

Novel Approaches for Vaccination Against HPV-Induced Cancers

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Abstract To date, more than 5 % of all cancers are as a result of human papillomavirus (HPV) infection, and this incidence is increasing. Early recognition of disease is associated with good survival, but late presentation results in devastating consequences. Prevention is better than cure, and there are now successful prophylactic vaccination programmes in place. We discuss these and the prospect of therapeutic vaccinations in the near future to address a growing need for improved therapeutic options.

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1 HPV Lifecycle—Exposure, Infection and Clearance

Infections cause more than 15 % of human cancers, and approximately one-third of these are as a result of human papillomavirus (HPV). HPV is a ubiquitous double-stranded DNA virus, and it is estimated that there are more than 100 different HPV types that can infect humans. They are subdivided into low and high risk (HR), pertaining to their malignant potential. It is estimated that a third of all primary school children are infected with low-risk HPV, resulting in cutaneous warts (Bruggink et al. 2012). Cervical smear samples in unvaccinated women suggest that approximately half of women aged 20–21 carry an infection with HR HPV types and 58 % are infected with any HPV type (Kavanagh et al. 2014). Prevalence studies using oral swabbing and testing for HPV suggest that 37–60 % of people are infected, although there are a number of caveats to consider with such prevalence studies (Steinau et al. 2014). Given that oropharyngeal and cervical cancers are relatively rare (although the former are increasingly incident), it is evident that most people who are exposed to HPV through sexual contact will clear the virus via cell-mediated immunity (Stern et al. 2000).

The HPV virus contains both ‘early’ and ‘late’ genes: the early genes, E1 and E2, are virus-encoded replication factors; E4 and E5 act to regulate late viral functions by mechanisms that are not yet well understood. Interestingly, E3 exists in only a few papillomavirus types, but not HPV16 (the HR subtype that is responsible for more than 97 % of HPV-driven malignancy in head and neck squamous cell carcinoma): the gene is not expressed as a protein and has no known function. Late genes L1 and L2 are synthesised following amplification and produce capsid proteins, which the virus needs for full assembly and release of infectious virions (Shulzhenko et al. 2014).

In the cervix (where most extensively studied), HPV virions infect the basal cell layer, as a result of microwounds that expose the basal layer. Viral capsids bind initially to the basement membrane and then to keratinocytes, as they move into the wound (Kines et al. 2009). Both Annexin A2 and S100 calcium-binding protein A10 are integral but with separate roles during HPV16 binding, entry and trafficking of HPV16 (Dziduszko and Ozbun 2013). Following cellular entry and uncoating, HPV is replicated in the nucleus to about 100 episomal copies per cell. This phase is orchestrated by the early genes, E1, E2, E6 and E7. E1 and E2 initiate viral DNA replication, and E6 and E7 maintain long-term replication competence (Frattini and Laimins 1994; Sedman and Stenlund 1995). In undifferentiated basal cells, the viral proteins are expressed at very low levels, favouring immune evasion. As the basal cells divide, each daughter cell contains HPV within the nucleus: E2 is central to this process as it binds to specific ACCN6GGT motifs in the viral genome and attaches it to mitotic chromosomes, tethering the virus genome to the host chromosomes during meiosis (Mcbride et al. 2006). In addition, E2 maintains a stable viral copy number, essential to allow continued persistence in basal cells; it can both positively and negatively regulate the early viral promoter that regulates E1, E6 and E7 as well as E2 itself (Steger and Corbach 1997).

Although the undifferentiated basal cells contain very low levels of viral proteins, as the infected host cell leaves the basal layer and differentiates, high levels of protein synthesis are induced. This delayed expression of viral proteins undoubtedly delays expression of viral antigens until they are in a less immune-competent compartment. However, sustaining long-term viral infection is a strategy not without problems: the virus does not produce its own replicative enzymes, host cell replication is required, and as the host cell differentiates, replication stops. To overcome this problem, the virus forces the differentiated cell to continue to replicate, largely under the control of E7 and its ability to inactivate retinoblastoma (Rb) protein (Felsani et al. 2006). The late viral promoter increases the copy numbers from hundreds to thousands, and then, the capsid proteins are produced. L1 and L2 are considered highly immunogenic, but are not expressed until the most differentiated strata, minimising immune impact. The virions are shed into the environment without lysis or necrosis, avoiding an inflammatory response. Thus, HPV infection is entirely intraepithelial with only limited transfer of antigen to adjacent lymph nodes, minimising an adaptive immune response.

The entry of HPV into epithelial cells is best documented in cervical cells. Data relating to HPV infection in the oropharynx and other genital areas are poorly documented.

Dependent upon type, HPV infection ranges from benign cutaneous warts (primarily due to HPV 1, HPV 2, HPV 27 and HPV 57) (Bruggink et al. 2012), to genital warts, recurrent respiratory papillomatosis (HPV 6 and HPV 11) and some HR ‘oncogenic’ HPV types can precipitate cancers; notably the majority of cervical cancers, 75 % of oropharyngeal cancer, 90 % of anal cancers and 40 % of vulval, vaginal and penile cancers are as a result of HR HPV (Anantharaman et al. 2013; Jemal et al. 2013).

Viral infection of the host can have several outcomes. Acute infection may develop, which is followed by recovery from the virus and total elimination of the virus mediated by cellular immunity. Alternatively, a chronic infection may persist, with prolonged carriage of the virus with or without further relapses of acute disease. Instead of immune-mediated clearance following infection, a latent stage may develop, during which clinical signs of disease are absent and new virions are not produced and released. It is possible that such latent infections may undergo subsequent reactivation leading to new virion synthesis, with or without the re-emergence of clinical disease: this late re-emergence may underlie disease recurrence including genital warts and may also contribute to the development of the aforementioned cancers (Maglennon and Doorbar 2012).

2 Malignant Transformation

In most patients, the host immune system prevails and the HPV infection resolves. However, in a significant minority, the infection is present for long periods allowing additional cellular changes and mutations to occur, leading to cancer. The ancient

relationship between HPV and its host relies on productive infection to maintain a continued viral existence: under this perspective, induction of malignant transformation is beneficial to neither host nor virus, but undoubtedly occurs. Although the role of E6 and E7 in the basal layer is uncertain during infection, it is crucial in malignant transformation. During malignant progression, the HPV genome can integrate into a host cell chromosome and as a result, E6 and E7 were thought to remain the only viral proteins that continue to be expressed. HPV genome integration is a terminal event and not a manifestation of the normal viral life cycle. Interestingly, viral integration is not a prerequisite for malignant transformation and episomal persistence can also result in malignant transformation (Olthof et al. 2014): It is likely that both episomal and integrated virus exists in the same cell. In anogenital carcinoma, there is a significant correlation between the frequency of integrated viral DNA and progression of dysplastic lesions to malignancy (Vinokurova et al. 2008). In addition, integration appears to be influenced by the subtype: in cervical malignancy, 55 % of HPV16 and 92 % of HPV18 cases have viral integration. Finally, in 39 % of oropharyngeal malignancies, viral integration was detected. Interestingly, in the latter cohort, there was no significant difference in the viral copy numbers of E2, E6 or E7 in either integrated or episomal cases (Olthof et al. 2014).

HPV-induced malignant transformation has been most extensively studied in the cervix: levels of both E6 and E7 increase in parallel to the increases in degree of dysplasia, i.e. cervical intraepithelial neoplasia 1(CIN) to CIN3. In CIN1 lesions, the virus can typically complete its life cycle and produce viral particles: clinically, patients have flat cervical warts. CIN2 lesions have elevated levels of E6 and E7 compared to CIN1, predisposing them to the accumulation of genetic changes as a result of diminished tumour suppressor gene (TSG) activity. Specifically, E6 downregulates p53, and E7 downregulates Rb.

Cancers arise secondary to the action of E6 and E7 oncoproteins, and only HR HPVs harbour significant malignant potential. Both E6 and E7 lack intrinsic enzymatic activity and function by associating with, and functionally reprogramming key components of the host cellular signal transduction networks. The E6 protein most frequently interacts with an E3 ubiquitin ligase, E6-associated protein (E6AP) (Huibregtse et al. 1991). The ‘ubiquitin cascade’ adds multiple ubiquitin monomers to the protein, destined to be degraded by proteosomal degradation, and this includes p53 (Scheffner et al. 1993). When p53 is bound by E6 and E6AP, it is unable to induce apoptosis and is degraded. E6 can also inhibit p53 activation by blocking the alternate reading frame p14 pathway and by interacting with histone acetyltransferase, hADA3 (Khoronenkova and Dianov 2011; Kumar et al. 2002). In addition, E6 in combination with E6AP can promote telomerase activity, via E6AP (Klingelhutz et al. 1996). Finally, HR E6 proteins (and not low-risk E6 proteins) can interact with PDZ domain-containing proteins (including hDIg) (Kiyono et al. 1997), MAGI-1 (Glaunsinger et al. 2000), hScrib (Nakagawa and Huibregtse 2000), MUPP1 (Lee et al. 2000) and PTPN3 (Jing et al. 2007) affecting epithelial cell polarity (McLaughlin-Drubin and Munger 2009; Muench et al. 2009) [ref].

Interestingly, E7 is considered the ‘main’ HPV oncoprotein and at low frequency is able to immortalise human epithelial cells. HPV16 E7, from both HR and LR HPV, binds the cullin 2 ubiquitin ligase complex and silences pRB and associated proteins (p105, p107, p130). The HR HPV E7 binds with much greater affinity than LR E7 (Munger et al. 2001). This binding results in E2F transcription factor repression, allowing entry into S-phase. pRb degradation results in p16 upregulation encoded by the sequence on the CDKN2a gene (Khleif et al. 1996). This results in high p16 expression in HPV-driven malignancy. Interestingly, p16 is a potent TSG, but E7 also directly activates cyclins A and E downstream of p16, negating the TSG effect (Zerfass et al. 1995). However, increased expression of p16 acts as a good biomarker for HPV-driven malignancy.

There are many additional host cell factors that HR E7 proteins bind to including HDACs 1, 2 and 3, p21, p27 (cell cycle inhibitory functions), ATM (DNA damage sensor) and p600 (anoikis). Interestingly, abrogation of pRb function by HR E7 protein leads to increased stabilisation of p53 potentially leading to increased apoptosis. As a result, the HR E6 proteins have evolved to induce degradation of p53 to block apoptosis (Howie et al. 2009). E5, E6 and E7 oncoproteins are all considered anti-apoptotic, and the main contributors to malignant transformation, E2 and E7 are also pro-apoptotic proteins, and a balance therefore exists (Garnett and Duerksen-Hughes 2006).

Interestingly, E6 and E7 also act as potent mitotic mutators, thereby increasing the occurrence of mutations that contribute to carcinogenic progression (McLaughlin-Drubin and Munger 2009). The E6 and E7 genes are located in the same open reading frame and are transcribed as a single transcript. E2 protein differentially regulates E6/E7 expression: the transcription of E6/E7 is controlled by E2. E2 binds to its promoter which upregulates p97, which in turn activates the transcription of E6. Interestingly, E2 protein stabilises p53 and maintains apoptosis in HeLa cells (Webster et al. 2000). HPV 16 and HPV 18 E2 proteins have been shown to activate transcription of HPV 16 E6 and E7 oncogenes (Bouvard et al. 1994); however, there are numerous other factors that affect the repression and activation of E6 and E7 oncoproteins. When HPV integrates into the host genome, E2 is inhibited, resulting in a loss of E2 apoptosis and E2-mediated regulation of E6 and E7 (Arisa-Pulido et al. 2006). Clearly, episomal virus does not lose E2-mediated regulation.

Although E6 and E7 are cited as the main oncogenes, E5 also has a role to play. E5 is the smallest HPV oncoprotein and in HPV16, E5 is primarily localised within the endoplasmic reticulum (ER) (Conrad et al. 1993; Borzacchiello et al. 2010). E5 expression in oropharynx malignancy is associated with high EGFR expression, which is linked with poor outcome (Um et al. 2014). E5 protects against apoptosis through inhibition of death receptor apoptosis and ER stress-induced apoptosis (Jiang and Yue 2014). E5 protein may cooperate with E6 and E7 to immortalise cells, and play an inhibitory role in apoptosis (Jiang and Yue 2014). E5 may contribute to the early stages of cancer initiation, but in integrated viruses, E5 is often lost and is not necessary for the maintenance of the transformed phenotype.

Interestingly, in low-risk subtypes, E5 is missing or lacks an ORF and/or a translation start codon (Schiffman et al. 2005).

We understand now that the host immune system is central to survival in many solid tumours, including HPV-induced head and neck malignancy (Ward et al. 2014a). It is likely that it is also important during the early stages of malignant transformation: in order to restrict an immune response, it is probable that both the adaptive and innate immune systems are affected by HPV-infected cells. Healthy keratinocytes constitutively express low levels of interferon-inducible genes in the absence of added interferon. However, cells infected by high-risk HPV E6 and E7 proteins repress the transcription of many interferon target genes including Stat-1, IRF-1 and IRF-3. In addition, both E6 and E7 can minimise the expression of TLR-9, important to sense double-stranded DNA (Ghittoni et al. 2010). Furthermore, keratinocytes constitutively express low levels of several proteins that are upregulated following viral infection. HPV infection does not result in upregulation of key pro-inflammatory cytokines including IL-1, IL-6, TNF α and TGF β , but does upregulate anti-inflammatory IL-10 (Alcocer-González et al. 2006). Clearance of HPV-infected lesions is via cell-mediated responses and cytolysis, and dendritic cells are central to this process. HPV L2 proteins have been shown to suppress maturation, migration and cytokine secretion by dendritic cells (Fahey et al. 2009). MHC class I is downregulated as a result of E6, E7 or E5. In addition, E7 has been reported to downregulate TAP, interfering with antigen presentation via MHC class I (O'Brien and Saveria Campo 2002). Clinically, immunosuppressed patients are at increased risk of both benign and malignant HPV infections. Rates of anal HPV infection are extremely high in HIV-positive patients, especially in those men that have sex with men, resulting in high rates of anal intraepithelial neoplasia (AIN) and anal cancer (Gami et al. 2014).

3 HPV-Related Cancer

The World Health Organization (WHO) published their 'position paper' on HPV in 2009. It identified that in 2005, there were 500,000 cases of cervical cancer and 260,000 related deaths worldwide. The rates were variable from 1 to 50 per 100,000 females, higher in Latin America and the Caribbean, sub-Saharan Africa and Asia. Most were diagnosed when older than 40 years. Up to 80 % can be prevented by screening programmes, and mortality rates remain significantly higher in the developing world.

Vulvar, vaginal, penile, anal and oropharyngeal cancers, and their precancerous lesions are all relatively rare, and most of these cancers occur in adults aged more than 50 years. HPVs are estimated to cause at least 80 % of anal cancer, 75 % of oropharyngeal cancer and 40–60 % of vulvar, vaginal and penile cancers, though prevalence rates do vary.

The estimated burden of non-cervical HPV-related cancers in Europe is higher in men than in women and is driven primarily by head and neck cancers. It has been

estimated that 17,403 cancer cases attributable to HPV (15,497 attributable to HPV 16/18) occur each year in *men* in Europe. This compares with an estimated 9,308 non-cervical cancer cases attributable to HPV 16/18 each year in *women* in Europe (Hartwig et al. 2012).

3.1 Cervical Cancer

HPV infections in the genital tract are the most common sexually transmitted infections. Although progression to malignancy is rare, the high prevalence of the virus makes HPV-related cancers among the most common malignancies. In the UK, cervical cancer is the second most common cancer in women under 35 years [1]. HPV types 16 and 18 are essential precipitants in at least 70 % of cervical cancers (Smith et al. 2007) but may contribute in excess of 80 % of cervical cancers in particular geographic areas, such as Scotland (Cuschieri et al. 2010). Worldwide, 0.5 million new cases of cervical cancer are reported with 274,000 associated deaths annually, making it the second most prevalent cancer in women (Brotherton et al. 2011). Certain HR HPV types, recognised as class I carcinogens by the WHO, are necessary risk factors for the development of cervical cancer.

Not all cervical precancers progress to an invasive cancer: Approximately 25 % of CIN2 and 3 lesions completely regress within a short time frame (4 months) (Trimble et al. 2005). Detection of antibodies against E6 and E7 in serum does not predict which lesions will regress (Trimble et al. 2009a). However, the presence of CD8+ T cells in cervical dysplastic lesions does predict dysplasia regression (Trimble et al. 2010).

High-risk viruses in the cervix include HPV 16, HPV 18, HPV 31, HPV 33 and HPV 45, and together they cause 97 % of cervical cancers worldwide. By contrast, HPV 6 and HPV 11 frequently infect the genital tract, but are rarely detected in malignancy (Lorincz et al. 1992). In all, there are more than 40 HPV subtypes frequently detected within the female genital tract (Schiffman et al. 2005): Schiffman et al. prospectively followed 10,000 women and identified HPV16 as uniquely likely to both persist and to cause neoplastic progression when it persisted. Remarkably, 20 % of HPV16-infected women were either diagnosed with CIN3 or cancer either at enrolment or within 5 years. Other carcinogenic types were not as persistent, but could induce malignancy at a less frequent rate, and many were persistent, without significant malignant potential. Most women clear the infection within 12–18 months. However, a 10 % minority fail to clear the infection, resulting in a persistent infection: if this is HR HPV, there is a risk of malignant progression.

HPV is not only implicated in squamous cell carcinoma (SCC). In the cervix, HPV DNA is detected in most adenocarcinomas, adenosquamous carcinomas and carcinomas with neuroendocrine differentiation (Howley and Lowy 2007). HPV16 is most commonly associated with squamous cell carcinomas, while HPV18 is the predominant type found in adenocarcinomas and neuroendocrine carcinomas.

The Papanicolaou (Pap) smear allows the recognition of cellular abnormalities in HPV-infected cells, and screening has reduced the number of cervical cancers by 80 % in the USA, over the past 50 years. Early stages (I–IIa) of cervical cancer can be treated successfully, but locally advanced cancers are characterised by high recurrence rates and a poor prognosis. The standard therapy of locally advanced cervical cancer is a combination of radiotherapy and cisplatin-based chemotherapy with an overall 5-year survival of less than 50 %. Patients with stage IV or recurrent cervical cancer treated with cisplatin alone or in combination with topotecan only have a median survival of less than 1 year [57].

3.2 Anogenital Cancer

Anogenital malignancies include those arising from the vulva, vagina, penis, scrotum and anus. The majority (90 %) of vulval carcinomas are SCC, representing 4–5 % of malignancies in women (Dittmer et al. 2012). The majority of these are in older women and not related to HPV. However, there is an increasing incidence in younger (<50) women, and 43 % are related to HPV 16 and HPV 18. Early lymph node spread is frequent due to the prominent lymphatic supply, and surgery is the primary mode of treatment. Survival varies from early- to late-stage disease from 90 to 18 %.

Vaginal cancer is rare with under 260 new cases diagnosed in the UK each year (CRUK), less than 1 out of every 600 cancers diagnosed in women. Seventy percentage of vaginal cancers are caused by HPV, the majority HPV16. Treatment is radiotherapy (with or without chemotherapy) and salvage surgery if required.

Penile cancer is rare in men in developed countries, but common in underdeveloped countries. Risk factors include non-circumcision and HPV infection. HPV DNA has been identified in both benign and malignant penile lesions including condylomata acuminata, Bowens disease and SCC (Schoeneich et al. 1999). Lymphadenopathy is present between 28 and 64 % of cases at presentation. Surgery is the main form of treatment.

Scrotal carcinoma is also associated with HPV, predominately subtype 18. In a recent study, 40 % of patients had HPV-driven scrotal SCC (Matoso et al. 2014). It is again treated surgically with wide local excision, and survival is related to stage at presentation.

Anal carcinoma is an uncommon malignancy. In the general population, the incidence is between 0.8 and 1.4 cases per 100,000 people-years. This rises to 35 and 128 cases per 100,000 in men practicing anal intercourse and those HIV-positive patients practicing anal intercourse, respectively. Risk groups include HPV16 infection and high-grade AIN. Most AIN is associated with HPV (6, 11, 16 and 18). The risk for progression to malignancy from AIN is 10 % at 5 years (Scholefield et al. 2011). HPV is responsible for approximately 3,000 anal cancer cases in the USA per year (Markowitz et al. 2007).

3.3 Oropharynx Cancer

Head and neck SCC is the sixth leading incident cancer worldwide. In the USA, there were 11,500 deaths in 2012 from this malignancy (Siegel et al. 2012). Of these, HPV is responsible for approximately 3,500 of the cases (Markowitz et al. 2007). There has been a significant increase (225 %) in HPV-driven head and neck malignancy over the past 15–20 years, and this is predicted to continue to rise (Chaturvedi et al. 2011), especially in the Western world, where smoking is in decline. By 2020, the number of HPV head and neck cancers will exceed cervical cancers if this trend continues. Compared to HPV-independent head and neck cancer, HPV-driven patients are younger, frequently white males, non- or light-smokers and with only moderate or light alcohol consumption. It is likely that HPV head and neck cancer is a sexually transmitted disease (Gillison et al. 2008). HPV-driven cancer in the head and neck is predominantly related to the oropharynx. The oropharynx is an anatomical subsite that includes both palatine and lingual tonsils, soft palate and lateral pharyngeal wall. Both distinct sets of tonsillar tissue consist of organised lymphoid tissue, surrounded by stratified squamous epithelium. The surface area is significantly increased due to multiple crypts, facilitating antigen capture and immunosurveillance. This epithelium has an incomplete basal cell layer and basement membrane (to facilitate antigen trafficking), dispensing with the need for microtrauma for viral access (Pai and Westra 2009).

Traditional risk factors for head and neck cancer were smoking and alcohol. This cohort of patients have a significantly worse prognosis than those with HPV-driven malignancy (Ward et al. 2014b). Treatment includes surgery, chemotherapy or radiotherapy, either alone or in combination. In the HPV-driven group, survival does not depend on treatment type, instead on the infiltration of lymphocytes into the tumour (Ward et al. 2014a, b).

4 Prophylactic Vaccination—Cohorts Vaccinated, Uptake, Serological Evaluation

Both the prophylactic bivalent (Cervarix [HPV16 and 18]) and quadrivalent (Gardasil [HPV 6, HPV 11, HPV 16 and HPV 18]) vaccines prevent infection using L1 virus-like particles (VLP). Both prevent cervical HPV 16 and HPV 18 infection and confer protection against subsequent virally induced CIN (Paavonen et al. 2009; Brotherton et al. 2011; Pollock et al. 2014). Low levels of neutralising antibodies against L1 are detectable in 50–70 % of patients, 6–18 months following HPV infection (Viscidi et al. 1997; Carter et al. 2000; Safaieian et al. 2010). E1, E2, E6 and L2 do not evoke any measurable antibody response following natural infection (Mariani and Venuti 2010).

Although it is assumed that the HPV vaccine protects via neutralising antibody, this mechanism has only ever been demonstrated in a preclinical model

using passive transfer of serum immunoglobulins (Suzich et al. 1995). More recent evidence suggests that AS04-adjuvanted vaccines (such as Cervarix) stimulate NF- κ B with increased cytokine production as a result of increased numbers of activated, antigen-loaded dendritic cells and monocytes in the lymph nodes draining the injection site, further increasing the activation of antigen-specific T cells (Didierlaurent et al. 2009). Systemic immunisations with L1 VLP generates antibody concentrations fourfold higher than following a natural infection, as a result of both route of administration and concentration of antigen (Harro et al. 2001). Although the commercial HPV vaccines have proven efficacy, the correlation between either antibody levels or B-cell memory has not been established (Stanley et al. 2012). Animal models suggest that only very low levels of antibody are required to be protective (Day et al. 2010).

Although both Gardasil and Cervarix use L1 VLP, quadrivalent vaccine produces neutralising antibodies to HPV L1, which are type restricted and possess limited cross-reactivity. However, bivalent vaccine confers a degree of cross-protection against some phylogenetically related types including HPV 31, HPV 33 and HPV 45 (Kavanagh et al. 2014). While L2 does not produce a neutralising antibody response in natural infections (it is not highly immunogenic, unlike L1), following deliberate immunisation with L2 protein, neutralising antibodies were protective against viral challenge in cows and rabbits. Strikingly, these antibodies could cross-neutralise a broad range of HPV subtypes (Karanam et al. 2009). However, this vaccine remains poorly immunogenic, consistent with other protein vaccines, compared to the L1 VLP. Attempts to improve its immunogenicity are currently being tested in animal models (Tyler et al. 2014).

Population-based surveillance data from countries such as Australia, Denmark and the United Kingdom provides early encouragement that prophylactic HPV vaccination is significantly associated with a reduction in both low- and high-grade cervical abnormalities (CIN1-3) in young women (Brotherton et al. 2011; Crowe et al. 2014; Baldur-Felskov et al. 2014; Pollock et al. 2014). Furthermore, the quadrivalent vaccine, which additionally includes HPV types 6 and 11 that are associated with 85–95 % of genital warts, has also been shown to be strongly associated with a reduction in genital warts in both females and heterosexual males (Ali et al. 2013). In Australia, the decrease in genital warts in heterosexual men was observed prior to the implementation of vaccination of boys and is likely due to herd immunity.

In the Western world, cancers of the anus, penis, scrotum, vagina and vulva (henceforth described as non-cervical genital cancers) are increasing in incidence (Parkin and Bray 2006). The increase in non-cervical genital cancers may be associated with a concomitant rise in HR oncogenic HPV infections, with HPV 16 and HPV 18 estimated to contribute between 74–93 % of these cancers (Olsen et al. 2012). Autoinoculation of HPV occurs both from cervix to anus and from anus to cervix in the same woman, and it appears to be relatively common (Mosicki et al. 2012). Although no natural history studies of anal intra-epithelial neoplasia (AIN) are available in women, women with other HPV-associated lesions, including high-grade CIN and vulvar cancer, have higher rates of anal cancer. Therefore, it seems

biologically plausible that girls vaccinated with the HPV vaccine will have a significantly reduced propensity in developing non-cervical genital cancers and oropharyngeal malignancy (Garland et al. 2009; Giuliano et al. 2011; Kreimer et al. 2011; Olsen et al. 2012; Herrero et al. 2013) with a successful prophylactic vaccination programme.

While the benefits of the HPV vaccine are now being realised (Crowe et al. 2014; Ali et al. 2013; Pollock et al. 2014), efficacy is dependent on a number of critical factors. National vaccine programmes, which target preadolescent girls through school-based delivery, are likely to be more successful in preventing HPV infection and disease (Sinka et al. 2014; Pollock et al. 2014). However, such coordinated programmes require a robust and well-governed infrastructure and tend only to be a feature of affluent countries. In spite of this, a high HPV vaccine uptake has been achieved in Rwanda, showing what can be achieved (Hopkins and Wood 2013). Nevertheless, the burden of disease attributable to HPV infection is significantly greater in deprived countries, where recognised barriers such as high vaccine cost must be overcome if global burden of disease is to be reduced (Campo and Roden 2010).

Gender-neutral vaccination has been recommended in the USA, Canada, Austria and Australia. Considered cost-effective modelling has preceded such decisions suggesting that when the burden of disease in men is included in the models, depending upon vaccine price and vaccine uptake as well as other factors, male vaccination can become cost-effective (Stanley 2014). Although the HPV vaccine is not currently offered to boys within the United Kingdom, the UK Joint Committee on Vaccination and Immunisation is appraising the evidence as to whether vaccination of boys would be cost-effective. In a recent Norwegian analysis, public health priority and cost-effectiveness appears to be directed towards increasing vaccine uptake in girls rather than expanding vaccination coverage to boys, but this is crucially dependent upon vaccine tender price (Burger et al. 2014).

There are indirect benefits of the HPV vaccine. Achieving high HPV vaccine uptake may reduce inequalities in cervical cancer prevention by mitigating inequalities observed in the cervical screening programme. Knowledge and awareness of HPV infection, cervical cancer and screening in young girls who have been vaccinated against the virus is surprisingly low (Bowyer et al. 2013). Thus, it may be assumed that in older women knowledge and awareness of the virus and its association with cervical cancer will be even lower. By having a high-profile HPV vaccine campaign with prompt dissemination of the realised benefits, it provides an opportunity to emphasise the importance of attendance at cervical screening for both vaccinated women and older, unvaccinated women. Given that there is a small minority of mothers and daughters from disadvantaged backgrounds who do not participate in either cervical screening or HPV vaccination, it is imperative that awareness of HPV is raised through targeted efforts to reach these deprived groups (Spencer et al. 2014).

4.1 Prophylactic Vaccines for Established Lesions

Although both licensed HPV vaccines are most effective in individuals with no prior exposure to HPV, there are reports of vaccination after HPV DNA was demonstrated in cervical specimens (Hildesheim et al. 2007). They demonstrated that the bivalent vaccine did not improve the clearance rate of the virus. In addition, there are reports of resolution of HPV-induced warts after quadrivalent HPV vaccination (Silling et al. 2014; Kreuter et al. 2010). These studies enrolled patients who were immunocompromised and suggest that there may be clinical benefit in post-exposure treatment of persistent warts. However, in a recent case series, there was no clinical improvement in immunocompetent patients with HPV 6-positive condylomatas who received quadrivalent vaccine (Kreuter and Wieland 2013). It is likely that the decreased expression of L1 in chronic premalignant lesions and loss of L1 expression in SCC (and the infected basal cells) is responsible for the unsuccessful outcome of prophylactic vaccine for established malignant disease (Yoshida et al. 2008). The majority of healthy controls and cervical cancer patients are able to mount a systemic Th1 response against L1 (van Poelgeest et al. 2006). Well-designed clinical trials are required to elucidate the immunological mechanisms required for wart clearance.

5 Therapeutic Vaccines

HPV-driven malignancy unlocks a unique opportunity for cancer immunotherapy. For this patient cohort, the viral oncoproteins (E5, E6 and E7) responsible for malignant transformation and progression are known, permitting targeted treatment. In addition, there is a known immunosuppressive environment that cancer ‘creates’ and this must also be overcome to permit an effective immune response following vaccination. Recently, regulatory T cells (Tregs) within head and neck cancer were shown to express more immunosuppressive molecules compared to circulating Tregs (Jie et al. 2013): These molecules included cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), T-cell immunoglobulin and mucin protein 3 (TIM-3) and programmed cell death ligand-1 (PD-1). In addition to the checkpoint receptors, cancer visibility to the immune system can also be lessened due to reduced expression of HLA I molecules and also transporters associated with antigen processing (TAP) (Li et al. 2010).

5.1 Proteins and Peptides

There are data to show the likely benefit of immunotherapy in HPV-driven lesions. In a small study, a HPV16 E6/E7-based peptide vaccine caused regression of

HPV-associated premalignant vulvar lesions (Kenter 2009). In addition, a second study in patients with vulval intraepithelial neoplasia (VIN) showed that using an E6E7L2 fusion protein, efficacy was associated with the induction of HPV-specific CD4 and CD8 T cells (Daayana et al. 2010; Welters et al. 2010). A number of other trials with a range of vaccines have shown variable clinical results, but none have shown a convincing correlation between clinical outcome and immunogenicity (Ressing et al. 2000; Baldwin et al. 2003; Smyth et al. 2004; Trimble et al. 2009b; Santin et al. 2008; Frazer et al. 2004; Matijevic et al. 2011; Brun et al. 2011; Maciag et al. 2009).

Current trials evaluating alternative peptide vaccines include a phase I/II study to determine the safety and immune modulating effects of the therapeutic HPV16 E6/E7 long-peptide vaccine (ISA101). This vaccine will be used at different doses with or without IFN α as combination therapy with carboplatin and paclitaxel in women with HPV16-driven advanced or recurrent cervical cancer (NCT02128126). In addition, another phase 1 trial is evaluating the treatment of high-grade squamous intraepithelial neoplasia of the cervix using a vaccine consisting of four HPV16 E6 peptides in combination with Candin® (NCT01653249). Candin is a yeast extract and has anti-HPV effects; it has been used to treat common warts caused by LR HPV. Furthermore, synthetic peptides (SLP-HPV-01® with or without interferon- α injections) are being evaluated in men with AIN (NCT01923116).

In addition, protein vaccines using HPV16-derived peptides presented as a Trojan-type construct to prevent proteolysis and facilitate HLAI processing have been trialled in head and neck patients (Voskens et al. 2012). They were combined with MAGE-A3 proteins (HLA restricted), and although a peripheral blood response could be detected (PBMCs from 4 of the 5 patients were able to recognise both the full Trojan constructs and constituent HLA-II peptides), there was no clinical response in any of the 5 patients with advanced cancer. In a study with vulval lesions, vaccination of HPV16 E6 and E7 long peptides with incomplete Freund's adjuvant in 20 women with VIN reported 50 % complete response and 75 % having a durable clinical response (Kenter et al. 2009). Unfortunately, when these long peptides were trialled in patients with high-grade CIN, the trial was terminated early due to unacceptable side effects including flu-like symptoms and injection site morbidity (de Vos van Steenwijk et al. 2012).

5.2 Viral Vectors

A modified vaccinia virus (TG4001), designed to express HPV16 E6 and E7 and IL2, was shown to induce a clinical response in 10 patients (48 %) with CIN 2 and 3 lesions following 3 weekly subcutaneous injections (Brun et al. 2011). A collaboration between transgene and EORTC was announced with a view to trial this vaccine in head and neck patients (EORTC: transgene collaborates with EORTC on phase 2b trial with TG4001 in head and neck cancer).

5.3 DNA Vaccines

DNA vaccines remain attractive due to their stability, ease of production and high expression of antigen in transfected cells, but their limited immunogenicity remains problematic (Huang et al. 2010). However, advances in delivery, including electroporation, are likely to significantly impact on immunogenicity (Sardesai and Weiner 2011). In a completed phase I trial, a microencapsulated DNA vaccine (ZYC-101) consisting of multiple HLA-A2-restricted E7 epitopes was evaluated in women with high-grade CIN. Thirty-three percentage of the patients had a complete response (Sheets et al. 2003). There is also a phase I trial currently recruiting head and neck patients testing pNGVL4a-CRT/E7 (Detox) DNA vaccine in combination with cyclophosphamide (NCT01493154). This same vaccine is being used in combination with topical imiquimod in a phase I trial for CIN 3 patients (NCT00788164). Another study (NCT00988559) is evaluating the efficacy and safety of different routes of administration of the same DNA vaccine [pNGVL4a-CRT/E7(detox)] in patients with HPV16+ CIN2/3. Patients will be enrolled in one of six treatment groups including intradermal vaccination (with a needle-free delivery device, a gene gun), intramuscular and intralesional vaccination.

NCT02172911 is an open-label study to evaluate the safety, tolerability and immunogenicity of VGX-3100 (2 separate DNA plasmids encoding E6 and E7 proteins of HPV 16 and HPV 18) and INO-9012 (DNA plasmid encoding human interleukin 12) delivered by electroporation (EP) in patients with biopsy-proven HPV 16 or HPV 18 cervical SCC.

5.4 Bacterial Vector Vaccines

Bacterial vector vaccines have been investigated, with *Listeria monocytogenes* generating the most interest due to its ability to infect APCs in the cytosolic compartment, permitting both MHC class I and II presentation (Wood and Paterson 2014). ADXS11-001 (ADXS-HPV) is a live-attenuated *L. monocytogenes* (Lm)-LLO immunotherapy in trial for the treatment of head and neck cancer HPV-associated dysplasia and malignancy as a window study prior to surgical resection (NCT02002182). This American study is due to complete recruitment ($n = 30$) in January 2015. A second study evaluating the same vaccine in CIN has just been terminated due to lack of enrolment (NCT01116245). Unfortunately, the UK head and neck trial REALISTIC has recently been terminated due to patient infection with *Listeria* resulting in a serious adverse reaction and withdrawal of the study by the sponsor (NCT01698792). In addition, there are 2 studies looking at the dose range (NCT01356823), efficacy and immunogenicity (NCT01735006) of recombinant HPV 16/18 bivalent vaccine expressed in *Escherichia coli* in vaginal–intraepithelial, vulval–intraepithelial, cervical–intraepithelial neoplasia and cervical cancer. Both trials are reported as ongoing but not recruiting.

For completeness, tumour cell and dendritic cell vaccines have been explored for HPV-driven disease, but production problems (including purity) and administration have remained problematic and will not be discussed further.

6 Summary

It is clear that HPV has relied on its host for both replicative machinery and productive infection for many generations. In the majority of cases, the host's immune system can clear the infection with limited morbidity. In a significant minority, a HR infection remains, providing the initial platform for malignant transformation. We do not yet understand what predisposes individual patients to harbour rather than clear the infection, but it is intriguing to understand which part of the virus/host interaction influences this outcome. It is apparent that this group of malignancies will provide insight into both prophylactic and therapeutic vaccination strategies and hopefully provide understanding that can be transferred to other solid malignancies where the obvious treatment target is not as clear-cut. It will also be interesting to observe what 'help' the immune system requires in addition to the vaccination model: whether in the form of an adjunct or discrete immunostimulatory molecules (i.e. anti-CD40 monoclonal antibodies). It is clear that this is a rapidly emerging field and data generated will facilitate our understanding of the host–tumour interaction.

Conflicts of Interest None declared.

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