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Vaccine Strategies against Human Papillomavirus: A Discussion Focused on Developing Countries

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Abstract

Cervical cancer is the second most common form of cancer among women, and responsible for 274,000 deaths each year, most of which occur in developing countries. Persistent infection with high-risk human papillomavirus (HR-HPV) is an essential factor in the development of cervical cancer and also a contributory factor in other types of cancer. The current prophylactic HPV vaccines provide protection against the -16 and -18 genotypes which are most commonly associated with cervical cancer worldwide. However, the increased costs of these vaccines inhibit their implementation in developing countries, affecting their viability. Moreover, a therapeutic vaccine is needed for women who are already infected by HPV and/or affected by HPV-related cancer. A number of innovative approaches to combat and treat HPV infection are currently being studied and some of these will be consider in this work, together with the development of new vaccines, especially in seriously affected areas located in developing countries. At the same time, there will be a discussion of the issues involved in carrying out effective HPV vaccination programs; these will take account of financial constraints, the lack of adequate infrastructure and the competing priorities, that are found in the surrounding social context of the developing countries.

Keywords: Human papillomavirus; Cervical cancer; Prophylactic vaccines; Therapeutic vaccines; HPV vaccination

Introduction

Every year about 500,000 women develop cervical cancer and 274,000 die from this disease throughout the world, resulting in a mortality rate of approximately 55% [1-4]. Over 80% of these deaths occur in developing countries [4,5]. Worldwide, cervical cancer affects around 1.4 million women, and the highest incidence rates are found in Africa and Latin America, while India has the largest number of cases (20%) [4]. While among developed countries the 5-years patient survival rate ranges from 51% to 66%, in developing countries, where cases only tend to the diagnosed in a relatively advanced stage, this survival rate is about 40%. The world average is estimated as being 49% [3].

According to World Health Organization (WHO), persistent infection by Human Papillomavirus (HPV) is the main risk factor for developing cervical cancer [6]. The relationship between cervical cancer and HPV infection has been established by epidemiological and functional studies [7,8], in which the virus was detected in more than 99.7% of squamous cell carcinoma [9,10] and in 94-100% of cervical adenocarcinoma and adenosquamous carcinoma [11,12]. However, it takes several years for the cervical cancer to become established. At least 15 oncogenic HPV types have been identified as high-risk (HR-HPV), among which -16 and -18 genotypes are detected in more than 70% of all cases of cervical cancer [13]. The monitoring of the uterine cervix and removal of premalignant lesions can result in significant decline in the mortality rates reported worldwide. Apart from skin cancer, cervical cancer shows the greatest scope for prevention and cure when diagnosed early [3].

It should be mentioned here that screening programs are necessary to ensure that cervical cancer is prevented to a satisfactory degree, but in developing countries these programs are performed in precarious manner. Prophylactic vaccination is a potential means of supplementing the screening programs, and helping to reduce the burden of cervical cancer by preventing HPV infection [14]. The search for preventive vaccines against HPV infection has been the object of studies for several decades [15]. Although the production of inactivated or attenuated HPV virions on a large scale is difficult to reproduce in vitro, because the productive life-cycle is dependent on epithelial differentiation [16,17], the emergence of new technologies has intensified the development of these vaccines [18]. The HPV prophylactic vaccines already licensed are based on these methodologies.

Since a large number of people are already infected with HPV, and current treatments have a low rate of effectiveness, a therapeutic approach is needed to treat patients with advanced lesions. Therapeutic HPV vaccines form a part of more recent studies, and are still undergoing preclinical testing and clinical studies [19].

The aim of this paper is to review the current status of vaccines that are being evaluated for HPV infection and hence for cervical cancer. These innovative technologies to combat infection are argued, especially given the challenges posed to its availability in developing countries. Finally, we focus on the need to discuss the importance of implementing an effective program involving currently licensed vaccines against HPV for the control of cervical cancer and other HPV-related cancers in developing countries and the poorest regions of the world.

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The Current Situation with Regard to Prophylactic HPV Vaccines

Principles and current formulations

About 120 HPV genotypes have been identified and more than 15 have been shown to induce tumors that can progress to carcinomas [20]. In particular, 53.5%, 17.2% and 6.7% of cervical cancer cases worldwide are attributed to HPV -16, -18 and -45 respectively. The remaining 12 oncogenic genotypes (-31, -33, -52, -58, -35, -59, -56, -51, -39, -68, -73 and -82, in descending order of importance) cause 30% of all the cases of cervical cancer [18].

At present, there are two licensed preventive vaccines against HPV, both composed of macromolecular structures formed by the L1 subunits, called virus-like particles (VLPs), which are morphologically similar to the virion. These current HPV vaccines comprise Gardasil® from Merck & Co., Inc. [21], composed of four VLP types (HPV-6, -11, -16, and -18), and Cervarix® from GlaxoSmithKline, UK. [22], which is bivalent and combines two VLP types (HPV-16 and -18). The need to produce multivalent vaccines is attributed to type-specific immune response, which is due to the L1 immunodominant epitopes that are located in the hypervariable regions of the loops that compose the protein [23]. In addition, neutralizing antibodies recognize conformational (nonlinear) epitopes, which result in a greater binding specificity [24]. Nevertheless, weak cross- reactivity is observed with other HPV types, leading to certain protective effects [25,26].

According to reports from the major phase III trials, the vaccines prevent from 97 to 98% of infections caused by HPV-16 and -18 [27]. Investigators from the FUTURE (Females United to Unilaterally Reduce Ecto/Endocervical Disease) II study found a 98% protection rate after a 3-year follow-up period. Gardasil® is recommended for 11-12 year old girls (who are expected not to have been sexually exposed) [28]. Females in the age range of 9-26 years may also be vaccinated and, recently, vaccination has been shown to be efficacious in women in the age range of 24-45 years who have not already been infected with the relevant HPV types. Cervarix®, on the other hand, is licensed for use among women up to 45 years old in Australia and an immunogenicity study showed 100% seroconversion in women up to the age of 55 [28]. Despite the efficacy that has been observed, the exact duration of antibody protection is unknown, although the longest follow up study so far has shown high antibody levels up to 7.3 years after vaccination with Cervarix® [28].

Attempts to develop alternatives for prophylactic HPV vaccines

Perhaps, the most serious concern about the licensed prophylactic vaccines is their high cost (up to $120 per dose, and 3 doses are required), which makes it difficult for them to be widely available in developing countries, where the vast majority of cervical cancer cases occur [29]. In view of this, several attempts have been made to establish other biotechnological HPV VLP production platforms that can fill the gap that exists in the demand and supply [30]. These measures can reduce the price of currently licensed vaccines, and increase their global penetration [30].

The VLPs can be obtained through the production of L1 protein in heterologous expression systems using mammalian cells [31], plants [32], bacteria [33], insects [34] and yeasts [24]. Both the bivalent and quadrivalent HPV vaccines contain VLPs produced from insect cells (*Trichoplusia ni Hi5* infected with recombinant baculovirus) or *Saccharomyces cerevisiae* yeast, respectively. Since 2007, several investigators have reported the expression and purification of HPV L1 protein in *Pichia pastoris*, and characterized the system as a potential vaccine platform in view of its low cost and the high levels of recombinant protein expression reported in the last 15 years [35]. In a similar way, our research group has been working with the HPV -16, -18 and -33 L1 expression in *P. pastoris* [36,37].

All these studies in the *Pichia* system employ commercial vectors and exploit the potential of the *AOX1* inducible promoter, with a wide range of expression results. In 2005, de Almeida and colleagues [38] isolated and characterized the *P. pastoris* constitutive *PGK1* promoter, which showed higher expression levels than the *AOX1* promoter. Currently, our group has been working on the expression of HPV proteins by employing a non-commercial vector based on this constitutive promoter and the partial results are promising (unpublished data). The main distinguishing feature of this work involves the use of a non-commercial vector and an alternative expression system to those already being employed or that adopted by Merck and GlaxoSmithKline. This avoids the payment of royalties tied to the use of commercial vectors and allows the possibility of making a patent application and thus, having exclusive rights for use in the future production of HPV vaccines.

The role of universities is essential in the production of medicines and vaccines and their participation has been critical in providing the basis for the creation of HPV vaccines [39-41]. The main problem is that, in the past, every public research entity involved in the initial development of these vaccines obtained licenses for the exclusive commercial use of this technology, and this has created a major obstacle to laboratories in developing countries that seek to draw on the new technology. Additionally, all of the business partners involved were based in the developed world [42]. This meant that there was little incentive to develop vaccine for low-cost sale in developing countries.

Technology transfer mechanisms are essential to ensure low-cost access to these new vaccines in developing countries, as well as for the funding of research by foundations with adequate resources. One study of manufacturers in developing country found that there were various arrangements between multi-national corporations (MNCs) and academic research institutions [42]. In the case of Indian and Brazilian vaccine manufacturers, this reliance on technology transfer allows a greater freedom to operate and more equitable benefits. These Indian and Brazilian vaccine manufacturers found that, on average 28 percent of new antigens were being produced, either by in-house research activities or by technology transfer [42].

With regard to this, the financial support by the Bill & Melinda Gates Foundation for the development of a new generation of HPV vaccines is also very important. Public institutions are taking the lead in developing these vaccines and among the participating research institutes is the Ludwig Cancer Center in Brazil [43].

A Pan-HPV vaccine

Another drawback of the available vaccines against HPV is their failure to provide protection against other carcinogenic genotypes which account for about 30% of cervical cancer cases reported worldwide. In addition, the prevalence of HR-HPV [44] is subject to geographical variation, which is the case, for example, in Brazil. Although there are no clear statistics on the prevalence of HPV infection in the sexually-active population in various states and regions, several studies confirm the high prevalence of HPV-16 in all Brazilian regions,

whereas in the case of the second most relevant HPV type, the degree of incidence differs in each region. For example, HPV-31 and -33 are more commonly found than -18 in the Northeast and Midwest regions, while in the North, South and Southeast regions, HPV-18 appears as the second most prevalent type [45-48]. This clearly shows that there is a need to develop a second generation of prophylactic vaccines against HPV, with a larger spectrum of action [49].

As well as the L1 protein, which is the main structural unit of the viral capsid, the PVs have another capsid protein called L2. This contains an N-terminal region that was found to be conserved among the HPV genotypes, and suggests that there is a potential peptide that can cause a cross-immune reaction. In confirmation of this, animals immunized with the HPV L2 protein produced neutralizing antibodies for a broad HPV spectrum [50,51]. This observation contrasts sharply with the type-specific protection induced by L1 and suggests the possibility of a simple pan-HPV prophylactic vaccine, based on the L2 protein [49,52,53].

However, some caveats must be noted. The neutralizing antibody titers produced by vaccination with L2 are much lower than those produced by vaccination with L1 VLPs. Since VLPs, as well as natural immunogens, have proved to be efficient carriers of peptides, DNA and small molecules to dendritic cells [52], some researchers have produced VLPs based on both L1 and L2 proteins to increase the immunogenicity of L2 and extend protection to more genotypes. Unfortunately, the immunogenicity of L2 is subdominant to L1 and, in the context of an L1/L2 VLP or even in serological studies of natural infection; the neutralizing antibodies are produced almost exclusively against the L1 protein [54].

In 2010, Jagu and colleagues [53] evaluated different vaccine formulations with L2 polypetides (different HPV fused neutralizing epitopes) in animals. In accordance with the findings of the authors, the formulations containing L2 polypeptides with L1 capsomeres were found to be the vaccine with the highest potential. This may be an inexpensive strategy, because lowered the production costs of the vaccine by employing a system based on *Escherichia coli,* to expand the immunity against HPV. However, the L2-based prophylactic vaccines have not been tested on patients and when providing protection, it was unclear how long this immunity would last [53].

Therapeutic Vaccine Against Cervical Cancer

A vaccine for the prevention of cervical cancer is very important in public health. However, many women (an estimated figure of 5 million) are already infected by HPV and several of them will develop invasive cervical cancer [54]. The two HPV vaccines currently in use cannot combat the lesion or cancer that has already established itself and, thus, it is essential to seek different strategies that address the major antigens that are active in the transformation by the virus, as well as the cellular pathways involved [55,56].

HPV oncoproteins that are early expressed in the viral cycle and responsible for malignant progression of the lesion, are the most suitable targets in developing therapeutic vaccines because they stimulate the cellular response to the transformed cells [57,58]. Several studies of therapeutic vaccines against cervical cancer are being conducted and almost all of them are using E6 and E7 proteins. Some of these are already undergoing pre-clinical and clinical trials and include the use of peptides or proteins, recombinant vaccines with live virus vectors, cell-based vaccines and DNA vaccine [59,60].

Two of the first therapeutic approaches to HPV vaccines were called TA-CIN and TA-GW and consisted of HPV-16 L2 protein fused to the E6 and E7 oncoproteins and HPV-6 L2 protein fused to E7, respectively, both produced in bacterial cells [61,62]. Although these approaches potentially have a preventive and therapeutic effect on cervical lesions and genital warts, it was only the results obtained from the TA-GW vaccine that were found to be promising due to the considerable amount of neutralizing antibodies, interferon gamma and IL5 produced, as well as the proliferation of antigen-specific T cells [63,64]. Two different therapeutic approaches examined the use of chimeric VLPs (cVLPs), which are structures formed of capsid proteins (L1 or L1/L2) fused to various viral epitopes or polypeptides [17]. Both protective and therapeutic responses were achieved through immunization with cVLP containing E7 [65] or a polypeptide composed of E1, E2 and E7 fused protein of HPV-16 [17].

Genetic immunization: promising results

Tests involving genetic immunization by introducing viral DNA into organisms have shown to be a very attractive candidate for antigen-specific immunotherapy, because it can express high levels of antigen in the cells where it was introduced [66,67]. After the vector vaccine has been injected in the body, the antigen is then produced through the transcriptional machinery of the host [68], and induce both types of immunity: humoral and cellular ($CD8^+$ T cells and $CD4^+$ T cells, respectively) [68,69].

However, some studies show low immunogenicity, which can be explained by the introduction of genetic material in non-specific cells and the difficulty found in replicating or spreading this to the neighboring cells *in vivo*. For this reason, there have been a large number of studies aimed at enhancing the DNA vaccine through the improving of the DNA delivery systems and DNA sequence (codon optimization and/or fusion with other genes for the improvement of vaccinal antigens) [70-78].

Recently, two vaccines have been tested for cervical cancer. One is the ZYC-101a vaccine (MGI Pharma, MA, USA), which encodes multiple E6 and E7 epitopes (for activation of cytotoxic T lymphocytes - CTLs) of HPV-16 and -18. This vaccine was tested through the administration of three intramuscular doses and the results showed that significantly more types of CIN-2/3 lesions were successfully treated in young women (under 25) than in those who received placebo doses [79,80]. Another example of DNA vaccine, called Sig/E7detox/HSP70, was administered in Phase I clinical trials. This vaccine encodes a signal sequence, fused to an E7 mutated form (E7detox) for loss of affinity for pRB, which in turn is fused to heat shock protein (HSP70). The results of the tests carried out with patients who had high-grade CIN-2/3 lesions showed an E7-specific immune response of cytotoxic cells ($CD8^+$ T), with no adverse effects. A lesion regression was observed in 3 of the 9 vaccinated patients [81].

The E5-based therapeutic vaccines also hold out good prospects. An adenovirus vector (AdV) carrying an E5 gene has been tested in mice with an E5-expressing tumoral cell line (TC-1/E5) and showed a reduction of the tumor [82]. Another vaccine, which comprises a potential Db-restricted CTL peptide of the HPV-16 E5 gene fused to CpG motifs (DNA sequences found in bacteria and used as vaccine adjuvant) showed better results than the previous recombinant adenovirus vaccine (rAd-E5) [83,84]. These studies show that E5 is recognized by the immune system as a tumor antigen and supports the hypothesis that this oncogene is a good candidate for the eradication

of premalignant lesions, since this viral protein is more active in the early stages of cervical cancer [85,86]. Following this line of argument, our group has been working on a therapeutic strategy based on genetic immunization with the HPV-16 E5 gene (unpublished data).

The Impact of HPV Vaccination on Developing Countries

Over 80% of new cases of cervical cancer diagnosed each year occur in developing countries and it is estimated that this percentage will rise to 90% by 2020 [4]. In developing world, cervical cancer is the biggest single cause of years of life lost from cancer, because affects relatively young women. The 5-year survival rate of patients observed in these countries is less than 50%, while in developed countries it reaches 66% [87].

According to the American Cancer Society, the introduction of Pap smear screening in prevention programs in the United States reduced deaths from cervical cancer by almost 75% between 1955 and 1992 [88]. Similarly, the incidence of cervical cancer and mortality rates sharply declined in Europe (particularly in Nordic countries) and Canada due to the implementation of cervical cytology in health care, most notably in population-based screening programs [27]. The establishment of cervical cancer trial programs requires a large number of well-trained professionals and persistent public funding to support the requisite infrastructure [30,89]. The cytology-based cervical screening needs to be conducted regularly and systematically using an organized approach, with quality systems ensuring sufficient uptake, laboratory services and continuous improvement, in order to have substantial impact [90]. Unfortunately, the developing countries have experienced great difficulty in doing this in a satisfactory manner.

In the developing countries (and even in some developed or middle-income countries), the situation in rural areas can cause people great difficulty in gaining access to the health services. The accessibility of health services in these areas is far too low to guarantee desired impact overall [27]. At the same time, it should be noted that the conventional methods of detection, such as Pap smears and liquid based cytology, have been found to yield contradictory results. In a clinically-controlled study, Clavel and colleagues [91] conducted tests that showed sensitivity of 68% and 88%, respectively, for HSIL. In view of this, it is essential to test for DNA hybridization of HR-HPV, as a supplementary test for women over 30 years old and in cases of dubious Papanicolaous test (atypical squamous cells of undetermined significance, ASCUS) [92], although this inflate even more the cost of the procedure.

The HPV vaccines provide different means of prevention, and theoretically, they are more practical in the poorest areas, because they make it easier to control cervical cancer [90]. Since 2007, there have been two prophylactic vaccines against HPV. Both have the potential to prevent 70% of cases of cervical cancer and 90% of genital warts in the next 10-20 years [93]. In a shorter period (5-10 years), it is estimated that the implementation of an effective HR-HPV vaccination program can prevent 30% of the infections caused by these virus types, as well as 40-50% of cytological abnormalities and 50-60% of HSIL [93]. Some mathematical models suggest that it would take 40-60 years of vaccination to see significant reductions in all HPV-related diseases worldwide, including non-genital disease [94]. However, the introduction of prophylactic vaccines in mass vaccination programs by developing countries is impracticable due to their high cost. As a result, the expected impact of these vaccines on the reduction of cervical cancer will not be achieved if current economic conditions persist.

The choice of a target group: cultural, ethical and educational factors

Another key issue is the choice of a target population for vaccination. First, it should be noted that the success of vaccination (or even a screening program) partly depends on the decision made by the objects of the intervention or other parties (such as parents, other family members, leaders of communities). In particular cases of HPV, attitudes toward vaccination tend to be more positive among people who have more information about the vaccine, and the causal link between HPV and cancer. People with a low social status in developing countries, living in the rural regions, are the least well off in the case of HPV-related diseases [27]. Although some experts regard HPV vaccination as a prevention strategy in low-income areas, a campaign to heighten awareness among the people is needed by the authorities to ensure it is provides effective coverage.

With regard to the specific case of HPV vaccination, with a strait window of applicability (before sexual debut), it is advisable to decide early on if the vaccine will be offered only to females or to both sexes. Additionally, delivering HPV vaccines effectively in primary prevention programs raises various issues and challenges, ranging from financial constraints regarding access to questions of cultural or religious acceptability, unlike those faced by other routine childhood vaccination programs [27,90].

While the average age of "sexual debut" differs across populations, most countries recommend primary HPV vaccination between the ages of 10 and 14. Several studies have shown that there is an increased immune response with high antibody titers during pre-puberty, compared to post-puberty [90]. Nevertheless, the vaccination of adolescents in some regions of the world raises moral, social, religious and ethical questions, such as in India, where pre-marital sex is socially unacceptable [95]. In that country, cervical cancer cases comprise 25% of cases worldwide and the implementation of these vaccines against HPV could reduce the incidence of cervical cancer by 60-100% [92]. However, a preliminary survey carried out with the parents of 9-16 year old girls showed that most of them believe that these vaccines could encourage early sex and promiscuity, which is disapproved in India. This could cause social stigmatizing and undermine the family´s prestige [95].

Other fundamental questions about the provision of vaccines to pre-adolescent/adolescent girls entail concerns about how best to access this group. Since routine vaccination of pre-adolescents and adolescents is less common than infant vaccination in developing countries, the health care provision of this group is generally less structured. While the delivery of public health interventions (including immunization) to this age group in many developed countries occurs most effectively through school-based services, developing countries often lacks any infrastructure or experience in school-based delivery vaccine [90]. Possible strategies to reach adolescent girls in school-based approaches are contracting out non-government organizations or the inclusion of the private sector. PATH (Program for Appropriate Technology in Health) is leading HPV vaccine implementation schemes in four countries – India, Vietnam, Uganda and Peru – and shows that a high coverage has been achieved, between 80 and 95% [90,96].

On the other hand, although boys cannot develop cervical cancer, they can be infected by HPV and suffer from other diseases related to the virus, such as anogenital and oropharyngeal cancers. Vaccinating males is currently not recommended by the WHO, but some experts believe that the impact of herd immunity on female cancers and other HPV-related cancers may need to be reconsidered. The cost benefits of vaccinating both sexes are still under investigation and there is evidence that the vaccination of males with the quadrivalent vaccine reduces the risk of HPV transmission to their partners, as well as the HPV-6/11-associated disease burden [27,97,98].

Type replacement

There has also been much discussion about the potential risk of new oncogenic genotypes emerging from the introduction of vaccination programs based on currently licensed vaccines. Previously work reported the increased risk of acquiring co-infections over time [99]. The herd immunity and resulting reduction in the prevalence of HPV-16 and -18 could create the required ecological niche for type replacement, and give to the HR-HPV non-targeted by the vaccines a competitive advantage over those two types [99]. Women who are vaccinated, in the belief that they are protected against all types of HPV, could adopt a high-risk sexual behavior and be at risk of infection by oncogenic genotypes, besides the HPV-16 and -18.

Data recently published by our group [48] suggest that, in Pernambuco (Brazil), while HPV-31 (15.49%) shows a much higher incidence than HPV-18 (2.82%), which has the same incidence as HPV-33, there was also a high percentage of concurrent infections between HPV -16/-31 (70.67%) and between HPV -16/-33 (18.66%). In addition, among women infected with HPV-31, there was a greater frequency of HSIL when compared with low-grade lesions, contrary to what was observed in women infected with HPV-16. Finally, it was observed that when the HPV-16 infection was combined with HPV-31, the lesions tended to be more severe. Thus, these results suggest that the vaccines currently available cannot meet the requirements of different populations such as those of Pernambuco (Brazil). Some studies have found that the bivalent vaccine provides little protection against HPV-31 and -33, but nothing can be inferred about its effectiveness when it is observed that these genotypes are, at least, as prevalent as those that it covers [48]. These vaccines could further allow the spread of other oncogenic genotypes, which in certain populations assume a more malignant form, as seems to be the case with HPV-31 in the population of Pernambuco (Brazil).

Some aspects of the Brazilian experience

The screening programs for the prevention of cervical cancer are widely available and the self-reported healthcare coverage by the Pap test is generally suited to the conditions in Brazil. The decline in the incidences of mortality attributable to cervical cancer in the last two decades has run parallel with an improvement in the screening practices, which were introduced in the 1980s and has been refined since 1998 [100-102]. From 2003 to 2008, the frequency rate of women between 25 and 59 years old who reported having had at least one Pap test in the previous three years, rose from 25% to 84.6% [101], despite the fact that there are wide variations in levels of income. However, in the rural areas of North and Northeast regions (areas with restricted access to screening programs), mortality rates are still rising. Overall, the incidence of cervical cancer is still very high, with rates close to those countries with the highest incidence, like Peru and some African countries [101,102].

In Brazil, the National Immunization Program has been very successful and achieved the highest immunization coverage rates in the world, without the need to resort to coercive strategies. All the vaccines are offered free of charge at the public health centers. This is largely due to the existence of centenary research centers, such as Adolfo Lutz Institute, Bio-Manguinhos and Butantan Institute, which produce most of the vaccines offered by the SUS (The Brazilian Unified Health System) [103]. The HPV vaccines, in contrast, are only available in private clinics, but recent public-private partnerships have increased the supply by offering large discounts to low-income and middle-income patients, with medical care provided by charity institutions that form part of the Holy House of Mercy Fraternity [103].

Although a constant improvement in screening programs is being made by the Brazilian authorities, in a desire to increase social equity, the number of women infected by HPV and with cervical lesions remains high [102]. Added to this is the fact that Brazil has one of the highest incidences of penile cancer in the world, especially in less developed regions of the country [104]. This situation is untenable and the Brazilian Ministry of Health has fostered the development of therapeutic strategies for HPV vaccines aimed at national needs. Apart from the fact that DNA vaccines have been showing promising results for immunotherapy, their application has an advantage, especially for developing countries, since this approach is cost-effective and simple to produce in large quantities, while at the same time, it can be distributed in poor areas of difficult access, due to the stability of DNA even in high temperatures [66,67]. Running with this, alternative prophylactic vaccines are being undertaken and take account the relevant HPV type that is prevalent in Brazil.

Conclusion and New Perspectives

The training of technical and scientific competence in the development of vaccines with the aid of advanced technologies, particularly those involving genetic engineering, is essential for the control of HPV infection and cervical cancer. As a preventive vaccine for cancer, there is community demand for the vaccine in many countries. To what extend decision-makers perceive and are able to respond to this demand will vary depending on competing priorities, available resources and how health-care decisions are made in particular countries [90]. While many governments, together with international health organizations and pharmaceutical companies, are urgently attempting to cooperate and increase the accessibility of the HPV vaccines that are currently available [92], great attempts have been made in many developing countries to establish alternative vaccine platforms, which not only increase competition and help drive down costs, but also enable them to bridge the demand and supply gap [30]. Researchers in South Africa, Brazil, China and India have experienced different expression systems with promising results for a second generation of prophylactic vaccines based on VLPs. The Brazilian Ministry of Health, which has an extensive National Immunization Program [105], estimates that U.S.$ 1.857 billion will be necessary to pay for vaccinating 11-12 year-old girls with the quadrivalent HPV vaccine, in addition to the $750 million currently being invested in the program. For countries with a gross domestic product of less than $1000 per capita, the cost of the HPV vaccine will not be cost-effective unless it is as low as $1 to $2 [106]. Each 5-year delay in establishing a global vaccination program against HPV can lead to the deaths of 1.5 to 2 million people in developing countries due to cervical cancer [107]. In view of this, the search for strategies to reduce the cost of HPV vaccination should become a priority.

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References

1. Parkin DM (2006) The global health burden of infection-associated cancers in the year 2002. Int J Cancer 118: 3030-3044.
2. Lowndes CM (2006) Vaccines for cervical cancer. Epidemiol Infect 134:1-12.
3. INCA (2009) Estimativa 2010: Incidência de cancer no Brasil. Rio de Janeiro, Ministério da Saúde, Instituto Nacional do Câncer, Brasil.
4. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre) (2010). Human Papillomavirus and Related Cancers in World. Summary Report 2010.
5. Frazer IH (2004) Prevention of cervical cancer through papillomavirus vaccination. Nat Rev Immunol 4: 46-54.
6. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV (2002) The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 55: 244-265.
7. Zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342-350.
8. Bosh FX, de Sanjose S (2003) Chapter 1: human papillomavirus and cervical cancer-burden and assessment of causality. J Natl Cancer Inst Monogr 31: 3-13.
9. Walboomers JMM, Jacobs MV, Manos MM, Bosh FX, Kummer JA, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189: 12-19.
10. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, et al. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518-527.
11. van Muyden RC, ter Harmsel BW, Smedts FM, Hermans J, Kuijpers JC, et al. (1999) Detection and typing of human papillomavirus in cervical carcinomas in Russian women: a prognostic study. Cancer 85: 2011-2016.
12. Zielinski GD, Snijders PJ, Rozendaal L, Daalmeijer NF, Risse EK, et al. (2003) The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. J Pathol 201: 535-543.
13. Muñoz N, Castellsague X, de Gonzalez AB, Gissmann L (2006) Chapter 1: HPV in the etiology of human cancer. Vaccine 24: S3-1-S310.
14. Schiller JT, Davies P (2004) Delivering on the promise: HPV vaccines and cervical cancer. Nat Rev Microbiol 2: 343-347.
15. Stanley M (2010) Potential mechanisms for HPV vaccine-induced long-term protection. Gynecol Oncol 118: S2-S7.
16. Stanley MA (2001) Human papillomavirus and cervical carcinogenesis. Best Pract Res Clin Obstet Gynaecol 15: 663-676.
17. Da Silva DM, Eiben GL, Fausch SC, Wakabayashi MT, Rudolf MP, et al. (2001) Cervical cancer vaccines: emerging concepts and developments. J Cell Physiol 186: 169-182.
18. Muños N, Bosch FX, Castellsague X, Diaz M, de Sanjose, S et al. (2004). Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int. J. Cancer 111: 278-285.
19. Huang CF, Monie A, Weng WH, Wu T (2010) DNA vaccines for cervical cancer. Am J Transl Res. 2: 75-87.
20. Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, et al. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518-527.
21. Merck & Co., Inc (2006) GARDASIL, Human Papillomavirus Quadrivalent (Types 6, 11, 16, and 18) Vaccine, RecombinantSuspension for intramuscular injection.
22. GlaxoSmithKline UK (2007) Cervarix suspension for injection in pre-filled syringe Human Papillomavirus vaccine [Types 16, 18] (Recombinant, adjuvanted, adsorbed).
23. Chen XS, Garcea RL, Goldberg I, Casini G, Harrison SC (2000) Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. Mol Cell 5: 557-567.
24. Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT (1992) Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci USA 89: 12180-12184.
25. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA et al (2005) Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 6: 271-278.
26. Harper DM Franco EL, Wheeler CM, Moscicki AB, Romanowski B et al. (2006) Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 367: 1247-1255.
27. Natunen K, Lehtinen J, Namujju P, Sellors J, Lehtinen M (2011) Aspects of Prophylactic Vaccination against Cervical Cancer and Other Human Papillomavirus-Related Cancers in Developing Countries. Infect Dis Obstet Gynecol 2011: 1-10.
28. Bello FA, Enabor OO, Adewole IF (2011) Human Papilloma Virus Vaccination for Control of Cervical Cancer: A Challenge for Developing Countries. Afr J Reprod Health 15: 25-30.
29. Kling M, Zeichner JA (2010) The role of the human papillomavirus (HPV) vaccine in developing countries. Int J Dermatol 49: 377-379.
30. Hanumantha Rao N, Baji Babu P, Rajendra L, Sriraman R, Pang YY, et al. (2011) Expression of codon optimized major capsid protein (L1) of human papillomavirus type 16 and 18 in *Pichia pastoris*: purification and characterization of virus-like particles. Vaccine 29: 7326-7334.
31. Nakauchi M, Kariwa H, Kon Y, Yoshii K, Maeda A, et al. (2008) Analysis of severe acute respiratory syndrome coronavirus structural proteins in virus-like particle assembly. Microbiol Immunol 52: 625-630.
32. Varsani A, Williamson AL, Rose RC, Jaffer M, Rybicki EP (2003) Expression of Human papillomavirus type 16 major capsid protein in transgenic *Nicotiana tabacum* cv. Xanthi. Arch Virol 148: 1771-1786.
33. Hou L, Wu H, Xu L, Yang F (2009) Expression and self-assembly of virus-like particles of infectious hypodermal and hematopoietic necrosis virus in Escherichia coli. Arch Virol 154: 547-553.
34. Zhou J, Sun XY, Stenzel DJ, Frazer IH (1991) Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. Virology 185: 251-257.
35. Sorensen HP (2010) Towards universal systems for recombinant gene expression. Microb Cell Fact 9: 27.
36. Coimbra EC, Souza HM, Leitão MCG, Santos JF, Beçak W, et al. (2007) Virus-Like Particles as a vaccinal strategy against tumors induced by papillomavirus infection. Communicating Current Research and Educational Topics and Trends in Applied Microbiology II: 689-696.
37. Coimbra EC, Gomes FB, Campos JF, D'arc M, Carvalho JC, et al. (2011) Production of L1 protein from different types of HPV in *Pichia pastoris* using an integrative vector. Braz J Med Biol Res 44: 1209-1214.
38. de Almeida JR, de Moraes LM, Torres FA (2005) Molecular characterization of the 3- phosphoglycerate kinase gene (PGK1) from the methylotrophic yeast Pichia pastoris. Yeast 22: 725-737.
39. Zhou J, Sun XY, Stenzel DJ, Frazer IH (1991) Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. Virology 185: 251-257.
40. Ghim SJ, Jenson AB, Schlegel R (1992) HPV-1 L1 protein expressed in cos cells displays conformational epitopes found on intact virions. Virology 190: 548-52.
41. Billich A (2003) HPV vaccine MedImmune/GlaxoSmithKline. Curr Opin Investig Drugs 4: 210-213.
42. Milstien JB, Gaulé P, Kaddar M (2007) Access to vaccine technologies in developing countries: Brazil and India. Vaccine 25: 7610-7619.
43. Bloom S (2005) Q & A with the man who can stop cervical cancer in its tracks. J Clin Invest 115: 2587-2588.
44. Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, et al. (1995) Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 87: 796-802.

45. Noronha V, Mello W, Villa L, Brito A, Macêdo R, et al. (1999) Human papillomavirus associated with uterine cervix lesions. Rev Soc Bras Med Trop 32: 235-240.

46. Lorenzato F, Ho L, Terry G, Singer A, Santos LC, et al. (2000) The use of human papillomavirus typing in detection of cervical neoplasia in Recife (Brazil). Int J Gynecol Cancer 10: 143-150.

47. Silva AMTC, Amaral AMVT and Cruz AD (2006) HPV e Câncer: O papel do papilomavirus humano na carcinogênese. Biotecnol Cien Desenvolv 29: 48-54.

48. Baldez da Silva MF, Chagas BS, Guimarães V, Katz LM, Felix PM, et al. (2009) HPV31 and HPV33 incidence in cervical samples from women in Recife. Brazil Genet Mol 8: 1437-1443.

49. Karanam B, Jagu S, Huh WK, Roden RBS (2009) Developing vaccines against minor capsid antigen L2 to prevent papillomavirus infection. Immunol Cell Biol 87: 287-299.

50. Roden RB, Yutzy WI, Fallon R, Inglis S, Lowy DR, et al. (2000) Minor capsid protein of human genital papillomaviruses contains subdomminant, cross-neutralizing epitopes. Virology 270: 254-257.

51. Pastrana DV, Gambhira R, Buck CB, Pang YY, Thompson CD, et al. (2005) Cross- neutralization of cutaneous and mucosal Papillomavirus types with anti-sera to the amino terminus of L2. Virology 337: 365-372.

52. Roy P, Noad R (2008) Virus-like particles as a vaccine delivery system: myths and facts. Hum Vaccin 4: 5-12.

53. Jagu S, Kwak K, Garcea RL, Roden RB (2010) Vaccination with multimeric L2 fusion protein and L1 VLP or Capsomeres to broaden protection against HPV infection. Vaccine 28: 4478-4486.

54. Day PM, Gambhira R, Roden RB, Lowy DR, Schiller JT (2008) Mechanisms of human papillomavirus type 16 neutralization by L2 cross-neutralizing and L1 type-specific antibodies. J Virol 82: 4638-4646.

55. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, et al. (2007) Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 356: 1928–1943.

56. Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, et al. (2007) HPV Vaccine Trial Group. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 298: 743-753.

57. Crook T, Morgenstern JP, Crawford L and Banks L (1989) Continued expression of HPV-16 E7 protein is required for maintenance of the transformed phenotype of cells co-transformed by HPV-16 plus EJ-ras. Embo J 8: 513-519.

58. Huang CF, Monie A, Weng WH, Wu T (2010) DNA vaccines for cervical cancer. J Transl Res 2: 75-87.

59. Bolhassani A, Mohit E, Rafati S (2009) Different spectra of therapeutic vaccine development against HPV infections. Human Vaccines 5: 671-689.

60. Palena C, Abrams SI, Schlom J, Hodge JW (2006) Cancer vaccines: Preclinical studies and novel strategies. Adv Cancer Res 95: 115-137.

61. de Jong A, O´Neill T, Khan AY, Kwappenberg KM, Chisholm SE, et al. (2002) Enhancement of human papillomavirus (HPV) type 16 E6 and E7-specific T-cell imununity in healthy volunteers through vaccination with TA-CIN, and HPV16 L2E7E6 fusion protein vaccine. Vaccine 20: 3456-3464.

62. Gambhira R, Gravitt PE, Bossis I, Stern PL, Viscidi RP, et al. (2006) Vaccination of healthy volunteers with human papillomavirus type 16 L2E7E6 fusion protein induces serum antibody that neutralizes across papilomavirus species. Cancer Res 66: 1120-1124.

63. Thompson HS, Davies ML, Holding FP, Fallon RE, Mann AE, et al. (1999) Phase I safety and antigenicity of TA-GW : a recombinant HPV6 L2E7 vaccine for the treatment og genital warts. Vaccine 17: 40-49.

64. Lacey CJ, Thompson HS, Monteiro EF, O´Neill T, Davies ML, et al. (1999) Phase IIa safety and immunogenicity of a therapeutic vaccine, TA-GW, in persons with genital warts. J Infect Dis 179: 612-618.

65. Schafer K, MuÈ ller M, Faath S, Henn A, Osen W, et al. (1999) Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumor protection. Int J Cancer 81: 881-888.

66. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA (1997) DNA vaccines. Annu Rev Immunol 15: 617-648.

67. Gurunathan S, Klinman DM and Seder RA (2000) DNA vaccines: immunology, application, and optimization. Annu Rev Immunol 18: 927-974.

68. Dunham SP (2002) The application of nucleic acid vaccines in veterinary medicine. Res Vet Sci 73: 9-16.

69. Henke A (2002) DNA immunization – a new chance in vaccine research? Med Microbiol Immunol 191: 187-190.

70. Ulmer JB, Wahren B, Liu MA (2006) Gene-based vaccines: recent technical and clinical advances. Trends Mol Med 12: 216-222.

71. Luo D, Saltzman WM (2000) Synthetic DNA delivery systems. Nat Biotechnol 18: 33-37.

72. Ko HJ, Ko SY, Kim YJ, Lee EG, Cho SN, et al. (2005) Optimization of Codon Usage Enhances the Immunogenicity of a DNA Vaccine Encoding Mycobacterial Antigen Ag85B. Infect Immun 73: 5666-5674.

73. Grujic M, Holst PJ, Christensen JP, Thomsen AR (2009) Fusion of a viral antigen to invariant chain leads to augmented T-cell immunity and improved protection in gene- gun DNA-vaccinated mice. J Gen Virol 90: 414-422.

74. Wang QM, Sun SH, Hu ZL, Zhou FJ, Yin M, et al. (2004) Epitope DNA vaccines against tuberculosis: spacers and ubiquitin modulates cellular immune responses elicited by epitope DNA vaccine. Scand J Immunol 60: 219-225.

75. Xu W, Chu Y, Zhang R, Xu H, Wang Y, et al. (2005) Endoplasmic reticulum targeting sequence enhances HBV-speciWc cytotoxic T lymphocytes induced by a CTL epitope-based DNA vaccine. Virology 334: 255-263.

76. Rinaldi M, Ria F, Parrella P, Signori E, Serra A, et al. (2001) Antibodies elicited by naked DNA vaccination against the complementary-determining region 3 hypervariable region of immunoglobulin heavy chain idiotypic determinants of B lymphoproliferative disorders specifcally react with patients' tumor cells. Cancer Res 61: 1555-1562.

77. Gollob JA, Mier JW, Veenstra K, McDermott DF, Clancy D, et al. (2000) Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-alfa induction is associated with clinical response. Clin Cancer Res 6: 1678-1692.

78. Perales MA, Yuan J, Powel S, Gallardo HF, Rasalan TS et al (2008) Phase I/II study of GMCSF DNA as an adjuvant for a multipeptide cancer vaccine in patients with advanced melanoma. Mol Ther 16: 2022-2029.

79. Sheets EE, Urban RG, Crum CP, Hedley ML, Politch JA, et al. (2003) Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. Am J Obstet Gynecol 188: 916-926.

80. Garcia F, Petry KU, Muderspach L, Gold MA, Braly, P et al. (2004) ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol 103: 317-326.

81. Trimble CL, Peng S, Kos F, Gravitt P, Viscidi R, et al. (2009) A phase I trial of a human papillomavirus DNA vaccine for HPV16+ cervical intraepithelial neoplasia 2/3. Clin Cancer Res 15: 361-367.

82. Liu DW, Tsao YP, Hsieh CH, Hsieh JT, Kung JT, et al. (2000) Induction of CD8 T cells by vaccination with recombinant adenovirus expressing human papillomavirus type 16 E5 gene reduces tumor growth. J Virol 74: 9083-9089.

83. Chen YF, Lin CW, Tsao YP, Chen SL (2004) Cytotoxic-T lymphocyte human papillomavirus type 16 E5 peptide with CpG-oligodeoxynucleotide can eliminate tumor growth in C57BL/6 mice. J Virol 78: 1333-1343.

84. Krieg AM (2002) CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 20: 709-760.

85. Kim SW, Yang JS (2006) Human papillomavirus type 16 E5 protein as a therapeutic target. Yonsei Med J 47: 1-14.

86. Kim MK, Kim HS, Kim SH, Oh JM, Han JY, et al. (2010) Human papillomavirus type 16 E5 oncoprotein as a new target for cervical cancer treatment. Biochem Pharmacol, 80: 1930-1935.

87. Parkin DM, Bray F (2006) Chapter 2: The burden of HPV-related cancers. Vaccine 24: S11-S25.

88. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, et al. (2004) Cancers statistics. CA Cancer J Clin 54: 8-29.

89. Sankaranaravanan R, Esmy PO, Rajkumar R, Muwonge R, Swaminathan R, et

al. (2007) Effect of visual screening on cervical câncer incidence and mortality in Tamil Nadu, India: a cluster-randomized trial. Lancet 370: 398-406.

90. Brotherton JML, Gertig DM (2011) Primary prophylactic human papillomavirus vaccination programs: future perspective on global impact. Expert Rev Anti Infect Ther 9: 627-639.

91. Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, et al. (2001) Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. Br J Cancer 84: 1616-1623.

92. Hakim AA and Dinh TA (2009) Worldwide impact of the Human Papillomavirus Vaccine. Curr Treat Opt Oncol 10: 44-53.

93. Kaufmann AM, Scheneider A (2007) New paradigm for prevention of cervical cancer. Eur J Obstet Gynecol Reprod Biol 130: 25-29.

94. Barnabas RV, Garnett GP (2004) The potential public health impact of vaccines against human papillomavirus. In: Prendivill W, Davies P, (edsn). The Clinical Handbook of Human Papillomavirus. Lancaster, United Kingdom: Parthenon Publishing/Parthenon Medical Communications.

95. Bharadwaj M, Hussain S, Nasare V, Das BC (2009) HPV & HPV vaccination: Issues in developing countries. Indian J Med Res 130: 327-333.

96. PATH and National AIDS Research Institute (2009) Shaping a Strategy to Introduce HPV Vaccines in India: Results from the HPV Vaccines: Evidence for Impact Project. Seattle, WA: PATH.

97. Goldie S (2006) A public health approach to cervical cancer control: considerations of screening and vaccination strategies. Int J Gynecol Obstet 94: S95-S105.

98. Stanley M, Lowy DR, Frazer I (2006) Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. Vaccine 24: S3 106-113.

99. Merikukka M, Kaasila M, Namujju PB, Palmroth J, Kirnbauer R, et al. (2011). Differences in incidence and co-occurrence of vaccine and nonvaccine human papillomavirus types in Finnish population before human papillomavirus mass vaccination suggest competitive advantage for HPV33. Int J Cancer 128: 1114-1119.

100. Brasil Ministério da Saúde, Instituto Nacional do Câncer. Viva Mulher. Câncer do colo do útero: Informações Técnico-Gerenciais e Ações Desenvolvidas. Rio de Janeiro: INCA, 2002.

101. Schmidt MI, Duncan BB, Azevedo e Silva G, Menezes AM, Monteiro CA, et al. (2011) Chronic non-communicable diseases in Brazil: burden and current challenges. Lancet 377: 1949-1961.

102. Ayres A, Azevedo e Silva G. Revisão Sistemática Sobre Estudos de Prevalência de Infecção por HPV no Brasil. Rev Saúde Pública.

103. Barreto ML, Teixeira MG, Bastos MI, Ximenes RAA, Barata Rb, et al. (2011) Successes and failures in the control of infectious diseases in Brazil: social and environmental context, policies, interventions, and research needs. Lancet 377: 1877-1889.

104. Favorito LA, Nardi AC, Ronalsa M, Zequi SC, Sampaio FJ, et al. (2008) Epidemiologic Study on Penile Cancer in Brazil. Int Braz J Urol 34: 587-593.

105. INCA (2009) HPV: Perguntas e respostas mais frequentes.

106. Agosti JM, Goldie SJ (2007) Introducing HPV vaccine in developing countries – key challenges and issues. N Engl J Med 356: 1908-1910.

107. GAVI (2009) The global alliance for vaccines and immunization.

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