome respectively. As discussed earlier E6 and E7 viral encoded genes degrade the tumor suppressor like p53 and Rb and thus induce aberrant cell cycle progression leading to carcinogenesis and malignancies. With an understanding of the aforementioned HPV vaccines such as Gardasil and Cervarix, it can be concluded that these have been successful in providing prophylaxis against various intraepithelial neoplastic lesions as well as carcinomas to varied levels. However, this approach is limited to cases with no prior history of HPV infection. Thus for cases with HPV induced lesions and carcinomas, these vaccines are still debatable and underscored.

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