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PROPHYLACTIC HUMAN PAPILLOMA VIRUS VACCINES

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ABSTRACT

Human papillomaviruses are the primary etiologic agents of cervical cancer. Thus, cervical cancer and other Human papillomavirus associated malignancies might be prevented by Human papillomavirus vaccines. Currently, two Human papillomavirus L1 Virus -Like Particle vaccines namely Gardasil® - quadrivalent (Merck) and Cervarix™ - bivalent (GlaxoSmithKline) are widely marketed internationally. These HPV vaccines are commercially available. The advisable period of Human papillomavirus vaccination is before the onset of sexual activity. Human papillomavirus vaccines should not be given to people who have experienced severe allergic reactions after a previous vaccine dose or to a component of the vaccine. These vaccines are not recommended for use in pregnant females. The mechanisms by which these vaccines induce protections are not fully defined but involve both cellular immunity and neutralizing immunoglobulin G antibodies. This review brings up to date information on the two commercially available prophylactic Human papillomavirus vaccines.

KEYWORDS: Prophylactic Human papillomavirus vaccines, Virus-like particles vaccine preparation, Competitive radioimmunoassay, Pseudovirion-based neutralization assay, Human papillomavirus vaccines clinical trials in INDIA.

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INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide with an estimated 5,29,409 new cases and 2,74,883 deaths in 2008. About 86% of the cases occur in developing countries and may constitute up to 25% of all female cancers[1]. Worldwide, mortality rates of cervical cancer are substantially lower than its incidence with a ratio of mortality to incidence being 52%[2]. According to WHO: Human Papillomavirus and Related Cancers Summary Report (2010), in India, cervical cancer is reported to be responsible for almost 20 percent of all female deaths and takes the lives of 8 women in India every hour. India recorded 1,32,000 new cases out of these cases 74,000 cases lost their lives[3]. In the early 1980s, Dr. Harald zur Hausen established the link between genital human papillomavirus (HPV) infections and cervical cancer. After that a number of molecular and epidemiological studies demonstrated a strong co-relation between HPV infection and cervical cancer[4]. Now developing and developed countries need to consider whether and how to use these HPV vaccines. Therefore, critical analysis for comparative status of HPV vaccines is warranted.

HUMAN PAPILLOMAVIRUS VACCINES

The development of any human vaccine is highly challenging, complex and an expensive process. However, the development of cancer vaccine is the most challenging of all because these vaccines need to boost the immune system's natural ability to protect the body from foreign invaders like viruses which causes cancer. Cancer vaccines are considered to belong to "Biological response modifiers" class[5]. Therefore, to prevent HPV infection, researchers at pharmaceutical companies, biotechnology firms around the world are actively developing candidates for both prophylactic and therapeutic HPV vaccines. The commercial development of HPV vaccines began around 1993 and within 15 years of intense research, academic researchers proved the causative link between HPV and cervical cancer and figured out the basic natural history of HPV virus. In the initial phase, research efforts involved teams of up to 20 scientists who were working on cloning and assay development but later stages of this vaccine development program involved more than ten times as many scientists to bring the vaccines through manufacture and clinical trials which have involved more than 60,000 subjects[6, 7].

Vaccination aims to produce neutralizing antibodies which are capable of preventing infection by binding tightly to the surface of virus and physically prevent them from the docking into the host cell. Human papillomavirus capsids structural proteins are ideal target for such antibodies but genetic diversity and inability to culture HPV in vitro have made development of prophylactic HPV vaccines difficult. It has been observed that, when L1 and L2 proteins are expressed in vitro, they self-assemble into a structure identical to viral capsid known as "virus-like particle"(VLP)[8, 9]. The VLP induces a humoral immune response which is similar to a live virion but it does not produce infection in the recipient because it lacks viral nucleic acid (Refer Figure.1). However, some recent developments over the last few years have the potential to change the way we address the problem of cervical cancer prevention[10]. One such significant development is the availability of human papilloma virus (HPV) vaccines[11].

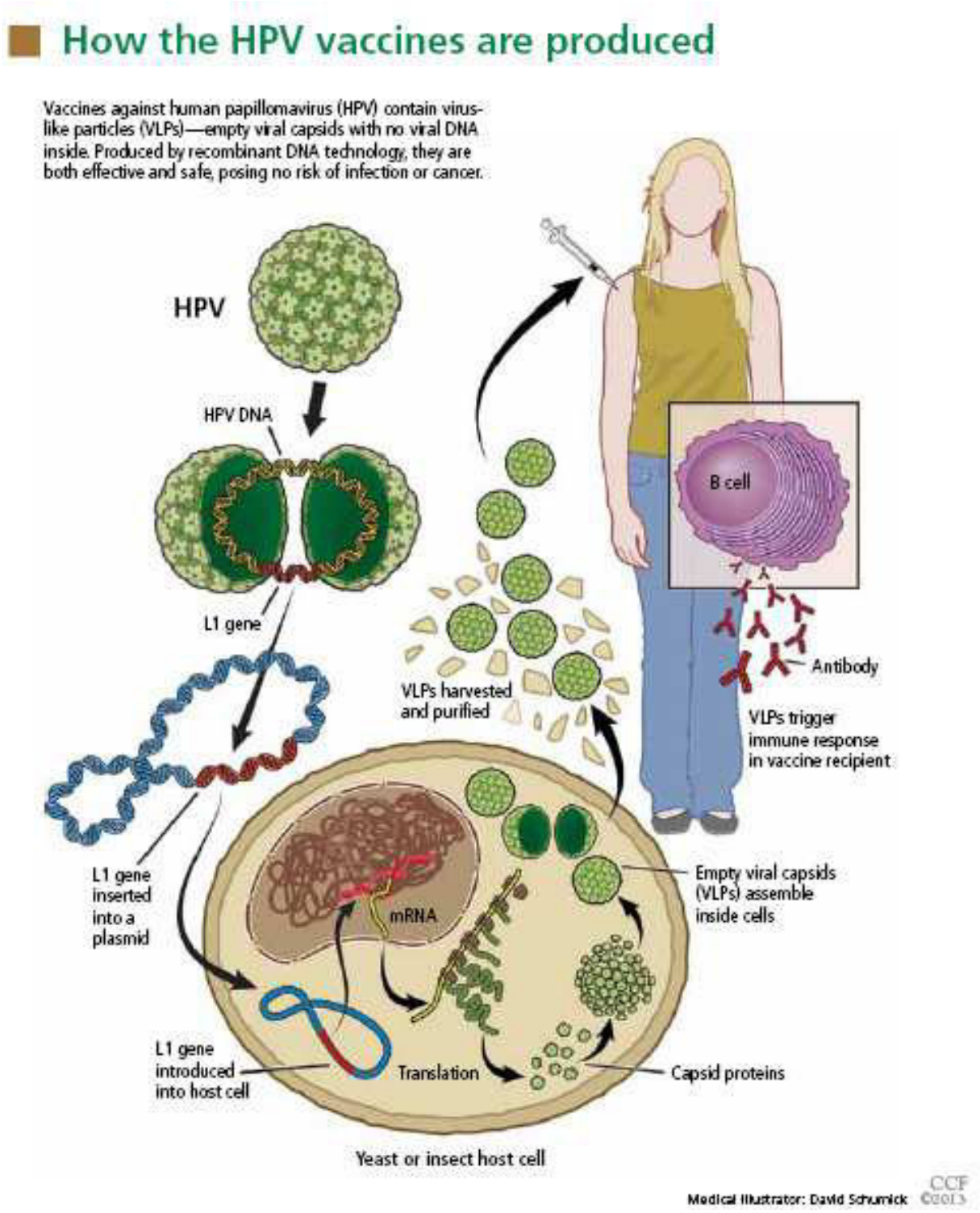

Figure1

Schematic representation of HPV vaccines produced[12] (Reprinted from Jin XW, Lipold L, Sikon A, Rome E. Human papillomavirus vaccine: Safe, effective, underused. Cleve Clin J Med 2013: 80:49-60. With permission from The Cleveland Clinic Foundation. © 2013 The Cleveland Clinic Foundation. All rights reserved).

Currently, two HPV L1 VLP vaccines namely Gardasil® - quadrivalent (Merck) and Cervarix™ - bivalent (GlaxoSmithKline) are widely marketed internationally and these HPV vaccines are commercially available. Both HPV vaccines markedly differ in their composition and their adjuvants. These HPV vaccines are formulated with adjuvant to enhance the desired immunogenicity to weak antigens. The quadrivalent vaccine was approved by the US Food and Drug Administration (FDA) in 2006 and the bivalent vaccine was approved in 2009[12]. Both the HPV vaccines have been designed for prophylactic use only. They cannot clear existing HPV infection or treat HPV related disease. However, the mechanisms by which these vaccines induce protections are not fully defined but involve both cellular immunity and neutralizing immunoglobulin G (IgG) antibodies[13].

Table I

Comparative characteristics of two commercial available prophylactic HPV vaccines.

Sr.	Parameter	HPV4	HPV2
1	Brand name	Gardasil®	Cervarix ™
2	Manufacturer	Merck & Co, Inc.	GlaxoSmithKline
3	Common Name	Human papillomavirus vaccine [Types 6, 11, 16, 18] (recombinant, adsorbed)	Human Papillomavirus vaccine Types 16 and 18 (Recombinant, AS04 adjuvanted
4	Vaccine composition (L1 protein)	20 µg HPV 6, 40 µg HPV 11, 40 µg HPV 16, 20 µg HPV 18	20 µg HPV 16, 20 µg HPV 18
5	Expression system	Saccharomyces cerevisiae expressing L1	Trichoplusia ni insect cell line infected with L1 encoding recombinant baculovirus
6	Adjuvant	AAHS 225 µg of aluminium hydroxyphosphate sulphate	AS04 50 µg of aluminium hydroxide
7	Preservatives	None	None
8	Other content	Sodium chloride, L-histidine, polysorbate 80, sodium borate and water for injection	Sodium chloride and sodium dihydrogen phosphate dehydrate, and water for injection
9	Volume per dose	0.5 mL	0.5 mL
10	Administration	Intramuscular in deltoid muscle of the upper arm or in the higher anterolateral area of the thigh	Intramuscular in deltoid muscle
11	Schedule / Intervals	Second and third doses 1 to 2 months and 6 months after first dose	Second and third doses 1 to 2 months and 6 months after first dose
12	Target Population	Females aged 9 through 26 years	Females aged 10 through 25 years
13	Targeted HPV types	HPV 6,11,16 and 18	HPV 16 & 18
14	Stability (Month) at 2-8	36	48

Sr.	Parameter	HPV4	HPV2
15	Immune response monitored	Competitive radioimmunoassay (cRIA) or a competitive ELISA method (cEIA)	Pseudovirion-based neutralization assay (PBNA)assay
16	Drug product	Sterile liquid suspension	White deposit and colourless supernatant after sedimentation, and appears as a turbid liquid after shaking
17	Sold in Countries	109	100
18	Co-administration of other vaccines	Permitted	Permitted
19	Licence to use in INDIA	Oct-08	Feb-09

1. CERVARIX™

Cervarix™ is manufactured by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium in alliance with MedImmune by using canine oral papillomavirus (COPV) model. It is also known as "Human Papillomavirus vaccine Types 16 and 18 (Recombinant, AS04 adjuvanted)". For this preparation, L1 protein of each HPV type is produced in *Trichoplusia ni* Rix4446 cell substrate using a baculovirus expression vector system (BEVS). Medimmune's chosen system requires an insect producer cell and a baculovirus strain engineered to carry the L1 gene. The product consists of purified L1 VLPs of HPV types 16 or 18 at 20/20 µg per dose which is formulated with ASO_4 adjuvant because VLPs alone might not be able to trigger T- cell responses, therefore adjuvants are added for the efficient uptake into antigen presenting cells and to boost T- cell responses. ASO_4 comprises of 500 µg of aluminium hydroxide and 50µg of 3-deacylated monophosphoryl lipid A. Finally, the product is delivered by intra-muscular injection in a three- shot immunization protocol as a 0.5 mL dose[6, 14]. In INDIA, the cost of this bivalent HPV vaccine is Rs. 3,300[15]. Cervarix™ is presented sterile turbid white suspension. Upon storage, a fine white deposit with clear colorless supernatant can be observed. This vaccine is available in market either in single-dose glass vials (type I glass) with stopper and in single use prefilled syringe (type I glass) with plunger that should be stored at 2^o - 8^o C[16].

Cervarix™ was first approved in September 2007, by the European Union for use in girls and women in the age group of 10-35 years. Indian regulatory licensed this bivalent HPV vaccine in 2008 for use in females. The advisable period of HPV vaccination is before the onset of sexual activity. However, bivalent vaccine is indicated in females from 10-45 years old. The bivalent vaccine is given at baseline and repeated at 1 and 6 months[15] (Refer Table. I). A randomized controlled trial was carried out using bivalent L1 VLP vaccine for the prevention of infection with human papillomavirus types 16 and 18 in young women. The measurement of specific serum immunoglobulin G (IgG) anti-L1 VLP antibodies by immunoassays in vaccinated and unvaccinated individuals is the main parameter in the current vaccine trials to monitor vaccine-induced immune responses. For the bivalent HPV-16/18 vaccine, measurement of serum antibody to HPV 16 and 18 VLPs was performed using a conventional enzyme-linked immuno sorbent assay (ELISA) and they claimed that HPV 16 and 18 bivalent vaccine is effective at preventing the acquisition of persistent HPV infection at very low doses. In 2007, this vaccine was licensed for use in females of 15-25 years of age to prevent HPV infections and cervical cancer[17, 18]. The protective efficacy duration for Cervarix™ is 8.4 years[15]. Cervarix™ vaccine should not administered in people who have latex allergy or allergy with vaccine component. The concomitant administration of Cervarix™ and other vaccines is not advisable. However, if

Cervarix™ is to be given at the same time with another vaccine, the vaccine should be administered at different injection sites. The enough data have not been generated for women who have received Cervarix™ and used contraceptive pills[16].

2. GARDASIL ®

Gardasil® is a quadrivalent HPV 16/18/6/11 L1 VLP vaccine developed by Merck and Co. Inc., West Point, Pennsylvania, USA in collaboration with the Australian company CSL by using cottontail rabbit papillomavirus (CRPV) model which had in-licensed VLP technology from Ian Frazer's group at the University of Queensland, Australia. It is also known as "Human papillomavirus vaccine [Types 6, 11, 16, 18] (recombinant, adsorbed)". L1 protein for each HPV VLP type is produced in *Saccharomyces cerevisiae*. During quadrivalent HPV vaccine program, following formulations were evaluated: 20/40/40/20 µg of HPV 6, 11, 16 and 18 L1 VLP including 225 µg of aluminum adjuvant hydroxyphosphate sulfate (AAHS) as an adjuvant, 40/40/40/40 µg of HPV 6, 11, 16 and 18 L1 VLP (including 225 µg of AAHS) or 80/80/40/80 µg of HPV 6, 11, 16 and 18 L1 VLP (including 395 µg of AAHS)[19]. The selected product consists of purified L1 VLPs of HPV types 6/11/16/18 at 20/40/40/20 µg per dose in an adjuvant of 225 µg of traditional aluminum hydroxyphosphate sulfate (alum) adjuvant and final product is delivered via intra muscular injection with 0.5 - mL dose in a three- shot immunization protocol. In INDIA, the cost of this bivalent HPV vaccine is Rs. 2,800[15] (Refer Table. I).

Gardasil® is presented sterile white, cloudy liquid. Upon storage, a fine particulate matter with clear colorless supernatant can be observed. This vaccine is available in market either in single-dose vials or in single use prefilled Luer lock syringe with tip caps that should be stored at 2^{o} - $8\ ^{o}\ C$[20]. Food and Drug Administration (FDA) licensed this quadrivalent HPV vaccine in 2006 while INDIA licensed this vaccine in October 2008 for use in females. The advisable period of HPV vaccination is before the onset of sexual activity. The quadrivalent vaccine is given at baseline and repeated at 2 and 6 months[15].

For the quadrivalent vaccine, serum antibodies to HPV 6, 11, 16 and 18 measured using a competitive radioimmunoassay (cRIA) or a competitive Luminex immunoassay (cLIA). Quadrivalent vaccine phase IIb trials were performed in young women of the age 15– 26 years from both developed and developing countries. HPV 16/18/6/11 L1 VLP vaccine shows protection against HPV 6/11 induced mucosal and cutaneous genital disease[21]. This vaccine contraindicated in people who have yeast allergy or allergy with vaccine component. Co-administration of the quadrivalent vaccine with a recombinant hepatitis B vaccine (in females aged 16-23 years) did not significantly impair the immune response to any of the involved antigens. Studies of the co-administration of both HPV vaccines with other vaccines are ongoing. The quadrivalent HPV vaccine may be administered to lactating females because available data do not indicate any safety concerns[20]. In 2006, quadrivalent vaccine has been licensed for use in young adolescent girls to prevent cervical precancers and cancers and anogenital warts. In addition, the quadrivalent vaccine is licensed for prevention of vulvar and vaginal precancers and cancers as well as anogenital warts in females. In some countries, the vaccine is also licensed for the prevention of anogenital warts in males[21, 6, 19, 22, 23, 14, 24]. The protective efficacy duration for Gardasil® is 5 years[15]. In case HPV vaccination schedule for *Gardasil ®* and Cervarix™ is interrupted, restarting three dose vaccine series is not necessary. However, remaining vaccine doses should be administered as close to the recommended schedule as possible. Till today no booster dose of HPV vaccine is recommended after completion of HPV vaccine series[16, 20].

HPV VACCINES EFFICACY TRIALS

In 2011, Lu et. al. conducted a systematic review and meta-analysis of 7 unique HPV vaccines (phase II and III) trials. These trials enrolled 44,142 women to access efficacy and safety of prophylactic HPV vaccines against cervical

cancer precancerous events. In this meta-analysis, male vaccination or therapeutic vaccinations are excluded. According to this study, efficacy against CIN+1 was in favour of vaccine. HPV vaccines were highly efficacious against 6-month persistent infection with HPV 16 and 18, both in per-protocol population cohort (RR: 0.06 [0.04-0.009] and 0.05[0.03-0.09], respectively) and the intention-to-treat analysis cohorts (RR: 0.15 [0.10-0.23] and 0.24[0.14-0.42], respectively. There was limited prophylactic effect against CIN+2 and 6 month persistent infections associated with non-vaccine oncogenic HPV types. However, long-term efficacy needs to be addressed in future trials[25].

HEAD TO HEAD TRIAL

Previously, direct comparison of the available clinical trial data for the two commercial HPV vaccines across different studies is not feasible given the absence of an established serological correlate of protection and differences in study design and methodology used to evaluate HPV-16/18 specific efficacy endpoints and immune responses. To overcome HPV vaccine immunogenicity comparison problem, GSK sponsored observer-blind head-to-head randomized controlled trial. This trial compared the two vaccines in a single, well-defined population of healthy women aged 18–45 years, using identical methodology for assessment of immunogenicity and safety. *Cervarix*™ and *Gardasil* ® were administered according to their recommended three-dose vaccination schedules (Months 0, 1, 6 and Months 0, 2, 6, respectively). The age range of 18–45 years was chosen to enable full characterization of the immune response to vaccination which included collection of cervicovaginal secretion (CVS) samples for assessment of mucosal HPV antibody levels. In this study, neutralizing antibody levels induced by the two vaccines were evaluated using Pseudovirion-based neutralization assay (PBNA) assay in order to objectively compare functional immune responses using an unbiased assay. According to this trial results, the incidence of adverse events was comparable between groups. *Cervarix*™ induced significantly higher serum neutralizing antibody titers and circulating HPV-16 and -18 specific memory B-cell frequencies. Both vaccines were generally well tolerated. The incidence of unsolicited adverse events was comparable between vaccinated groups. The incidence of solicited symptoms was generally higher after *Cervarix*™, injection site reactions being most common. The advantage of *Gardasil*® over *Cervarix*™ is the additional protection available for HPV-6 /11[15, 21].

CLINICAL TRIALS STATUS OF HPV VACCINES IN INDIA

For marketing approval of HPV vaccines two trials conducted on small sample sizes in India. One was a phase IIIb double-blind, randomized, controlled study to evaluate the immunogenicity and safety of *Cervarix*™ on 354 healthy Indian female subjects aged 18-35 years (NCT00344032) which was completed in November 2007. The other was a phase III trial looking at the safety, tolerability and immunogenicity of *Gardasil*® in 110 healthy females 9 to 15 years of age in India (NCT00380367). This trial was completed in February 2008[26]. Then around mid-2009, two HPV vaccine trials were initiated in INDIA. One was a post-licensure observational study which was conducted in the Khammam district (Andhra Pradesh) for *Gardasil* ® and in (Gujrat) for *Cervarix*™ among school based and community based for operational feasibility. This study was conducted by state govt. in collaboration with Indian Council of Medical Research and Program for Appropriate Technology in Health (PATH) with support from Milinda Gates Foundation[27]. The other was multicentric clinical trial to investigate immunogenic efficacy of two doses compared with conventional 3- doses of *Gardasil* ®. This study was reported in clinical trial-registry of India (CTRI/2009/091/000137)[28]. However, following media allegations of vaccine induced deaths of four girls in Khammam, clinical trials on HPV vaccines are have been suspended by the union government in 2010. The deaths are found to be vaccine unrelated. However, these studies have not been resumed till the time writing this article[29]. Further, in October 2009, Merck announced another phase

III trial of 600 women to study the tolerability and immunogenicity of a three-dose regimen of *Gardasil* ® administered to healthy females between 16 and 23 years of age in India (CTRI/2009/091/000787). CTRI lists the status of this trial (CTRI/2009/091/000787) as temporary halt or suspended. Another trial of HPV vaccine on HIV positive women (CTRI/2009/091/000298), described as phase I in CTRI has been completed[28].

FUTURE TRENDS

There are a lot of obstacles when it comes to making this theory into a reality of introducing a successful cervical cancer vaccine in the market. Corporate interest could be generated by developing a prophylactic vaccine against a sexually transmitted disease. However, it would be difficult to obtain funding for new approaches of developing a therapeutic cervical cancer vaccine after an effective prophylactic cervical cancer vaccine is available in the market. Obtaining government funding for alternative approaches in developed countries would prove to be very difficult as the government would think that the problems would have been already solved in these countries. Private companies, on the other hand, would prefer to rely on existing vaccines rather than investing on new approaches even if they are more profitable in the future. Intellectual property rights further make it difficult for private pharmaceutical companies to commercialize a second generation VLP based vaccine in developed countries. Also, since pharmaceutical companies like Merck and GlaxoSmithKline (GSK) have hugely invested in their current vaccines Gardasil and Cervarix respectively, it is doubtful that they will invest in second- generation VLP based vaccine. However, investment in a totally different approach of cervical cancer therapeutic vaccine is possible.

CONCLUSION

In developing countries, cervical cancer is the leading cause of cancer death in women. HPV vaccines are safe and efficacious against prevention of cervical cancer and other HPV associated malignancies. Currently, Gardasil® - quadrivalent (Merck) and Cervarix™ - bivalent (GlaxoSmithKline) are widely marketed internationally and these HPV vaccines are commercially available. The advisable period of HPV vaccination is before the onset of sexual activity. HPV vaccines should not be given to people who have experienced severe allergic reactions after a previous vaccine dose or to a component of the vaccine. Observation of vaccinees for 15 min after the injection is administered is recommended. HPV vaccines are not recommended for use in pregnant females. However, the quadrivalent HPV vaccine may be administered to lactating females because available data do not indicate any safety concerns. On other hand, safety data for lactating women are not available for the bivalent vaccine. For successful HPV vaccine immunization program and for prevention of cervical cancer, the knowledge and awareness of HPV, cervical cancer and HPV vaccine should be raised.

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