Papillomavirus vaccination for prevention and treatment of cervical carcinoma

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Some human cancers are associated with human papillomaviruses, particularly cervical carcinoma, representing the second most frequent cancer in women worldwide, and with an increasing incidence in women of childbearing age in developed countries. The association to some viral types as human papillomavirus (HPV) 16 makes it feasible to develop prophylactic or therapeutic vaccines that can have an efficacy of at least 70%. The combination of epitopes from the L1 and E7 proteins are the most immunogenic, inducing neutralising antibodies to control virus spread, as well as cytolytic T cells, which are detectable in the genital tract. These vaccines have no side effects and have passed phase II clinical trials. Phase III clinical trials are underway in several countries where viral infection and high-grade lesions are used as endpoints parameters for vaccine evaluation in the follow-up.

Key words: Vaccine, papillomavirus, cervical

To justify the prevention of the human papillomavirus (HPV) infection by vaccination it is necessary to identify what pathology is going to be prevented, what viral types are implicated, and what are the characteristics of the host immune response against these viruses. Clearly the pathology behind the attempts to develop an HPV vaccine is cervical carcinoma. Infections by human papillomavirus are a very common sexually transmitted disease, approximately 11% of women are infected by human papillomavirus, but

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the majority of viral associated lesions regress spontaneously. Cervical carcinoma is on a worldwide basis the second cancer in women, and the first in developing countries. According to the World Health Organization there are approximately 400,000 new cases annually¹, of which 2,000 are diagnosed in Spain. Lately it has been reported that the incidence of invasive cervical carcinoma in fertile women, in their thirties, has increased in several European countries, such as UK, Finland, Israel and Slovakia^{2,3}. Therefore cervical carcinoma represents a major public health problem⁴. This type of cancer has the particularity of being associated with some human papillomaviruses, which appear to play a very important role in cancer development^{5,6}, increasing the risk by more than one order of magnitude. The viral types more frequently implicated are 16 and 18, which together

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account for approximately 70% of the cases⁵, although other less frequent types should not be forgotten. The role of HPV in this tumour, and because of that, as a source of tumour specific antigens, suggests that a vaccine can be developed that might reduce the incidence of this carcinoma, or help to its management. This cancer burden is the cause of an intensive effort to develop effective vaccines, either prophylactic or therapeutic. The development of a vaccine for HPV will represent the second major vaccine aiming at prevention or treatment of a human cancer, following the success of the hepatitis B vaccine for prevention of hepatocellular carcinoma⁷.

CHARACTERISTICS OF HUMAN PAPILLOMAVIRUS

HPV constitute a group of more than 80 viral genotypes, that infect epithelial cells, probably by binding to the $\alpha 6\beta 4$ integrin⁸⁻¹⁰. The epithelial pathology caused by HPV is very heterogeneous and is viral-type specific, ranging from benign lesions, such as warts and condylomas, to carcinomas¹¹. HPV are not only associated to cervical carcinoma, but also to a significant number of cases of vulvar, anal and oropharynx carcinomas¹²⁻¹⁴. Thus the vaccine may also contribute to reduce the burden of these tumors.

The virus contains a circular DNA molecule of 8 kilobases, with all the viral genes having the same transcriptional orientation. Gene transcription is initiated in a regulatory region, known as long control region (LCR) or upstream regulatory region (URR), followed by the early and late genes. There are eight early genes (E1 to E8) that code for regulatory proteins, and two late genes (L1 and L2) coding for structural proteins (Fig. 1). Viral replication and early genes expression take place in the basal layer of the epithelium, and late genes expression and viral particle assembly occurs in the non-dividing and differentiating middle layers of the epithelium where these cells appear as koilocytes. Thus, the HPV life cycle is linked to the epithelial differentiation¹⁵. Viral E6 and E7

proteins interact with the p53 and retinoblastoma tumour suppressor proteins, thus the expression of two early viral proteins might functionally mimic the inactivation of two cellular tumour suppressor¹⁵. These viral-cellular protein interactions cause premature progression of the cell cycle from the G1 to the S phase, before DNA repair takes place, and thus induce genomic aberrations¹⁶. The interaction of these two viral proteins, E6 and E7, with two tumour suppressor proteins, p53 and Rb, is a characteristic of malignant HPV, mainly types 16 and 18^{17,18}. E6 and E7 proteins from benign HPV types interact either weakly, or do not interact, with p53 and Rb proteins, thus this interaction constitutes a major functional difference between malignant and benign HPV types which is very relevant for their implication in tumour development.

In benign lesions the viral DNA is in an episomal form or extrachromosomal, while in carcinomas the viral DNA is integrated in the host genome. Because of the genetic damage resulting from the integration some viral genes are deleted or inactivated, and no viral particles are made in cancer cells, which are not infectious. However the presence of only the viral DNA is not sufficient for cancer development, other additional factors are required, such as host genetic damage and evasion of host immune response¹⁹.

RATIONALE FOR HUMAN PAPILLOMAVIRUS VACCINE DESIGN

HPV infections, particularly those associated with cervical cancer are very persistent. In women that develop cervical cancer and for which biopsies from early lesions were available, the same viral type was detected in the lesions taken 20 years before cancer development²⁰. HPV presents two important problems to have in mind for the development of a vaccine. The first is a consequence of the high number of viral types, currently more than 80; of which at least eight are high-risk types¹¹, and their relative prevalence

Fig. 1. Genomic structure of human papillomavirus type 16. There are two types of genes early (E) and late (L). There is a unique transcriptional orientation from E to L that starts in two different promoters (P). Transcription terminates in the polyadenylation sites (Poly A). The different proteins are made by a combination of promoter use that leads to translation of different reading frames.

varies depending on the country⁵. The genetic differences among HPV types might allow escaping immune reactions, which are type-specific. Secondly, the integration of viral DNA in the host genome represents the other major problem in cancer cells harbouring viral DNA, because it is accompanied by the loss of expression of some viral proteins, and their epitopes, thus making ineffective any vaccine against the lost proteins, such as the late proteins^{19,21}. However, cervical carcinomas always express the viral E6 and E7 genes that are retained. The other viral genes are either damaged or deleted as a result of the recombination event necessary for integration, and if they are retained as DNA sequences they are not expressed. This antigenic loss will have to be considered for vaccine development, particularly for treatment purposes.

Before designing an HPV vaccine it is necessary to consider the characteristics of the HPV infection. There are two major HPV groups, low risk and high-risk viruses. The low risk viruses are not the aim of most of the vaccine development projects, because they are only implicated in benign lesions of the skin and genital tract, such as warts and condylomas, although they are responsible for a very high number of medical consultations, in this group only HPV11 is the target of some vaccine development. On the other hand, the so called high risk viruses, despite being a more reduced group, are the target of all the efforts to develop an HPV vaccine, particularly against the types most frequently detected in invasive carcinomas²², most of the current work is directed towards HPV16. Such specific use of viral type is based on the observation that when a carcinoma

develops, the viral type present is of the same genotype, as the one detected in the same patient almost 20 years before²⁰, which strongly supports its role in early stages of the disease. This is a very important point for vaccine design, since the loss or change of viral type in carcinomas would make the vaccine ineffective from the start. The most common viral type in carcinomas is HPV16, detected in 50% to 70% of the cases, followed by HPV18, and to a lesser extent HPV31 and HPV45 (table 1); but their relative prevalence varies in populations from different geographic areas⁵.

TYPES OF IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS PROTEINS

The development of an HPV vaccine is based on the possibility of inducing specific immune reactions against viral components to prevent either infection or elimination of virus-containing cells. The natural history of the diseases suggests that these reactions exist and that may be able to control the infection, most cervical lesions regress spontaneously, thus it is reasonable to expect that the immune system could be manipulated to avoid the disease. In natural HPV infections, the immune response is very weak. Like in most processes, the host immune response against HPV might be mediated by either humoral or cellular. The mucosae, which are infected by HPV, are organs with a poor immunogenic capacity due to their relative isolation from the rest of the organism. The local immunity has a cellular component mediated by dendritic cells, that are antigen-presenting cells, and by a humoral response that expresses immunoglobulin A²³. The weakness of the response probably reflects the location of the infection in mucosae, the limitation of keratinocytes as antigen presenting cells, and the coevolution of the virus and the host cell, which represents one of the most specialised infections, since specific viral types are specific for very specific types of cell in a specific location.

Some HPV infections are very persistent, but most infections regress spontaneously, which suggests that the host has a natural immunity implicated in this control²⁴.

Experimentally the immune reaction has been ex-

TABLE 1. Prevalence of major HPV types in invasive cervical carcinomas from different parts of the world (percentage)

Country	HPV positive	HPV16	HPV18	HPV31	HPV45
Algeria	75	33	41	0	0
Morocco	95	53	8	3	5
Mali	90	35	12	7	17
Argentina	95	60	14	5	5
Panama	93	47	15	3	10
Cuba	93	57	7	4	7
Indonesia	95	32	49	0	9
Thailand	100	59	23	4	8
Canada	93	58	17	4	10
USA	90	54	9	9	27
Germany	94	76	0	0	0
Spain	94	54	7	7	4

Based on Bosch et al (1995)⁵. HPV: human papillomavirus.

TABLE 2. Types of antigens utilised for vaccination

Purified viral proteins: E6, E7, E5, E2, L1, L2
VLP (virus like proteins): L1, L1-L2 chimera, L1-E7 chimera
Peptides: L1, E7, E6
Naked plasmid DNA: L1, E7

tensively studied using several viral antigens in different forms (table 2). The antibody response is directed against the L1 and L2 capsid proteins. Antibodies against these two HPV proteins are detected in most infected people, suggesting that they are markers of viral exposure, without conferring protection against it. The existence of previous infections by HPV, such as warts, may affect the type of risk. It has been observed that in women who are seronegative for HPV 6 and 11 and seropositive for HPV16 have a 5 fold increase in risk of cervical cancer compared to those who are also seropositive for types 6 or 11, suggesting that there might be some viral cross protection^{25,26}.

Once a cell is infected, the elimination of infected cells is a consequence of a T-cell response. Viral proteins are degraded intracellularly into short peptides, which are presented as antigens bound to class I major histocompatibility complex (MHC) molecules. The cytotoxic immune response against HPV appears to be influenced by the MHC haplotype. Some of them confer some predisposition to infection. This suggests that some individual have a better antigen presentation than others. This response is mainly directed against epitopes from early viral proteins²⁷. Experimental immunisation with the E7 and E5 proteins elicits strong and specific immune responses, mainly mediated by cytotoxic T lymphocyte (CTL), and this response might result in tumor clearance of transplanted tumor cells. However, the E5 protein can only be useful only in the early phases of the infection, when it is still expressed, but not in carcinomas where it is deleted. The genetic background of the host conditions the immune reaction. Some human haplotype of the MHC can confer a higher susceptibility to the infection, probably because they are more efficient for antigen presentation. But the long persistence of some infections points out that the control mechanism is not always effective, and that there are immune evasion mechanisms that might be conditioned by the

viral epitopes and the genetic constitution of the infected individual. Furthermore, in cervical carcinoma, to avoid the elimination of tumor cells by the host immune system, there is a down regulation of the MHC class I antigens²⁸, in that way antigen presentation is significantly reduced, and the host does not recognise the tumour cells. In fact the virus has evolved to evade the immune system²⁹.

It has also been shown experimentally that dendritic cells, loaded with viral antigens, are very potent stimulators of the response against HPV epitopes. This immune response is mainly mediated by Th1 cells and cytotoxic lymphocytes³⁰⁻³² and is also accompanied by an overproduction of interferon gamma and interleukin-4 by helper lymphocytes^{33,34}.

NATURAL ANIMAL MODELS OF PAPILLOMAVIRUS INDUCED LESIONS

To characterise the immune response to papillomavirus there is the possibility of analysing animal lesions caused by papillomavirus (table 3). There are several animal models for papillomavirus infection, however due to the species specificity of the virus, the models contain an animal papillomavirus and not human HPV. The first animal model was the rabbit where the cotton tail rabbit papillomavirus (CRPV) causes warts that may persist and cause skin carcinomas³⁵. The bovine system has two models, the bladder cancer induced by bovine papillomavirus (BPV2), and the papillomas of the upper alimentary tract induced by BPV4. In both systems progression to carcinomas requires the presence of cofactors, such as fern³⁶. However in these models the problem is the lack of viral DNA in the tumor lesions, which makes them different from human carcinomas, since there will be no viral epitopes in the tumor cells. In this case vaccines could only have a preventive role. The canine model is characterised by the mucosotropic canine oral papillomavirus (COPV) that causes oral papillomas in dogs, but it has received very little attention as experimental model compared to other systems²².

The rodent model might eventually be the best model because the murine immune system is very well characterised. In this model the response against cells expressing particular proteins from HPV can be studied. In this model it is possible to obtain clearance of tumors expressing viral epitopes if the animal has been immunised against the E6 and E7 proteins. This tumor elimination is mediated by cytotoxic T lymphocytes^{37,38}. This model is proving useful for development of strate-

TABLE 3. Natural and experimental models to study immune reaction to papillomavirus

Animal models

Natural models:

- Skin carcinomas in rabbits induced by CRPV
- Esophageal carcinomas in cattle by BPV4
- Bladder carcinoma in cattle by BPV2
- Oral papillomas in dogs by COPV

Murine models:

- Transgenic mice expressing E6 or E7
- In vivo* challenges with viral proteins, VLP or cells

CRPV: cotton tail rabbit papillomavirus; COPV: canine oral papillomavirus; VLP: virus like particle.

gies for a therapeutic vaccine.

These animal models have been used to delineate what are the viral proteins that are candidates for vaccine development, and the type of immune response they induce before proceeding to studies in humans. Papillomaviruses are species-specific viruses and do not infect cells from animal models. Therefore to study experimentally the immune response against HPV some genetic manipulations are necessary, or alternatively use as model an animal pathology associated with papillomaviruses, such as carcinomas in cows or rabbits. However the best model to study immune reactions is the mouse, because its immune system is the best characterised. To overcome the species restriction of HPV, immunisations are performed using three different systems, purified viral proteins prepared in vitro, empty viral particles, the so called virus like particle (VLP), and cell lines from cervical carcinomas expressing some viral proteins or from cells transfected with specific viral proteins that can be used for cytotoxicity assays.

ANIMAL EVALUATION OF HUMAN VACCINE MODELS AND STRATEGIES IN THE DESIGN OF HUMAN PAPILOMAVIRUS VACCINES

The different strategies utilised for HPV vaccination depend on three parameters, the nature of the viral epitopes, the type of host immune response implicated, as well as the purpose of the vaccine, either for prevention of HPV infection or treatment of HPV-induced pathology.

The first strategy consists in the induction of neutralising antibodies against structural proteins of the capsid. Theoretically, the HPV specific antibodies would prevent the entry of the virus in the epithelial cells, and thus limit, or control, the spread of the viral infection. In this case the viral epitopes have to be present on the surface of the viral capsid. Usually the L1 and L2 proteins are used for this purpose using recombinant DNA methods. Currently the most widely used approach consists in the use of VLP³⁹. VLP are empty viral particles that are assembled in cells that only express capsid proteins. The VLP system is a suitable approach for a preventive vaccine that aims to prevent the transmission of viral particles from one cell to another³⁹.

A second approach is represented by attempts to develop cell-mediated immunity in order to get rid of infected cells, or cells containing viral antigens, as are tumor cells, and consequently the vaccine will be therapeutic. The selected proteins for this type of immunisation are more frequently derived from the early region genes. Most of the

studies have been performed with E1, E2, E6 and E7 proteins. However, because in cancer cells the E1 and E2 proteins are frequently deleted^{21,40,41}, today most attempts are performed only with the on-cogenic proteins, E6 and E7, for which several different methods are used. The idea is to eliminate, induce a regression, or prevent proliferation of cells that contain viral DNA; this approach is useful for developing therapeutic vaccines.

But for any vaccine development effort, it is necessary to define clearly the antigenic material to be used. Among the purified proteins, the most commonly utilised is L1 from HPV16^{38,42}, and also E6 and E7, because they are expressed in invasive carcinomas^{27,43,44}. The L1 protein elicits a good response of neutralising antibodies³⁸. For these viral antigens the epitopes responsible for the immune reaction have been identified, and they can be used even as synthetic peptides to elicit the immune response. It is important to draw the attention to the fact that in carcinomas, the viral epitopes are associated to histocompatibility leukocyte antigen (HLA) class I antigens, a group of antigens which is usually down-regulated

in tumor cells²⁸. Furthermore, HPV epitope presentation varies depending on the host cell haplotype^{33-35,45,46}.

An alternative approach is a combination of the previous strategies. By means of recombinant DNA the idea is to express, in chimeric molecules or VLP, epitopes that are derived both from early and late region genes, so that both viral transmission from cell to cell and elimination of infected cells can be achieved simultaneously. The most common system implicates the generation of virus like particles³⁹. These particles, which are very similar to empty viral capsids, are obtained by mixing L1 or L1 and L2 viral proteins. VLP are empty viral particles resulting from expression of individual late proteins in a cell line. These VLP present the proper epitopes to trigger the specific immune response. The L1 protein by itself is capable of forming VLP that can be used for immunisation and induces a good response of neutralizing antibodies³⁹. The expression of these proteins can be obtained in vitro using bacterial or baculovirus expression systems, or in mammalian cells by expressing the proteins using vaccinia virus as vector^{22,47}.

Also the VLP system permits the co-expression of L1 with L2, and the modification with E7, by fusion of E7 epitopes to L1. This L1-E7 protein is very promising because after internalisation, the MHC class I pathway processes this fusion protein, and both an antibody and a cytolytic T cell response can be obtained⁴⁸. This system can thus be preventive of HPV infection, and therapeutic for E7 con-

taining cells. Recently, protection against a different L1 protein variant from HPV16 has been achieved using VLP⁴⁹.

These approaches cannot be used to study the effects on virus transmission because the lack of infectivity of animal cells by HPV. However they are very useful to study reactions against cervical carcinoma cells expressing viral antigens. These cells induce tumors in the animal and this is a quantitative parameter that can be measured, as well as the CTL response. However most studies on immune reactions have been studied in murine models, and there might be some variation with respect to human responses, because of the difference in the MHC system^{28,42,45,46,50,51}.

Preventive vaccines are being developed by use of empty viral particles synthesized in culture as an immunogen³⁹, this approach requires type specific viral particles, otherwise protection will be very limited, and there is a risk that the void will be occupied by another viral type. As adjuvant for viral epitopes, some authors have used mycobacterium and heat shock proteins; in these cases there is an enhanced immune response³⁷. VLP particles induce a strong response of neutralizing antibodies that are highly specific for the type employed. Because of that recombinant VLP with different epitopes are being developed. Vaccination of both women and men is necessary in order to break the cycle of sexual transmission.

L1 based vaccines demonstrated that the use of the full-length protein or L1 incorporation into VLP is necessary in order to be protective. If VLP were disrupted or L1 peptides were used, these did not protect. The titre of antibody corresponded well with the level of protection. The L2 protein has given contradictory results. In some cases it has no effect, while in others it triggers a strong reaction characterised by a massive infiltration of the wart, such as in BPV4-induced papillomas³⁶.

The use of non-structural proteins, E2, E6 and E7 enhanced the regression of CRPV lesions in rabbits. The BPV4 E7 also induces a strong cellular reaction; this E7 is more similar to the HPV16 E7. More recently, the use of VLP is being extended to generate proteins that also contain and additional epitope, from E6 or E7, incorporated within the L1 protein³⁹. These chimeric proteins have been shown to induce strong CTL reactions, and at the same time retaining their ability to induce neutralizing antibodies⁵². Tumor protection was lost if the animals were depleted of CD8 lymphocytes, but the loss of CD4 lymphocytes did not interfere with tumor protection, suggesting the importance of the CTL response³⁸.

HUMAN PAPILLOMAVIRUS PROPHYLACTIC VACCINES

The aim of prophylactic vaccines is to prevent genital HPV infections, mainly of HPV types most commonly associated with carcinomas. These vaccines in general are based on the induction of neutralizing epitopes. These antibodies to be effective have to be present in the mucosa where infection takes place. Most of the approaches rely on the use of peptides or VLP particles using either L1 or L1 in combination with L2. These particles maintain the conformational epitopes required to induce a protective response. L1 induces a strong reaction of neutralizing antibodies that are type specific. On the other hand L2 does not induce a strong reaction, but its neutralizing antibodies confer some cross protection to different viral types. VLP and peptide-based vaccines have been developed for several HPV types including^{6,11,16,18,31,33,39,45,58,59}. Although most of the work has been done on HPV16. Several phase I studies have started to assess safety, immunogenicity, dose, schedule, route of administration and adjuvants. Thus for HPV 11 peptides a good response is obtained with 9 µg with a good tolerance, for HPV16 VLP it was necessary to reach a dose of 50 µg. Intramuscular injection of these viral genes, as DNA vaccines, can also trigger a good response of neutralizing antibodies. To obtain a more efficient vaccine, chimeric VLP (CVLP) molecules are being designed, which have the HPV16 E7 sequence fused to the carboxy terminus of L1⁵². In that way, both, humoral and cytotoxic responses can be obtained efficiently, and the vaccine will be bifunctional for prevention or therapeutic purposes³⁸. HPV VLP enter the MHC class-I pathway and induce cytotoxic T cells in the genital tract⁵³. At the moment all vaccines tested have been well tolerated and without side effects⁵⁴.

The ideal situation will require the use of a polyvalent vaccine that will protect against all viral types. Unfortunately the responses are type specific, and it is not likely that this will be achieved. However because most carcinomas are associated with HPV16 and 18, a vaccine against these types could significantly reduce the burden of this carcinoma. In the case of condylomas, where more than 90% of the cases are associated with HPV 6 and 11, a vaccine against these two types will be of help for the prevention of anogenital warts. No effort is being dedicated to a vaccine for cutaneous lesions.

Regarding the route of administration, one has to consider that the target cells are in a mucosa, and although initial approaches have used intramuscu-

TABLE 4. **Prophylactic HPV vaccines under development**

HPV type	Antigen	Type of vaccine	Status	Organization
HPV11	L1	VLP	Phase II, passed safety and immune response	MedImmune, SKF
HPV16,18	L1	VLP	Phase I trials	MedImmune, SKF
HPV16	L1	VLP	Phase II in several countries; phase III soon	Merck, CSL Ltd
HPV16	L1	VLP	Phase II underway. Efficacy trial in 2001	NCI, NIAID
HPV16	L1-E7	Chimeric VLP	Phase I/II under way	MediGene
HPV16	L1-E7	Chimeric VLP	Preclinical studies in mice	University of Queensland
HPV16	L1-E7	Recombinant BCG	Preclinical studies in guinea pigs	University of Cape Town
HPV16	L1-E7	Recombinant vaccinia virus	Preclinical	European Commission, China
HPV16	L1-E7	Recombinant BCG	Preclinical	University of Queensland
HPV16	L1	Recombinant adenovirus	Preclinical	Wistar Institute USA
		Recombinant Venezuelan equine encephalitis	Preclinical	Wyeth
	L1	DNA	Preclinical, antibodies in rabbits	Merck, Vical
		DNA	Preclinical	Wistar Institute USA
		DNA	Preclinical	Wyeth
		Edible (tomatos)	Preclinical	University of Cape Town

Based on Kols and Sherris. HPV vaccines: promise and challenges⁵⁵. HPV: human papillomavirus; VLP: virus like particle; SKF: Smith-Kline-French; CSL: Cambridge Scientific Limited; NCI: National Cancer Institute, NIAID: National Institute of Allergy and Infectious disease.

lar or subcutaneous routes, a more optimal route of administration may be needed in the future.

To determine the efficacy of a prophylactic vaccine at least two end points should be used, one is the determination of the specific HPV type, and the other is the effect on morphological lesions, of which the low-grade lesions, as low-grade squamous intraepithelial lesion (LSIL), are the more appropriate candidates.

A summary of current prophylactic vaccines under different stages of development is shown in table 4⁵⁵.

HUMAN PAPILLOMAVIRUS THERAPEUTIC VACCINES

The aim of therapeutic vaccines is to promote the elimination of tumour cells from lesions induced by papillomavirus. They might be developed against cells that contain replicating virus of against cells that only express viral epitopes, independent of any other characteristic related to the viral biology. In this area most efforts are directed for the treatment of cervical carcinoma. These vaccines have to rely on the utilisation of viral epitopes that belong to proteins that are re-

TABLE 5. **Therapeutic HPV vaccines under development**

HPV type	Antigen	Type of vaccine	Status	Organization
HPV6	L2, E7	Peptide (fusion protein)	Phase II trials with adjuvants	Cantab, SKF
HPV16/18	L2, E6, E7	Peptide (fusion protein)	Phase I trials started	Cantab
HPV16	E6, E7	Various peptides	Phase I and II trials	NCI
HPV16	E7	Peptide	Phase I and II trials	University of South Carolina
HPV16	E7	Peptide, protein	Phase II trials	StressGen Biotechnologies
HPV16	E7	Peptide	Phase I. Dose escalation completed without adverse effects	University of Leiden
HPV16	E7	Peptide	Phase I/II. Antibodies and T cells in some patients	University of Queensland
HPV16/18	E6, E7	Recombinant vaccinia virus	Phase II	Cantab
HPV58	E7	Recombinant vaccinia virus	Preclinical. Prevents tumour growth in mice	European Commission, China
HPV16		Recombinant vaccinia virus	Phase I trials	Transgene
HPV16	E6, E7	Recombinant adenovirus and vaccinia virus	Preclinical studies	Wistar Institute USA
HPV16	L1-E7	Chimeric VLP	Phase I/II	MediGene, Schering

Based on Kols and Sherris. HPV vaccines: promise and challenges⁵⁵. HPV: human papillomavirus; VLP: virus like particle; SKF: Smith-Kline-French; NCI: National Cancer Institute.

tained in carcinomas; therefore most studies have used the E6 and E7 proteins. Several vaccine candidates have been used, ranging from the use of synthetic peptides, recombinant proteins, or expression using viral vectors such as vaccinia. The peptide vaccine has the additional problem of its dependence on the MHC haplotype; therefore the individual variability is likely to be large^{34,42,56}. Several studies have been performed as part of phase I clinical trials. These vaccines were used as adjuvants to other treatments, such as radiation or chemotherapy. Some patients will present the antigen much better, and thus elicit a better immune response. Several studies have been performed using these approaches, in all there is a good antibody response, and a CTL response was obtained only in some patients, but there was no improvement of their clinical situation, that of advanced invasive carcinomas⁵⁶.

A summary of current prophylactic vaccines under different stages of development is shown in table 5⁵⁵.

VACCINE DEVELOPMENT AND CLINICAL TRIALS

To develop an HPV vaccine we need to take into consideration several issues. First we must know what is the fraction of the population that will respond to the vaccine. Thus for HPV, approximately 40% of the cases of HPV infections will be by HPV-16, thus the vaccine will only have a prevention role on this subpopulation. Defining the end point of the vaccine trials is very important both the sample of the size and the follow-up time. There are two major endpoints, one is the number of HPV type specific cases, and the other is the cumulative rate of cervical intraepithelial neoplasia (CIN) lesions. In Finland they are about to start the field trial of such a vaccine, because although there has been an overall decrease in the incidence of cervical cancer due to screening programs, there has also been an increase in the incidence of cases at younger age-groups due to change in sexual habits⁵⁷. Also in Costa Rica an efficacy trial will be conducted with a VLP L1 vaccine for HPV16 in view that this vaccine has been shown to induce a good specific antibody titre and confers protection from a later challenge by the same type⁵⁴.

Any vaccine against HPV might have several potential problems that may limit its utility. These are related to the divergence of different HPV types and their individual variation⁵⁸. The ideal vaccine should cover a wide range of HPV types, but that is not feasible at the moment, therefore all efforts are focused on the main type, HPV16, and

some in HPV18. Thus taking into account the distribution of different viral types in the world (table 1), only about 70% of the cases might be preventable. These vaccines will confer protection in so far as there are no escape mutants, HPV variants, as a consequence of the new immune selection pressure on the viral types used for vaccine development. Also there is the additional risk than elimination of one viral type could lead to the occupation of the biological niche by a different viral type⁵⁸. However in studies where the HPV type and variant distribution is compared between early lesions and carcinomas, there is a marked reduction in the number of types, and even variants, as the lesion is more severe⁵⁹. Several phase I clinical trials are currently underway. Some of them have already been reported: a group of eighteen patients with high grade cervical lesions, all belonging to the MHC A2 subgroup, were vaccinated with a peptide derived from the HPV16 E7 protein⁵⁶. The patients were analysed for the presence of dysplasia, and cellular infiltration by lymphocytes and dendritic cells. In only three patients the dysplasia cleared, in another six cases there was a significant infiltration by dendritic cells. Furthermore in ten of the patients there was cytokine release and cytolysis with E7 specific reactivity. These initial observations suggest that further improvements on an HPV vaccine might obtain important results. However, in these patients total loss of viral DNA was not achieved⁵⁶.

In Scandinavian countries several clinical trials are underway in Finland⁵⁷ and Estonia⁶⁰. In the latter they are already planning a phase III trial⁶⁰. This trial aims to reduce the burden of infection by HPV in young people. For this trial they are planning to enrol 20,000 women that will be divided in vaccinated and control groups. The vaccinated people will be monitored periodically, and it is expected that if the vaccine has a 70% efficacy, in five years there should be a very significant difference in the incidence of HPV infection between the two groups. However the effect on cervical carcinoma will not be detectable till twenty years after vaccination.

Although these vaccines will be mainly used in industrialised countries, where they will be more useful is in developing countries⁶¹. In these countries due to the lack of suitable screening programs, the burden of the disease is much higher, and will be the reservoir for the potential reintroduction of HPV. To achieve a high level of protection, particularly in developing countries, that are the virus reservoir, new and more effective vaccines than the ones currently being developed will be necessary. However, if it is not feasible

to obtain a vaccine that protects against all the major types, due to the variation in prevalence in different countries (table 1), it is possible that the biological niche left empty by the vaccine, may be occupied by a minor oncogenic type several years after vaccination^{62,63}. Thus, in the future, the problem might reemerge.

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Vacunas para la prevención y tratamiento del carcinoma de cérvix

Varios tipos de carcinoma están asociados a la infección por el virus de papiloma. El carcinoma de cuello uterino es el segundo cáncer más frecuente en mujeres a escala mundial, y su frecuencia está aumentando en mujeres en edad fértil en países desarrollados con programas de detección precoz. La asociación de al menos la mitad de los casos con un tipo de virus específico (HPV16) hace posible el desarrollo de vacunas profilácticas y terapéuticas. La combinación de epítomos de las proteínas L1 y E7 son las más inmunogénicas, induciendo una fuerte producción de anticuerpos neutralizantes para prevenir la diseminación del virus y una respuesta de linfocitos T citotóxicos, que son detectados en la mucosa genital. Estas vacunas han superado sin efectos secundarios la fase II, y actualmente se están desarrollando estudios en fase III utilizando la infección vírica y las lesiones de alto grado como referencias para el seguimiento de las cohortes.

Palabras clave: vacuna, virus de papiloma, carcinoma
de cérvix, prevención.

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