8. Impact of prophylactic HPV vaccination on the cervical cancer incidence and mortality. Perspectives for development of therapeutic HPV vaccines

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Abstract. In this Chapter we will discuss the current status of the prophylactic HPV vaccination, the introduction of HPV prophylactic vaccination and its impact on the cervical cancer incidence and mortality, challenges of the HPV vaccination in the developing world, screening post vaccination and the second-generation HPV prophylactic vaccines, as well as, the development of new therapeutic vaccines and its use at different stage of the Cervical Cancer (CC). Two commercial vaccines against HPV (Gardasil® and Cervarix®) are currently in the market in many countries around the world. Both are produced

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with recombinant technologies, consist of self-assembled Viral-Like Particles, the so-called VLPs, and had shown high immunogenicity.

More important, they had been found highly efficient in preventing persistent infections and lesions not only from the uterine cervix, but also from the anus, vagina, and vulva. The development of prophylactic HPV vaccines and proof of efficacy as described in this chapter represents a landmark in the field of preventive medicine. The challenge now is to implement use of these vaccines and in so doing to see a significant impact on incidence and death rates from CC. This needs to be most effectively achieved in resource-poor countries, where cancer rates are higher but obstacles to vaccine implementation will be greatest. Unfortunately, in a world of competing health-care priorities, having the means and know does not always translate into success. Even where HPV vaccine will be available, implementation needs to address some issues. Foremost is education, so that women and their daughters understand the potential benefit of primary prevention. Despite the success of screening, around 20% of women do not access screening for a variety of reasons, including socio-cultural barriers because some groups are socio-economically deprived women, those from ethnic minorities, and of course, there are anxious individuals who just cannot cope with the idea of cervical screening.

Unhappily, HPV vaccines are very expensive, and unaffordable for many Public Health initiatives in developing countries. They include two types involved in cancer development (16 and 18), and so we can only expect a partial protection against cancer (70/100), making it necessary to implement novel strategies to detect precursor lesions and cancer in the post vaccination era. Strategies that include education and organized screening programs with detection of persistent infections should be implemented in developing countries if a reduction of cancer of the uterine cervix is expected next years. Ongoing trials will document the precise degree of cross-protection and influence on preventing high-grade cervical intraepithelial neoplasia (HG-CIN) caused by other HPV types. In order to increase the protection provided, vaccines that contain multiple high-risk HPV types and which could prevent 90% of cancer are being developed. Issues of stability and cost will continue to drive the testing and development of alternatives to VLPs for second-generation vaccines. Another aspect of vaccination requiring greater clarity over time will be the duration of protection. It will take 15-20 years before a vaccinated girl reaches an age when CC becomes a significant risk and by that time we need to know whether booster vaccination will be required. The current generation of vaccinations will prevent not more than 60% of CIN 3, so a considerable amount of HG-CIN will inevitably occur for many years to come. A therapeutic vaccine to treat a persistent HPV infection, or even CIN 2+, would be an important advance in reducing morbidity, but much progress is requires to achieve significant clinical efficacy at a level that can challenge current therapy.

Introduction

We now are living a historical period in relation to CC control, because there is a new paradigm for primary and secondary prevention of disease
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from the association found between the presence of HPV and this disease. Therefore foresee a rising trend in the use of HPV vaccines and prospects for control of infection [1]. Thus, vaccination against HPV serves a public health need and is the only feasible preventive intervention at the population level, which limits exposure to persistent HPV infection [1,2]. HPV prophylactic vaccine development has been possible after assembly, by genetic engineering, of VLP or virus-like particles, formed by the capsid L1 protein of HPV but lack DNA, so they are not contagious. VLPs have intense antigenic capacity and produce high neutralizing antibody response [1]. Thus far there have been two prophylactic vaccines: a tetravalent produced in yeast containing VLP of two high-risk genotypes, HPV 16 and 18 responsible of 65% of the cervical neoplasm in Latin American, and two low-risk, HPV 6 and HPV 11, responsible of 90% of genital warts and recurrent respiratory papillomatosis. Bivalent vaccine containing VLPs HPV 16 and 18 is produced in insect cells with baculovirus as expression system.

Based on results from phase three clinical trials, in which assessed the efficacy and immunogenicity of HPV vaccines, the Food and Drug Administration (FDA) U.S. granted license for the use of these vaccines in women between 9 and 26 years of age [3,4]. In clinical trials, the greatest impact was seen in women without previous exposure to HPV. Therefore, the average age of onset of sexual intercourse in different populations is an important element in the recommendations of the age of vaccination. What is still a matter of controversy is vaccination in women over 26 years of age. The possibility that protection in women between 15 and 26 years is not conferred by the vaccine-induced antibodies but antibodies acquired as a result of previous infection and lack of therapeutic effect of the vaccine [5], not reveal the benefit of vaccination in this group of women and, in contrast, favors investment of resources in public health programs to vaccinate adolescents before their sexual debut. The vaccine availability to women over age 26 should only be done through private clinical practice [6].

Companies producing prophylactic vaccines for HPV quadrivalent recommend the vaccine in three doses intramuscularly with the pattern of 0, 2 and 6 months, and the bivalent to the scheme 0, 1 and 6 months. The only way to achieve effective guarantee of 100% in preventing HPV16-18-related lesions, in women before the onset of their sexual life or free of infection and seroconversion for both HPV types 16 and 18, after the third dose (month seven), according to clinical studies [7,8]. However, the vaccine current price no government in the region of the Americas can add their programs because the cost is eight times higher than other vaccines included. The cost of such vaccines is because the pharmaceutical producers, techniques and technologies needed, are "very new" for production. Based
on the principle that health is a universal right that transcends borders, the current immunization policy in Latin America is insufficient for the challenges seen in the context of public health. Additionally, the existing mechanisms of economic and financial multilateral cooperation for vaccination are ineffective and have been overwhelmed by an increasingly complex problem [1].

On the other hand, the high prevalence of malignant tumors and lesions associated with HPV worldwide generates the need to develop effective therapeutic vaccine against the disease. Current strategies for the development of such vaccines are based on live vectors, based on nucleic acids, cell-based and based on peptides and proteins. These strategies seek to eliminate pre-existing lesions and even HPV-associated malignancies, by activating cell-mediated immunity [9]. However, to achieve the development of therapeutic vaccines is necessary to face several challenges, including: a) the choice of target antigen, which is directed against the vaccine and allow choosing between infected cells and cancer cells, b) the choice and/or vehicle design safe, efficient and economically viable to transport therapeutic molecules, c) a better understanding of tumor micro-environment enabling the identification of new targets for therapeutic intervention, and d) development of new adjuvant [10]. Recently has been developed several strategies for therapeutic vaccines, which have shown great potential when were tested in both pre-clinical trials and in clinical trials. Additionally, it has been observed that many of the new strategies can be combined together or with others that are used in clinical practice, in order to create combination therapies that work in a synergistic way, the proposal seems very promising in eliminating lesions pre-existing HPV associated malignancies [11].

1. **Prophylactic HPV vaccination: current status**

The rationale of prophylactic HPV vaccination is based on the necessity this primary event, secondary changes which result in cytological abnormalities will also be prevented. It is the prevention of pathological change in the cervix which is the purpose of the vaccine, because the infection itself is asymptomatic and does not cause acute damage. Most cervical infections clear spontaneously within 12 months, but in around 40% of cases the virus will persist in the cervix for over a year and in some cases longer [12], which increases the risk of CIN developing in future years.

The principle of prophylactic vaccination to prevent CC relies on the generation of neutralizing antibodies, which prevent subsequent infection with high-risk types of HPV. Both vaccines have been tested in large randomized phase II trials and subsequently in pivotal phase III trials. The
phase II studies were designed to confirm sustained immunogenicity and some evidence of efficacy relevant to definitive endpoints of a pivotal efficacy trial. The phase III trials were designed to demonstrate the level of conclusive evidence of efficacy required to obtain a marketing license and convince funders that the vaccine is sufficiently effective and cost effective for public health programmes. This requires the prevention of CIN 3 as surrogate for cancer prevention. Table 1 summarizes some key properties of the two vaccines and their recommended uses.

Table 1. Vaccines properties and uses. Taken from Stern P et al. 2008[13].

<table>
<thead>
<tr>
<th>Clinical Effectiveness</th>
<th>CERVARIX (Glaxo Smith Kline-GSK)</th>
<th>GARDASIL (Merck &amp; Dohme-MSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevention of HG-CIN 2/3 and CC</td>
<td>Prevention of HG-CIN 2/3, high-grade Vulval Intraepithelial Neoplasia (VIN) 2/3, and genital warts causally related to HPV types 6/11/16 and 18 in girls/women aged 9-26 years based on efficacy studies in women 15-26 as well as bridging studies demonstrating immunogenicity in girls and boys aged 9-15.</td>
</tr>
<tr>
<td>Active ingredients</td>
<td>Each dose HPV 16 L1 protein (20mog), HPV 18 L1 protein (20mog)</td>
<td>Each dose: HPV 6 L1 protein (20mog), HPV 11 L1 protein (40mog), HPV 16 L1 protein (40mog), HPV 18 L1 protein (20mog)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>AS04-monoethylphosphoryl lipid A adsorbed on aluminium hydroxide</td>
<td>Alum-amorphous aluminium hydroxyphosphate sulphate</td>
</tr>
<tr>
<td>Dosage and schedule</td>
<td>One 0.5ml dose at 0, 1, and 6 months by IM injection in the deltoid region</td>
<td>One 0.5ml dose at 0, 2, and 6 months by IM injection in the deltoid region or anterolateral thigh</td>
</tr>
<tr>
<td>Side effects</td>
<td>Injection site reactions, headache, myalgia</td>
<td>Injection site reactions, fever</td>
</tr>
<tr>
<td>Very common</td>
<td>Gastrointestinal symptoms, itching/pruritus, rash urticearia, arthralgia, and fever &gt;38°C</td>
<td>Bleeding, itching at the site of injection</td>
</tr>
<tr>
<td>Common</td>
<td>Dizziness, URTI, injection site induration/paraesthesia</td>
<td>Urticaria bronchospasm</td>
</tr>
<tr>
<td>Uncommon</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.1. Highly immunogenic and induce memory

Cervarix® and Gardasil® had shown to be highly immunogenic in clinical trials, resulting in essentially 100% seroconversion. Peak geometric mean antibody titers (GMTs) obtained is approximately 50 to 100 fold higher than those generated after natural infection. Titers generally peak at month 7, one after the third dose (given at month 6 in both cases), decline over the next year and then remained relatively stable for around 7.3-9.5 years for both vaccines. At the plateau stage, titers remained above GMT observed in natural infections [14-16]. However, so far has been not identified a correlation of immune protection in vaccinated: no one knows the minimum required level of antibodies to protect against infection [1].
Cervarix® and Gardasil® have both shown to induce production of memory B cells. An additional dose of Gardasil®, at year five showed to induce a strong recall response, with titers for each type at least as high as the peak titer following the initial series of vaccinations [17]. Specific memory T lymphocytes against type 16 VLPs in women vaccinated four years before had found both with Cervarix® [18]. Modeling estimates assuming long term memory indicate that detectable antibody levels will remain at least for 12 years, and perhaps life-long in 99% of vaccines [19].

1.2. High efficacy in clinical trials

Several large clinical trials, including more than 40,000 individuals had been conducted to analyze efficacy of both vaccines (see Table 2). The majority of them showed high rates of efficacy, from 90 to 98% [20,21]. Merck and Co. reported 100% accumulated vaccine efficacy (95% CI) in preventing CIN 2/3 for 8.5 years of follow-up in adolescents and young women (15-26 years old), under the According-to-Protocol (ATP) group since there was not a single case among the vaccinated women as opposed to 8 cases in the placebo group. In relation to preventing type 16 infections they reported 95% (95% CI) since only one vaccinated women was found positive for this type as opposed to 21 cases in the placebo group [22]. A follow-up study (44 months) of 17622 women vaccinated with Gardasil® indicated, in the intention-to-treat group, reduction of the risk of any high-grade cervical lesions (19.0% reduction; rate vaccine = 1.43, rate placebo = 1.76, difference = 0.33, 95% confidence interval [CI] = 0.13 to 0.54), vulvar and vaginal lesions (50.7% reduction; rate vaccine = 0.10, rate placebo = 0.20, difference = 0.10, 95% CI = 0.04 to 0.16), genital warts (62.0% reduction; rate vaccine = 0.44, rate placebo = 1.17, difference = 0.72, 95% CI = 0.58 to 0.87), Pap abnormalities (11.3% reduction; rate vaccine = 10.36, rate placebo = 11.68, difference = 1.32, 95% CI = 0.74 to 1.90), and cervical definitive therapy (23.0% reduction; rate vaccine = 1.97, rate placebo = 2.56, difference = 0.59, 95% CI = 0.35 to 0.83), irrespective of causal HPV type [23].

A study conducted in a population (N=433) of women enrolled in Brazilian centers from an initial placebo-controlled study demonstrated during the most recent year of follow-up, approximately 7 years after initial vaccination, no cases of infection or cytohistological lesions associated with HPV-16/18 were observed in the vaccines. Vaccine efficacy (95% confidence interval) up to 7.3 years was 94.5% (82.9, 98.9) for incident infection, 100% (55.7, 100) for 12-month persistent infection and 100% (-129.8, 100) for cervical intraepithelial neoplasia grade 2+, antibody titers for total IgG and neutralizing antibodies remained several folds above natural infection levels.
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and >or=96% of women were seropositive [24]. The final analysis of efficacy of AS04-adjuvanted vaccine in the phase III randomized, double-blind, controlled PApiloma TRIal against Cancer In young Adults (PATRICIA) realized in women (15-25 years) vaccinated at months 0, 1, and 6 with mean follow-up of 34.9 months (SD 6.4) after the third dose, reported a vaccine efficacy against CIN2+ associated with HPV-16/18 of 92.9% (96.1% CI 79.9-98.3) in the primary analysis and of 98.1% (88.4-100) in an analysis in which probable causality to HPV type was assigned in lesions infected with multiple oncogenic types (ATP-E cohort). Vaccine efficacy against CIN2+ irrespective of HPV DNA in lesions was 30.4% (16.4-42.1) in the TVC and 70.2% (54.7-80.9) in the TVC-naive. Corresponding values against CIN3+ were 33.4% (9.1-51.5) in the TVC and 87.0% (54.9-97.7) in the TVC-naive. Vaccine efficacy against CIN2+ associated with 12 non-vaccine oncogenic types was 54.0% (34.0-68.4; ATP-E). Individual cross-protection against CIN2+ associated with HPV-31, HPV-33, and HPV-45 was seen in the TVC (Total Vaccinated Cohort). The HPV-16/18 AS04-adjuvanted vaccine showed high efficacy against CIN2+ associated with HPV-16/18 [25].

The prophylactic efficacies in five clinical trials against persistent infection and genital disease associated with vaccine-targeted types had been reported, and range from 98 to 100% [7,26-28]. Remarkably, efficacy was greater than 95% against all reported vaccine-type specific endpoints in the according-to-protocol analyses. In most instances, efficacy was lower in the modified intention-to-treat analyses, perhaps reflecting, at least in part, less protection after one dose of vaccine than after three. Some cases in the modified intention-to-treat analyses could also be due to prevalent infection reaching the minimum level for detection in the first month after enrollment [21]. As Schiller et al, reported notably lower efficacy in intention-to-treat analyses of FUTURE I and FUTURE II largely reflects two aspects of the study design and analyses. The first is the inclusion of prevalent infections in the analysis. The second is the presumably relatively high frequency of women who progress to disease from vaccine type-associated prevalent infection, compared to the number of women who develop disease from new infections during the relatively short duration of the follow up on which the analyses are based [21]. It is worth noting that the single CIN 2/3 case in which a vaccine type HPV DNA was detected in the according-to-protocol analysis of one of the Gardasil® trials (FUTURE II), and in two CIN 2/3 cases in the modified intention-to-treat analysis of the Cervarix® phase III trial (PATRICIA), HPV-16 or 18 DNA was detected on a single occasion and another high-risk type was persistently detected and/or specifically detected in the lesion. Although these cases fall under the pre-specified definition of vaccine-type associated cases, they could well represent
cervical dysplasia induced by non-vaccine types. These examples highlight the difficulties of assigning a case to a specific type when a women is infected with multiple types during follow-up [21].

The principal finding from these trials are that the VLP vaccines are highly effective at preventing type 16/18 specific lower genital tract neoplasia, that the quadrivalent vaccine prevents genital warts, and that the vaccines appear safe and free of concern in relation to pregnancy. It is also quite clear that the level of protection afforded by these vaccines depends on the likelihood of having had a previous type 16 or 18 infection. The impact of preventing cancer by preventing type 16 associated diseases will be proportionately stronger [13]. The principal characteristics and end-points used in clinical trials are summarized in Table 2.

The authorization to administer HPV vaccines has been granted in many countries worldwide, taking into account both advantages and disadvantages of these vaccines and their benefits and possible side effects in vaccinated women. The pros and cons of these vaccines are presented in the Table 3.

Table 2. Summary of principal characteristics and end-points used in clinical trials. Taken from Madrid-Marina V et al, 2009 [1].
Table 3. Pros and cons of current prophylactic HPV vaccines. Taken from Madrid-Marina V et al, 2009 [1].

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly immunogenic</td>
<td>High cost; most expensive so far produced</td>
</tr>
<tr>
<td>High clinical efficacy</td>
<td>Limited protection; only two oncogenic types</td>
</tr>
<tr>
<td>Induce memory B cells</td>
<td>Decay of antibody response?</td>
</tr>
<tr>
<td>Cross protection</td>
<td>Need for a booster?</td>
</tr>
<tr>
<td>Extended age for vaccination</td>
<td>Cost effective for preventing cancer only when used in children and young adolescents</td>
</tr>
<tr>
<td>Acceptable safety profile</td>
<td>Safety concerns and public perception in developed countries</td>
</tr>
<tr>
<td></td>
<td>Can create a false expectation in some sectors in developing countries</td>
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</table>

2. Introduction of HPV prophylactic vaccination and its impact on the CC incidence and mortality

Cervarix® and Gardasil® are approved vaccines for prevention of HPV infection and CIN, and their use should reduce CC. A number of national governments have now decided on whether to introduce and HPV vaccine into their national immunization schedules. Vaccine introduction requires complex decision making across a range of epidemiological, cost, social, and health system dimensions, and is a country-specific process. Cost-effectiveness is generally a pre-requisite for government-funded vaccine programmes. Pre-adolescent girls are the primary target population for vaccination because immunization should ideally precede the onset of sexual activity. The optimum age for vaccination can be derived from the age distribution for reported onset of sexual intercourse. The vaccination of older adolescents is less cost-effective because sexual activity increases with age, and a proportion of girls will have acquired HPV 16/18 infections. Vaccination to induce herd immunity will not be easily achieved because some young women are currently and persistently infected, and males will continue to transmit HPV infection.
A large number of European countries, United States, Canada and Australia have recommended HPV vaccination. Their policies are diverse in terms of the target age group and the recommendations for catch-up vaccination at older ages. In the United Kingdom 12 to 14 year old girls will be vaccinated with catch-up to age 18 phased over 3 years. In Italy the vaccine will be given to 12 year old girls, possibly with catch-up. In Switzerland to 11 to 14 year olds; in Belgium to 10 to 13 year olds; in France to 14 year olds with catch-up for women up to 23 years of age who are not yet sexually active; in Austria to pre-adolescents and in Germany, to girls aged 12-17. The Advisory Committee on Immunization Practices in the United Stated recommends HPV vaccination for various groups, including women up to 26 years, and Canada has similar recommendations [13].

Passive surveillance systems should be in place to monitor low incidence, serious adverse events, or unexpected vaccine side effects. The impact of the HPV vaccination on the CC incidence and mortality will only be evident after 20 years average of monitoring and surveillance of vaccinated cases, because of the long lead time in the development of CC. Systems may also need to be introduced to monitor vaccine effectiveness that to provide details on participation rates, to record vaccine information that can be compared with cervical screening outcomes and cancer registries to assess the long-term effectiveness of HPV vaccination, to determine vaccination status or identify women who may require a booster dose and to collect statistic for general reporting, among other.

3. Challenges of the HPV vaccination in the developing world

3.1. The most expensive vaccines

Cervarix® and Gardasil® are the most expensive vaccines so ever produced. In addition, they confer only a limited protection against cancer development, since although they target the two major HR HPV types, we can only expect at best a 70-80% protection against disease, in particular cancer of the uterine cervix [29]. Factors such as political awareness, public demand, availability of resources, donor pressure, and competing priorities are major determinants for the successful introduction of any new vaccine in developing countries. In developing countries, expensive and new vaccines are often available only in the private sector and the high cost is the major barrier for the rapid introduction and widespread use of HPV vaccine in immunization programmes in poor countries.

During the recent years, prices had been dropping and although it is clear that they will continue doing so, and new alternatives for financing and
making them available to poor countries will be seen, it is necessary that they do not take resources from existing screening programs, and that their introduction must be accompanied by the implementation of organized massive screening programs in developing countries. Vaccine policies balance political and economic realities with the scientific evidence base. Sometimes the introduction of new vaccines will be delayed or restricted because the cost to the health service, the insurance companies, or the tax payer, is too high. The risk is that recommending expensive vaccines without adequate public or social funding may lead to inequitable access and widen disparities in health between those who can afford private health care and those who cannot [13].

The initial recommendations for HPV vaccination in Mexico replicated the HPV vaccination extended scheme of Canada of 0, 6 and 60 months (according to the results of immunogenicity and published), but as a priority and targeted at girls aged 9 and 12 years of indigenous and marginal urban communities rather than a universal scheme, given the cost that the health system for mass vaccination, the limited budget available for this item [30]. Nevertheless, the HPV vaccination policy current consists in a universal extended scheme of 0, 6 and 60 months since 2013. In fact, an open-label nonrandomized clinical trial evaluating immune response to the HPV–16/18 AS04-adjuvanted vaccine administered on a standard (months (M) 0–1–6) versus extended schedule (M 0–6–60) at 7, 21, 60, 72 and 120 months post-vaccination was conducted in females recruited in Morelos, Mexico: 474 girls aged 9–10 years and 500 women aged 18–24 years receiving a standard schedule, and 1026 girls aged 9–10 years receiving an extended schedule (currently the girls in the extended schedule had received only the first 2 doses). This report presents the interim analysis results for non-inferiority between the regimes conducted with the current available data at 21 months after the first dose, with serum antibodies assessed by ELISA. A pre-stated margin of non-inferiority was defined by post-vaccination geometric mean titer (GMT) ratio (upper 95% confidence interval [CI] ≤ 2.0) between the standard and the two-dose schedule in girls at month 21. Immune response to the vaccine was strongest in adolescent girls and in the 3-dose group. Statistical non-inferiority of the two-dose versus three-dose groups was demonstrated. At 21 months, comparing the adolescent 2-dose versus 3-dose groups, the GMT ratio and 95% CI were 1.66(1.55–1.81) and 1.67 (1.51–1.86) for HPV16 and 18, respectively. The two-dose regimen was non-inferior when compared to the three-dose response in same-age girls and with women aged 18–24 years after 21 months of follow-up. The reduction in the number of doses from the current three-dose schedule in Mexico may lower overall costs associated with the vaccination and increase accessibility and compliance with the recommended dosing of the HPV vaccine [31].
3.2. Feasibility, accessibility and logistics of vaccine delivery

The integration of HPV vaccines into existing vaccine platforms could prove challenging in resource-poor settings since a comprehensive vaccination delivery infrastructure for adolescent vaccines is poorly developed or almost absent in most of these countries and, in many settings, new systems will be needed to reach young adolescents. The logistics of supplies of sterile syringes and needles, supplies for infection prevention, storage and transport of vaccines, and of teams in the context of an adolescent vaccination programme may also prove complex [13]. In developing countries, schools are often used as a focus for adolescent vaccination. This can be problematic because school attendance during later adolescence may be low; girls may be less likely to be in school than boys and often leave school early. Therefore, the poor who need the vaccine most are most likely not to be in school in many of the poorer countries. In countries with limited resources and health care infrastructure, coverage for re-vaccination/booster is often lower than those requiring a single shot of the vaccine. Thus, the current regime of three doses of HPV vaccine over 6 months for those aged over 9 makes it difficult to achieve high coverage [13].

3.3. HPV vaccine acceptability and sociocultural challenges

Parenteral consent is a critical factor, such as that a vaccine against a sexually transmitted infection will encourage children to become sexually promiscuous and that children will not have sex before marriage, and therefore do not need the vaccine, exist and may interfere with vaccine coverage. For better acceptance, HPV vaccine should be promoted as a CC prevention vaccine rather than a vaccine for prevention of a sexually transmitted viral infection for to avoid considerable controversies on moral issues.

4. Screening post vaccination

Notwithstanding the establishment of a vaccination programme in current teenagers, there will be a need for cervical screening to continue. There are several reasons to justify this. First, although the clinical trials have suggested that efficacy for the primary composite endpoint of protection against HPV 16 and HPV 18 associated high grade disease and cancer is over 90%, follow-up has only reached 5 years and there are, as yet, insufficient data to confirm that this protection will be maintained. Second, the protection against HPV 16 and HPV 18 high grade disease and cancer in the intention to treat population was only 44% and therefore, although
vaccinated, many of these women will remain at risk for the development of disease and will require regular screening. Furthermore, the relative efficacy of the vaccine against all high-risk HPV type associated CIN 2, CIN 3 and cancer in the intention to treat analysis of the whole trial population was less than 20%. It is therefore essential that vaccinated women are offered cervical screening [13]. All women, whether vaccinated or not, will need to be offered screening in the foreseeable future. Those women who have been vaccinated will be at reduced risk of developing cancer, although the risk of acquiring a non-16, non-18 oncogenic HPV type will not be affected unless cross-protection can be demonstrated. Current trials of HPV screening may well provide strong evidence for HPV screening, with scope for increased screening intervals; however strategies for HPV-positive tests, especially in young women, would need to be developed. The challenge of adapting cervical screening accounting vaccination will need to be addressed prior to the onset of screening of the vaccines [13].

5. Second-generation HPV Prophylactic vaccines

The approved HP vaccines Cervarix® and Gardasil® prevent infection by a subset of oncogenic HPV types, necessitating the development of highly multivalent L1 VLP vaccines or developing L2 as a single broadly protective antigen. The demonstrable efficacy of Cervarix® and Gardasil® vaccines has triggered multiple efforts to develop second-generation L1-based vaccines to address some of its shortcomings for global use, notably cost, type restriction, requirement for refrigeration, needle delivery and three immunizations, and inability to induce the clearance of pre-existing infections. Highly multivalent L1 VLP vaccines are currently being evaluated, but cost remains a primary issue preventing global realization of the benefits of HPV vaccination [13]. Potential second-generation HPV preventive vaccines nearing clinical study include: L1 expressed in Salmonella typhi for low-cost manufacture and oral delivery, L1 capsomeres purified from Escherichia coli for low-cost manufacture and greater temperature stability and L2 protein purified from E. coli for low-cost manufacture and broad protection. A combination of tiered pricing, local manufactures, and new technology is required to realize the full potential of HPV vaccination worldwide. Vaccines targeting L2 induce broadly neutralizing antibodies, capable of blocking infection by a wide range of HPV types. Several vaccine designs have been developed to optimize the display of L2 epitopes to the immune system and to reduce the cost of manufacture and distribution. L2-based vaccines show considerable promise as a potential next-generation HPV vaccine [32].
6. HPV therapeutic vaccines

Various strategies are being developed in order to eliminate pre-existing lesions or HPV-associated disease, including malignant tumors. Most of the therapeutic vaccines have been probe in preclinical and clinical stage focus on interacting with professional antigen presenting, to stimulating the production and T cells activation. Also been shown to the therapeutic vaccines can generate antigen-specific CD8+ T cells and CD4+ T cells. Th-1 CD4+ cells are able to stimulate and augment the immune response of cytotoxic CD8+ T cells [33-34]. The choice of target antigen is very important for designing therapeutic HPV vaccine in order to eliminate existing lesions, as a therapeutic vaccine should target HPV antigens that are continuously expressed in the infected cells and cancer cells. The viral oncoproteins E6 and E7 are expressed through-out the viral life cycle and are critical for the induction and maintenance of cellular transformation in HPV-infected cells; it is unlikely that the tumor cells can escape immune attack through antigen loss [35]. In addition E6 and E7 are constitutively expressed in all levels of the epithelium of HPV-infected cells and HPV-associated tumors, and are foreign proteins; immunization against HPV-associated tumors circumvents some common cancer-vaccine-associated problems such as immune tolerance. Hence, E6 and E7 oncoproteins appear to be excellent candidates for HPV therapeutic vaccination strategies. By other side the viral proteins E1 and E2 are expressed early in the progress of an HPV infection before the integration of the viral genome into the host DNA. Indeed, vaccinations against E1 and E2 have been reported in animal models and humans to induce natural and vaccine-induced T-cell response in patients with persistent cervical neoplasia [10, 36]. A number of strategies about therapeutic vaccines against HPV have been developed; include proteins and peptides, viral vectors, bacterial vectors, DNA, RNA, genetically modified tumors cells and dendritic cells. Attenuated bacteria and viral vectors have been evaluated as tools for HPV vaccine development or used to generate a potent immunogenic response with minimal toxicity. In the case of DNA the strategies have been focused on gene therapy and its application on CC, from the point of view of the alterations of the immune system focused on the tumor microenvironment, and the use of the cytokines as immunomodulators [37-39]. Recently, Maldonado et al developed a therapeutic DNA vaccine expressing the HPV16 E6/E7 antigens, and they demonstrated that intramuscular administration of therapeutic vaccine can elicit a T cell response, and stimulate an immune response and regression of high-grade cervical dysplasia [40]. In the following sections, we will discuss
the current status of therapeutic HPV vaccines for control of HPV associated malignancies.

6.1. Live Vector-based Vaccines

These vaccines have advantages including the possibility of choosing a desirable vector from a wide range of vectors to deliver antigens and can be tailored or engineered for a desired effect and can be classified into: bacterial vectors and viral vectors. The live vector-based vaccines are highly immunogenic, and can be used for the delivery of antigen E6 and E7 to dendritic cells (DC). Moreover, replication within host cells of live vectors, facilitate intracellular spread of antigen and can enable antigenic spread from cell to cell. This strategy is capable of stimulating antigen expression through Major Histocompatibility Complex (MHC) class I [to CD8+ cytotoxic T lymphocyte (CTL)] and MHC class II [(to CD4+ helper T lymphocyte (HTL)]. Additionally, the production of neutralizing antibodies as well as the possibility of pre-existing vector-specific immunity in the host during vaccination could reduce the potency of repeat immunizations; eliminating these factors may improve the efficacy of live vector-based vaccines [10, 37-39].

6.1.1. Bacterial-vector-based

Bacterial vectors can deliver desired genes or proteins to professional antigen-presenting cells (APCs). Attenuated bacteria have been explored in HPV therapeutic vaccines including: Listeria monocytogenes (Lm), Lactobacillus lactis, Lactobacillus plantarum, Salmonella enterica, and bacillus Calmette-Guérin [41,42]. Lm is the bacterial vector that has generated most interest, it has the ability to replicate in the cytosol of APCs and infect monocytes and macrophages; also it evades phagocytosis by macrophages by secreting a factor, listeriolysin O. This unique feature allows peptide antigens derived from Lm to be processed and presented via both MHC class I and class II pathways, in potent CD4+ and CD8+ T-cell-mediated immune responses. Moreover, the sensitivity of Lm to antibiotics means the vector can be easily killed if the patient shows severe adverse effects. Lm–based vaccine potency can be further enhanced by the means of encoding recombinant proteins composed of HPV E6/E7 antigen fused to immunostimulatory molecules. In preclinical trials Lm-based vaccines against E7 can induce the regression of solid implanted tumors in transgenic mice with tissue specific expression of HPV 16 E6 and E7 oncoproteins and overcome central tolerance by expanding low avidity CD8+ T cells specific
for E7 [43]. Intravaginal immunization with live attenuated *Salmonella enterica* serovar Typhimurium expressing HPV-16 antigens induced transient inflammatory responses in the genital mucosa and conferred protection against subcutaneously implanted HPV-16 tumors; however *Salmonella* has yet to enter clinical trials for therapeutic HPV vaccine. Hence, there is potential for bacterial vectors to not only serve as vaccine vectors but possibly as cancer immunotherapeutics as well, however it is necessary to analyze the immunogenicity of the vector and limiting vector-associated toxicity for further bacterial vector-based therapeutic HPV vaccine development [39,44].

6.1.2. Viral-vector-based

The high immunogenicity, highly efficient infection rates and expression of encoded antigen in the infected cells are attractive option in therapeutic HPV vaccines. There are many preclinical studies on the efficacy of live viral vectors which include: vaccinia virus [45,46], adenoviruses [47,48], vesicular stomatitis viruses [49], alphaviruses such as the Venezuelan equine encephalitis virus [50], adeno-associated [51], virus and fowlpox viruses [39,49,52]. The vaccinia virus is considered to be particularly promising for antigen-specific immunotherapy due to its high efficiency of infection. In mice model vaccinia virus constructs encoding E7 that enhance antigen presentation in DCs have shown to generate E7-specific immune responses that can cause regression of E7–expressing tumors. In phase I/II clinical trials has been evaluated a recombinant vaccinia vector encoding an HPV-16/18 E6 and E7 fusion protein, termed TA-HPV [53,54]. The therapeutic HPV vaccine candidate was evaluated in patients with therapy-unresponsive late stage CC, and was observed the patients developed HPV 18 E7-specific antibodies and HPV–specific CD8+ T-cells, showing that TA-HPV was capable of eliciting immune responses. Also in safety and immunogenicity study in patients with stage Ib or Ia CC, the vaccine was well tolerated and HPV–specific CTL response was observed. TA-HPV has also been tested with some success in patients with other HPV associated malignancies including VIN (vulvar intraepithelial neoplasia) and VAIN (vaginal intraepithelial neoplasia) [53]. In a clinical study in VIN and VAIN patients vaccinated with TA-HPV, elicited increased antibody and T-cell responses, and 5 out of 12 patients showed at least a 50% reduction in lesion diameter over a 24-week period, and 1 patient showed complete regression of the lesion, reduced viral load or viral clearance and increased antigen specific immune responses. Actually, an ongoing multi-center phase II clinical trial is investigating TA-HPV in combination with surgery to treat women with Stage Ib or Ia CC [53].
Currently, a recombinant vaccinia virus derived from modified vaccinia Ankara (MVA) encoding E2 protein of bovine papillomavirus, has been tested in patients with CIN and flat condyloma lesions. MVA E2 was designed for gene therapy, because MVA E2 expresses E2, so E2 can bind to HPV genome and prevent the upregulation of E6 and E7 oncogenic proteins for the potential control of HPV-associated CIN lesions. MVA E2, has been evaluated in phase II clinical trial in CIN 2/3 patients. The therapeutic scheme includes intrauterinely injected once a week, over a 6 week period. MVA E2 has demonstrated efficacy, all patients presenting lesion regression, reduction in lesion size and eliminating all HPV DNA. MVA E2 vaccine was well-tolerated and generated a specific CTL response against papilloma-transformed cells [55]. MVA E2 vaccine has been probed in a phase I/II study

**Table 4.** Clinical trials on virus-based vaccines.

<table>
<thead>
<tr>
<th>Vaccine / Organization</th>
<th>Strategy</th>
<th>Target / Phase</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVA E2 / Instituto Mexicano del Seguro Social (IMSS).</td>
<td>Recombinant vaccinia derived from MVA encoding E2 of bovine papillomavirus</td>
<td>HPV-16 E2 / Phase I/II</td>
<td>Patients with CIN 1-3. Patients with HG-CIN. Men with flat condyloma lesions</td>
</tr>
<tr>
<td>TG4001/R3484 (MVA-HPV-IL2 / Transgene/Roche</td>
<td>Recombinant vaccinia virus derived from MVA expressing HPV-16 modified E6 and E7 proteins and IL-2</td>
<td>HPV-16 E6/E7 / Phase Iia</td>
<td>women with CIN 2/3</td>
</tr>
<tr>
<td>TA-HPV /Xenova/ Celtic Pharma.</td>
<td>Live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV-16 and HPV-18</td>
<td>HPV-16/18 E6/E7. Phase I/II</td>
<td>Patients with late-stage CC. Patients with Stage Ib or IIa CC. Patients with high-grade VIN or VAIN: (11 VIN 3, 1 VAIN 2). Patients with HPV-16-positive VIN 3.</td>
</tr>
<tr>
<td>TA-HPV/European Organization for Research and Treatment of Cancer</td>
<td>Live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV-16 and HPV-18</td>
<td>HPV-16/18 E6/E7. Ongoing II.</td>
<td>Patients with Stage Ib or IIa CC.</td>
</tr>
</tbody>
</table>
of men with intraurethral flat condyloma. The patients developed antibodies against MVA virus E2 protein over a 4-week period, and had no detectable viral DNA after treatment; also the patients did not show any recurrence of lesions after 1 year of treatment. Therefore MVA E2 has demonstrated efficacy in controlling HPV-associated lesions, it is not clear how much of these effects occurred. Neither has shown the mechanism by which the vaccine can generate an E2-specific immune response [52,56,57]. In table 4, resumes the currently clinical trials on virus-based vaccines.

6.2. Peptide-based Vaccines

Peptide-based vaccines, involves the direct administration of peptides derived from HPV antigens for uptake by DCs, and presented in association with the MHC class I and/or class II pathway on human leukocyte antigen (HLA) molecules [10]. The peptide-based vaccines are easy to produce, stable and safe and suffer from low immunogenicity. Furthermore the polymorphic nature of the HLA molecules in genetically diverse populations makes it difficult to identify one immunogenic epitope which would cover all individuals, and it may be difficult to produce an effective vaccine in a variety of patients with different HLA haplotypes, making it impractical for large scale vaccination treatments [39]. This can be overcome through the use of overlapping long peptides that contain several epitopes of E6/E7 [58]; so is necessary developed immunogenic peptides or peptides that direct CD4+ HTL or CD8+ CTL immune responses; therefore the development of peptide-based vaccines, have been made possible by the identification of various MHC-restricted CD4+ HTL and CD8+ CTL epitopes of HPV early proteins in murine and human models. Another difficult in use of peptide-based vaccines is the poor immunogenic, so the most of the research in this area has focused on the use of adjuvants to enhance vaccine potency [33,59,60]. Other strategy for potentiating peptide-based vaccines includes employing the intranasal route of administration and linkage of peptides to lipids and the enhancement of epitopes to prevent peptide degradation; some of the most employed adjuvants in peptide-based vaccines on preclinical trials are: 4-1BB ligand [61], mutant cholera toxin [62] and CpG oligodeoxynucleotide (CpG ODN) [63], which mimic bacterial danger signals for Toll-like receptor 9 [64]. The strategy to increase and sustain levels of CTL response has been studied using DC-activating agents such as 4-monophosphoryl lipid A and granulocyte-macrophage colony-stimulating factor (GM-CSF). Also has been probed the linkage of peptides to lipids and the enhancement of epitopes to prevent peptide degradation; the therapeutic
Impact of Prophylactic and therapeutic HPV vaccines

vaccination with E6 and E7 long peptides has been shown to result in the control of both established virus-induced lesions and lately infected sites; so several peptide-based vaccines have been found to be safe and well tolerated in preclinic trials [65]. In table 5, resumes the actually clinical trials on peptide-based vaccines.

**Table 5.** Clinical trials on Peptide-based vaccines.

<table>
<thead>
<tr>
<th>Vaccine / Organization</th>
<th>Strategy</th>
<th>Target / Phase</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overlapping long peptide &amp; Montanide ISA- 51 adjuvant / Dutch Cancer Society, ISA Pharmaceuticals.</td>
<td>13 peptides together (nine E6 and four E7 peptides of 25-35 aa long with an overlap 10-14 aa) representing the entire sequence of HPV-16 E6 and E7, formulated in Montanide ISA 51 adjuvant.</td>
<td>HPV-16 E6/E7. Phases I and II.</td>
<td>Phase I: patients with HPV-16-positive VIN 3 and end-stage CC patients. Phase II: patients with resected HPV-16 positive stage IB1 CC and patients with HPV-16-positive VIN 3 [58,59].</td>
</tr>
<tr>
<td>Peptide &amp; Montanide ISA-51 adjuvant / NCI.</td>
<td>HLA-A*0201-restricted HPV-16 E7 epitopes (aa 12 - 20 ± aa 86 - 93) linked to non-specific helper peptide known as PADRE, emulsified in Montanide ISA 51 adjuvant.</td>
<td>HPV-16 E7. Phase I.</td>
<td>Patients with HG-CIN 2/3 or VIN 2/3 [58,59].</td>
</tr>
<tr>
<td>Peptide &amp; Montanide ISA 51 adjuvant / IDM Pharma.</td>
<td>HLA-A*0201-restricted HPV-16 E7 peptide (aa 11 - 20 &amp; 86 - 93) and PADRE HTL peptide, emulsified in Montanide ISA 51 adjuvant.</td>
<td>HPV-16 E7. Phases I / II.</td>
<td>HLA-A*0201-positive patients with recurrent or residual CC [58,59].</td>
</tr>
<tr>
<td>Lipopeptide / IDM Pharma.</td>
<td>Lipidated E7 (HLA-A*0201-restricted epitope, aa 86–93 lipopeptide) linked to PADRE HTL peptide.</td>
<td>HPV-16 E7. Phase I.</td>
<td>HLA-A2- positive patients with HPV-16-positive recurrent or refractory CC [66-68].</td>
</tr>
</tbody>
</table>
6.3. Protein-based vaccines

The protein-based vaccines against CC and associated lesions to HPV are an excellent strategy, because they have several advantageous properties.

**Table 6. Clinical trials on Protein-based vaccines.**

<table>
<thead>
<tr>
<th>Vaccine / Organization</th>
<th>Strategy</th>
<th>Target / Phase</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGN-00101 (HspE7), which is a fusion of <em>Mycobacterium bovis</em> variant bacilli Calmette-Guérin heat shock protein (Hsp65) and HPV-16 E7 [72], &amp; Poly ICLC adjuvant / Akela Pharma.</td>
<td>SGN-00101 adjuvanted in Poly ICLC.</td>
<td>HPV-16 E7. Ongoing, phase I</td>
<td>Women with CIN 1, 2, or 3. HspE7 is well tolerated in patients with HG-CIN, and to generate lesion regression in several HPV-associated diseases including genital warts, recurrent respiratory papillomatosis and HG-CIN [73,74].</td>
</tr>
<tr>
<td>PD-E7 / GlaxoSmithKline</td>
<td>Fusion protein comprising mutated HPV-16 E7 linked to first 108 aa of <em>Haemophilus influenza</em> protein D, in AS02B adjuvant.</td>
<td>HPV-16 E7. Phases I/II</td>
<td>Patients with CIN 1 or CIN 3.</td>
</tr>
<tr>
<td>TA-CIN, a genetically engineered fusion of L2, E6 and E7 proteins from HPV-16 / Celtic Pharma</td>
<td>Recombinant HPV-16 L2/E6/E7 fusion protein</td>
<td>HPV-16 L2/E6/E7. Phase I.</td>
<td>Healthy volunteers. TA-CIN induces antibody responses against L2 and T cell immunity against HPV-16 E6 and E7 and proving to be safe and immunogenic [75].</td>
</tr>
<tr>
<td>Therapeutic Antigen-Genital Warts (TA-GW), a fusion of HPV-6 L2 and E7 absorbed onto Alhydrogel / Celtic Pharma.</td>
<td>Recombinant HPV-6 L2/E7 fusion protein adjuvanted with 2% Alhydrogel.</td>
<td>HPV-6 L2/E7. Phases I and IIA</td>
<td>Healthy male volunteers and patients with genital warts. TA-GW has been tolerated by patients and was effective in clearing HPV genital warts in a subset of patients [76].</td>
</tr>
</tbody>
</table>
They are safe and easy to produce. Protein antigens can be processed and presented on the surface of DCs and contain all possible HLA epitopes of an antigen. However, the protein-based vaccines presented low immunogenicity, and as a result, adjuvant and fusion protein strategies are often used to enhance vaccine potency. Another limitation of protein-based vaccines is that proteins may elicit better antibody responses than CTL responses and APCs may only occasionally encounter and engulf an injected protein for MHC class I presentation [10]. The use of adjuvants, such as the liposome-polycation-DNA [65] or the saponin-based adjuvant ISCOMATRIX [69] and fusion with other immunostimulatory molecules, can improve CTL responses. Fusion HPV-16 E7 proteins with bacterial proteins such as the Bordetella pertussis adenylate cyclase (CyaA), a protein that targets APCs through specific interaction with αMβ2 integrin, or with the translocation domain of bacterial exotoxin Pseudomonas aeruginosa exotoxin A (EXA) [70,71], induce E7-specific CTL responses, or the Mycobacteria-derived heat-shock proteins (Hsp), enhance CTL responses and inhibit angiogenesis in tumors. In table 6, resumes the actually clinical trials on Peptide-based Vaccines.

6.4. DNA-based vaccines

DNA-based vaccines have emerged as a potentially and attractive promising approach against CC and associate lesions to HPV, due to naked DNA is safety, stable and ease of preparation. However, since DNA does not have the intrinsic ability to amplify or spread in transfected cells in vivo like viral vectors. DNA vaccines do not elicit neutralizing antibody production in the patient, so can have limited immunogenicity, and thus can be repeatedly administered. Since DCs are essentials in the generation of antigen-specific immune responses, it is important to develop strategies to modify the properties of the DNA-transfected DCs and enhanced the DNA potency. These strategies include increasing the number of antigen-expressing/antigen-loaded DCs, improving antigen processing and presentation in DCs, and enhancing the interaction between DCs and T cells (Table 7) [10,37-39, 77,78].

The strategies listed in Table 7 are being tested in preclinical phase (Table 8). However, other strategies are under clinical phase (Table 9) [10, 37-39,78-80].
Table 7. Strategies to enhance DNA-based vaccines potency.

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Via</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration by Gene gun.</td>
<td>Intradermal administration delivers gold particles coated in DNA directly to the immature DCs of the skin, the Langerhans cells.</td>
<td></td>
</tr>
<tr>
<td>Laser treatment</td>
<td>Treatment with femtosecond laser immediately after delivery of DNA vaccines can enhance the transfection efficiency of the DNA vaccine.</td>
<td></td>
</tr>
<tr>
<td>Electroporation</td>
<td>The recipe of intramuscular injection with electroporation enhances DNA uptake, creating large numbers of muscle cells expressing the desired antigen and increasing release of antigen, which local DCs can then process and present through the MHC class I pathway.</td>
<td></td>
</tr>
<tr>
<td>Intercellular antigen spreading as a strategy to increase the number of antigen-expressing DCs</td>
<td>Linkage of HPV antigen with proteins capable of intercellular transport in the context of DNA vaccination allows this spread of antigen in cells transfected with DNA.</td>
<td></td>
</tr>
<tr>
<td>Linkage of antigen to molecules capable of binding to DCs as a method to target antigen to DCs</td>
<td>Linkage of HPV antigen to molecules that target the antigen to the DC surface, such as FMS-like tyrosine kinase 3 (Flt3) ligands and heat shock protein, which binds with scavenger receptors on DCs, such as CD91.</td>
<td></td>
</tr>
<tr>
<td>Employment of chemotherapeutic-induced apoptotic cell death to increase the number of antigen-loaded DCs</td>
<td>Co-administration of therapeutic HPV DNA vaccines with chemotherapeutic agents (EGCG, (epigallialcatechin gallate), cisplatin, bortezomib, DR5 (death receptor 5), and agonist) release HPV E6/E7 antigens from apoptotic tumor cells, which may potentially facilitate antigen uptake by local DCs, resulting in enhancement of DNA vaccine potency.</td>
<td></td>
</tr>
<tr>
<td>Codon optimization as a strategy to enhance antigen presentation in DCs</td>
<td>Replaces codons infrequently used by the host cells with more commonly used codons to enhance translation of encoded antigens in cells transfected with DNA.</td>
<td></td>
</tr>
<tr>
<td>Employ of demethylating agents to increase antigen expression</td>
<td>There is reduced expression of DNA when methylated, thus demethylating agents upregulate gene expression.</td>
<td></td>
</tr>
<tr>
<td>Employment of intracellular targeting strategies:</td>
<td>The linkage of antigen to proteins that target the antigen for proteasomal degradation or entry into the endoplasmic reticulum can improve MHC class I presentation of linked antigen in DCs. MHC class II processing can also be enhanced, resulting in greater CD4+ HTL responses to rise CD8+ CTLI responses.</td>
<td></td>
</tr>
<tr>
<td>- to enhance MHC class I antigen presentation in DCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- to enhance MHC class II antigen presentation in DCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhancing the expression of MHC class II molecules</td>
<td>Cells transfected with DNA encoding MHC GHI, a master regulator of MHC class II expression, can lead to higher expression of MHC I and II molecules on transfected cells, leading to enhanced antigen presentation through the MHC I/II pathways.</td>
<td></td>
</tr>
<tr>
<td>Bypassing antigen processing as a method for generating stable Ag presentation in DCs</td>
<td>MHC class I single-chain trimer technology is a strategy to bypass the antigen processing/presentation.</td>
<td></td>
</tr>
<tr>
<td>Employing cytokines and costimulatory molecules to enhance T-cell activation</td>
<td>The enhancement of immunogenicity of a therapeutic CC DNA-based vaccine by co-application of sequence-optimized genetic adjuvants including DNA encoded cytokines (IL-2, IL-12, GM-CSF, IFN-gamma) and the chemokine MIP-1-alpha.</td>
<td></td>
</tr>
<tr>
<td>Prolonging DC survival to enhance T cell interaction</td>
<td>Employ of DNA encoding antia apoptotic proteins or with siRNA or shRNA targeting the key proapoptotic proteins Bak, Bax and Fas ligand or coadministration of DNA vaccine with the antia apoptotic protein Bcl-2.</td>
<td></td>
</tr>
<tr>
<td>Induction of CD8+ CTL as a strategy for augmenting CD8+ CTL responses</td>
<td>DNA vaccine, which encodes MHC class II-associated invariant chain (Ii) replaced with the pan HLA-DR binding epitope (PADRE) (Ii-PADRE).</td>
<td></td>
</tr>
<tr>
<td>Eliminating immunosuppressive regulatory T cells</td>
<td>The interaction between DCs and T cells may be better by eliminating immunosuppressive regulatory T cells. Using an anti-CD25 monoclonal antibody, PC51, to eliminate regulatory T cells before E7/HSP70 DNA vaccination potentiate effects of HPV DNA vaccines.</td>
<td></td>
</tr>
</tbody>
</table>
Table 8. Preclinical trials with DNA-based vaccines.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Model</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCDNA-HSP60/E6/E7</td>
<td>C57BL/6 mice</td>
<td>Strong immune response, antitumor effects.</td>
</tr>
<tr>
<td>IL-6/HPV16E7 DNA</td>
<td>Mice</td>
<td>Increased immune responses; antitumor effects.</td>
</tr>
<tr>
<td>pCDNA3-Sig/E7/LAMP-I and pSG5-Bclx-xL DNA vaccines enhanced by pcDNA3-li-PADRE DNA</td>
<td>C57BL/6 mice</td>
<td>Antitumor effects; enhancement of E7-specific CD8+ effector and memory T cells.</td>
</tr>
<tr>
<td>pCDNA3-IL2-E7</td>
<td>C57BL/6 mice</td>
<td>High E7-specific CD8+ T cells; protective and therapeutic antitumor effect.</td>
</tr>
</tbody>
</table>

Table 9. Clinical trials on DNA-based vaccines.

<table>
<thead>
<tr>
<th>Vaccine / Organization</th>
<th>Strategy</th>
<th>Target / Phase</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYC101 / Eisai (formerly MGI Pharma, formerly Zycos)</td>
<td>Plasmid DNA encoding HLA-A2- restricted epitopes derived from HPV-16 E7 protein (aa 83-95), encapsulated in 1-2 µm biodegradable poly(lactide coglycolide) microparticles</td>
<td>HPV-16 E7 / Phase I</td>
<td>HLA-A2- positive men with HPV-16-positive HG-AIN. patients with CIN 2/3</td>
</tr>
<tr>
<td>ZYC101a (Amolimogene beiplasmid) / Eisai (formerly MGI Pharma, formerly Zycos)</td>
<td>Plasmid DNA encoding fragments derived from HPV-16 and 18 E6 and E7 proteins, encapsulated in 1-2 um biodegradable poly-(D,L-lactideco glycolide) microparticles</td>
<td>HPV-16/18 E6/E7 / Phase I and II</td>
<td>Patients with CIN 2/3</td>
</tr>
<tr>
<td>pNGVL4a-Sig/E7(detox)/Hsp70 / NCI</td>
<td>Plasmid DNA expressing HPV-16 E7 mutated to abolish Rb binding site linked to sequences coding for Sig and for heat shock protein 70</td>
<td>HPV-16 E7 / Phase I</td>
<td>Patients with CIN 2/3</td>
</tr>
<tr>
<td>pNGVL4a-CRT/E7(detox) / NCI</td>
<td>Plasmid DNA expressing HPV-16 E7 mutated to abolish Rb binding site linked to sequence coding for calreticulin</td>
<td>HPV-16 E7 / Phase I</td>
<td>Patients with CIN 2/3</td>
</tr>
<tr>
<td>VGX-3100 / Inovio Biomedical Corp / VGX Pharmaceuticals</td>
<td>Plasmid DNA expressing HPV-16 and HPV-18 E6 and E7 proteins</td>
<td>HPV-16/18 E6/E7 / Phase I</td>
<td>Adult females, postsurgical or ablative treatment of CIN 2/3</td>
</tr>
<tr>
<td>DNA HPV16 E6/E7 / Johns Hopkins Medicine in Baltimore</td>
<td>DNA vaccine expressing the HPV16 E7 antigen, twice every 4 weeks, followed by recombinant vaccinia boost that expressed HPV16 and HPV18 E6 and E7 antigens another 4 weeks later.</td>
<td>HPV16 E6/E7 / Phase I and II</td>
<td>Patients with high-grade cervical intraepithelial neoplasia from an HPV infection. Patients were between 22 and 49 years of age.</td>
</tr>
</tbody>
</table>
6.5. RNA based-vaccines

The RNA replicons are naked RNA that can replicate in a self-limiting fashion within the transfected cell. Therefore, they can sustain the cellular antigen expression and a result; produce more antigenic protein than convectional DNA vaccines. RNA replicons may be derived from alphaviruses, such as Venezuelan Equine Encephalitis [81], Semiliki Forest virus [82] and Sindbis virus [83] and can replicate in a wide range of cell types. Many replicons are designed to lack the structural genes, and thus no infection particles are produced and the host and prevents the formation of neutralizing antibodies against the viral capside. Further-more, RNA replicons minimizing the risk of potential chromosomal integration and cellular transformation associated with DNA vaccines. In addition, the expression of inserted genes in RNA based-vaccines is transient and thereby reducing their effectiveness in stimulating the immune system [10,38,39].

RNAi may silence the expression of genes that encode for tumoral antigens or viral oncoproteins, in order to repress the specific proliferation of cancerous cells. The administration of siRNAs led to mRNA cleavage and the specific silencing of HPV16 E6 and E7 oncogenes expression. Besides this, E6 silencing induced the expression of gene p53, transactivation of the inhibiting gene of p21-CIP1/WAF1 cyclin-kinase, and decrease of cellular proliferation, whereas silencing of E7 induced cellular death by apoptosis. Additionally, have been analyzed the use of siRNAs for HPV E6/E7 oncogenes, which is their ability to silence the HPV E6-E7 bicistron. The effect of synthetic siRNA for HPV16 E6 oncogene on SiHa cells (HPV16+) has been reported and the silencing of both E6 and E7 oncogenes has been observed [84]. RNA replicons and siRNAs thus shown promising results in preclinical models; however are needed to evaluate efficacy and safety before investigated in clinical trials.

6.6. Tumor cells based-vaccines

The manipulation and isolating ex vivo of tumor cells to express immunomodulatory proteins can enhance their immunogenicity in vivo by expressing cytokines such as IL-2, IL-12 and GM-CSF [38]. The use of tumor cells based-vaccines is an interesting strategy, because is not necessary a clear identification of tumor antigens; however tumor antigens associated with HPV are largely know. In a murine model the vaccination with GM-CSF-expressing tumor cells led to an E7-specific CTL response and potent antitumor effects against tumors in vivo [85]. Furthermore, vaccination with irradiated E6/E7-positive tumor cells expressing IL-12 significantly decreased the size of E6/E7-expressing tumors [86]. In other pre-clinical model in mice, expression in tumors of the ligand for the
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lymphotoxin-beta receptor, generate in an increased expression of INF-γ and chemoattractant cytokines such as IL-1α, MIG and MIP-2. This result conduce an increased frequency of tumor-infiltrating CD8+ CTLs and eradication of large established tumors. However, the tumor cell-based vaccines are costly and difficult to produce on a large scale without introducing variations in purity and efficacy, so this strategy have limited scope for HPV vaccine development [10, 37,39].

6.7. Dendritic cells based vaccines.

This strategy is created to enhance T-cell mediated immunity by loading DCs with HPV antigens ex vivo and delivering them in those with HPV-associated lesions, so the DC acts as natural adjuvant. DCs can be prepared ex vivo for various methods include the usage of viral vectors, transfection with DNA or RNA encoding antigen and pulsation of DCs with antigenic protein, peptide or tumor cell lysates. Moreover, reintroduction of mature DCs bearing HPV antigens allows for more effective antigen presentation and thus a stronger immune response. For effective loading of tumor antigen into DCs, can be achieved through gene delivery to DCs by targeting adenoviral vectors to CD40 with specific antibodies. Intramuscular delivery has been shown the most effective method for generating large numbers of E7-specific CD8+ T cell precursors [37,39]. Several clinical trials in humans has been developed (Table 10). A study of DCs loaded with HPV-16 or HPV-18 E7 co-administered with IL-2 in HPV-16/18+ refractory CC patients showed E7-specific CD4+ and CD8+ responses. In a case report,

**Table 10.** Clinical trials on DC based vaccines.

<table>
<thead>
<tr>
<th>Vaccine / Organization</th>
<th>Strategy</th>
<th>Target / Phase</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC / National Taiwan University Hospital.</td>
<td>Autologous mature, monocyte-derived DCs pulsed with HPV-16 E7 peptide.</td>
<td>HPV-16 E7 / Ongoing, I.</td>
<td>Patients with recurrent CC.</td>
</tr>
<tr>
<td>DC / Deutsche Forschungsgemeinschaft, BMBF</td>
<td>Autologous, mature, monocyte-derived DCs loaded with recombinant HPV-16 E7 or HPV-18 E7</td>
<td>HPV-16 E7 or HPV-18 E7. Pilot study.</td>
<td>Patients with progressive or recurrent CC.</td>
</tr>
<tr>
<td>DC + IL2 / Italian Institute of Health (ISS).</td>
<td>Autologous, mature, monocyte-derived DCs pulsed with recombinant HPV-16 E7 or HPV-18 E7</td>
<td>HPV-16 E7 or HPV-18 E7. Case series.</td>
<td>CC patients with recurrent disease refractory to standard treatment.</td>
</tr>
<tr>
<td>DC + IL2 / Autologous mature monocytes derived DCs pulsed with HPV-18 E7 protein.</td>
<td>HPV-18 E7. Case report.</td>
<td>Patient with Stage IB2 CC.</td>
<td></td>
</tr>
<tr>
<td>DC + KLH / NIH</td>
<td>Autologous mature monocyte-derived DCs pulsed with recombinant HPV-16/18 E7 antigens and keyhole limpet hemocyanin (KLH), an immunological carrier protein.</td>
<td>HPV-16/18 E7. Phase I.</td>
<td>Patients with Stage Ib or IIa CC.</td>
</tr>
</tbody>
</table>
Table 11. Therapeutic HPV vaccine advantages and disadvantages.

<table>
<thead>
<tr>
<th>Vaccine Approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Viral vector-based | • High immunogenicity.  
• Wide variety of vectors available.  
• Can facilitate intracellular antigen spreading.  
• Different immunological properties of viruses. | • Risk of toxicity in using live viruses.  
• Potential pre-existing immunity may inhibit repeated administration.  
• Possible dominance of immune response to viral vector rather than HPV antigen. |
| Bacterial vector-based | • High immunogenicity.  
• Can deliver either engineered plasmids or HPV tumor proteins to APC  
• Wide variety of vectors available | • Risk of toxicity in using live bacteria  
• Potential pre-existing immunity  
• Inhibited repeat immunization |
| Peptide-based | • Easy to produce. Stable, Safe  
• Can combine multiple epitopes.  
• Can engineer peptides for enhanced MHC binding. | • Low Immunogenicity.  
• Epitopes must be determined.  
• HLA-restriction.  
• Difficult to have one-fits-all peptide. |
| Protein-based | • Stable, Safe. Easy to produce.  
• No HLA restriction.  
• Multiple known adjuvants. | • Low Immunogenicity; requires adjuvant.  
• Usually better induction of antibody response than CTL response. |
| DNA-based | • Safe, easy to produce, stable for storage and transportation.  
• Capacity for repeated administration.  
• Easy to prepare at high purity  
• Several delivery methods possible.  
• Sustained expression of antigen on MHC-peptide complex.  
• Can be engineered to add targeting and/or co-stimulatory genes | • No intercellular spreading immunogenicity  
• Risk of integration into genome or cellular transformation. |
| RNA-based | • Non-infectious, no risk of genomic integration or cellular transformation.  
• Transient.  
• Can administer multiple times.  
• Enhanced antigen expression.  
• Multiple vectors are available. | • Unstable, difficulty in long-term storage.  
• Labor-intensive preparation.  
• Difficult to prepare large amounts.  
• No intercellular spreading. |
| Dendritic cell-based | • High immunogenicity, uses the most potent APC.  
• Multiple methods available to load antigen.  
• Efficient antigen presentation.  
• Potency can be enhanced by gene transduction or cytokine treatment. | • Labor intensive, expensive, ex vivo, individualized cell processing.  
• Variable quality control and a lack of agreed standards for quality of vaccines.  
• Difficult to produce on a large scale.  
• DCs do not necessarily home to lymph nodes. |
| Tumor cell-based | • Useful if tumor antigen unknown.  
• Likely to express tumor antigens.  
• Potency can be enhanced by cytokine treatment. | • Safety concerns about injecting tumor cells into patients.  
• Labor-intensive as individualized.  
• Costly, difficult to produce on a large scale.  
• Requires availability of tumor cell lines or autologous tumor cells. |

Subcutaneous injection of HPV 18 E7-pulsed DCs in a patient with metastatic CC led to inhibition of tumor progression, but not causes complete remission. The success of DC based vaccines have serious limitations; these vaccines cannot be produced at a large scale, they are
complicated to produce and expensive, additionally is necessary elucidating the most effective delivery route and developing methods to enhance antigen loading [38,39,87,88].

7. **HPV-immunotherapy**

Various strategies have been evaluated to reverse the effect of immunosuppression in tumor microenvironment, including: inhibition of HPV oncogenic proteins, activation of the host specific immune response against HPV antigens by the use of specific HPV induction of expression of costimulatory molecules (B7, CD3 ζ chain, MHC class I) and the administration of Th1 cytokines to activate the immune response cell type. In several CC preclinical models, has been tested to interferons (IFN) and cytokines IL-2, IL-12 and GM-CSF, which are considered as major modulator of cellular immune response. Also in several murine tumor models associated with HPV, have been analyzed several immunization routes and vehicles. In fact we found a relationship between the antitumor effect of the IL-12 gene expression and expression of Th1 cytokines in an HPV16-positive murine tumor model. [10,38,39,77,78,84,89]. The table 11 describes the advantages and disadvantages of therapeutic HPV vaccines.

**Conclusions**

Cervarix® and Gardasil® had shown to be highly immunogenic in clinical trials, resulting in essentially 100% seroconversion. In this chapter we discuss that prophylactic HPV vaccines provide promise as a key component of future CC prevention programs in the Latin America and the Caribbean region. The successful introduction and acceptance of these vaccines depend on a range of factors including awareness of CC as a problem, affordability of the vaccine, political will, and competition with other vaccines, feasibility of vaccine delivery and acceptability of the vaccine among the range of groups who will influence uptake. Price is currently a major barrier to vaccine acceptability and a priority for advocacy; however, is necessary that health policymakers and vaccines providers to recognize the need for more widespread vaccination against HPV, it could result in unnecessary HPV-related cancer deaths [86]. This included mandates changes in the current HPV-vaccination program to include males as well as females beyond age 26 years, in order to achieve wide-spread immunity against oncogenic strains of the virus. Current arguments continue to call for vaccination solely of “high risk” populations, point out the high cost of expanding coverage beyond this group and raise social and political concerns of advocating promiscuity by making the vaccine
more widely available [90]. Additionally, two doses in 9-10 year old girls are better than three doses in 25 years old women [31]. On the other hand, we discuss the main strategies involved in the development of therapeutic vaccines, and we concluded that is necessary evaluated several strategies in clinical trials, furthermore is necessary the combination of different therapeutic strategies and developed new adjuvants. In addition to understanding the molecular biology of the tumor may favor the development of more effective therapeutic vaccines. HPV prophylactic vaccines represent an important strategy to prevent infection with HPV, however they are very expensive. The high prevalence of HPV malignancies and HPV-associated lesions worldwide represents a pressing need to develop of therapeutic vaccines for CC.

References

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