REVIEW

Vaccine against Papilloma virus: a review of the clinical studies

G. GABUTTI
Department of Clinical and Experimental Medicine, Section of Hygiene and Occupational Health, Univeristy of Ferrara, Italy

Key words

HPV • Vaccine • Clinical studies

Introduction

Human papillomaviruses (HPV) are ubiquitous agents with a double-stranded circular DNA consisting of around 8,000 base pairs. In terms of structure, HPVs consist of a capsid without an outer envelope consisting of 72 pentagonal capsomers. The genome is able to code for two proteins (L1 and L2) which make up the outer envelope and which have a high immunogenic power and for 6 early proteins (E1, E2, E4, E5, E6 and E7) necessary for the replication of the viral DNA and for the assembly of the new virions in the infected cells. Phylogenetically HPVs are classified on the basis of the level of homology of the L1 capsid gene, which is genetically stable [1, 2].
Based on a genomic homology of less than 90%, over 100 types of HPV have been identified and are classified according to their oncogenic potential as high and low risk HPVs. The strains with a high oncogenic potential have a marked tendency to become integrated in the cell genome, leading to the interruption of the E2 gene and the consequent loss of its control function on the expression of the E6 and E7 genes, whose products interact with cell cycle regulatory proteins (p53 and pRB) inhibiting the function and causing a high degree of genomic instability of the infected cells and loss of control of cell growth [3].
The causal role of high-risk HPVs in uterine cervical carcinoma has been confirmed from both a biological and epidemiological point of view [4].
In addition to cervical carcinoma, HPVs are responsible for other forms of anogenital (vulva, penis, vagina, anus) and oropharyngeal cancer [5].
The genotypes most frequently detected in all cervical tumours are HPV16, 18, 45 and 31; in particular HPV16 and HPV18 cause over 70% of cervical tumours, while HPV16, HPV18, HPV45 and HPV31 are responsible for more than 90% of cases of cervical adenocarcinoma [6].
HPVs are ubiquitary viruses and up to 80% of women acquire an infection during their lifetime, with the highest incidence in 20-40 year-olds [7, 8].
In most cases the infection clears and the mechanism of persistent infection is not triggered. However, HPV16, HPV18 and other oncogenic type infections tend to persist more frequently than low-risk HPV infections. Persistent oncogenic HPV infection represents the precursor of invasive cervical cancer. Once the uterine cervix has been infected, low-grade squamous intraepithelial lesions can occur (ASCUS/LSIL, CIN1) which generally subside spontaneously. If they do not, high-grade squamous intraepithelial lesions can occur (HSIL, CIN2 and CIN3), which are more likely to develop into invasive cancer [9]. The time interval between the infection and the possible onset of cancer is 10-20 years [10].
It is estimated that 500,000 new cases of cervical cancer and about 270,000 deaths occur worldwide every year [6, 11, 12].
Although not all the details are known, the immune response is known to play an important role, involving both humoral and cell-mediated immunity. Oncogenic HPVs are agents that can elude the host immune response. This occurs because the virus is not cytolytic, does not cause inflammation, eludes recognition of the capsid antigens by Langerhans cells and inhibits type I interferons (alpha and beta).
Previous oncogenic HPV infections do not necessarily cause immunity to subsequent infections; the level of protection provided by natural infection varies and reinfections or new infections are possible.
It is believed that humoral immunity, mainly to L1, prevents the anchorage and entry of the virus into the cells; cell-mediated immunity is important in eliminating most natural infections [13-15].
On the basis of this knowledge, preventive vaccines have been developed with the objective of preventing HPV infection by induction of the humoral response (antibodies against L1). The development of preventive vaccines began after the demonstration that the expression of L1 capsid proteins in eukaryotic cells lead to the self-assembly in virus-like particles (VLPs). VLPs are similar in structure to HPVs, but do not contain any genetic material [16-18].
Antibody-mediated immunity against L1 is type-specific and antibodies against L1 VLP protect against the infection and the disease. This fact was historically established by providing protection through the transfer of serum from vaccinated to non-vaccinated animals [19, 20].
As a result of the particular characteristics of HPVs, the level of antibody response that can be obtained by means of vaccination is higher than what is seen after

natural infection. Vaccination prevents infection by the induction of neutralizing antibodies that bind to sites present on the capsid and prevent infection of the host cell. The WHO believes that neutralizing antibodies are fundamental for post-vaccine protection against HPV infection even though there is no demonstration of either short or long-term immune correlate for protection [21, 22].
Modern technology makes it possible to modulate the quality and quantity of antigen-specific immune response through the use of adjuvants [23, 24].
In the case of HPV infection it is important to elicit the production of high levels of serum neutralizing antibodies by vaccination, since a certain amount of these must be available at cervical-vaginal level (by transudation or exudation) in order to prevent and block new infections [25].
On the basis of these scientific findings, two preventive vaccines for HPV were developed with the main objective of preventing the onset of cervical-vaginal cancer.
Since the two products were developed on the basis of different rationales and were evaluated in trials with different end-points, different structures and different laboratory methods, it is currently difficult to make a direct comparison of the results obtained with the two products. It is, however, possible to examine the scientific evidence acquired during the phase II and III studies for each vaccine, with particular reference to immunogenicity and clinical efficacy, protection duration, any cross-protection acquired, tolerability and safety.
As far as the evaluation of clinical efficacy is concerned, it should be pointed out that in addition to the "classic" clinical-histological end-points (CIN1, CIN2 and CIN3) it is important to consider the persistence of the infection as a key marker and to also therefore use virological type assessments when examining the results of the clinical trials [26].

The bivalent vaccine

As a result of the first indications on the potential impact of the L1 VLPs on cervical cancer, GSK developed a bivalent vaccine (Cervarix) containing HPV16 and HPV18 type L1 VLPs with the rationale of intervening against the types responsible for around 70% of all cases of CIN2/3 and of cervical cancer and for around 25% of all cases of CIN1. The L1 VLPs contained in the vaccine are produced by means of recombinant DNA technology using a Baculovirus expression system in cells derived from *Trichoplusia ni* (Hi-5 Rix4446). The vaccine contains a new adjuvant system, named AS04, consisting of monophosphoryl lipid A (MPL), i.e. a detoxified lipopolysaccharide obtained from *Salmonella minnesota* adsorbed on aluminium hydroxide $Al(OH)_3$ [27]. The vaccine schedule foresees 3 doses to be administered at 0, 1 and 6 months; each dose contains 20 µg of HPV16 L1 VLP, 20 µg of HPV18 L1 VLP, 50 µg of AS04 and 0.5 mg of Al^{3+}.

The use of the AS04 adjuvant system optimizes the immunological response; specifically, AS04 stimulates the antigen-presenting cells (APC) through the toll-like receptors TLR-4. The stimulated dendritic cells (APC) secrete cytokines and present L1 VLP antigens, optimizing the adaptive response, involving both B and T cells and causing the development of a clone of antigen-specific memory cells [28-30].
The potential of the AS04 adjuvant was previously evaluated for the development of an anti-HBV vaccine [31, 32] and then integrated in the anti-HPV16 and 18 vaccine. This new adjuvant was also assessed with respect to the immune response induced with HPV16/18 L1 VLPs in formulations containing only aluminium hydroxide. During a follow-up lasting up to 4 years, the AS04 adjuvant system was shown to induce an antibody response to HPV16/18 L1 VLPs in humans that was significantly higher and more persistent with respect to aluminium salts. In particular, these high antibody levels after the use of vaccine containing AS04 were confirmed by means of various methods, such as ELISA (to determine total Ab), pseudo-neutralization tests (for specific assessment of neutralizing Ab) and inhibition ELISA (to determine just epitope-specific Ab) [33]. The same study also showed that one month after the third dose the frequency of the B memory cells specific for HPV16 L1 VLPs in the vaccinated subjects was significantly higher than the one obtained with aluminium salts. A similar, albeit not significant trend was seen for HPV18 L1 VLPs.
The first efficacy study was performed by means of a double blind, randomized and controlled clinical trial in healthy women enrolled in Brazil, Canada and the USA [34]. The women were aged 15-25 years, had not had more than 6 sexual partners and did not have a positive history of abnormal PAP tests. All these women were also cytologically negative, seronegative for anti-HPV16 and anti-HPV18 antibodies (in ELISA) and HPV-DNA-negative at PCR for 14 types of high-risk HPV (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). According to randomization, one group of women was vaccinated with three doses of bivalent vaccine (at 0, 1 and 6 months) while the second group received placebo (500 µg of aluminium hydroxide/dose).
The study consisted of two stages: an initial stage with a post-vaccine follow-up of up to 18 months and a second stage with an extended blind follow-up of up to 27 months.
The primary objective of the study was evaluation of the vaccine's efficacy in preventing HPV16 and HPV18 infection at 6 and 18 months. The secondary end-points included: evaluation of the vaccine's efficacy in preventing persistent HPV16/18 infection and cytologically confirmed low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and CIN1, CIN2/3 lesions and HPV16/18-associated carcinoma (squamocellular or adenocarcinoma) between months 6 and 18 and months 6 and 27. A total of 1,113 women were enrolled and randomized (560

received vaccine and 553 placebo). Compliance with the treatment was excellent (85.7% in the vaccine group and 86.4% in the placebo group) and the drop-out rate was similar in the two groups.
As far as efficacy against incident infection is concerned, the vaccine was 100% effective against HPV16 and 91.6% effective against HPV16/18 in the ATP (according to protocol) group. With regard to persistent infection, efficacy was 100% (for HPV16 and HPV16/18) in the ATP group; for HPV18 statistical significance was reached in the ITT (intention-to-treat) cohort (100%).
The efficacy in preventing abnormal cytology associated with HPV16/18 infection was 95.2%, 91.2% and 92.9% respectively for HPV16, HPV18 and HPV16/18 in the ITT cohort.
In the ATP group, the percentage of seroconversion after 3 doses (month 7) was 100% and 99.7% respectively for HPV16 and HPV18. At month 18 all the immunized women had seroconverted for both HPV16 and HPV18. The level of antibodies (expressed as geometric mean titre, GMT) was significantly higher at month 7 in the immunized women compared to the placebo group. In particular, the GMTs the vaccinated women were over 80-100 times higher than the antibody level detected in subjects with previous HPV16 and HPV18 natural infection. In vaccinated women the antibody levels were still high at month 18, being 10 to 16-fold higher than observed after natural infection.
As far as safety and tolerability are concerned, no serious adverse events were reported in either the vaccine or the placebo group. Local adverse reactions at the site of the vaccination were reported more frequently in the vaccinated women than in the placebo group. The local adverse reactions were, however, mild and short-lasting and did not reduce compliance to the treatment.
An extended follow-up study was also carried out in women enrolled in this trial and who had received three doses of vaccine or placebo and for whom double blind conditions continued (extended follow-up, multicentre, double blind, randomized and controlled study) [35]. The primary and secondary end-points were identical to the basic study. The extended follow-up, up to 4.5 years, included 776 women. More than 98% of the vaccinated women were persistently seropositive for anti-HPV16 and HPV18 antibodies throughout the follow-up. The humoral response peaked a month after the third dose; the peak was followed by a slight decline of the GMT, reaching a plateau from month 18 onwards. At the end of the extended follow-up (months 51-53) the antibody level in the vaccinated women was around 17 and 14 times higher, for HPV16 and HPV18 respectively, than post-infection levels detected in women with a previous natural infection. In the ATP group, the efficacy of the vaccine against incident infection was 95.8%, 100% and 96.9%, respectively for HPV16, HPV18 and HPV16/18. As regards persistent infection, the efficacy at 12 months was 100% against HPV16, HPV18 and HPV16/18.
A combined analysis of the initial and follow-up stages showed the bivalent vaccine to be highly effective against HPV16/18-associated cytological abnormalities (ASCUS, LSIL) and 100% effective against HPV16/18-associated histological abnormalities (CIN1 and CIN2) (no HPV18-associated lesions were detected). The vaccine was also found to be considerably effective with respect to the cytological and histological outcomes associated with any other high-risk HPV infection and regardless of the HPV DNA status. Efficacy against HPV45 and HPV31 incident infections was also respectively found to be 94.2% and 54.5%. The level of tolerability and safety of the vaccine was excellent in this study too.
The women included in the extension of the phase II trial continued their follow-up and the data at 6.4 years are now available. In particular, after that period the antibody levels reached after vaccination were still at least 11-fold higher than levels observed after natural infection. This was observed by determining the antibody titre by means of both the ELISA test and the pseudo-neutralization test. There is evidence that the ELISA test used in these trials to detect the anti-HPV16 and -HPV18 antibodies had, in all the age groups examined, a high degree of correlation with the levels detected with the pseudo-neutralization test, considered to be extremely reliable; this makes it possible to declare the ELISA test an excellent surrogate for evaluating the response induced by the L1 VLPs [36].
It should be pointed out that an additional extension of the follow-up period is foreseen for the phase II trial, taking it up to 9.5 years post-vaccination.
While the phase II extended trial was reaching its final stages, a phase III multicentre clinical trial began. This study, named PATRICIA, involved a very large population (more than 18,000 women), aged between 15 and 25 years and with less than 6 sexual partners. The criteria for exclusion from the trial were limited to women with a positive history of colposcopy, women who were pregnant or breast-feeding at the time of the study, who had chronic or autoimmune diseases or were immunodeficient. Unlike the previous studies, at the time of enrolment some of the women were seronegative and DNA negative, seropositive and DNA negative, seronegative and DNA positive and seropositive and DNA positive for HPV16 or HPV18. This protocol made it possible to carry out a study that examined a population that was more similar to the general population, compared to the naïve population previously studied [37].
A total of 18,644 women were enrolled, 9,319 of whom were vaccinated with bivalent vaccine and 9,325 with HAV vaccine (control group). The primary end-point of the study was evaluation of the vaccine's efficacy against CIN2+ associated with HPV16/18 in women who were initially seronegative and DNA negative for HPV 16 and 18. Secondary end-points were evaluation of the vaccine's efficacy against CIN1 and CIN2+ associated with HPV16/18, against persistent infection (6 and 12 months) for HPV16/18 or for other oncogenic HPV types (6 months), and of the immunogenicity and safety of the vaccine.
The protocol included a predefined intermediate evaluation on reaching at least 23 cases of CIN2+ associated

with HPV16/18 DNA in the total vaccinated cohort (bivalent vaccine and control group) for efficacy. On the basis of these criteria, the efficacy of the vaccine against CIN2+ was 90.4%; efficacy against CIN1+ was 89.2%. However, additional analyses made it possible to demonstrate that in the two cases detected in the vaccinated group and in the case of the control group it was not possible to identify HPV 16 or HPV18 in any previous cervical cytology sample, while other types of non-vaccine HPV had been identified in all the histological sections of the CIN2+ cases and in the previous cytological samples. Based on these findings, a causal role of HPV 16 or HPV18 in the onset of the detected lesions was therefore excluded and the efficacy of the vaccine against CIN2+ for HPV16, HPV18 and HPV16/18 became consequently equal to 100%.
As far as cross-protection is concerned, this was statistically significant against persistent infection at 6 months for HPV45 (59.9%) and HPV31 (36.1%), considered individually; a significant level of protection against persistent infection at 12 months was also detected for another 12 oncogenic HPV types considered, excluding HPV 16 and HPV18 (27.1%).
A total of 99.5% of the women who were seronegative at enrolment seroconverted both for HPV16 and HPV18 after the second and third dose. At month 7 the antibody titres reached in the vaccinated group were significantly and consistently higher with respect to the levels detected after natural infection.
As regards safety and tolerability, the data that emerged during the phase II trials were confirmed; mild and short-lasting local adverse events at the inoculation site were recorded more frequently in the vaccinated group with respect to the control group.
The percentage of serious adverse events was marginal (0.1%), both in the vaccinated women and in the control group. There were no significant differences in pregnancy outcomes in the overall study population (bivalent vaccine and control group).
Another clinical trial evaluated the possibility of immunising girls aged 10-14 years. In order to carry out this comparison, a total of 773 subjects were enrolled: 458 aged 15-25 years and 158 aged 10-14 years. All subjects were immunised with three doses of bivalent vaccine at 0, 1 and 6 months [38]. One month after the third dose, the seroconversion rate was 100% for both antigens in all subjects. The immunogenicity in the 10-14-year-old girls was not lower than the level found in the 15 to 25-year-olds in terms of seroconversion. As regards the antibody titres reached, the GMTs in the girls aged 10-14 years were substantially higher than those observed in the women aged 15-25 years and in the women enrolled in the first phase II efficacy study.
The safety profile was similar in the two groups of subjects.
A study to evaluate the immunogenicity and safety of the bivalent vaccine in women aged between 26 and 55 years is currently underway. The multicentre open study consists of two stages; the first with a follow-up at 1 year after the first dose and the second with a follow-up extended up to 48 months. The results relative to the follow-up at 24 months have already been published [39].
The objective of the study was to demonstrate that the seroconversion rates detected in women aged 26-45 and 46-55 years are not lower than those in women aged 15-25 years. The inclusion criteria consisted of a negative pregnancy test and the use of appropriate contraceptive measures. A total of 667 women were enrolled in the first stage of the study; it is worthy of note that at month 24, 531 of these women were included in the extended follow-up period. Over 70% of the women evaluated in the ATP cohort were seronegative for HPV16 and HPV18 at time 0. All the seronegative women, in both age groups, seroconverted for HPV16 and HPV18 one month after the third dose. The finding that the seroconversion rates were not lower in the women aged 26-45 and 46-55 years than in those aged 15-25 years was confirmed; the seroconversion rate was 100% in all the women. At month 24 all the women were still positive for anti-HPV16 and HPV18, regardless of age group.
The antibody response peak in terms of GMT was observed at month 7 and the antibody kinetics were similar to what was observed in the phase II and III trials with a slight decrease at month 18, then reaching a plateau at month 24, in line with what was observed in women aged 15-25 years and in any case significantly higher than the antibody titres induced by natural infection.
At month 24, the correlation between the serum antibody level and the level found in the cervicovaginal secretions was evaluated in a total of 250 women. The correlation coefficients were high, regardless of the age group considered. This finding merits careful evaluation since it is believed that the main mechanism of protection against HPV infections is mediated by the neutralizing antibodies, available at cervicovaginal level. The fact that, thanks to the action of the AS04 adjuvant, the bivalent vaccine induces a high antibody response also means a high level of antibodies available at the cervicovaginal level, where they can act effectively against HPV infections [25].
In this study too, the bivalent vaccine proved to be safe and well tolerated in all the age groups considered.
A further aspect worthy of note was the evaluation of the immunogenicity and safety of the bivalent vaccine in male subjects. A total of 270 healthy boys was enrolled, aged between 10 and 18 years, and vaccinated with HPV vaccine (3 doses) or HBV vaccine (3 doses). The exclusion criteria were a positive history of HPV or HBV vaccination, immunosuppression therapy in the 6 months prior to the study or immunoglobulins or blood products in the 3 previous months, a clinical history of HBV infection, exposure to HBV in the 6 previous weeks and immunosuppression [40]. At month 2, 100% of the initially seronegative subjects presented antibodies against HPV16 and HPV18 and all subjects were seropositive 1 month after completing the vaccination cycle.
The immune response in the boys aged 10-18 years was similar to that found in women aged 15-25 years. Local reactions, pain and swelling in particular, were more frequent in the subjects immunised with the bivalent

vaccine than in the control group, without however reducing compliance with the treatment.
Starting from the results of the first study on the efficacy of the bivalent vaccine, now at 6.4 years of observation, the long-term persistence of the antibody response to HPV16 and HPV18 infection was very recently estimated [41].
These results were then analysed, applying 3 different statistical models (power-law and modified power-law) in order to obtain projections on the long-term duration of the antibody titres. It was seen that with all three models the levels of the antibodies against HPV16 and HPV18 remain significantly higher than those detected after natural infections for at least 20 years and, in the "most optimistic" model, for the entire lifespan. It is important to stress that these results were achieved without resorting to any recall dose which, in the prospect of a public health initiative, would have a negative impact in terms of organisation and costs.
Since a crucial point to be considered for the use of a vaccine is represented by its safety and tolerability, a group of researchers evaluated 11 different studies carried out using the bivalent vaccine [42]. The analysis collected the data on safety from a cohort of around 30,000 girls and women aged > 10 years, 16,142 of whom had received at least one dose of vaccine and 13,811 at least one of the three control doses (aluminium hydroxide or HAV vaccine). The data available therefore related to over 45,900 doses; local and general adverse effects were recorded for 7 days after each dose. Any serious adverse events, pregnancies, significant medical conditions and signs of new cases of chronic-degenerative disease, including autoimmune diseases, were also carefully monitored. The data were analysed by dividing them into age groups (10-14, 15-25 and > 25 years) and time of reporting (0-7, 7-12 and > 12 months). The data reported in this study were therefore taken from a large database and refer to subjects for whom the vaccination schedule and safety monitoring system were the same. The considerable size of the database also made it possible to evaluate the appearance of any rare adverse events. The bivalent vaccine demonstrated a favourable safety profile, similar to that of other licensed and commonly used vaccines. In particular, compliance with vaccination was high at all ages, the number of women who abandoned the trials due to adverse events or serious adverse events was low and not significantly different to the number recorded in the controls. The incidence of symptoms not specifically monitored was similar for all age groups in both the vaccinated and the control groups; there was no report of an increased risk of onset of chronic-degenerative diseases or of autoimmune diseases. No difference was detected in pregnancy outcomes between vaccinated and control subjects in any age group.
These data therefore demonstrate the favourable safety profile of the bivalent vaccine in women of all ages, confirming what was previously found by Verstraeten et al. [43], who evaluated the safety of vaccines containing the AS04 adjuvant system, with particular attention to adverse events with a potential autoimmune etiology.

The quadrivalent vaccine

The quadrivalent vaccine (Gardasil) was developed by Merck/Sanofi Pasteur MSD with the aim of acting against HPV16, HPV18, HPV6 and HPV11 and thus against 70% of all CIN2/3 infections and cases of cervical cancer, 90% of all genital warts and around 35-50% of all cases of CIN1. The vaccine schedule consists of 3 doses to be administered at 0, 2 and 6 months. Each dose contains 40 µg of HPV16 L1 VLP, 20 µg of HPV18 L1 VLP, 20 µg of HPV6 L1 VLP and 40 µg of HPV11 L1 VLP adsorbed on adjuvant amorphous aluminium hydroxyphosphate sulfate (AAHS) (225 micrograms of Al). The L1 VLPs are produced by means of recombinant DNA technology in *S. cerevisiae*.
As far as the adjuvant is concerned, aluminium salts have been historically used to potentiate the immune response in many vaccines [44]. With respect to adjuvants consisting of aluminium phosphate ($ALPO_4$) and aluminium hydroxide (ALOH), the amorphous aluminium hydroxyphosphate sulphate used in the quadrivalent vaccine makes it possible to optimize the quality and quantity of the immune response to the L1 VLPs. This was confirmed during animal model (mouse) experiments, obtaining significantly higher antibody levels with respect to other aluminium salts; in particular, it is believed that AAHS allows a better uptake of L1 VLPs by the immune system than aluminium hydroxide [45].
The development of the quadrivalent vaccine has followed the evaluation of the results obtained in the phase II trial with a monovalent prototype vaccine containing 40 µg of HPV16 L1 VLP [46]. This was a randomised, double-blind clinical trial, administering three doses of vaccine or placebo in non-pregnant women aged 16-23 years, with a negative history for previous abnormal PAP tests and with not more than 5 sexual partners during their lifetime.
The primary end-point was the evaluation of the efficacy against persistent HPV16 infection and the analysis involved women who were seronegative and HPV DNA negative (for HPV16) at the time of enrolment and HPV DNA negative at month 7.
Of the 2,392 women enrolled in the study, 1,194 received the vaccine and 1,198 received placebo (containing 225 µg of adjuvant). The mean follow-up period was 17.4 months and the efficacy against persistent HPV16 infection was 100%. At month 7, the geometric mean titre (GMT) in the vaccinated group was higher than in the women who were seropositive at the time of enrolment (respectively 1,510 and 337 mMU/ml in competitive RIA). The vaccine was also well tolerated and no differences were observed in the incidence of adverse events between the vaccinated subjects and the placebo group.
A phase II trial was subsequently carried out to define which of the three prepared formulations was better

in terms of safety, immunogenicity and efficacy [47]. The randomised, double-blind multicentre study used three different formulations of HPV16, HPV18, HPV6 and HPV11 L1 VLPs while for the placebo 2 different doses of aluminium-based adjuvant (225 and 450 µg) were used. The different formulations of L1 VLPs were derived from the phase I trials previously carried out.
The study consisted of two stages: the first (stage A) evaluated the dose and was not completely blind. The second stage (B) was completely blind and the aim was to evaluate safety, efficacy and immunogenicity in relation to the dosage of L1 VLPs used. In stage B, 1,106 healthy women were enrolled, aged 16-23 years, not pregnant, with a negative history for abnormal PAP tests and with less than 4 sexual partners during their lifetime. The study did not exclude the possibility of enrolling anti-HPV seropositive and/or HPV DNA positive women. The vaccine was administered at 0, 2 and 6 months. An intermediate analysis of the data was carried out when around 50% of the post-dose 3 responses were available, with the aim of selecting the quadrivalent vaccine dose to be used in the phase III studies.
At the time of the intermediate analysis, the anti-HPV GMT in the three immunised groups for each of the four vaccine components was higher (from 27 to 145 times) than the levels observed in the women treated with placebo and seropositive at enrolment. At month 7, the GMT reached for each of the 4 vaccine components was similar for the three formulations and 100% of the vaccinated women were seropositive. Determination of the antibodies was carried out both by means of a competitive RIA method and a competitive Luminex Immunoassay (cLIA) which measures the neutralizing antibodies against specific conformational epitopes of the different HPV types (H6.M48 for HPV6, K11.B2 for HPV11, H16.V5 for HPV16 and H18.J4 for HPV18). On the basis of these data, the formulation with the lowest content of L1 VLPs (40 µg of HPV16 L1 VLP, 20 µg of HPV18 L1 VLP, 20 µg of HPV6 L1 VLP and 40 µg of HPV11 L1 VLP) was chosen for use in the phase III trials.
Considering the dose chosen for the phase III trials, the antibody response was evaluated both in the randomised HPV-naïve women and in the women who were seropositive and DNA negative at enrolment [48].
In general, the maximum antibody peak was reached one month after completing the vaccination cycle; this was followed by a decline in the antibody level until month 18 when a plateau was reached, remaining stable for at least 2.5 years after administration of the third dose.
Administration of the vaccine induced higher antibody levels in the seropositive women compared to those who were HPV-naïve at enrolment; this suggests the induction of a booster response in the seropositive women.
Evaluation of the long-term immunogenicity (month 36) demonstrated that the antibody levels induced with the vaccination against HPV18, HPV6 and HPV11 returned to the levels observed after natural infection; the anti-HPV16 antibodies, on the other hand, remained around 16 times higher compared to the levels observed after natural infection.
Evaluation of the long-term immunogenicity also involved establishing the number of subjects who seroconverted during the follow-up period. At month 7, 100% of the HPV-naïve vaccinated women were seropositive for all 4 vaccination components; at month 18 and at month 36 the seropositivity levels were 98%, 98%, 100% and 86%, and 94%, 96%, 100% and 76% respectively for anti- HPV6, -HPV11, -HPV16 and -HPV18 antibodies.
The vaccine proved to be safe and well tolerated. The number of subjects who reported any adverse event was slightly higher in the vaccinated group compared to the control group. The adverse events most frequently associated with the vaccine were headache and fever; local adverse events (redness, pain and swelling) were also more frequently recorded in the vaccinated women than in the control group.
Evaluation of the immunogenicity carried out during the phase II study reported above up to month 36 was subsequently extended up to month 60, involving 241 women and including determination in the vaccinated group of the antibodies against the vaccination components by means of the cLIA test [49]. A number of the immunised women were found to be seronegative for one or more of the HPV vaccinal strains at month 60, confirming the downward trend of the antibody titre shown in the previous stage of the study. All the women included in the extended follow-up period were immunised with an additional dose of vaccine at month 60 and the antibody response was evaluated one month later [50]. This dose induced an anamnestic response, making it possible to reach high antibody levels, in some cases higher than the levels observed at month 7 after the administration of dose 3. This anamnestic response was also observed in women who were seropositive and PCR negative at enrolment, and then immunised with three doses of the vaccine.
During the phase II study, the characteristics of which have already been described, the vaccine efficacy was also evaluated [49]. A first assessment was performed during the follow-up period at 36 months, showing vaccine efficacy against the incidence of HPV6, HPV11, HPV16 and HPV18 infection or cervical or external genital disease (persistent infection, detection of HPV at the last examination, CIN, carcinoma, external genital lesions sustained by HPV types present in the vaccine). Overall efficacy was 90%.
The extended follow-up in the women enrolled in this study demonstrated 95.8% efficacy against infection or disease in the ATP cohort (which included both the women initially enrolled for the study with a 3-year follow-up and those included in the extended 5-year follow-up) and 95.1% efficacy in the ATP cohort limited to the women in the extended follow-up period.
In the MITT (modified intention to treat) cohort, which included subjects who were HPV-naïve for HPV6, HPV11, HPV16 and HPV18 treated with at least one dose, regardless of any protocol infringements, efficacy was 93.7%.
In 2006, the results were published of a study aimed at determining whether the antibody response induced by

L1 VLPs in subjects aged 10-15 years was comparable with what had been observed in women aged 16-23 years. This was therefore a bridging immunogenicity study, its rationale being based on the legal and ethical impossibility of evaluating the clinical efficacy of the vaccine in preadolescents and adolescents [51]. The multicentre study involved the enrolment of 3 groups of subjects: males aged 10-15 years, females aged 10-15 years and females aged 16-23 years. The first two groups had to be healthy subjects who had not started sexual activity and did not do so during the study; the older subjects had to have a negative history for abnormal PAP tests, genital warts or CIN and not have had more than 4 sexual partners. A negative pregnancy test was required for all the women/girls. The three doses of vaccine were administered at 0, 2 and 6 months. Immunogenicity was evaluated by means of cLIA.

A total of 1,529 subjects were enrolled, 94.8% of whom completed the immunisation protocol. Evaluation of the anti-HPV GMTs at month 7 in the HPV-naïve subjects showed that the results obtained in the 10-15 year-olds were not lower with respect to the levels observed in the 16-23 year-olds. The GMTs observed in the males and the younger females after dose 3 were consistently higher than in the women aged 16-23 years. More than 99% of the enrolled subjects had seroconverted at month 7. The vaccination was also safe and well tolerated in all the groups of subjects included in the study.

From June 2002 to May 2003 a phase III, multicentre, double-blind, randomised, controlled study (FUTURE II) was performed in women aged 16-26 years. The women were eligible for the study if they were not pregnant, had a negative history for abnormal PAP tests and had not had more than 6 sexual partners. The women were treated with vaccine (3 doses at 0, 2 and 6 months) or with placebo (containing aluminium). A total of 12,167 women were enrolled, 6,087 of whom received the vaccine. The results were published in two separate articles in 2007.

In the first publication, the primary end-point was evaluation of the efficacy of the vaccine against high-grade CIN for HPV16 and HPV18 [52]. The vaccinated women were followed for a mean period of three years after administration of the first dose of vaccine or placebo. The intermediate evaluation of the study was based on identification of at least 19 cases of high-grade CIN while the final analysis required identification of at least 29 cases. In the per protocol cohort (women serologically HPV16 and HPV18 DNA negative at enrolment and HPV16 and HPV18 DNA negative up to month 7) vaccine efficacy was 98% against high-grade HPV16 or HPV18 lesions (CIN2-3). The only vaccinated subject presenting a case of HPV16 CIN3 was positive for HPV52 both at enrolment and in another 5 histological samples taken during the follow-up period; HPV16 DNA was, on the other hand, detected only in one histological sample. In the unrestricted cohort, which included seronegative women with negative PCR at enrolment, vaccine efficacy for the same end-points was 95%. Evaluation of vaccine efficacy against high-grade HPV16 or HPV18-related lesions in a population that included women with and without infection or CIN at enrolment and treated with at least 1 dose was performed in the intention-to-treat cohort. In this case, efficacy against high-grade HPV16 and HPV18-related lesions was 44%. Considering the appearance of any HPV type high-grade lesions, vaccine efficacy was 17%.

In the same study, a group of 1,512 women were included in an immunogenicity evaluation; at month 24 the seropositive rate for neutralizing antibodies in the protocol cohort was 96%, 97%, 99% and 68% respectively for HPV6, HPV11, HPV16 and HPV18.

The vaccine presented an excellent safety and tolerability profile; no significant differences were observed in the outcome of pregnancies occurring in the vaccinated women or in those treated with placebo.

The second published article reported the data relative to two end-points: reduction with respect to the placebo group of the incidence of anogenital warts, grade 1-3 vulvar or vaginal lesions or neoplasia associated with any type of HPV and reduction with respect to the placebo group of the combined incidence of CIN1-3, adenocarcinoma in situ or carcinoma associated with the HPV types included in the vaccine [53]. Identification of 38 cases of anogenital or vaginal lesions and at least 38 cases of cervical lesions associated with the HPV types included in the vaccine were required to ensure the power of the study. An intermediate analysis was performed when 38 cases of anogenital and vaginal lesions and 37 cases of cervical lesions had occurred at around 1.5 years after administration of the third dose. Evaluation of the efficacy was carried out considering the per protocol, unrestricted and intention-to-treat cohorts described above.

A total of 2,723 women were vaccinated and 2,732 received placebo; the mean follow-up period was 3 years after administration of dose 1.

In the per protocol cohort, immunisation proved 100% effective in preventing vaginal, vulval, perineal or perianal lesions or warts in association with vaccine-type HPV; efficacy against CIN1-3 lesions caused by HPV types included in the vaccine was also 100%.

In the unrestricted cohort, the combined efficacy against any type of external anogenital or vaginal lesions and against cervical lesions of any grade was, respectively, 95% and 98%.

Finally, in the intention-to-treat group, efficacy against any external anogenital or vaginal lesions and against cervical lesions of any grade was 73% and 55%.

A second analysis of the intention-to-treat cohort was performed to evaluate the vaccine efficacy regardless of the type of HPV involved (whether included in the vaccine or not); efficacy against the incidence of external anogenital or vaginal lesions and against cervical lesions was, respectively, 34% and 20%.

At least 99.5% of the women included in the per protocol cohort had seroconverted at month 7. The safety and tolerability profile was confirmed as satisfactory. Local adverse events at the inoculation site and fever were

reported more frequently among the vaccinated women than in the control groups (87% vs 77% and 13.3% vs 10.3%).

In 2007, two studies were published, one on the combined analysis of 4 clinical trials on the efficacy of the quadrivalent vaccine against CIN2-3 lesions and adenocarcinoma in situ and the other on the combined analysis of 3 clinical studies on the vaccine efficacy against high-grade vulval and vaginal lesions.

The first combined analysis evaluated over 20,000 women aged 16-26 years, randomised to receive the quadrivalent vaccine, the monovalent HPV16 vaccine or placebo [54]. The primary end-point was evaluation of the combined efficacy against CIN2-3, AIS or cervical cancer caused by HPV16/18. After a mean follow-up period of three years after the first dose, vaccine efficacy in HPV-naïve women was 99%; in the intention-to-treat cohort it was 44%. A second intention-to-treat analysis showed an 18% reduction of CIN2-3 or AIS regardless of the type of HPV considered.

The second combined analysis involved over 18,000 women (16-26 years) randomised to receive quadrivalent vaccine or placebo [55]. The primary end-point was evaluation of the combined efficacy for VIN2-3 or VaIN2-3 caused by HPV16 or HPV18.

After three administrations and a mean follow-up period of 3 years, efficacy against VIN2-3 and VaIN2-3 was 100% in the HPV16 and HPV18 naïve women (per protocol cohort) and 71% in the intention-to-treat cohort; efficacy against VIN2-3 and VaIN2-3, regardless of the type of HPV considered, was found to be 49%. The quadrivalent vaccine was found to be safe and effective in this study too.

A clinical trial carried out within the phase III study described above evaluated the safety and immunogenicity of co-administered quadrivalent vaccine and HBV vaccine [56]. The trial involved 1,877 women, aged 16-23 years, and demonstrated that co-administration induced high GMT levels against the various HPV vaccine components and a seroconversion rate (> 99%) that was not lower than could be achieved by administering the quadrivalent vaccine alone. As far as the HBV vaccination was concerned, high antibody titres were observed at month 7, albeit lower on the whole than those achieved with the HBV vaccination alone. The co-administration was generally well tolerated.

Two reviews were very recently published on the impact of the quadrivalent vaccine on infection and disease associated with oncogenic non-vaccine HPV types in HPV-naïve women aged 16-26 years.

The first review refers to 17,622 enrolled women with a mean follow-up of 3.6 years [57]; the primary end-point was verification of the prevention of the combined incidence of HPV31, HPV45 and HPV-31/33/45/52/58-related infections and disease. Other end-points were verification of the prevention of the combined incidence of infections and disease related to non-vaccine HPV types (HPV31, 33, 35, 52, 58, 39, 45, 59, 51, 56). The analysis was carried out in women who had received > 1 dose and who were seronegative and HPV DNA negative for the HPV types included in the vaccine, DNA negative for each of the 10 non-vaccine HPVs indicated above and who had a normal PAP test at enrolment.

Efficacy (evaluated in 2,068 women) was 40.3% and 25% respectively against the incidence of HPV31/45 and HPV-31/33/45/52/58 infection; the analysis performed to evaluate efficacy against each individual type of HPV investigated was statistically significant only for HPV31 (46.2%).

The analysis of 9,296 subjects to evaluate the efficacy against cervical disease showed values of 23.4% and 32.5%, respectively, against CIN1-3/AIS and for high-grade lesions (CIN2-3/AIS) against 10 non-vaccine HPV types. The efficacy against the incidence of CIN1-3/AIS caused by HPV31/45 was 43.6% while the efficacy against the incidence of CIN1-3/AIS correlated with HPV-31/33/45/52/58 was 29.2%. In general, a significant level of efficacy against infection and disease caused by HPV45 was not demonstrated.

The second review reports the data on the evaluation of quadrivalent vaccine efficacy on the incidence of persistent infection (> 6 months) or on cervical disease due to HPV31 and HPV45 and to HPV31/33/45/52/58 in an intention-to-treat cohort [58]. Other end-points included evaluation of the efficacy against persistent infection (> 6 months) or disease associated with species A9 (HPV31, 33, 52, 58) and A7 HPV types (39, 45, 59) and with all non-vaccine HPV types that can be tested (HPV31, 33, 35, 39, 45, 51, 52, 58, 59). The women (16-26 years and enrolled for the phase III studies) were included in the analysis regardless of the presence of HPV infection or HPV-correlated disease at enrolment; the mean follow-up was 3.6 years after administration of dose 1.

The quadrivalent vaccine reduced the incidence of persistent HPV31/45 infection by 31.6% and of HPV31/33/45/52/58 infection by 17.7%. A significant level of efficacy against the incidence of persistent infection was observed for HPV31 (33.6%) and HPV59 (24.6%).

With regard to efficacy against HPV-related disease, the quadrivalent vaccine reduced the incidence of HPV31/45-related CIN1-3/AIS by 22.2% and of HPV31/33/45/52/58-related CIN1-3/AIS by 18.8%. The combined efficacy for the 10 non-vaccine HPV types against CIN1-3/AIS was 15.1%; a significant level of efficacy was observed against HPV31, HPV58 and HPV59-related CIN1-3/AIS (26%, 28.1%, 37.6% respectively).

The ECDC Newsletters on vaccines and immunization recently published a comment on these last two studies pointing out: "In summary, the potential of cross-protection was very small if all women were included regardless of status of infection or disease at enrolment [58]. The modest reduction of CIN2 lesions or worse was not statistically significant. As expected, the efficacy of the vaccine against HPV infection and HPV related disease was much higher in the group of women that were HPV negative at study entry, and the predominant non-

vaccine type was HPV31. Both here-presented studies used the study population recruited for the Future I and II studies, which were designed to estimate the impact of the quadrivalent vaccine on CIN1-3 and AIS associated with the HPV types included in the vaccine. Around 70% of cervical cancer cases as well as the majority of cervical high-grade lesions are associated with HPV types 16 and 18. Therefore both studies were probably underpowered to estimate the vaccine impact on disease associated with nonvaccine HPV types, and the results have to be interpreted carefully. Even if both studies may contribute to the answer, the question of possible cross-protection remains unsolved, and should be looked at further as it plays an important role in estimating the effectiveness and impact of available measures including vaccination for the prevention of cervical cancer …" [59].

As far as evaluation of long-term immunogenicity is concerned, the application of two different mathematical models using the antibody levels detected two years after the administration of the monovalent vaccine containing HPV16 L1 VLPs produced different indications [60]. The conventional mathematical model indicates that the anti-HPV16 antibodies decrease in < 20 years below the antibody level reached after natural infection. The modified model, which presumes a long-term immune memory, indicates instead that the anti-HPV16 antibodies are persistently high, above the level detected after natural infection.

As far as safety and tolerability are concerned, the quadrivalent vaccine was well tolerated with a favourable safety profile [61, 62]. The most frequent adverse events were fever, erythema, pain, swelling and itching at the inoculation site. Administration of the quadrivalent vaccine during pregnancy did not lead to any reports; nevertheless, the data currently available are not sufficient to recommend use of the vaccine during pregnancy. During the clinical studies, the subjects included in the safety analysis reported any new clinical condition that occurred during the follow-up.

According to the CDC the overall evaluation of more than 20 million doses passively monitored and more than 375,000 doses actively monitored demonstrated a number of reports that were coherent with the results of the clinical trials [62]. The data available do not support the causal association between the vaccine and Guillain Barré syndrome or venous thromboembolism nor any evidence of a high risk of syncope after administration of the vaccine, except in adolescents. The data available in the USA do not support the preliminary indications reported in Australia on some cases of demyelinising disease or post-vaccination anaphylaxis.

Concluding remarks

Evaluation of the data emerging from the numerous clinical trials carried out on the two HPV vaccines confirms the high preventive value of vaccination.

While not directly comparable, the data obtained with the two available vaccines indicate that the immune response can be effectively modulated in terms of quality and quantity as well as duration by using different adjuvants which make it possible to maximise the immune action.

We are therefore waiting for a direct comparison between the two products, based on homogeneous populations, with the same end-points and performed using unambiguous laboratory methods, in order to show the existence of "real" differences regarding the activity of the two vaccines currently available.

Despite considerable and confirmed clinical efficacy and established tolerability and safety, there are some aspects that still require further investigation:

- significance of the immune response and identification of a protective serological correlate;
- duration of the protection;
- cross-protection;
- the possibility of co-administration with other vaccines;
- update of the cost-efficacy profiles;
- long-term impact of the vaccination on the ecology of HPVs.

The WHO has very recently published a position paper on HPV vaccination, recommending that this vaccination be included in the immunisations programmes of countries in which the prevention of cervical cancer represents a priority in terms of public health. This intervention must be sustainable from an organisational and economic point of view, and in-depth cost-efficacy studies are therefore necessary. Since the HPV vaccines currently available are highly effective in girls who are naïve for the vaccine HPV types, the primary target of immunization programmes should be girls aged between 9-10 and 13 years. The vaccination programmes should also be included in a coordinated strategy of health education (on behaviour at risk for HPV infection) and information on the need for integration with cervical cancer screening programmes [63].

Declaration of conflict of interest

G.G. has previously participated at speaker's bureau and advisory board meetings sponsored by GlaxoSmith-Kline Biologicals and Sanofi Pasteur MSD. G.G. has previously received research funding from GlaxoSmith-Kline Biologicals and Sanofi Pasteur MSD.

References

[1] De Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. *Classification of papillomaviruses*. Virology 2004;324:17-27.

[2] Munoz N, Castellsagué X, Berrington de Gonzalez A, Gissmann L. *HPV in the etiology of human cancer*. Vaccine 2006;24S3:1-10.

[3] Stanley M. *Immunobiology of HPV and HPV vaccines*. Gynecol Oncol 2008;109:S15-S21.

[4] Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. *The causal relation between papillomavirus and cervical cancer*. J Clin Pathol 2002;55:244-65.

[5] Parkin DM, Bray F. *The burden of HPV-related cancers*. Vaccine 2006;24S3:11-25.

[6] Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, et al. *Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia*. Vaccine 2008;26S:K1-16.

[7] Baseman JG, Koutsky LA. *The epidemiology of human papillomavirus infections*. J Clin Virol 2005;32S:S16-24.

[8] Burchell AN, Winer RL, de Sanjose S, Franco EL. *Epidemiology and transmission dynamics of genital HPV infection*. Vaccine 2006;24S3:S52-62.

[9] Einstein MH. *Acquired immune response to oncogenic human papillomavirus associated with prophylactic cervical cancer vaccines*. Camper Immunol Immunother 2008;57:443-51.

[10] ECDC. *Guidance for the introduction of HPV vaccines in EU countries*. http://ecdc.europa.eu/en/Latest_publications.aspx.

[11] Bosch FX, de Sanjosé S. *Human papillomavirus and cervical cancer – Burden and assessment of causality*. J Natl Cancer Inst Monographs n°31, 2003:1-13.

[12] Bosch FX, Castellsagué X, de Sanjosé S. *HPV and cervical cancer; screening or vaccination?* Br J Cancer 2008;98:15-21.

[13] Stanley M. *Immune responses to human papillomavirus*. Vaccine 2006;24S1:S16-22.

[14] Stanley M, Lowy DR, Frazer I. *Prophylactic HPV vaccines: underlying mechanisms*. Vaccine 2006;24S3:S106-13.

[15] Frazer I. *Correlatine immunity with protection for HPV infection*. Int J Infect Dis 2007;11S2:S10-6.

[16] Stanley M. *Human papillomavirus vaccines*. Rev Med Virol 2006;16:139-49.

[17] Stanley M. *Prevention strategies against the human papillomavirus: the effectiveness of vaccination*. Gynecol Oncol 2007;107:S19-23.

[18] Stanley M. *Prophylactic HPV vaccines*. J Clin Pathol 2007;60:961-5.

[19] Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA, et al. *Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas*. Proc Natl Acad Sci USA 1995;92:11553-7.

[20] Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, et al. *Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection*. J Virol 1995;69:3959-63.

[21] WHO. *Human papillomavirus and HPV vaccines: technical information for policy-makers and health professionals*. www.who.int/reproductive-health/publications/hpvvaccines_techinfo/.

[22] Ferguson M, Wilkinson DE, Zhou TQ. Meeting report. *WHO meeting on the standardization of HPV assays and the role of the WHO HPV Laboratory network in supporting vaccine introduction held on 24-25 January 2008, Geneva, Switzerland*. Vaccine 2009;27:337-47.

[23] Schijns VE, Degen WG. *Vaccine immunopotentiators of the future*. Clin Pharmacol Ther 2007;82:750-5.

[24] Stanley M, Gissmann L, Lardelli-Haefliger D. *Immunobiology of human papillomavirus infection and vaccination – Implications for second generation vaccines*. Vaccine 2008;26S:K62-7.

[25] Schwarz TF, Oberdan L. *Immune response to human papillomavirus after prophylactic vaccination with AS04-adjuvanted HPV-16/18 vaccine: improving upon nature*. Gynecol Oncol 2008;110:S1-10.

[26] Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. *Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis*. Am J Epidemiol 2008;168:123-37.

[27] Garcon N, Van Mechelen M, Wettendorff M. *Development and evaluation of AS04, a novel and improved immunological adjuvant system containing MPL and aluminium salt*. In: Schijns V, O'Hagan D, eds. *Immunopotentiators in modern vaccines*. London; Elsevier Academic Press 2006, pp. 161-77.

[28] De Becker G, Moulin V, Pajak B, Bruck C, Francotte M, Thiriart C, et al. *The adjuvant monophosphoryl lipid A increases the function of antigen-presenting cells*. Int Immunol 2000;12:807-15.

[29] Ismaili J, Rennesson J, Aksoy E, Vekemans J, Vincart B, Amraoui Z, et al. *Monophosphoryl lipid A activates both human dendritic cells and T cells*. J Immunol 2002;168:926-32.

[30] Martin M, Michalek SM, Katz J. *Role of innate factors in the adjuvant activity of monophosphoryl lipid A*. Infect Immun 2003;71:2498-507.

[31] Thoelen S, Van Damme P, Mathei C, Leroux-Roels G, Desombere I, Safari A, et al. *Safety and immunogenicity of a hepatitis B vaccine formulated with a novel adjuvant system*. Vaccine 1998;16:708-14.

[32] Boland G, Beran J, Lievens M, Sasadeusz J, Dentico P, Nothdurft H, et al. *Safety and immunogenicity profile of an experimental hepatitis B vaccine adjuvanted with AS04*. Vaccine 2004;23:316-20.

[33] Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F, et al. *Enhanced humoral and memory B cellular immunity using HPV16/128 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only*. Vaccine 2006;24:5937-49.

[34] Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. *Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial*. Lancet 2004;364:1757-65.

[35] 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.

[36] Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MPM, Poncelet SM, et al. *Correlation between direct ELISA, single epitome-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine*. Hum Vaccines 2008;4:425-34.

[37] Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron 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.

[38] Pedersen C, Petaja T, Strass G, Rumke HC, Poder A, Richardus JH, et al. *Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant*. J Adolesc Health 2007;40:564-71.

[39] Schwarz TF, Spaczynski M, Schenider A, Wysocki J, Galaj A, Perona P, et al. *Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical vaccine in women aged 15-55 years*. Vaccine 2009;27:581-7.

[40] Petaja T, Keranen H, Karppa T, Kawa A, Lantela S, Siitari-Mattila M, et al. *Immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in healthy boys aged 10-18 years*. J Adolesc Health 2009;44:33-40.

[41] David MP, Van Herck K, Hardt K, Tibaldi F, Dubin G, Descamps D, et al. *Long-term persistence of anti-HPV-16 and –18 antibodies induced by vaccination with the AS04-adjuvanted cervical cancer vaccine: modelling of sustained antibody responses*. Gynecol Oncol (2009), doi:10.1016/j.ygyno.2009.01.011.

[42] Descamps D, Hardt K, Spiessens B, Izurieta P, Verstraeten T, Breuer T, et al. *Safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for cervical cancer prevention: A pooled analysis of 11 clinical trials*. Hum Vaccines 2009;5:1-9.

[43] Verstraeten T, Descamps D, David MP, Zafah T, Hardt K, Izurieta P, et al. *Analysis of adverse events of potential autoimmune aetiology in a large integrated safety database of AS04 adjuvanted vaccines*. Vaccine 2008;26:6630-8.

[44] Lindblad EB. *Aluminium adjuvants – in retrospect and prospect*. Vaccine 2004;22:3658-68.

[45] Caulfield MJ, Shi L, Wang S, Wang B, Tobery TW, Mach H, et al. *Effect of alternative aluminium adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice*. Hum Vaccines 2007;3:139-46.

[46] 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.

[47] Villa LL, Costa RLR, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. *Prophylactic quadrivalent human papillomavirus (types 6,11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial*. Lancet Oncol 2005;6:271-8.

[48] Villa LL, Ault KA, Giuliano AR, Costa RLR, 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.

[49] Villa LL, Costa RLR, 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.

[50] Olsson SE, Villa LL, Costa RLR, Petta CA, Andrade RP, Malm C, et al. *Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine*. Vaccine 2007;25:4931-9.

[51] Block SL, Nolan TN, Sattler C, Barr E, Giacoletti KED, Marchant CD, et al. *Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6,11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women*. Pediatrics 2006;118:2135-45.

[52] The FUTURE II Study Group. *Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions*. N Engl J Med 2007;356:1915-27.

[53] Garland SE, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. *Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases*. N Engl J Med 2007;356:1928-43.

[54] The FUTURE II Study Group. *Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials*. Lancet 2007;369:1861-8.

[55] Joura EA, Leodolter S, Hernadez-Avila M, Wheeler CM, Perez G, Koutsky LA, et al. *Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials*. Lancet 2007;369:1693-702.

[56] Wheeler CM, Bautista OM, Tomassini JE, Nelson M, Sattler CA, Barr E, on behalf of the Protocol 11 study Investigator. *Safety and immunogenicity of co-administered quadrivalent human papillomavirus (HPV)-6/11/16/18 L1 virus-like particle (VLP) and hepatitis B (HBV) vaccines*. Vaccine 2009;26:686-96.

[57] Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernadez-Avila M, Wheeler CM, et al. *The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years*. JID 2009;199:926-35.

[58] Wheeler CM, Kjaer SK, Sigurdsson K, Iversen OE, Hernadez-Avila M, Perez G, et al. *The impact of quadrivalent human papillomavirus (HPV; types 6, 11,16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16-26 years*. JID 2009;199:936-44.

[59] ECDC. V&I News. Number 62 – 26 March 2009. ecdc.Europa.eu/en/Health_Topics/vi/VI_news.aspx

[60] Fraser C, Tomassini JE, Xi L, Golm G, Watson M, Giuliano AR, et al. *Modeling the long-term antibody response of a human papillomavirus (HPV) virus-like particle (VLP) type 16 prophylactic vaccine*. Vaccine 2007;25:4324-33.

[61] WHO. Global Advisory Committee on vaccine safety, 17-18 December 2008. Wkly Epidemiol Rec 2009;84:37-40.

[62] CDC. *Reports of health concerns following HPV vaccination*. www.cdc.gov/vaccinesafety/vares/gardasil.htm.

[63] WHO. *Human papillomavirus vaccines WHO position paper*. Wkly Epidemiol Rec 2009;84:118-31.

■ Received on March 3, 2009. Accepted on May 15, 2009.

■ Correspondence: G. Gabutti, Dept. of Clinical and Experimental Medicine, Section of Hygiene and Occupational Health, Univeristy of Ferrara, via Fossato di Mortara 64b, 44100 Ferrara, Italy - E-mail: giovanni.gabutti@unife.it