Vaccination against human genital papillomaviruses. More than a hypothesis

G. De Palo[1], L. Mariani[2]

[1]National Tumor Institute, Milan; [2]Regina Elena Cancer Institute, Rome (Italy)

Summary

Human genital papillomaviruses are the necessary cause of cervical cancer. A prophylactic vaccine designed to prevent genital HPV disease by inducing virus neutralizing antibodies has been proposed. Studies on animal models have produced relevant data on the efficacy of HPV vaccine. The results of HPV clinical studies suggest that it will be possible to develop an effective vaccine. Nevertheless the number of subjects analyzed in the full text published clinical studies is still poor. Although a recently presented phase III study appears satisfied, it is probably necessary for a larger phase III study. Besides the choice of the geographical area for a very large clinical trial, there are different aspects to consider, such as the identification of the target population, identification of the endpoints, composition of the vaccine and marketing of the vaccine. Furthermore there are two open questions: the duration of protection and the behavioral modifications. All these issues are discussed in this review.

Key words: Papillomavirus, Cervical Cancer, Vaccination.

Introduction

The ascertained carcinogenetic role that high-risk human papillomaviruses (HPV) have on cervical cancer represents the background on which the hypothesis of a vaccine for the primary prevention of cervical carcinoma is structured.

The high incidence of such neoplasm creates a true social health problem for public health services. The worldwide incidence [1, 2] of cervical carcinoma is nearly 500,000 women/year with a geographic distribution directly associated with social and economic levels.

In 2002 the incidence of invasive cervical cancer was 83,437 new cases in developed countries and 409,404 in less developed countries [1]. Therefore more than 80% of the total number of cases of cervical carcinoma are found in less developed countries, where the disease is usually diagnosed at an advanced stage, with a consequent poor outcome.

The developing areas (Central and South America, the whole of Africa, and parts of the Asiatic region) due to many reasons, have great difficulty in planning cytological screening programs. The absence of preclinical diagnoses and treatment of preneoplastic cervical disease is at the basis of the high incidence of invasive cervical cancer. In these countries where cervical cancer is a very important cause of death, the effect of HPV vaccination could be substantial. However also in developed countries where the diagnoses and treatment of preneoplastic cervical disease are common, a vaccination program would be important. Indeed, despite screening programs the annual number of cervical cancer cases in developed countries still remains too high.

This article concentrates on prophylactic genital HPV vaccines. It is a critical review that, on the basis of the excellent reviews of Lowy and Schiller [3], Schiller [4], Hilleman [5], Stern *et al.* [6], Schiller and Lowy [7], Stanley [8, 9], Galloway [10], Jansen and Shaw [11] and clinical studies, tackles the problems related to a HPV vaccination program.

HPVs are small DNA viruses. Thus far 96 types of HPV have been fully characterized [12]. Of these 96 types, 30 have been detected in the genital tract. Genital HPVs are divided in oncogenic (high-risk) and non oncogenic (low-risk) types. The high-risk HPVs are 15-18 and the low risk are 12 [13, 14]. The viral genome encodes six early proteins and two late proteins.

Early proteins (E1, E2, E4, E5, E6, E7) have a role in cervical carcinogenesis. E6 and E7 genes of the high-risk HPV, following genome integration, interact with cellular proteins whose normal role is the negative regulation of the cell cycle. Thus E7 binds pRB and E6 binds and degrades p53.

Late proteins constitute the viral capsid (L1 major and L2 minor viral capsid) and are the first to interact during contact with the genital epithelium, generating an immune specific response.

The capacity of the L1 major capsid protein of HPV to self-assemble in what are known as virus-like particles (VLPs) structurally and antigenically identical to natural virions, but lacking the viral DNA core, and thus inducing, when inoculated into a host, a virus-specific antibody response conferring specific protection, has been the key to producing the prophylactic HPV vaccine [15]. Therefore L1 VLP is an empty viral capsid lacking any viral-genetic material and, thus,

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can be safely administered in the healthy population to induce type-specific immunity without any oncogenic risk. L1 VLPs are able to induce neutralizing antibody levels often 40 times [16], 50 times [10] and 60 times [17] higher than the titers seen in naturally occurring infection. VLPs have the limitation of inducing type-specific immunity [3, 7]. Since neutralizing antibodies are type-specific, immunization with heterologous VLPs is not to be expected to confer protection.

In clinical terms, an increase in HPV-related pathology (both benign and malignant) in immune-depressed patients [18-20], as in renal transplant patients receiving immuno-suppressive therapy [21, 18], has been described. For example, a relationship between immunologic status and frequency and/or severity of cervical abnormalities in women positive for HIV antibodies was studied by Garzetti *et al.* [19]. In this study HIV infected women had a ten-fold higher prevalence of both HPV infection and cervical dysplasia than the outpatient population. An increased risk of invasive cervical cancer among HIV infected women was confirmed by the study of Serraino *et al.*, [20]. In the study of Halpert *et al.* [21] the rate of HPV infection in immunosuppressed renal transplant recipients was nine times greater and the rate of cervical neoplasia 16 times greater than in the general population. Patients with HIV infection are at higher risk compared to immunosuppressed patients with renal transplants [18]. The fact that cervical precancer and cancer are more frequent in immunodepressed women than in the general population underlines the importance of cellular immunodeficiency in the genesis of these lesions [22]. Moreover, regression of viral lesions accompanied by an efficient cellular response has been well documented. The study of Coleman *et al.* [23] on genital wart spontaneous regression provides evidence that effective host responses to HPV in the genital tract are characterized by an active cell-mediated immune response.

Furthermore, the natural history of genital HPV infection, with a high prevalence in young sexually active women, but very transitory, suggests a role for the host immune status. There is a spontaneous regression of infection in the majority of the infected population and only a proportion of women develop persistent infection. Some of these persistent infections with high-risk HPV progress to CIN and some high-grade CIN progress to invasive cancer.

A successful vaccination program theoretically requires the induction of a humoral as well as a cellular response. While the first one comes with an antibody production useful to neutralize free-viruses at the moment of contact with the host, the cell-mediated response is aimed at the destruction of infected cells.

Prophylactic vaccine is designed to prevent genital HPV infection and HPV associated genital lesions by inducing virus neutralizing antibodies. The target antigens are the capsid proteins L1 and L2. Delivery of these antigens results in the mobilization of the humoral arm of the immune system with a neutralizing antibody response.

Therapeutic vaccine should clear an established infection through the induction of a cell-mediated response against viral proteins, such as oncoproteins E6 and E7.

Besides prophylactic and therapeutic vaccines, there is also a *chimeric vaccine* that incorporates other papillomavirus proteins into the L1 VLP and has the objective to induce humoral response and also cell-mediated response to non structural viral proteins such as E7, E6. In experimental animals a chimeric VLPs show success as reported by some authors [24-26]. In humans the chimeric VLP vaccine will probably be initiated as clinical trial in the near future [7].

Indeed, the combined intervention of specific-neutralizing antibodies and the cell immunity is the theoretical basis for the success of a preventive program [8] which uses a combination of late and early proteins inducing a total combined humoral and cellular response.

Animal models

Encouraging results from species-specific HPV VLP vaccinations have been obtained in three animal models: bovine papillomavirus type 4 (BPV4), canine oral papillomavirus (COPV), and cottontail rabbit papillomavirus (CRPV) [27-31]. The study designs were similar. Animals were parenterally injected with VLPs and boosted once or twice with a similar dose of VLPs. Subsequently the animals were challenged by the application of high doses of virus to an epithelium that was abraded to expose the proliferating basal cells to virus infection [4].

From the animal studies it appears that L1 VLP was effective in protecting against the experimental challenge [4]. For COPV complete protection has been reported [29] similarly for rabbits and cows excellent protection against CRPV and BPV4 papillomas was obtained with systemic vaccination. Furthermore one year after VLP vaccination rabbits still displayed considerable protection [31]. It is worthy of note that the efficacy was limited to prophylaxis since vaccination with BPV4L1/L2 VLPS was able to protect against homologous virus but did not induce regression of existing papillomas [30]. Passive transfer from immunized rabbits or dogs conferred protection against the homologous virus [27, 29].

In summary, preclinical studies on animal models have produced scientifically relevant data on the efficacy of VLP vaccines that may be summarized as follows:

– induction of specific neutralizing serum antibodies;

– presence of anti-HPV neutralizing antibodies not only in the serum, but also in blood-free cervico-vaginal secretions of primates (African green monkeys) immunized with HPV 11 VLP [32];

– protection one year after vaccination in the rabbit model [31];

– protection after vaccination with the homologous VLP type [27, 29, 31];

– possibility of passively transferring immunity [27, 29] from immunized rabbits or dogs to naïve animals via immune sera.

All these data make likely that VLPs will perform similarly in humans. The success achieved in animal models justified the clinical trials in humans.

Human clinical studies

Human studies have shown a high immunogenicity of VLP vaccines with significant specific-type antibody production.

At the beginning of 2005 there were a total of eight studies; in chronological order the studies are as follows:

The immunogenecity of HPV 11 LP vaccine was evaluated in the study of Brown *et al.* [33]. In this study 104 young women (18-25 years old) negative for HPV 6/11 were randomized to receive placebo or HPV 11 L1VLP vaccine. Intramuscular injections of vaccine or placebo were administered at enrollment and after two and six months. The results showed that vaccination with HPV 11 VLP elicited a vigorous serum immune response in a high percentage of women.

Harro *et al.* [16] published a small study of a vaccine on 72 patients (58 women and 14 men) at Johns Hopkins University in Baltimore. This was a randomized double-blind placebo-controlled trial using a HPV16 L1 VLP vaccine administered by IM injection, dose-escalated (10 μg vs 50 μg). The frequency and intensity of the side-effects (pain and redness at the site of inoculation, slight and transitory hyperthermia) were the same as the placebo and resolved spontaneously within 48-72 hours. All the patients vaccinated were seroconverted within a month of the second injection. A 50 μg dose of vaccine gave the best immunogenic results (IgG with titers 40-fold higher compared to that observed in natural infection).

The first clinical trial was that of Koutsky *et al.* [34]. It was a double-blind randomized placebo controlled trial using a HPV-16 L1 VLP vaccine. The endpoint of the study was the rate of incidence/persistence of HPV16 infection.

In the study, 2,392 women, aged 16-23 years, were randomized to receive three IM injections (0.5 ml) of 40 μg of HPV16 L1 VLP vaccine or placebo at month 0, month 2 and month 6. Genital samples for HPV-DNA were obtained at enrollment, one month after the immunization and after every six months. Out of 2,392 eligible women, 859 were excluded because they were seropositive or PCR positive. Therefore 1,533 subjects (768 in the vaccine group and 765 in the placebo group) were eligible.

Biopsy tissue from participants with abnormal pap smears was evaluated for cervical intraepithelial neoplasia and analyzed by PCR for HPV16 DNA. With a median duration of follow-up of 17.4 months following completion of the vaccination, in the placebo group there were 41 subjects with HPV16 infection including nine cases of HPV related CIN, while in the vaccinated group there was no one resulting in an efficacy rate of 100%. The study concluded that administration of HPV16 L1 VLP vaccine reduced the incidence of HPV16 infection and HPV16 related cervical intraepithelial neoplasia.

The study of Brown *et al.* [35] is an analysis performed using combined data from two placebo-controlled dose ranging studies on tolerability/immunogenicity of HPV11 or HPV16 L1 VLP vaccines. Data from the two protocols were combined since the protocols had identical inclusion/exclusion criteria, similar visit schedules and identical collection techniques. As a consequence the placebo group plus HPV11 L1 VLP accounted for 167 subjects and the HPV16 L1 VLP group for 82 subjects; 129 out of 167 subjects in the control group and 66 out of 82 subjects receiving HPV16 L1 VLP vaccine were included in the analysis. The results showed the efficacy of the vaccine against HPV 16 infection.

A randomized double-blind placebo-controlled trial was performed to assess the efficacy, safety and immunogenecity of a bivalent HPV 16/18 L1VLP vaccine by Harper *et al.* [36]. A total of 1,113 healthy women from Brasil and the USA aged 15-25 years, without any history of an abnormal pap smear, cytologically negative, seronegative for HPV 16 and 18 antibodies and HPV DNA negative by PCR for 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) were randomized to receive three doses of vaccine IM or placebo at month 0, month 1 and month 6. Subjects were assessed every three months for HPV infection by cervical Thin prep cytology and HPV DNA testing with a self obtained cervical sample for up to 27 months. Out of 1,113 randomized subjects, 560 were randomized to vaccine and 553 to placebo. The results showed that the bivalent HPV vaccine, safe and well tolerated, was highly efficacious against persistent HPV16/18 cervical infection, cytological abnormalities and histological development of HPV16/18 associated CIN.

More recently Villa *et al.*, [37] published the preliminary results of a multinational, multicenter randomized double-blind placebo-controlled study of a quadrivalent HPV (HPV types 6, 11, 16, 18) L1VLP vaccine. The objective of the study was to establish if a quadrivalent L1VLP HPV 6, 11, 16, 18 vaccine was effective against persistent infection due to these virus types.

The study was preceded by two small reports by the same research group on the safety and immunogenecity of the monovalent HPV 11 and HPV16 L1 VLP [38] and HPV 18 L1VLP [17] vaccines. Both studies reported high immunogenicity of the HPV L1 VLP.

Healthy women, aged 16-23 years, not pregnant and without any previous abnormal pap smear were recruited in Brazil, the USA, Norway, Finland, and Sweden. The subjects were treated with the quadrivalent anti HPV 6, 11, 16,

Table 1. — Estimated incidence of invasive cervical cancer in the world (Globocan 2002 - Ferlay *et al.*, 2004).

Region	No. of cases 2002	Crude rates per 100,000
World	49,3243	16.0
More-developed countries	83,437	13.6
Less-developed countries	409,404	16.6
America - Central	17,165	24.4
America - South	48,328	26.9
USA - Canada	14,670	9.1
Europe - Central/Eastern	30,897	19.6
Europe Northern	5,647	11.7
Europe Southern	10,641	14.4
Europe Western	12,744	13.6
Australia/New Zealand	1,063	9.1
Africa	78,896	9.2 (North) - 30.2 (South)
Asia	265,885	4.6 (West) - 21.2 (Central South)

Table 2. — Number of subjects analyzed in clinical trials of vaccination.

Study	L1 VLP vaccine	No. of evaluable subjects (vaccinated and control)
Harro *et al.*, 2001 [16]	HPV 16	72
Brown *et al.*, 2001 [33]	HPV 11	104
Koutsky *et al.*, 2002 [34]	HPV 16	1533
Harper *et al.*, 2004 [36]	HPV 16/18	1113
Brown *et al.*, 2004 [35]	HPV 16	195
Ault *et al.*, 2004 [17]	HPV 18	33
Fife *et al.*, 2004 [38]	HPV 11	140
	HPV 16	109
Villa *et al.*, 2005 [37]	HPV 6/11/16/18	552
Total		3851
Ault, 2005 [39]	HPV 16	17.829
	HPV 6/11/16/18	(8.487* + 9.342**)
Total		21.680

*Regular protocol; **Irregular protocol.

18 vaccine or placebo. Vaccine or placebo was administered intramuscularly at the dose of 0.5 ml at day 1, month 2 and month 6. Subjects were followed-up with a gynecological examination, Thinprep Pap test, hybrid capture II, PCR analysis of HPV, biopsy samples of external genital lesions, colposcopy, and serum samples for serum concentration of antibodies. The preliminary results on 552 subjects, 277 in the vaccine group and 275 in the placebo group, showed that with a follow-up of 35 months the incidence of persistent HPV infection or genital disease decreased by 89% in subjects allocated vaccine compared with those allocated placebo. Therefore the quadrivalent HPV vaccine was highly immunogenic since induced high titers of serum antibodies to HPV 6, 11, 16, 18, prevented the acquisition of infection and clinical disease caused by these HPV types and was well tolerated.

The results of HPV clinical studies are encouraging suggesting that it will be possible to develop an effective prophylactic vaccine. Nevertheless the number of subjects analyzed in full-text published clinical studies is still poor, for a total of 3,851 evaluable subjects: 1,533 in the study by Koutsky *et al.* [34], 1,113 in the study by Harper *et al.* [36], 552 in the study by Villa *et al.* [37], 249 in the study by Fife *et al.* [38], 195 in the study by Brown *et al.* [35], 104 in the study by Brown *et al.* [33], 72 in the study by Harro *et al.* [16], and 33 in the study by Ault *et al.* [17] (Table 2).

Nevertheless, a consistent phase III study has recently been presented by Ault [39] at the 13th European Cancer Conference in Paris. In this multinational and multicenter study, 20,541 women aged 16-26 years recruited from the Americas, Europe and Asia were enrolled in one of four trials. In one trial subjects were randomized to either a HPV 16 L1VLP vaccine or placebo. In the other three trials subjects were randomized to either quadrivalent HPV 6, 11, 16, 18 L1 VLP vaccine or placebo. The endpoint of the studies was the incidence of HPV 16/18-related CIN II-III, AIS, or cancer; in the HPV 16 vaccine study only HPV 16 related cases were considered. Vaccination was performed at day 1, month 2 and month 6. Subjects were followed-up with a Pap test and HPV DNA tests at 6-12 month intervals for a maximum of 48 months. Analysis was done for two different situations: regular protocol (subjects received three doses, had no protocol violations, were HPV 16-18 seronegative at day 1 and HPV 16-18 DNA negative day 1 through month 7) and irregular protocol. Of a total of 17,829 evaluable subjects (8,487 in the regular protocol and 9,342 in the second group) the results showed that a prophylactic quadrivalent HPV vaccination prevents HPV 16/18-related CIN II-III and AIS.

Transferring these preliminary studies to the concept of vaccination of the general population calls for some reflections.

First of all, is the number of subjects analyzed in the clinical trials sufficient to use the vaccine in the general population or is it still necessary for a large clinical trial on tens of thousands of volunteers to be able to define the utility of the prophylactic vaccine?

Secondly, if a further large trial is considered in which area should the trial be performed?

For the first point the response would be when the data reported by Ault [39] is published and only if these data are satisfactory. For the second point, if a further large trial is necessary under the aegis of an official institution such as UICC, IARC or IFCPC, where should the site for the trial be?

Besides these two points, there are other different aspects to consider such as the identification of the target population, identification of the endpoint, the composition of the vaccine and marketing of the vaccine.

Choice of geographical area

There are two possible areas where such a program could be carried out: the first one is in the Nordic regions of Europe, characterized by a female population highly sensitive to prevention programs and by the existence of orga-

nized mass screening. In the Nordic regions of Europe the prerequisites for a large scale trial on HPV vaccination appear to be fulfilled [40, 41]. The second one would be to perform a clinical trial in developing countries [42], such as South-East Asia, Africa, and Central and South American nations where there is a very high incidence of cervical carcinoma.

Wherever planned (developed or developing areas) there are organizational problems for such vaccinations. This is particularly evident in developing countries.

HPV vaccine could have the greatest utility in developing countries but there are many difficulties for an extended vaccination program in these areas. The difficulties include the recruitment of subjects, follow-up, organization and the cost of the program.

Recruitment and follow-up: The target group is difficult to recruit because in developing countries young females are unlikely to be in school; the initiation of clinical trials with HPV vaccine targeted to teenagers has to be accepted by the parents; educational programs aimed at the mothers in which the knowledge of the etiology and risk factors of cervical cancer, and the usefulness of a preventive vaccine should be emphasized.

Nevertheless research on the acceptability of a HPV vaccine has been performed in Cuernavaca (Mexico) on 880 women 15-49 years old. They were interviewed on their knowledge of risk factors for cervical cancer and the general usefulness of vaccines. Whereas their knowledge regarding cervical cancer was little, 84% know the general usefulness of vaccines. After information on cervical cancer risk factors and on future availability of a HPV vaccine, 84% referred consent for their daughters' participation in a trial to evaluate the effectiveness of the vaccine to protect against cervical cancer [43].

Another problem is the long follow-up. Special models have to be developed to ensure that subjects included in a trial, many of whom may be illiterate, can be followed and the endpoints of interest ascertained many years after.

Furthermore there may be ethical difficulties. The fact that institutions of "rich" countries perform the trial in "poor" countries where the normal medical care is hugely different might be considered unethical.

Organization and cost: Conducting trials of vaccination programs in developing countries may be difficult since the global health services might be insufficient. Moreover, it should not be forgotten that the World Health Organization in 1996 [44] recognized that cytology screening programs are not feasible as a widespread policy in the developing world. They do not attain adequate coverage and often lack the expertise to guarantee quality results.

Finally the high cost is the main obstacle to vaccinations in developing countries.

Based on this situation, a phase III study in which the endpoint is the appearance of cervical invasive cancer or rather high-grade CIN may be possible only in countries in which a population-based cytological mass screening for cervical cancer exists. As a consequence not a developing country but the Nordic countries (Finland, Denmark, Norway, Sweden) with comparable mass screening for cervical cancer represent an unique venue for evaluation of a HPV vaccination trial.

Identification of the endpoint

The main endpoint is related to the natural history of the disease and therefore should require a long-term study since cervical HPV infection precedes cancer development by many years. The follow-up should be very long given the length of time necessary to confirm the hypothesis of the vaccine itself. This situation will incur considerable cost to public health systems. Thus it is necessary to choose *surrogate intermediate endpoints*, such as high-grade squamous intraepithelial lesions (CIN II-III) – the immediate and fixed precursor of cervical cancer – to determine vaccine efficacy in prospective trials. Low-grade intraepithelial lesions, in other words CIN I, will be the clinical endpoint that provides an indication of the efficacy of a vaccine, but unfortunately low-grade intraepithelial lesions are poor surrogate endpoint markers because of the variable natural history of such lesions.

Identification of the target population

Age and the sex should be considered. When to vaccinate? From a study [45] showing the rate of diagnosis of genital warts in England and Wales by age, vaccination of adolescents aged 10-12 years would be most desirable to control HPV infection and disease since it is most likely that this population is still uninfected. It is worthy of note that a vaccination at this age could be very difficult with follow-up difficult as well. On the other hand, performing a HPV vaccination within the scholastic vaccine program at a more advanced adolescent age (13-15 years) might make it more feasible.

Should vaccination be performed in both sexes or only in females? Females are the main subjects with HPV pathology, whereas males may develop genital warts but very rarely develop HPV-associated malignancy in the penis-anus. However males are the counterpart of the infection, and therefore also males need to be vaccinated to block the venereal disease cycle.

Anti-HPV vaccination should be as compulsory as anti-diptheria, anti-tetanus, antipoliomyelitis and antiviral hepatitis B, in Italy.

What should be the timing of a vaccination program? The design may be summarized as follows: vaccination of adolescents before they become exposed to HPV; monitoring possible side-effects; long period of follow-up; no measurement of cervical outcome until the subjects are at the age to benefit from screening. Therefore if HPV vaccination is at age 15, a 5-year interval between vaccination and first screening examination with high-grade CIN as the endpoint occurs.

Composition of the vaccine

Since there are many oncogenic HPV types, how many should be considered? Recent studies have shown that HPV types 16, 18, 45, 31, 33, 52, 58 are in descending order of frequency as the seven most common types in cervical cancer [13, 14]. Theoretically a polyvalent HPV vaccine with the seven most common types should be established to suppress development of the majority of cervical cancers.

However would a common prophylactic vaccination be suitable for worldwide distribution or would a vaccine tailored to the HPV types prevalent in a given geographic area be necessary?

The international biological study on cervical cancer of the IARC performed an analysis on 3,085 cases of cervical cancer from 25 countries around the world to determine geographic variation and prevalence of HPV types [14].

A total of 30 different HPV types were detected. Two types predominated: 57.4% of cases were associated with HPV 16 and 16.6% with HPV 18. These two types as either single or multiple infections were detected in 74% of all cases.

Concerning geographic variation, HPV 16 and HPV 18 were the first and second most common types in all regions, but showed significant differences in distribution. The highest prevalence of HPV 16 was observed in Europe/North America (69.7%) and in Northern Africa (67.6%) and the lowest in Sub Saharan Africa (47.7%). HPV 18 was more common in South Asia (25.7%) than in Europe/North America (14.6%). The third most common type was HPV 45 in Europe, North America, Africa, South Asia and HPV 31 in Central/South America.

Marketing of the vaccine

Public heath authorities should recommend a vaccine that would prevent cancer but this cancer is considered as a female cancer, therefore why a vaccination of both sexes? The term *a vaccine that prevents cancer* might be justified by the fact that other cancers besides cervical cancer may be induced by HPV, for example other anogenital cancers as vulvar, anal, penis cancer and furthermore some cancers of the upper aerodigestive tract (tonsil, tongue and probably esophagus) [46]. Therefore it would be a prophylactic vaccine against oncogenic types of mucosatropic HPVs. Since the pathogen is sexually transmitted it might be helpful to use the term indogy a *vaccine against a sexually transmitted disease*. In favor of the use of polyvalent viral mixtures (high and low-risk types) in the vaccine composition is the fact that the vaccine could be more attractive to public opinion, eliminating also an important sexually transmitted disease as common in women as in men [7].

Nevertheless a mixture of high and low-risk types, the seven most common high-risk out of 15-18 high risk and at least two out of 12 low-risk, might be difficult to realize.

As clearly reported by Jansen and Shaw [11] "the most effective strategy will be to maintain philosophical distance from the sexual aspects of the question and focus on the prevention of a common cause of cancer".

Open questions

The HPV vaccination program has at least two points to reflect upon and which require deep investigation.

The degree to which vaccination effect persists overtime

Because women are at risk of HPV infection for as long as they are sexually active, protection induced by a HPV vaccine must endure from adolescence for several decades. At this time the duration of protective immune response after immunization with VLP-based vaccines is not known. A study [47] on 1,656 primiparous women followed until their second pregnancy showed that the correlation between the first and second pregnancy HPV 16 serum antibody levels of the same woman was high even when > 4 years had elapsed between pregnancies. These data indicate that HPV 16 antibody levels are generally stable over several years of follow-up. Nevertheless the long persistence of antibodies should be confirmed.

Behavioral modifications

A vaccination program could generate behavioral modifications. Women vaccinated may perceive that are fully protected from HPV infection and cervical cancer, with the consequence of an increase in risky sexual activity and in delay of gynecological controls. Gynecological controls and screening can not be eliminated because the duration of protection from the vaccine might not be lifelong and because women who have been vaccinated would still be at risk of developing cervical cancer caused by other high-risk types not present in the vaccine mixture [48].

Conclusion

The concept of a HPV vaccination program has to be considered not as a hypothesis, a dream, a hope, but as a concrete possibility. Studies on the immunogenicity of HPV suggest that it will be possible to develop a prophylactic vaccine; the results of preliminary clinical trials confirm this suggestion.

It should be to pointed out that such a vaccination could be a very complicated preventive objective and that a long time is necessary for the impact of HPV vaccination to become evident in vaccinated populations. Considering the start of a HPV vaccination program, the period of enrollment and the long period of follow-up, the impact of HPV vaccination on the total number of cervical cancer cases will be visible after at least ten years from the start of the vaccination program.

There is no doubt that HPV vaccine could represent the "*beginning of the end*" of cervical cancer.

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Address reprint requests to:
L. MARIANI, M.D.
Gynecol. Oncol. Unit
Regina Elena Cancer Institute
Rome (Italy)