Anticancer Vaccine GARDASIL® and Cervical Cancer

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About Author: Dr. Li Shi is currently a Senior Director leading the efforts of analytical, characterization, and formulation support of process scale-up, clinical supplies manufacturing, and commercial process development of protein and viral vector therapeutics in Genzyme Corporation. Before joining Genzyme in early 2008, Dr. Shi accumulated 12+ years of industrial experience at Merck & Co., Inc. in the general areas of therapeutic protein and vaccine characterization, stabilization, formulation design, analytical testing, and process development support, as well as adjuvant and delivery system evaluations. He has been involved in the entire Gardasil® vaccine product development from basic research of discovery, preclinical development, through clinical phase I, II, and III trials, and all the way to the technology transfer, manufacturing process validation, and the product license application as well as product commercialization. The Gardasil® vaccine reached a sale of about $1.5 billion in 2007 and Merck win the 2006 ASC Industrial Biotechnology Award and 2007 Pre-Galen Prize (The "Nobel Prize" of Pharmaceutical industry).

Dr. Shi received his B.S. and M.S. from Peking University, Ph.D. from University of California, and postdoctoral training from The Scripps Research Institute. He has a total of over 120 scientific paper, patent, and conference abstract publications and is an active speaker in addressing protein product stability and dosage form development international conferences. He has been serving as a reviewer and/or editor for over 15 international technical and scientific journals.

GARDASIL® is an anticancer vaccine developed by Merck & Co. Inc. and the US Food and Drug Administration (FDA) approved GARDASIL® on June 8, 2006. The vaccine prevents human against certain types of human papillomavirus (HPV) that causes cervical cancer and genital warts.

What is Human Papillomavirus?

Human Papillomavirus (HPV) is a common virus that is highly contagious and spreads by skin-to-skin contact. It can infect the skin, the mouth, the rectum and the anus. It can also infect both male and female genital areas, including the skin on the penis or the vulva (area around the vagina), as well as the lining of the vagina and the cervix (the lower part of the uterus that is connected to the vagina).

HPV infection is one of the most common sexually transmitted infections around the world. As many as 75% of people (women and men) who are sexually active will have at least one type of HPV infections in their lifetime with the majority of exposure taking place in adolescence or early adulthood. According to the Centers for Disease Control and Prevention (CDC) estimated, by the age of 50 more than 80% of American women will have contracted at least one strain of HPV. HPV is highly communicable. College freshmen women who used condoms consistently had a 37.8% per patient-year incidence of genital HPV, compared to an incidence of 89.3% among those who did not.[1][2][3] In 2005, the CDC estimated that 20 million people in the United States had this virus.

There are many different types of HPV. Some cause no harm and some can cause diseases of the genital area. So far, over 120 types of HPV have been identified. About 40 of which are easily transmissible through sexual contact. About 10% of those HPV types can lead to changes in the cervix that may become cancerous. Some of these types of HPV can eventually result in cancer of the vulva, vagina, anus or penis.

HPV can live on the skin or mucous membranes, including those in the genital tract, without causing any signs or symptoms. Most people do not know that they are infected with HPV, and most people infected with HPV will eventually clear the infection on their own without treatment. HPV can cause warts, or papillomas. Certain types of HPV (called "low-risk" types) cause common skin warts that often appear on the hands and feet. Other types of low-risk HPV can cause genital warts. Genital warts are single or multiple growths or bumps that often resemble a bumpy piece of cauliflower and can appear on the vulva, vagina, cervix, penis, scrotum, groin

or thigh. These warts may appear weeks to months after sexual contact with a person who is infected.

In 2005, the CDC estimated that at least 50% of sexually active people catch HPV during their lifetime. A male or female of any age who takes part in any kind of sexual activity that involves genital contact is at risk. Many people who have HPV may not show any signs or symptoms. This means that they can pass on the virus to others and not know it.

What is Cervical Cancer?

Cervical cancer is a serious disease that is life-threatening and the second leading cause of cancer death in women worldwide [4][5][6]. Virtually all cervical cancers are caused by some type of human papillomavirus (HPV) [7,8] that infects about half of all people at some point in their lives [9,10] and more than two-thirds of cervical cancer cases are attributed to high-risk HPV types 16 and 18 [11,12]. The infection of these HPV types causes the cells in the lining of the cervix to change from normal to precancerous lesions. According to the US fact sheet by Merck & Co., Inc., Oct 2005, cervical cancer develops in the cervix, the cone-shaped part [4,14] of the uterus that connects the upper part of the uterus (the womb) and the vagina [15,16] when abnormal cells in the lining of the cervix begin to multiply out of control in response to HPV infection [17,18]. Abnormal cervical cells can gather to form a lump called a tumor. Benign (non-cancerous) tumors do not spread and usually are not harmful. Malignant (cancerous) tumors, however, spread from their sources and grow into life-threatening cancer [19,20].

Worldwide, cervical cancer is the second-most common cancer affecting women [21,22] and the leading cause of cancer-related deaths in developing countries.[15,16]. Almost 80 percent of all cervical cancer cases occur in the developing world [23,24].. Globally, approximately 500,000 women are diagnosed each year [25,26] and almost 300,000 die from the disease [27,28].. In the United States, about 10,000 women will develop cervical cancer each year [29,30] resulting in almost 3,000 deaths [30,32].

Cervical cancer tends to occur in women in their 40s and 50s [33,34] when many are still raising children and contributing to their families' livelihoods and security [35,36]. Women in underserved communities are less likely to undergo recommended cervical cancer screening, which could account for the greater risk for cervical cancer among these groups [37,38]. In the United States, women have limited access to screening tend to have the highest incidence rates of cervical cancer [39-44].

Cervical cancer is currently diagnosed by the Pap test (also called Pap smear) that is a simple test – usually conducted in a doctor's office or clinic – that detects abnormal or cancerous cervical cells [45,46]. Because the Pap test can detect cervical changes before they progress to cancer [47,48], it is credited with significantly reducing cervical cancer deaths since its widespread use began in the 1950s [49,50]. Between 1950 and 1970, the number of cervical cancer deaths in the United States decreased 70 percent [49,51]. Every year, 3.5 million Pap tests find cervical abnormalities that require medical follow-up with high cost [52,53]. However, Pap tests aren't perfect and sometimes they do not find cervical abnormalities that actually exist or cause unnecessary anxiety for the patient [54-57].

According to the August 2007 Public Health Fact sheet of Canada, cervical cancer will develop in about 1 in 138 (0.7%) Canadian women in their lifetime. Every year there are 1300 new cases diagnosed in Canada alone. Among Canada's 11 million women aged 15–64, 400 will die from cervical cancer each year, making it the 13th most common cause of cancer related deaths [58]. About 11,000 American women are diagnosed with cervical cancer every year, and about 3,700 die per year of the disease[11,12].

HPV infection causes nearly all cases of cervical cancer [13]. Most cervical cancers result from persistent infection with human HPV. Out of the more than 120 serotypes of HPV, about 10% of which can lead to changes in the cervix that become cancerous. Although, the majority of HPV infections are cleared without causing any disease or symptoms, some will result in persistent infections that can result in anogenital warts, cervical cancer, and less frequently, other anogenital cancers, or recurrent respiratory papillomatosis, in which warts develop in the respiratory tract.

There are over 500,000 new cases of cervical cancer worldwide each year and 270,000 deaths. Eighty percent of new cervical cancer cases occur in developing countries. In the U.S. it is estimated that 10,000 new cases of cervical cancer occur annually, resulting in approximately 3,700 deaths. Of the approximately forty sexually transmissible varieties of HPV, two of them – serotypes 16 and 18 – cause about 70% of all cases of cervical cancer attributable to HPV, while serotypes 6 and11 cause approximately 90% of genital warts cases. These four serotypes have been the focus of vaccine development efforts to date [59].

Cervical cancer is second to breast cancer as the most common cancer in women worldwide and nearly all cases of cervical cancer (20% in women worldwide, and 80% in the developing world) and other mucosal, cutaneous, and oral cancers stem from HPV infection [60]. The death rate of cervical cancer is 50% (Figure 1).

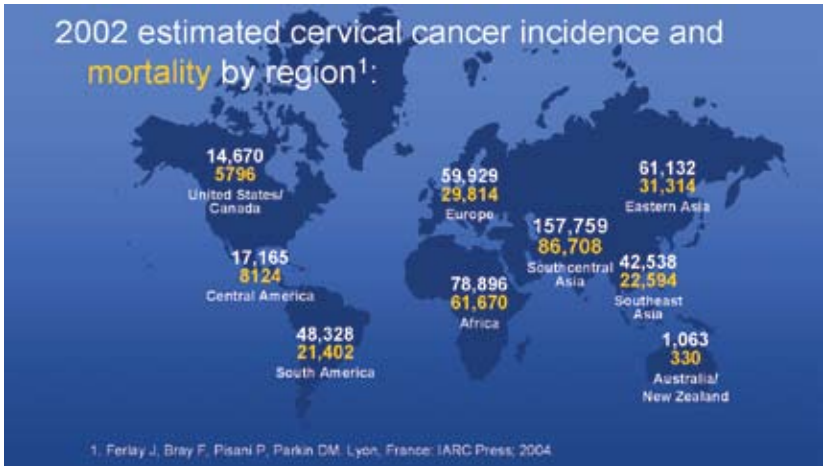


Figure 1. *2002 Estimated cervical cancer incidence and mortality by region.* [60]

Currently, one in four teenage girls in the U.S. had at least one common sexually transmitted disease, according to the first national study to assess combined rates of the most common STDs among young women. About 3.2 million women between ages 14 and 19 had human papillomavirus, chlamydia, genital herpes or trichomoniasis according to the report released by the U.S. Center's for Disease Control and Prevention on March 12, 2008. Sexually transmitted diseases cost almost $15 billion to treat annually in the U.S., and more than half of those infected are under 24 years old, according to the Atlanta-based CDC. About half the young women in the study reported having sex, and 40 percent of sexually active women had STDs. HPV that causes cervical cancer was the most common infection and prevalent among 18 percent of the teen girls [61, 62].

When talking about the importance of vaccination for public health, the National Cancer Institute writes, "Widespread vaccination has the potential to reduce cervical cancer deaths around the world by as much as two-thirds, if all women were to take the vaccine and if protection turns out to be long-term. In addition, the vaccines can reduce the need for medical care, biopsies, and invasive procedures associated with the follow-up from abnormal Pap tests, thus helping to reduce health care costs and anxieties related to abnormal Pap tests and follow-up procedures." [4,14,21,22]

The GARDASIL® vaccine

GARDASIL® is an anticancer vaccine developed, manufactured, and distributed by Merck & Co., Inc. Its generic name is Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine.

GARDASIL® is a vaccine that helps protect against the following diseases caused by HPV types in the vaccine (6, 11, 16, and 18):

- Cervical cancer (cancer of the lower end of the uterus or womb) [37, 38].
- Abnormal and precancerous cervical lesions.
- Abnormal and precancerous vaginal lesions.
- Abnormal and precancerous vulvar lesions.
- Genital warts.

GARDASIL® is given in three injections over six months, namely at enrollment, then 2 and 6 months later. The 3 doses are required to boost the immune response to an optimal level to get the full benefits of GARDASIL®. The main ingredients are purified inactive proteins that come from HPV Types 6, 11, 16, and 18. It also contains amorphous aluminum hydroxyphosphate sulfate, sodium chloride, L-histidine, polysorbate 80, sodium borate, and water for injection [5]. The aluminum salt is used to stimulate human immune system to achieve a high level immune response while other non-active ingredients are for the stabilization of the antigens.

The GARDASIL® vaccine has been launched in USA, countries of the European Union, Canada, Australia, New Zealand, Hong Kang, Taiwan, Southern Korea, Malaysia, Brazil, Serbia, Israel, Croatia, Mexico, and many other counties since June 2006 for women age of 9-26 in all countries [37, 38]. The vaccine is currently not approved yet for males in most countries although Australia[46][48] and South Korea[53] have approved the vaccine for boys aged 9 to 15 years.

The HPV major capsid protein, L1, can spontaneously self-assemble into virus-like particles (VLPs) that resemble authentic HPV virions. GARDASIL® contains recombinant VLPs assembled from the L1 proteins of HPVs 6, 11, 16 and 18. Since VLPs lack the viral DNA, they cannot induce cancer. They do, however, trigger an antibody response that protects vaccine recipients from becoming infected with the HPV types represented in the vaccine. The vaccine does not contain any antibiotics or preservatives such as thimerosal, mercury, or live virus[23].

The GARDASIL® contains purified, HPV virus like particles composed with recombinant proteins that come from the 4 most common types of HPV: HPV types, 6 and 11 (which cause 90% of genital warts) and HPV types 16 and 18 (which cause 70% of cervical cancer). A cross protection for other types HPV strains that are not included in

the vaccine is expected and continue effort in getting supporting data is ongoing now [63].

The vaccine is primarily effective in females who have not yet been exposed to HPV; thus, it is recommended that females be vaccinated before they become sexually active. The vaccine offers protection for at least 5.5 years, and studies are being performed to determine whether a booster dose will be required. Even with the vaccine, regular Pap smears are essential because the vaccine does not protect against all types of HPV that can cause cervical cancer. Although the vaccines are not a treatment or cure for cervical cancer, it has demonstrated efficacy in preventing infection by specific types of HPV if administered to girls before the start of sexual activity [52, 53].

People will still benefit from GARDASIL® if the people already have HPV. This is because most people are not infected with all four types of HPV contained in the vaccine. In clinical trials, individuals with current or past infection with one or more vaccine-related HPV types prior to vaccination were protected from disease caused by the remaining vaccine HPV types and show certain boost immune response to those other HPV types of past infection.

In addition to GARDASIL® (Merck and Co.), another distinct HPV vaccine, Cervarix (GlaxoSmithKline) is now approved in 2007 in the European Union and Australia for women aged 10–25 years .Table 1 shows the comparison of the two products.

GARDASIL® is designed to prevent infection with HPV types 16, 18, 6, and 11. HPV types 16 and 18 cause about 70% of HPV-related cervical cancer cases. In addition, some types of HPV, particularly type 16, have been found to be associated with oropharyngeal squamous-cell carcinoma, a form of throat cancer[61, 62]. HPV types 6 and 11 cause about 90% of genital wart cases. The National Cancer Institute says, "FDA-approved GARDASIL® prevented 100 percent of the precancerous cervical cell changes caused by the types of HPV targeted by the vaccine for up to 4 years after vaccination."[39]

Table 1: *Cervarix & Gardasil: Comparative Product Profiles*

	Cervarix (GSK)	Gardasil (Merck)
HPV Serotypes	16, 18	6, 11, 16, 18
Adjuvant	AS04 (aluminum hydroxide + monophosphoryl Lipid A)	Amorphous aluminum hydrophosphate sulfate
Dosing Schedule	0, 1, 6 months	0, 2, 6 months
Storage/Transport	Refrigeration	Refrigeration (2-8 °C)
Regulatory Status	Submitted in US for Cervarix (human papillomavirus vaccine, AS04 adjuvant-adsorbed), with data from clinical trials in almost 30,000 females 10 to 55 years of age and from the largest Phase III cervical cancer vaccine efficacy trial to date.[67] European Union (women aged 10–25 years) Approved in Australia for women ages 10 to 45.[68] and Philippines.[69]	Approved for women in US, EU, Canada, Brazil, Mexico, Australia, NZ, Russia, others; approved for men in EU, Australia, Russia. United States, Canada (women aged 9–26 years), European Union (women aged 9–26 years and boys aged 9–15 years);
Price	Undetermined	$120/dose; $360/course

Source: *Company reports and JPMorgan estimates.*

The market and cost of GARDASIL® vaccine

According to the American Centers for Disease Control, getting as many girls vaccinated as early and as quickly as possible will reduce the cases of cervical cancer among middle-aged women in 30 to 40 years and reduce the transmission of this highly communicable disease. Unfortunately, there are hurdles to getting this done. These include the limited understanding by many people that HPV causes cervical cancer, the difficulty of getting preteens and teens into the doctor's office to get a shot, and the high cost of the vaccine ($120/dose, $360 total for the three required doses, plus the cost of doctor visits).[39][41]

One way to bring down the cost of the vaccine and to educate the public on the benefits of vaccination is to make it mandatory for girls entering school. This approach has been taken with vaccines for mumps, measles, rubella, and hepatitis B (which is also sexually transmitted) so many state legislators have penned bills that do this. The vaccination is recommended for women aged 9 to 26 and is approved for funding through the U.S. Vaccines for Children program in all 50 states. Since

the vaccine is against Human Papillomavirus (HPV) that causes virtually all cases of cervical cancer and genital warts, on June 29, 2006, a panel of experts, the Advisory Committee on Immunization Practices (ACIP), gave their approval for the vaccination of GARDASIL® on children as young as nine years old. The ACIP recommended that GARDASIL® be placed on the childhood immunization schedule at the 11 to 12 year old visit. They also recommended that the vaccine be included in the federal Vaccines for Children Program, which would provide the vaccines free of charge to children under the age of eighteen who are uninsured.[37] Since then debate is heating up about whether or not to require girls to be vaccinated. Currently, many states have legislation currently pending that would make the vaccine mandatory for school entrance [50]. Texas Governor Rick Perry issued an executive order on February 2, 2007 mandating the vaccine be given to all school girls entering sixth grade, beginning September 2008 althought the Texas legislature overruled Governor Perry's order later on April 25, 2007, forbidding mandatory vaccination until at least 2011.[57] New Hampshire has adopted a voluntary program, in which it supplies the vaccine free of charge to girls between the ages of 11 and 18. Alaska has also adopted a similar voluntary program for girls between the ages of 9 and 18. [64] This has met with wide acceptance, with many young women requesting the vaccine. South Dakota and Washington are considering similar measures.[65] Kentucky, Virginia, and New Mexico require 9- to 14-year-old girls entering school to be vaccinated and allow parents to opt their daughters out. Nevada requires health insurance companies to cover the cost of the vaccine [66].

According to the 2006 market research report, JP Morgan has modeled HPV vaccine sales through 2015, and project worldwide revenues in that year to reach $7.1 billion. They expect Merck to account for 53% of this, the Merck-sanofi-aventis JV which sells the product in the EU to earn 14% of the total, and GSK to receive the remaining 33%. They believe the Merck product's lead time and greater number of protective serotypes (4 versus 2 in GSK's Cervarix) will make it the market leader. What could change this is data demonstrating that GSK's Cervarix provides better cross protection against HPV serotypes not included in the vaccine than does Merck's GARDASIL®. Such data are not yet available, however, and it may turn out that Cervarix does not have such superiority since GARDASIL® may also well demonstrate such cross protection [59]. In Canada and the United States, the GARDASIL® vaccine costs between $400 and $500 for the 3 required doses, at about $150 per dose. In the United Kingdom, each dose costs about £80, for a total cost of about £240 [58]. So far the vaccine is the most expensive vaccine product according to CDC and as reported Philadelphia Inquire in 2006 as shown by Table 2.

Table 2. *Recommended Pediatric/Adolescent Vaccines*
Approximate costs for vaccines recommended for all youngsters. Physicians' administrative costs are not included. Some brands of combination vaccines and catch-up immunizations are not included.

Vaccine	Doses/child	Manufacturer(s)	Price/dose
Human papillomavirus	3	Merck	$120
Meningococcal	1	Sanofi pasteur	$82
Varicella	2	Merck	$71
Rotavirus	3	Merck	$63
Hepatitis B	3	Merck., Sanofi Pasteur, GSK	$23-60
Measles, Mumps, Rubella	2	Merck	$43
Diphtheria, Tetanus, Pertussis Booster	1	Sanofi Pasteur, GSK	$36
Hepatitis A	2	Merck, GSK	$29
Haemophilus Influenza B	2	Merck, Sanofi pasteur	$23
Polio	2	Sanofi pasteur	$23
Diphtheria, Tetanus, Pertussis	5	Sanofi Pasteur, GSK	$21

Source: *Centers for Disease Control and Prevention by The Philadelphia Inquier*

About 10 percent of women ages 18 to 26 have received the shot, according to the CDC. Impact of GARDASIL® Vaccine sales commenced in June 2006 and the vaccine is already in the top 10 vaccines in the world. GARDASIL® has become one of Merck's top-selling products, with $1.48 billion in sales in 2007 (ref). According to Citibank report forecasting, the Australia company, CSL, will have about 28% earnings from GARDASIL® royalties by 2010 and the world vaccine market will nearly double between now and 2012, an increase from $US10 billion this year to US$23 billion by 2012. According to GSK's estimate, the world vaccine market will be $21 to $31 billion in 2010 and HPV vaccines could account at least for 13% to 19% of this total [59]. Critical to this growth is cancer vaccines which are forecast to rise from their current level of $US135m to more than US$8 billion by 2012. CSL has made an a $80m investment to double vaccine manufacturing capacity from 20m to 40m doses from 2008 to 2009 [70].

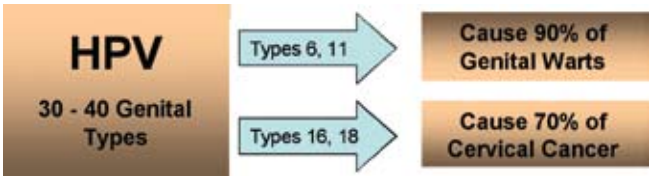
To provide quantitative insight into current U.S. policy choices for cervical cancer prevention, Goldhaber-Fiebert and colleagues [71] developed a model of human papillomavirus (HPV) and cervical cancer, explicitly incorporating uncertainty about the natural history of disease. Their stochastic microsimulation of cervical cancer distinguishes different HPV types by their incidence, clearance, persistence, and progression. Through systematic reviews and formal data synthesis, they established multiple epidemiologic targets for model calibration, including age-specific prevalence of HPV by type, age-specific prevalence of cervical intraepithelial neoplasia (CIN), HPV type distribution within CIN and cancer, and age-specific cancer incidence. According to their study, the expected reductions in lifetime risk of cancer with annual or biennial screening were 76% and 69%, respectively. The reduction from vaccination alone was 75% reflecting considerable parameter uncertainty about the natural history of type specific HPV infection. The uncertainty surrounding the model-predicted reduction in cervical cancer incidence narrowed substantially when vaccination was combined with every-5-year screening, with a mean reduction of 89% and range of 83% to 95% [71].

The Development of GARDASIL® vaccine:

GARDASIL® vaccine is a very complex biological product. It takes scientists more than 14 years to develop and more than 10 years of clinical studies which are still ongoing up to today. A total of more than 30000 patients and 33 counties including Taiwan and China have been involved in the clinical trials.

The **first challenging** is to identify which HPV types of are responsible for the majority of cases of infection and cancer. This kind of work heavily relies on many years of frontier basic research work which is still on going globally. The conclusion also relies on systematic and very extensive epidemiological research to identify the leading types of HPV that cause the cervical cancers and genital infections. The great attention of scientists has been focucing on at least 4 types of the virus that cause the most cases of HPV-related diseases in women. HPV Types 16 and 18 cause 70% of cervical cancer cases, HPV Types 6 and 11 cause 90% of genital warts cases as shown by Figure 2 below.

Figure 2. *HPV types 6, 11, 16 and 18 that cause most genital warts and cervical cancer cases [72].*



The **second challenge** is to develop a suitable candidate antigen that can be developed into vaccine and an antigen production system that possesses the ability to product high quality and efficacious antigens with yield meeting the needs of commercial production. Many systems have been tested for the expression of recombinant HPV antigens in the past decade. Systems that have been shown to produce sufficient quantities of HPV virus-like particles (VLPs) for research and/or clinical studies include baculovirus vectors[73], vaccinia virus[74], yeast, [75], and Escherichia coli [76].

The research that led to the development of the vaccine began in the 1980s by groups at the University of Rochester, Georgetown University, the University of Queensland, and the US National Cancer Institute. In 1991 investigators at the University of Queensland found a way to form non-infectious virus-like particles (VLP), which could also strongly activate the immune system. However, these VLPs assembled poorly and did not have the same structure as infectious HPV. In 1993, a laboratory at the US National Cancer Institute was able to generate HPV VLPs that were morphologically correct. These VLPs were the basis for the HPV vaccine antigen component of the HPV vaccines. More recently, Merck and GSK successfully achieved commercial manufacturing of HPV VLP antigens using yeast and baculovirus systems respectively. Vaccines manufactured with HPV VLP antigens expressed from yeast and baculovisus have been shown to protect Human against natural HPV disease with 100% efficacy. This is the most significant milestone toward to development of a HPV vaccine.

The history of the discovery of this vaccine is described by McNeil [17]. Upon commercialization of the vaccine, controversy involving intellectual property arose between the various groups that played a role in developing the vaccine. If it is asked who should be the original inventor of HPV vaccine, the answer is not simple. As reviewed by McNeil [17]., the press releases from the University of Rochester in New York, Georgetown University in Washington, D.C., and Queensland University in Brisbane, Australia, have claimed responsibility for original work leading to the Merck or GlaxoSmithKline vaccine. The two vaccines are all based on the ability of the L1 protein of the papillomavirus to self-assemble into virus-like particles (VLPs) that elicit neutralizing antibodies. According to McNeil's review, the U.S. Patent Office recognized four claimants to the basic technology — the National Cancer Institute, Georgetown, Queensland, and Rochester. After a 10-year "interference" to adjudicate the overlapping claims, Georgetown won the dominant patent for its contribution to the "background science". Ironically, of the four, Georgetown was the only one not to have developed VLPs. Other U.S. patents are held by NCI's parent institution, the National Institutes of Health. Rochester, NIH, and Queensland hold patents in other countries. Merck and GSK have cross-licensed the patents of all parties. All the above work reviewed by McNeil only reached to the level of candidate expression system. Tremendous amount of process development work have been carried out since then to research the level for the system can be used for clinical supply production and for commercial manufacturing of the vaccine products.

The **third challenge** is the preclinical development for a stable vaccine dosage form for safety assessment and clinical studies. Like many other biological materials or proteins, HPV VLP antigens share the general instability features of most other natural biological materials that will lose activity soon after purification without extensive protection in solution. Those features of instability are due to the isolation of the proteins from its optimal natural environment in the cell. There are multiple major jobs that have to be done before the vaccine is ready for clinical studies. First of all, an optimal HPV VLP purification process has to be developed. Tremendous challenges were faced during the process development. It is not only required to reach a very high level of purity, the purified protein must also maintain full activity and integrity with all correct primary, secondary, and tertiary as well as quaternary structural properties. Merck engineer and scientist teams successfully developed the engineering process of expressing four types of HPV vaccine antigens and purifying all of the four types of antigens to the desired purity and functional level.

The success of purification process development involved with extensive pre-formulation research including protein basic property characterization and stabilization approach. The success development for a promising purification process only means the achievement of bulk drug substance production. The final achievement of preclinical development requires another key success in formulation development that defines the final dosage form for safety assessment, clinical studies, and commercial use. The formulation research and development for GARDASIL® vaccine was a continue process in parallel with clinical trial with multi-years of constant effort of laboratory research and scale up process testing. The Merck team moved step-by-step and finally achieved the maximum in optimizing the bioprocess and the dosage form goal as reflected by Merck Research Laboratories' multiple publications and patents for HPV vaccine development. During this process, the scientists successfully stabilized the antigen against the aggregation in bulk solution, which ensured the success of process development [77]. In addition to antigen inherent stability issue, almost all recombinant protein based vaccines require adjuvant in the vaccine formulation to stimulate immune response of animal or human system. The inherent instability of HPV vaccine antigens becomes even more complicated with the involvement of adjuvant. This is very typical for GARDASIL® vaccine. To balance interactions among buffer components, salt, excipients/stabilizers and adjuvant as well as the four antigens, it needs very precise screening, adjusting and long time stability testing of the complex mixture. Among all recorded achievements of Merck research team, the most critical achievement is the dramatic enhancement of HPV VLP antigen's inherent stability by adjusting the inherent structure of the VLPs through a disassembly and then reassembly process [78].

The joint effort of engineers and scientists result in a dramatic improvement in GARDASIL® vaccine. The antigens' stability in the selected formulation is enhanced to a surprising level with from half life increased from hours to months under accelerated condition, and 6 month to more than 130 months under refrigeration condition [79]. The achievement in formulation development again significantly benefit manufacturing process by significantly improving the consistency or repeatability of producing the same quality GARDASIL® vaccine product with the lowest process variation.

The Merck teams honorably received American Chemical Society's 2006 Industrial Biotechnology Award "in recognition of the development of the manufacturing process for GARDASIL®, a quadrivalent vaccine for Human Papillomavirus" [80] as shown by the Figure 3 below.

Figure 3. *The Merck teams honorably received ACS 2006 Industrial Biotechnology Award.*

The **last challenge** is the clinical development of GARDASIL® vaccine. Due the special feature of vaccine products that are used for mostly infant, youth, and healthy people in a mass population, regulatory agency is very cautious in reviewing or release a vaccine product. More extensive data is required for convincing the regulatory for the safety and efficacy of a vaccine product. All components especially those called novel excipients or adjuvants used in a vaccine formulation could be a target or trigger that causing regulatory agency's attention and results in an extensive evaluation. Merck team has been carrying out more than two dozen different clinical studies for the past over 10 years and multiple clinical studies are still going on. The total patents participate in the clinical studies are about 30,000 from more than 30 countries. Regardless the cost of those clinical studies, the design of clinical protocols is a very challenging job. Those clinical studies not only address safety and efficacy, but also address dose ranging, patient population and epidemiology history, patient age and sex, and any manufacturing process related factors such as process changes. In addition, there are also two most critical challenges that are the duration of the clinical studies and surrogate markers for cervical cancer vaccine.

Due to the fact that GARDASIL® is an anticancer vaccine, the vaccine must provide strong evidence of prevent cervical cancer. Such evidence can not be collected with a normal vaccine clinical study time frame such as 3 years. This is due to the long hidden time of cervical cancer. However, even the clinical study can be carried out long enough to obtain the required evidence of preventing cervical cancer, no patient who is given the placebo vaccine is allowed to develop cancer to give the evidence. Therefore a close monitoring of all patients with continue testing and a surrogate marker to signal the precursor of cancer must be in place during all studies and all patients show the precursor should receive treatment before they develop cancer. Multiple clinical studies have demonstrated the effective of preventing cervical cancer in human with 100% efficacy as demonstrated by examples shown in Table 3.

Data of more than 5 years of studies have also demonstrated GARDASIL® induces strong immune memory since the system memory effectively responded to challenge conducted with the vaccine (mimic the virus infection) by successfully boost the system 5 years later after vaccination. Some of the clinical studies are still on going to target 10 year of data collection. An example of reported clinical trials is as follows: Merck & Co. conducted a Phase III study named FUTURE II. This clinical trial was a randomized double-blind study with one controlled placebo group and one vaccination group.[27, 31, 85] Over 12,000 women aged 16–26 from thirteen countries participated in the study. Each woman was injected with either GARDASIL® or a placebo on Day 1, Month 2, and Month 6. In total, 6,082 women were given GARDASIL® and 6,075 received the placebo.[27] Merck has tested the vaccine in only a few hundred

Table 3. *Eamples of Gardasil efficacy in young women (aged 16–23) (Date adapted from ref. 81-83)*

Phase/ protocol number	Primary end point	Number of women enrolled	Efficacy (CI)	Cases (Vaccine/ Placebo)
IIa/V501-007	HPV 6-, 11-, 16-, or 18-related disease	552	100% (12–100)	0/6
III/V501-013	HPV 6-, 11-, 16-, or 18-related CIN 1–3 or AIS	5,442	100% (87–100)	0/37
III/V501-013	HPV 6-, 11-, 16-, or 18-related vulvar or vaginal lesions or warts	5,442	100% (88–100)	0/40
III/V501-015	HPV 16-, or 18-related CIN 2/3 or AIS	12,157	100% (76–100)	0/21

Figure 4. *Prix Galien Prize.*

Figure 5. *Merck HPV vaccine development team leaders who attended the 2007 ceremony of Prix-Galien Prize. Back 2nd to the right is the author of this article. Front 2nd to the right is Dr. Peter Kim, the president of Merck Research Lab.*

Figure 6. *Prix-Galien Prize review committee members. All of them except Richard Learner (right) are all former Nobel Prize winners.*

11- and 12-year-old girls.[31] On February 27, 2006, the independent Data and Safety Monitoring Board recommended the clinical trials be terminated on ethical grounds, so that young women on placebo could receive GARDASIL®.[85]

Some of the on-going or finished studies also tested in men and aged women. When more clinical data become available, it may provide further evidence to suggest cross protection against HPV types that are not included in the vaccine dosage form. This cross protection is theoretically reasonable due to the high conservative structure composition of various HPV L1 proteins. To recognize the great effort, success, and contribution, the Pre-Galen Prize committee awarded Merck Team the 2007 Pre-Galen Prize for the team's success development of GARDASIL® vaccine (Figures 4 and 5). The pre-Galen award is called the Nobel Prize of Pharmaceutical Industry. The Award is given once in about four year world wide. Majority of the review committee members are former noble prize winners (Figure 6).

Other aspects and path forward of HPV vaccines:

Some outstanding medical issues remain: The current HPVL1 vaccines have been shown with an efficacy of at least 5 years and they are under clinical studies with boys and men. The vaccines can reduce the incidence of benign and malignant genital HPV infections, but their type-restricted protection means that infections by other type of HPV will still occur in vaccinated women although clinical data may show certain cross protection. And it is unclear when the HPV vaccine will be widely implemented in the developing world, where most cases of cervical cancer occur. The need for second-generation vaccines with more coverage is widely recognized and effort is being made.[86] Also, both men and women are carriers of HPV. To eradicate these particular strains, men would eventually need to be widely vaccinated.[87] Studies are being conducted now to determine the efficacy of vaccinating boys with the current vaccine.

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