

Review Article

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Human papillomavirus vaccines: current issues & future

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Cervical cancer is the leading cause of cancer mortality among women in worldwide. Some 99 per cent of cervical cancer cases are linked to genital infection with human papillomaviruses (HPVs) comprised of approximately 15 oncogenic genital HPV types. Most HPV infections resolve spontaneously. But, the remainder persist and may then progress to cervical cancer in some women. In high-resource countries, the best way to prevent cervical cancer is to implement organised gynaecological screening programs with appropriate treatment of the detected pre-cancerous lesions. However, in developing countries, this method is not practicable because of cost and complexity of proper screening. Vaccines against HPV infections hold promise to reduce incidence of cervical cancer cost-effectively. Two Prophylactic HPV vaccines have been thus far developed: Gardasil®, a quadrivalent vaccine targeting HPV-6, -11, -16 and -18) and Cervarix®, a bivalent vaccine which targets HPV-16 and -18. Both vaccines contain L1 virus-like particles (VLPs) derived from HPV-16 and -18 which are most frequently associated with cervical cancer. The L1-VLP vaccines are HPV type-specific and therefore can effectively prevent infection of a HPV type in question alone. Therefore, the L1-VLP vaccines are hoped to be multivalent for 15 oncogenic HPV types, which comes at a price. Otherwise, costly cytologic screening for cervical cancer is still necessary. The current HPV vaccines thus may not be ultimate strategy and study on new HPV vaccines is needed. Broad-spectrum prophylactic vaccines against all oncogenic HPV types and therapeutic vaccines for clearance of HPV-related cervical lesion are being developed.

Key words Cervical cancer - human papillomavirus (HPV) - HPV vaccines

Epidemiology of HPV infection

At present, there are about 100 identified genotypes (types) of human papillomavirus (HPV), of which about 40 are genital HPV types that invade the genital organs such as the uterine cervix, vaginal wall, vulva, and penis. Genital HPV types are classified into high-risk types commonly associated with cervical cancer and low-risk types known causative pathogens of condyloma acuminatum. This classification varies among researchers, but, in general, types 16/18/31/33/35/39/45/51/52/56/58/66/68 are classified as

high-risk and 6/11/40/42/43/44/54/61/72 as low-risk types¹. Interestingly, the HPV type distribution varies depending on the discrete stage of cervical neoplasia (Fig. 1).

The HPV-DNA detection rate in the genital organs of healthy adult females varies between advanced and developing countries, being approximately 20-40 per cent collectively^{2,3}. In Japan, the HPV-positive rate in pregnant women aged 20-29 yr has been reported to be 20-30 per cent similar to, or higher than in the same age group in the US⁴. The World Health Organization

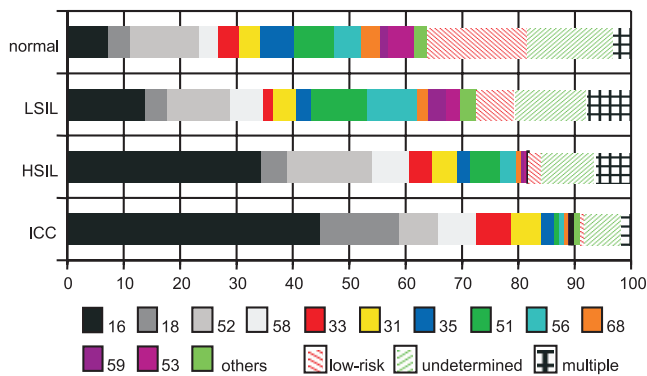


Fig. 1. HPV type distribution in cervical neoplasia in Japan¹⁸. HPV16 and 18 are the most common types in invasive cervical cancer (ICC) while more than 40 per cent of the invasive cancer is associated with the other types in Japan. HPV52 is the most common type in female with normal cytology in Japan¹⁸.

(WHO) estimated an annual increase of 3 hundred million in the number of HPV carriers in the world^{5,6}. Overall HPV prevalence in 157,879 women with normal cervical cytology was estimated to be 10.4 per cent⁶. In the US, epidemiological data show HPV infection at least once in life in 3 out of every 4 females³. Thus, HPV infection is a common disease affecting any female but not an event those in particular populations. High sexual activity has been reported to increase the risk of HPV infection⁷; in some women.

Risk factors for the progression of cervical neoplastic diseases

The incidence of cervical epithelial dysplasia (corresponding to squamous intraepithelial lesion: SIL) is about 1 per 10 females with HPV infection⁸. The incidence of high-SIL (corresponding to cervical intraepithelial neoplasia 2 and 3: CIN2 and CIN3, respectively) is about 3 per 10 females with low-SIL, and that of CIN3 is about 1-2 per 10 females with low-SIL⁹. Since therapeutic interventions are performed for CIN3, the actual incidence of cervical cancer is about 1 per 600 females with HPV infection. Without treatment, the incidence of the progression of CIN3 to cervical cancer is about 30 per cent¹⁰. Therefore, the incidence of the spontaneous development of cervical cancer is about 1 per 200-300 females with HPV infection.

Factors associated with progression to cervical cancer in females with HPV infection have been extensively studied¹. Many prospective studies have identified persistent HPV infection as the most important risk factor, and also showed that the persistent infection tends to occur in high-risk type HPV. Persistent HPV infection generally involves

persistent virus proliferation, as verified by the detection of virus DNA from cervical exfoliated cells. Chronic virus proliferation induces the active proliferation/differentiation of infected epithelial cells, and some infected cells incidentally immortalize, which is the first step of carcinogenesis¹.

On the other hand, transient infection involves short-term virus proliferation followed by long-term latent presence of low copies of the viral genome in the basal cells of the genital epithelium. A fate of HPV infection leading to transient, but not persistent, is determined by cellular immunocompetence against HPV. It is unlikely that transient infection progresses to cervical cancer¹.

Prophylactic vaccines

Development of the current L1-VLP vaccines

HPV is the causative virus (requirement) for genital cancers with cervical cancer being most prevalent. Thus, theoretically, if HPV infection could be completely eradicated, most of genital cancers could be prevented. The study of HPV vaccines began about 10 years ago. In 2002, Koutsky *et al* were the first to show the clinical prophylactic effects of an HPV vaccine¹¹. Merck in the US and Glaxo Smith Kline (GSK) in Europe launched full-scale development of prophylactic vaccines against HPV, and their vaccines were approved and commercially available a few years ago. The vaccine antigens of the two companies are virus-like particles (VLP) produced using HPV type16 L1 protein overexpressed in yeasts or insect cells. These particles externally have a 3-dimensional structure similar to that of virus particles, but have no contents, and, therefore, are not infective. The vaccine reported by Koutsky *et al*¹¹ also uses HPV16L1-VLP as an antigen.

However, the main problem of the L1-VLP vaccine is its negligible prophylactic effects on other HPV types¹². Therefore, GSK and Merck developed cocktail vaccines composed of L1-VLPs corresponding to HPV types as targets. The vaccine developed by Merck is a tetravalent vaccine against types 6, 11, 16, and 18 (Gardasil®)¹³ and that developed by GSK was a bivalent vaccine against types 16 and 18 (Cervarix®)¹⁵. A follow-up after inoculation with the quadrivalent vaccine showed the prevention of persistent infection with all 4 HPV types in 96 per cent¹⁴. Though the antibody titers have been maintained for 4-5 years¹³⁻¹⁵, whether the antibody titers can be maintained for longer periods is unknown.

Clinical trials led by the two companies are ongoing in Japan and elsewhere.

Issues regarding the currently prevailing L1-VLP vaccines

The current HPV vaccines developed by GSK and Merck are used for uninfected females to prevent HPV infection/spread. For mass prophylactic vaccination in uninfected females, vaccination should be performed at the age of about 10 years before sexual activity begins. A recent phase III clinical study (FUTURE 1 & 2) in which females aged about 20 years were randomly inoculated with Gardasil® revealed prophylactic effects on the development of CIN2-3 associated with HPV types 16 and 18 in more than 98 per cent of females who completed the vaccination protocol^{16,17}. However, prophylactic effects were observed in only 13-22 per cent of females inoculated just once or twice or by intention-to-treat analysis including prophylactic effects on other HPV types^{16,17}.

At present, antibody titers induced by L1-VLP vaccines are confirmed to be maintained for only 5 yr. There is no guarantee that the prophylactic effects of the vaccine inoculated at the age of 10 yr will be maintained, beyond the sexual activity period. Even if the prophylactic effects of the current HPV vaccines continue for life, only cases of cervical cancer due to HPV types 16 and 18, which constitute less than 60 per cent of all invasive cervical cancer cases in Japan¹⁸, can be prevented (Fig. 1). Indeed, the HPV type distribution in cervical cancer varies depending on regions in the world¹⁹. HPV16 and 18-associated cervical cancer is more than 70 per cent in North America, Europe and Australia, about 65 per cent in Africa, about 60 per cent in South and Central America, and less than 60 per cent in Asia including Japan^{18,19}. Therefore, females who undergo vaccination and receive the current vaccine may have a risk for the development of cervical cancer and thereby need not undergo cervical cancer screening. Providing such information to females undergoing this vaccination is the most important for the introduction of the current HPV vaccines. A single dose of the present HPV vaccines costs about 100 USD. There is need for reduction of this high cost. In addition, the L1-VLP vaccines are highly protective against infection corresponding to the papillomavirus type used to derive the immunogen, but are ineffective against all but the most closely related HPV types. Therefore, the L1-VLP vaccines should be ultimately multivalent for

15 oncogenic HPV types. This makes the prophylactic vaccine more expensive than the current vaccines.

In some countries and states, the current HPV vaccines are distributed for free, or inoculation is covered by public expenses²⁰. However, considering the progression of HPV infection to cervical cancer in only 1 per 300 females, vaccines effecting the prevention of only limited types, the relatively widespread cancer screening, and the high cost of such vaccines, it mandatory mass preventive inoculation with the current HPV vaccines is of value in developed country such as Japan may not be feasible. In addition, the current HPV vaccines targeting only HPV types 16 and 18 do not enable the omission of cancer screening, and vaccination at public expenses has no advantage in terms of medical economics. In Japan, voluntary inoculation during the sexual activity period should be performed first at the expense of each woman. The mass prevention employing the current HPV vaccines is a matter of debate.

Second generation HPV prophylactic vaccines

The main problem regarding the current L1-VLP vaccines is the induction of type-specific immunity. To overcome this, broad-spectrum vaccines that are also effective for the prevention of high-risk type HPV infection are under development. L2 as the other structural proteins of virus particles contains many conserved regions among all HPV types (Fig. 2). We

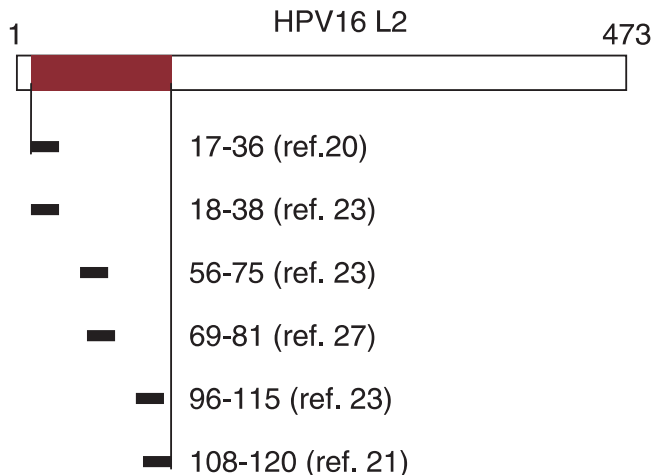


Fig. 2. Broad-spectrum neutralization epitopes of HPV16 L2 capsid protein. Many studies reveals linear epitope cross-neutralizing infection with many HPV types in L2 capsid protein. Each epitope includes amino acid conserved regions between genital HPV types and has potential of neutralization of HPV infection. These are candidates for type-common prophylactic vaccines to HPV^{21,23,26,27}.

and Kondo *et al* have sought a way to develop novel vaccines using partial regions of L2 containing type-common neutralization epitope^{21,22}. Recently, Kondo *et al* identified a vaccine candidate for the prevention of all types by developing newly type-common neutralization epitopes of L2 and optimizing the regions²³. Roden *et al.* also studied the type-common neutralization epitope of L2²⁴⁻²⁶. They devised strategies to use the entire L2 for vaccines, and their joint study with Christensen *et al*²⁰. confirmed its suppressive effects on infection with a broad spectrum of HPV types in animal experiments²⁵. Furthermore, they discovered a new region (17-36 amino acid of HPV16 L2) of L2 which contains broad-spectrum neutralization epitopes²⁶. It is certain that L2 will be a vaccine antigen candidate for common-type vaccines for the prevention of HPV infection.

The problem of L2 is its lower antigenicity than that of L1-VLP²². To apply L2 to humans, there are various problems such as the incidence of non-responders to the vaccine and the necessity for adjuvants. Several groups have recently revealed that chimeric VLP in which the cross-neutralization epitope of L2 inserted induce cross-neutralizing antibodies more effectively^{27,28}. If high-risk type HPV infection can be suppressed using L2, the benefits of mass prevention by prophylactic HPV vaccine should be increased.

Other vaccine strategies for cervical cancer

Vaccine and cancer prevention strategies for cervical cancer depend on the medical/economic situations of each country. In low-resource settings, prophylactic vaccines against HPV infections have clearly the potential to reduce incidence of cervical cancer cost-effectively. By contrast, in developed countries, where precursor lesions of cervical cancer can be detected early based on well-established cancer screening program, the following diverse vaccine strategies warrant consideration (Fig. 3): (i) vaccines for the prevention of infection in uninfected females, (ii) vaccines for the reduction of viral load at the cervical mucosa in females with low-SIL and prevention its progression, (iii) vaccines for treatment in females with high-SIL, and (iv) immunotherapy for cervical cancer. The current HPV vaccines are those for the prevention of infection described in (i). On the other hand, (iii) and (iv) are considered to be therapeutic vaccines used for females with disease, and many clinical studies on such vaccines have been performed worldwide²⁹. However, none of the vaccines exhibited statistically significant clinical effects with

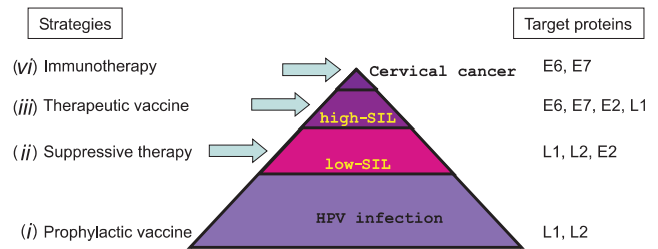


Fig. 3. Possible strategies utilizing immunological responses to HPV proteins for HPV-associated lesion and the target viral proteins for each strategy. (i) vaccines for the prevention of infection in uninfected females, (ii) vaccines for the reduction of viral load at the cervical mucosa in females with low-SIL to prevent from progression, (iii) vaccines for treatment in females with high-SIL, and (iv) immunotherapy for cervical cancer.

adequate cellular immunological responses induced by the vaccines. Since prophylactic vaccines such as the current HPV vaccines are preceding, the development of the latter seems to be delayed at present.

Possible suppressive therapy for cervical neoplasia

Long-term effects of the current HPV vaccines on HPV infection are still unclear. Clinical studies were already initiated by inoculating females aged about 20 yr with this HPV vaccine irrespective of the presence/absence of HPV infection. A recent study revealed that the current HPV vaccines tended to protect women who had already oncogenic HPV infection as well as cytological abnormalities from progression to high-grade CIN at 15 months follow-up³⁰. We reported that HPV16-associated CIN1-2 tends to regress at 24 months follow-up in patients positive for serum high-titer neutralizing antibodies to HPV16³¹. Both evidences were not based on long-term follow-up. The current HPV vaccines are known to have a marked ability to induce neutralizing antibodies. Given these considerations, current HPV vaccines are likely to eliminate persistent HPV infection and subsequent malignant transformation. This raises the expectation that the vaccines can work so as to suppress HPV infection as described in 2). The results of further clinical studies are awaited.

Therapeutic vaccines

Because of limitations of the current HPV vaccines as mentioned above, necessity of therapeutic vaccines for the treatment of HPV-associated lesions is still in demand even after the prophylactic vaccine program are implemented in the world²⁹. Development of the HPV therapeutic vaccines has been performed for the

Table. Clinical trials of therapeutic vaccine for HPV-associated cervical lesion

Trial phase	Target proteins	Vaccine vectors	Inoculation	Target HPVs
Ph-I/II ³⁴	L1, E7	Chimera-VLP	sc	16
Ph-II ³³	E7	Hsp (SGN-00101)	sc	16
Ph-II ³⁵	E6, E7	Vaccinia virus (TA-HPV)	sc	16, 18
Ph-II ³⁶	L2, E6, E7	Fusion protein L2E6E7 (TA-CIN)	im	16, 18
Ph-II ³⁷	BPV E2	Vaccinia virus (MVA-E2)	intrauterine	all
Ph-III ³⁸	E6, E7	Plasmid vaccine (ZYC101a)	im	16, 18

sc, subcutaneous injection; im, intramuscular injection; BPV, bovine papillomavirus

last two decades. The following vaccines have been well evaluated in clinical studies (Table).

1. SGN-00101 (sc) is a fusion protein consisting of heat shock protein (Hsp) of *Mycobacterium bovis* and HPV type 16 E7. The Ph-II study looking at effect of SGN-00101 in cases with CIN3 revealed histological CR in 13 (22.5%) of 58 cases, although immunological responses was not determined³². Another Ph-II study in cases with CIN showed 7 (35%) of 20 patients. In 5 of the 7 cases, the induction of CTL against HPV16E7 in peripheral monocytes was shown³³.
2. L1VLP-E7 (sc) is a vaccine using chimera particles composed of HPV type 16 L1-VLP and E7. In the Ph-I/II study in CIN2-3 cases, histological PR was shown in 39 per cent of vaccine recipients compared with 25 per cent of placebo recipients although there was no significant difference³⁴. The clinical efficacy was coupled with cellular immune responses in some cases.
3. TA-HPV (im) is a recombinant vaccinia virus expressing HPV16/18 E6 and E7. The Ph-II study of TA-HPV in VIN cases revealed PR was shown in 8 of 13 cases and reduction of viral load was also shown in 6 of 8 lesion responders. The responders showed increase of lesion-infiltrating CD4 and 8-positive cells³⁵.
4. TA-CIN (im) is a fusion protein consisting of E6, E7 and L2 of HPV types 16 and 18. The Ph-II study in VIN cases revealed that CR or PR was shown in only 6 of 29 cases. CTL against E6/E7 was induced in 4 of 29 cases³⁶. The correlation between clinical efficacy and cellular immune responses to the vaccine are unclear.
5. MVA-E2 (TGA4001) (intrauterine) is also a recombinant vaccinia virus expressing bovine papilloma virus (BPV) E2. The Ph-II study in

cases with CIN2-3 confirmed antibody responses in serum, CTL induction in peripheral blood, and the regression of CIN in some cases (19/34 cases). There was no significant clinical efficacy³⁷.

6. ZYC-101a (im) is a DNA vaccine synthesized from some proteins containing CTL epitopes against E6 and E7 of HPV types 16 and 18. The Ph-III test was performed in subjects with CIN2-3. CR or PR was observed in 41 per cent in the vaccination group and 27 per cent in the placebo group, with no significant difference. When the cases were limited to those aged ≤ 25 yr, the percentage showing CR or PR was significantly higher in the vaccination (72%) than in the placebo (23%) group. However, no correlation between CTL induction against E6/E7 and clinical effects was shown³⁸.

Thus, there are no therapeutic HPV vaccines so far with apparent clinical efficacy based on enhanced cellular immune responses induced by vaccines. The current therapeutic vaccines elicit systemic cellular immunity by intramuscular or subcutaneous injection and thereby the clinical trials have shown cellular immune responses to the vaccines in peripheral monocyte, but not mucosal immunity at cervical mucosa.

We consider that CTL induction in the cervical mucosa is indispensable for treating cervical mucosal lesions such as CIN. In addition, vaccination is an effective method in the induction of mucosal immunity. Therefore, we have attempted induction of mucosal T cell responses by stimulating intestinal mucosal immunity through mucosal administration, particularly oral administration. Bermudez-Humaran *et al*³⁹. produced gene-recombinant type lactic acid-expressing HPV16E7 and IL-12 from live lactobacillus, and evaluated the induction of CTL activity following its nasal or oral administration as a live vaccine in an experiment using mice, and also its preventive and

reductive effects in a tumor challenge test. They also found more marked mucosal induction after nasal than oral administration and a more effective induction of immunity using *Lactobacillus plantarum* than *Lactococcus lactis*⁴⁰. No information on clinical studies of this vaccine is available. We have worked with a lactobacillus HPV vaccine using the *Lactobacillus casei* strain showing of inflammatory immune responses. We noted marked induction of mucosal T cells possessing CTL activity to HPV E7 at intestinal mucosa after its oral administration of *Lactobacillus casei* expressing HPV16 E7 to mice (Kawana *et al*, unpublished data). Further studies are necessary to get a detailed picture of this approach.

Summary

The usefulness of the current HPV vaccines cannot be underestimated. These vaccines are a valuable step toward the control of cervical cancer. The mass prevention strategy by use of the current HPV vaccine is ongoing in many countries. However, a conclusion cannot be drawn until the results of large-scale clinical studies in progress and long-term follow-up data are available. In addition, the development of the next generation HPV vaccines is also essential.

References

- zur Hausen H. Papillomavirus and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; 2 : 342-50.
- Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, *et al*. Prevalence of HPV infection among females in the United States. *JAMA* 2007; 297 : 813-9.
- Bosch FX, de Sanjose S. Human papillomavirus and cervical cancer – burden and assessment of causality. *J Natl Cancer Inst Monogr* 2003; 31 : 3-13.
- Masumoto N, Fujii T, Ishikawa M, Mukai M, Ono A, Iwata T, *et al*. Dominant human papillomavirus 16 infection in cervical neoplasia in young Japanese women; study of 881 outpatients. *Gynecol Oncol* 2004; 94 : 509-14.
- The current status of development of prophylactic vaccines against human papillomavirus infection. Report of a technical meeting. WHO, Geneva; February 16-18, 1999.
- Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998; 338 : 423-8.
- de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, *et al*. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis* 2007; 7 : 453-9.
- Koutsky L. Epidemiology of genital human papillomavirus infection. *Am J Med* 1997; 102 : 3-8.
- Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine* 2006; 24S1: S1/4-S1/15.
- Holowaty P, Miller AB, Rohan T, To T. Natural history of dysplasia of the uterine cervix. *J Natl Cancer Inst* 1999; 91 : 252-8.
- Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, *et al*. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; 347 : 1645-51.
- Ochi H, Kondo K, Matsumoto K, Oki A, Yasugi T, Furuta R, *et al*. Neutralizing antibodies against human papillomavirus types 16, 18, 31, 52, and 58 in serum samples from women in Japan with low-grade cervical intraepithelial neoplasia. *Clin Vaccine Immunol* 2008; 15 : 1536-40.
- Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, *et al*. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine* 2006; 24 : 5571-83.
- Villa LL, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE, *et al*. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer* 2006; 95 : 1459-66.
- Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, *et al*. 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* 2006; 367 : 1247-55.
- Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, *et al*. FUTURE I investigators. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007; 356 : 1928-43.
- FUTURE II study group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007; 356 : 1915-27.
- Miura S, Matsumoto K, Oki A, Satoh T, Tsunoda H, Yasugi T, *et al*. Do we need a different strategy for HPV screening and vaccination in East Asia? *Int J Cancer* 2006; 119 : 2713-15.
- Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003; 88 : 63-73.
- Wright TC Jr. Current status of HPV vaccination recommendation. *HPV Today* 2008; 14 : 8-9.
- Kawana K, Yoshikawa H, Taketani Y, Yoshiike K, Kanda T. Neutralizing epitope of L2 minor capsid protein common to human papillomavirus type 16 and 6. *J Virol* 1999; 73 : 6188-90.
- Kawana K, Yasugi T, Kanda T, Kino N, Oda K, Okada S, *et al*. Safety and immunogenicity of a peptide containing the cross-neutralization epitope of HPV16 L2 administered nasally in healthy volunteers. *Vaccine* 2003; 21 : 4256-60.
- Kondo K, Ishii Y, Oshi H, Matsumoto T, Yoshikawa H, Kanda T. Neutralization of HPV16, 18, 31, and 58 pseudovirions with antisera induced by immunizing rabbits with synthetic peptides representing segments of the HPV16 minor capsid protein L2 surface region. *Virology* 2007; 358 : 266-72.
- Roden RB, Yutzy WH 4th, Fallon R, Inglis S, Lowy DR, Schiller JT. Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitopes. *Virology* 2000; 270 : 254-7.
- Gambhira R, Jagu S, Karanam B, Gravitt PE, Culp TD, Christensen ND, *et al*. Protection of rabbits against challenge

- with rabbit papillomaviruses by immunization with the N terminus of human papillomavirus type 16 minor capsid antigen L2. *J Virol* 2007; 81 : 11585-92.
26. Alphas HH, Gambhira R, Karanam B, Roberts JN, Jagu S, Schiller JT, *et al*. Protection against heterologous human papillomavirus challenge by a synthetic lipopeptide vaccine containing a broadly cross-neutralizing epitope of L2. *Pro Natl Acad Sci USA* 2008; 105 : 5850-5.
 27. Slupetzky K, Gambhira R, Culp TD, Shafti-keramat S, Schellenbacher C, Christensen ND, *et al*. A papillomavirus-like particle (VLP) vaccine displaying HPV16 L2 epitopes induces cross-neutralizing antibodies to HPV11. *Vaccine* 2007; 25 : 2001-10.
 28. Kondo K, Ochi H, Matsumoto T, Yoshikawa H, Kanda T. Modification of human papillomavirus-like particle vaccine by insertion of the cross-reactive L2-epitopes. *J Med Virol* 2008; 80 : 841-6.
 29. Kanodia S, Da Silva DM, Kast WM. Recent advances in strategies for immunotherapy of human papillomavirus-induced lesions. *Int J Cancer* 2008; 122 : 247-59.
 30. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmerón J, Wheeler CM, *et al*. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007; 369 : 2161-70.
 31. Kawana K, Yasugi T, Kanda T, Kawana Y, Hirai Y, Yoshikawa H, *et al*. Neutralizing antibodies against oncogenic human papillomavirus (HPV) as a possible determinant of the fate of low-grade cervical intraepithelial neoplasia. *Biochem Biophys Res Commun* 2002; 296 : 102-5.
 32. Einstein MH, Kadish AS, Burk RD, Kim MY, Wadler S, Streicher H, *et al*. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. *Gynecol Oncol* 2007; 106 : 453-60.
 33. Roman LD, Wilczynski S, Muderspach LI, Burnett AF, O'Meara A, Brinkman JA, *et al*. A phase II study of Hsp-7 (SGN-00101) in women with high-grade cervical intraepithelial neoplasia. *Gynecol Oncol* 2007; 106 : 558-66.
 34. Kaufmann AM, Nieland JD, Jochmus I, Baur S, Friese K, Gabelsberger J, *et al*. Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *Int J Cancer* 2007; 121 : 2794-800.
 35. Davidson EJ, Boswell CM, Sehr P, Pawlita M, Tomlinson AE, McVey RJ, *et al*. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. *Cancer Res* 2003; 63 : 6032-41.
 36. Fiander AN, Tristram AJ, Davidson EJ, Tomlinson AE, Man S, Baldwin PJ, *et al*. Prime-boost vaccination strategy in women with high-grade, noncervical anogenital intraepithelial neoplasia: clinical results from a multicenter phase II trial. *Int J Gynecol Cancer* 2006; 16 : 1075-81.
 37. García-Hernández E, González-Sánchez JL, Andrade-Manzano A, Contreras ML, Padilla S, Guzmán CC, *et al*. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. *Cancer Gene Ther* 2006; 13 : 592-7.
 38. Garcia F, Petry KU, Muderspach L, Gold MA, Braly P, Crum CP, *et al*. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2004; 103 : 317-26.
 39. Bermúdez-Humarán LG, Cortes-Perez NG, Lefèvre F, Guimarães V, Rabot S, Alcocer-Gonzalez JM, *et al*. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. *J Immunol* 2005; 175 : 7297-302.
 40. Cortes-Perez NG, Lefèvre F, Corthier G, Adel-Patient K, Langella P, Bermúdez-Humarán LG. Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria. *Vaccine* 2007; 25 : 6581-8.

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