CERVICAL CANCER VACCINE DEVELOPMENT

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**HPV and cancer**

Papillomaviruses were the first infectious agents to be unequivocally associated with tumour formation. While studies had linked animal Papillomavirus infection to induction of warts and of cutaneous cancer, human papillomaviruses proved extremely difficult to work with as they could not productively infect cells in culture, and therefore there was little material available to support serology and pathogenesis studies and test hypotheses concerning their oncogenicity, and it was unclear whether there was just one or a whole family of human papillomaviruses. Electron microscopy studies confirmed that human skin and genital warts contained virus particles similar in morphology to those found in rabbit warts, but as human skin warts “never” turned malignant, there was no evidence that human papillomaviruses might be involved in malignancy.

Prof Harold zur Hausen was nevertheless intrigued by the epidemiology of cervical cancer, which had long suggested an association of this cancer with sexual activity, and by the occasional malignant transformation of giant genital warts, which were clearly transmitted sexually. Development of tools for DNA analysis in the 1970s made it possible for his team to examine genital lesions including genital cancers for DNA homologous with the HPV DNA found in genital warts. These studies eventually revealed that there was genetic material partly homologous with that of wart papillomaviruses in cervical cancer and precancerous lesions(1;2). These findings, initially greeted with some scepticism, were eventually replicated by many teams, and during the 1980s there was increasing acceptance that papillomaviruses were likely causative agents for at least some anogenital cancer. This work led to the award of the Nobel Prize for Physiology and Medicine in 2008. Careful epidemiological work by many groups world wide has subsequently established that of the over 200 now known human papillomaviruses some 20 are associated with anogenital cancer(3), and that practically all cervical cancer and a substantial proportion of other anogenital squamous cancers are a consequence of persisting infection with one or more so called “high risk” HPV genotypes. From early studies, it was clear that two type, HPV16 and HPV18, were particularly associated with cervical cancer, and it is now accepted that across the globe approximately 70% of cervical cancer is attributable to persistent infection with
one of these viruses. Further, the relative risk of cancer given infection with these two viruses is significantly higher than for other genital HPV infections, perhaps because of their better ability to persist, to suppress immune function locally, and to promote induction and fixation of genetic errors within replicating epithelial cells.

**Preclinical studies leading to HPV vaccine development**

Recognition that HPVs contributed to cervical cancer renewed interest during the 1980s in the study of the pathogenesis of HPV associated cell proliferation, and of the host immune response to this unusual viral infection which, rather than killing infected skin cells, enabled them to proliferate and survive better than uninfected cells. However, Papillomavirus could still not be propagated in vitro, and research reagents for human papillomaviruses were limited to fragments of the viral genome propagated as recombinant plasmids in bacteria, the viral proteins that could be expressed from these in bacteria, and antibodies raised to these proteins in animals. These reagents allowed further progress on the understanding of viral pathogenesis. Research was in large part undertaken with bovine papillomaviruses, a relatively abundant source of viral material. However nothing resembling the Papillomavirus itself had been produced in vitro. A fortuitous meeting that I had in 1989 in Cambridge England with the late Dr Jian Zhou, when we were both visiting the lab of Dr Lionel Crawford, led us to use some new molecular biology techniques developed by Dr Zhou to try to make a Papillomavirus in vitro. Our initial interest was more in how Papillomavirus worked as a virus, and how it was seen by the immune system, than in whether we could make a vaccine, for at that time the connection between HPV infection and cervical cancer was still subject to debate. There was a feeling that cervical cancer was a common outcome from a rare virus infection, rather than, as we now recognise, a rare outcome of persistence of infection with a common infectious agent. These considerations and the possibility that the virus involved was oncogenic hindered enthusiasm for vaccine development. However, we needed to make the virus capsid, or coat, to make a virus in vitro. We chose to attempt this using a clone of HPV16 derived from a clinical lesion (a fortunate choice) and expressed using a viral vector in epithelial cells (another fortunate choice). When we didn’t get much protein, we experimented with whether we were starting the translation of the gene at the correct site and (again a fortunate choice) located another potential start site somewhat upstream from what seemed the logical start position. These strategies enabled us to produce in vitro for the first time something that
looked like the virus on electron microscopic examination (4). While the yield wasn’t great, the first electron micrographs of the synthetic virus shell, which had self assembled from the capsid protein building blocks, suggested that a vaccine based on this technology might be possible. We and others then refined the expression technology, produced higher yields of the virus like particles in better expression systems, and demonstrated that they were immunogenic (5-8). The vaccines available today are based on these virus like particles (4;9), empty shells comprising 360 copies of the viral capsid protein L1 which self assemble to look like the virus to the immune system. These virus like particles, or VLPs, are now made in yeast or in insect cells. They are combined with a mineral adjuvant, Alum, with or without monophosphoryl lipid A, a bacterial cell wall component, to help stimulate a strong immune response (10). The vaccines currently include either two (HPV16 and HPV18) or four (HPV 6, 11, 16, 18) HPV types. The two extra types in the quadrivalent vaccine are responsible for about 90% of genital warts.

Future vaccines may contain more HPV types but will likely continue to be based on virus like particles. VLP based vaccines incorporating multiple HPV types are currently in clinical trial. However, other potential strategies in preclinical development that may eventually reach the clinical are worth noting. VLPs are assembled from 72 copies of a basic building block, the pentamer, which is assembled from 5 L1 molecules, and many of the neutralising epitopes presented by the VLP are also presented by the pentamer. Thus simpler vaccines based on pentamers have been suggested (11), though no simple process for their production and purification has yet been promulgated. An alternative vaccine strategy is based on the minor capsid protein, L2, which includes a number of neutralising epitopes. While the virus neutralisation potency of antisera raised against whole length L2 does not appear as great as for antisera raised against intact L1 capsids, some L2 epitopes are shared across many HPV types and a vaccine incorporating these epitopes together with immunogenic carrier protein may therefore provide extended coverage against the HPV types not included in VLP based vaccines (12;13).

Clinical Vaccine development studies
Production and testing of virus like particle based Papillomavirus vaccines was undertaken commercially from the late 1990s by two pharmaceutical companies, Merck and GSK. Initial trials to demonstrate the safety and efficacy of vaccines designed to prevent infection with genital HPV infections and also to prevent the premalignant consequences of these
infections were undertaken in young sexually active women, though more recently studies have been extended to include older women, and men. Phase 1 studies are designed to demonstrate safety and immunogenicity in human subjects, and Phase 2 studies extended these data to include data on prevention of infection with relevant HPV types. Pivotal licensing studies address efficacy in prevention of HPV associated disease, including cervical and other anogenital pre-cancer (CIN2,3) and, for the quadrivalent vaccine, genital warts. Subsequent phase 4 post marketing studies have addressed efficacy in particular target populations, and duration of protection.

Phase 1 studies, in subjects with and without HPV infection, confirmed the safety and immunogenicity of VLP-based vaccines, given intramuscularly with or without adjuvant (14-16). Three administrations of VLPs with adjuvant induce peak antibody levels at least 10 times those seen in subjects naturally infected with HPV, and levels of antibody above those produced by natural infection have been sustained for at least 8 years post vaccination.

Phase 2 studies (17-19) further addressed immunogenicity. They also tested ability to prevent infection in women naïve to the HPV types in the administered vaccine at recruitment, as assessed by failure to detect of HPV DNA in genital samples, and by absence of serum antibody to viral capsids of the relevant type. These studies have shown nearly 100% efficacy of both the bivalent and the quadrivalent vaccines in prevention of acquisition of HPV infections caused by the types present in the vaccines, amongst sexually active young women. Phase 3 studies (20-25) have demonstrated near 100% efficacy in “according to protocol analyses” at preventing HPV associated anogenital disease due to vaccine HPV types, in young sexually active women, with efficacy data now extended to 4-5 years in most studies. In these studies, if a new cytological or clinical abnormality was detected which included a vaccine HPV DNA type, even if other HPV types were also present in the same lesion, the vaccine HPV type was held to be causal. This interpretation biases study results away from effectiveness, and may be responsible for the few cases of disease associated with vaccine HPV types in these studies, as infection with multiple HPV types are now increasingly recognised. For the phase III efficacy studies, young sexually active women were recruited whether or not they had prior HPV infection, so long as they did not have, or have a history of, anogenital disease that might be HPV related. The studies therefore
included many women infected with vaccine HPV types at recruitment. Immunisation did not appear to impact on the natural history of these existing HPV infections, which regressed or progressed at similar rates in vaccine and placebo recipients (26;27). In consequence, vaccine efficacy is much less in “intention to treat” analyses of prevention of all HPV related disease, particularly where these included the 30% of disease due to non-vaccine HPV types, as well as women already infected with a vaccine HPV type but without disease at recruitment.

In the major efficacy studies, immunisation with HPV 16 and HPV18 provided some protection against infection with high risk HPVs of other types, and against disease attributable to these infections. In a pivotal trial of a bivalent HPV16/18 vaccine, significant protection was seen against HPV45 infection, and partial protection against HPV33 infection (24). For the quadrivalent HPV 6/11/16/18 vaccine, generally HPV naïve subjects showed some protection against new anogenital pre-malignancy (CIN 2/3 and AIS) associated with 10 non-vaccine HPV types (28). In each of these studies, actual case numbers were quite low, and the true degree of protection therefore hard to assess accurately – however the overall efficacy would not be sufficient to alter current clinical practice and the need to continue assessing women for cervical pre-cancer through conventional screening. Nevertheless field effectiveness in a vaccine population can be inferred from the significant reduction in genital wart disease observed amongst the target vaccine female population in Australia (27;29) where up to 50% coverage has been achieved in 18-25 year olds.

**Vaccine Safety**

Adverse events after vaccination are assessed from the placebo controlled efficacy studies. However, rare events can be assessed effectively only through post marketing surveillance. In the placebo controlled studies, local reactogenicity at the site of immunisation and systemic malaise was more common than with placebo, but was generally mild and did not lead to discontinuation of vaccination. No serious adverse events were attributable to vaccination in the placebo controlled trials.
Over 40 million doses of the quadrivalent vaccine have been delivered to young women subsequent to vaccine licensure. According to the vaccine adverse events reporting service, no rare, serious events have been seen with more frequency in vaccine recipients than might be expected in an unvaccinated age matched community. Fainting after vaccination is the most commonly reported event. HPV vaccines also seems safe in pregnancy, with no increased frequency of adverse pregnancy outcomes. While vaccination during pregnancy is not recommended, women who become pregnant while undergoing vaccination can be reassured that there is unlikely to be any consequence for the pregnancy. Allergic reactions likely attributable to vaccination are rare, with reported frequency of 1 -2 per million vaccine doses delivered.

**Duration of protection**

Most viral vaccines work by inducing neutralising antibody to conformational determinants on the surface of the virion. Antibody to conformational determinants on the Papillomavirus capsid is sufficient to convey protection against challenge with live Papillomavirus when passively transferred in either a dog or a rabbit model, and, somewhat surprisingly, serum IgG antibody seems sufficient to convey protection.

A surrogate marker for virus induced protection against infection would be useful for assessing duration of protection post immunisation. There are no standardised assays for antibody to HPV virions through though the World Health Organisation in collaboration with the National Centre for Biological Standards is attempting to produce one for HPV16. Antibodies to HPV are measured in human serum in a range of in vitro and in vivo assays. Each assay measures different specificities of antibody, and a different proportion of the total virus neutralising capacity of a serum, and thus direct comparisons of antibody titre are only valid for antibody to one virus type, measured in one assay.

Antibody assays have nevertheless been used as bridging assays for the introduction of vaccine into age groups where new infection with HPV is uncommon, including male and female children aged 9-15, who produce on average higher levels of antibody than the 16-24 year old women in whom vaccine efficacy studies were undertaken, and in women
aged 25-45, whose antibody response is on average somewhat lower. The quadrivalent HPV vaccine has been shown at least 90% effective at preventing new infections with HR HPVs in 25-45 year old women(37).

Duration of protection following vaccination can also be inferred from serology studies. Antibody levels peak after three doses of vaccine at levels at least 20 times higher than those seen after natural infection, and fall significantly during the first two years after immunisation, and then remain fairly constant out to five years, at a level well above that seen with natural infection(38), and these levels are associated with continuing protection against infection. Further, immunological memory is retained, as a single re-immunisation five years after the primary immunisation results in a substantial boost to antibody titres, not seen in non-immune subjects(39). These data can be modelled to suggest that protection from these vaccines is likely to be long lasting.

Declaration of Conflict of Interest
Ian Frazer derives royalties from the sale of VLP based vaccines, and consults for and receives speaker fees from Merck, GSK, and CSL Ltd.

Reference List


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