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Chapter 11: HPV vaccines: Commercial Research & Development

Stephen Inglis [a,*], Alan Shaw [b], Scott Koenig [c]

[a] *National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK*
[b] *Vaxinnate, Cranbury, NJ, USA*
[c] *MacroGenics Inc., Rockville, MD, USA*

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Abstract

We are now in the fortunate position of having two highly promising human papillomavirus (HPV) vaccines in the pipeline. Amidst the excitement of anticipating what these vaccines may be able to offer, it is worth pausing to look back at how the vaccine development story unfolded from an industrial perspective, since without the massive commitment shown by manufacturers over the last decade, without any guarantee of success, there would be no such prospect.

This chapter focuses on the two HPV prophylactic vaccines, produced independently by Merck & Co., Inc. and GlaxoSmithKline (GSK), that are in advanced clinical development, and it aims to provide an insight into the key considerations for initiating the programmes in a commercial context as well as some of the research and development hurdles that needed to be surmounted to bring them to the point where efficacy has been demonstrated and the licensing process is well underway.
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1. Introduction

Development of any human medicine is an extremely complex, arduous and expensive process, and it is fair to say that vaccines are amongst the most challenging of all. They have a good track record in terms of their success, but the hurdles they must pass as medicines for healthy people are formidable, and the difficulties in running meaningful clinical studies to prevent diseases that may be relatively uncommon make them hugely demanding.

Development of HPV vaccines in a commercial context began in earnest around 1993, following a decade and a half of intense academic research that proved beyond doubt the causative link between HPV and cervical cancer and elucidated the basic natural history of the virus. From that point onwards, the investment of time and effort grew almost exponentially. The earliest research efforts involved teams of up to 20 working on cloning and assay development, and later stages of the programme involved more than ten times as many scientists to bring the vaccines through manufacture and clinical trials that have involved over 60,000 subjects. Over some 15 years, this adds up to a massive investment set against very high-risk. This kind of investment is simply not within reach of the public sector, underlining the crucial role of the private sector in such development programmes.

2. Getting to the starting post

2.1. Scientific case

The most crucial factor in stimulating commercial interest in HPV vaccine development was undoubtedly the scientific

* Corresponding author. Tel.: +44 1707 641400
E-mail address: singlis@nibsc.ac.uk (S. Inglis).

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evidence that protection against infection caused by papillomaviruses could be accomplished successfully. Both vaccine development programmes began on the basis of the discovery by several academic groups that the L1 coat protein of papillomaviruses could assemble into a virus-like particle (VLP) when expressed as a recombinant protein in a heterologous eukaryotic system. Collaborative studies with academic groups quickly showed that immunization with these L1 VLP structures could protect against viral challenges with cottontail rabbit papillomavirus (CRPV), bovine papillomavirus (BPV) and canine oral papillomavirus (COPV) [1–3]. CRPV studies were particularly influential on the Merck program (a collaboration with the Australian company CSL, which had in-licensed VLP technology from Ian Frazer's group at the University of Queensland). Wart formation could be completely abrogated by pre-incubation of the challenge stock of CRPV with antibodies from animals vaccinated with native VLPs, and all of the virus-neutralizing activity in the serum could be absorbed out by pre-incubation with an excess of native VLPs [4]. MedImmune, which subsequently formed a co-development alliance with GSK, was significantly influenced by COPV studies, which showed that oral papillomas could be prevented with a L1-based VLP vaccine alone, (VLPs containing L1 and L2 did not appear to offer additional protection), that very low doses (nanogram levels) were sufficient, and that protection could be generated by passive transfer of antibody [2]. Overall, the findings from the animal models served to convince both groups that native L1 VLPs were immunogenic and that the resulting protective immune response depended on conformation-specific antibodies.

2.2. Business case

It was clear from the outset that the development programme would be long and hugely demanding. From the beginning, therefore, there needed to be a justifiable business case to support the commitment of investment. Preparing such a case more than 10 years before likely introduction of a new product is not easy, as it has to account for all sorts of uncertain factors, such as price, competition, regulatory environment and likelihood of cost reimbursement, set against the risks of failure. As the level of investment required for a development programme goes up, this analysis becomes more and more sophisticated, but even at the outset, it is essential to be able to make a convincing case that the product, if successful, has a good chance of being profitable.

2.2.1. Medical need and marketability

Since genital disease caused by HPV (particularly precancerous cervical lesions, cervical cancer and genital warts) was clearly a significant public- and personal-health problem, it seemed from the start that there should be a strong demand for a vaccine on the grounds that prevention is better than a cure and that a prophylactic vaccine could be justified in terms of cost-effectiveness when weighed against the cost of medical interventions.

The question of marketability of a vaccine against HPV disease was complicated by the fact that effective cervical screening programmes were in place already in many developed countries and that cervical cancer rates were falling as a result. Offsetting this, however, was the growing evidence that HPV infection was extremely common, with a high incidence in the late teens and early 20s, and the recognition that precancerous cervical disease was itself a significant economic and psychosocial burden. With a regular cohort of about four million entering the sexually active pool each year in the US alone, the potential for "catch-up" vaccination in an older age group and the possibility of vaccinating males as well as females made the overall market size potentially very substantial.

A further, less concrete but nevertheless important factor, was a growing appreciation in both the public and private sector of the real value of vaccines (illustrated by the financial success of hepatitis B vaccines), which challenged the traditional view of them as low-cost "commodity" products with poor profit margins, high development risks and substantial liability issues and created renewed commercial enthusiasm for novel vaccine opportunities.

2.2.2. Intellectual property

The importance of intellectual property for successful commercialization of a product cannot be overstated. Companies need to be as sure as possible that they will be able to sell their product without insurmountable intellectual property obstacles when development is complete, which may be many years hence. They also need to be confident that if the product does come to market, it will garner sufficient sales to make the original investment worthwhile. In this case, holding intellectual property which prevents other companies from entering the market is a huge commercial advantage. Relevant patents can cover not only the product itself, but any element of the process by which it is produced.

In the case of the development of a HPV prophylactic vaccine, competing intellectual property was a critical issue. Several academic groups had filed patent applications for inventions relating to VLP technology around the same time. The experiments performed by the various groups were slightly different, as were the proposed patent claims, and the precise timing of the inventions was not publicly disclosed. Therefore, this was a highly complex landscape to negotiate through. In fact, the circumstances around these inventions were so unusual that the US Patent Office eventually declared a "four-party interference" whereby the patent applications from each of the groups would be examined for their distinct content and dates of invention, and even now, deliberations on these thorny issues continue.

Recently, Merck, GSK, and MedImmune agreed to settlement terms whereby each party would be granted access to relevant inventions independent of which of the four patent applications to the VLP structure ultimately proved to be dominant.

3. Development strategy: from preclinical to clinical development

Following the successful completion of proof-of-concept studies with the animal papillomavirus-based protection models, and a positive assessment of the business opportunity, Merck and Medimmune independently made the decision to initiate human clinical trials as quickly as possible to determine if neutralizing antibody responses could be generated in volunteers vaccinated with HPV L1 VLPs. Since the human papillomavirus could not be propagated readily in animal model systems, the amount of informative preclinical data that could be generated using HPV VLPs was limited. The observation that pre-incubation of antibodies raised against HPV-11 VLPs with infectious HPV-11 virus could prevent tumour formation in the challenge model developed by John Kreider was very important; this model involved implantation of HPV-11-infected neonatal human foreskin fragments under the sub-renal capsule of a nude mouse, which led to formation of large tumours and represented the first neutralization assay to be developed for HPV [5].

3.1. Vaccine design

The successful animal studies suggested that, at a minimum, a vigorous antibody response to the L1 protein of HPV would be required for a successful vaccine and that sustained detectable levels of antibody at the site of primary infection would be necessary for continued protection. The question of how to generate such a response in humans, and indeed whether it was likely to be sufficient to block the development of malignant genital disease, was less easy to answer. Parenteral vaccination with L1 VLPs induced specific IgG that protected dogs against an oral mucosal challenge with COPV [2], but it was by no means certain that this would translate to protection against tumour formation at a genital mucosal site in humans. Most of the animal models for protection were not based on malignant disease and so did not accurately reflect the situation in humans, where a long latency period exists between the time of the initial HPV infection, development of a pre-malignant lesion, and transformation to a frank malignancy.

There was also a substantial body of literature at that time, however, pointing to the importance of T-cell responses in clearing HPV infection; for example, infiltrating T-cells were clearly visible at sites of regressing papillomas and pre-malignant lesions. Resolution of HPV lesions is accompanied by a CD4 T lymphocyte-dependent response, although details of the effectors involved were, and are still, poorly understood [6,7]. It appeared quite likely, therefore, that an effective T-cell response could add to the efficacy of a prophylactic vaccine by "mopping up" any infected cells resulting from the virus breaking through antibody defences. Additional viral proteins (e.g. E6 and E7) could have been included in the vaccine composition as specific targets for T-cells, but the additional development hurdles would have been very significant. Nevertheless, the possibility of developing a vaccine formulation that could result in T-cell responses from the antigens already included was an important consideration. The VLPs alone might trigger T-cell responses on the basis of their efficient uptake into antigen presenting cells, but an additional option was to seek an adjuvant formulation that would boost T-cell responses specifically. This was one of the critical factors behind the Medimmune/GSK decision later on in their vaccine development programme to formulate the VLP vaccine using a new adjuvant. This choice was based on a desire to promote sustained protection through induction of high and persistent antibody titers while also inducing cell-mediated immunity. Although the selection of a novel adjuvant can always present a risk, promising safety data had already been generated with this adjuvant and the hepatitis B surface antigen [8]. Merck, on the other hand, chose to proceed with a traditional aluminium-based adjuvant, taking the view that it would offer a satisfactory compromise between tolerability and performance [9].

L1 VLPs appeared to induce very limited cross-neutralization against other genotypes [10] and it seemed most likely that multiple vaccine components would be needed to provide good coverage against diseases caused by more than one virus type. Merck and MedImmune each made a strategic decision early on to develop a multivalent vaccine composed of L1 VLPs corresponding to HPV types 6, 11, 16 and 18 in order to prevent both genital warts and the majority of cervical cancers. Following their development alliance later in the programme, MedImmune/GSK elected to concentrate specifically on prevention of cervical cancer, focusing on the two most common oncogenic HPV types found in tumours, 16 and 18, and incorporating the adjuvant AS04 for reasons mentioned previously.

3.2. Vaccine manufacture

Having selected the appropriate antigens to produce, it was then necessary to choose a suitable expression system. The choice needs to be based on factors which are both scientific and commercial. An expression system for large-scale vaccine development needs not only to produce high levels of the required proteins, but also to satisfy stringent regulatory requirements. Merck selected a yeast-based system comprising of just a single biological component (the producer cell carrying the L1 gene) [11] with which it already had substantial experience for vaccine production. MedImmune chose a baculovirus expression system, based on a number of factors including product yield. This decision was innovative yet also presented some risks from a regulatory perspective, as any new system will be subject to particularly close scrutiny. Although it had been used to produce candidates for clinical development outside Medimmune, none had been licensed at that point. Medimmune's chosen system required two components: an insect producer cell and a baculovirus strain engineered to carry the L1 gene. Various *Spodoptera* insect cell-lines were tested but found wanting

with respect to VLP yield and so a *Trichoplusia ni* cell line (Hi 5) was selected; this required substantial characterization and process development work by Medimmune and subsequently by GSK, taking account of substantial input from regulatory authorities, to make it a robust and consistent process for high-yield manufacturing and to meet the necessary standards.

Developing the expression systems to the point where they could support large-scale production was not straightforward. Medimmune encountered one particularly vexing issue related to the production of HPV-16 VLPs, a critical component of their vaccine composition. VLPs produced using a L1 gene sequence obtained from a clinical isolate (GU-2) provided by the Georgetown University group proved to be much less immunogenic than VLPs prepared from a C-generated truncated form of a different version of the gene (114 K) generated originally by a group at the Deutsches Krebsforschungszentrum (DKFZ). Detailed molecular analysis eventually traced this effect to a single amino acid difference between the two sequences at position 50. The L1 gene from a further clinical isolate obtained from the Georgetown group (GU-1) turned out to lack one of the antibody neutralization sites that had been identified by monoclonal antibody mapping. For this reason, the Medimmune program was ultimately based on the 114 K HPV-16 reference sequence, which contained all HPV-16 neutralization sites identified at the time.

3.3. Initial clinical trials

The complexity and risk of developing a quadrivalent formulation directly from the outset would have been very considerable, and so initial 'proof-of-principle' clinical trials in both programmes were based on a single aluminium-formulated VLP type, with the aim of using the resulting data and experience as a springboard for the next phase. The first was carried out using HPV-11 L1 VLPs by Medimmune. This strategy was strongly influenced by the fact that, at the time, HPV-11 was the only VLP type for which a practical neutralisation assay existed to assess the immunogenicity of the virus in vaccinated individuals; Bonnez at the University of Rochester had developed a method for generating small quantities of HPV-11 in SCID mice [12], and Lloyd Smith had used this to work up an *in vitro* neutralization assay based on infection of an immortalized human keratinocyte cells line (HaCaT) and subsequent analysis of intracellular production of HPV-specific RNA [13]. The success of these initial studies with HPV-11 laid the foundation for the clinical evaluation of the other HPV types.

3.4. Going it alone versus partnering

Development of any new biological product is very challenging, even for the biggest pharmaceutical companies, but for a small or medium-size biotechnology company developing a new prophylactic vaccine to prevent a cancer presents a particularly daunting landscape. In the early/mid-90s, key medical questions related to HPV and cervical cancer pathogenesis were unanswered, reagents for testing prototypic vaccines were scarce or absent, surrogates for immunological protection were speculative, competition for commercialization was intensifying, and huge resources were needed that involved substantial financial risk. Recognising these issues, CSL elected to license the intellectual property it held to Merck from the outset. Medimmune chose to take on the initial stages of product development itself, in order develop more value in the programme, but its situation in the mid-90s, with limited capital and human resources, a large commitment to clinical development of a monoclonal antibody to prevent respiratory syncytial virus infection in infants, and minimal commercial experience in vaccine development, was such that it made sense for them to enter into partnership with one of the other major vaccine development companies, GSK, for late-stage development.

4. Product development

Selecting a system to manufacture a defined biological product is just the first stage in the immensely time consuming and complex task of developing a process that can produce the product at the required scale and to the quality standards needed for licensure and sale. It is often said that for every scientist needed to carry out the research to create a product candidate, a further 10 will be needed to complete the subsequent development work. This stage of the project can be divided more or less into three parts: process development, formulation and analytical development.

4.1. Scale-up and process development

MedImmune's first clinical trial with HPV-11 VLPs in December 1996, the first prophylactic HPV trial to be initiated, was a great success and demonstrated the safety and immunogenicity of the VLPs. The process for manufacture of the trial material was, however, small-scale and labour intensive, involving purification of VLPs by density centrifugation. This initial process was inadequate for large-scale production, and later trials conducted using HPV-16 and -18 VLPs developed in conjunction with GSK were based on a radically different process designed to provide robust and consistent large-scale production while ensuring the safety and purity of the vaccine. This new process involved a variety of chromatographic and filtration techniques, including ion exchange, hydrophobic interaction and hydroxyapatite and gel filtration. Development of these techniques posed some unique challenges, given the large size of the VLPs. The ability to dissociate the VLPs into their constituent capsomers through the use of reducing agents, and to reassemble them subsequently by removing the reducing agent, proved very useful for the purification process. For example, the relatively small size of the capsomers allowed the addition of a

nanofiltration step to remove viruses that could theoretically contaminate the unpurified product.

Merck's process development programme also presented considerable challenges and took up a great deal of time, particularly the development of fermentation processes and solving the problem of significantly different yields between the engineered yeast strains expressing different HPV L1 gene types.

4.2. Formulation

The success of a biological medicine product depends critically on its final formulation, which needs to be such that the product can be manufactured consistently and that the "active substance" is stable enough to be useful in a real-world setting. Developing such a formulation is, in itself, a major undertaking and can throw up unexpected problems.

In MedImmune's first clinical study, the L1 VLP and the alum adjuvant components were stored in separate vials and mixed prior to the vaccine administration. The subsequent development programme, however, required the AS04-adjuvanted product to be formulated in a single vial. Because AS04 contains an aluminium salt as one of its components, it was not possible to perform a sterile filtration immediately before vialing, and so the decision was taken to add a widely used (at that time) preservative, thiomersal, to the vaccine, to ensure prevention of microorganism growth in the final vialed product. In initial trials with this material, very few of the vaccinated subjects developed neutralizing antibody responses against the appropriate VLP type, in spite of significant increases in ELISA titers. Thus, the vaccine preparations were immunogenic but failed to induce biologically relevant antibody responses. This was a significant setback to the vaccine development programme. It transpired that the incubation of HPV-16 L1 VLPs with thiomersal had resulted in the destruction of the structural epitope recognized by the V5 virus-neutralizing monoclonal antibody. Some four months after the original vaccination, a number of subjects from the phase I study were re-immunised with an AS04-formulated VLP preparation lacking thiomersal. All developed strong HPV-16 neutralizing antibodies following this booster. Consequently, thiomersal was removed from subsequent formulations of the HPV-16/18 vaccine.

4.3. Analytical development

The availability of high-quality analytical methods for product characterisation and testing is crucial to the development of any pharmaceutical product and this also requires enormous effort. Robust and fully validated tests need to be in place to ensure that each production step is performing as it should, and that the intermediates in the process, as well as each batch of the final product, meet the appropriate specifications consistently. One of the most important and fundamental aspects of the quality of any biological product is its potency, and for a vaccine, this equates to its immunogenicity. In the case of HPV L1 VLPs, immunogenicity was known to be dependent on the correct conformational structure of the VLPs, and so the development of a sensitive and practical assay for potency was a major goal. Both companies chose to develop new ELISA-based assays to measure the integrity of epitopes on the VLPs required for inducing neutralizing antibodies. Identifying these epitopes was, in itself, a substantial project. The Merck approach took advantage of the substantial serological cross-reactivity between HPV-11 and HPV-6, together with the availability of a set of neutralizing MAbs (monoclonal antibodies) from Neal Christensen, specific for either HPV-6 or HPV-11. Through immunological analysis of a series of chimeric type 6 and type 11 VLPs, made by swapping short sequences from one virus to the other, they were able to create an approximate map of the neutralising epitopes on the VLPs [14]. This proved extremely valuable for development of assays for measuring immune responses to these VLPs.

5. Measuring success in the clinic

As important as developing the product itself was developing techniques to measure its success. It was clear from the outset that ultimately an efficacy trial would be necessary to prove that the vaccine worked. Exactly what constituted efficacy was, however, a different question altogether, and this is dealt with in detail elsewhere in this monograph (see Chapters 12 and 13). Two crucial laboratory tests needed to be developed in a form that could provide accurate and valuable information to support clinical trials on a very large scale. The first was required to measure the presence of virus DNA (infection) in the genital tract following vaccination in a meaningful way, and the second to measure immune responses against the vaccine that related to its protective effect. These assays needed to be designed to give the best chance of answering unambiguously the many kinds of question that would be raised by the scientific community and by regulators, as not only part of the licensing process, but also of the post-implementation surveillance, should the vaccine be successful. The sheer scale of the assay work required to support the clinical trials is mind boggling. The phase 3 trials being conducted by the two manufacturers so far involved over 60,000 women, and with multiple time-point sampling and a large number of tests carried out on each sample, the number of assays required runs into many millions.

5.1. Detection of infection: PCR analysis

Prior work in academia had generated polymerase chain reaction (PCR) systems that relied on broadly cross-reactive primer sets to allow amplification of different genital HPV types. Typing was then achieved by applying type-specific probes to the primary PCR products. Different HPV detection methods were shown to vary in sensitivity and to potentially misclassify HPV type status in multiple infections, so

selection of a robust system was crucial. GSK elected to use the SPF_{10} PCR system in combination with type-specific PCR testing [15], aiming for sensitivity and specificity across a broad range of high-risk HPV types. Merck chose to develop a set of type-specific and gene-specific primers for HPV types 6, 11, 16 and 18 covering the L1, E6 and E7 genes; this meant carrying out 14 separate PCR reactions (including controls) on each sample, which was a huge undertaking. Initially, this was done using a dot-blot method, but eventually it was possible to reduce the resources required through an automated multiplex method [16].

5.2. Serological assays

Developing good assays to measure human immune responses against the vaccine was vital. Although antibody against the L1 VLPs was generally recognized to be the most likely protective mechanism, it was not clear what type or level of antibody response would constitute a serological correlate of protection for HPV infection. Selection of any kind of assay for anti-HPV antibody as a surrogate for vaccine efficacy was therefore fraught with difficulty. The scale of the clinical trials required to demonstrate efficacy directly against disease was such that a practical and robust form of serological analysis was needed. Furthermore, it was clear that in the long term the ability to link protective efficacy to a robust serological measurement would be hugely valuable for monitoring consistency, longevity of protection, predicting likely efficacy in different populations and the development of possible new vaccine formulations. Consequently, a great deal of effort was devoted to establishing serological assays for measuring antibody responses against the VLPs.

The assessment of neutralizing antibodies to HPV-16 and HPV-18 was hampered by the difficulties in producing infectious virus in the laboratory and in quantifying infectious events. High-throughput neutralizing assays have only recently been developed [17], and so the most widely used serological assays were ELISA based. Merck's initial serological assay involved the use of plate-immobilised HPV-6, -11, -16 and -18 L1 VLPs [18]. However, as more information emerged about likely neutralizing epitopes on the VLPs, a new assay was introduced, which relied on competition between antibodies raised in the vaccine and "tagged" monoclonal antibodies targeted at neutralising epitopes on the VLPs. Initially, measuring each HPV type required its own assay. Once again, the company developed an automated assay system based on the use of Luminex beads, in which multiple assays for different serotypes could be carried out simultaneously with the same sensitivity and specificity as the single-type assays, to reduce this huge workload [19].

The other major issue in the development of serological assays was the threshold signal that should be considered positive in the assay. Setting this too high could lead to underestimation of seroconversion rates following vaccination, but setting it too low might mean inappropriate exclusion of subjects from the clinical trial analysis (on the grounds that they appear to be already HPV-positive) and reduction in the statistical power of the trial. Typically, a serology assay uses a cut-off of three standard deviations above the mean of the background and is calibrated against a relevant international reference serum. At the time, however, there were no such standards available (see Chapter 23) and so it was necessary to make difficult internal decisions about what was the most reasonable approach.

6. Conclusions

The development of any prophylactic vaccine against any viral disease represents a mammoth task, but human papillomavirus represented a particularly daunting challenge at the outset for many reasons. The target was a persistent infection, thereby implying that natural infection was not consistently protective. Very little was known about the immune response to the virus, and progress was severely hampered by the lack of a simple cell-culture-based system to propagate the virus. Multiple types of virus were known to be involved in causing disease, and the disease end-point of major concern, i.e., cervical cancer, usually takes more than 20 years to develop from primary infection.

Along the way, extremely difficult decisions had to be made, with less than ideal information on which to base them. Committing huge resources to a programme that ultimately fails, although part of the landscape of pharmaceutical development, can damage even the largest company, and may pose significant professional risks to those involved. Though success is not yet assured, getting this far is a very considerable achievement.

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