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Tumour virus vaccines: hepatitis B virus and human papillomavirus

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Two of the most important human oncogenic viruses are hepatitis B virus (HBV) and human papillomavirus (HPV). HBV infection has been preventable by vaccination since 1982; vaccination of neonates and infants is highly effective, resulting already in decreased rates of new infections, chronic liver disease and hepato-cellular carcinoma. Nonetheless, HBV remains a global public health problem with high rates of vertical transmission from mother to child in some regions. Prophylactic HPV vaccines composed of virus-like particles (VLPs) of the L1 capsid protein have been licensed since 2006/2007. These target infection by the oncogenic HPVs 16 and 18 (the cause of 70% of cervical cancers); a new vaccine licensed in 2014/2015 additionally targets HPVs 31, 33, 45, 52, 58. HPV vaccines are now included in the national immunization programmes in many countries, with young adolescent peripubertal girls the usual cohort for immunization. Population effectiveness in women is now being demonstrated in countries with high vaccine coverage with significant reductions in high-grade cervical intra-epithelial neoplasia (a surrogate for cervical cancer), genital warts and vaccine HPV type genoprevalence. Herd effects in young heterosexual men and older women are evident. Cancers caused by HBV and HPV should, in theory, be amenable to immunotherapies and various therapeutic vaccines for HPV in particular are in development and/or in clinical trial.

This article is part of the themed issue 'Human oncogenic viruses'.

1. Introduction

The evidence that some cancers are caused by infection with a specific virus implies that intervention against these infections, by prevention or treatment, should be able to prevent the vast majority of such cancers worldwide and have a major public health impact. With respect to HBV (hepatitis B virus) and HPV (human papillomavirus) associated cancers, this presumption is now underpinned by solid evidence with the development and implementation over the past 3–4 decades of effective prophylactic vaccines.

2. Hepatitis B virus

Cancer caused by infection with the HBV is a major global health problem (http://www.who.int/mediacentre/factsheets/fs204/en/). It is estimated that 257 million people are infected with HBV (defined as hepatitis B surface antigen positive–HBVsAg+), and in 2015 HBV resulted in 887 000 deaths predominantly from cirrhosis and hepatocellular cancer. Most cases occur in low- and middle-income countries with the highest prevalence in the WHO Western Pacific and African regions, 6.2% and 6.1%, respectively. The virus is highly infectious, abundantly found in blood and body fluids of infected persons many of whom are asymptomatic and unaware of their disease. HBV is transmitted via the parenteral route, infusion of infected blood or blood products, needle sharing, penetration of micro-wounds on skin or mucosae, etc. [1]. Child to child transmission causes many HBV infections in low- and middle-income countries, but also, many infections in these settings are vertically transmitted from an infected mother to her child during parturition. Infection acquired by this route of transmission is probably the major contributor to the development of hepatocellular carcinoma (HCC) in the

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long term. Ninety per cent of those infected at birth and 30–50% infected between 1 and 4 years of age develop chronic hepatitis (HBsAg +ve) and have a 25% risk of dying prematurely from cirrhosis and/or HCC [2]. Current strategies for controlling HBV infection include active immunization, passive immunization and chemoprophylaxis with antiviral drugs [1], but although passive immunization and chemoprophylaxis can contribute to the control of HBV infection, the only preventive strategy is active immunization.

3. Hepatitis B virus prophylactic vaccines

Two types of vaccine, plasma-derived and recombinant, are available for active immunization against HBV. HBV cannot be grown efficiently in tissue culture and the first-generation vaccines were inactivated and purified HBsAg particles derived from the plasma of chronic carriers. Subsequently, this was replaced in virtually all countries by second-generation vaccines generated by expression of the gene sequence encoding the HBsAg in recombinant vectors, mainly the yeast *Saccharomyces cerevisiae*. Both plasma and recombinant vaccines are comparable in terms of efficacy and durability, contain the HBsAg and elicit the development of specific antibodies, anti-HBs, which alone confer protective immunity [3]. The protective titre (immune correlate) is accepted as more than 10 IU ml^{-1} of anti-HBs antibody [4]. The recommended immunization schedule is a three-dose: prime, prime, boost at 0, 1 or 2 and 6 months delivered intramuscularly [2], although a two-dose immunization schedule is used for adolescents in some countries. The antibody response to the recombinant vaccine is robust in children and adults under 40 years, but older adults, the immunocompromised and those with chronic conditions, such as coeliac disease, diabetes and renal disease, respond less well. The anti-HB antibody persists in most subjects for many years ($\geq$20 years) after immunization, but even when titres fall below 10 IU ml^{-1}, most subjects continue to be protected and show strong recall responses to a booster dose of the vaccine. Vaccine-conferred protection is probably lifelong and booster doses are not recommended.

Initially the strategy for HBV vaccination was to immunize at-risk groups such as babies born to infected mothers, intravenous drug users, sex workers and healthcare professionals. This strategy was not successful and it was recognized that to reduce virus prevalence and block transmission, the infant reservoir of virus had to be targeted by vaccinating babies soon after birth to prevent infection and carriage in early life. In 1992, WHO advised all countries to include HBV in all universal vaccination programmes and the implementation of this, particularly in low- and middle-income countries with high virus prevalence, has resulted in a worldwide decrease in carrier rate and disease burden and has shown the first evidence of impact on the incidence of HCC [5].

4. Hepatitis B virus vaccine impact

Widespread HBV immunization of neonates has had a major impact on the incidence of chronic HBV infection in children 5 years of age and under globally, and the estimated prevalence now in this age group is 1.3% compared to 4.7% in the pre-vaccination era. There is now evidence that universal hepatitis B immunization in infancy can effectively reduce the incidence of HCC in children and adolescents [2,6,7]. However, the incidence rate of liver cancer increases with age and HCC incidence is much higher in adults than children [8] so is there formal evidence that HBV immunization at birth provides protection against HCC in adults? Taiwan is endemic for chronic HBV infection and HCC; it implemented the world's first universal HBV immunization programme for infants starting in July 1986, and in a recent study the long-term effects of the Taiwan infant immunization programme in preventing HCC were reported [9]. In this analysis, 1509 patients 6–26 years of age diagnosed with HCC between 1983 and 2011 were identified, their HBV vaccination history and prenatal maternal levels of HBVsAg collated, and the relative risks (RRs) of vaccinated versus unvaccinated calculated. The RRs for different birth cohorts 6–9, 10–14, 15–19 and 20–26 years were, respectively, 0.26 (CI 0.17–0.40), 0.34 (CI 0.25–0.48), 0.37 (CI 0.25–0.51) and 0.42 (0.32–0.36). The incidence of HCC/10 [5] person-years in the unvaccinated cohort was 0.92 compared with 0.23 in the vaccinated cohort, leading the authors to conclude that 'universal HBV immunization in infants has successfully prevented liver cancer both in children and young adults'.

Routine neonatal, infant and adolescent vaccination programmes have significantly changed the epidemiology of HBV infection [10,11] and this has now reduced and will continue to reduce the disease burden of HCC globally [12]. Nonetheless, about 5% of immunocompetent individuals are non-responders and fail to seroconvert after the regular three-dose regimen or a fourth booster dose of the HBV vaccine. Various strategies have been or are under development, to develop vaccines that elicit a more potent immune response. The current licensed yeast recombinant second-generation vaccines contain partially misfolded HBsAg and lack the preS1 antigen that carries the major HBV attachment site and neutralizing epitopes. Third-generation vaccines produced in mammalian cells contain correctly folded HBsAg and the neutralizing epitopes of the preS antigens (reviewed in [7]). These vaccines increase seroconversion among non-responders [13,14] and may provide better protection for neonates born to highly infectious mothers. Other strategies to enhance immunogenicity focus on novel adjuvants [6] such as MPL (3-deacylatedmonophosphoryl lipid A), an agonist of toll-like receptor 4, which has been shown to induce immune responses in non-responders [15] and the polysaccharide adjuvant delta inulin [16].

Therapeutic vaccines against HBV infection have proved disappointing despite heavy investment in time and money [1]. Any immunotherapeutic strategy that elicited robust HBV antigen-specific cytotoxic effectors would have inherent risks because HBV is non-cytopathic, and immunological mechanisms contribute significantly to the chronic inflammation that underpins HBV-induced cirrhosis and HCC.

5. Human papillomavirus

HPVs are a very large branch of the papillomavirus family with DNA of more than 170 HPV types, many of which have been fully sequenced, having been isolated from tissue biopsies [17]. Despite the daunting number of HPVs, they fall basically into two groups: those that infect skin, or cutaneous surfaces, and those that infect the internal wet squamous mucosal surfaces, particularly the genital tract. Within these groups, there are low-risk types (LRHPV), which generate benign lesions, in other words warts, and high-risk or oncogenic types

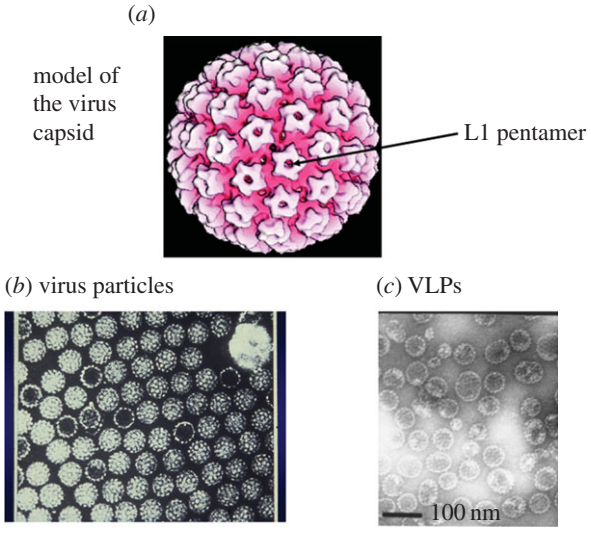

Figure 1. (*a*) A model of the papillomavirus coat or capsid. There are two coat proteins L1 and L2. The rosette-like surface structures (arrowed) are pentamers, each consisting of five molecules of L1; one molecule of L2 fits into the central dimple of each pentamer. (*b*) Papillomavirus particles; both full (contain DNA) and empty particles can be seen. (*c*) HPV 16 L1 virus-like particles, VLPs, made by expressing the HPV 16 L1 gene in baculovirus. The L1 protein so expressed spontaneously assembles into empty capsids or VLPs that are morphologically similar to the empty virus particles seen in (*b*). From Stanley *et al.* [24] with permission. (Online version in colour.)

(HRHPV), which are associated with cancers and their precursor lesions [18]. HPV-associated cancer in the genital tract is dominated by HPV16 and HPV18, which, with their close relatives 31, 33, 35, 52, 58, 39, 45, 59, 56 and 51, are the cause of virtually all cervical cancers and the majority of the high-grade cervical cancer precursor lesions CIN2/3 (cervical intra-epithelial neoplasia grades 2 and 3) [19]. Thus, 99% or more of biopsies of invasive cervical cancer worldwide, and approximately 80% of CIN 2/3 contain HRHPV DNA sequences [20]. HPV16 dominates, and is present in at least 50% of cancers irrespective of geographical location, followed by HPV18, 7–20%. However, invasive cervical cancer is not the only malignant disease associated with HRHPV infection; HPV DNA sequences are found in most anal and tonsil carcinomas, and a proportion of vulvar, vaginal, penile, and head and neck cancers [21]. HPV16 again is the dominant oncogenic type [19,22,23]. Overall, the global malignant burden attributable to HPV infection is calculated to be 5.2% of all cancers.

6. Human papillomavirus prophylactic vaccines

HPV prophylactic vaccines are subunit vaccines consisting of virus-like particles (VLPs) made of only one protein—the major HPV coat or capsid protein L1. The VLPs are made using sophisticated recombinant technology in which the L1 gene is expressed in recombinant yeast or baculovirus vectors. The chemistry of the expressed protein is such that it spontaneously assembles into VLPs that are morphologically (and more importantly) immunologically similar to the native virus but lack DNA and are therefore non-infectious (figure 1). Currently, there are three licensed VLP vaccines. These are Cervarix®, a bivalent HPV16/18 product (2vHPV) from GlaxoSmithKline Biologicals, Rixensart, Belgium; Gardasil® also known as Silgard, a quadrivalent HPV16/18/6/11 product (4vHPV); and Gardasil 9, a nonavalent 6, 11, 16, 18, 31, 33, 45, 52, 58 product (9vHPV), both from MSD, Whitehouse Station, New Jersey, USA; the details of these vaccines are given in table 1. All vaccines have undergone large, randomized, placebo-controlled, double-blind trials (RCTs) and have demonstrated more than 90% efficacy against HPV vaccine type associated anogenital disease and against persistent infection in individuals naive for vaccine HPV types at trial entry and at the completion of the three-dose immunization regimen [25,26]. In the optimal scenario, the 2vHPV and 4vHPV vaccines should reduce cervical cancer incidence by 70%; and the 9vHPV vaccine, by ≤90% [27].

7. Vaccine impact and effectiveness

The 4vHPV vaccine and 2vHPV vaccines were licensed in 2006/2007 and, in the subsequent 10 years, have been introduced into the National Immunization Programmes of more than 62 countries. In general, HPV immunization programmes target adolescent girls 9–15 years of age, with or without catch-up for older adolescents and young women. Anogenital HPV infections are sexually transmitted with the first infection occurring most frequently within the 6–12 months following the start of sexual activity [28]. To achieve optimal vaccine effectiveness, immunization ideally should be completed before the start of sexual activity and this is why the target group for immunization are young adolescents. Cervical cancer in women is the most prevalent HPV-associated cancer and this plus the cost of cervical cancer screening programmes made the cost-effectiveness case initially for immunizing perimenarchal girls [29].

The impact, at the population level, on the incidence of vaccine HPV type infection and disease of a three-dose female-only HPV vaccination has been assessed recently [30,31]. In countries achieving more than 50% vaccine coverage, HPV 16 and 18 infections decreased by 68%; significant reductions in infections with the non-vaccine HPV types 31, 45, 33 were also recorded, suggesting some cross protection. In populations immunized with the 4vHPV vaccine, anogenital warts fell by 61% in girls 13–19 years of age, and reductions in warts were reported in men less than 20 years of age and in women 20–29 years, implying herd effects. In countries with less than 50% vaccine coverage, significant reductions in 16/18 infection and anogenital warts occurred in women less than 20 years of age, but no cross protection or herd effects were demonstrated.

Reductions in cervical cancer will only be seen in the long term—decades after vaccination—but a reduction in high-grade cervical intra-epithelial lesions CIN2/CIN3 caused by HPV 16/18 in vaccinated cohorts should be a surrogate for the long-term effects on cervical cancer incidence. Approximately 50% of CIN2/CIN3 are attributable to HPV16 and 18 [18]. The Australian National Vaccination Programme, a school-based programme targeted to girls aged 12–13 years with a catch-up over 2 years for 13–26 year old young women, was introduced in 2007 and achieved ≥70% coverage in the school cohort. Four years after the implementation of vaccination with the 4vHPV vaccine, high-grade cervical abnormalities (CIN2/3, AIS) in the vaccinated cohorts (age 12–26 years) receiving three doses of vaccine decreased by about 48% [32]. Comparable data have been reported from several countries using the 4vHPV vaccine (for review see [31]) and from Scotland with the 2vHPV vaccine [33]. In all studies,

Table 1. Prophylactic HPV VLP vaccines.

	Cervarix bivalent vaccine		Gardasil quadrivalent vaccine		Gardasil9 nonavalent vaccine	
manufacturer	Glaxo Smith Kline		Merck		Merck	
volume	per dose	0.5 ml	per dose	0.5 ml	per dose	0.5 ml
adjuvant	AS04:		amorphous	225 mg	amorphous	500 mg
	$Al(OH)_3$	500 mg	aluminium		aluminium	
	MPL®	50 mg	hydroxyphosphate sulphate		hydroxyphosphate sulphate	
antigens	L1 HPV16	20 µg	L1 HPV6	20 µg	L1 HPV6	30 µg
	L1 HPV18	20 µg	L1 HPV11	40 µg	L1 HPV11	40 µg
			L1 HPV16	40 µg	L1 HPV16	60 µg
			L1 HPV18	20 µg	L1 HPV18	40 µg
					L1 HPV31	20 µg
					L1 HPV33	20 µg
					L1 HPV45	20 µg
					L1 HPV52	20 µg
					L1 HPV58	20 µg
expression system	Hi-5 baculovirus		yeast: *Saccharomyces cerevisiae*		yeast: *Saccharomyces cerevisiae*	
immunization schedule	intramuscular		intramuscular		intramuscular	
>15 years age	0, 1, 6 months		0, 2, 6 months		0, 2, 6 months	
<15 years age	0, 6 or 12 months		0, 6 or 12 months		0, 6 or 12 months	
FDA-licensed	2009		2006		2014	
EMA-licensed	2007		2007		2015	

the largest declines in cervical precancers were shown in the youngest vaccinated birth cohorts [31,33].

8. Gender-neutral human papillomavirus vaccination

In economically developed countries, the incidence of cervical cancer in women has been significantly reduced as a result of cervical screening programmes to detect and then treat high-grade cervical intra-epithelial neoplasms (CIN), the obligate precursor to invasive cancer [34]. However, the other cancers associated with HPV are not amenable to screening and the incidence of these is rising in both men and women [35] and in developed countries, such as those in Western Europe, the burden of HPV-associated cancers in men is comparable to that in women. There are interesting differences between men and women in the immune response to natural genital HPV infection. HPV infection varies with age in women, with the peak prevalence in the late teens and twenties declining steadily throughout the subsequent decades [36]; in contrast, men acquire infection in the late teens and the prevalence remains constant throughout the subsequent decades [37,38]. The majority of women, ≥70%, seroconvert after detectable cervical HPV infection with antibody to the major coat protein L1 [39], but only ≥20–30% of men do so [40]. Despite the poor antibody response in natural infection, men make robust humoral immune responses to VLP vaccines with virtually 100% seroconversion [41]. Trials with the 4vHPV vaccine have shown efficacy against infection and disease in men who have sex with women (MSW) [42] and men who have sex with men (MSM) [43], preventing 6/11+ve genital warts and 6/11/16/18+ve anal intra-epithelial neoplasia, respectively. Men clearly will benefit from HPV vaccination, but despite the widespread introduction of national HPV immunization programmes for girls and women, few countries have recommendations for boys and men. Gender-neutral vaccination is a controversial issue. The rationale for immunizing only one gender (females) against a sexually transmitted infection is that where immunization coverage is high enough, this generates herd protection by blocking transmission to effectively protect the sexual partners [44]. Female-only vaccination by definition cannot achieve herd immunity because heterosexual men are not immune but protected if their sex partners are immune or uninfected, but remain susceptible to infection if this scenario changes. Men who have sex with men (MSM) are left entirely unprotected by the female-only approach and HPV genoprevalence remains high in this group. MSM can be immunized as a high-risk group, but there are many challenges and immunizing at-risk groups does not have a good track record. HPV vaccines are highly effective and, in common with most vaccines, depend for their impact at the population level upon the indirect effects of reducing transmission and carriage. Gender-neutral vaccination is the required strategy for optimal epidemiological benefit because it is the most effective way to break the chain of infection.

9. Mechanism of protection of human papillomavirus vaccines

The current assumption is that neutralizing antibody is the mechanism of protection afforded by HPV VLP vaccines [45]. In contrast to natural HPV infection, in which the humoral

immune response is slow and weak and not all individuals seroconvert, systemic immunization with L1 VLP vaccines generates high serum-neutralizing antibody concentrations at least 50–1000 times greater than those measured in natural infections and virtually all vaccines seroconvert [46,47]. Following the three-dose immunization schedule, geometric mean titres (GMTs) for antibodies to the vaccine HPV types peak at month 7; GMTs then wane until 18–24 months at which there is a plateau level at about 10 times that of natural infection, and this level persists for at least 10–12 years after the primary immunization [48]. Antigen challenge at ≥60 months post dose 1 results in a rapid, and robust, anamnestic response with antibody concentrations rising within a week to levels greater than that achieved at peak (one month post dose 3) in the initial immunization schedule, demonstrating the presence of reactive memory B cells [49–51]. Mathematical modelling predicts slow decay of antibody over a 30–50 year period and potentially, therefore, protection over that time [52,53]. However, there is no immune correlate of protection against infection or disease; the minimum level of antibody, the affinity/avidity of such antibody needed for protection and the role of B cell memory if the antibody wanes have yet to be established.

So a central question of contemporary interest and debate is how much antibody is needed and how many doses are really essential for protection? In the RCTs that led to licensure for the current products, an immunization schedule of three doses at 0, 1–2 and 6 months was used. As the intended cohorts for immunization were young adolescents, immunobridging studies were undertaken in 9–15 year olds and showed that GMTs in these cohorts were twice those achieved in the 16–26 year old women in whom efficacy had been demonstrated in the RCTs [54–56]. Subsequent studies investigated the impact on immunogenicity in the young adolescent cohort (9–14 year old girls and boys) of changing from the three-dose 'prime, prime, boost' to a two-dose 'prime, boost' at 0 and 6 months and showed that two doses of vaccine at 0 and 6 months resulted in antibody responses (GMTs) that were non-inferior over a 3 or 4 year period to those achieved after three doses in 16–26 year old women. As antibody concentration and quality for two doses given at 0 and 6 months in adolescents are as good as or better than for three doses given at 0, 1/2 and 6 months in older women, vaccine-mediated protection and duration of protection should be comparable. The Strategic Advisory Group of Experts on Immunization (SAGE) of WHO in April 2014 reviewed the evidence [57] and 'recommended a two-dose schedule for girls, if vaccination is initiated prior to 15 years of age. A three-dose schedule remains necessary if immunization is initiated after the girls' 15th birthday. The recommended minimal interval between the two doses is six months. This interval may be extended to 12 months if this facilitates administration. A three-dose schedule (i.e. at 0, 1–2 and 6 months) remains recommended for immunocompromised individuals, including those known to be HIV-infected'.

The immunological evidence is robust in 9–14 year old girls for a two-dose schedule with a minimum interval of six months between doses, but at present there are no published data on vaccine effectiveness either for infection or disease in the adolescent two- and three-dose cohorts in any randomized control trial. These cohorts are being prospectively followed. A post hoc analysis of data from two trials using the 2vHPV vaccine show that 4 years after vaccination of women aged 15–25 years [58] or 18–25 years [59], one and two doses of vaccine protected against persistent infection with HPV 16 and 18 (a prerequisite for the development of high-grade CIN), raising the provocative suggestion that one dose of vaccine might be enough for protection, a notion supported by data with the 4vHPV vaccine from an observational cohort study sponsored by IARC/WHO in India [60].

A central issue for the one-dose scenario is the duration of protection. Protection will have to be maintained in adolescents who are immunized at 12/13 years of age for the following 2–4 decades. In all studies reported to date, GMTs after two doses 0, 6 or 0, 12 months are non-inferior to the standard three-dose schedule and the expectation is that, in view of this, protection will be equivalent to the standard three-dose schedule. However, after one dose, GMTs are inferior to two or three doses although the kinetics of antibody persistence do not differ, with antibody concentrations in all dosage regimens achieving a plateau after 18–24 months that remains stable for up to 48 months [61]. Importantly, in the WHO India study all the geometric mean avidity indices after fewer than three doses in any group were non-inferior to those after three doses of vaccine, suggesting that antibody quality was as good as that achieved after two or three doses [60]. Furthermore, in a recent study [62] a single dose of the 4vHPV vaccine elicited high titres of neutralizing antibody and potent B memory cells, confirming the remarkable immunogenicity of the VLPs. This, it is suggested [63], is due to the optimal combination, in the VLP, of particle size and geometry with the dense repetitive paracrystalline display of epitopes on the L1 pentamers activating B cells [64] and other key immunocytes generating potent immune responses. All these findings are very encouraging, but the issue of whether one dose of vaccine will be enough for protection against infection and disease will only be addressed to the satisfaction of public health and regulatory bodies by randomized controlled trials with infection or disease endpoints.

10. Human papillomavirus immunotherapy

Prophylactic HPV VLP vaccines are highly effective and in the long term should control HPV infection and disease against the HPV types in the vaccine. In the interim, however, therapeutic intervention to enhance or induce effective immune targeting and clearance of established infections is an attractive strategy. Such therapies have the potential for treating inapparent infection and/or disease in addition to clinically visible lesions. A Th1-biased cell-mediated immune response is critical for regression of HPV-induced disease [65]. Agents therefore that enhance or induce strong cell-mediated immune responses would be predicted to be effective HPV therapies. In HPV-associated cancers and the associated high-grade intraepithelial neoplasias (HGINs), oncogenic viral gene expression is deregulated and the E6 and E7 genes are constitutively expressed. The continued expression of these oncogenes is essential for progression to, and maintenance of, the malignant phenotype. In effect therefore, there are only two possible antigenic targets, E6 and E7, because these are the only viral proteins that will be expressed in all cancers and HGINs. However, the formidable problems facing vaccines for HGIN and invasive cancer relate to the neoplastic phenotype. These lesions are genetically unstable with the potential to rapidly evolve immune escape mechanisms, and an

immunosuppressive mucosal environment with tolerance to viral antigens, modulation of the cytokine milieu and down-regulation of MHC Class I alleles on the neoplastic keratinocytes is associated with progression of CIN and cancer [66,67]. Having said this, it is important to remember that only 30–50% of CIN 3 progress to invasive carcinoma [68] and lesions classified as CIN 3 regress, presumably by immune mechanisms, which implies that therapeutic vaccination is feasible.

The approach of deliberate immunization with E6 and/or E7 of HPV 16 and HPV 18 predominantly, and the generation of antigen-specific cytotoxic T cells (CTL) as an immunotherapy for HPV-associated cancer has been tested with a wide array of potential vaccine delivery systems in transplantable rodent tumour models. It turns out from these studies that these transplantable HPV-expressing cancers in rodents are relatively easy to cure, but human HPV-induced cancers and their high-grade precursors have been largely refractory to the approaches successful in rodents [69].

All the vaccines tested in clinical trials in humans have been safe and well tolerated; they induced robust peripheral blood vaccine-specific T cell responses, but these did not necessarily correlate with clinical responses [69]. However, the many published studies showing that systemic T cell responses to HPV infections are weak and transient but that regressing lesions have an intense intra-lesional and stromal T cell infiltrate implies that local responses are crucial for lesion regression [70]. The cellular effectors in these local responses are still not unequivocally identified, but regression of CIN 1 in longitudinal studies has been shown to be correlated with the presence at study entry of functional cytotoxic CD8+ cells producing Granzyme B [71]. Intra-epithelial CD8+ T cells in the cervix express the intra-epithelial homing receptor $\alpha4/\beta7$ [72] and, significantly, in a retrospective analysis, CIN regression correlated with the expression on the stromal microvascular endothelium in dysplastic lesions of the mucosal addressin cell adhesion molecule 1 (MadCAM1), the ligand for $\alpha4/\beta7$ [73], implying that, with increasing disease severity, cytotoxic effector lymphocytes could not exit the lymphatics and vessels and enter the local lesion. These data suggest that inducing T cell responses that overcome the local immunosuppressive environment to target and localize in the infected mucosa would be a prerequisite for therapeutic vaccines against mucosal HPV infection and disease. Such a notion also implies that analysis of the effector responses at disease sites after vaccination would be a more informative predictor of vaccine efficacy [74].

This notion was tested in a landmark study [75] in which women with HPV 16+ve CIN2/3 received a heterologous HPV 16 E7 DNA prime (weeks 0, 4) followed by a recombinant vaccinia (TA-CIN, week 8) boost delivered intramuscularly to the deltoid muscle. All patients subsequently (seven weeks post vaccination) underwent standard therapeutic resection of the lesions at the squamocolumnar junction. Systemic T cell responses were modest, but histological and molecular analysis of the excised lesional tissue revealed a marked local effector response with intense T cell infiltrates in stroma and epithelium, evidence for clonal T cell expansion and expression of immune activation (CXCR3) and effector function (Tbet and IFNβ) genes.

These and other recently published studies [76–78] provide reasons for cautious optimism and there is now the outline of a blueprint that could lead to successful therapeutic immunization of HPV infection and associated disease. Firstly, vaccines must be highly immunogenic. The HPV DNA vaccine VGX-3100 delivered by electroporation in a three-dose schedule elicited robust adaptive immune responses to HPV 16 and 18 E6 and E7 at least 10× greater than those previously reported for an HPV therapeutic vaccine [79]. Subsequently, a Phase II double-blind randomized controlled trial assessed the efficacy of VGX-3100 delivered by electroporation at 0, 4 and 7 weeks [80]. The primary endpoint in this trial that enrolled women with CIN2/3 was regression to CIN 1 or normal pathology 36 weeks after the first dose; 49.5% of vaccine recipients compared to 30.6% of placebo had histopathological regression. The importance of robust immunogenicity and adjuvantation was demonstrated in a Phase II trial in which patients with longstanding and refractory (vulval intra-epithelial neoplasia grade 3) VIN3 were immunized with a highly immunogenic vaccine comprising HPV 16 E6 and E7 overlapping long synthetic peptides (HPV16 SLP) with an oil in water adjuvant: 47% showed a durable and complete regression histologically and clinically. Secondly, the local immune milieu must be manipulated [76]. Some support to the notion of local immunomodulation is given from studies combining topical Imiquimod, a TLR7 agonist (functioning as a local immunomodulator/adjuvant), with systemic immunization with an HPV 16/18 E6/E7 protein delivered via a recombinant vaccinia virus (TA-CIN) resulting in 32% of patients with VIN3 showing complete regression of lesions [77]. A clinical trial of peripheral vaccination with the heterologous prime-boost regimen, described in the study by Maldonado and colleagues [75], combined with direct immunomodulation with topical Imiquimod in women with CIN2/3 is ongoing (NCT00788164).

Thirdly, immune regulation in the tumour environment must be biased to activation, not tolerance to permit recruitment, retention and activation of the effector cytotoxic vaccine-induced response. Therapeutic vaccines cannot cure large established malignancies or large persistent precancerous lesions and the evidence to date is that this is due, at least in large part, to the dominance of T regs over cytotoxic effectors in the lesions [81,82]. If therapeutic vaccine success is to be improved, then either T regs should be depleted or the pool of cytotoxic effectors increased or both. There is considerable interest in exploiting monoclonal antibody-based therapies (checkpoint inhibitors) that target the co-inhibitory receptors CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) or PD-1 (programmed death receptor 1) and the ligand PD-L1. Such therapies have achieved dramatic results in metastatic melanoma and some other solid tumours, and many clinical trials are in the planning stage or in progress to incorporate these therapies into the algorithms for HPV-associated cancers, alone or in combination with HPV E6/E7 vaccines.

11. Conclusion

The development and implementation of prophylactic vaccines against HBV and HPV are of seminal importance in preventing the cancers caused by these viruses and represent major public health interventions. Both vaccines are highly effective in reducing disease and blocking transmission, and in the long term, providing high population vaccine coverage in the targeted cohorts can be sustained, then significant reduction in the cancers caused by these viruses should be

seen. As the overwhelming burden of HBV- and HPV-caused cancers is in low-resource countries with poor medical infrastructure, the only effective preventive anti-cancer strategy for HBV- and HPV-associated cancers is global vaccination.

So could the success of HBV and HPV immunoprophylaxis be replicated for other oncogenic human viruses? The answer unfortunately is probably not. Viable vaccine candidates have been and new ones could be developed for the oncogenic DNA viruses, but translating this from bench to clinic would face huge challenges. In view of the low burden of disease (not cost-effective) and the difficulty in demonstrating efficacy against infection and/or disease endpoints in clinical trials, it is extremely unlikely that such developments would be undertaken by the pharmaceutical industry.

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