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Mini-review

Papillomavirus and treatment

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Human papillomaviruses (HPVs) are small DNA viruses responsible for a broad range of clinical presentations, characterized histologically by the proliferation of epithelial cells. HPVs are responsible for benign as well as malignant lesions, the most frequent of the latter being cervical carcinoma. A better knowledge of the immunobiology of these lesions allowed the development of prophylactic vaccines (for the most frequent genital types) that are presently under evaluation. The present paper describes different approaches for the treatment of HPV lesions, still mostly based on surgery, and underlines the importance of developing adjuvant therapies.
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Keywords: Papillomavirus; Cervical carcinoma; Warts; Vaccines; Cidofovir

Contents

1. Introduction

Human papillomaviruses (HPVs) are small DNA viruses that belong to the *Papillomaviridae*, a homogenous taxonomic family, recently recognized by the International Committee on Taxonomy of Viruses (ICTV). Prior, papillomaviruses were grouped with polyomaviruses in one family, the *Papovaviridae*, based on the fact that they have electronmicroscopically similar viral capsids, no envelope and double-stranded circular DNA genome. Nevertheless, it is now established that polyoma- and papillomavirus have different genome sizes, different transcriptional

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strategies, and that their proteins are not homologous (Bernard, 2006).

Papillomaviruses are widespread in nature, most of them being isolated from humans (de Villiers et al., 2004). The other papillomaviruses described to date cover a broad range of host species, including domestic mammals, but also a number of wild and exotic mammals, reptiles and two bird species (Sundberg et al., 1997, 2000; Tachezy et al., 2002). Phylogenic analysis of papillomaviruses from mammals and birds suggest that the split between mammalian papillomaviruses and bird papillomaviruses is as old as that between their hosts, probably more than 100 million years ago (Bernard, 1994; Bernard et al., 1994).

Papillomaviruses of animals are clearly related to some pathologies. They are mostly responsible for chronic diseases and/or lesions usually with little economic or health consequence, with the possible exception of cow warts, which affect the value of the hide and may cause discomfort to the animal. Animal papillomaviruses have been studied as surrogate models for human diseases as well as for molecular and phylogenic studies.

In contrast, HPV-associated diseases in humans have taken an increased importance over the years and are now a major concern for public health. Most of the HPVs responsible for significant diseases in human belong to the genus alpha-papillomavirus (all genital PVs), beta-papillomavirus (PVs, i.e. responsible for epidermodysplasia verruciformis), and gamma PVs (most of the viruses responsible for cutaneous lesions) (Bernard, 2005, 2006). Prior to this classification, HPVs were grouped as mucosal or cutaneous types, based on their preferred tissue tropism. The mucosal HPV types were further classified into low-risk and high-risk types, according to their association with anogenital cancers including those of the cervix.

The clinical manifestations of HPV (mostly genital and cutaneous warts) have been known and described since the Antiquity. It is only at the beginning of the 20th century that the infectious origin of such lesions was demonstrated (Ciuffo, 1907) and virus particles could be visualized by electron microscopy in skin warts in 1949 (Strauss et al., 1949) and soon after, the suspected sexual transmission of condylomata acuminata was confirmed (Barret et al., 1954). More importantly, HPV is definitely associated with cancer of the cervix which is the second most frequently occurring cancer in women worldwide and is as for condylomata acuminata, recognized as a sexually transmitted disease (Baseman and Koutsky, 2005; Bosch et al., 2006; IARC, 1995; NIH consensus development panel, 1996; de Sanjose et al., 1994). HPV infections are frequent, but in most instances the virus becomes undetectable without causing diseases. Only a small percentage of the infected persons will retain the virus and become chronic or persistent carriers, leading to the possibility for the virus to induce epithelial cell proliferation, malignant or not.

This reflects the complexity of the relations between HPVs and their hosts: on one hand the infected cell where the virus can interfere with the mechanism of cell replication, mostly by driving for its own benefit the regulation of the different cellular polymerases and, on the other hand, the cellular environment that includes the different immune reactions as well as the possible external cofactors (Bosch et al., 2006).

The replicative cycle of HPV takes place in epithelial cells (Doorbar, 2005) and unlike some animal papillomaviruses types, they do not infect or express their gene products in the underlying dermis. Initial infection requires access of infectious particles to cells in the basal layer, which for some HPV types is thought to require a break in the stratified epithelium and this may occur under conditions where the skin is exposed to microtraumas. There are some evidences that for a lesion to be maintained, the virus needs to infect a stem cell, a type of cell that is abundant in the hair follicles (Egawa, 2003; Ohyama et al., 2006; Blanpain et al., 2004). The formation of mucosal lesions could be facilitated by the infection of columnar cells, which can go to form the basal layer of the stratified epithelium of the transformation zone (Doorbar, 2005). As soon as the virus enters the cell and that the different viral proteins are synthesized, there is a precise and complex control of the cell machinery by the virus, mostly via two proteins, E6 and E7 that are able to interfere with the p53 and pRb cell replication regulatory proteins, respectively, and the cascade controlled by them. The interaction of the viral oncoproteins E6 and E7 with cell cycle regulatoty proteins, as well as the role of the viral proteins E1 and E2, responsible for the initiation of the viral DNA replication, have been widely studied and the different aspects have been summarized elsewhere (Doorbar, 2005; Chow and Broker, 2006; Howley and Lowy, 2001; Fehrmann and Laimins, 2003; Zhang et al., 2006). Each step of this complex relation between the virus and its host cell is a potential target for the development of therapeutics that could interfere with virus replication (antiviral) and/or with cell proliferation (antiproliferative). Antiproliferative is probably more appropriate than anticancer for lesions caused by HPV because all HPV infections lead to cell proliferation, benign or malignant. Even so, the example of recurrent respiratory papillomatosis (RRP) underlines once more the complexity of this family of viruses. This apparently benign lesion (at least induced by a non-malignant HPV type) can kill the patient by widespread dissemination mostly at the level of the lungs and in some cases might transformed, mostly in smokers, into laryngeal or pulmonary malignant tumors (Gerein et al., 2005).

The different approaches for the control and the prophylaxis of HPV-induced infections include a better knowledge of the known and/or possible risk (co)factors. The risks factors linked to HPV infections have been extensively studied for the cervix carcinoma (Baseman and Koutsky, 2005; Bosch et al., 2006). Age and number of sexual partners have been consistently associated with HPV infections, a decrease in genital HPV infections being observed with increasing age worldwide (Burk et al., 1996). This seems to be independent of changes in sexual behavior, suggesting a role for immune response. While most studies indicate a decrease in HPV prevalence with age, a number of epidemiological studies conducted in different international regions have shown a second peak of prevalence at older ages. The prevalence of HPV increases with the number of lifetime sexual partners and with the number of recent sexual partners. These findings support the sexual transmissibility of HPV infections of the cervix and this is independent of other

risk factors such as age, race/ethnic groups, use of oral contraception, and other characteristics of sexual behavior.

Other possible risk factors, mostly oral contraception, condom use, smoking, HLA polymorphisms, other sexually transmitted diseases and nutrition have been also widely studied, giving variable results and interpretations from study to study (Rohan et al., 1991; Stone et al., 2002; Wang et al., 2003). Nevertheless, there are some trends that can be summarized: there is a positive association for the use of oral contraception (mostly the duration of oral contraception) and smoking while there is no association with nutrition and a variable association regarding HLA polymorphisms. The use of condoms is protective against genital HPV diseases as indicated by several studies (Hogewoning et al., 2003; Bleeker et al., 2003). In a recent meta-analysis of the efficacy of condoms use in prevention of HPV infection it was found that there is no consistent evidence that condom use reduced the risk of acquiring a HPV infection, although condoms appear to reduce the risk of disease (Manhart and Koutsky, 2002). The importance of the risk factors for cervix carcinoma as well as for other HPV-related diseases has been extensively reviewed and discussed elsewhere (Trottier and Franco, 2005; Scheurer et al., 2005; Gerein et al., 2005; Castellsague et al., 2002; Frazer et al., 2006).

These general reviews do not consider a group of patients of increasing importance and for which HPV-induced lesions, not only cervix carcinoma, is becoming a serious problem: the immunocompromised patients and among them, particularly, AIDS patients. The diversity and the severity of these lesions point to the major role played by the immune system to control such established infections, particularly the specific CD4-T cell population. It underlines also the necessity of developing therapeutical strategies independent of immune stimulation, such as specific chemotherapies (Stanley, 2006).

The immune response to HPV is counterbalanced by viral strategies elaborated to evade or subvert the immune attack. In most individuals the immune response against HPV is capable of controlling genital HPV infections. Under certain conditions the virus or the infected cells may escape these immune control mechanisms even in immunocompetent individuals leading to the persistence of the infection in the host. The interaction between viral and host factors appears to play an important role.

Innate as well as adaptive immune responses of the host are regulated by the expression and signaling events of cytokines, chemokines and their receptors. Cytokines can be produced by the mucosal keratinocytes, the hosts of HPV, or the immunocompetent cells present in the local environment. Viral infection does not only alter the cytokine expression patterns of keratinocytes, but also interferes with cytokine signaling and thus, perturbs local cell–cell communication via cytokine network. Moreover, changes of cytokine expression and receptor-mediated signaling during malignant progression may further contribute to carcinogenesis.

The most spectacular improvement in the prevention of HPV infection has been done the last years by a much better knowledge of this immune response, leading to the successful development of prophylactic vaccines that are now tested in extensive clinical trials (Stern, 2005; Giles and Garland, 2006).

2. Prophylactic and therapeutic vaccines

In the natural history of an HPV infection, there are basically three points at which the immune system can be boosted to control the virus: (i) a prophylactic vaccine given before infection that can prevent viral entry, (ii) a therapeutic vaccine given during viral replication that can eliminate cells expressing late genes, and (iii) a therapeutic vaccine given after viral integration that can control growth of invasive tumors.

Prophylactic vaccination needs to generate virus-neutralizing antibodies directed against the L1 and L2 capsid proteins that have a role in viral entry. Candidate vaccines for HPV consist of virus-like particles (VLP) generated by recombinant expression of the major capsid protein (L1) from eukaryotic expression vectors such as recombinant vaccinia, baculovirus or yeast under conditions that preserve the conformation of the synthesized protein, leading to its self-assembly into capsomers and full capsids. These noninfectious VLP particles are empty capsids (i.e. lack viral DNA) that contain the major neutralizing epitopes of the native virion necessary for the induction of neutralizing antibodies (Kirnbauer et al., 1992; Rose et al., 1993). Production of neutralizing antibodies following vaccination with VLPs exceeded that seen in a natural infection (Christensen et al., 1994a). Preclinical studies using several animal models, including cottontail rabbit papillomavirus, bovine papillomavirus, and canine oral papillomavirus, have demonstrated that VLP vaccines induced protection of animals upon viral challenge (Breitburd et al., 1995; Kirnbauer et al., 1996; Suzich et al., 1995). The only problem with this approach arises in that L1 VLPs vaccines give type-specific protection (White et al., 1998; Christensen et al., 1994b). For this reason, vaccine development to date has focused on HPV types 16 and 18, given that approximately 70% of cervical squamous carcinomas have been attributed to HPV 16 and 18. Therefore, the number of HPV high-risk types in the vaccine will have to be increased in order to prevent 80–90% of cancers.

Phase I studies have shown the vaccines to be safe and highly immunogenic (Harro et al., 2001; Evans et al., 2001) and vaccine efficacy was demonstrated in three phase II randomized controlled trials. Vaccination (3 intramuscular injections in a period of 6 months) of young girls with adjuvant HPV16L1 (Koutsky et al., 2002) or HPV16 and 18L1 (Harper et al., 2004) VLPs have shown that 100% of vaccinees in the per-protocol cohort were protected against persistent infection with the homologous HPV type, whereas the placebo group had persistent infections with both HPV types. Recently, Villa et al. (2005) presented results with a quadrivalent (low risk HPV types 6 and 11, and high-risk HPV types 16 and 18), aluminium-adjuvant vaccine in young women negative for HPV and as in previous reports, peak antibody concentrations were much higher in vaccinees than in seropositive non-vaccinated individuals at seroconversion. Patients were protected against infection associated with HPV 6, 11, 16 and 18 (89% efficacy) and disease associated with these HPV types (100% efficacy). The primary end-point in these studies was the prevention of cervical infection and larger studies having the prevention of CIN 2/3 as the primary endpoint are still necessary. On the basis of these phase III studies,

the U.S. Food and Drug Administration has approved on June 8, 2006, Gardasil, Merck's quadrivalent vaccine. GlaxoSmithKline is developing a bivalent vaccine (Cervarix).

To date, clinical trials with HPV vaccines have enrolled women, but considering the sexual transmission of HPV infection, men will also need to be vaccinated if the goal is to get the whole population developing immunity (Stanley, 2005). Considering that seroconversion rates in men with anogenital warts who are infected with HPV6 or 11 are consistently lower than those in women (Eisemann et al., 1996; Carter et al., 1995), L1 VLP vaccines will also need to enrol men to confirm that responses in men and women are similar.

There are several questions related to the benefits of prophylactic vaccines. It is unknown how long neutralizing antibodies induced by vaccination against the target HPV type will persist *in vivo*; therefore, further trials will need to be performed to define the duration of the immunity. It has also been suggested that elimination of some HPV types through vaccination may select for other HPV types that are now less prevalent (Lunec, 2005; Scheurer et al., 2005). The initial target population for prophylactic vaccines will likely be adolescents, which raises several issues regarding social acceptance, public health infrastructure and economics since successful vaccination will not eliminate the requirements for cervical screening (Bonnez, 2005). The vaccines that will be available soon will be able to protect women against only two high-risk HPV types and they will be still at risk for other HPV types and will therefore need still to attend screening for cervical cancer. In addition, the effects of immunization on incidence will take decades to develop. Indeed, both vaccination and screening will be difficult to accomplish in less-developed countries, where cervical disease continues to be a public health burden.

Therapeutic vaccines can be used against infected cells expressing the viral oncoproteins E6 and E7. Following viral genome integration into the cellular genome, HPV-infected cells no longer express late genes and would not bind antibodies directed against capsid proteins. The continuous expression of E6 and E7 is necessary to escape apoptosis and cell cycle arrest and to maintain cells in a transformed state. Therefore, the generation of specific CTLs against high-risk HPV E6 and/or E7 peptides would lead to the destruction of these infected tumor cells. These concepts support the rationale for the development of therapeutic vaccines targeting the viral oncoproteins. Studies with several animal models revealed that these types of vaccines can induce cellular immunity and help in regression of lesions and eradication of infected cells (Feltkamp et al., 1993; Chen et al., 1992). Several therapeutic vaccine candidates aimed at the treatment of precancerous lesions and invasive carcinoma of the cervix, anus and vulva, and juvenile respiratory papillomatosis are in various stages of development (Scheurer et al., 2005) and have produced encouraging as well as discouraging results (Govan, 2005; Kenneth, 2005). A possible reason for the disappointing results is that patients enrolled in the studies were at an advanced stage of cervical disease, which is known to be associated with genetic instability, viral immune escape, viral antigen tolerance, and downregulation of MHC class I alleles (Govan, 2005). A vaccine designated HspE7 that contains the HPV16 E7 protein fused to the heat shock protein Hsp65 from Mycobacterium bovis BCG, was reported to significantly improve the clinical course in pediatric patients with RRP (Derkay et al., 2005). In a trial of HspE7 for genital warts, a reduction in the size of the warts was observed but they usually did not totally disappear within 6 months (Goldstone et al., 2002).

Chimeric vaccines are also being developed to bridge the gap between prophylaxis and immunotherapy. In these vaccines a fragmented E7 protein is attached to the L1 VLP, allowing the stimulation of antibodies against the capsid protein to prevent infection and treatment of tumors expressing the E7 protein with one vaccine. A number of candidate chimeric vaccines are in early stages of development (Scheurer et al., 2005). Recently, therapeutic vaccination with an HPV type 16 L2E7 vaccine failed to increase the efficacy of conventional therapies for external anogenital warts (Vandepapeliere et al., 2005).

3. Surgery

The treatment of HPV lesions is based on surgery, local or systemic applications of medications which are more or less specific, being cytodestructive or immunomodulatory or a combination of all of them (Fox and Tung, 2005; Lacey, 2005; Snoeck et al., 1998a).

Surgery remains the basic approach for the treatment of the most frequently observed HPV lesions, the anogenital lesions, the warts, as well as the RRP. The excision of the lesions is performed using cold knife, electrosurgery or laser.

An important development in the treatment of cervical intraepithelial neoplasia (CIN) was the application of the large loop excision of the transformation zone (LLETZ) that has been shown to be safe and effective, with a 95% cure rate at one year (Bigrigg et al., 1994). The diagnostic approaches as well as the treatment of the different presentations of CIN is now well codified (Spitzer et al., 2006; Alexander, 2005). For the other anogenital lesions, the surgery based on the use of cryosurgery, cryotherapy, laser ablation or electrocautery is the standard approach. Anal dysplasia and malignancies are challenging clinical problems and there are no standardized recommendations as for CIN lesions (Alexander, 2005). A similar remark can be made for other lesions of the genital tract such as vulval (VIN), vaginal (VAIN), and penile (PIN) intraepithelial neoplasia as well as for Bowen lesions.

For laryngeal papillomatosis, surgery is the mainstay of treatment. Often, patients require many debulking procedures, since the lesions might relapse in the most severe case on a weekly basis. The goal of surgical treatment is to completely remove the papillomas while maintaining the surrounding normal structure and so to maintain the airways free, avoiding tracheostomy as a last resort, since, this usually lead to pulmonary spread. The CO_2 laser is not without problems since it can be responsible for thermal injuries, aerosolization of HPV viral particles into the laser plume and the risk of laser-induced airway fire. More recently, patients suffering of laryngeal papillomatosis can benefit of the development of the laryngeal microdebrider as well

as of the use of the 585-nm pulsed dye laser and argon plasma coagulation (Alexander, 2005; Kimberlin, 2004; Lacey, 2005; Pasquale et al., 2003; Vambutas and Steinberg, 2006).

Most of the warts usually seen by dermatologists or gynecologists are usually treated in first intention using small surgery techniques accomplished by scalpel, sharp curette or scissors under local anesthesia. For a significant proportion of patients a relapse occurs and adjuvant therapies are then needed (Lacey, 2005).

4. Adjuvant therapies

Since papilloma regrowth is often the rule for the patients with HPV-induced lesions, a series of adjuvant therapies have been proposed and tested, in order to complete or to replace the surgery, whenever necessary.

4.1. Immunomodulators

For RRP, independent of the technique used the high rate of recurrence and severe outcome of the disease has justified the use of a variety of adjuvant therapies. Among the proposed treatments, interferon α-2a is certainly the most commonly accepted for this indication (Alexander, 2005; Kimberlin, 2004). The different trials have shown variable efficacy in such patients with severe RRP, approximately one third of the responders experiencing a rebound phenomenon at the end of the treatment (Kimberlin, 2004). A recent study, designed to investigate the putative mechanism of anti-tumor activity of interferon α and the predictive markers for interferon α treatment, could define some possible markers for response prediction. Viral load, as determined by quantitative real-time PCR, and proliferation rate, evaluated as the percentage of Ki-67-positive cells, were used as markers of response to interferon therapy. There was a tendency for HPV-6 induced lesions to respond better than those induced by HPV-11 (Szeps et al., 2005). Interferons have been used in several other presentation of HPV, given topically or systemically, but the costs and also the side effects (fever, headache, fatigue, myalgia . . .), particularly when given systemically have narrowed the number of indications of these molecules (Baker and Tyring, 1997; Lacey, 2005).

By means of immune response stimulation, imiquimod is a member of a new class of imidazoquinolines that demonstrated potent immunomodulating, antiviral and antitumor activities in different animal models. In clinical trials, imiquimod has been evaluated for the treatment of different viral infections including herpes and molluscum contagiosum with variable results. More convincing results were obtained for the treatment of external anogenital warts (Edwards et al., 1998; Gollnick et al., 2001). In this indication the mechanism of action of the compound has been shown to involve tissue production of interferons α, β and γ as well as tumor necrosis factor-α. It could act also by direct stimulation of immune cells. There is now a clear interest for the activity of imiquimod for the treatment of actinic keratosis, where it acts by stimulation of dendritic cells and T-lymphocytes (Ooi et al., 2006; Arican et al., 2004; Lacey, 2005; Fox and Tung, 2005).

Intralesional immunotherapy using injection of mumps, candida and trichophyton skin test antigens has been shown to have some efficacy in the treatment of warts in a single-blinded randomized controlled trial (Horn et al., 2005). The delayed-type hypersensibility reaction induced by these antigens appears to increase the ability of the immune system to recognize and clear HPV. More clinical trials are necessary to further evaluate its effectiveness.

Another approach to stimulate the host's immune system is duct tape occlusion therapy (DTOT). In a randomized study, DTOT proved to be more effective than cryotherapy in achieving complete resolution of verruca vulgaris (common warts), although no difference in the average time to resolution was observed (Focht et al., 2002). No data were collected on wart recurrence after completion of the therapy. Larger randomized studies are needed to assess the effectiveness of DTOT on warts in different anatomic locations.

4.2. Antiproliferative agents

4.2.1. Podophyllin and podophyllotoxin

Podophyllin is a crude resin extract prepared from the roots of different species of *Berberiaceae*. Podophyllin exerts an antimitotic effect by reversibly binding tubulin, but also works in part by destroying the HPV virions. Both compounds are already known for years for the treatment of warts. Podophyllin has been abandoned in favor of podophhyllotoxin, locally less toxic (inflammation, necrosis) and more active, the use of large amounts of podophyllin being associated with severe systemic side effects.

The indication of podophyllotoxin is the treatment of external lesions, mostly warts (von Krogh and Longstaff, 2001; Fox and Tung, 2005; Lacey, 2005; Fox, 2006).

4.2.2. 5-Fluorouracil (5-FU)

5-FU is a cytotoxic agent, pyrimidine analog that acts via inhibition of nucleic acid synthesis. 5-FU is used as a 5% cream mostly for the treatment of genital warts. Its use is restricted by local irritation and inflammation (Lacey, 2005; Snoeck et al., 1998a).

4.2.3. Bleomycin

This molecule that acts by binding to the DNA has been used mostly for the treatment of plantar warts (Fox and Tung, 2005). Intralesional injection of bleomycin is painful and followed by swelling and finally by eschar formation. Bleomycin is not a treatment of choice for HPV lesions.

4.3. Photodynamic therapy

Photodynamic therapy is based on the selective uptake of hematoporphyrins by tumor cells, including HPV-induced lesions. Local applications of photosensitizers have been evaluated for the treatment of CIN and VIN lesions giving a response rate of up to 50% (Martin-Hirsch et al., 1998, 2002). Most of the experience with the use of photodynamic therapy for HPV has been accumulated for patients suffering of

RRP. The results have not been conclusive. In a recent study of meso-tetra(hydroxyphenyl)chlorine as photosensitizer, remission was short-lasting casting doubt on the usefulness of this treatment (Shikowitz et al., 2005). In addition, it has been reported that photosensitivity can persists for weeks after treatment (Kimberlin, 2004; Snoeck et al., 1998a; Shikowitz et al., 2005).

4.4. Aspecific destructive therapies

Cryotherapy is used to freeze external warts by means of liquid nitrogen or dry ice applied directly on the lesions. Two cycles of freezing and thawing are usually performed. There is a variable response rate, and about one-fourth of the treated patients relapses. Cryotherapy requires special equipment, but is inexpensive and safe for the treatment of pregnant women.

Other modalities used for aspecific destruction of external lesions, mostly warts, are based on the local application of different acids (salicylic acid, mono-, bi-, and trichloracetic acids) and glutaraldehyde or formaldehyde or the combination thereof. Lithium succinate creams have been used too. The results are similar to those obtained for the cryotherapy, with a response rate ranging between 50 and 80% (Lacey, 2005; Snoeck et al., 1998a; Jennings et al., 2006)

4.5. Antivirals

4.5.1. Acyclovir and ribavirin

Both drugs have been used systemically (acyclovir) or injected locally (ribavirin) for the treatment of severe RRP. For both, there are only limited reports and no control studies have been designed to date (Kimberlin, 2004).

4.5.2. Cidofovir

The (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl) cytosine (HPMPC, cidofovir, Vistide®) is a nucleotide analog with broad spectrum antiviral activity against DNA viruses, including all herpesviruses, poxviruses, polyomaviruses, papillomaviruses, adenoviruses and iridoviruses. The different indications and clinical applications of cidofovir have been extensively reviewed and commented elsewhere (De Clercq, 2003; Snoeck et al., 2001a; Snoeck and De Clercq, 2002; De Clercq et al., 1999; De Clercq and Holy, 2005).

The rational to use cidofovir for the treatment of HPV infections was not obvious since HPV has a replicative cycle completely different from other DNA viruses, against which the molecule was shown to be active. Particularly, HPV does not encode for its own DNA polymerase, the final target of cidofovir diphosphte in the case of other DNA viruses that code for their own DNA polymerase. Preliminary results in the cottontail rabbit papillomavirus model indicated that cidofovir could offer some therapeutic solution for the treatment of HPV-induced lesions (Christensen et al., 2000). The first patient ever treated with cidofovir was reported in 1995 (Van Cutsem et al., 1995). A 69-year old woman with hypopharyngeal/esophageal HPV lesions induced by types 16 and 18, that failed to respond to a treatment with laser was locally injected with a solution of cidofovir, first weekly and then every other week. The lesions regressed slowly and finally disappeared. A localized relapse was successfully treated more than five years later. Three additional patients with a similar pathology were treated and all three showed a remarkable regression of their tumor upon local injection of cidofovir. We were then prone to extend our experience with cidofovir to patients with severe RRP. We reported the first series of patients with RRP, treated with local injections of cidofovir (Snoeck et al., 1998b). RRP is probably the HPV disease that has mostly benefit of the off-label use of cidofovir. The results obtained have been summarized in recent publications and reviews. It appears from these studies that the general favorable trend that is observed with this approach would benefit of large scale randomized studies, in order to answer different questions on the treatment modalities, the different patients groups and their response rate (Kimberlin, 2004; Lee and Rosen, 2004; Silverman and Pitman, 2004; Mandell et al., 2004; Shehab et al., 2005; Peyton and Wiatrak, 2004). Such studies would allow also answering the concern of the potential carcinogenicity of the molecule, by integrating into the analysis, other risk factors for carcinomas, such as alcohol and/or smoking habits, mostly in the older population concerned by the disease (Kimberlin, 2004; Wemer et al., 2005).

Cidofovir has also been used systemically for the treatment of pulmonary lesions of RRP alone or combined with other adjuvant therapies (Dancey et al., 2000; Van Valkenborgh et al., 2001; Armbruster et al., 2001; de Bilderling et al., 2005).

The use of cidofovir has been also reported for the local treatment of external HPV lesions in normal as well as in immunocompromised patients. These are limited series or case reports (Hengge and Tietze, 2000; Toro et al., 2003; Coremans et al., 2003; Snoeck et al., 1995; Zabawski et al., 1997; Zabawski and Cockerell, 1998; Snoeck et al., 2001b; Snoeck and De Clercq, 2002). Only one placebo-controlled study demonstrate cidofovir superior to a placebo for the treatment of condylomata acuminata in a group of non-immunocompromised patients (Snoeck et al., 2001c). One study reports on the application of cidofovir on the cervix in an attempt to treat CIN lesions (Snoeck et al., 2000).

The question remains whether the activity of cidofovir against HPV is more an antiproliferative than an antiviral effect. The strong relations between the virus and the different cellular mechanisms responsible for cell replication suggest that the activity of cidofovir could be related to its interference with the viral control of cell replication.

Cidofovir has been demonstrated to have an antiproliferative activity and to induce apoptosis in HPV-positive cell lines *in vitro* (Andrei et al., 1998a,b, 2000; Abdulkarim et al., 2002; Johnson and Gangemi, 1999). Further studies are needed to define the exact mechanism of action and to explain the apparent selectivity of cidofovir for HPV-transformed cells compared to normal epithelium.

4.6. Miscellaneous

Indol-3-carbinol (I3C) is a compound derived from cruciferous vegetables (broccolis, cabbages or cauliflowers) and it

interferes with estrogen metabolites which are known to have anti-papilloma activity (Yuan et al., 1999; Rogan, 2006). I3C has been found effective in treating some cases of recurrent laryngeal papillomatosis either alone or as adjuvant therapy (Rosen and Bryson, 2004; de Bilderling et al., 2005). No large study has been published to date to confirm the possible role of the molecule in such indications. I3C has been shown some efficacy in treatment of CIN in a small placebo-controlled clinical trial (Bell et al., 2000).

Large randomized trials are also missing for two adjuvant therapies, mostly used for patients with severe RRP, retinoic acid and cimetidine (Kimberlin, 2004; Snoeck et al., 1998a).

HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a folding variant of human alpha-lactalbumin in an active complex with oleic acid. It has been shown that HAMLET selectively enters tumor cells, accumulates in their nuclei and induces apoptosis-like cell death (Duringer et al., 2003; Svensson et al., 2003). HAMLET interacts with histones and chromatin in tumor cell nuclei and this interaction locks the cells into the death pathway by irreversibly disrupting chromatin organization. In a randomized, placebo-controlled, double-blind study, treatment with topical HAMLET had a beneficial and lasting effect on skin papillomas (Gustafsson et al., 2004).

5. New strategies

Since the combined actions of the high-risk E6 and E7 oncoproteins are essential for the maintenance of the neoplastic phenotype and evasion of apoptosis, abrogation of either E6 or E7 function (or both) in neoplastic cells by targeting gene expression or protein-protein interaction should therefore induce apoptosis and be an effective oncotherapy for HPV associated diseases (Stanley, 2002).

Among therapeutic nucleic acid strategies, antisense oilgodeoxynucelotides (ASO) and ribozymes (Rz) targeted to inhibit expression of HPV E6 or E7 oncogenes showed effect to some degree, but problems such as low efficiency, short-period maintenance and high cost still remain (DiPaolo and Alvarez-Salas, 2004). In general, successful attacks on E6/E7 have been restricted to the world of cell culture; the reason for the rather limited success in targeting E6/E7 may be due to the target *per se* ('low accessibility' of most sites on HPV16 E6/E7 RNA) (Venturini et al., 1999). In a latter study, Clawson et al. (2004) used ASO targeted to accessible sites in the HPV11 E6/E7 RNA, which proved to be effective in blocking progression of HPV-induced papillomas in human foreskin grafts on immunodeficiency mice.

Recently, RNA interference-mediated gene silencing has been used as another approach to block HPV-16 E6 gene expression. The inhibitory effects of HPV16 E6 small interfering RNA (siRNA) in vitro and *in vivo* have been reported (Niu et al., 2006; Tang et al., 2006).

In another approach, HPV expressing cervical carcinoma cells have been infected with recombinant viruses expressing full length E2 protein. E2 binds the HPV early promoter and represses transcription of E6 and E7 resulting in the expression

Table 1
Strategies for treatment of HPV infections

Strategy	Treatment	Reported clinical efficacy
Surgery	LLETZ (large loop excision transformation zone	Intraepithelial neoplasia
	Scalpel, curette, scissors	Warts
	Electrosurgical techniques	Warts
	Laser therapy	RRP
Destructive therapies	Cryotherapy	External warts
	Photodynamic therapy	RRP, intraepithelial neoplasia
	Salicylic acid, trichloroacetic acid	External warts
	Glutaraldehyde and formaldehyde	External warts
Antiproliferative agents	Podophyllin/podophyllotoxin	Warts
	5-Fluorouracil	Genital warts
	Bleomycin	Plantar warts
Antiviral agents	Cidofovir	Anogenital warts. Verruca vulgaris, RRP, intraepithelial neoplasia
Immunotherapies	Interferon	RRP
	Imiquimod	Anogenital warts
	Mumps, Candida and Trichophyton skin antigens	External warts
	Duct tape occlusion therapy (DTOT)	Verruca vulgaris
	Therapeutic vaccines	Intraepithelial neoplasia, RRP, anogenital warts
Miscellaneous	Indole-3-carbinol	RRP, CIN
	Micronutirents	CIN
	HAMLET (human α-lactalbumin made lethal to tumor cells)	Warts
Molecules targeting HPV transcripts	Ribozymes	No clinical data available
	siRNA	
	antisense oligonucleotides	

and stabilization of p53 and reactivation of pRb mediated cell cycle checkpoint control (Goodwin and DiMaio, 2000; Goodwin et al., 2000; Wells et al., 2000) (Table 1).

6. Conclusion

The recent breakthrough in the development of prophylactic vaccines against HPV has opened new perspectives for the future. Together with this new implementation, new policies and new public health approaches have to be elaborated. Nevertheless, the prophylaxis and certainly the treatment of HPV lesions remain multidisciplinary, including surgery, systemic and local immune therapy, as well as chemotherapy. The development of novel specific approaches to treat HPV-associated diseases as well as a better understanding of the complex relationship between HPV, the host cell and the immune response is needed to improve the management of HPV lesions in the future. Also, it becomes increasingly important to consider the needs of developing therapeutical strategies independent of immune stimulation, such as specific chemotherapies, for immunosuppressed patients that are prone to persistent and widespread HPV infections.

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