Developing an HPV vaccine to prevent cervical cancer and genital warts

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Abstract

The challenges of the journey from target identification through development of a prophylactic quadrivalent human papillomavirus (HPV) vaccine have been met in Gardasil®. Cervical cancer is the second leading cause of cancer-related death in women worldwide. Approximately 70% of cervical cancer is caused by infection with HPV types 16 and 18 and ~90% of genital warts are caused by HPV types 6 and 11. The quadrivalent HPV vaccine was generated by expression of the major capsid protein (L1) of HPV types 16, 18, 6 and 11 in yeast. L1 proteins self assemble into pentamer structures and these pentamer structures come together to form virus-like particles (VLPs). The VLPs are antigenically indistinguishable from HPV virions. The VLPs contain no viral DNA and therefore the vaccine is non-infectious. Gardasil® is composed of VLPs of HPV types 16, 18, 6 and 11 conjugated to a proprietary amorphous aluminum hydroxyphosphate sulfate adjuvant. The results of a rigorous clinical program have demonstrated that the vaccine is safe and highly efficacious in preventing dysplasias, cervical intraepithelial neoplasias (CIN 1–3) the precursors of cervical cancer and external genital lesions caused by vaccine-HPV types. In conclusion, Gardasil® addresses a major medical need, that is, reduction of HPV-related disease including cervical cancer as a safe, immunogenic, and highly efficacious vaccine.

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1. Rationale and design of the quadrivalent HPV vaccine

Human papillomavirus (HPV) related morbidity and mortality from cytologic abnormalities of the cervix, surrounding genitalia and cervical cancer occur with high frequency. Cervical cancer has been shown to be caused by HPV infection in over 99% of cases [1]. There are over 100 HPV types with 30–40 types associated with anogenital disease. Approximately two-thirds of these are considered “high-risk” for oncogenic progression whereas the remaining one-third are considered “low-risk”. Cervical cancer is the second leading cause of cancer-related death in women worldwide [2,3]. The low-risk HPV types are primarily associated with condyoma acuminata, external genital lesions that typically manifest as benign genital warts. The morbidity associated with the low-risk HPV types results in physical and emotional discomfort treated by ablative or cryo therapy, often followed by recurrence of lesions. Thus, there is a significant medical need to address HPV-related disease.

Although there are a number of HPV types which cause anogenital disease, the vast majority are caused by four HPV types [4]. A number of studies have suggested that ~55% of high-grade dysplasias and cervical cancer are caused by HPV type 16 and approximately 10–15% are caused by HPV type 18. HPV 16 and 18 also cause a significant proportion of vaginal, vulvar, anal and penile cancers [5–8]. Therefore, although HPV disease is commonly referred to as a woman’s disease via the cervical cancer connection, HPV disease is clearly evident in both genders. Low-risk HPV disease is seen equally in both male and female populations. HPV types 6 and 11 infections are responsible for over 90% of external genital lesions and the majority of recurrent respiratory papillomatosis (RRP) lesions [9–12].
HPV 16, 18, 6 and 11 cause the majority of HPV-related anogenital disease. Therefore, a quadrivalent vaccine design was adopted to have the greatest impact on HPV-related disease. The vaccine itself was developed in Merck Research Laboratories (MRL). A number of academic and institution investigators, in a very similar timeframe, discovered that the major capsid protein of HPV, L1, could be expressed as a recombinant protein [13–16]. The L1 molecules were found to self-associate and form virus-like particles (VLPs). The VLPs appeared to be empty capsids virtually identical to HPV viral capsids. Thus the challenge was to produce VLPs of HPV types 16, 18, 6 and 11 as simple, safe antigens which would induce a robust immune response and to demonstrate that the immune response was efficacious.

To produce VLPs that could be used as a vaccine required a method of expression that would allow large-scale production in a benign background host system that would be amenable to large-scale purification techniques providing highly purified VLPs. At MRL, we had considerable experience utilizing Saccharomyces cerevisiae, a yeast host system, to produce vaccine quality recombinant hepatitis B proteins [17]. This experience was applied to the expression of HPV VLPs. A yeast expression vector, pGAL110, was constructed to allow regulated expression of the recombinant protein by galactose induction [18]. Two commercially available cell lines, CaSki and SW756, which have been shown to contain HPV 16 or HPV 18 sequences respectively, were obtained and the L1 sequences subsequently cloned into pGAL110 [19]. HPV 6a DNA isolated from a large vulvar condyloma acuminatum lesion [20] was used as the source material to clone the HPV 6 L1 gene [18]. As HPV 6 and 11 L1 proteins are 92% identical, the HPV 6 L1 DNA sequence was used as a backbone upon which modifications were made to generate the HPV 11 L1 amino acid sequence [21]. The four pGAL110-L1 plasmids were individually transformed into S. cerevisiae and expressed by galactose induction. Initial purification experiments yielded highly purified VLPs that, as expected, appear to be well-formed empty capsids [22]. Subsequent modifications have been put in place to enhance large-scale fermentation and purification yields including a disassemble/reassemble VLP step performed via alterations in salt concentration for HPV types 6, 11, and 16 [23]. The HPV vaccine was expected to be safe and highly immunogenic based upon substantial pre-clinical data generated using animal papillomavirus infections (reviewed by Dillner and Brown [24]). In clinical trials, monovalent, single HPV VLP type vaccines (11, 16 or 18) were tested initially demonstrating safety and immunogenicity. These studies were subsequently followed by dose-ranging studies [25–29].

2. Immunogenicity

Ideally, a prophylactic vaccine would be highly immunogenic and stable at a low dose providing the subject with maximal response and limiting any adverse experiences. In the development and storage of VLPs it was found that under certain conditions and concentrations, VLP aggregation might occur. To provide optimal stabilization of HPV VLPs in solution required the presence of both non-ionic surfactants and sufficient levels of salt. PS80 was incorporated into the VLP storage buffer in addition of NaCl to ensure high quality, conformationally consistent, stable VLPs for vaccine formulation [30].

In addition, adjuvants have been traditionally used to bolster the immune response to the antigen. Greater than 100 compounds or formulations exhibiting adjuvant properties have been described [31] however, the primary adjuvants in widespread use in humans, are mineral-based compounds containing aluminum [32]. Pre-clinical quadrivalent HPV vaccine experiments were conducted to determine if antibody responses to HPV VLPs could be enhanced using a proprietary amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant. AAHS has been conjugated to a number of vaccine compounds and has a proven safety profile in humans with over a million doses delivered [17,35]. Ruiz et al. showed that in non-human primates the immune response to the quadrivalent HPV VLP vaccine conjugated to AAHS was 1–2 logs greater than the titers reached for the non-adjuvanted quadrivalent vaccine [34].

It should be noted that the chemical composition of aluminum-containing adjuvants can have a significant effect on the ability on a given antigen to associate with the adjuvant and induce an immune response. In a comparison of three major forms of aluminum adjuvants in clinical use, it was found that not all aluminum-containing adjuvants are the same, in fact, each had substantially different physical and chemical properties. The first type, aluminum hydroxide (AlOH) carries a net positive charge at neutral pH [35]. The second, aluminum phosphate (AlPO4) has a net negative charge at neutral pH [36,37], and the third type, Merck’s AAHS, carries approximately zero charge at neutral pH. Under physiological formulation conditions it was found that AAHS had the greatest capacity to bind HPV VLPs and that mice immunized with HPV 16 VLPs adsorbed to AAHS generated substantially higher antibody titers than mice immunized with VLPs adsorbed to AlOH [38]. Therefore, the final formulation of the quadrivalent HPV VLP vaccine, Gardasil®, contains HPV 16, 18, 6 and 11 VLPs stabilized with PS80 and NaCl during purification and storage and adsorbed to AAHS.

The primary mechanism of action of prophylactic vaccines is protection induced by antibodies generated through the humoral immune response to the vaccine antigen. To measure seroconversion, the production of vaccine-induced antibodies, a total IgG immunoassay is traditionally performed. Such an assay measures total antibody binding to the antigen. A complex antigen may contain many epitopes. The total IgG immunoassay measures end-point dilution titers to any antibody-antigen epitope binding. Not all antibody bound epitopes have the capacity to prevent or neutralize infection. Thus the total IgG immunoassay demonstrates
seroconversion or the induction of antibodies which recognize the antigen, but does not provide information on the ability of those antibodies to function in the prevention of infection or disease [39]. As there is no commercially available HPV serological assay nor serology standard by which to compare immunoassays, great care must be applied in understanding the limits of the kinds of data an immunoassay can provide and making comparisons between assays.

There is no known immune correlate of protection against HPV. In other words, the minimum antibody titer required to prevent or protect against HPV infection and disease is not known. In developing the quadrivalent HPV vaccine it was important to establish that upon vaccination subjects seroconverted to all four VLP components of the vaccine. In addition, an assay that would provide some link between the antibodies induced and protection was desired. The L1 proteins of HPV 16, 18, 6 and 11 share significant homology at the amino acid level (64–92%) and thus in a total IgG HPV 6 immunoassay for example, heterologous anti-HPV 11 antibodies would likely bind HPV 6 VLPs in addition to the homologous anti-HPV 6 VLP antibodies. This cross-reactivity would make it impossible to clearly establish HPV 16, 18, 6 and 11 type-specific seroconversion and antibody titers.

To ensure seroconversion of each of the four VLP types, a competitive Luminex immunoassay (cLIA) was developed to monitor a type-specific neutralizing, conformational epitope on the VLP of each of the four VLP types present in Gardasil® [40,41]. Four mAbs, H6.M48 for HPV 6, K11.B2 for HPV 11, H16.V5 for HPV 16 and H18.J4 for HPV 18 [41–44] have been shown to recognize neutralizing epitopes on the associated HPV VLPs. The ability of vaccine-induced serum antibodies to displace the phycoerythrin (PE) labeled mAbs from conformational, neutralizing epitopes on the VLPs is monitored in the assay read-out. Therefore, only a subpopulation of the total immune response is evaluated but it is a population of vaccine-induced antibodies that have the potential to provide protection against HPV [39].

Clinical trials of the quadrivalent HPV VLP vaccine Gardasil® have assessed seroconversion and cLIA geometric mean titers (GMT) in a number of populations [45–47]. Block et al. describes a study comparing the immunogenicity of Gardasil® in women 16–23 years of age to adolescent girls and boys between ages of 10 and 15 years. As has been typically found in our clinical trials, greater than 99% of all subjects generated antibodies to neutralizing epitopes on each of the four VLP types [47]. This outcome, high seroconversion rates, as assessed by antibodies with the potential to provide protection, was exactly what one would hope the quadrivalent HPV VLP vaccine could provide.

3. Efficacy and immune memory

We have demonstrated that we can produce a highly immunogenic quadrivalent HPV vaccine, the question now is whether the vaccine is in fact efficacious and what might the duration of protection be? Prior to initiating the Phase III clinical trials for Gardasil®, the question of which clinical endpoint should be monitored, was addressed. Clearly the most significant HPV-related disease endpoint is cervical cancer, however, progression to cervical cancer can take many years. Ethically, it is not a viable option to knowingly allow a woman to progress to cervical cancer. Typical progression is HPV infection then clearance or progression to CIN1. One may remain at CIN1, regress and possibly clear or progress to CIN2. One may remain at CIN 2, regress or progress to CIN3. One may remain at CIN3, regress or progress to cervical cancer. There are many intermediate steps on the way to cervical cancer. In discussions which included Merck, the FDA and WHO it was determined that although monitoring prevention of the first step, HPV infection, is useful, monitoring a disease stage that is a necessary direct precursor (CIN2/3) to cervical cancer would provide the best, most direct correlate of vaccine efficacy to prevent cervical cancer. For the low-risk types, a demonstration of prevention of external genital lesions would be the vaccine efficacy endpoint. Merck implemented these thoughts into the clinical plan to demonstrate the quadrivalent HPV VLP vaccine efficacy.

HPV-related disease is determined in standard practice by biopsy, followed by histological evaluation of the tissue. To ensure standardization of evaluation, a pathology panel of four independent pathologists was assembled and a diagnosis algorithm applied. H&E slides of each biopsy were sent in a blinded fashion to two pathologists for initial diagnosis [45,48]. All pathologists were blinded to the diagnoses results of the other members of the panel. If the diagnoses were in agreement then that diagnosis was accepted. If there was disagreement, then the slides were sent to a third pathologist. If two of the three diagnoses were in agreement then that diagnosis was accepted. If there was disagreement between all three then the slides would be sent out for evaluation by the fourth pathologist. In very rare cases there was no overall agreement. In that situation, all four pathologist were brought together to simultaneously evaluate the slides and come to an agreement on diagnoses.

To determine vaccine efficacy however required more information than just HPV-related histology. There needed to be a way to determine that the vaccine HPV types were or were not the cause of the HPV-related disease. This was determined by testing for the presence of vaccine-type (HPV 16, 18, 6 or 11) DNA in the biopsy tissue. As there was a desire to directly link the histological evaluation to the HPV genotype assessment each tissue was prepared and evaluated in a predetermined, structured way. The paraffin-embedded biopsy tissue was cut in 4 µm sections. The first two sections were placed on slides and stained by H&E. The next 9 sections were individually placed in sterile tubes and the final sections 12 and 13 were placed on slides and stained by H&E. The pathology panel received the H&E “bookend” slides to make their histological evaluation as described above and

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the HPV genotyping lab received sections 3–11 in individual tubes. The sections were tested in triplicate, i.e. DNA was isolated independently from sections 3–5 and HPV genotype determined. A consensus genotype of the three sections was reported as a single result. As was true for serology, there is no universally accepted standardized genotyping assay for HPV. Most genotyping assays utilize a two-step process of PCR amplification with degenerate or consensus primers followed by hybridization to a type-specific HPV sequence usually within the L1 gene. These assays seek to “type” up to 37 HPV types simultaneously. Utilizing just one amplification and just one hybridization condition to establish the presence of a specific HPV type via sequences of just one gene did not provide us with the sensitivity, specificity or confidence to ensure detection of the quadrivalent vaccine HPV types. Multiplex real-time PCR assays were developed by MRL for each of the HPV vaccine types (16, 18, 6 and 11) in which a portion of the E6, E7 and L1 genes were simultaneously amplified and detected utilizing type-specific probes for each of the three genes. A β-globin assay was also performed to ensure quality DNA had been recovered from the biopsy tissue [49]. The HPV genotyping lab was blinded to all biopsy identifier and to the results of the Pathology panel. The results of the pathology panel and the HPV genotyping lab were merged and results evaluated after input into the database was frozen.

The efficacy of the quadrivalent HPV VLP vaccine, Gardasil®, in the population of subjects with no protocol violations (PPE, pre-protocol evaluation), was 100% [45,46]. These subjects were naive to the vaccine types upon enrollment and remained naive to the vaccine types throughout the 7-month vaccination period. They received all three immunizations within the pre-specified intervals. Therefore, all HPV 16, 18, 6 or 11-disease endpoints were found to occur within the population that receive the placebo and none were attributable to disease in the vaccine group. The evaluation of the duration of efficacy of the quadrivalent vaccine is ongoing.

With 100% quadrivalent vaccine efficacy against disease caused by the vaccine types and no known minimum titer required for protection, it may take some time determine the duration of efficacy of the vaccine. There is evidence however that the quadrivalent HPV VLP vaccine produces a sustained antibody response through 5 years [46]. The antibody titers of a population within the P007 clinical trial were determined at month 60. These subjects were then given a fourth dose of Gardasil® and their antibody titers determined 1 week and 1 month post immunization. It was shown that the antibody titer levels had stabilized and were maintained from approximately month 12 through month 60. After the fourth immunization the antibody titers increased rapidly (1 week) and continued to climb (1 month) [50]. This strongly suggests that an amnesic response occurred and that the quadrivalent HPV VLP vaccine had induced B-cell memory both key components to the long-term duration of the vaccine.

4. Conclusions

A quadrivalent HPV 16, 18, 6 and 11 VLP vaccine has been effectively produced. It can be produced consistently at large scale and is stable. It has been packaged and delivered safely to thousands of subjects. Through the use of highly sensitive and specific HPV immunomasys and HPV genotyping assays it has been demonstrated that the vaccine is immunogenic and highly efficacious. The efficacious duration of the vaccine is not known, however it appears to be greater than 5 years. Pharmacovigilance plans are in place to monitor duration and determine possible future needs for a booster dose. HPV 16, 18, 6 and 11 cause the majority of HPV-related anogenital disease and cervical cancer. Gardasil® is the first cervical cancer vaccine. It has been licensed in many countries and efforts are on-going to expand its availability throughout the world. HPV-related disease is associated with significant morbidity and mortality. The quadrivalent HPV VLP vaccine, Gardasil®, has the potential to significantly reduce the HPV-related disease burden throughout the world.

Uncited reference

[33].

References


