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Advances in therapeutic vaccines for treating human papillomavirus-related cervical intraepithelial neoplasia

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Abstract

Aim: Human papillomavirus (HPV) is the etiologic agent of the majority of cervical intraepithelial lesions (CIN) and cervical cancers. While prophylactic HPV vaccines prevent infections from the main high-risk HPV types associated with cervical cancer, alternative nonsurgical and nonablative therapeutics to treat HPV infection and preinvasive HPV diseases have been experimentally investigated. Therapeutic vaccines are an emerging investigational strategy. This review aims to introduce the results of the main clinical trials on the use of therapeutic vaccines for treating HPV infection and -related CIN, reporting the ongoing studies on this field.

Methods: Data research was conducted using MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID and Cochrane Library querying for all articles related to therapeutic vaccines for the treatment of HPV-related CIN. Selection criteria included randomized clinical trials, nonrandomized controlled studies and review articles.

Results: Preliminary data are available on the evaluation of therapeutic vaccines for treating cervical HPV infections and CIN. Despite having *in vitro* demonstrated to obtain humoral and cytotoxic responses, therapeutic vaccines have not yet clinically demonstrated consistent success; moreover, each class of therapeutic vaccines has advantages and limitations. Early clinical data are available in the literature for these compounds, except for MVA E2, which reached the phase III clinical trial status, obtaining positive clinical outcomes.

Conclusion: Despite promising results, to date many obstacles are still present before hypothesize an introduction in the clinical practice within the next years. Further studies will draw a definitive conclusion on the role of therapeutic vaccines in this setting.

Key words: cervical intraepithelial neoplasia, HPV infection, immunotherapy, therapeutic vaccines.

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Introduction

Human Papillomavirus (HPV) is the etiologic agent of the majority of cervical dysplasias and cancers.[1] Cervical cancer is the fourth most common cancer of women in the world, with an estimated global incidence of 528 000 new cases and 266 000 deaths in 2012. In general, it represents also the second cause of cancer mortality in developing countries[2]; moreover, the overall burden of disability-adjusted life years (DALY) lost attributed to this cancer was 6.9 million years in 2013, globally being considered the third cause of lost DALY in women, after breast and lung cancers.[3]

Evidence is emerging about a complex interplay between infection by high-risk (HR) HPV types and the immune system, which may be critical for cervical carcinogenesis: in fact, the majority of sexually active women are infected by HPV during their life; anyway, these infections often remain asymptomatic, cleared by the immune system; nevertheless, a group of women, having persistent HPV infections, may develop low or high-grade cervical intraepithelial neoplasia (CIN) and eventually an invasive disease.[4,5] Even if the majority of HPV infections are transient and subclinical because of rapid immune clearance, persistent HPV infection in presence of compromised immune response may be responsible for the development of pre- and cancerous lesions.[6]

Overall, the most common HR HPV types are HPV-16 and 18, which represent the etiological agents of around 70% of cervical cancers in the world.[7] In addition to cervical cancer, HPV is responsible for a variable fraction of cancers of vulva, vagina, penis and anus.[8]

The burden of HPV related diseases is mainly due to cervical lesions because the majority of cancers occur in the cervix and these localizations are the main source of data about current prophylactic vaccination against HPV.[9] Even if the vaccine uptake in females between 9 and 45 years of age is less than 2% and despite the lack of vaccination programs in lower- and middle-income countries (where the incidence of cervical cancer is the highest), a recent review estimated indicates that 444 627 cervical cancers will have been averted by the program of vaccination.[8,9]

For low-grade cervical intraepithelial neoplasia (CIN 1), the therapeutic management consists in a follow-up without therapeutic intervention, because most of these dysplasias can spontaneously regress; on the other hand, high-grade CIN (CIN 2–3) needs to be treated by conservative surgical treatments, such as cryotherapy, loop electrosurgical excision procedure (LEEP), or cone biopsy.[10] However, these surgical therapies are associated with recurrences,[11] likely related to persistent infection[12] and other reproductive morbidities.[13]

In recent years, research is focalized in finding alternative noninvasive therapeutic options for treating cervical HPV infection and dysplasia. Even if prophylactic HPV vaccines can prevent HR HPV infections associated with cancer, there still is a need for nonablative solutions to control HPV related diseases. In particular, the interest in immunotherapy has grown: this approach consists in the treatment of disease by modulating the immune response, enhancing its action against infected cells. Among immunotherapeutic options, therapeutic vaccines are an emerging investigational strategy. Although their use has been particularly studied for advanced cervical cancer,[14] also the treatment of CIN has been a focus of the development of these vaccines.[15] This narrative review aims to give an overview of the role of therapeutic vaccines for the treatment of cervical HPV infection and preinvasive lesions.

Methods

The data research was conducted using the following databases MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID and Cochrane Library querying for all articles related to therapeutic vaccines for the treatment of HPV-related CIN from the inception of the database up to November 2019. The studies were identified with the use of a combination of the following text words: CIN; immunotherapy; therapeutic vaccines; HPV infection. The selection criteria of this narrative review included randomized clinical trials, nonrandomized controlled studies (observational prospective, retrospective cohort studies, case–control studies and case series) and review articles of therapeutic vaccines in women affected by HPV-related CIN. A review of articles also included the abstracts of all references retrieved from the search. Article not in English language, conference papers and reviews and studies with information overlapping another publication were excluded. In the event of overlapping studies, we selected the most recent and/or most comprehensive manuscript (Figure 1).

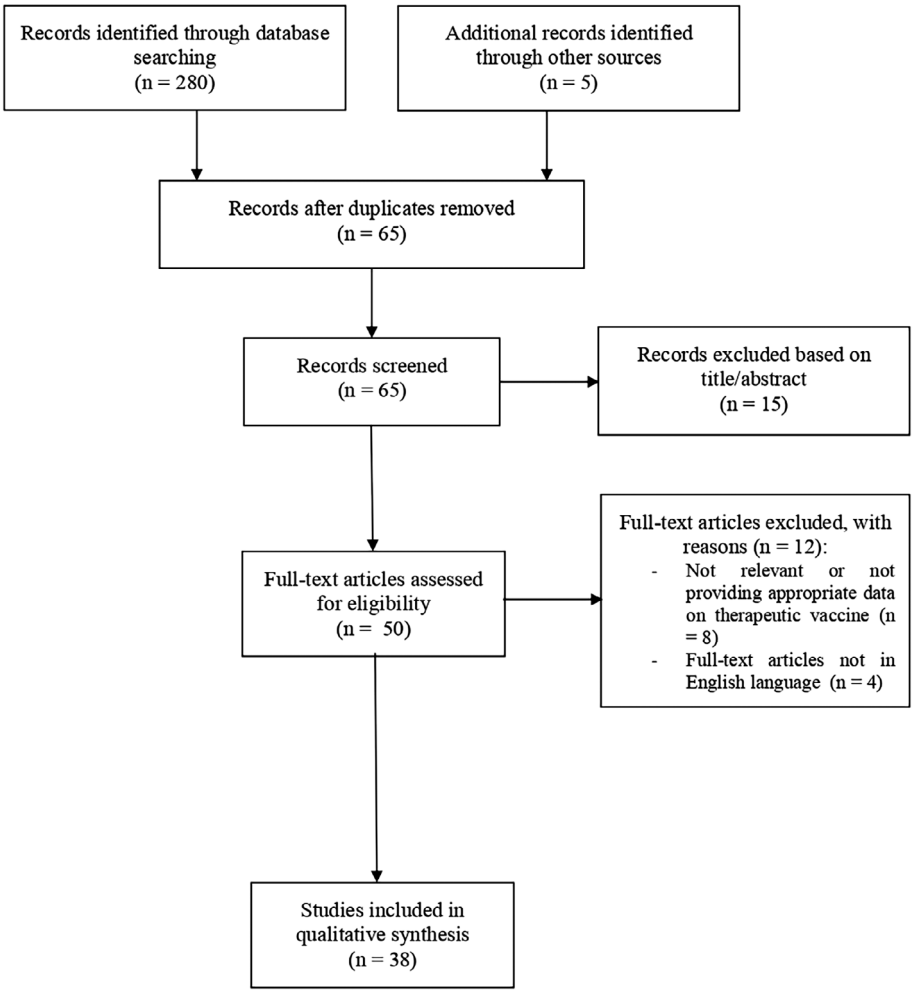

Figure 1 Flow diagram of studies identified in the review.

Discussion

HPV infection and immune system

HPV has a double-stranded DNA, which is characterized by eight open reading frames, six early genes (E1, E2, E4, E5, E6 and E7) encoding for early proteins and two late genes (L1 and L2) encoding for late proteins (Figure 2).[16,17] Cervical HPV infection occurs by the interaction between the virus and the basement epithelial membrane in the presence of microabrasions areas.[18] E1 protein supports viral replication whereas E2 exhibits a regulatory function for the transcription of E6 and E7, which are considered tumorigenic proteins as they block the apoptosis of infected cells and stimulate their oncogenic transformation.[16] Moreover, E5, E6 and E7 proteins have been associated with virus immune escape.[19] On the other hand, L1 and L2 proteins form the structural components of the HPV capsid.[20]

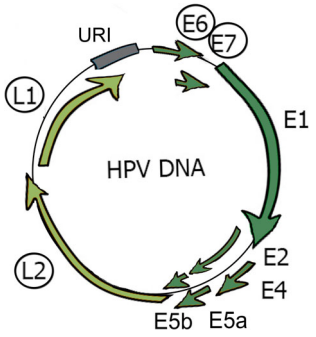

Figure 2 Human papillomavirus genome.

Proteins like E1 and E2 are highly expressed during the infection and in low-grade CIN; in these situations, the expression of E6 and E7 is low and it reaches high levels only after the establishment of the transformed phenotype.[21]

The alterations induced by HR HPV types in the site of infection are influenced by immune system response, which in some patients tends to create a proper microenvironment for persistent infection and lesion progression.[6,21] Moreover, despite stimulating immune cell migration to the dermis, HPV makes these cells more tolerant to the infection through different mechanisms: for instances, as HPV remains silent for a long time and its replication does not cause cytolysis, a decrease the action of immune response occurs; moreover, HPV seems to be able to inhibit interferon (IFN) synthesis through E6 and E7 oncoproteins and reduce the expression of major histocompatibility complex (MHC) class I, which normally activates T cytotoxic cells against infected cells.[22,23] With this regard, it has been also demonstrated that HPV can induce the accumulation of anergic CD4 and CD8 T cells in cervical lesions.[24]

In the last years, all the evidence on the critical role of the immune system in the pathogenesis of HPV related diseases led researchers to investigate immunotherapeutic options for treating HPV related cervical infection and preinvasive lesions. The idea of enhancing the host's immune system against these diseases is based on the insight that lymphocytes T and natural killers (NK) can specifically identify and eliminate infected cells based on expression of specific antigens or molecules induced by cellular stress within a process called immune surveillance.[25]

Nowadays, the immunotherapeutic approach includes administration of antibodies or recombinant proteins that either co-stimulate cells or block the so-called immune checkpoint pathways and therapeutic vaccines, oncolytic viruses, adoptive transfer of *ex vivo* activated T and NK cells. The growth of interest in this field has been inspired by the success of immune checkpoint inhibitors, such as antibodies targeting programmed cell death protein 1 (PD-1) or cytotoxic T lymphocyte-associated transmembrane receptor 4 (CTLA-4) and adoptive cell therapy with chimeric antigen receptor T cells.[26,27] These two types of immunotherapy reached late clinical development for treating advanced cancers.

Today, the study of therapeutic vaccines has a growing interest in treating both advanced cancers and precancerous lesions. They differ from prophylactic vaccines as they aim to generate cell-mediated immunity rather than neutralizing antibodies. The ideal therapeutic vaccine can trigger the activation and maturation of dendritic cells (DC) to promote the immune generation of tumor-reactive, CD8+ cytotoxic T cells.[28]

Therapeutic vaccines for treating cervical cancer

In general, antigen-presenting cells (APC), such as DC, have the role to enhance T cell activity, presenting foreign antigens released from infected and tumor cells. Therapeutic vaccines specifically aim to trigger the activation and maturation of DC to promote the immune generation of tumor-reactive CD8+ cytotoxic T cells.[28] For eliciting this strong immune response against cells infected by HPV, E6 and E7 proteins have been considered ideal molecular targets. These molecules are not expressed in normal cells but tend to be constitutively expressed on cervical cells infected by HPV.[29]

In the future, the therapeutic vaccine may have a role for the treatment of preinvasive disease, or alongside other therapies, such as surgery, chemo and radiotherapy for the treatment of invasive cancer (and in particular advanced cervical cancer).[14]

Currently, live vector, protein and nucleic acid, cells-based therapeutic vaccines are suitable experimental options for treating HPV-related cervical infection and CIN (Table 1).

Live vector-based therapeutic vaccines

Live vectors based on the use of bacteria have been one of the first class of therapeutic vaccines developed. These vaccines have a high efficacy in delivering antigens, being able to directly replicate in the host.[6] However, it should be taken into account that these vaccines inherently represent a potential risk to immunocompromised individuals.[30]

In a single-arm phase I/IIa study, GLBL101c (consisting of *Lactobacillus casei* expressing E7) was administered orally (six capsules per day) to 17 women with CIN 3. Specific E7 immune response, evaluated by enzyme-linked immunospot (ELISPOT), was detected in all the patients. After 9 weeks from the administration, histologic regression to CIN 1 or less occurred in 8 of 27 patients (30%) undergone LEEP; 70% of them had a downgrade of the lesion to CIN 2. No adverse side events were experienced by any women.[31] These results seemed to give strong evidence between disease regression and HPV E7 cellular immunity.

Listeria monocytogenes is a promising live vector due to the ability to infect macrophages without undergoing

Table 1 Overview of main therapeutic vaccines for cervical cancer

Class	Main characteristics	Types of vaccine	References
Live attenuated bacteria and viruses	• Highly immune stimulant (directly replicate in the host) agents • Potential risk of immune system hyperstimulation (subsequent high rate of adverse events)	• GLBL101c • TA-HPV • MVA • Lm-LLO-E7 • BLS-M07	Hancock *et al.*[6] Ma *et al.*[30] Kawana *et al.*[31] Flickinger Jr *et al.*[32] Maciag *et al.*[33] Kaufmann *et al.*[34] Baldwin *et al.*[35] Brun *et al.*[36] Rosales *et al.*[37] Goepfert *et al.*[38] Garcia-Hernandez *et al.*[39]
Peptide vaccines	• Low antigenicity (need adjuvant compounds and selection of the most antigenic peptides or proteins) • Easier to be produced than other types of vaccines	• PDS0101 • Pepcan • ISA 101 • TG4001(Tipapkinogen Sovacivec)	Hancock *et al.*[6] Kenter *et al.*[40] van Poelgeest *et al.*[41] van Poelgeest *et al.*[42] Greenfield *et al.*[43] Coleman *et al.*[44]
Protein vaccines	• Not MHC restricted (all antigens derive from APC intracellular process) • Low antigenicity (need adjuvant compounds)	• SGN-00101 • TA-CIN • TVGV-1 • GTL001 • GTL002	Hancock *et al.*[6] Greenfield *et al.*[43] Van Damme *et al.*[46] Einstein *et al.*[47] Daayana *et al.*[48] Davidson et al[49] de Jong *et al.*[50]
DNA vaccines	• Low antigenicity sustained cellular gene expression	• pNGVL4a-CRT-E7 • GX-188 • VGX-3100 • ZYC 101	Hancock *et al.*[6] Keane-Myers *et al.*[52] Ledwith *et al.*[53] Sheets *et al.*[54] Alvarez *et al.*[55] Zhang *et al.*[56] Kim *et al.*[57] Bagarazzi *et al.*[58] Trimble *et al.*[59] Garcia *et al.*[60] Lundstrom *et al.*[61]
Cell vaccines	• High manufacturing challenge	• HPV E6-E7 loaded monocytes (no data on CIN)	Chang *et al.*[62] Indrova *et al.*[63] Kozlowska *et al.*[64] Santin *et al.*[65] Santin *et al.*[66] Kim *et al.*[67]

ACT, T cell-based adoptive cell transfer, APC, antigen-presenting cell, CIN, cervical intraepithelial neoplasia, HPV, human papillomavirus, MHC, major histocompatibility complex.

phagocytosis and to allow antigen processing via MHC I and MHC II pathways.[32]

Lm-LLO-E7, an E7-based vaccine based on these bacteria, has been tested for treating advanced cervical cancer showing an acceptable safety profile.[33] Patients received two doses 3 weeks apart as an intravenous infusion. Three dose levels of Lm-LLO-E7 (1×10^9 CFU, 3.3×10^9 CFU or 1×10^{10} CFU) were used. Patients treated with dose levels of 1×10^9 CFU and 3.3×10^9 CFU, experienced a tolerable safety, while episodes of grade 2 diastolic hypotension within hours after the infusion were developed with dose levels of 1×10^{10} CFU, so this has been considered as the dose-limiting toxicity (DLT). The main adverse effects observed after vaccine administration were a self-limiting flu-like syndrome (with pyrexia, vomiting, chills, headache, nausea and tachycardia) and an increase of liver enzymes. However, this increase in liver enzymes was transient and not clinically significant, so no medical intervention was necessary and the vaccine was considered safe and well-tolerated.[33] No trials on this vaccine have been organized for treating CIN.

BLS-M07 (BLS-ILB-E710c) is based *L. casei* as vector expressing E7 protein and it is orally administered. This vaccine can induce an immune response in the gut-associated lymphoid tissue and humoral antibodies against antigens with homology to HPV E7 thanks to epitope spreading and cross-reactivity.[33] This vaccine was tested in phase I/IIa open-label dose-escalation study among subjects with HPV-16 infection and a diagnosis of CIN 3. The primary endpoints of this study were to evaluate BLS-M07 safety and efficacy; regression was evaluated by colposcopic biopsies. The secondary endpoints were systemic production of immunoglobulin G against HPV E7 and lesion grade modification evaluated by Reid Colposcopic Index. Among three cohorts of subjects receiving different vaccine doses but the same schedule of administration (five times a week, on weeks 1, 2, 4 and 8; 4 patients 500 mg, 3 patients 1000 mg and 3 patients 1500 mg) no events of DLT were reported. The colposcopic biopsies were performed at 4,9,12 and 16 weeks after the vaccine injection. Seventy-five percent of the patients experienced a clinical response; notably, in half of these, it was observed a complete lesions disappearance and in the other half a remission to CIN 1. There was a significant decrease in mean RCI score in eight patients ($P < 0.05$); moreover, a correlation between E7-specific IFN-γ-producing cells and pathological responses was observed.[33]

Viral vectors can infect directly host's cells, leading to cellular expression of targeted antigens.

TA-HPV is a recombinant Vaccinia virus (double-stranded DNA virus), expressing HPV-16-18 E6 and E7. A phase II clinical trial was organized for testing TA-HPV plus pNGVL4a-Sig/E7, a DNA-based therapeutic vaccine, in patients affected by high-grade CIN (NCT00788164, see below).[33] TA-HPV succeeded in generating CD8 antigen-specific T-cells in the cervicovaginal tract.[33] TA-HPV has been tested in several studies, using a dose of 20 μL administered through a dermal scarification technique. No serious systemic adverse events due to vaccination were observed: the most common ones were malaise, myalgia and headache. A mild to moderate local reaction to the site of scarification with erythema and swelling, followed by ulceration that resolved by 17 days is possible.[34,35]

TG4001 consists of a recombinant virus Ankara containing the sequence coding for HPV-16 E6 and E7 early genes and human interleukin (IL)-2 gene. In a single-arm, multicenter phase II trial, it has been tested in women with CIN showing promising results: at 6-month follow-up after three subcutaneous injections of TG4001 at the dose of 5×10^7 pfu, 48% of patients were clinical responders (complete eradication of HPV lesions at colposcopy with subsequent cytology; efficacy rate per protocol 24%); the 6-month efficacy rate was 24%. In particular, HPV-16 mRNA clearance was associated with CIN regression at cytology and colposcopy in 70% of the study population.[36] All biochemical and hematologic parameters were normal throughout the study. Adverse effects were mild or moderate, including inflammation, pruritus, edema at the injection site, lymphadenopathy, fever, headache, asthenia, bone pain and vaginal discharge.

MVA E2 is based on vaccinia virus Ankara containing the bovine papillomavirus E2 protein[12] and it was the only therapeutic vaccine reaching the phase III clinical trial status for treating HPV-related cervical lesions. The trial was conducted on 1356 patients (1176 female and 180 male) affected by HPV related lesions (CIN 1, CIN 2, CIN 3 and condyloma lesions). MVA E2 (injected directly into the uterus of patients in a radial clockwise fashion at 3, 6, 9 and 12 o'clock once a week for 6 weeks) at a dose of 10^7 virus particles, induced complete regression (at 8, 14 weeks after the beginning of the protocol) in 825 (94.8%) out of 870 female patients with low-grade CIN and 220 (73.3%) out of 300 patients with high-grade CIN; among male patients, 100% of condyloma lesions were eliminated. No description of E2-specific T cells was provided in this study. Only 5 patients of 141 (3.54%) affected by high-grade CIN showed disease recurrence within 5 years of follow-up. All the adverse events experienced during the trial were considered moderate.[37]

Among the total patients, the adverse events observed were: headaches, flu symptoms, chills, abdominal ache and joint pain, all of grade 1 (very mild). Several studies in which MVAE2 has been administered, both intramuscularly and directly in the cervix, have demonstrated its safety: only mild local side reaction and flu-like symptoms have been reported.[38,39]

Peptide/protein-based vaccines

Peptide-based vaccines

The majority of available data on therapeutic vaccines derives from studies on peptide/proteins-based compounds. These vaccines tend to have low antigenicity and for this reason, have been often tested in combination with immunogenic adjuvants.[6] Peptide vaccines have however the advantages of safety and stability.[6] The main side effects consist of local reactions at the

injection site and the peptides are likely to be the cause. They are divided into two classes concerning their biological structure, which may be characterized by short (<15) and synthetic long chains of amino acids (>20). The first ones do not need to be biologically processed by professional APC while the second must be processed and presented by professional APC.[6]

A vaccine containing nine HPV-16 E6 and four HPV-16 E7 synthetic peptides was tested in 22 patients with a histologic diagnosis of HPV-16 related VIN 3. Vaccine-induced specific T cells were found in 85% of the patients by an assay based on IFN-γ ELISPOT. The vaccine was administered subcutaneously at 3-week intervals, at the dose of 0.3 mg for each peptide in a total volume of 2.8 mL. All patients reported injection site reaction, like swelling, redness, increased skin temperature and local pain. Systemic adverse events observed include influenza-like symptoms, chills and tiredness; all of these symptoms typically started after the second vaccination and were all of grade 2 (moderate). A complete response (CR) was observed in five patients at 3 months (25%; 95% confidence interval [CI], 9–49) and nine patients at 12 months (47%; 95% CI, 24–71). Partial response was observed in seven patients at 3 months (35%; 95% CI, 15–59) and in six patients at 12 months (32%; 95% CI, 13–57). All patients with CR were still free after 2 years of follow-up.[6,40] ISA 101 is a vaccine consisting of two mixtures of 13 synthetic long peptides covering the entire amino acid sequence of HPV-16 E6 and E7. In an open-label, randomized controlled trial this vaccination was tested with or without application of topic 5% imiquimod among patients with HPV-16 positive VIN/VaIN. Each dose contains 300 μg per peptide in a volume of 1.4 mL, injected subcutaneously four times with a 3-week interval between doses. Notably, it induced determined a clinical response in 18 of 34 (53%; 95% CI 35.1–70.2) patients at 3 months and in 15 of 29 (52%; 95% CI 32.5–70.6) patients at 12 months. Eight patients showed a complete histological response; seven of them displayed a well viral clearance.[6] ISA101 vaccination has determined local reactions that in some patients were long-lasting, with swelling and ulceration of the skin, still present after 12 months.[41] A previous study, by the same Authors, have shown that the vaccine was well tolerated, none of the systemic and local adverse events exceeded grade 2.[42] The reduction of side effects may be achieved by using alternative adjuvants and by the combination of vaccination with imiquimod on the lesion.

A single-arm dose-escalation phase I clinical trial evaluated a therapeutic vaccine (Pepcan) composed by peptides covering the HPV-16 E6 protein and candida skin test reagent as a novel adjuvant. Overall, 24 patients with biopsy-proven CIN 2/3 received this vaccination (four intradermal injections every 3 weeks with dose-escalation: 50, 100, 250 and 500 μg) before undergoing LEEP. The best histological response was seen at the 50 μg dose level with a lesion regression rate of 83%. Moreover, vaccine-induced immune responses were demonstrated in 65% of women and systemic T-helper type 1 cells within these cervical lesions were significantly increased after the four vaccinations. The most common adverse events were injection site reactions; none of the patients experienced dose-limiting toxicities. More grade 2 immediate and delayed injection site reactions were recorded at the higher doses. Other vaccine-related adverse events were myalgia, headache nausea, fatigue, hypokalemia, flu-like symptoms, feeling feverish, body pain, agitation, vomiting, hot flushes, muscle spasm, photophobia, vertigo, dizziness, neutropenia, thrombocytopenia and increased g-glutamyl transpeptidase. None of these AE were more than grade 2.[43] A subsequent study by the same group has confirmed that Pepcan was safe.[44] An ongoing randomized phase II trial is comparing the use of Pepcan plus candida skin test reagent as adjuvant. The primary endpoint of this study is represented by a 12-month clinical response by colposcopy-guided quadrant biopsies (NCT02481414).

PDS0101 is a liposomal nanoparticle-based vaccine composed of the cationic lipid R-DOTAP (R-enantiomer of 1, 2-dioleoyl-3-trimethylammonium-propane chloride) encapsulating six human HPV-16 E6 and E7 peptides.[45] No results on its use on humans are available in the literature. An ongoing open-label, escalating dose phase I trial is evaluating PDS0101 (three vaccinations SC given approximately 21 days apart) in female subjects with HR HPV infection and biopsy-proven CIN 1 (NTC02065973).

Protein-based vaccines

GTL001 is a vaccine constituted by HPV 16 and HPV 18 E7 fused with detoxified adenylate cyclase from Bordetella Pertussis (CyaA). The CyaA specific interaction with CD11b/CD18 integrin helps to deliver E7 antigens to CD11b APC. In the mouse model, it has been shown to induce response and to eliminate tumor cells expressing HPV-16 E7.[43] A phase I trial was conducted to examine the safety, tolerability and immunogenicity of GTL00. Forty-seven women

received two intradermal GLT001 doses of 100 or 600 μg with the addition of topical imiquimod cream at the injection site. Administrations were carried out 6 weeks apart. The study showed that intradermal vaccination with GTL001 is associated with injection-site reactions mild to moderate (pain, swelling, induration, tenderness and itching). Headache, myalgia and fatigue were the most common systemic reactions. All reactions were transient and only in few cases needed interventions, so the Authors concluded that GLT001 has an acceptable safety profile.[46] In phase II randomized, double-blind, placebo-controlled trial, GTL001was tested with imiquimod as an adjuvant on 239 HPV-16 or −18 positive patients with either normal or mildly abnormal cervical cytology (ASC-US/LSIL). The vaccination was well tolerated and no unexpected event was observed.[43] Despite its acceptable safety profile and its positive induction of antigen-specific cellular immune response, there was no statistically significant difference in viral clearance and lesions progression to high-grade between GTL001and placebo groups (NCT01957878).[47]

GTL002 is a recently developed second-generation vaccine, which comprises modified E7 proteins from HPV-16, 18, 45, 31, 33 and 52. Models in mice and Beagle dogs demonstrated the induction of E7-specific T-cell response against each of the genotypes.[43] No data on human exist until now about its use.

SGN-00101 is another therapeutic protein-based vaccine consisting of the entire sequence of HPV-16 E7 linked to BCG heat-shock protein. In an open-label phase II trial, its administration (subcutaneous 500 μg/ proteins three times 1 month apart) to 58 patients with CIN 3, obtained a histologic CR in 13 (22.5%) and a partial response (PR) in 32 (55%) women. Only mild, self-limiting injection site-related side effects were encountered. No patient had a serious drug-related adverse event during the observation period supporting the safety and tolerability of SGN-00101.[47]

TA-CIN consists of HPV-16 L2, E6 and E7 single fusion protein. Including structural (L2) and functional (E6 and E7) proteins, this vaccine has investigated for its combined prophylactic and therapeutic proprieties. In phase II clinical trial, topical imiquimod, a toll-like receptor 7 agonists with antiviral activity, was administered for 8 weeks, followed by 3 doses (1 mL of 128 μg at weeks 10, 14 and 18) of TA-CIN in patients affected by VIN 2–3. In particular, TA-CIN was administered intramuscularly into the deltoid muscle and was well tolerated, not occurring relevant treatment-related adverse events. Local reactions at injection sites were associated with imiquimod. One year after this vaccination, there was 63% (12 out of 19 women) of histologic CR.[48] Despite the promising results, the combination of TA-CIN and imiquimod has been never tested for treating CIN lesions. TA-CIN has been proven safe in previous clinical trials; tenderness and pain at the injection site, headache and fatigue of moderate severity were the most frequently reported events.[49,50]

TVGV-1 is a fusion protein consisting of a peptide sequence of HPV-16 E7 fused to the *Pseudomonas aeruginosa* exotoxin A (PE) and endoplasmic reticulum (ER) retention signal. After promising results from an *in vitro* study,[51] an ongoing open-label double-blind phase II trial is testing TVGV-1 with or without GPI-0100 adjuvant (0.6 mg ± 0.6 mL) in patients with CIN 2–3. Another clinical trial is testing TVGV-1 safety and efficacy against HPV-induced cervical HSIL (NCT02576561). The histological results will be evaluated at day 270 after treatment; injection-site toxicity and any serious adverse event will be reported, as secondary-outcome of this trial. To date, no studies are demonstrating the safety of this vaccine in humans.

Nucleic acid-based vaccines

DNA vaccines involve the injection of plasmid DNA encoding the specific viral antigens into the host's cells, such as myocytes (in case of intramuscular [IM] injection) or DC. Differently from live vector and protein vaccines, DNA vaccines do not produce neutralizing antibodies directed against the vector, theoretically allowing for repeated vaccination without risking a progressive reduction of immune response. Delivery by electroporation, encapsulation and gene gun has been reported as strategies to enhance immunogenicity by using DNA vaccines.[6,52] Moreover, the safety of this class of vaccines tends to be higher than most of the therapeutic vaccines; at least, they can be obtained by an easier process of production.[6,52]

The most concern is the possible integration of DNA into chromosomes; however, some studies have shown that the risk of mutation due to the integration of DNA vaccines, following IM injection, is negligible.[53,54]

Twelve subjects with HPV-16 positive CIN2/3 were enrolled in a clinical study designed to assess the safety, tolerability and immunogenicity of priming vaccination with DNA vaccine targeting HPV16 E7, followed by escalating doses of a boost vaccination with recombinant vaccinia targeting HPV-16 and HPV-18 E6 and E7 (TA-HPV). Patients were divided

into three treatment arms characterized by different doses. This study reported qualitative and quantitative changes regarding frequency, intensity and localization of immune cells within the cervical lesions but there were no findings of antigen-specific T cell responses and/or signs of regression of disease.[6,52]

In a clinical phase I trial pNGVL4a-CRT-E7, a DNA vaccine linked to calreticulin, was administered either intradermally, intramuscularly, or directly into the cervical lesions in 32 patients with HPV-16 associated CIN 2–3. The vaccine doses used were different in the different patient cohorts (8 μg, 16 μg, 1 mg, 3 mg) and were administered at study weeks 0, 4 and 8. Histological regression to CIN 1 or less was obtained in 8 of 27 (30%) women.[55] However, 69% of patients had vaccine-specific related adverse events, the most frequent of which were constitutional (fatigue and headache) and local injection (discoloration, pain and reaction) site events.[55] At the moment, pNGVL4a-CRT-E7 is under clinical investigation in several clinical trials: an ongoing open-label nonrandomized pilot study is evaluating it (via gene gun at weeks 0, 4, 8 before therapeutic resection of their lesion at week 15) for treating women with CIN 2–3 (NCT02596243). Moreover, an ongoing open-label nonrandomized multiarm phase I clinical trial is testing pNGVL4a-Sig/E7 linked to HSP70 with or without TA-HPV and topical imiquimod in patients who are positive for HPV-16 CIN 3 (NCT00788164).

GX-188 is another DNA vaccine engineered to express HPV-16 and -18 E6 and E7 fused to the extracellular domain of Fms-like tyrosine kinase-3 ligand (Flt3L) to enhance the presentation of antigens by DC to T cells, as previously described.[56] In a clinical phase I trial, GX-188 was administered to nine patients with CIN 3 lesions through electroporation technique (EP). All subjects received three injections of GX-188E, with the last two injections given at 4 and 12 weeks after the first injection. Patients were divided into three cohorts receiving three different doses of GX-188: 1, 3 and 4 mg. HPV-16 specific CD8 T-cell response was observed in seven out of nine patients (7.8%) and, notably, it was correlated to HPV clearance and histological CR. A total of 49 adverse events were recorded during all visits, including chills, injection site pain, swelling, hypoesthesia, headache, fatigue and rhinitis; however, all these events were considered to be mild.[57] Two clinical trials are evaluating the use of GX-188 for treating patients with CIN lesions: an ongoing double-blinded randomized phase II clinical trial is testing this vaccine (1 mg IM using EP at day 0, week 4 and week 12) among HPV-16 and/or −18 positive women affected by CIN 2–3 (NCT02596243). The primary endpoint of this study is represented by the number of participants with histopathological regression of cervical lesions to CIN 1 or less. At least, an ongoing randomized, open-label, multicenter, phase II trial is determining the optimal dose and the safety of GX-188E (1 mg or 4 mg IM using EP at 0, 4, 12 weeks) in patients with HPV-16 and/or −18 CIN 3 (NCT02139267).

VGX-3100 is a plasmid DNA vaccine encoding E6–E7 genes, which has been tested in a preliminary dose-escalation study (0.3, 1 and 3 mg per plasmid by EP) to 18 women previously treated for CIN 2–3. Overall, 10 out of 18 patients (56%) generated antibodies specific for the vaccine antigens. VGX-3100 was well tolerated with no observed dose-limiting toxicities. Adverse effects included injection site reaction, fever, pain during electroporation and tenderness.[58] Subsequently, in a two-arm randomized phase IIb trial, VGX-3100 was administered to 167 women with CIN 2–3: patients were randomized to receive VGX-3100 (6 mg) or placebo (1 mL), given intramuscularly at 0, 4 and 12 weeks. This study showed 49.5% of lesion regression with vaccine, compared to 30.6% of the placebo group. A significantly higher viral clearance among the VGX-3100 group (80%) when compared with the placebo group (50%) was observed. Post-hoc efficacy analyses confirmed histopathological regression to normal in 40.2% of VGX-3100-treated patients (vs 16.7% in patients belonging to the placebo group). The most common adverse events were site reactions, but only injection-site erythema had a statistically higher incidence in the VGX-3100 group (98/125, 78.4%) than in the placebo group (24/42, 57.1%). Other adverse events were fatigue, headache, myalgia, nausea, arthralgia.[59] An ongoing phase III randomized, double-blind, placebo-controlled study (REVEAL 1) in women with confirmed CIN 2 or 3 is underway to evaluate the efficacy, safety and tolerability of VGX-3100 administered by IM injection followed by electroporation (NCT03185013).

ZYC101 is a vaccine constituted by a residue of a human MHC class I antigen (HLA-DRα) fused to a peptide derived from the E7 protein of HPV-16. This vaccine was tested against anal dysplasia and HPV-16-related cervical dysplasia in an open-label uncontrolled trial, in which three out of 12 patients (40.0%) with anal intraepithelial neoplasia showed partial regression and 5 out of 15 patients with CIN showed

complete regression.[59] A previous phase 2 multicenter, double-blind, randomized, placebo-controlled trial has shown the safety of ZYC-10. One hundred twenty-seven subjects were randomized to three IM doses of either placebo or vaccine (100 or 200 μg). Local injection site reactions, including pain, erythema and induration, were more frequently reported by women receiving ZYC101 compared with placebo. No differences were observed between the two doses, so the Authors concluded that ZYC101 was well tolerated in all the patients.[60] Although some RNA-based therapeutic vaccines have been developed, none of them have been tested for treating CIN or advanced cervical cancer. RNA-based vaccines have similar characteristics of DNA-bases vaccines, nevertheless not posing the risk of chromosomal integration or cellular transformation. However, they are more difficult to make and also cannot spread intercellularly.[61] Shortly, the first data on the application of this class of vaccine will be obtained in this setting.

Cell based vaccines

Whole cell-based HPV vaccines have emerged as a potential therapeutic vaccine against HPV-associated diseases. DC-based vaccines are obtained by loading these immune cells on HPV antigens.[62] *In vitro*, they have shown a strong ability to initiate and control T-cell response.[63] For this reason, they are theoretically ideal candidates for immunotherapy strategies as they stimulate recognition of specific tumor-associated antigens, which are not normally present on human cells. These cells are often loaded by genes encoding cytokines like IL-2, IL-12 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in order to increase immune response. These therapeutic vaccines have been preliminarily investigated for treating advance cancers.[64] In a phase I dose-escalation trial (5, 10 and 15×10^6 cells for injection), autologous monocyte-derived DC were pulsed with recombinant HPV16E7 or HPV18 E7 oncoprotein and administered in cervical cancer patients. The vaccine was applied to the patient by subcutaneous injection. No significant local or systemic reactions were detected at the time of, or after, DC vaccinations.[65] Same results were obtained in a previous study by the same group.[66]

The drawbacks of DC-based vaccines are a discrepancy of vaccine quality caused by variations in cell culture strategies, difficulty in obtaining large numbers of autologous DC from the patient, short half-life and absence of proliferation that limit long-lasting immune response. Moreover, DC-based vaccines, while being able to induce serological and cellular immunity against HPV, gave no clinical responses.[29,67] In conclusion, although DC-based vaccines may be used in advanced cases of cervical cancer, it is unlikely they will be used to treat CIN lesions because the procedures involved are labor-intensive and costly.

Conclusion

Current treatment strategies such as LEEP or cone biopsy aim to destroy visible CIN. In these patients, the conservative use of therapeutic vaccines alone or in combination with other immunomodulators appears theoretically promising. Obtaining the lesion regression and the downgrading from CIN 3 to CIN 2–1 would allow these patients to avoid surgical treatment (such as cone biopsy).

Some preliminary considerations on the requirements of therapeutic vaccines should be done. First, an ideal foreign antigen to be targeted should not only be expressed at high levels in a significant percentage of patients with the disease but should also be at lower levels within normal tissues to ensure an adequate safety profile. Moreover, the antigen should be essential for infected cell proliferation and survival to minimize the progressive immune escape due to the downregulation of antigen expression. More importantly, it has to be characterized by high immunogenicity for obtaining an adequate immune response. Overall, no many antigens fulfill these criteria so that they cannot assure the production of a durable and efficacious immune response against infected and tumor cells.[68,69]

Despite having *in vitro* demonstrated to obtain humoral and cytotoxic responses, the majority of therapeutic vaccines have not yet clinically demonstrated remarkable success.[67] Each class of therapeutic vaccines has advantages and limitations (Table 1): live vector-based vaccines have a high efficacy in delivering antigens, being able to directly replicate in the host, but they may potentially have safety concerns if the patient is immunocompromised. Peptide and protein vaccines are generally stable, easier to be produced compared to live vector-based vaccines, but their action is not MHC restricted, because all antigens are intracellularly obtained by APC process. Moreover, these vaccines tend to have low immunogenicity. DNA vaccines do not tend to induce high immune responses, although they can lead to sustained

Table 2 Summary of clinical trials on therapeutic HPV vaccines

Class	Vaccine	Antigen(s)	Trial phase	Patients (*n*)	Outcome	Side effect
Live attenuated bacteria and viruses						
	Lm-LLo-E7	HPV-16 E7	Phase I in patients with metastatic, refractory or recurrent, advanced squamous cell carcinoma of the cervix	15	Increase in E7-specific T cells detected in PBMC of three patients. Reduction in tumor size observed in four patients.	Pyrexia, vomiting, chills, headache, anemia, nausea, tachycardia, muscle and skeletal pain
	GLBL101c	HPV16-E7	Phase I/IIa in HPV16+ CIN3 patients	17	Significant increase in E7-CMI in cervical/ vaginal tract. Nine patients experienced disease regression to CIN2 and 5 further regressed to LSIL	No major side effects observed
	TA-HPV	HPV-16/18 E6/E7	Phase I/II in patients with advanced stage of cervical cancer	8	Vaccination induced HPV-specific cytotoxic T lymphocyte immune response in 28% of participants. 2 patients showed tumor-free condition at 15 and 21 months after vaccination	Single-dose generated mild and tolerable toxicity
			Phase I in patients with FIGO stage Ib or IIa cervical cancer who will undergo radical hysterectomy	29	After a single vaccination HPV-specific CTL were found in 4 patients. 8 patients (28%) developed HPV-specific serological responses	Mild to moderate local toxicity
			Phase II in patients ages 42–54 with high-grade HPV-positive vulval or vaginal intraepithelial neoplasia of up to 15 years duration	12	Five out of 12 patients showed at least 50% reduction in total lesion diameter over 24 weeks with 1 patient showing complete regression of the lesion. Overall, 83% of women showed some average decrease in lesion size of 40%. All patients showed an increased immunoglobulin G titer and T-cell response to the vaccinia virus	A local reaction at the site of vaccination between day 7 and 10 was common and 2 patients had temporarily limited arm movement

(*Continues*)

Table 2 Continued

Class	Vaccine	Antigen(s)	Trial phase	Patients (*n*)	Outcome	Side effect
	MVA E2	HPV-16 E2	Phase III in patients with HPV-induced AGIN	1176 female 180 male	90% lesion clearance in female treated patients and 100% lesion clearance in male treated patients. Antibody and T cell responses observed in all tested patients	Headache, flu-like symptom, fever, chills, abdominal pain, joint pain
	BLS-M07	HPV-16 E7	Phase I/IIa in patients with HPV-16 infection and a diagnosis of CIN 3.	19 in phase I (dose-limiting toxicity) 8 in phase IIa	Evaluate safety and efficacy (primary endpoint); systemic production of immunoglobulin G (IgG) against HPV E7 and lesion grade modification evaluated by Reid Colposcopic Index (RCI) (secondary endpoints)	No major side effects observed.
Peptide vaccines						
	ISA101	HPV-16 E6/E7	Phase II in patients with HPV-16+ Incurable solid tumors (oropharyngeal squamous cell carcinoma, cervical, vulvar, vaginal, anal and penile cancer)	34	Clinical response in 18 of 34 (53%; 95% confidence interval [CI] 35.1–70.2) patients at 3 months and in 15 of 29 (52%; 95% CI 32.5–70.6) patients at 12 months. Eight patients showed a complete histological response.	Local reactions that in some patients were long-lasting, with swelling and ulceration of the skin, still present after 12 months
	PDS0101	HPV-16 E6/E7	Ongoing phase I in female patients with high-risk HPV infection or CIN1	18 estimated patients		
	Pepcan	HPV-16 E6	Phase I in patients with biopsy-proven CIN 2/3	24	Lesion regression rate of 83%. After the four vaccinations, vaccine-induced immune responses in 65% of women	Injection site reactions; none of the patients experienced dose-limiting toxicities
			Ongoing phase II		Clinical response by colposcopy-guided quadrant biopsies	
	TG4001	HPV-16 E6 and E7 early genes and human interleukin (IL)-2 gene	Phase II trial in women with CIN	40	48% of patients were clinical responders; the 6-month efficacy rate was 24%. HPV-16 mRNA clearance was associated with CIN regression at cytology and	Adverse effects include: inflammation, pruritus, edema at the injection site, lymphadenopathy,

Table 2 Continued

Class	Vaccine	Antigen(s)	Trial phase	Patients (*n*)	Outcome	Side effect
					colposcopy in 70% of the study population	fever, headache, asthenia, bone pain and vaginal discharge.
Protein vaccines						
	SGN-00101	HPV-16 E7 linked to BCG heat-shock protein	Phase II trial in patients with CIN 3	58	Histological complete response (CR) in 13 (22.5%) and a partial response (PR) in 32 (55%) women	Only mild, self-limiting injection site-related side effects were encountered
	TA-CIN +	HPV-16 E6/E7/L2	Phase I in healthy patients	40	TA-CIN specific IgG in 24 of 32 vaccinated patients. 25 of 32 vaccinated patients generated CMI	Injection site reaction, tenderness. Headacheand fatigue
			Phase II with VIN2/3 patients	19	63% lesion response 1 year after vaccination. Significant increase. Significant CMI observed in lesion responders	Local reaction associated with imiquimod
	TVGV-1	Peptide sequence of HPV-16 E7 fused to the *Pseudomonas aeruginosa* exotoxin A (PE) and endoplasmic reticulum (ER) retention signal	Ongoing phase IIa trial in patients with CIN 2–3	51 estimated patients		
	GTL001	HPV 16 and HPV 18 E7 fused with detoxified adenylate cyclase from Bordetella Pertussis (CyaA)	Phase I trial in HPV-16 or – 18 positive patients with either normal or mildly abnormal cervical cytology (ASC-US/LSIL)	47	Examine the safety, tolerability and immunogenicity of GTL00	Injection-site reactions mild to moderate (pain, swelling, induration, tenderness and itching). Headache, myalgia and fatigue were the most common systemic reactions.
		Tested with imiquimod as adjuvant	Phase II HPV-16 or –18 positive patients with either normal or mildly abnormal cervical cytology (ASC-US/LSIL)	239	No statistically significant difference in viral clearance and lesions progression to high-grade between GTL001and placebo groups	The vaccination was well tolerated and no unexpected event was observed

(*Continues*)

Table 2 Continued

Class	Vaccine	Antigen(s)	Trial phase	Patients (*n*)	Outcome	Side effect
	GTL002	modified E7 proteins from HPV-16, 18, 45, 31, 33 and 52	Models in mice and Beagle dogs	No data on human exist until now about its use.	Induction of E7-specific T-cell response against each of the genotypes	
DNA vaccines						
	pNGVL4a-CRT/E7(detox)	HPV-16 E7	Phase I with HPV16+ CIN2/3 patients	32	30% vaccinated patients experienced histological regression to CIN1 or less. Increase in intraepithelial C8+ T-cells infiltrate after vaccination	Injection site reaction
	GX-188E	HPV-16/18 E6/E7	Phase I in patients with HPV 16/18+ CIN3	9	All patients displayed enhanced HPV-specific CMI. Seven patients demonstrated complete lesion regression by the end of the trial.	Chills, injection site pain, swelling, hypoesthesia, headache, fatigue, rhinitis
	VGX-3100	HPV-16/18 E6/E7	Phase I with HPV16/18 + CIN2/3 patients	18	HPV-specific CMI observed in 78% of patients and HPV-specific humoral response observed in all patients	Injection site reaction, pain, fever, tenderness
			Phase IIb with HPV16/18 + CIN2/3 patients	167	49.5% vaccinated patient demonstrated regression compared to 30.6% in placebo group. Vaccinations enhance T cell and humoral response	Injection site reaction, fatigues, headache, myalgia, nausea, arthralgia, erythema
Cell vaccines						
	DC vaccinations	HPV antigens	Phase I in patients with HPV+ advanced, recurrent cervical cancer	14	No significant increase in lymphocyte proliferation observed. Lack of biopsy sample and small sample size prevent definite conclusions	Local site reaction, fever, chills, abdominal discomfort, vomiting

cellular gene expression and thus antigens production. All these issues require a great effort to find innovative solutions to improve outcomes related to the use of therapeutic vaccines for HPV: for peptides vaccines, it could be advisable to select high immunogenic antigens, derived from the targeted protein. Moreover, it should be considered the addition of specific adjuvants (liposome-polycation-DNA, the saponin-based adjuvant or imiquimod) or the use of peptide conjugate vaccines (in particular, by employing bacterial proteins such as the *Bordetella pertussis* adenylate cyclase, the translocation domain of *P. aeruginosa* exotoxin A, or the *Mycobacteria*-derived HSP proteins). These strategies aim to elicit stronger DC response and antigenic presentation to T cells.[6]

At the moment, no therapeutic vaccine has been approved in the clinical practice. Moreover, the studies available in the literature are often uncontrolled and included a small sample size; we deem that there is an urgent need to compare therapeutic vaccines to the conventional management for HPV-related lesions (or expectant management for low-grade preinvasive disease; conization for high-grade disease).

Until now, the majority of therapeutic vaccines have been tested in phase I–II clinical trials. MVA E2 has been the only vaccine tested in a multicenter phase III clinical trial conducted on more than 1300 patients (Table 2).[37] The results of this trial were surprising (reduction of around 95% of CIN 2/3 and 70% of CIN 1). However, comparative studies between it and surgical management of high-grade CIN have been not published yet and thus are strongly awaited within the near future. Moreover, it would be of interest to know if the addition of this vaccine to the conventional surgical approach for CIN could reduce the risk of disease recurrence.

The economic aspect related to therapeutic vaccines represents another important topic; one study tried to explore the potential pricing of a therapeutic HPV vaccine for women identified with HPV-induced cervical lesions in the Netherlands. The maximum vaccine price was found to be lower than the average treatment cost for patients with CIN 2/3 and FIGO 1A cervical cancer.[14]

Particular attention should be addressed to the promising combination of structural (L1/L2) with functional (E6/E7) HPV protein. The availability of combined prophylactic and therapeutic vaccines might be a novel opportunity to target adolescents with preventive intent, but also adults already infected by HPV by the clearance of productive infections. Hypothetic strategies would be to combine a licensed virus-like participle (VLP) vaccine with a therapeutic one in a co-formulation, or to generate a chimeric VLP vaccine, including E protein sequences. As reported above, TA-CIN, a fusion protein vaccine composed of HPV-16 L2, E6 and E7 represents an innovative solution in this setting; until now, this vaccine administered with topical imiquimod succeeded in obtaining CR in more than half of patients affected by high-grade VIN.[48] No data on this vaccine is available in patients affected by CIN, although it is being tested in women with advanced cervical cancer (NCT02405221). If the development of combined prophylactic and therapeutic vaccines will obtain success, a greater impact on HPV transmission rates, with possible implications even on herd immunity, could be likely obtained.

To date, despite promising results, many obstacles exist before hypothesizing an introduction of therapeutic vaccines into clinical practice within the next few years, such as the need for further phase III clinical trials, the identification of the optimal population (age and gender) and also the accurate definition of the role of therapeutic vaccines for the treatment of cervical HPV infection and preinvasive lesions. Further studies will draw a definitive conclusion on the role of therapeutic vaccines in this context.

Disclosure

The authors have not conflict of interest to disclose.

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