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REVIEW

Immune therapy for human papillomaviruses-related cancers

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Abstract

Human papillomaviruses (HPVs) are a large family of double strand DNA viruses comprising more than 180 types. Infection with HPV is very common and it is associated with benign and malignant proliferation of skin and squamous mucosae. Many HPVs, considered low-risk such as HPV 6 and 11, produce warts; while high-risk viruses, such as HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58, induce tumors. About 5% of all cancers in men and women are associated with HPV infection. Because there are not antiviral drugs for HPV infection, current therapies for low-risk HPV infections involve physical removal of the lesion by cryotherapy, trichloracetic acid, laser, or surgical removal. Surgical procedures are effective in the treatment of precancerous lesions, however after these procedures, many recurrences appear due to new re-infections, or to failure of the procedure to eliminate the HPV. In addition, HPV can inhibit recognition of malignant cells by the immune system, leading to the development of cancer lesions. When this occurs, radiotherapy and chemotherapy are then used. Unfortunately, about 50% of the HPV-cancer patients still die. In the past decade, a better knowledge of the natural history of the virus-host interaction and of the immune response against this viral infection has brought new therapeutic strategies geared to modulate the immune system to generate an efficient virus-specific cytotoxic response. Novel HPV protein-expressing vaccines have shown some significant clinical efficacy and systemic HPV-specific cytotoxic T cell responses. This review will describe the current status of the several therapeutic strategies used to treat HPV-induced lesions, and discuss the various new therapies now being tested.

Key words: Human papillomavirus; T cell; Immunoglobulin; Antibody; Vaccinia virus

Core tip: Infection with human papillomavirus (HPV) is very common and it is associated with benign and malignant proliferation of skin and mucosae. Low-risk HPV produce warts; while high-risk HPV induce tumors. Because there are not antiviral drugs for HPV infection, current therapies involve surgical removal of the lesion. Unfortunately, after surgery many recurrences still appear and about 50% of the HPV-cancer patients die. In the past decade, new therapeutic strategies geared to generate an efficient virus-specific cytotoxic response have been developed. This review describes the current status of the several therapeutic strategies used to treat HPV-induced lesions.

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INTRODUCTION

In the mid 70s human papilloma virus (HPV) was described as a mild viral infection that caused benign warts, that in most cases would regress spontaneously[1]. In few years however, it became evident that there are many HPV with selective tropism for various cutaneous mucosae, and that HPV infection could cause severe diseases. Especially, some HPVs infecting the ano-genital area are real oncogenic viruses that lead to cervical carcinoma[2], the seventh most common cancer in the world[3] and the second most common cancer among women[4].

More than 180 types of HPV have been identified from different lesions, classified by DNA sequences, and named sequentially[5]. HPV have selective tropism for cutaneous or mucosal epithelia and are divided into two groups: low-risk that cause benign lesions, named papillomas (small wart-like neoplasias) that usually regress on their own; and high-risk that cause malignant transformation and develop into large tumors. The two most common low-risk HPV are HPV 6 and HPV 11. They are responsible for 90% of genital warts and recurrent respiratory papillomas[6]. There are about 15 high-risk HPV causing around 95% of all cervical carcinomas[7]. The types HPV 16 and HPV 18 account for about 50% and 14% of all cases respectively[4]. High-risk HPV are also involved in other types of cancer. Around 80% of tumors of the anus, 60% of the vagina, and 40% of vulva and penis are induced by HPV, mainly the type HPV 16[8]. Also, around 60% of tongue, trough[9], and tonsil tumors are also caused by HPV[10].

Natural history studies of HPV show that 60% of sexually active people will be infected with at least one high-risk HPV during their lifetime[11]. Most of these infections are eliminated by the immune system in about 1 or 2 years from exposure[12]. However, in the remaining cases, the virus persists for a long time causing lesions that can progress into cancer[13]. Early detection of HPV-induced lesions becomes important in order to prevent the development of cancer. The best way to diagnose an HPV infection is to confirm the presence of HPV DNA in the lesion by hybridization or by PCR[14,15]. This type of testing is expensive and difficult to implement in poor parts of the world[16]. Therefore, regular screening of cytological (Pap smear) or colposcopic abnormalities, which are signs of HPV infection, continues to be an effective preventive strategy for cervical cancer[17] particularly in developing countries. Unfortunately, this strategy is not without problems. There is great variability due to the lack of trained personnel resulting in high rate of false negative results[18]. Clearly a combination of screening strategies is needed to detect as early as possible HPV-induced lesions.

Once HPV lesions are detected, the main therapeutic approach involves physical elimination of the lesion by cryotherapy, trichloracetic acid, laser or surgical removal[19]. In pre-cancerous lesions however, surgical procedures alone are not very effective, since recurrences occur at rates of 20%-30% or more with lesions both at previously treated sites due to failure of the procedure to eliminate the HPV, and at new sites due to new infections[20]. In addition, HPV can inhibit recognition of malignant cells by the immune system[21], leading to the development of cancer lesions. When this occurs, radiotherapy and chemotherapy are then used with relative success, since about 50% of the HPV-cancer patients still die[22]. Clearly, new therapeutic strategies are in urgent need to control the burden of HPV-related cancer[23].

The fact that most HPV infections are cleared spontaneously shows that the immune system can effectively eliminate virus-infected cells. But, in the case of persistent infections, the immune system clearly has failed. HPV has evolved several mechanisms to evade the immune system. First, HPV infects keratinocytes, which are distant form immune cells, presents a minimal expression of viral proteins in the keratinocyte, and thus does not induce lysis of keratinocytes. In addition, HPV down-regulates the expression of major histocompatibility complex (MHC) class I molecules, Toll-like receptor (TLR) 9, and cytokines such as, interferon and interleukin (IL)-8[21]. New therapeutic approaches take advantage of our knowledge on how the immune system deals with viral infections and eliminates virus-infected cells mainly through cytotoxic activity of various leukocytes, mainly T cells[21]. These new therapies involve the use of anticancer vaccines and intralesion immunotherapy with the idea to activate specific cytotoxicity towards HPV-infected cells[24,25]. In this review, we describe the current status of the several therapeutic strategies used to treat HPV-induced lesions, and discuss the various new therapies now being tested.

PAPILLOMAVIRUS

Papillomavirus belong to the Papovaviridae family of DNA viruses. These viruses contain a double strand DNA with 8000 pb approximately arranged in an 8 well-defined genes (Figure 1). Six early genes are involved in virus replication and two late genes are involved in virus assembly. HPV infect the epithelium of the cervix, and their replication is closely linked to the differentiation of the epithelium (Figure 2)[26,27].

IMMUNE RESPONSE TO HPV

Protection against viral infections is provided by both arms of the immune system. First, HPV can infect cells through damaged skin tissue. When the damage reaches the basal layer of the epidermis, the virions can infect dividing keratinocytes. The initial inflammatory response induced by tissue damage leads to infiltration of immune cells mainly neutrophils, followed by macrophages and later lymphocytes. These nonspecific innate immune cells detect "danger" by recognizing viral molecules, such as the double-stranded DNA HPV genome or the L1 and L2 capsid proteins. These molecules are detected by pat-

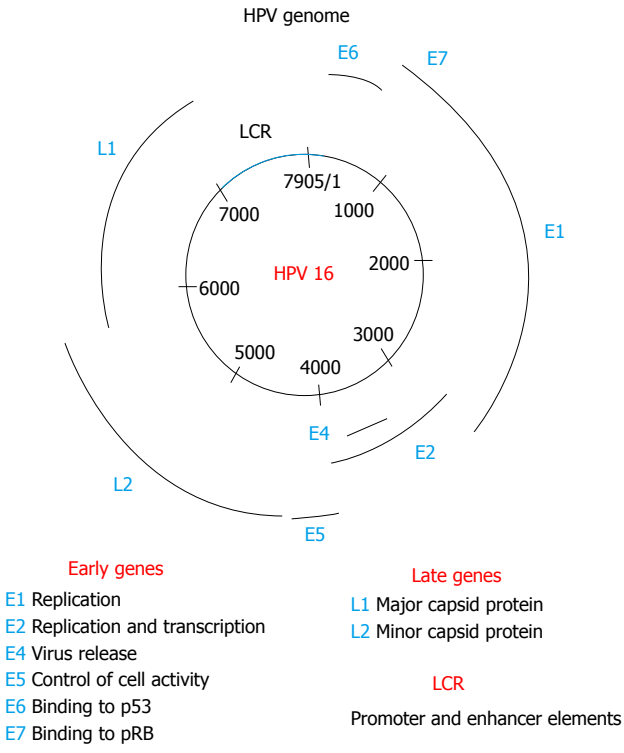

Figure 1 Human papilloma virus genome. The genomic organization of the human papilloma virus 16 (HPV 16) is shown. The double strand DNA is close to 8000 base pairs. The early genes code for proteins involved in viral replication and transcription: *E1*, *E2*, *E6*, and *E7* genes. The *E4* and *E5* genes are expressed a little later and have functions in immune evasion and virus release. The late genes code for the virus structural proteins: the major capsid protein L1, and the minor capsid protein L2. The E6 and E7 proteins of the high-risk HPV types have oncogenic properties. The long consensus repeat (LCR) sequence contains the promoter and enhancer elements of the virus.

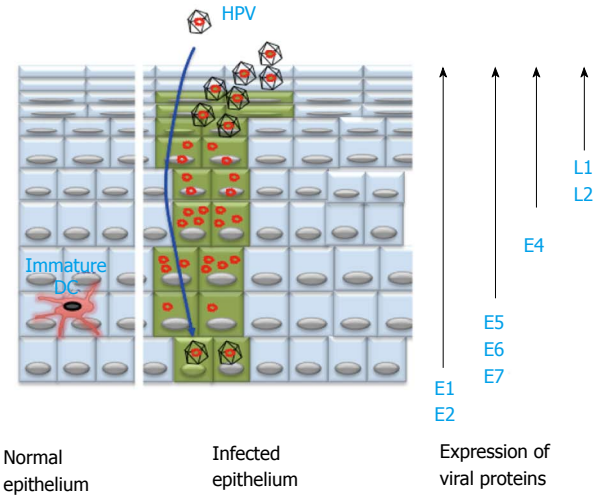

Figure 2 Papilloma virus replication is tissue specific. The human papilloma virus (HPV) infects a keratinocyte in the basal layer of the epithelium after a micro trauma (a small cut of the epithelium that uncovers the basal membrane). The virus DNA is maintained in the proliferating cells at a low-copy number (E1 and E2 viral proteins are expressed). When the infected cells begin to differentiate into mature keratinocytes, the virus activates other genes (E4, E6, E7 viral proteins are expressed) and replicates its DNA to a high-copy number. In the top layers of the epithelium all viral proteins (including E4 and the capsid proteins L1 and L2) are expressed. Thousands of new virions are formed and released from the cells without causing cell death.

tern recognition receptors, such as Toll-like receptors[28], which signal to activate defense mechanisms *via* the production of inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12, and α-, β- and λ-interferon (IFN) to activate natural killer (NK) cells[29] and other immune cells. Later, antigen presenting cells (APCs), such as Langerhans cells or dendritic cells (DCs) in the skin and mucosa[30], can uptake viral antigens and process them into small peptides that are presented together with MHC (HLA in humans) molecules on the cell membrane, to lymphocytes for initiation of an adaptive immune response (Figure 3). For this process, DCs migrate to lymph nodes where they undergo maturation through the expression of co-stimulatory molecules. The processed viral antigen/MHC complex on DCs binds to antigen-specific T cell receptors on naïve $CD4^+$ and $CD8^+$ T cells. This binding induces T cell proliferation, IL-2 production, and activation of T cells. Activated $CD4^+$ helper T cells can differentiate into Th1, Th2, or Treg/Th3 phenotypes depending on the cytokines they produce. Th1 produce IL-2, IL-12, IL-15, tumor necrosis factor (TNF)-α, and λ-IFN; Th2 produce IL-4, IL-5, IL-6, IL-10, and IL-13; and Treg/Th3 produce IL-10, transforming growth factor (TGF)-β, and λ-IFN. On the other hand, activated $CD8^+$ T cells differentiate into cytotoxic T lymphocytes (CTL) which secrete the proteolytic enzymes, granzyme and perforin[31]. CTLs migrate back to the infected sites and destroy HPV-infected cells (Figure 3).

The majority of HPV infections are cleared spontaneously within two years from infection and without any clinical manifestation by immune-competent individuals[12,26]. In spontaneously regressing HPV-related lesions, infiltration of $CD4^+$ and $CD8^+$ T cells has been detected, while in persistent lesions these immune cells are not seen[32]. In addition, in immunosuppressed individuals such as organ transplant recipients[33] or human immunodeficiency virus (HIV)-infected people a higher incidence of HPV-related lesions are observed[34]. These observations indicate that the adaptive immune response against the virus is important and for the most cases effective. This adaptive response comprises elements of humoral and cellular immunity[21]. However, HPV has also evolved mechanisms both to avoid initial detection and to interfere with adaptive response that allow the virus to persist and lesions to progress into cancer.

Humoral response

The majority of patients with HPV infections present a humoral immune response that is detected by the presence of antibodies against different papillomavirus proteins such as L1, E2, and E4 proteins in the first stage of infection. At later times when viral DNA gets integrated into cellular genome, antibodies against E6 and E7 proteins can be detected in low- and high-grade lesions as well. However, this antibody response is usually weak and variable and it does not seem to protect from future re-

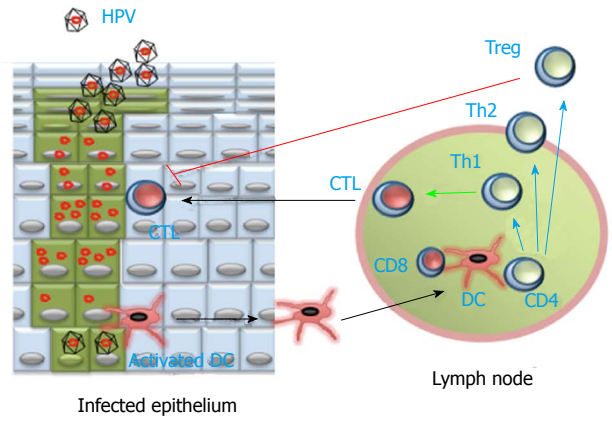

Figure 3 Cellular immune response against human papilloma virus. Dendritic cells (DC) can take human papilloma virus (HPV) antigens from the infected epithelium, and then migrate (black arrows) to lymph nodes. There, DC present the processed antigen together with MHC class I and class II molecules, to CD8+ T cells and CD4+ T cells, respectively. CD4+ T cells proliferate and differentiate (blue arrows) into T helper cells, either Th1 or Th2, depending on the type of cytokines they produce. CD8+ T cells differentiate (blue arrow), with help (green arrow) from Th1 cells, into cytotoxic T cells (CTL). Then, CTL migrate (black arrow) back to the infected epithelium to destroy virus-infected cells. CD4+ T cells can also differentiate (blue arrow) into regulatory T cells (Treg), which inhibit (red line) the cytotoxic activity of CTL.

infections[35]. For high-risk HPVs, the seroconversion rate is about 50% within 18 mo of detecting the corresponding HPV DNA[36], and important differences have been found in the proportion of seropositivity among HPV 16 DNA-positive individuals, depending on the site of the cancer around the ano-genital area, indicating that cancer development may lead to changes in antibody responses in a site-specific fashion[37]. Thus, humoral responses are not efficient at eliminating established HPV lesions. In addition, antibody titers can persist for many years even after the virus is cleared, so seropositivity is a useful marker for past infection rather than current infection.

Cellular response

Cell-mediated immune responses are more important in clearing HPV-related lesions. HPV-specific $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells are generated to fight and eliminate infected cells (Figure 3). Patients who successfully eliminated previous HPV 16 infections present memory T cell responses to viral proteins[38], and in patients with spontaneous regression of grade 3 vulvar intraepithelial neoplasia strong $CD4^+$ and $CD8^+$ T cell responses are found[32]. A high ratio of $CD4^+$ to $CD8^+$ T cells[39,40] with a predominance of Th1 cytokines[41,42] is also observed during regressing lesions. Thus both $CD4^+$ and $CD8^+$ T cells are important for elimination of HPV infection. In contrast, patients with cervical intraepithelial neoplasia or cervical cancer present deficient T cell responses[39]. A low ratio of $CD4^+$ to $CD8^+$ T cells[42,43] and a strong shift to Th2 cytokine profile[44-46] are observed in persistent lesions. Also, responses that include HPV-specific Treg that inhibit cytotoxic activity[38]. Thus, an efficient cytotoxic cell-mediated immune response is critical for elimination of HPV-related lesions. Unfortunately, the virus has also evolved mechanisms to interfere with the immune response.

HPV mechanisms to evade the immune system

The best mechanism against the immune system is to avoid detection. HPV replication takes place in areas where immune surveillance is poor. In the stratified squamous epithelium of the uterine cervix, surveillance by DCs greatly declines towards the keratinized layers (Figures 2 and 3). In addition, the virus links its own replication with the differentiation state of the keratinocyte. At the beginning of the infection, in the keratinocytes of the basal layer, expression of viral genes is minimal. Expression of viral gene products up-regulates progressively with differentiation and upward migration of keratinocytes (Figure 2). In this way, HPV late proteins, which are the most immunogenic, are expressed at areas of poor immune surveillance (Figure 3). In addition, new virions are released through the normal rupture of surface epithelium, reducing any possible inflammatory response and avoiding uptake by DCs. Therefore, HPV replication is a local phenomenon with minimal activation of the immune system.

In addition of keeping a low profile during replication, HPV has other mechanisms that help the virus interfere with the immune response[21]. The E6 and E7 proteins block IFN production by the infected cell. E6 inhibits the transcription factor IFR-3 that binds to the -IFN promoter, downregulating expression of interferon-responsive genes[47]. E7 also inhibits the expression of -IFN responsive genes[48,49]. In addition both E6 and E7 reduce the expression of TLR 9[50], and cytokines, such as IL-8[51], and IL-18[52], which are pro-inflammatory molecules. The proteins E5, E6, and E7 downregulate the expression of MHC class I molecules, reducing recognition of the HPV-infected cell by NK cells and by specific CTLs[53].

Moreover, a reduced inflammation state is found in persistent lesions and in tumors. This condition correlates with a change in the cytokine profile produced at the site of infection. A shift to Th2 cytokines is also common in persistent lesions[45,54]. This leads to an inhibitory state for helper $CD4^+$ T cells. In addition, Tregs have been found infiltrating tumors, especially in the early stage of tumor progression[55]. Tregs are able to strongly inhibit activation of $CD4^+$ T cells, cytokine production, and activation of CTLs[56]. In patients with cervical cancer, larger numbers of Tregs and increased suppressive activity have been observed, both in the tumor and in draining lymph nodes[57,58]. Presence of these Tregs associates with an increased risk for progression of premalignant lesions to cancer[59]. Thus, when trying to activate the immune system special attention should be paid to the possible development of HPV-specific Tregs that may block the cytotoxic response needed to eliminate virus-infected cells[60]. Indeed, it has been proposed that immunogenic tumors (those with abundant tumor-infiltrating lymphocytes) may display potent immunosuppressive potential[61]

and should be treated with Tregs-depleting agents or with inhibitors of co-stimulatory molecules such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) with the therapeutic monoclonal antibody ipilimumab[62].

There is much information in the literature regarding cellular immune responses in patients with HPV infection, with cervical lesions, and with cervical cancer. These reports are beyond the limits of this review, but a general description of the cell responses from HPV infection to development of cancer is as follows. During the initial infection an inflammatory state favors activation of innate immune cells[63,64] and secretion of cytokines that induce $CD4^+$ T cell activation[39]. As mentioned before in regressing lesions, both $CD4^+$ and $CD8^+$ T cells are found with a high ratio of $CD4^+$ to $CD8^+$ T cells, and also cytokines of the Th1 type are produced[42,43]. However, when the $CD4^+$ to $CD8^+$ T cell ratio is low and a shift to Th2 type cytokines is observed a persistent infection is found in lesions[45,46] and a progression to cervical cancer is more likely[44,54,65]. Finally the presence of Tregs in lesions seems to increase the risk for progression of premalignant lesions to cancer[59,66]. Thus, any therapeutic approach must be able to induce a strong HPV-specific immune cell response that involves $CD4^+$, $CD8^+$ cells, and Th1 type cytokines.

PREVENTION

In principle, if HPV infection could be prevented, HPV-associated cancers would disappear. Following this idea, HPV vaccines that could prevent infection were developed in the last decade. Taking advantage of the fact that capsid L1 proteins spontaneously assemble in virus-like particles (VLP) without viral DNA, it was possible to have the immunogenic L1 proteins in a non-infective form. Two pharmaceutical companies, Merck in the United States and Glaxo Smith Kline (GSK) in Europe, used VLP produced by overexpressing HPV L1 protein in yeast or insect cells, to create prophylactic vaccines. Gardasil® (Merck) is a quadrivalent vaccine against HPV types 6, 11, 16, and 18[67], and Cervarix® (GSK) is a bivalent vaccine against types 16 and 18[68]. Both vaccines have been approved and are commercially available. They induce an antibody response that prevents new infections with high efficacy against HPV 16 and 18 (Cervarix®)[69,70] and HPV-6, 11, 16, and 18 (Gardasil®)[71]. They promise, in the long-term (30-50 years) to reduce the incidence of disease associated with the vaccine HPV types[72]. However, this type of protection could only be achieved with a large vaccination coverage (larger than 50%) of uninfected people, which are boys and girls 10 years old, to predate the debut of sexual activity. Unfortunately, full vaccination coverage of large populations will no be easy in many parts of the world[73], and a large unvaccinated population will remain at a high risk of HPV-related disease, and in need of treatment.

In addition, other factors indicate that current vaccines will not be useful to prevent all types of HPV-related cancers. These vaccines target only HPV 16 and 18 and in the case of Gardasil® HPV 6 and 11. Despite some cross-reactivity[69], these vaccines show a small prophylactic effect on many HPV subtypes not included in the vaccine[74,75]. Also, the HPV subtype distribution in cervical cancer varies throughout the world[76,77]. For example, in Japan less than 60% of cervical cancer cases are due to HPV 16 and 18[24]. Thus, current vaccines cannot cover all oncogenic types even in a single population, and their general use in other parts of the world is questionable[75]. To be completely effective, future vaccines would need to be multivalent for all described oncogenic HPV types. But these formulations will certainly be much more expensive than current vaccines. Despite government subsidies trying to achieve full vaccination coverage, weaknesses in current prophylactic vaccines dictate that even vaccinated females must continue cervical cancer screening[78]. Clearly, large numbers of people remain at risk of developing HPV-related disease, and thus virus-specific therapies remain a priority.

CURRENT THERAPIES

HPV infections that are not cleared by the immune system usually persist in latent form as cells of the basal layer maintain low viral copy numbers indefinitely. Tissue damage or immunosuppression can then induce an active infection that depending on the HPV type, may progress to neoplasias. HPV infection of the ano-genital area produces two types of lesions: warts (condyloma acuminata) and squamous intraepithelial lesions. Condylomata are mostly related to low-risk HPV infections, and represent a low risk of malignant transformation. In contrast, squamous intraepithelial lesions are related to high-risk HPV infections. These malignant lesions display various degrees of histological abnormalities. For the cervix these lesions are classified as cervical intraepithelial neoplasia (CIN) 1, mild, CIN 2, moderate, and CIN 3, severe. A similar classification is used for vaginal intraepithelial neoplasia, vulvar (VIN), penis (PIN) and anus (AIN) intraepithelial neoplasias. All of these lesions can progress to invasive cancer. Treatment for CIN and similar lesions has predominantly involved ablative therapies which purpose is to eliminate the damaged HPV-infected tissue, leaving the healthy tissue of the cervix intact[79]. Hysterectomy is not acceptable as primary therapy for high-grade CIN because most patients are women of reproductive age[80]. Ablative therapies include cryotherapy, excision procedures (conization), laser therapy, and electrosurgery[81].

Ablative therapies

Cryotherapy: Also known as cryosurgery, it is a surgical treatment to freeze and destroy abnormal tissue in the cervix. It is performed with a cryoprobe, which is a metal rod cold enough to freeze and eliminate the tissue. The procedure is more effective when the tissue is frozen for 3 min, allowed to thaw for about 5 min, and frozen again

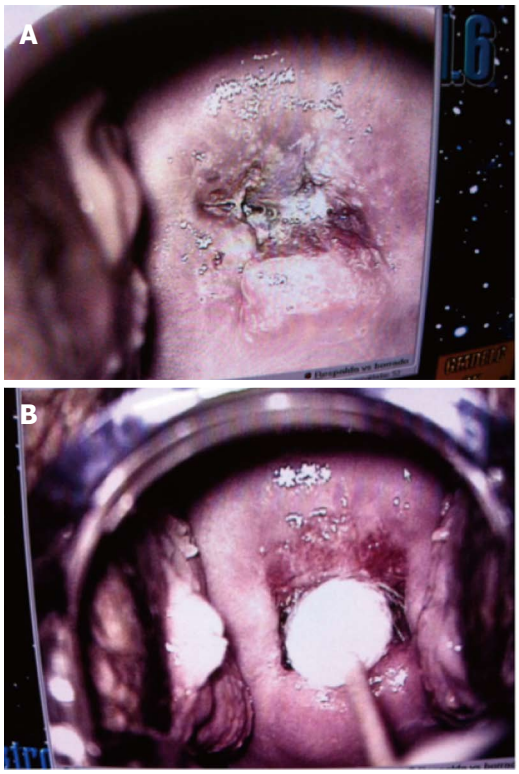

Figure 4 Conization of human papilloma virus cervical intraepithelial neoplasia 3 lesions. Colposcopy of papillomavirus cervical intraepithelial neoplasia 3 lesions from a patient treated with conization. Photographs of cervix before (A) and immediately after conization (B).

for another 3 min. Cryotherapy is widely used because it is easy of use and can be performed at the doctor's office. In fact, in many countries it is the only option available outside surgical settings. So, it is important that diagnosis and visualization of lesions be accurate before the cryotherapy is performed, in order to avoid missing small or deep lesions. During colposcopy, vinegar (acetic acid) or iodine (Lugol's solution) are applied to vagina and cervix with a swab or cotton balls to see areas of abnormal tissue more clearly, prior to cryotherapy. In summary cryotherapy is used mainly when ectropion (cervical eversion), cervicitis, or an HPV infection is present. If low- and high-grade lesions are detected after histology, then conization is recommended.

Excision procedures: For larger lesions excision procedures are used. The most commonly procedures are the loop electrosurgical excision procedure (LEEP) and the cold knife cone biopsy (conization). These methods are relatively inexpensive (between United States $ 500 and United States $ 1000[82]) and can be performed at the doctor's office (Dysplasia clinic) in an outpatient setting. LEEP uses a thin, low-voltage electrified wire loop to cut out abnormal tissue, which is either visible during colposcopy, or hidden in the cervical canal and not visible during colposcopy. Conization is an extensive form of cervical biopsy used to remove abnormal tissue. It is called a cone biopsy because a cone-shaped piece of tissue is removed. Conization removes abnormal tissue, usually high in the cervical canal, and also some normal tissue around the damaged tissue, so that a perimeter of normal cells is left in the cervix. Usually conization is the standard treatment if invasive disease (CIN 1, CIN 2, and CIN 3 lesions) is suspected, because it can remove tissue deep in the endocervical canal and can avoid diathermy artifacts (Figure 4).

Laser therapy: In this type of surgery a highly focused beam of light is used to obliterate the damaged tissue. Commonly a carbon dioxide laser is used to create the light beam. The laser light destroys the tissue and cauterizes the wound at the same time, reducing bleeding and pain, and shortening the healing time.

Electrosurgery: Electrofulguration is a type of electrosurgery used to burn the cancerous tissue[83]. It consists of applying a moderately damped alternating electrical current through an electrode that is held close to the tissue so that sparks hit and destroy the lesion.

Hysterectomy: In this surgery, complete removal of the uterus, and other reproductive organs, such as ovaries, fallopian tubes and surrounding structures, is performed. This radical procedure is done when initiation of invasive cancer in female patients is diagnosed.

The choice to use one treatment over another depends mainly on infrastructure and is usually made by the provider. All these ablative therapies are very effective in initial treatments where elimination of lesions can be as high as 80%. However, removal of damaged tissue, does not always guarantee elimination of viral DNA, and high rates of recurrence (up to 40%) are found[20] even after several treatments[84].

Non-surgical therapies

Non-surgical therapies that kill cells on contact are also used to eliminate HPV-infected lesions[85]. Some of these alternative treatments can be self applied by the patient, and generally are the first line of treatment for genital warts[86].

Trichloracetic acid: Trichloracetic acid is applied topically by the physician directly on warts. The acid action produces a chemical burn of the wart. This treatment is effective in clearing up to the 50% of lesions; it does not present systemic toxicity and can be used during pregnancy. However, it is associated with adverse effects such as ulceration, dermal scarring and secondary infections.

Podophyllotoxin: Podophyllotoxin is a non-alkaloid lignan extracted from the roots of *Podophyllum* species (*e.g.*, *P. peltatum*-Mayapple)[87]. This toxin is used as an initial treatment for genital warts. It is found as a gel (Condylox) or as a solution or cream (Wartec), that can be self-applied by the patient. The mechanism of action is not clear, but it may be related to the binding of lignans to micro-

tubule proteins, inducing cell cycle arrest at metaphase. Podophyllotoxin treatment can achieve 50% clearance, but recurrence rates are 25%-30%[84]. Podophyllotoxin is contraindicated in pregnancy.

Imiquimod: Imiquimod is a low-molecular-weight imidazoquinoline compound that modulates the innate immune system. In the form of a patient-applied cream (Aldara) it is used to treat genital warts. Reduction in genital warts was observed in 50% of patients that used the 5% cream for 12 wk, with only 19% recurrence[88]. Imiquimod was also reported to have certain therapeutic effects on basal cell carcinoma[89], vaginal[90], vulvar[91], and anal intraepithelial neoplasia[92], but the drug is not licensed for these treatments. Imiquimod has important inflammatory side effects, including itching, erythema, burning, irritation, tenderness, ulceration, and pain, thus its use on mucosal tissue is limited[93].

The mechanism of action of Imiquimod is not completely clear, but it is known that Imiquimod is an agonist for TLR 7, commonly involved in pathogen recognition[94]. Imiquimod binding to TLR 7 activates immune cells such as dendritic cells, macrophages, and keratinocytes to produce γ-IFN[95] and other pro-inflammatory cytokines, such as IL-6 and TNF-α[96,97]. There is also evidence that Imiquimod, when applied to skin, can activate antigen presenting cells, which then migrate to local lymph nodes to induce an adaptive immune response[95]. Because Imiquimod induces IFN production, it should be used with caution on intra-epithelial lesions infected with high-risk HPV types. Both α-IFN and β-IFN inhibit cell growth of keratinocytes with episomal HPV DNA, leading to virus elimination. However, IFNs do not affect cell growth of cells containing integrated HPV DNA. The result is that it selects for keratinocytes with integrated HPV DNA[98,99]. The continued use of Imiquimod may then select for transformed cells with integrated HPV, and induce progression to high-grade intra-epithelial neoplasia.

Polyphenon: Polyphenon is a high-grade extract of green tea leaves from *Camellia sinensis,* manufactured by the Mitsui Norin Co., Ltd. of Japan. In the form of a topical ointment it can be self applied by the patient for treatment of genital warts. It has shown efficacy (54%) to reduce warts in treated patients with fewer recurrences[100]. The main component in polyphenol is the catechin (a plant natural phenol belonging to the flavonoids family) (-) Epigallocatechin gallate (EGCG). EGCG is a potent antioxidant that reduces nitric oxide production by inhibiting inducible nitric oxide synthase *via* inactivation of the transcription factor nuclear factor-B (NF-B)[101,102]. EGCG has also been shown to induce inhibition of growth and apoptosis[103].

Cidofovir: Cidofovir is an acyclic nucleoside phosphonate analog to cytidine. It is used for the treatment of cytomegalovirus infections[104], and it is also used off-license as a topical treatment against benign low-risk HPV infections[105]. Cidofovir is phosphorylated in the cell and then blocks incorporation of cytidine into viral DNA thus arresting virus replication. Small trials with CIN patients have shown promising efficacy[106], but safety issues are a concern[107].

NOVEL IMMUNOTHERAPIES

Prophylactic vaccination as discussed above, concentrates on preventing virus infection of epithelial cells, by generating a humoral immune response with antibodies that recognize viral capsid proteins and neutralize the virus. However, this approach is not effective for treating existing infections or established HPV-related diseases. Thus, a high prevalence and mortality of cervical cancer continues around the world, especially in developing countries. An effective therapy for established disease should stimulate a cellular immune response, with activation of both $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells, which can recognize and eliminate virus infected cells[108]. Cytotoxic cells need to recognize a viral antigen expressed in the infected cells. Several of the HPV proteins are potential targets to induce a good immune cytotoxic response. Capsid proteins L1 and L2 are poor antigens for a therapeutic vaccine, since these proteins are expressed mainly in terminally differentiated keratinocytes and in advanced-stage infections of cervical tissue low levels of these protein are detected (Figure 2). In contrast, HPV early proteins, such as E1, E2, E6, and E7, are expressed in multiple stages of the virus infection, and in HPV-associated cancers the E6 and E7 proteins are constitutively expressed (Figure 2). Thus, these proteins are all good antigenic targets for a therapeutic vaccine.

E2 is a DNA-binding protein involved in activation or repression of different HPV promoters[109], and it also has a relevant role in controlling migration of viral DNA to daughter cells during mitosis of infected cells[110]. Due to its importance in the whole virus replication cycle, E2 protein expression is maintained throughout most stages of infection (Figure 2) and thus, it becomes an excellent target for stimulating the immune system to eliminate infected cells independently of the stage of pathogenesis. Based on this, earlier studies using dogs immunized against papilloma E1 and E2 proteins, found that dogs were protected from papilloma growth after viral challenge, or had complete regression of papilloma after post challenge immunization[111,112]. These results in animal models led to the design of new vaccines intended to induce a cellular immune response to the E2 protein. In clinical trials very encouraging results have been obtained with this approach (see next section)[113,114].

The oncogenic HPV E6 and E7 proteins are essential for progression to, and maintenance of, the malignant phenotype. Therefore, these two proteins are also possible antigenic targets for therapeutic vaccines. The high-risk HPV 16 and 18 E6 and E7 proteins have been selected predominantly for induction of a cellular response.

Table 1 Immune therapies for human papilloma virus-related lesions in clinical trials

Name	Target protein	Platform	Immune response/clinical efficacy	Ref.
Peptide-based				
	E7	Synthetic peptides restricted to HLA-A2	T helper cell response, but no CTL response	[118,152]
HPV16-SLP	E6, E7	Synthetic overlapping long peptides	HPV-specific T cell response. Neither tumor regression nor prevention of progressive disease	[120-122]
Protein-based				
L1VLPE7	E7	Chimeric L1-E7 VLP	Histological reduction to CIN 1 in 39% of treated patients compared to 35% of placebo recipients. This trend was not significant	[123]
SGN-00101	E7	Fusion protein of M. bovis Hsp and HPV 16 E7	Complete regression in 35% of patients with CIN 3	[125,126]
HPV16 E6/E7	E6, E7	Fusion protein of HPV 16 E6 and E7 proteins administered with the adjuvant ISCOMATRIX	Demonstrated important CD8+ T cell responses	[128]
TA-CIN	E6, E7, L2	Recombinant fusion protein consisting of E6, E7, and L2 from HPV	Imiquimod, for 8 wk, followed by 3 doses of TA-CIN induced complete regression in 32% of patients with VIN	[131]
DNA vaccine				
pConE6E7	E6, E7	DNA vaccine that encodes a HPV 16 or 18 consensus E6/E7 fusion gene	Cellular immune response against HPV E6 and E7 antigens and protection against HPV E6 and E7-expressing tumors	[133,134]
ZYC101a	E7	Microencapsulated DNA vaccine encoding E7-specific CTL epitopes	In younger patients a 70% reduction of lesions was detected	[135]
Amolimogene	E6, E7	A poly (lactide co-glycolide) encapsulated plasmid DNA encoding T cell epitopes from HPV 16 and 18	Increase in HPV-specific T cells to epitopes from HPV 16, 18, 6 and 11. No correlation between cellular immunity and clinical response	[136]
Sig-E7(detox)-HSP70	E7	Fusion protein between HPV 16 E7 and heat shock protein 70	Low frequency and weak HPV E7-specific T-cell responses with no correlation to clinical results	[137]
Recombinant virus				
TA-HPV	E6, E7	Vaccinia virus encoding modified HPV 16/18 E6 and E7 proteins	60% patients with high-grade VIN had partial reduction in lesion diameter, and an increase in lesion-infiltrating CD4+ and CD8+ T cells	[142,143]
TG4001	E6, E7	Vaccinia virus encoding modified HPV 16 E6 and E7 proteins, and the human IL-2	Ten patients (48%) with CIN 3 showed promising clinical responses at 6 mo, but no data on related immune response	[144]
MVA E2	E2	Vaccinia virus (MVA) encoding bovine papilloma virus (BPV) E2	19 out of 34 patients with high-grade CIN 3 lesions had complete regression. Specific cytotoxic activity against cancer cells correlated with clinical outcome	[113,114]
MVA-E1	E1	Vaccinia virus encoding the E1 sequence of HPV 16	MVA-E1 into C57BL/6 mice resulted in sustained HPV 16 E1-specific T cells with cytotoxic activity	[147]

HPV: Human papillomavirus; CTL: Cytotoxic T lymphocytes; VLP: Virus-like particles; VIN: Vulvar; CIN: Cervical intraepithelial neoplasia.

Immunization has been tried with different delivery systems in transplantable rodent tumor models[115] and in some clinical trials. The types of therapeutic vaccines tested so far in clinical trials can be grouped into five categories: peptide-based, protein-based, DNA vaccination, viral vectors, and DC-based immunization (Table 1). These therapeutic approaches are described next.

Peptide-based vaccines

Peptides are easy to use, inexpensive, and safe, but they are in general weakly immunogenic and they need to be mixed with adjuvants to improve their recognition by the immune system. The design of peptides involves identification of what parts of the HPV antigen are immunogenic. Short peptides may miss the relevant epitope needed for stimulation of an efficient immune response. Thus, current preparations contain mixtures of peptides. In addition, the polymorphic nature of the MHC may result in some peptides not being presented because the corresponding HLA molecule is missing in particular patients. Therefore, the peptide vaccine approach has also used restricted HLA-binding peptides. A potential problem with this strategy is that exogenously added peptides may load onto MHC class I molecules on cells other than antigen presenting cells, leading to tolerance instead of stimulation because non-APC lack co-stimulatory molecules[116]. The best approach seems to be the use of long overlapping peptides, which appear to be processed and presented better than whole proteins by DC[117].

A vaccine consisting of two synthetic HPV 16 E7 peptides (representing HLA-A*0201-restricted cytotoxic T lymphocyte epitopes) and one helper peptide (a pan-HLA-DR-binding T-helper epitope) emulsified in adjuvant was tried in a phase I - II clinical trial with cervical carcinoma patients. The peptide preparation was safe, and induced proliferative immune responses in 4 out of 15 patients. Unfortunately, no cytotoxic T lymphocyte responses against the HPV 16 E7 peptides were detect-

ed[118]. In a different study, 20 patients with HPV 16-positive, high-grade vulvar intraepithelial neoplasia were immunized with a mix of long peptides from the HPV 16 viral oncoproteins E6 and E7 in incomplete Freund's adjuvant. Five patients had complete regression, and HPV 16 was not longer detected in four of them. These patients also presented an increased T cell response[119].

Another peptide vaccine (HPV 16-SLP) consists of a mix of 9 HPV16 E6 and 4 HPV 16 E7 overlapping long peptides. This vaccine was used to immunize patients with resected HPV 16-positive cervical cancer. HPV 16-specific T-cell immune responses were found with a preference of $CD4^+$ -IFN-producing T cells. Interestingly, there was also proliferation of T cells with a $CD4^+$, $CD25^+$, $Foxp3^+$ phenotype that is associated with Treg, suggesting that the response against HPV was not completely effective[120]. In another study, this vaccine was given to women with high-grade cervical squamous intraepithelial lesions. Immunization induced increased HPV 16-specific T-cell immunity, but not infiltration of HPV 16-specific T cells into the lesions, nor HPV clearance at the time of LEEP excision[121]. More recently, in a small trial including 20 patients with HPV 16-positive advanced or recurrent gynecological carcinoma, HPV 16-SLP was subcutaneously administered with Montanide ISA-51 adjuvant. A good HPV-specific T cell response was detected. However, no tumor regression nor prevention of progressive disease were found[122].

Recombinant proteins

Another approach for HPV immunization is the use of complete or recombinant proteins. Recombinant proteins have the advantage of providing all potential epitopes of an antigen, after processing by APC; but they are also more difficult to produce than peptides. Despite their longer size, proteins present low immunogenicity and they need to be mixed with adjuvants. For this reason, the E6 and E7 protein vaccines are made of viral proteins fused to proteins with more immunogenicity such as capsid proteins or bacterial heat shock proteins (Hsp).

L1VLPE7: L1VLPE7 is made of chimeric VLPs consisting of a carboxyl-terminally truncated HPV 16 L1 protein fused to the amino-terminal part of the HPV 16 E7 protein. The recombinant fusion proteins self-assembles into VLPs. In a small placebo-controlled clinical trial with 39 HPV 16-infected CIN 2/3 patients, these chimeric VLPs were able to induce antibodies with high titers against HPV 16 L1 and low titers against HPV 16 E7. Also cellular immune responses against both proteins were detected. Unfortunately, only a small no significant trend for histological improvement to CIN 1 or normal was observed in 39% of the patients[123].

Another similar vaccine made with a recombinant HPV 16 L1(ΔN26)-E7(ΔC38) protein, expressed in *E. coli* has been tested in a murine of cervical cancer. This chimeric protein also assembles into chimeric VLPs. Immunization with these chimeric VLPs induced neutralizing antibodies and triggered cell-mediated immune responses[124].

SGN-00101: SGN-00101 is a fusion protein consisting of a Hsp from *Mycobacterium bovis* and HPV 16 E7 protein. This protein was given to patients with CIN 3, *via* three monthly subcutaneous immunizations. In 22% of patients regression to CIN 1 was observed. However, it was unclear whether this response was due to natural regression rather than the treatment[125]. In another study, SGN-00101 was given in four injections 3 wk apart to patients with high-grade CIN. With this protocol seven of 20 patients (35%) had a complete regression that correlated with immune response[126]. This vaccine also showed a partial response in 60% HIV-infected men with anal intraepithelial neoplasia[127].

HPV16 E6/E7: A fusion protein formed by HPV 16 E6 and E7 proteins was mixed with the adjuvant ISCOMATRIX and given to patients with CIN. A specific HPV 16 E6 and E7 immune response was detected with an important contribution of $CD8^+$ CTL response in immunized individuals than in placebo recipients. Elimination of HPV 16 DNA from lesions was detected in some treated patients and placebo recipients, but it did not correlate to immune status of patients[128].

TA-CIN: Tissue antigen-cervical intraepithelial neoplasia (TA-CIN) is a recombinant fusion protein consisting of E6, E7, and L2 from HPV 16 and HPV 18. This fusion protein was tested in 29 patients with ano-genital intraepithelial neoplasia. They received three intramuscular doses of TA-CIN at four weekly intervals. Several immune parameters indicated an HPV-specific response. However, there was not a correlation between induction of systemic immunity and clinical outcome[129]. Another study examined immunization of patients with vulval intraepithelial neoplasia with TA-CIN followed by one dose of a recombinant vaccinia virus encoding HPV 16 and 18 E6/E7 (TA-HPV). Only six out of 29 patients presented partial regression, and CTL activity was found in only four patients. Due to the poor outcome, this protocol has been abandoned[130]. More recently a different protocol with TA-CIN was examined in patients with vulval intraepithelial neoplasia. In a phase Ⅱ clinical trial 19 patients were given a topical application of the immunomodulator, Imiquimod, for 8 wk, followed by 3 doses of TA-CIN at four-week intervals. Complete regression was observed in 32% (6 out of 19) patients at week 10, increasing to 58% (11 out of 19) at week 20. At this time, there was also a significant local infiltration of $CD8^+$ and $CD4^+$ T cells in lesions of responding patients. Interestingly, non-responders had an increase of Treg cells. It seems that the inflammatory state induced by Imiquimod enhances the immune response, but the therapeutic effect still depends on the individual immune response of patients[131].

DNA-based vaccines

Another interesting approach for immunization against HPV early proteins has been the use of plasmid DNA that

codes for the corresponding viral proteins. It is known that plasmid DNA, when injected into the skin or muscle can induce immune responses to encoded antigens. The process is relatively inefficient, but new technological advancements such as improved physical methods of delivery can induce more-potent cellular and humoral responses[132].

pConE6E7: A DNA vaccine that encodes a HPV 16 or 18 consensus E6/E7 fusion gene (pConE6E7) has been tested in mice and rhesus monkeys. Immunization induced a potent cellular immune response against HPV 18 E6 and E7 antigens[133]. Moreover, prophylactic immunization with this vaccine also induced complete protection against HPV E6 and E7-expressing tumors. In the case of established HPV-tumors, the vaccine was able to delay the growth of tumors[134].

ZYC101a: ZYC101a is a microencapsulated DNA vaccine. It consists of plasmid DNA encoding E7-specific CTL epitopes from HPV 16 and 18 embedded in biodegradable micro particles. In a placebo-controlled trial, patients with histologically confirmed CIN 2/3 neoplasia were treated with three intramuscular doses of ZYC101a. Forty three percent of patients presented regression, compared to 27% of patients receiving placebo, but the difference was not statistically significant. However, in a subset of younger patients (less than 25 years of age) a significant reduction of lesions was detected in treated patients (70%) *vs* patients receiving placebo (23%). Unfortunately, no correlation between cytotoxic activity and clinical outcome was detected[135].

Amolimogene: Amolimogene is another encapsulated plasmid DNA proteins of HPV types 16 and 18. In a phase II trial of patients with HPV-associated high-grade CIN, 11 out of 21 patients had elevated $CD8^+$ T cell responses to HPV 16 and/or 18 peptides, and seven of these patients also had increases to corresponding HPV 6 and/or 11 peptides. No correlation between cellular immunity and clinical response was reported[136].

Sig-E7 (detox)-HSP70: Sig-E7(detox)-HSP70 is a DNA vaccine encoding a fusion protein between HPV 16 E7 protein and heat shock protein 70. This vaccine was evaluated in patients with HPV 16 positive CIN 2/3. Patients received three intramuscular immunizations with increasing doses of plasmid DNA. Low frequency and weak HPV E7-specific T-cell responses were detected after treatment. Regression of lesions was seen in only 3 out of 9 patients, but the difference was not significant. Thus, no correlation was found between immune response and clinical outcome[137].

Recombinant virus

The therapeutic approaches described above have given interesting results at the level of *in vitro* or animal models. Clinical results are at best slightly better than those expected for spontaneous regression, and in many cases no clear correlation between the immune response and the clinical outcome were found. Another approach that has shown better results for treatment of HPV-induced lesions is the use of recombinant viruses. With this method, the HPV early gene products can be delivered directly into the cells. All the immune system responses against viruses get activated and a much better presentation of antigens takes place leading to a stronger cellular immune response. The highly attenuated poxvirus strain modified vaccinia virus Ankara (MVA) has become the vector of choice for novel HPV therapeutic vaccines[115]. MVA is a non-replicating derivative of the uniquely successful smallpox vaccine, thus its use in humans is completely safe. In addition MVA is genetically stable, easy to manufacture, and very immunogenic[138,139], in part due to cross-presentation of dying vaccinia virus-infected cells by DC to $CD8^+$ T cells[140]. Several MVA vectors against various diseases are now being evaluated in phase I/II clinical trials[141].

TA-HPV: TA-HPV is a vaccinia virus encoding modified versions of the E6 and E7 genes from HPV 16 and HPV 18. In a phase II trial of HPV 16-positive high-grade vulval intraepithelial neoplasia, patients were immunized intramuscularly with TA-HPV. Forty two percent of patients showed partial reduction in total lesion diameter at 24 wk. Although, there was no increase in cytotoxic activity against selected individual HLA class I-restricted HPV 16 E6/7 peptides[142]. In another study, eight out of 13 patients with high-grade VIN presented a partial reduction in lesion diameter, and an increase in lesion-infiltrating $CD4^+$ and $CD8^+$ T cells, however no increase in cytotoxic activity was detected[143].

TG4001: TG4001 is a recombinant vaccinia virus (MVA) encoding a modified sequence of HPV 16 E6 and E7 proteins, and the human IL-2 gene. In a clinical study, 21 patients with HPV 16-related CIN 2/3 received three weekly subcutaneous injections of TG4001. Ten patients (48%) showed promising clinical responses at six months after treatment, but the related immune response was not reported[144].

MVA E2: MVA E2 is also a recombinant vaccinia virus (MVA) encoding bovine papilloma virus E2 protein[145]. In a series of studies, MVA E2 was evaluated in patients who had established HPV-induced CIN lesions. In a phase I / II clinical trial, for CIN 1 to CIN 3 lesions, 36 women received the recombinant virus vaccine at a total of 10^7 MVA E2 virus particles injected directly into the uterus once every week over a 6-wk period. Ninety four percent (34) of patients showed complete elimination of precancerous lesions after treatment. In the other two patients, precancerous lesions were reduced from grade CIN 3 to CIN 1. In addition, 50% of patients eliminated completely the HPV, and in the remaining 50% of patients, HPV DNA was only 10% of the original viral load[113]. Later, in a phase II clinical trial for high-grade

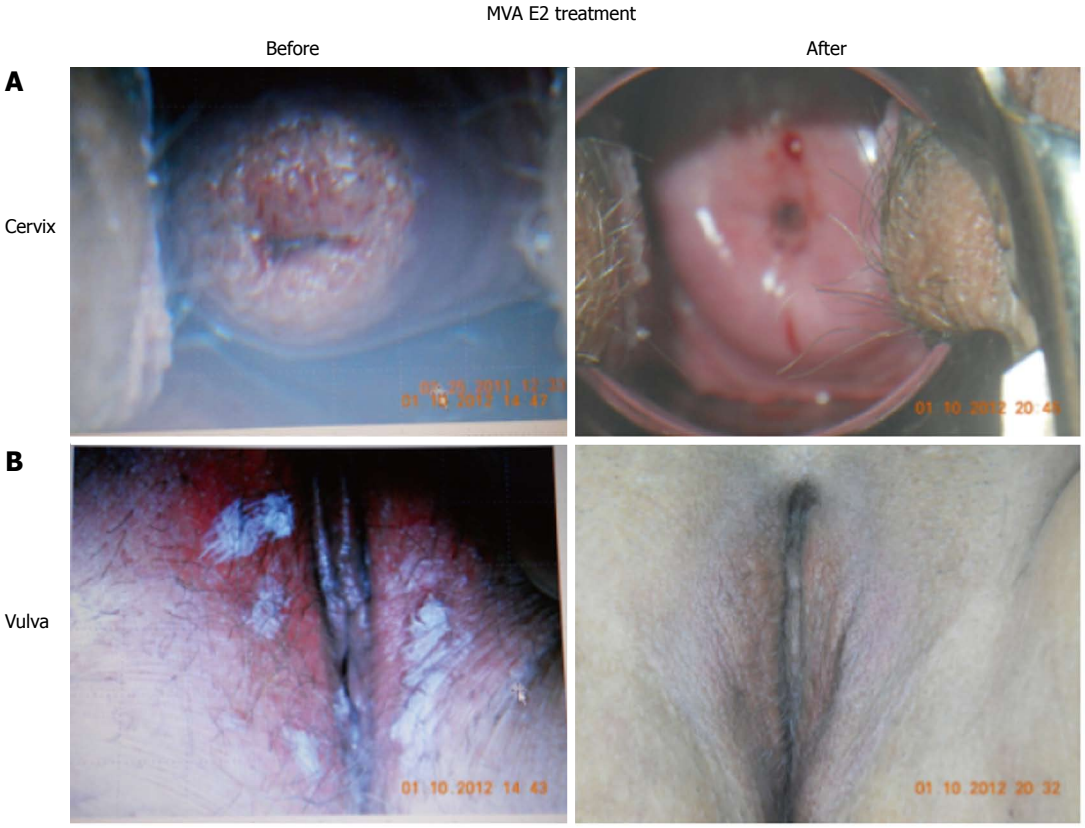

Figure 5 Effects of vaccinia virus Ankara E2 on cervix and vulva. Colposcopy of papillomavirus-induced intraepithelial lesions from patients treated with the therapeutic recombinant vaccinia virus Ankara E2. Photographs of (A) cervix with a cervical intraepithelial neoplasia 3 lesion, and (B) vulva with a condyloma lesion, before and after treatment. MVA: Modified vaccinia virus Ankara.

lesions (CIN 2 and CIN 3), 19 out of 34 (56%) patients had a complete regression, while in 11 (32%) more patients the lesions were reduced by 90%-60%. Specific cytotoxic activity against cancer cells correlated with clinical outcome[114]. More recently, in a phase Ⅲ clinical trial, 1176 female patients with ano-genital intraepithelial lesions were injected directly into the uterus, vulva, or anus lesions with 10^7 MEL-1 (MVA E2) virus particles. One thousand and forty-five out of 1176 (89%) treated patients showed complete elimination of lesions, and generated a specific cytotoxic response against papilloma-transformed cells (Figure 5) (Rosales, R. Manuscript in preparation). Thus, local application of MEL-1 vaccine is an excellent therapy for stimulating the immune system and creating regression of HPV-induced intraepithelial lesions. In support to this conclusion, a recent report, using the murine model of cervical cancer with HPV 16 E6- and E7-expressing TC-1 tumor cells, indicated that another recombinant HPV vaccine (TA-HPV) increased its efficacy when it was administered directly into the tumor. An augment in E7-specific $CD8^+$ T cells was found in the blood, together with a significant decrease in tumor size[146].

MVA-E1: As described above, both HPV E1 and E2 proteins are expressed early in the HPV life cycle, and are necessary to maintain coordinated viral replication and gene expression during differentiation of keratinocytes along the epithelium. Thus, E1 is also a good candidate target for immunotherapy. Recently a new recombinant vaccinia virus has been reported, MVA-E1. This new vaccine consists of the MVA vector encoding the E1 sequence of HPV 16. Multiple injections of MVA-E1 into C57BL/6 mice resulted in sustained HPV 16 E1-specific cellular immune response involving T cells with cytotoxic activity[147].

DC-based vaccines

Another approach to develop a cellular therapeutic vaccine is the use of dendritic cells pulsed with HPV antigens. Autologous monocytes are differentiated into DCs *in vitro* and then loaded with recombinant HPV proteins. The cells are then administered back to the patient in order to stimulate a better cellular immune response. Previous studies used autologous DCs loaded with HPV 16 or HPV 18 E7 protein to induce *in vitro* a specific T cell response. In 18/20 T cell lines from healthy blood donors E7-specific T cells were detected[148]. This approach was also evaluated in patients with cervical cancer. Autologous monocyte-derived DCs were pulsed with recombinant HPV 16 E7 or HPV 18 E7 oncoprotein and administered to 15 stage IV cervical cancer patients[149]. Four out of 11 patients had T cell proliferative responses, and three out of 11 patients showed γ-IFN production by ELISpot assay. Treatment was well-tolerated with no side effects Unfortunately, an objective clinical response

was not observed[149]. In a different study, a pilot trial gave autologous DCs pulsed with HPV E7 to four cervical cancer patients. E7-specific γ-IFN secreting $CD8^+$ T cells were detected in all patients after vaccination[150]. Later, in a phase I trial, autologous DCs pulsed with HPV 16/18 E7 protein and keyhole limpet hemocyanin were injected five times subcutaneously at 21-d intervals into 10 cervical cancer patients. Eight out of 10 patients had elevated counts of E7-specific γ-IFN secreting $CD8^+$ T cells. Contribution of this immune response to therapy could not be evaluated since all patients were treated by surgery[151]. These results show that this approach may be useful in the future for some difficult advanced cervical cancer patients. Another autologous DC vaccine for prostate cancer treatment, Provenge® (first FDA-approved therapeutic cancer vaccine) has given good results with metastatic patients. Thus, a great interest for new DC vaccines exists. However, this kind of treatment is labor-intensive and expensive, and has to be performed individually for each patient.

CONCLUSION

HPV infections remain an important public health issue because they are associated to cervical carcinoma, the second most common cancer among women[4]. Two approved prophylactic vaccines, promise in the long run a reduction in HPV-related cancer incidence. However, the fact that full vaccination coverage of large populations will no be easy in many parts of the world[73], and that these vaccines target only HPV 16 and 18 (HPV types responsible for about 60% of all cervical cancers), a large population will remain at a high risk of HPV-related disease, and in need of treatment.

Treatment for CIN and similar lesions has predominantly involved ablative therapies which purpose is to eliminate the damaged HPV-infected tissue, leaving the healthy tissue of the cervix intact[79]. Ablative therapies include cryotherapy, excision procedures, laser therapy, and electrosurgery[81]. Unfortunately, these surgical procedures are only effective in the treatment of pre-cancerous lesions, since after surgery many recurrences appear due to new re-infections.

Novel HPV protein-expressing vaccines are being evaluated for their potential to stimulate the immune system and generate a cellular cytotoxic response against the HPV-infected tissue or tumor. These new therapeutic vaccines can be grouped into five categories: peptide-based, protein-based, DNA vaccination, viral vectors, and DC-based immunization. They have shown variable results, but the ones using recombinant virus have demonstrated significant clinical efficacy and systemic HPV-specific cytotoxic T cell responses, particularly when the recombinant vaccinia virus is administered directly to lesions. Thus, recombinant vaccinia therapies are today the best candidates for a successful treatment of HPV-induced cancers.

REFERENCES

1 **Jablonska S**, Dabrowski J, Jakubowicz K. Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. *Cancer Res* 1972; **32**: 583-589 [PMID: 5061309]

2 **zur Hausen H**, de Villiers EM, Gissmann L. Papillomavirus infections and human genital cancer. *Gynecol Oncol* 1981; **12**: S124-S128 [PMID: 6273261 DOI: 10.1016/0090-8258(81)90067-6]

3 **Forman D**, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M, Franceschi S. Global burden of human papillomavirus and related diseases. *Vaccine* 2012; **30** Suppl 5: F12-F23 [PMID: 23199955 DOI: 10.1016/j.vaccine.2012.07.055]

4 **Arbyn M**, Castellsagué X, de Sanjosé S, Bruni L, Saraiya M, Bray F, Ferlay J. Worldwide burden of cervical cancer in 2008. *Ann Oncol* 2011; **22**: 2675-2686 [PMID: 21471563 DOI: 10.1093/annonc/mdr015]

5 **Bernard HU**. Taxonomy and phylogeny of papillomaviruses: an overview and recent developments. *Infect Genet Evol* 2013; **18**: 357-361 [PMID: 23523816 DOI: 10.1016/j.meegid.2013.03.011]

6 **Lacey CJ**, Lowndes CM, Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine* 2006; **24** Suppl 3: S3/35-S3/41 [PMID: 16950016]

7 **Muñoz N**, Bosch FX. The causal link between HPV and cervical cancer and its implications for prevention of cervical cancer. *Bull Pan Am Health Organ* 1996; **30**: 362-377 [PMID: 9041748]

8 **Parkin DM**, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine* 2006; **24** Suppl 3: S3/11-S3/25 [PMID: 16949997]

9 **Cantley RL**, Gabrielli E, Montebelli F, Cimbaluk D, Gattuso P, Petruzzelli G. Ancillary studies in determining human papillomavirus status of squamous cell carcinoma of the oropharynx: a review. *Patholog Res Int* 2011; **2011**: 138469 [PMID: 21772959 DOI: 10.4061/2011/138469]

10 **D'Souza G**, Fakhry C, Sugar EA, Seaberg EC, Weber K, Minkoff HL, Anastos K, Palefsky JM, Gillison ML. Six-month natural history of oral versus cervical human papillomavirus infection. *Int J Cancer* 2007; **121**: 143-150 [PMID: 17354235 DOI: 10.1002/ijc.22667]

11 **Schiffman M**, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007; **370**: 890-907 [PMID: 17826171 DOI: 10.1016/S0140-6736(07)61416-0]

12 **Woodman CB**, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* 2007; **7**: 11-22 [PMID: 17186016]

13 **Trimble CL**, Piantadosi S, Gravitt P, Ronnett B, Pizer E, Elko A, Wilgus B, Yutzy W, Daniel R, Shah K, Peng S, Hung C, Roden R, Wu TC, Pardoll D. Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. *Clin Cancer Res* 2005; **11**: 4717-4723 [PMID: 16000566 DOI: 10.1038/nrc2050]

14 **Nuovo GJ**, Friedman D, Richart RM. In situ hybridization analysis of human papillomavirus DNA segregation patterns in lesions of the female genital tract. *Gynecol Oncol* 1990; **36**: 256-262 [PMID: 2153624 DOI: 10.1158/1078-0432.CCR-04-2599]

15 **Cuzick J**, Bergeron C, von Knebel Doeberitz M, Gravitt P, Jeronimo J, Lorincz AT, J L M Meijer C, Sankaranarayanan R, J F Snijders P, Szarewski A. New technologies and procedures for cervical cancer screening. *Vaccine* 2012; **30** Suppl 5: F107-F116 [PMID: 23199953 DOI: 10.1016/j.vaccine.2012.05.088]

16 **Poljak M**, Cuzick J, Kocjan BJ, Iftner T, Dillner J, Arbyn M. Nucleic acid tests for the detection of alpha human papil-

lomaviruses. *Vaccine* 2012; **30** Suppl 5: F100-F106 [PMID: 23199952 DOI: 10.1016/j.vaccine.2012.04.105]
17 **Solomon D**, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T, Young N. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002; **287**: 2114-2119 [PMID: 11966386 DOI: 10.1001/jama.287.16.2114]
18 **Cuzick J**, Meijer CJ, Walboomers JM. Screening for cervical cancer. *Lancet* 1998; **351**: 1439-1440 [PMID: 9593447 DOI: 10.1016/S0140-6736(05)79490-3]
19 **Martin-Hirsch PL**, Paraskevaidis E, Kitchener H. Surgery for cervical intraepithelial neoplasia. *Cochrane Database Syst Rev* 2000; **(2)**: CD001318 [PMID: 10796771 DOI: 10.1002/14651858.CD001318.pub2]
20 **Lacey CJ**, Woodhall SC, Wikstrom A, Ross J. 2012 European guideline for the management of anogenital warts. *J Eur Acad Dermatol Venereol* 2013; **27**: e263-e270 [PMID: 22409368 DOI: 10.1111/j.1468-3083.2012.04493.x]
21 **Stanley M**. Immune responses to human papillomavirus. *Vaccine* 2006; **24** Suppl 1: S16-S22 [PMID: 16219398 DOI: 10.1016/j.vaccine.2005.09.002]
22 **American Cancer Society.** Survival rates for cancer of the cervix. Available from: URL: http: //www.cancer.org/cancer/cervicalcancer/overviewguide/cervical-cancer-overview-survival-rates. Accessed: November 24 2013
23 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
24 **Kawana K**, Adachi K, Kojima S, Kozuma S, Fujii T. Therapeutic Human Papillomavirus (HPV) Vaccines: A Novel Approach. *Open Virol J* 2012; **6**: 264-269 [PMID: 23341862 DOI: 10.2174/1874357901206010264]
25 **Stern PL**, van der Burg SH, Hampson IN, Broker TR, Fiander A, Lacey CJ, Kitchener HC, Einstein MH. Therapy of human papillomavirus-related disease. *Vaccine* 2012; **30** Suppl 5: F71-F82 [PMID: 23199967 DOI: 10.1016/j.vaccine.2012.05.091]
26 **Doorbar J**, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA. The biology and life-cycle of human papillomaviruses. *Vaccine* 2012; **30** Suppl 5: F55-F70 [PMID: 23199966 DOI: 10.1016/j.vaccine.2012.06.083]
27 **Doorbar J**. Latent papillomavirus infections and their regulation. *Curr Opin Virol* 2013; **3**: 416-421 [PMID: 23816390 DOI: 10.1016/j.coviro.2013.06.003]
28 **Kawai T**, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 2011; **34**: 637-650 [PMID: 21616434 DOI: 10.1016/j.immuni.2011.05.006]
29 **Woodworth CD**. HPV innate immunity. *Front Biosci* 2002; **7**: d2058-d2071 [PMID: 12165480 DOI: 10.2741/woodworth]
30 **Degli-Esposti MA**, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat Rev Immunol* 2005; **5**: 112-124 [PMID: 15688039 DOI: 10.1038/nri1549]
31 **Zhang N**, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. *Immunity* 2011; **35**: 161-168 [PMID: 21867926 DOI: 10.1016/j.immuni.2011.07.010]
32 **Bourgault Villada I**, Moyal Barracco M, Ziol M, Chaboissier A, Barget N, Berville S, Paniel B, Jullian E, Clerici T, Maillère B, Guillet JG. Spontaneous regression of grade 3 vulvar intraepithelial neoplasia associated with human papillomavirus-16-specific CD4(+) and CD8(+) T-cell responses. *Cancer Res* 2004; **64**: 8761-8766 [PMID: 15574788 DOI: 10.1158/0008-5472.CAN-04-2455]
33 **Paternoster DM**, Cester M, Resente C, Pascoli I, Nanhorngue K, Marchini F, Boccagni P, Cillo U, Ribaldone R, Amoruso E, Cocca N, Cuccolo V, Bertolino M, Surico N, Stratta P. Human papilloma virus infection and cervical intraepithelial neoplasia in transplanted patients. *Transplant Proc* 2008; **40**: 1877-1880 [PMID: 18675077 DOI: 10.1016/j.transproceed.2008.05.074]
34 **Moscicki AB**, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis* 2004; **190**: 37-45 [PMID: 15195241 DOI: 10.1086/421467]
35 **Tjiong MY**, Zumbach K, Schegget JT, van der Vange N, Out TA, Pawlita M, Struyk L. Antibodies against human papillomavirus type 16 and 18 E6 and E7 proteins in cervicovaginal washings and serum of patients with cervical neoplasia. *Viral Immunol* 2001; **14**: 415-424 [PMID: 11792070 DOI: 10.1089/08828240152716655]
36 **Carter JJ**, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, Galloway DA. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000; **181**: 1911-1919 [PMID: 10837170 DOI: 10.1086/315498]
37 **Carter JJ**, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC, Porter P, Daling JR, McDougall JK, Galloway DA. Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. *Cancer Res* 2001; **61**: 1934-1940 [PMID: 11280749]
38 **Welters MJ**, de Jong A, van den Eeden SJ, van der Hulst JM, Kwappenberg KM, Hassane S, Franken KL, Drijfhout JW, Fleuren GJ, Kenter G, Melief CJ, Offringa R, van der Burg SH. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. *Cancer Res* 2003; **63**: 636-641 [PMID: 12566307]
39 **de Jong A**, van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, Kenter G, Offringa R, van der Burg SH. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Res* 2004; **64**: 5449-5455 [PMID: 15289354 DOI: 10.1158/0008-5472.CAN-04-0831]
40 **Monnier-Benoit S**, Mauny F, Riethmuller D, Guerrini JS, Căpîlna M, Félix S, Seillès E, Mougin C, Prétet JL. Immunohistochemical analysis of CD4+ and CD8+ T-cell subsets in high risk human papillomavirus-associated premalignant and malignant lesions of the uterine cervix. *Gynecol Oncol* 2006; **102**: 22-31 [PMID: 16427684 DOI: 10.1016/j.ygyno.2005.11.039]
41 **Stanley MA**. Immune responses to human papilloma viruses. *Indian J Med Res* 2009; **130**: 266-276 [PMID: 19901436]
42 **Xu Y**, Zhu KJ, Zhu N, Jiang DH, Chen XZ, Cheng H. Expression of Foxp3+CD4+CD25+ regulatory T cells and Th1/Th2, Tc1/Tc2 profiles in the peripheral blood of patients with condyloma acuminatum. *Clin Exp Dermatol* 2009; **34**: 229-235 [PMID: 19077104 DOI: 10.1111/j.1365-2230.2008.03001.x]
43 **Scott M**, Stites DP, Moscicki AB. Th1 cytokine patterns in cervical human papillomavirus infection. *Clin Diagn Lab Immunol* 1999; **6**: 751-755 [PMID: 10473530]
44 **Konya J**, Dillner J. Immunity to oncogenic human papillomaviruses. *Adv Cancer Res* 2001; **82**: 205-238 [PMID: 11447764]
45 **Bais AG**, Beckmann I, Ewing PC, Eijkemans MJ, Meijer CJ, Snijders PJ, Helmerhorst TJ. Cytokine release in HR-HPV(+) women without and with cervical dysplasia (CIN II and III) or carcinoma, compared with HR-HPV(-) controls. *Mediators Inflamm* 2007; **2007**: 24147 [PMID: 18288269 DOI: 10.1155/2007/24147]
46 **Bais AG**, Beckmann I, Lindemans J, Ewing PC, Meijer CJ, Snijders PJ, Helmerhorst TJ. A shift to a peripheral Th2-type cytokine pattern during the carcinogenesis of cervical cancer becomes manifest in CIN III lesions. *J Clin Pathol* 2005; **58**: 1096-1100 [PMID: 16189158 DOI: 10.1136/jcp.2004.025072]
47 **Nees M**, Geoghegan JM, Hyman T, Frank S, Miller L, Woodworth CD. Papillomavirus type 16 oncogenes downregulate expression of interferon-responsive genes and upregulate

proliferation-associated and NF-kappaB-responsive genes in cervical keratinocytes. *J Virol* 2001; **75**: 4283-4296 [PMID: 11287578 DOI: 10.1128/JVI.75.9.4283-4296.2001]
48 **Barnard P**, Payne E, McMillan NA. The human papillomavirus E7 protein is able to inhibit the antiviral and anti-growth functions of interferon-alpha. *Virology* 2000; **277**: 411-419 [PMID: 11080488 DOI: 10.1006/viro.2000.0584]
49 **Li S**, Labrecque S, Gauzzi MC, Cuddihy AR, Wong AH, Pellegrini S, Matlashewski GJ, Koromilas AE. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha. *Oncogene* 1999; **18**: 5727-5737 [PMID: 10523853 DOI: 10.1038/sj.onc.1202960]
50 **Hasan UA**, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, Mansour M, Vincent I, Gissmann L, Iftner T, Sideri M, Stubenrauch F, Tommasino M. TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. *J Immunol* 2007; **178**: 3186-3197 [PMID: 17312167 DOI: 10.4049/jimmunol.178.5.3186]
51 **Huang SM**, McCance DJ. Down regulation of the interleukin-8 promoter by human papillomavirus type 16 E6 and E7 through effects on CREB binding protein/p300 and P/CAF. *J Virol* 2002; **76**: 8710-8721 [PMID: 12163591 DOI: 10.1128/JVI.76.17.8710-8721.2002]
52 **Lee SJ**, Cho YS, Cho MC, Shim JH, Lee KA, Ko KK, Choe YK, Park SN, Hoshino T, Kim S, Dinarello CA, Yoon DY. Both E6 and E7 oncoproteins of human papillomavirus 16 inhibit IL-18-induced IFN-gamma production in human peripheral blood mononuclear and NK cells. *J Immunol* 2001; **167**: 497-504 [PMID: 11418688 DOI: 10.4049/jimmunol.167.1.497]
53 **Heller C**, Weisser T, Mueller-Schickert A, Rufer E, Hoh A, Leonhardt RM, Knittler MR. Identification of key amino acid residues that determine the ability of high risk HPV16-E7 to dysregulate major histocompatibility complex class I expression. *J Biol Chem* 2011; **286**: 10983-10997 [PMID: 21321113 DOI: 10.1074/jbc.M110.199190]
54 **Rosenthal DW**, DeVoti JA, Steinberg BM, Abramson AL, Bonagura VR. T(H)2-like chemokine patterns correlate with disease severity in patients with recurrent respiratory papillomatosis. *Mol Med* 2012; **18**: 1338-1345 [PMID: 23019074 DOI: 10.2119/molmed.2012.00284]
55 **Piersma SJ**, Welters MJ, van der Burg SH. Tumor-specific regulatory T cells in cancer patients. *Hum Immunol* 2008; **69**: 241-249 [PMID: 18486758 DOI: 10.1016/j.humimm.2008.02.005]
56 **Khazaie K**, von Boehmer H. The impact of CD4+CD25+ Treg on tumor specific CD8+ T cell cytotoxicity and cancer. *Semin Cancer Biol* 2006; **16**: 124-136 [PMID: 16443370 DOI: 10.1016/j.semcancer.2005.11.006]
57 **van der Burg SH**, Piersma SJ, de Jong A, van der Hulst JM, Kwappenberg KM, van den Hende M, Welters MJ, Van Rood JJ, Fleuren GJ, Melief CJ, Kenter GG, Offringa R. Association of cervical cancer with the presence of CD4+ regulatory T cells specific for human papillomavirus antigens. *Proc Natl Acad Sci USA* 2007; **104**: 12087-12092 [PMID: 17615234 DOI: 10.1073/pnas.0704672104]
58 **Visser J**, Nijman HW, Hoogenboom BN, Jager P, van Baarle D, Schuuring E, Abdulahad W, Miedema F, van der Zee AG, Daemen T. Frequencies and role of regulatory T cells in patients with (pre)malignant cervical neoplasia. *Clin Exp Immunol* 2007; **150**: 199-209 [PMID: 17937675 DOI: 10.1111/j.1365-2249.2007.03468.x]
59 **Adurthi S**, Mukherjee G, Krishnamurthy H, Sudhir K, Bafna UD, Umadevi K, Jayshree RS. Functional tumor infiltrating TH1 and TH2 effectors in large early-stage cervical cancer are suppressed by regulatory T cells. *Int J Gynecol Cancer* 2012; **22**: 1130-1137 [PMID: 22872166 DOI: 10.1097/IGC.0b013e318262aa53]
60 **von Boehmer H**, Daniel C. Therapeutic opportunities for manipulating T(Reg) cells in autoimmunity and cancer. *Nat Rev Drug Discov* 2013; **12**: 51-63 [PMID: 23274471 DOI: 10.1038/nrd3683]
61 **Schreiber TH**, Raez L, Rosenblatt JD, Podack ER. Tumor immunogenicity and responsiveness to cancer vaccine therapy: the state of the art. *Semin Immunol* 2010; **22**: 105-112 [PMID: 20226686 DOI: 10.1016/j.smim.2010.02.001]
62 **Wolchok JD**, Hodi FS, Weber JS, Allison JP, Urba WJ, Robert C, O'Day SJ, Hoos A, Humphrey R, Berman DM, Lonberg N, Korman AJ. Development of ipilimumab: a novel immunotherapeutic approach for the treatment of advanced melanoma. *Ann N Y Acad Sci* 2013; **1291**: 1-13 [PMID: 23772560 DOI: 10.1111/nyas.12180]
63 **Karim R**, Meyers C, Backendorf C, Ludigs K, Offringa R, van Ommen GJ, Melief CJ, van der Burg SH, Boer JM. Human papillomavirus deregulates the response of a cellular network comprising of chemotactic and proinflammatory genes. *PLoS One* 2011; **6**: e17848 [PMID: 21423754 DOI: 10.1371/journal.pone.0017848]
64 **Patel S**, Chiplunkar S. Host immune responses to cervical cancer. *Curr Opin Obstet Gynecol* 2009; **21**: 54-59 [PMID: 19125004 DOI: 10.1097/GCO.0b013e32831a9890]
65 **Feng Q**, Wei H, Morihara J, Stern J, Yu M, Kiviat N, Hellstrom I, Hellstrom KE. Th2 type inflammation promotes the gradual progression of HPV-infected cervical cells to cervical carcinoma. *Gynecol Oncol* 2012; **127**: 412-419 [PMID: 22828962 DOI: 10.1016/j.ygyno.2012.07.098]
66 **Molling JW**, de Gruijl TD, Glim J, Moreno M, Rozendaal L, Meijer CJ, van den Eertwegh AJ, Scheper RJ, von Blomberg ME, Bontkes HJ. CD4(+)CD25hi regulatory T-cell frequency correlates with persistence of human papillomavirus type 16 and T helper cell responses in patients with cervical intraepithelial neoplasia. *Int J Cancer* 2007; **121**: 1749-1755 [PMID: 17582606 DOI: 10.1002/ijc.22894]
67 **Villa LL**, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE, Olsson SE, Høye J, Steinwall M, Riis-Johannessen G, Andersson-Ellstrom A, Elfgren K, Krogh Gv, Lehtinen M, Malm C, Tamms GM, Giacoletti K, Lupinacci L, Railkar R, Taddeo FJ, Bryan J, Esser MT, Sings HL, Saah AJ, Barr E. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer* 2006; **95**: 1459-1466 [PMID: 17117182 DOI: 10.1038/sj.bjc.6603469]
68 **Harper DM**, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA, Dubin G. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006; **367**: 1247-1255 [PMID: 16631880 DOI: 10.1016/S0140-6736(06)68439-0]
69 **Paavonen J**, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet* 2009; **374**: 301-314 [PMID: 19586656 DOI: 10.1016/S0140-6736(09)61248-4]
70 **Kjaer SK**, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, Perez G, Brown DR, Koutsky LA, Tay EH, García P, Ault KA, Garland SM, Leodolter S, Olsson SE, Tang GW, Ferris DG, Paavonen J, Lehtinen M, Steben M, Bosch FX, Dillner J, Joura EA, Majewski S, Muñoz N, Myers ER, Villa LL, Taddeo FJ, Roberts C, Tadesse A, Bryan J, Maansson R, Lu S, Vuocolo S, Hesley TM, Saah A, Barr E, Haupt RM. A pooled analysis of continued prophylactic efficacy of quadrivalent human papillomavirus (Types

6/11/16/18) vaccine against high-grade cervical and external genital lesions. *Cancer Prev Res* (Phila) 2009; **2**: 868-878 [PMID: 19789295 DOI: 10.1158/1940-6207.CAPR-09-0031]
71 **Dillner J**, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M, Perez G, Brown DR, Koutsky LA, Tay EH, García P, Ault KA, Garland SM, Leodolter S, Olsson SE, Tang GW, Ferris DG, Paavonen J, Lehtinen M, Steben M, Bosch FX, Joura EA, Majewski S, Muñoz N, Myers ER, Villa LL, Taddeo FJ, Roberts C, Tadesse A, Bryan JT, Maansson R, Lu S, Vuocolo S, Hesley TM, Barr E, Haupt R. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. *BMJ* 2010; **341**: c3493 [PMID: 20647284 DOI: 10.1136/bmj.c3493]
72 **Dillner J**, Arbyn M, Unger E, Dillner L. Monitoring of human papillomavirus vaccination. *Clin Exp Immunol* 2011; **163**: 17-25 [PMID: 21062269 DOI: 10.1111/j.1365-2249.2010.04268.x]
73 **van Bogaert Lj**. Are the currently existing anti-human papillomavirus vaccines appropriate for the developing world? *Ann Med Health Sci Res* 2013; **3**: 306-312 [PMID: 24116304]
74 **Muñoz N**, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, Perez G, Brown DR, Koutsky LA, Tay EH, Garcia PJ, Ault KA, Garland SM, Leodolter S, Olsson SE, Tang GW, Ferris DG, Paavonen J, Steben M, Bosch FX, Dillner J, Huh WK, Joura EA, Kurman RJ, Majewski S, Myers ER, Villa LL, Taddeo FJ, Roberts C, Tadesse A, Bryan JT, Lupinacci LC, Giacoletti KE, Sings HL, James MK, Hesley TM, Barr E, Haupt RM. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. *J Natl Cancer Inst* 2010; **102**: 325-339 [PMID: 20139221 DOI: 10.1093/jnci/djp534]
75 **Tomljenovic L**, Shaw CA. Human papillomavirus (HPV) vaccine policy and evidence-based medicine: are they at odds? *Ann Med* 2013; **45**: 182-193 [PMID: 22188159 DOI: 10.3109/07853890.2011.645353]
76 **Clifford GM**, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1157-1164 [PMID: 15894666]
77 **Smith JS**, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007; **121**: 621-632 [PMID: 17405118]
78 **American Cancer Society.** Can cancer of the cervix be prvented? Available from: URL: http: //www.cancer.org/cancer/cervicalcancer/overviewguide/cervical-cancer-overview-prevention. Accessed: November 24 2013
79 **von Krogh G**, Lacey CJ, Gross G, Barrasso R, Schneider A. European course on HPV associated pathology: guidelines for primary care physicians for the diagnosis and management of anogenital warts. *Sex Transm Infect* 2000; **76**: 162-168 [PMID: 10961190]
80 **Wright TC**, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 2007; **197**: 346-355 [PMID: 17904957]
81 **Sonnex C**, Lacey CJ. The treatment of human papillomavirus lesions of the lower genital tract. *Best Pract Res Clin Obstet Gynaecol* 2001; **15**: 801-816 [PMID: 11563874]
82 **Saidi MH**, Setzler FD, Sadler RK, Farhart SA, Akright BD. Comparison of office loop electrosurgical conization and cold knife conization. *J Am Assoc Gynecol Laparosc* 1994; **1**: 135-139 [PMID: 9050476]
83 **Roy MC**, Mayrand MH, Franco E, Arseneau J, Ferenczy A. Electrofulguration for low-grade squamous intraepithelial lesions of the cervix (CIN 1). *J Low Genit Tract Dis* 2004; **8**: 10-15 [PMID: 15874830]
84 **Maw R**. Critical appraisal of commonly used treatment for genital warts. *Int J STD AIDS* 2004; **15**: 357-364 [PMID: 15186577]
85 **Viera MH**, Amini S, Huo R, Konda S, Block S, Berman B. Herpes simplex virus and human papillomavirus genital infections: new and investigational therapeutic options. *Int J Dermatol* 2010; **49**: 733-749 [PMID: 20618491 DOI: 10.1111/j.1365-4632.2009.04375.x]
86 **Lacey CJ**. Therapy for genital human papillomavirus-related disease. *J Clin Virol* 2005; **32** Suppl 1: S82-S90 [PMID: 15753016]
87 **Xu H**, Lv M, Tian X. A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: 2003-2007. *Curr Med Chem* 2009; **16**: 327-349 [PMID: 19149581 DOI: 10.2174/092986709787002682]
88 **Beutner KR**, Tyring SK, Trofatter KF, Douglas JM, Spruance S, Owens ML, Fox TL, Hougham AJ, Schmitt KA. Imiquimod, a patient-applied immune-response modifier for treatment of external genital warts. *Antimicrob Agents Chemother* 1998; **42**: 789-794 [PMID: 9559784]
89 **Beutner KR**, Geisse JK, Helman D, Fox TL, Ginkel A, Owens ML. Therapeutic response of basal cell carcinoma to the immune response modifier imiquimod 5% cream. *J Am Acad Dermatol* 1999; **41**: 1002-1007 [PMID: 10570388]
90 **Buck HW**, Guth KJ. Treatment of vaginal intraepithelial neoplasia (primarily low grade) with imiquimod 5% cream. *J Low Genit Tract Dis* 2003; **7**: 290-293 [PMID: 17051086 DOI: 10.1097/00128360-200310000-00011]
91 **Terlou A**, van Seters M, Ewing PC, Aaronson NK, Gundy CM, Heijmans-Antonissen C, Quint WG, Blok LJ, van Beurden M, Helmerhorst TJ. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: seven years median follow-up of a randomized clinical trial. *Gynecol Oncol* 2011; **121**: 157-162 [PMID: 21239049 DOI: 10.1016/j.ygyno.2010.12.340]
92 **Fox PA**, Nathan M, Francis N, Singh N, Weir J, Dixon G, Barton SE, Bower M. A double-blind, randomized controlled trial of the use of imiquimod cream for the treatment of anal canal high-grade anal intraepithelial neoplasia in HIV-positive MSM on HAART, with long-term follow-up data including the use of open-label imiquimod. *AIDS* 2010; **24**: 2331-2335 [PMID: 20729710 DOI: 10.1097/QAD.0b013e32833d466c]
93 **Beutner KR**, Spruance SL, Hougham AJ, Fox TL, Owens ML, Douglas JM. Treatment of genital warts with an immune-response modifier (imiquimod). *J Am Acad Dermatol* 1998; **38**: 230-239 [PMID: 9486679]
94 **Hemmi H**, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol* 2002; **3**: 196-200 [PMID: 11812998 DOI: 10.1038/ni758]
95 **Miller RL**, Gerster JF, Owens ML, Slade HB, Tomai MA. Imiquimod applied topically: a novel immune response modifier and new class of drug. *Int J Immunopharmacol* 1999; **21**: 1-14 [PMID: 10411278]
96 **Bilu D**, Sauder DN. Imiquimod: modes of action. *Br J Dermatol* 2003; **149** Suppl 66: 5-8 [PMID: 14616337]
97 **Stanley MA**. Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential. *Clin Exp Dermatol* 2002; **27**: 571-577 [PMID: 12464152]
98 **Herdman MT**, Pett MR, Roberts I, Alazawi WO, Teschendorff AE, Zhang XY, Stanley MA, Coleman N. Interferon-beta treatment of cervical keratinocytes naturally infected with human papillomavirus 16 episomes promotes rapid reduction in episome numbers and emergence of latent integrants. *Carcinogenesis* 2006; **27**: 2341-2353 [PMID: 16973673]
99 **Pett MR**, Herdman MT, Palmer RD, Yeo GS, Shivji MK, Stanley MA, Coleman N. Selection of cervical keratinocytes

containing integrated HPV16 associates with episome loss and an endogenous antiviral response. *Proc Natl Acad Sci USA* 2006; **103**: 3822-3827 [PMID: 16505361]
100 **Tatti S**, Stockfleth E, Beutner KR, Tawfik H, Elsasser U, Weyrauch P, Mescheder A. Polyphenon E: a new treatment for external anogenital warts. *Br J Dermatol* 2010; **162**: 176-184 [PMID: 19709100 DOI: 10.1111/j.1365-2133.2009.09375.x]
101 **Ahmad N**, Gupta S, Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Arch Biochem Biophys* 2000; **376**: 338-346 [PMID: 10775421]
102 **Khan N**, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. *Cancer Res* 2006; **66**: 2500-2505 [PMID: 16510563]
103 **Beltz LA**, Bayer DK, Moss AL, Simet IM. Mechanisms of cancer prevention by green and black tea polyphenols. *Anticancer Agents Med Chem* 2006; **6**: 389-406 [PMID: 17017850]
104 **Hakki M**, Chou S. The biology of cytomegalovirus drug resistance. *Curr Opin Infect Dis* 2011; **24**: 605-611 [PMID: 22001948 DOI: 10.1097/QCO.0b013e32834cfb58]
105 **Coremans G**, Snoeck R. Cidofovir: clinical experience and future perspectives on an acyclic nucleoside phosphonate analog of cytosine in the treatment of refractory and premalignant HPV-associated anal lesions. *Expert Opin Pharmacother* 2009; **10**: 1343-1352 [PMID: 19463071 DOI: 10.1517/14656560902960154]
106 **Van Pachterbeke C**, Bucella D, Rozenberg S, Manigart Y, Gilles C, Larsimont D, Vanden Houte K, Reynders M, Snoeck R, Bossens M. Topical treatment of CIN 2+ by cidofovir: results of a phase II, double-blind, prospective, placebo-controlled study. *Gynecol Oncol* 2009; **115**: 69-74 [PMID: 19647859 DOI: 10.1016/j.ygyno.2009.06.042]
107 **Donne AJ**, Hampson L, He XT, Day PJ, Salway F, Rothera MP, Homer JJ, Hampson IN. Potential risk factors associated with the use of cidofovir to treat benign human papillomavirus-related disease. *Antivir Ther* 2009; **14**: 939-952 [PMID: 19918098 DOI: 10.3851/IMP1421]
108 **Best SR**, Niparko KJ, Pai SI. Biology of human papillomavirus infection and immune therapy for HPV-related head and neck cancers. *Otolaryngol Clin North Am* 2012; **45**: 807-822 [PMID: 22793854 DOI: 10.1016/j.otc.2012.04.005]
109 **McBride AA**. The papillomavirus E2 proteins. *Virology* 2013; **445**: 57-79 [PMID: 23849793 DOI: 10.1016/j.virol.2013.06.006]
110 **Ibeanu OA**. Molecular pathogenesis of cervical cancer. *Cancer Biol Ther* 2011; **11**: 295-306 [PMID: 21239888]
111 **Moore RA**, Walcott S, White KL, Anderson DM, Jain S, Lloyd A, Topley P, Thomsen L, Gough GW, Stanley MA. Therapeutic immunisation with COPV early genes by epithelial DNA delivery. *Virology* 2003; **314**: 630-635 [PMID: 14554090]
112 **Moore RA**, Santos EB, Nicholls PK, White KL, Anderson DM, Lloyd A, Topley P, Romanos M, Thomsen L, Parmar V, Walcott S, Gough GW, Stanley MA. Intraepithelial DNA immunisation with a plasmid encoding a codon optimised COPV E1 gene sequence, but not the wild-type gene sequence completely protects against mucosal challenge with infectious COPV in beagles. *Virology* 2002; **304**: 451-459 [PMID: 12504584]
113 **Corona Gutierrez CM**, Tinoco A, Navarro T, Contreras ML, Cortes RR, Calzado P, Reyes L, Posternak R, Morosoli G, Verde ML, Rosales R. Therapeutic vaccination with MVA E2 can eliminate precancerous lesions (CIN 1, CIN 2, and CIN 3) associated with infection by oncogenic human papillomavirus. *Hum Gene Ther* 2004; **15**: 421-431 [PMID: 15144573]
114 **García-Hernández E**, González-Sánchez JL, Andrade-Manzano A, Contreras ML, Padilla S, Guzmán CC, Jiménez R, Reyes L, Morosoli G, Verde ML, Rosales R. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. *Cancer Gene Ther* 2006; **13**: 592-597 [PMID: 16456551]
115 **Su JH**, Wu A, Scotney E, Ma B, Monie A, Hung CF, Wu TC. Immunotherapy for cervical cancer: Research status and clinical potential. *BioDrugs* 2010; **24**: 109-129 [PMID: 20199126 DOI: 10.2165/11532810-000000000-00000]
116 **Melief CJ**, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nat Rev Cancer* 2008; **8**: 351-360 [PMID: 18418403 DOI: 10.1038/nrc2373]
117 **Rosalia RA**, Quakkelaar ED, Redeker A, Khan S, Camps M, Drijfhout JW, Silva AL, Jiskoot W, van Hall T, van Veelen PA, Janssen G, Franken K, Cruz LJ, Tromp A, Oostendorp J, van der Burg SH, Ossendorp F, Melief CJ. Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. *Eur J Immunol* 2013; **43**: 2554-2565 [PMID: 23836147 DOI: 10.1002/eji.201343324]
118 **Ressing ME**, van Driel WJ, Brandt RM, Kenter GG, de Jong JH, Bauknecht T, Fleuren GJ, Hoogerhout P, Offringa R, Sette A, Celis E, Grey H, Trimbos BJ, Kast WM, Melief CJ. Detection of T helper responses, but not of human papillomavirus-specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma. *J Immunother* 2000; **23**: 255-266 [PMID: 10746552]
119 **Kenter GG**, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, Essahsah F, Fathers LM, Offringa R, Drijfhout JW, Wafelman AR, Oostendorp J, Fleuren GJ, van der Burg SH, Melief CJ. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med* 2009; **361**: 1838-1847 [PMID: 19890126 DOI: 10.1056/NEJMoa0810097.]
120 **Welters MJ**, Kenter GG, Piersma SJ, Vloon AP, Löwik MJ, Berends-van der Meer DM, Drijfhout JW, Valentijn AR, Wafelman AR, Oostendorp J, Fleuren GJ, Offringa R, Melief CJ, van der Burg SH. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. *Clin Cancer Res* 2008; **14**: 178-187 [PMID: 18172269 DOI: 10.1158/1078-0432.CCR-07-1880]
121 **de Vos van Steenwijk PJ**, Ramwadhdoebe TH, Löwik MJ, van der Minne CE, Berends-van der Meer DM, Fathers LM, Valentijn AR, Oostendorp J, Fleuren GJ, Hellebrekers BW, Welters MJ, van Poelgeest MI, Melief CJ, Kenter GG, van der Burg SH. A placebo-controlled randomized HPV16 synthetic long-peptide vaccination study in women with high-grade cervical squamous intraepithelial lesions. *Cancer Immunol Immunother* 2012; **61**: 1485-1492 [PMID: 22684521 DOI: 10.1007/s00262-012-1292-7]
122 **van Poelgeest MI**, Welters MJ, van Esch EM, Stynenbosch LF, Kerpershoek G, van Persijn van Meerten EL, van den Hende M, Löwik MJ, Berends-van der Meer DM, Fathers LM, Valentijn AR, Oostendorp J, Fleuren GJ, Melief CJ, Kenter GG, van der Burg SH. HPV16 synthetic long peptide (HPV16-SLP) vaccination therapy of patients with advanced or recurrent HPV16-induced gynecological carcinoma, a phase II trial. *J Transl Med* 2013; **11**: 88 [PMID: 23557172 DOI: 10.1186/1479-5876-11-88]
123 **Kaufmann AM**, Nieland JD, Jochmus I, Baur S, Friese K, Gabelsberger J, Gieseking F, Gissmann L, Glasschröder B, Grubert T, Hillemanns P, Höpfl R, Ikenberg H, Schwarz J, Karrasch M, Knoll A, Küppers V, Lechmann M, Lelle RJ, Meissner H, Müller RT, Pawlita M, Petry KU, Pilch H, Walek E, Schneider A. Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *Int J Cancer* 2007; **121**: 2794-2800 [PMID: 17721997]
124 **Sharma C**, Dey B, Wahiduzzaman M, Singh N. Human papillomavirus 16 L1-E7 chimeric virus like particles show prophylactic and therapeutic efficacy in murine model of cervical cancer. *Vaccine* 2012; **30**: 5417-5424 [PMID: 22717329

DOI: 10.1016/j.vaccine.2012.06.010]
125 **Einstein MH**, Kadish AS, Burk RD, Kim MY, Wadler S, Streicher H, Goldberg GL, Runowicz CD. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. *Gynecol Oncol* 2007; **106**: 453-460 [PMID: 17586030]
126 **Roman LD**, Wilczynski S, Muderspach LI, Burnett AF, O' Meara A, Brinkman JA, Kast WM, Facio G, Felix JC, Aldana M, Weber JS. A phase II study of Hsp-7 (SGN-00101) in women with high-grade cervical intraepithelial neoplasia. *Gynecol Oncol* 2007; **106**: 558-566 [PMID: 17631950]
127 **Palefsky JM**, Berry JM, Jay N, Krogstad M, Da Costa M, Darragh TM, Lee JY. A trial of SGN-00101 (HspE7) to treat high-grade anal intraepithelial neoplasia in HIV-positive individuals. *AIDS* 2006; **20**: 1151-1155 [PMID: 16691066]
128 **Frazer IH**, Quinn M, Nicklin JL, Tan J, Perrin LC, Ng P, O'Connor VM, White O, Wendt N, Martin J, Crowley JM, Edwards SJ, McKenzie AW, Mitchell SV, Maher DW, Pearse MJ, Basser RL. Phase 1 study of HPV16-specific immunotherapy with E6E7 fusion protein and ISCOMATRIX adjuvant in women with cervical intraepithelial neoplasia. *Vaccine* 2004; **23**: 172-181 [PMID: 15531034]
129 **Smyth LJ**, Van Poelgeest MI, Davidson EJ, Kwappenberg KM, Burt D, Sehr P, Pawlita M, Man S, Hickling JK, Fiander AN, Tristram A, Kitchener HC, Offringa R, Stern PL, Van Der Burg SH. Immunological responses in women with human papillomavirus type 16 (HPV-16)-associated anogenital intraepithelial neoplasia induced by heterologous prime-boost HPV-16 oncogene vaccination. *Clin Cancer Res* 2004; **10**: 2954-2961 [PMID: 15131030]
130 **Fiander AN**, Tristram AJ, Davidson EJ, Tomlinson AE, Man S, Baldwin PJ, Sterling JC, Kitchener HC. Prime-boost vaccination strategy in women with high-grade, noncervical anogenital intraepithelial neoplasia: clinical results from a multicenter phase II trial. *Int J Gynecol Cancer* 2006; **16**: 1075-1081 [PMID: 16803488]
131 **Daayana S**, Elkord E, Winters U, Pawlita M, Roden R, Stern PL, Kitchener HC. Phase II trial of imiquimod and HPV therapeutic vaccination in patients with vulval intraepithelial neoplasia. *Br J Cancer* 2010; **102**: 1129-1136 [PMID: 20234368 DOI: 10.1038/sj.bjc.6605611]
132 **Ferraro B**, Morrow MP, Hutnick NA, Shin TH, Lucke CE, Weiner DB. Clinical applications of DNA vaccines: current progress. *Clin Infect Dis* 2011; **53**: 296-302 [PMID: 21765081 DOI: 10.1093/cid/cir334]
133 **Yan J**, Harris K, Khan AS, Draghia-Akli R, Sewell D, Weiner DB. Cellular immunity induced by a novel HPV18 DNA vaccine encoding an E6/E7 fusion consensus protein in mice and rhesus macaques. *Vaccine* 2008; **26**: 5210-5215 [PMID: 18455277 DOI: 10.1016/j.vaccine.2008.03.069]
134 **Yan J**, Reichenbach DK, Corbitt N, Hokey DA, Ramanathan MP, McKinney KA, Weiner DB, Sewell D. Induction of antitumor immunity in vivo following delivery of a novel HPV-16 DNA vaccine encoding an E6/E7 fusion antigen. *Vaccine* 2009; **27**: 431-440 [PMID: 19022315 DOI: 10.1016/j.vaccine.2008.10.078]
135 **Garcia F**, Petry KU, Muderspach L, Gold MA, Braly P, Crum CP, Magill M, Silverman M, Urban RG, Hedley ML, Beach KJ. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2004; **103**: 317-326 [PMID: 14754702]
136 **Matijevic M**, Hedley ML, Urban RG, Chicz RM, Lajoie C, Luby TM. Immunization with a poly (lactide co-glycolide) encapsulated plasmid DNA expressing antigenic regions of HPV 16 and 18 results in an increase in the precursor frequency of T cells that respond to epitopes from HPV 16, 18, 6 and 11. *Cell Immunol* 2011; **270**: 62-69 [PMID: 21550027 DOI: 10.1016/j.cellimm.2011.04.005]
137 **Trimble CL**, Peng S, Kos F, Gravitt P, Viscidi R, Sugar E, Pardoll D, Wu TC. A phase I trial of a human papillomavirus DNA vaccine for HPV16+ cervical intraepithelial neoplasia 2/3. *Clin Cancer Res* 2009; **15**: 361-367 [PMID: 19118066 DOI: 10.1158/1078-0432.CCR-08-1725.]
138 **Gilbert SC**. Clinical development of Modified Vaccinia virus Ankara vaccines. *Vaccine* 2013; **31**: 4241-4246 [PMID: 23523410 DOI: 10.1016/j.vaccine.2013.03.020]
139 **Cottingham MG**, Carroll MW. Recombinant MVA vaccines: dispelling the myths. *Vaccine* 2013; **31**: 4247-4251 [PMID: 23523407 DOI: 10.1016/j.vaccine.2013.03.021]
140 **Iborra S**, Izquierdo HM, Martínez-López M, Blanco-Menéndez N, Reis e Sousa C, Sancho D. The DC receptor DNGR-1 mediates cross-priming of CTLs during vaccinia virus infection in mice. *J Clin Invest* 2012; **122**: 1628-1643 [PMID: 22505455 DOI: 10.1172/JCI60660]
141 **Gómez CE**, Perdiguero B, García-Arriaza J, Esteban M. Clinical applications of attenuated MVA poxvirus strain. *Expert Rev Vaccines* 2013; **12**: 1395-1416 [PMID: 24168097 DOI: 10.1586/14760584.2013.845531]
142 **Baldwin PJ**, van der Burg SH, Boswell CM, Offringa R, Hickling JK, Dobson J, Roberts JS, Latimer JA, Moseley RP, Coleman N, Stanley MA, Sterling JC. Vaccinia-expressed human papillomavirus 16 and 18 e6 and e7 as a therapeutic vaccination for vulval and vaginal intraepithelial neoplasia. *Clin Cancer Res* 2003; **9**: 5205-5213 [PMID: 14614000]
143 **Davidson EJ**, Boswell CM, Sehr P, Pawlita M, Tomlinson AE, McVey RJ, Dobson J, Roberts JS, Hickling J, Kitchener HC, Stern PL. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. *Cancer Res* 2003; **63**: 6032-6041 [PMID: 14522932]
144 **Brun JL**, Dalstein V, Leveque J, Mathevet P, Raulic P, Baldauf JJ, Scholl S, Huynh B, Douvier S, Riethmuller D, Clavel C, Birembaut P, Calenda V, Baudin M, Bory JP. Regression of high-grade cervical intraepithelial neoplasia with TG4001 targeted immunotherapy. *Am J Obstet Gynecol* 2011; **204**: 169.e1-169.e8 [PMID: 21284968 DOI: 10.1016/j.ajog.2010.09.020.]
145 **Rosales C**, Graham VV, Rosas GA, Merchant H, Rosales R. A recombinant vaccinia virus containing the papilloma E2 protein promotes tumor regression by stimulating macrophage antibody-dependent cytotoxicity. *Cancer Immunol Immunother* 2000; **49**: 347-360 [PMID: 10999461]
146 **Lee SY**, Kang TH, Knoff J, Huang Z, Soong RS, Alvarez RD, Hung CF, Wu TC. Intratumoral injection of therapeutic HPV vaccinia vaccine following cisplatin enhances HPV-specific antitumor effects. *Cancer Immunol Immunother* 2013; **62**: 1175-1185 [PMID: 23615841 DOI: 10.1007/s00262-013-1421-y]
147 **Remy-Ziller C**, Germain C, Spindler A, Hoffmann C, Silvestre N, Rooke R, Bonnefoy JY, Préville X. Immunological characterization of a modified vaccinia virus Ankara vector expressing the human papillomavirus 16 E1 protein. *Clin Vaccine Immunol* 2014; **21**: 147-155 [PMID: 24307238 DOI: 10.1128/CVI.00678-13]
148 **Nonn M**, Schinz M, Zumbach K, Pawlita M, Schneider A, Dürst M, Kaufmann AM. Dendritic cell-based tumor vaccine for cervical cancer I: in vitro stimulation with recombinant protein-pulsed dendritic cells induces specific T cells to HPV16 E7 or HPV18 E7. *J Cancer Res Clin Oncol* 2003; **129**: 511-520 [PMID: 12905010]
149 **Ferrara A**, Nonn M, Sehr P, Schreckenberger C, Pawlita M, Dürst M, Schneider A, Kaufmann AM. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. *J Cancer Res Clin Oncol* 2003; **129**: 521-530 [PMID: 12898233]
150 **Santin AD**, Bellone S, Palmieri M, Ravaggi A, Romani C, Tassi R, Roman JJ, Burnett A, Pecorelli S, Cannon MJ. HPV16/18 E7-pulsed dendritic cell vaccination in cervical cancer patients with recurrent disease refractory to standard treatment

modalities. *Gynecol Oncol* 2006; **100**: 469-478 [PMID: 16249018]
151 **Santin AD**, Bellone S, Palmieri M, Zanolini A, Ravaggi A, Siegel ER, Roman JJ, Pecorelli S, Cannon MJ. Human papillomavirus type 16 and 18 E7-pulsed dendritic cell vaccination of stage IB or IIA cervical cancer patients: a phase I escalating-dose trial. *J Virol* 2008; **82**: 1968-1979 [PMID: 18057249]
152 **van Driel WJ**, Ressing ME, Kenter GG, Brandt RM, Krul EJ, van Rossum AB, Schuuring E, Offringa R, Bauknecht T, Tamm-Hermelink A, van Dam PA, Fleuren GJ, Kast WM, Melief CJ, Trimbos JB. Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. *Eur J Cancer* 1999; **35**: 946-952 [PMID: 10533477]

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