Introduction

Vaccines are an effective public health measure. Papillomavirus research and introduction of vaccines to prevent one of the major cancers in women—cervical cancer, is an excellent example of translational research, wherein the collective knowledge of molecular biology, virology, immunology and biotechnology from bench has been brought into the clinic. The WHO has stated that they will work in consultation with expert advisors to develop guidelines to assist countries in integrating HPV (human papillomavirus) vaccination into their immunization, cancer control, reproductive health and adolescent health programmes\(^1\). The impact of this new vaccine will be realized only if and when it is effectively delivered to the populations that need it most. Considerations for policy makers include the disease burden, health infrastructure, capacity for initiating and sustaining an immunization program for adolescents, affordability, cultural acceptability, political will and of course public support. But, we should also take into account that while the current vaccines confer type-specific immunity against two most commonly prevalent “high-risk” (HR) or oncogenic types, at least 15 types of HR HPV have been associated with progression to invasive cervical cancer (ICC). Do we have sufficient data on the HPV type distribution in India? Will the implementation of the current vaccine programme be adopted to the Indian...
conditions? This review will discuss cervical cancer with respect to HPV infection, with special emphasis on the prevalence and distribution of the major HPV types in Indian population for introducing in India the currently available HPV vaccine.

Cervical cancer

Cancer of the cervix uteri, is the second most common cancer among women worldwide, with an estimated 493,000 new cases and 274,000 deaths in 2002. The disease represents a major health inequity; approximately 83 per cent of the cases occur in developing countries; for the reason that cervical cancer affects relatively young women, it is an important cause of lost years of life in the developing world.

The majority of cases of cervical cancer are squamous cell carcinomas (SCCs); adenocarcinomas are less common. However, in various countries, over the past two to three decades, for reasons not yet known, the trend is shifting towards more in the cervical adenocarcinoma cases. The rates of incidence and mortality of cervical cancer, has declined in the last 40 years in many western countries, primarily due to screening. In developing countries, the incidence and mortality rates have been relatively stable or have shown modest declines. The absence of overall decline - as observed in resource-rich populations- probably reflects the lack of screening programmes, or where programmes have been introduced, their low population coverage and poor cytology.

Cervical cancer detection: role of screening

Existing patterns of incidence worldwide, reflects the underlying risk and prevention of its manifestation as invasive cancer by effective screening. The goal of cervical cytologic screening programs is the detection of cervical cancer and precursor lesions. The Pap (Papanicolaou) cytological test relies on the microscopic examination of exfoliated cervical cells. But, the sensitivity and specificity of cytological screening vary widely. Improved, liquid-based cytology, visual inspection with 3-5 per cent acetic acid, magnified visual inspection with acetic acid and visual inspection with Lugol’s iodine have been evaluated as alternative tests. More recently, since infection with oncogenic HPV has been identified as the underlying cause of cervical cancer, there is interest in the use of HPV testing by hybridisation procedures or polymerase chain reactions, as a primary screening test for cervical cancer.

Cervical cancer: role of human papillomavirus

Genital HPV infections, commonly sexually transmitted, have strongly and definitely been linked as the primary cause of cervical cancer in women. A landmark study has shown that HPV DNA can be found in 99.7 per cent of cervical cancer specimens. HPV fulfils the criteria for a carcinogenic agent defined by the International Agency for Research on Cancer (IARC) and in a few instances can also cause cancer of the anal canal, vulvae, penis and oropharynx. Recognition of the association between cervical cancer and a subset of HR genital papillomavirus infections, first postulated by Zur Hausen and colleagues, was confirmed through painstaking research over 25 years by epidemiologists.

Studies have reported that HPV infection occurs shortly after the onset of sexual debut. When a woman is infected with oncogenic HPV types, a spectrum of cellular and molecular events can result. It begins by primary infection of the proliferating basal cells of the squamous epithelium. Most of the HPV infections are benign, but, women with persistent oncogenic infections have the greatest risk of developing cervical precancer and cancer. The longer the HPV infection persists, the less likely a patient can clear her infection. It is unclear why HPV infections resolve in certain individuals while it results in more severe lesions in others, but individual susceptibility and other enabling factors may play a role. When the infections resolve whether it is by complete viral clearance or by maintenance of a latent state in the basal-cell epithelium, in which the virus replicates at extremely low levels without full viral expression, is also unclear.

CIN 1 (cervical intra epithelial neoplasia), most likely the initial infective and potential progressive state, develops from the infected normal cervical epithelium in the vulnerable transformation zone. SCCs are the most commonly occurring form of cervical cancer and develop from CIN 1. The progression from HPV infection to HPV persistence to the development of high-grade CIN (cervical intraepithelial neoplasia) and ultimately cervical cancer appears to take, on average, up to 15 yr, although cases of rapid-onset cancers do occur. Further, the time of invasion reflects the time needed for additional genetic events to occur such as integration of HPV into host genome, inactivation of host tumour suppressor genes and ultimately the realization of effects of proteins produced by oncogenic HPV which allow the accumulation of irreparable and
irreversible mutations in the host genome. “Precancer” is the intraepithelial precursor to invasive cancer. Progression through each step involves a number of known and unknown external and internal co-factors including host immune responses.

**Papillomaviruses: human papillomavirus**

The papillomaviruses were initially known as members of the Papovaviridae family but considering the genetic diversity now they have been reclassified as independent Papillomavirus family. Papillomaviruses are small, non-enveloped, viruses that contain a double stranded, circular DNA genome. They are ubiquitous infectious agents characterized by strict species specificity - thus HPVs infect only humans. They are also tissue tropic with a preference for infection of either cutaneous or internal squamous mucosal surfaces. Papillomaviruses are classified by genotype and approximately 130 HPV types have been identified by sequencing. More than 40 of these types infect the epithelial and mucosal lining of the anogenital tract and other areas following sexual transmission, and about 15 of them are highly carcinogenic\(^{15-17}\). The HPV genome can be divided into three domains: a non-coding URR (upstream regulatory region), an early region with ORFs (open reading frames) E6, E7, E1, E2, E4 and E5, and a late region encoding two genes - L1, the major capsid protein, and L2, the minor capsid protein. E6 and E7 play a significant role in HPV mediated carcinogenesis\(^{14}\). L1 and L2 are capsid proteins produced for assembly of complete virions. L1 self-assembles to form VLPs (virus-like particles), which can also incorporate L2 if co-expressed. However, L2 protein does not form a VLP when expressed on its own.

Based on the individual viruses’ predilection for either cutaneous or mucosal surfaces, HPVs are classified into cutaneous and mucosal types. Cutaneous types of HPV are epidermitrophic and target the skin of the hands and feet. Mucosal types infect the lining of the mouth, throat, respiratory tract or anogenital epithelium. Within the groups of skin or mucosal viruses, depending on their oncogenic potential, they can be separated into HR or LR (low-risk) types\(^{17,18}\).

**Human papillomavirus types and types in histology of cervical cancer: a worldwide perspective**

In women, the prevalence of genital HPV ranges from 7 to 37 per cent depending on the country and age of the study group\(^{19}\), and it is a necessary cause for the development of cervical and other anogenital malignancies. Infection with HPV, considering all possible types, achieves a lifetime cumulative incidence of up to 70 per cent, whereas cervical cancer is a relatively rare disease, with a lifetime incidence range of 1.1 to 3.0 per cent across the world\(^{20,21}\). By far, the most common histological type of cervical cancer is squamous cell carcinoma constituting approximately 80 per cent of cervical cancers\(^{22}\). Adenocarcinoma is the second most common histologic type and, as noted above, shows a rising incidence, even in developed countries\(^{23-29}\).

More than 99 per cent of cervical cancers, including both squamous and adenocarcinoma histologies have identifiable HPV sequences\(^{7,26}\).

The HPV are classified into types based on their DNA sequence. By definition, the nucleotide sequences of the E6, E7 and L1 ORFs of a new HPV type should be no more than 90 per cent homologous to the corresponding sequences of known HPV types. HPVs have further been classified into subtypes, when they have 90 to 98 per cent sequence similarity to the corresponding type and variants when they show no more than 98 per cent sequence homology to the prototype\(^{8}\). Table I shows HPV type and disease association other than that in cervical cancer.

Although over 99 per cent of cervical cancers possess HPV DNA, the simple detection of HPV DNA is a poor predictor for the risk of cancerous transformation\(^{7,29}\). However, there is a better correlation between certain HR HPV types and cervical cancer\(^{30}\). As a result, genital HPV types have been subdivided into LR types, which typically produce benign genital warts, and HR types, which are more frequently associated with invasive cervical cancer and other malignancies\(^{31}\). A large epidemiologic study has identified 15 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 probable high-risk HPV types (26, 53, and 66), and 12 LR HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108)\(^{30}\). It is interesting to note that most of HR types are phylogenetically related to either HPV 16 (31, 33, 35, 52 and 58) or HPV 18 (39, 45, 59, 68)\(^{32}\).

IARC sponsored study named the HPV types involved in cervical cancer (Table II). This study compared the epidemiological classification (based on their study) with the phylogenetic grouping. A discrepancy was observed only in two types: HPV type 70, which was classified as a HR type phylogenetically but as a LR type by their epidemiologic study; and type 73, which was classified as a LR type phylogenetically but a HR type epidemiologically\(^{30}\).
Preliminary data from 9 countries (India not included) indicate the prevalence of the four most common HPV types among 1545 cases with single infections were: HPV 16- 59 per cent, OR- (Odd’s ratio) 182, HPV-18 -12 per cent, OR- 231, HPV-45 - 4.8 per cent OR- 148, HPV- 31 - 3.7 per cent OR-71.552. Other less common types showing equally high odd’s ratio were- HPV-33, -35, -51, -52, -58, -5933.

In a meta-analysis of epidemiological studies on HPV type distribution in women with and without cervical neoplastic diseases34, HPV-16/18 were estimated to account for 70 per cent of all cervical cancers worldwide, although the estimated HPV-16/18 fraction was slightly higher in more developed (72-77%) than in less developed (65-72%) regions. About 41-67 per cent of high-grade squamous intraepithelial lesion (HSIL), 16-32 per cent of low-grade squamous intraepithelial lesion (LSIL) and 6-27 per cent of atypical squamous cells of undetermined significance (ASCUS) were also estimated to be HPV-16/18-positive, thus highlighting the increasing relative frequency of HPV-16/18 with increasing lesion severity.

A comprehensive meta-analysis16 comprised of 10,058 cervical cancer cases. India was one of the countries included in the region ‘Asia’ – for the study along with Mainland China, Indonesia, Japan, Korea, Malaysia, Philippines, Taiwan and Thailand. Asia (31%) and Europe (33%) had the majority of the cases in the study. The HPV prevalence among the cases did not vary significantly with regions, but HPV DNA was less likely detected in ADC (collective term representing both adeno carcinoma and adenosquamous carcinoma) - 76.5 per cent than in SCC- 87.3 per cent16.

In a meta-analysis of epidemiological studies on HPV type distribution in women with and without cervical neoplastic diseases34, HPV-16/18 were estimated to account for 70 per cent of all cervical cancers worldwide, and eight most...
common types (HPV-16, -18, -33, -45, -31, -58, -52, and -35) accounted for 90 per cent of the cases. The same eight types were most frequent in each of the world regions – Africa, Asia, and Europe, North America and south and Central America, with HPV-56 being the eighth most common type instead of HPV-52 in Europe. In Asia, HPV-58 prevalence was particularly high, and HPV 52 was also more frequently identified. HPV-16 prevalence varies from 52 per cent in Asia to 58 per cent in Europe, and HPV-18 prevalence varies from 13 per cent in South/Central America to 22 per cent in North America.

HPV type prevalence and histology -worldwide: general picture

After HPV 16, HPV-18 is the type most clearly shown by epidemiologic data to be a human carcinogen. The evidence is limited to cervix, in which HPV-18 appears to be strongly linked to a substantial minority of squamous cancers and approximately half of the adenocarcinomas. The meta-analysis also showed that HPV type-distribution varies significantly between SCC and ADC. HPV-16 being identified more often in SCC than in ADC. The same was found for HPV 16 phylogenetically related types 31, 33, 52 and 58, but not 35. Conversely, HPV-18 was more prevalent in ADC than in SCC. The HPV 18 phylogenetically related type 45 was also more prevalent in ADC than in SCC.

To sum up, HPV-type distribution is broadly consistent across cervical cancer in world regions, particularly with respect to HPV-16 (HPV-16 prevalence is estimated to be the highest among European cases although the differences are not very relevant when compared to the rest of the world) and -18. Nevertheless, some inter- and intra regional variations in the relative importance of the next most common types namely HPV-31, -33, -45, -52, -58 and -35 have been reported16.

The most consistently observed geographical variation concerns the prevalence of HPV-16 relative to non-HPV-16 types in cytologically normal women. In a study16, by the IARC on the distribution of HPV types in cytologically normal women, 15613 sexually active women aged between 15 and 74 yr, all with valid HPV test results and normal cytological findings, were included. The study included 1799 subjects from India, in the ‘Asia’ group. The Fig. shows the percentage of HPV infections by different types of HPV in Asian region.

Overall HPV prevalence was lowest in Spain and highest in Nigeria with intermediate prevalence in South America and Asia. The most common HPV type, in either single or multiple infections, was HPV16, followed by HPV42, HPV58, HPV31, HPV18, HPV56, HPV81, HPV35, HPV33, and HPV45. HPV16 was twice as frequent as any other high-risk type in all regions except sub-Saharan Africa, where HPV35 was equally common. The next most common HR-HPV

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Proportion of cervical cancers caused (in %)</th>
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<tr>
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<tr>
<td>HPV18</td>
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<td>HPV33</td>
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<td>HPV45</td>
<td>3.7</td>
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<td>HPV52</td>
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<tr>
<td>HPV59</td>
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<tr>
<td>HPV31</td>
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</tr>
<tr>
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Data adapted from Ref. 35

![Fig. Infection by different HPV types in Asian region. Adapted from Ref. 36.](image-url)
types were HPV33 and HPV56 in Asia, HPV58 in South America, and HPV31 in Europe. With respect to other HR HPVs (3.7%) and LR HPVs (4.2%), HPV 16 showed only a prevalence of 1.7 per cent in women in Asia. HPV-positive women in Asia had proportions of HPV16 infection in between those for Europe and sub-Saharan Africa, but heterogeneity was significant across areas in Asia. HPV-positive women in Europe were significantly less likely to be infected with HR types of HPV other than HPV16 than were women in sub-Saharan Africa. HPV-positive women from Asia and from South America had intermediate risks of such infection36.

Risk of HPV18 infection among HPV-positive women was similar for all regions, although some heterogeneity for HPV18 was evident within the regions of South America and Europe. Heterogeneity was significant between regions for HPV31, HPV33, and HPV35 (which was particularly prevalent in sub-Saharan Africa compared with other regions), HPV45, and HPV58. Heterogeneity was also evident within South America for HPV35 and within Asia for HPV58. The pooled data analyses provide evidence for regional variation in distribution of HPV type for women without cytological abnormalities36.

Pretet et al in his two recent reports37,38 on studies conducted in France-for the EDITH group (Etude la Distribution des types d’ HPV en France), HPV 31 as the most prevalent HPV type after HPV 16 in high grade lesions while in invasive cancers it was the most common HPV type only after HPV 16 and HPV 18. Likewise, another study39 reported the prevalence of HPV genotypes -16 and -31 as most commonly distributed in cervical specimens from French women with or without abnormalities. Similarly, Antonishyn et al 40 reported HPV-31 as a major type contributing significantly to the proportion of women with CIN in Saskatchewan, Canada.

The relative type prevalence seems to fluctuate and HPV-31 was the most common HPV infection in women that were referred for colposcopy. This observation is not typical, as most studies have found HPV-18 as the second most prevalent HR HPV type in clinical populations40,41,42. However, a high prevalence of HPV-31 types has been observed in some populations42-44, and studies on European populations have also shown HPV-31 as the second most common HPV type in low-grade cervical lesions45. HPV-58 or -52 is the second most common type in Asia46,47.

**Human papillomavirus types, histology: state of affairs in India**

Despite the high incidences of cervical cancer reported from India, large-scale population based studies on the HPV prevalence and genotype distribution are very few.

The HPV type distribution in a rural community was studied48 in women attending the regional cancer centre in Hyderabad. Among the HPV positive cancers, (n=41) the overall type distribution of the major HR HPV types was as follows: HPV 16 (66.7%), HPV 18 (19.4%), HPV 33 (5.6%), HPV 35 (5.6%), HPV 45 (5.6%), HPV 52 (2.8%), HPV 58 (2.8%), HPV 59 (2.8%) and HPV 73 (2.8%). In the women participating in the community screening programme, belonging to the Medchal community, the most frequently detected HPV types are HPV 52 and 16.

This differs slightly from that of the study in a rural area in Dindugal, Tamil Nadu district, where the major types were HPV 16 and 56, followed by HPV 31, 33, and HPV 1849. The same group conducted a hospital-based case-control study in Chennai, Southern India49. In a total of 205 invasive cervical cancer cases, and 213 frequency age-matched control women, 23 different HPV types were found. HPV 16 was the most common type in either case or controls, followed by HPV 18, HPV 33, HPV 35 and 45. This was consistent with the type distribution shown in cervical carcinoma worldwide16,30. Similarly another study from Mumbai reported HPV prevalence statistics which broadly matched the existing literature, HPV 16 and HPV 18 accounted for the bulk of the HPV infections followed by HPV 33, -31, and -45. They also reported considerably large number of HPV16/18 coinfections from the non-malignant group50.

Clare et al reported an overall prevalence of HPV in two communities near Trivandrum, in Southern India. The overall prevalence of HR-HPV in this south Indian population was 3.9 per cent. HPV 16 was the most common type, accounting for 47 per cent of the HR infections, either alone or with another type. The next most common type was HPV33, accounting for 10 per cent51.

A cross-sectional biopsy study from 100 patients from South India and 30 patients from East India showed 60 per cent HPV 16 infection, 14 per cent HPV 18 infection as the most frequent HP types. This constitutes only 70 per cent of the total cases. 16 other
types were identified: HPV- 26, -31, -33, 35, -42, -45, -51, -52, -53, -56, -58, -61, -62, -64, -81 and -82.

A study from North India reported that in HPV positive samples, exfoliated cervical swabs collected from unscreened married women aged 16-24 yr, type 16 was seen in 36.2 per cent and type 51 and 59, 13 per cent each. A single HPV type was found in 58 per cent cases and mixed infection was seen in 42 per cent. But yet another hospital-based study in New Delhi, North India, found that, in 106 ICC cases found that HPV 16 type was the commonest type, seen in 73.6 per cent of the cases, followed by HPV 18 (14.2%) and HPV 45 (11.3%)..

**Distribution of HPV types: Implication in the effectiveness of the currently available vaccine**

HPV 16 appear to be believed the most prevalent type of HPV in cervical cancer, worldwide and in India, although with minor regional differences. The prevalence of HPV-16 over other types may be related to the complex geographical and biological interplay between virus and host immunogenetic factors. Regardless of the underlying mechanism, trying to identify which types account for geographical variation in the non-HPV-16 fraction of cervical lesions, is difficult because of their lower frequency and the variability in sensitivity of different assays to detect them; it is even more difficult in case of India, since the information available is so less and meagre considering the vast, widespread geography of the sub-continent.

**Human papillomavirus infection: host immune interventions**

The viral replication and assembly occur in a cell already destined for death by natural causes, far from the sites of immune surveillance. So, there is no cytolysis as a consequence of HPV replication, assembly or release and inflammation or “danger signal” to alert the immune system to the virus’s presence. Despite HPV’s ability to impede host defences, a successful immune response to genital HPV infections is established in most cases. This seems to be characterised by strong, local, cell-mediated immunity that is associated with lesion regression and the generation of serum neutralising antibody. Such antibody is generated in most, but not all, infected individuals and is directed against conformational epitope(s) on the L1 protein displayed on the outer surface of the intact virus particle. Serum neutralizing antibody levels following natural HPV infections, even at peak titres, are low. This probably reflects the exclusively intraepithelial infectious cycle (the absence of a viremia), as well as the production of virus particles in the superficial epithelial cells, distant from APCs (antigen presenting cells) and patrolling macrophages. Despite these low antibody levels, seropositive animals are protected against further viral challenge and this protection can be transferred from resistant to native animals by passive transfer of serum. A vaccine that will generate neutralizing antibody to the major capsid protein L1 of genital HPV’s would be protective against infection, L1 protein must be in the tertiary or native form and assembled as a multimer for neutralizing antibody to be generated these observations formed the basis for vaccine development against HPV.

**HPV vaccine: prophylactic vaccines**

Vaccines against HPV may be prophylactic or therapeutic. Prophylactic vaccines must be given to individuals prior to virus exposure and to be effective, must be able to stimulate a high neutralising antibody response, preferably at the mucosal surface. A therapeutic vaccine however, would be utilised once infection has occurred to induce specific T-cell-mediated response, leading to regression of an existing lesion.

While most anti-viral vaccines are based upon virions to induce anti-virion antibodies, it is difficult to produce sufficient quantities of HPV virions in cultured cells to induce a host response. It was discovered that by inducing expression of the major HPV capsid protein L1 in cultured eukaryotic cells, with or without the presence of the minor capsid protein L2, it is possible to produce what are known as HPV virus-like particles, or VLPs. These VLPs are morphologically identical to the native HPV virions, though they lack the viral DNA core. Thus, they can be injected into a host, to induce an antibody response, without any oncogenic risks. The L1 protein is highly conserved antigenically and bears epitopes that are broadly cross-reactive or even group specific among all the papillomaviruses of man and animals, but they are exposed only on disrupted virions or on capsid monomers. By contrast, intact virions and virus-like particles expose mainly a type-specific conformational epitope and there are few or no cross-reactions between the intact viruses.

Clinical trials of multivalent L1 VLP vaccines show safety, immunogenicity and high efficacy. The mechanisms by which VLPs elicit protection are not completely understood. At present there are two vaccines: A bivalent that includes antigens of HPV 16
and 18 and a quadrivalent that includes HPV 16, 18, 6 and 11.\textsuperscript{68,66,67} Types 16 and 18 cause 70 per cent of cervical cancers and 6 and 11 cause about 90 per cent of genital warts.

**Bivalent vaccine**

Cervarix\textsuperscript{TM}, developed by GlaxoSmithKline Biologicals, Rixensart, Belgium, is a bivalent HPV-16/18 L1 VLP vaccine. The L1 protein of each HPV type is expressed by a recombinant baculovirus vector, and the VLPs are generated separately and then combined.\textsuperscript{68} Cervarix\textsuperscript{TM} consists of purified L1 VLPs of HPV types 16/18 at 20/20 µg/dose, respectively, formulated on an ASO4 adjuvant consisting of aluminum hydroxide 500 µg and 3-deacylated monophosphoryl lipid A 50 µg. It is administered as a 0.5 mL intramuscular injection in a three-dose immunization protocol at 0, 1 and 6 months.

Clinical trial data indicate that vaccine protection is maintained over a period of 4.5 years with the Cervarix\textsuperscript{TM} bivalent vaccine.\textsuperscript{67} A multicenter, randomized follow-up trial (n=776) demonstrated that 98 per cent seropositivity was maintained for HPV-16/18 antibodies at 4.5 years. The bivalent vaccine was 96.9 per cent effective against incident HPV-16/18 infection and 100 per cent effective against 12-month persistent infection. A combined analysis of the initial and follow-up studies showed 100 per cent vaccine efficacy against CIN lesions associated with HPV-16/18.

**Quadrivalent vaccine**

Gardasil\textsuperscript{®}, a quadrivalent HPV-16/18/6/11 L1 VLP vaccine, has been developed by Merck & Co. Inc.\textsuperscript{69} For each HPV VLP, the L1 protein is expressed via a recombinant Saccharomyces pombe vector and the vaccine is comprised of purified L1 VLPs of HPV types 6/11/16/18 at 20/40/40/20 µg/dose, respectively, formulated on a proprietary alum adjuvant.\textsuperscript{68} Gardasil\textsuperscript{®} is available as a 0.5 mL intramuscular injection in a three-dose immunization protocol at 0, 2 and 6 months.

Data from a phase II randomized, multicenter study (n=552) that followed women aged 16 to 23 yr for up to 5 yr demonstrated that vaccination of adolescents and young adults with Gardasil\textsuperscript{®} at 0, 2 and 6 months resulted in 100 per cent vaccination coverage and effectively prevented persistent infection and disease caused by HPV types 6/11/16/18.\textsuperscript{70} Pooled data from four studies in 20,583 women aged 16 to 26 yr, who were followed for a mean of 3 yr, indicate that the quadrivalent vaccine has the potential to substantially reduce the incidence of HPV-16 and -18-related cervical precancers and cancers.\textsuperscript{71} In the per-protocol analysis, women who were negative for HPV-16 or -18 (n=17,129) demonstrated 99 per cent vaccine efficacy for the primary endpoint of the combined incidence of HPV-16 and -18-related CIN 2/3, adenocarcinoma in situ, or cervical cancer. In the intention-to-treat analysis, which included women who were infected with HPV-16 and/or -18 at day 1, vaccine efficacy for the primary endpoint was 44 per cent.

The implementation of HPV vaccination is predicted to have wide ranging impact with regard to a reduction in the use of healthcare resources. Cost-effective vaccination strategy is thought to reduce pap screening, work load at the sexually transmitted diseases clinics. The Markov model predicted that, over the lifetime of a cohort of 12-yr old females, a 100 per cent vaccination coverage and 95 per cent vaccination efficacy against HPV-16 and -18 infections result in a 76 per cent reduction in cervical cancer deaths and a 66 per cent reduction in high-grade cervical lesions.

Thus, the available vaccines are effective and safe, and can protect against HPV infections responsible for about 70 per cent of cervical cancer. They need to be given before infection occurs, and are, for countries where cervical cancer screening programs are already in place, an adjunct to, rather than a replacement for these existing screening programs. Their general introduction will perhaps reduce considerable amount of surgical intervention to treat precancerous lesions and should substantially reduce the economic and social burden of cervical cancer in the developing and the developed world.

**Introducing the prophylactic HPV vaccine in India: what are the issues for implementation?**

Vaccines can be optimally effective and successful only if their coverage, \textit{i.e.}, the number of people vaccinated against a disease in a given population, continuously remains at a sufficiently high level. The community benefits of vaccination are thus directly related to the percentage of the population that has been immunised and this percentage varies according to the vaccine in question. Can India achieve this goal with the implementation and widespread introduction of the current vaccines- Cervarix and Gardasil? A vital issue requiring careful consideration is the cost of the vaccine(s), social, cultural and economical factors and geographical variation in the HPV type-distribution.

The current vaccines available - both bivalent and quadrivalent - offer protection against only two of the
HR HPV types most commonly seen—HPV-16 and HPV-18. Although there is some evidence of cross reactivity among certain HPV types, it accounts for less than 1 per cent of the antibody reactivity, indicating that protection to infection is type-specific.

**Economics of vaccination in India**

Even as the developed world countries are making mandatory, the vaccination programmes for their adolescent girls and contemplating on the age and group to be vaccinated, Indian scientific community and policy makers should think whether these new expensive vaccines will really help India? Mass immunization with these vaccines at this critical juncture might greatly reduce the cervical cancer incidence. Theoretically, a 100 per cent efficient vaccine with 100 per cent coverage on populations will reduce the cervical cancer burden by 70 per cent. But the spectrum of HPV types targeted in current vaccine trials is based largely on the prevalence of HPV types in cancers from the developed world, especially the European population. Heterogeneity in HPV type distribution should be taken into account when predicting the effect of vaccines on the incidence of infection or in developing screening tests for virus. HPV types other than HPV-16 and -18 still account for 25 to 30 per cent of cervical cancer cases, and this percentage maybe higher in certain populations. The efficacy of the current vaccine formulations being type-specific, it may not be possible at all to achieve the true purpose of vaccination programs in India. The most annoying barrier in discerning the effectiveness if the existing vaccine is implemented in India, is lack of knowledge, lack of data, about the HPV types affecting the Indian women. Despite high encumbrance from cervical cancer, there are few large population studies on cervical cancer cases, unscreened populations or cytologically normal women, from India, describing either HPV prevalence or type distribution. HPV being a sexually transmitted disease, the obvious cultural and social diversity existing in various parts of India, will have an immense impact on HPV prevalence in the entire country. Hence, it is important that each jurisdiction will need the knowledge of the baseline HPV causing disease in their community, region, to implement vaccine programs and uncover their efficiency. These components make it all the more apparent to describe and find out by multi representative population studies, the HPV genotypes in cancer case and community samples. Baseline studies will be compromised if HPV is only categorized as either HR or low risk without discriminating as to which actual HPV types are present. So, the study should be well monitored, sufficiently quality assured and unbiased. These data can then be generalized for application in national cancer prevention strategies or in devising the optimum strategy for vaccination in India. Based on the huge database regarding the incidence and frequency of the dominant HPV types in the west, Merck has already performed clinical trials in Western nations with an octavalent HPV VLP vaccine, including benign L1 VLP types 6 and 11, and six oncogenic HPV types (16, 18, 31, 45, 52, and 58). However, data have not yet been released and it is unclear how this program will advance. An L2-based vaccine is in early phase clinical trials at the University of Alabama at Birmingham, as preclinical studies have demonstrated that L2-based vaccines can be used to generate broad spectrum cross neutralizing antibodies and could reduce the number of VLP types required for protection.

Knowledge on different HPV types may have clinical utility as well, like in the risk assessment in cervical cancer. The data on prevalent HPV types in a population will also help in determining the extent of cross immunity among the different HPV types once the vaccination takes place. Further, since HR HPV contributes to a significant fraction of head and neck, anal, penile, vulvar and vaginal cancers, such a broad study will also be helpful in determining whether the benefits of HPV preventive vaccine extend beyond cervical cancer.

Most cancer epidemiology studies involve people living in North America and Europe which represent only a fraction of the global population. Taking into account, the fact that, approximately 14 per cent of women in the world live in rural India, the new study from regions where data is lacking and a collaborative reanalyses of all existing data will have substantial potential to be of worldwide significance in cervical cancer prevention including development of efficient second generation vaccines. Such an epidemiology research need sustained support in a developing country like India with outreaching educational programmes, better infrastructure like development of common accessible cancer registries with the ability to follow up on the study subjects. Also, a central analysis centre which ensures uniformity in the experiments and analyses performed, and, most importantly the fostering of fair and effective collaborations between clinicians, researchers and technicians are needed.

The other minor issues affecting the outcome of the multivalent vaccines in question maybe the presence of HPV L1 variants, differences in immune response haplotypes and multiple HPV co infections.
and redistribution of the HR HPV types, post vaccine implementation.

(i) The presence of HPV L1 variants

Recent research has focused on the nucleic acid sequence variation within HPV 16 long control region, E6 and L1 regions. HPV variants differ in biological and chemical properties and pathogenicity. Based on sequence variation of the L1, L2, and LCR regions of HPV-16, five naturally occurring phylogenetic clusters have been defined for HPV-16: European (E), Asian (As), Asian-American (AA), African-1 (Af1), and African-2 (Af2). Intratypic sequence variation has also been found in the E2, E4, E5, E6, and E7 genes of HPV-16. Since the LCR contains several E2 binding sites in addition to binding sites for several transcription factors, nucleotide sequence variation in the LCR, E2, E6, and E7 genes may be of functional significance. The oncogenicity of specific HPV variants appears to vary geographically and also with the ethnic origin of the population studied. One study suggested that because of increased transcriptional activity and changes in the progesterone response elements, Asian-American variants might have enhanced oncogenic activity compared to European isolates. The L1 variants with a 2 per cent sequence divergence have been observed in the L1 neutralising epitopes, which suggests selection to escape neutralization. The geographical distribution of HPV variants and its relevance in HPV testing for vaccine development are still uncertain. But given the HPV sequences are very stable over time, and with evidence of cross-neutralization with variants of the same genotype, immune escape is unlikely to be a significant issue.

(ii) Differences in immune response haplotypes

A statistically significant association of the non-European variant with the presence of HLA class II alleles was reported. Other studies have reported the association of HLA class II alleles with cervical HPV disease although these associations appear to be relatively weak. These studies however, suggest that host genetics may need to be investigated in concert with HPV typing to fully understand geographic variation in type-specific HPV prevalence.

(iii) Multiple HPV co infections and redistribution of the HR HPV types, post vaccine implementation

The presence of multiple HPV coinfections has been reported. The majority of multiple coinfections contain two HPV genotypes, but samples of three, four or five genotypes have also been reported.

In a multiple coinfection, especially, when two or more HPV types belong to the HR types, it is not clear as to the persistence of which HR type leads to the progression of lesions. It is still ambiguous whether they coexist with synergy or competition. But, the major disadvantage of the multiple coinfection along with the introduction of type-restricted protection (like the current vaccines) against HPV seems to be the possibility of genotype replacement as HPV vaccination is implemented.

When the multiple HPV types are present in the same system, each of them coexists with the other in a delicate balance of individual ‘ecological niches’. Alternatively, infection by a single type of HPV might be also because one of them has been able to completely competitively exclude other types. When type-specific vaccination against the most common cancer causing types becomes effective, a large ‘potential niche’ created, opens for the nontargeted types and allows them to proliferate. Using a mathematical model, Lipsitch found that in a system with two or more serotypes, elimination of one of those serotypes by a monovalent vaccine may cause an increase in the other serotypes that is actually greater than the reduction of the targeted serotype. While Lipsitch’s studies are based upon bacterial systems, replacement is a potential problem for viral systems as well. Nevertheless, studies indicate that in the short term, there is no expansion of other HPV types implying that HPV infections are independent of each other which does not suggest a probable genotype replacement. In the unlikely event that this does occur over time, additional VLP types, or perhaps an L2 cross-protective antigen, could be included in the vaccine.

Conclusions

Research on HPV has progressed rapidly and we have reached a point where prevention of cervical cancer by vaccination against HPV infection will be possible in the foreseeable future. Evidence suggests that risk for progression to invasion is closely related to the infecting human papillomavirus type. Studies indicate a geographical variation in the HPV types’ distribution in the population and in patients with cervical cancer. The implications of such variability on HPV vaccination is unknown and not clear. The outcome would even be more confusing in India, where, understanding about presence and prevalence of variable HPV types in the diverse Indian population is very scanty. Broad, extensive and far-reaching studies
involving large population samples from diverse and distinct locales covering the Indian peninsula are needed.

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Reprint requests: Dr M. Radhakrishna Pillai, Director, Laboratory of Translational Cancer Research, Rajiv Gandhi Centre for Biotechnology Thycaud PO, Poojappura, Thiruvananthapuram 695 014, India
e-mail: director@rgcb.res.in, mrpillai@rgcb.res.in