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The current state of therapeutic and T cell-based vaccines against human papillomaviruses

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Abstract

Human papillomavirus (HPV) is known to be a necessary factor for many gynecologic malignancies and is also associated with a subset of head and neck malignancies. This knowledge has created the opportunity to control these HPV-associated cancers through vaccination. However, despite the availability of prophylactic HPV vaccines, HPV infections remain extremely common worldwide. In addition, while prophylactic HPV vaccines have been effective in preventing infection, they are ineffective at clearing pre-existing HPV infections. Thus, there is an urgent need for therapeutic and T cell-based vaccines to treat existing HPV infections and HPV-associated lesions and cancers. Unlike prophylactic vaccines, which generate neutralizing antibodies, therapeutic, and T cell-based vaccines enhance cell-mediated immunity against HPV antigens. Our review will cover various therapeutic and T cell-based vaccines in development for the treatment of HPV-associated diseases. Furthermore, we review the strategies to enhance the efficacy of therapeutic vaccines and the latest clinical trials on therapeutic and T cell-based HPV vaccines.

Keywords

human papillomavirus; immunotherapy; therapeutic vaccine; T cell-based vaccine; HPV E6; HPV E7

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Disclosure of Potential Conflicts of Interest

The authors declared that no potential conflict of interest exists.

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1. Introduction

Human papillomavirus is the causative agent of a multitude of conditions including warts, cancer, and other diseases. In particular, HPV is responsible for nearly 100% of cervical cancer cases, which remains the fourth most common female cancer (Forman et al., 2012; Wakeham and Kavanagh, 2014). Furthermore, HPV has been shown to be a human biological carcinogen for five other types of cancers: penile, vaginal, vulvar, anal, and oropharynx including base of the tongue and tonsils (Forman et al., 2012; Maxwell et al., 2015; Mehanna et al., 2013; Wakeham and Kavanagh, 2014). Over 200 types of HPV had been identified and categorized into high risk and low risk groups according to their degree of oncogenic capacity (Egawa et al., 2015). Among high-risk HPV types, type 16 and 18 are the most carcinogenic and are responsible for inducing over 70% of cervical cancer cases (Walboomers et al., 1999). To date, HPV infections remain extremely common and represent a significant global health burden (Forman et al., 2012).

Most sexually active women will be infected by HPV during their lifetime; the majority of these infections will remain asymptomatic and be cleared by the immune system. However, for a handful of infected women whose immune system fails to clear the infection, these can develop into persistent HPV infections, which may further progress into low and high-grade cervical intraepithelial neoplasia (CIN) and consequently cervical carcinoma, or regress at any stage of this process (for review see (Ghittoni et al., 2015; Ostor, 1993)).

The identification of HPV as an etiological factor for HPV-associated malignancies allows for the opportunity to control these cancers through immunization and other target therapies. Vaccines have been traditionally used as a preventative measure against infectious disease. Several successful prophylactic vaccines have been developed to prevent disease-causing HPV types targeting the major capsid protein L1 of the viral particle (for review see (Harper, 2009; Kash et al., 2015)). Prophylactic vaccines have been effective in preventing vaccinated, healthy patients from acquiring HPV infections. They have also been effective in preventing previously infected patients who do not have active infections from being re-infected by the same HPV type. However, there is no strong evidence demonstrating any therapeutic effects of these prophylactic vaccines in treating and clearing established HPV infections and HPV-associated lesions (for review see (Harper and Williams, 2010; Ma et al., 2012)).

While there have been giant strides in the prevention of HPV infections and HPV-associated malignancies, there is still a need to develop treatments for the control of existing HPV infections and its associated diseases. Our review will cover various therapeutic and T cell-based vaccines in development for the treatment of HPV-associated cancers and lesions. Moreover, we will review strategies designed to enhance the efficacy of these vaccines and review the latest clinical trials on both therapeutic and T cell-based HPV vaccines.

2. Therapeutic Vaccines

Due to the prevalence of HPV infections worldwide, there is an urgent need to develop effective treatments for established HPV infections and HPV-associated diseases. One

potential treatment method involves the use of therapeutic vaccines. Unlike preventative vaccines, which are intended to generate neutralizing antibodies against viral particles, therapeutic vaccines are intended to stimulate cell-mediated immune responses to specifically target and kill infected cells. In many occasions, as HPV-associated lesions develop into cancer, the HPV viral DNA will be integrated in to the host's genome (Klaes et al., 1999). Typically, the integration process leads to the deletion of many early (E1, E2, E4, and E5) and late (L1 and L2) genes. The deletion of L1 and L2 genes during HPV DNA integration renders the prophylactic vaccines ineffective in targeting infected cells. In addition, E2 is a negative regulator of the HPV oncogenes E6 and E7; therefore, the deletion of the E2 gene during integration leads to the increased expression of these oncoproteins (for review see (Doorbar, 2016; zur Hausen, 2002)). This process is believed to contribute to the carcinogenesis of HPV-associated lesions, and the uncontrolled expression of E6 and E7 is considered a biological hallmark of HPV-associated cancers. Since HPV oncoproteins E6 and E7 are necessary for the generation and maintenance of HPV-associated malignancies, they are, consequently, constantly expressed and remain present and transcriptionally active in the transformed cells in HPV-induced cancer and precancerous lesions. Furthermore, since E6 and E7 are foreign proteins they are able to circumvent the setback of immune tolerance against self-antigens, a challenge presented by many other cancers. Thus, E6 and E7 serve as ideal targets for therapeutic HPV vaccines (for review see (Yang et al., 2016)). These findings have initiated many efforts to create an optimal immunotherapeutic treatment against HPV infections and diseases.

There are several types of therapeutic HPV vaccines that have been developed and tested in preclinical and clinical trials, most of which mainly target HPV E6 and E7. These include live vector, protein or peptide, nucleic acid, and cell-based vaccines. Most therapeutic vaccines contain E6 and E7 antigens in various forms, and aim to deliver these antigens to antigen-presenting cells (APCs) to stimulate antigen presentation through major histocompatibility complex (MHC) class I and MHC class II. This leads to the generation of CD8⁺ cytotoxic T cell or CD4⁺ helper T cell responses, respectively. Before the E6 and E7 antigens can be presented on the MHC class I molecule for the stimulation of CD8⁺ T cell responses, they are processed and digested in to smaller peptides by the proteasome in the APCs. Not all peptide fragments can be successfully loaded on to the MHC molecule and recognized by antigen-specific T cells (Yao et al., 2013). Only some of the short peptides that contain the sequence of antigenic fragments (epitopes) can bind to the MHC molecule with high affinity and interact with the T cell receptor (TCR) of antigen-specific T cells for the elicitation of an immune response (Feltkamp et al., 1993; Kumar et al., 2015; Riemer et al., 2010). Most therapeutic vaccines focus on provoking immune responses against the E7 antigen because it is better characterized immunologically than E6 in preclinical models. This section discusses the various therapeutic HPV vaccines being developed and tested. Figure 1 presents the process of antigen presentation and T cell activation upon therapeutic HPV vaccination.

2.1 Live vector-based

Live vector-based vaccines can be categorized as bacterial or viral vectors, depending on the vector platform. Live vector-based vaccines can induce strong cellular and humoral immune

responses (for review see (Yang et al., 2016)). Live vector-based vaccines are highly immunogenic and there are many vectors to choose for antigen delivery. However, the main drawback for live vector-based vaccines is they pose a safety risk, especially for immunocompromised individuals.

2.1.1 Bacterial vectors—Several bacterial vectors have been widely selected for the development of therapeutic HPV vaccines, including *Listeria monocytogenes*, *Lactobacillus lactis*, *Lactobacillus plantarum*, and *Lactobacillus casei* (Adachi et al., 2010; Bermudez-Humaran et al., 2004; Cortes-Perez et al., 2005; Sewell et al., 2008). Among these, *Listeria* is a promising vector because it is able to infect macrophages and secrete a pore-forming toxin, listeriolysin O (LLO) to escape phagosomal lysis (Schnupf and Portnoy, 2007). By evading phagosomal lysis, *Listeria* is able to replicate in the cytoplasm of the host cell. This allows the bacteria to be present in both the cytoplasm and endosomal compartments, therefore the antigen peptides in the bacteria can be presented via MHC class I to cytotoxic T cells and MHC class II to helper T cells (Peters and Paterson, 2003). *Listeria*-based E7 vaccines have been shown to reduce implanted, E6/E7-expressed solid tumors as well as limit the growth of spontaneous E6/E7-expressing tumors in HPV-16 E6/E7 transgenic mice (Sewell et al., 2008). Additionally, LLO was shown to induce a decrease in regulator T cells, resulting in the augmentation of the therapeutic and antitumor effects of the vaccine against E6/E7-expressing TC-1 tumors (Chen et al., 2014). Other attenuated bacterial vectors can be generated by vector transformation with plasmids containing selected genes of interest. Several examples include mutant strains of *Salmonella*, *Shigella*, and *Escherichia coli*, all of which can deliver plasmid-encoding genes of interest to ACPs.

2.1.2 Viral vectors—Viral vectors are able to infect and express encoded antigens, which makes them an attractive candidate for therapeutic HPV vaccines. Several viral vectors have been explored to deliver HPV E6 and E7 antigens, including adenoviruses, adeno-associated viruses, alphaviruses, and vaccinia viruses (Casseti et al., 2004; Gomez-Gutierrez et al., 2007; Hsieh et al., 2004; Liu et al., 2000). Vaccinia virus is an enveloped, double-stranded DNA virus belonging to the *Poxviridae* family. Vaccinia makes a promising viral vector due to its large genome, highly infectious nature, and low likelihood of irregular integration of foreign DNA into its genome (Borysiewicz et al., 1996). Vaccinia-based vaccines include vaccinia encoding the E7 protein fused to calreticulin (CRT), vaccinia-expressing E7 fused to LLO, and vaccinia-expressing E7 linked to sorting signals and lysosomal-associated membrane protein (SigE7-LAMP-1) (Hsieh et al., 2004; Lamikanra et al., 2001). Adenoviruses have been used as potential vectors in preclinical studies (for review see (Yang et al., 2016)). One study showed that vaccination with replication-deficient adenoviruses encoding CRT/E7 fusion protein successfully eliminated E7-expressing tumors in mice (Gomez-Gutierrez et al., 2007). In addition, an adenovirus vaccine encoding chimeric hepatitis B virus surface antigen (HBsAg) and HPV-16 E7 proteins has also been shown to increase E7 specific antibody and cytotoxic T lymphocyte (CTL) response in vaccinated mice (Baez-Astua et al., 2005). An alphavirus vector-based vaccine encoding a fusion protein of HPV-16 E6 and E7 packaged into the recombinant Semliki Forest virus (SFV) particles (SFVeE6,7) may be a potential candidate for treatment of HPV because it may not require additional immune interventions to modulate regulatory T cell activity (Walczak et

al., 2011). A recent study enhanced the potency of recombinant Semliki Forest virus (rSFV) vaccine by fusing rSFV replicon particles expressing HPV E6 and/or E7 to helper T cell epitopes and an ER targeting signal (Ip et al., 2015). This vaccine was reported to generate potent antigen CD8⁺ T cell responses, leading to TC-1 tumor clearance and prevent formation of TC-1 tumors in mice. Lastly, lentiviruses are able to transduce a variety of cell lines including tumor cells and dendritic cells (DCs) (for review see (Yang et al., 2016)).

One setback to using live vector-based vaccines is the generation of antiviral immune responses and neutralizing antibodies upon initial exposure to the vaccine. This limits the effectiveness of subsequent vaccine administrations. Another concern is the pathogenic potential of viral and bacterial vectors, which can be a safety risk for individuals with impaired immune functions (for review see (Ginaldi et al., 2001)).

2.2 Peptide-based and Protein-based

Peptide-based and protein-based vaccines are safe, stable, and easy to produce. Peptides and proteins derived from HPV antigens are processed by DCs and presented on either MHC class I or class II molecules to stimulate CD8⁺ or CD4⁺ T cell responses (for review see (Yang et al., 2016)).

2.2.1 Peptide-based vaccine—While peptide-based vaccines are stable, safe, and easy to produce, they have poor immunogenicity. Therefore, to enhance vaccine potency peptides are linked to lipids and adjuvants such as chemokines, cytokines, and Toll-like receptor (TLR) ligands (for review see (Yang et al., 2016)). These methods help to generate a stronger innate and adaptive immune response. Peptide-based vaccines are MHC specific making them an unlikely candidate for large-scale production and treatment (Hung et al., 2008; Su et al., 2010). One solution to improve potency is to apply overlapping long-peptide vaccines, which have been shown to induce antigen-specific T cell responses in several preclinical models (for review see (Lin et al., 2010; Su et al., 2010)).

2.2.2 Protein-based vaccine—Protein-based vaccines contain all possible human leukocyte antigen (HLA) epitopes, which represents a significant concern in peptide-based vaccines (for review see (Lee et al., 2016)). However, protein-based vaccines also have poor immunogenicity and most are presented through the MHC class II pathway, which activates the production of antibodies rather than generate a CTL response (for review see (Su et al., 2010)). Adjuvants and immunostimulating molecules are added to protein-based vaccines to enhance their endogenous processing and resultantly enhance their uptake and presentation by MHC class I molecules (Lin et al., 2010).

2.3 Nucleic acid-based

2.3.1 DNA vaccine—DNA vaccines have emerged as an attractive and potentially effective approach for antigen-specific immunotherapy. Much like peptide-based vaccines, they are safe, stable, easy to produce, and do not induce neutralizing antibodies permitting repeated vaccination (for review see (Lee et al., 2016)). However, there is a potential risk that administration of DNA encoding HPV oncogenes E6 and E7 may lead to cellular transformation, which can be addressed by modifying E6 and E7 DNA that results in

expression of proteins that are incapable of oncogenic transformation (Kim et al., 2004). DNA vaccination involves the injection of plasmid DNA encoding the antigen of interest (in this case, HPV E6 and E7) into the host's cells.

DNA vaccines are typically administered via intramuscular injection. After injection the DNA may be taken up by myocytes or DCs (for review see (Lee et al., 2016)). While myocytes transfected with injected DNA will express the target antigen, they are not professional APCs and therefore cannot fully activate a strong immune response (Fu et al., 1997). DCs play a critical role in presenting the antigen to naïve CD8+ cytotoxic T cells and do so through exogenous antigen uptake or direct transfection by vaccination (Chattergoon et al., 1998; Dupuis et al., 2000; Porgador et al., 1998). One important limitation to the use of DNA vaccines is that naked DNA has low immunogenicity because it is unable to amplify and spread from transfected cells to surrounding cells *in vivo*. To overcome this setback several strategies to enhance DNA vaccine potency have been developed (for review see (Yang et al., 2016)).

2.3.2 RNA vaccine—Naked RNA replicon vaccines can be derived from RNA viruses such as Sindbis virus, Venezuelan Equine Encephalitis virus, and SFV (Berglund et al., 1997; Cassetti et al., 2004; Cheng et al., 2002). RNA replicon is capable of self-replication, leading to sustained antigen expression and increased immunogenicity. Moreover, RNA replicon vectors do not form viral particles, and therefore, do not lead to the generation of neutralizing antibodies, allowing for repeated administration. RNA replicons also alleviate the potential risk of chromosomal integration and cellular transformation associated with DNA vaccines. One drawback of RNA replicons is low stability. Some attempts to overcome this issue have been made by combining RNA replicons and DNA vaccine in to a DNA-launched RNA replicon, or 'suicidal DNA.' 'Suicidal DNA' triggers apoptosis in cells that uptake the injected DNA to prevent potential integration and transformation of the infected cells (Hsu et al., 2001). 'Suicidal DNA' has been applied to a therapeutic HPV vaccine in preclinical models, but it has been shown to lead to poor immunogenicity. Several strategies have been developed to overcome this problem including the incorporation of genes encoding anti-apoptotic proteins and the use of a flavivirus Kunjin (KUN) vector to deliver the replicons (for review see (Yang et al., 2016)). Despite promising results in preclinical models, RNA vaccines targeting HPV antigens and HPV-associated diseases have not been tested in any clinical setting.

2.4 Whole cell-based

2.4.1 Dendritic cell-based—DC-based HPV vaccines have emerged as a potential therapeutic vaccine against HPV-associated malignancies. DC-based HPV vaccines involve loading the DCs with HPV antigens *ex vivo* and subsequent delivery of DCs in to the patient (Adams et al., 2003; Benencia et al., 2008; Kim et al., 2009; Mackova et al., 2004; Murakami et al., 1999; Peng et al., 2005; Wang et al., 2000). DCs are also able to serve as natural adjuvants to enhance the potency of antigen-specific immunotherapy against cancer (for review see (Santin et al., 2005)). In order to prolong DC survival and enhance the efficacy of DC-based vaccines several strategies have been developed, including transfecting DCs with siRNAs against pro-apoptotic molecules (Kim et al., 2009; Peng et al., 2005).

While progress has been made to improve the efficacy of DC-based vaccines, they have several limitations. DC-based vaccines are technically demanding making them difficult to produce at a large-scale for the general population. Secondly, varying culture techniques may lead to inconsistencies in vaccine quality and standard evaluation criteria. Lastly, the most effective route for DC vaccine administration to amplify the effects elicited by the vaccine has not yet been determined.

2.4.2 Tumor cell-based—Tumor cell immunogenicity *in vivo* has been improved through the increased expression of immune modulatory proteins. Cytokine genes IL-2, IL-12, and granulocyte macrophage colony stimulating factor (GM-CSF) have been used in transformed tumor cell-based vaccines for HPV (Chang et al., 2000; Mikyskova et al., 2004). One advantage of using tumor cell-based vaccines is that tumor antigens do not need to be well identified; therefore, these vaccines have the potential to provide protection against more tumor antigens. Unfortunately, HPV-associated cervical cancers have well known tumor-specific antigens rendering this approach impractical. Additionally, tumor-based vaccines run the risk of implanting new cancers in to patients. For these reasons tumor cell-based vaccines against HPV and HPV-associated malignancies have not yet been utilized and tested in clinical trials.

2.5 Methods to enhance therapeutic effects of vaccines

Many of the therapeutic HPV vaccine designs listed above have low immunogenicity. Several strategies have been developed and investigated to enhance the therapeutic effects of these vaccine types. Such strategies include immunostimulating molecules or adjuvants, combinatorial approaches with prime-boost regimens, immunomodulatory agents and other therapeutic modalities, and identifying ideal methods and location of vaccine administration (for review see (Yang et al., 2016)).

3. T cell-based Vaccines

Efforts to target HPV oncoproteins (E6 and E7) using therapeutic vaccines have yet to demonstrate remarkable success in treating advanced cervical cancer. Thus, there is still an urgent need for treatment of HPV and HPV-associated malignancies, which therapeutic vaccines have not yet met (Stevanovic et al., 2015). As the knowledge and understanding of tumor immunology has expanded exponentially over the last three decades, we have begun to shed light on to the complicated relationships between tumor cells, the tumor microenvironment, and immune cells such as cytotoxic T cells, helper T cells, and regulatory T cells. T cells are often considered to be the final effectors of immune-mediated cancer regression. For this reason, therapies that directly use tumor-reactive T cells have been developed. This therapy is referred to as adoptive cell transfer (ACT). ACT involves T cell expansion outside of the tumor microenvironment and the infusion of autologous tumor-reactive T cells (Draper et al., 2015; Stevanovic et al., 2015). ACT has been shown to facilitate complete clinical responses in some patients with B-cell malignancies and metastatic melanomas; however, its evaluation in epithelial malignancies has been limited and it is still unknown whether ACT can mediate regression of metastatic cervical cancer (Stevanovic et al., 2015). Although ACT is a promising cancer treatment strategy and has

shown encouraging results in preclinical and clinical trials (Draper et al., 2015), there is still much work to be done.

There are several types of T-cell based vaccines, or adoptive T-cell therapies (ACTs) that have been tested in preclinical and clinical trials. These include adoptive transfer of tumor-infiltrating lymphocytes (TIL), engineered T-cell receptors (TCR), and chimeric antigen receptor (CAR) T cells (Draper et al., 2015). T-cell based therapies bypass the immune reliance on DCs to activate T cells and instead modifies T cells directly. Additionally, T-cell based therapy can be designed to overcome immune tolerance and incorporate modified tumor-specific T cells, which may not be generated from conventional therapeutic vaccination (Jeanbart and Swartz, 2015). Furthermore, T cell-based therapies can be improved through engineering technologies and represent highly personalized therapeutic treatments with significant clinical potential (Stevanovic et al., 2015). This section discusses the various T-cell based vaccines/therapies being developed and tested. Figure 2 presents a schematic diagram depicting the process of T-cell based vaccination.

3.1 Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TIL) are T cells isolated from a metastatic tumor, expanded *ex vivo*, and infused back into the patient (Draper et al., 2015) (Kelderman et al., 2016). Since the late 1980s TILs have been shown to control tumor regression in patients with metastatic melanoma (Schober and Busch, 2016). In more recent years, TIL therapy has been shown to induce tumor reactivity in several cancer types including melanoma, GI, lung, and HPV-associated malignancies (Klebanoff et al., 2016). Furthermore, TILs selected for HPV oncogenes E6 and E7 have been shown to cause tumor regression in patients with metastatic cervical cancer (Stevanovic et al., 2015). In some situations, cervical cancers house somatic gene mutations, which can lower TIL therapy efficacy. Further studies on TIL reactivity against non-HPV tumor antigens may shed light on the mechanisms of tumor regression using TIL therapy (Stevanovic et al., 2015).

The efficacy of TIL therapy can be further improved by enhancing the reactivity toward defined tumor antigens (Kelderman et al., 2016). Advancements in gene engineering technologies have overcome many practical barriers that have impeded the use of TIL cells (Klebanoff et al., 2016). Moreover, through advancements in genomics and immunology TIL therapy has the potential to be applied to a wider range of cancers (Draper et al., 2015). In addition, genetically engineered antitumor T cells may have the potential to be produced on a large scale using commercial production methods (Klebanoff et al., 2016).

While there is a wide range of tumor types that respond to ACT, it is still unknown whether HPV-TILs can mediate regression of other HPV-associated malignancies including oropharynx, anus, vulva, vagina, and penis (Stevanovic et al., 2015).

3.2 Engineered T-cell receptor T Cells

A more “off-the-shelf” approach to ACT is the use of engineered T-cell receptors (TCR). Engineered-TCR T cell therapy is achieved by administering peripheral blood T cells that have been genetically engineered to express a tumor-targeting TCR (Draper et al., 2015). Furthermore, engineered-TCRs can also be developed to help overcome immune tolerance.

Engineered-TCR T cells have recently received widespread attention after the FDA labeled them as “breakthrough status” (Jeanbart and Swartz, 2015). Two types of approaches can be employed when genetically redirecting T cell specificity toward a particular cancer. The first type involves the introduction of cloned, tumor-specific TCR into circulating lymphocytes that recognize specific antigen peptides bound to both classes of MHC molecules (Klebanoff et al., 2016).

Engineered-TCRs are most efficient at targeting surface or intracellular/nuclear proteins that are processed and presented on the cell surface by MHC molecules (Hossain et al., 2016). Some tumor-associated antigens (TAAs) targeted by engineered-TCRs include tissue-specific differentiation antigens, cancer-testis antigens, overexpressed antigens, and mutated self-proteins that form neoantigens (Hossain et al., 2016). Engineered-TCRs trigger T cell activation through the same signal transduction machinery utilized by native TCR (Klebanoff et al., 2016). In HPV associated malignancies, oncoproteins E6 and E7 are ideal targets for engineered-TCR gene therapy because they are always expressed in transformed cells and play an important role in the survival of the HPV+ cancers mentioned above (Draper et al., 2015). Unfortunately, there is a lack of supporting evidence demonstrating that engineered-TCR T cells targeting E6 and E7 can recognize HPV+ tumor cells (Draper et al., 2015).

Immunotherapy using engineered-TCRs relies on the generation of TCR α and β chains recognizing tumor targets and expressing these engineered-TCR molecules in autologous T cells (Hossain et al., 2016). Not only does the ability of engineered-TCR cells to identify intended tumor cells depend on the cell surface abundance of the TCR α/β heterodimer, it also depends on the receptors affinity for the target antigen (Hossain et al., 2016). Several strategies have been developed to optimize expression of engineered-TCR including the use of modified promoters and the introduction of mutations in the α and β chains (Hossain et al., 2016). While several clinical trials have demonstrated the safety of engineered-TCRs without significant adverse effects, some data suggests TCR-engineered cells can cause adverse on-target/off-tumor effects as well as off-target effects. These effects can lead to morbidity and even mortality (Hossain et al., 2016). While engineered-TCR cells have been shown to have a potential role in the treatment of hematologic malignances, their effects on solid tumors as well as HPV and HPV-associated malignances still need to be explored.

3.3 Chimeric antigen receptor (CAR) T cells

As mentioned earlier, manipulation of host immune cells can be one effective strategy for immunotherapy and personalized medicine. Collected lymphocytes can be genetically engineered to express specific T-cell receptors (Subramaniam et al., 2016). The second method to genetically redirect T cell specificity to cancer is through the introduction of a synthetic recognition structure called a chimeric antigen receptor (CAR) (Klebanoff et al., 2016). The design of CAR aims to provide the appropriate co-stimulatory signaling to activate effector T cells (Dai et al., 2016). CARs consist of an extracellular antigen recognition domain, a hinge domain, a transmembrane domain (TM), and an intracellular domain (Zhang et al., 2016). The extracellular antigen recognition domain is derived from a single chain variable fragment (scFv) isolated from an antigen-specific monoclonal antibody

(mAb). This is what enables the T cell to bind to antigens with preserved specificity and affinity. The hinge region facilitates CAR flexibility, ensures suitable positioning of the binding domain during scFv-antigen interaction, and transduces crucial signals. The TM can influence function of CAR-T cells, and is derived from CD3- ζ , CD4, CD8, OX40, H2-K^b. Lastly, the intracellular domain, which contains a signaling domain, is responsible for signal delivery within CAR-T cells. The signaling domain is derived from lymphocyte signal-initiating molecules (Dai et al., 2016) and is critical for CAR-T cells to accomplish anti-tumor functions (Zhang et al., 2016).

CARs only recognize antigens present on the cell's surface; however, this recognition occurs independently of MHC molecules. MHC-independent antigen recognition allows CAR-T cells to work on any patient who has a tumor expressing the target antigen (Klebanoff et al., 2016). Because CAR recognition is MHC-independent CAR-T cells can overcome tumor evasion caused by MHC molecule down-regulation (Dai et al., 2016; Haji-Fatahaliha et al., 2016), and potentially be used for the treatment of tumors that have defects in antigen processing and presentation (Klebanoff et al., 2016).

Currently, four generations of CAR-T cells exist. The first generation of CARs showed modest clinical activity and poor persistency *in vivo*. To increase its efficacy, the second and third generation CARs incorporate one and two co-stimulatory molecules, respectively (for review see (Zhang et al., 2016)). Second generation CARs have been shown to enable increased T cell antitumor activity, while third generation CARs have been shown to enhance cytokine production and tumor growth inhibition in mice (Dai et al., 2016). Currently, second generation CAR-T cells have been more widely used in clinical trials than third generation because the reduced activation threshold in third generation CAR-T cells may induce on-target/off tumor side effects in normal tissue (Zhang et al., 2016). Lastly, fourth generation CAR-T cells, which are termed T cells redirected for universal cytokine-mediated killing (TRUCKs), have a cytokine expression cassette on the vector encoding the CAR construct (Dai et al., 2016).

Much like engineered-TCR, CAR-T cells have recently received widespread attention (Jeanbart and Swartz, 2015). Clinical trials using second generation CD19 CAR-T cells to treat hematological malignancies have had promising effects. As a result, CD19 CAR-T cell therapy (CTL019) has been approved as the “breakthrough” therapy by the FDA (Zhang et al., 2016). Although CAR-T cell therapy has been successful as a novel treatment for hematologic malignancies, it has been less successful in treating solid tumors and has not yet been used in the treatment of HPV infections or HPV-associated malignancies. The histopathological structure and strong immunosuppressive environment of solid tumors have significantly limited CAR-T cell therapy. These limitations have inspired several advancements in CAR-T cell therapy against solid tumors, including cytokine release, and target TAA modification (Zhang et al., 2016).

Due to the mechanisms of HPV mentioned in Section 2, CAR-T cell therapy may not be the most suitable T cell-based vaccine treatment method for HPV and HPV-associated diseases. For this reason, no clinical trials have been performed using CAR-T cell therapy to treat HPV-associated malignancies. Nonetheless, we believe CAR-T cells were an important T

cell-based vaccine treatment with promising therapeutic results against hematologic malignancies. We believe that these successes warrant further investigation. Furthermore, it is possible that future generations of CAR-T cells may be developed to better treat solid tumors and HPV-associated malignancies; therefore, we chose to discuss them in this review.

4. Clinical Trials

Clinical trials are crucial steps for evaluating the efficacy of therapeutic and T cell-based vaccines in curing HPV infections and HPV-associated diseases in humans. Here, we summarize a number of recent clinical trials that implemented various forms of therapeutic and T cell-based HPV vaccines against HPV-associated lesions and malignancies. Table 1 and Table 2 summarize the clinical trials on therapeutic and T cell based HPV vaccines described in this section, respectively. In addition, Table 3 lists several ongoing clinical trials evaluating the efficacy of therapeutic and T cell based HPV vaccines against HPV-associated diseases.

4.1 Therapeutic Vaccines

4.1.1 Live vector-based—In 2009, the first clinical trial of a *Listeria*-based therapeutic HPV vaccine was reported (Maciag et al., 2009). The vaccine Lm-LLO-E7 (also known as ADXs11-001 or ADXS-HPV) is a fusion vaccine consisting of a *prfA* defective LM, HPV-16 E7 antigen and LLO (Gunn et al., 2001). The phase I trial of Lm-LLO-E7 tested the safety of this vaccine in 15 patients with recurrent, metastatic, or refractory advanced squamous cell carcinoma of the cervix. Patients were vaccinated intravenously, followed by IV supplementation with 500mg ampicillin 5 days after vaccination, followed by a 10-day oral course of ampicillin. Common adverse effects included pyrexia, vomiting, chills, headache and anemia, nausea and tachycardia, and musculoskeletal pain. Reduction in total tumor size was observed in four patients suggesting Lm-LLO-E7 may have therapeutic effects in controlling the progression of cancer.

A more recent study tested the safety and efficacy of oral vaccination of GLBL101c, a bacterial vector-based therapeutic HPV vaccine in a phase I/IIa study involving 17 patients with HPV-16+ CIN three lesions (Kawana et al., 2014). GLBL101c is generated from a recombinant of *L. casei* that expresses a modified version of HPV-16 E7 antigen (Adachi et al., 2010). None of the patients experienced seriously adverse effects and a significant increase in E7-specific cell-mediated immune response was observed in all patients who received the vaccine.

Several MVA-based therapeutic HPV vaccines have been designed and tested in clinical settings. One such vaccine, TG4001, is a suspension of MVATG8042 particles with attenuated recombinant MVA carrying sequences encoding modified HPV-16 E6/E7 and human IL-2. The safety and efficacy of this vaccine was evaluated in 21 patients with HPV-16 related CIN2/3 lesions (Brun et al., 2011). Both HPV-16 DNA and mRNA clearance was observed in responders and no recurrence of high-grade lesions was observed 12 months after treatment. Another MVA-based vaccine, MVA E2, was created to target HPV E2 protein in lieu of E6 or E7 oncoproteins (Rosales et al., 2000). Recently, the MVA E2 vaccine was used in a phase III clinical study for the treatment of HPV-induced ano-

genital intraepithelial lesions (Rosales et al., 2014). MVA E2 demonstrated a strong therapeutic effect against HPV-induced CIN lesions and is a promising therapeutic vaccine.

TA-HPV is a live recombinant vaccinia virus vaccine that expresses oncoproteins E6 and E7 of HPV16 and 18. The first use of TA-HPV in clinical trial was done in eight patients with advanced stages of cervical cancer (Borysiewicz et al., 1996). TA-HPV induced an HPV-specific cytotoxic T cell response in three patients and two patients were tumor free at 15 and 21 months after vaccination. Two additional clinical studies were performed using TA-HPV vaccine. In the first clinical study eight out of 29 patients displayed HPV specific serological responses; however, HPV-specific cytotoxic T cell response was short-lived (Kaufmann et al., 2002). The second clinical study administered TA-HPV to 12 patients with HPV-16+ vulval intraepithelial neoplasia (VIN) grade III and a single patient with HPV-16+ vaginal intraepithelial neoplasia (VAIN) grade II (Baldwin et al., 2003). Patients showed a reduction in HPV-associated lesion size and there was a significant increase of HPV 16 E6 and E7-specific T-cell immunity.

4.1.2 Peptide and protein-based—The therapeutic effects of HPV16 synthetic long-peptide vaccine (HPV16-SLP) have been studied extensively in numerous clinical trials (de Vos van Steenwijk et al., 2012; Kenter et al., 2009; van Poelgeest et al., 2013). A recent study explored whether HPV16-SLP vaccination combined with standard carboplatin and paclitaxel (CarboTaxol) chemotherapy improved immunity (Welters et al., 2016). This study aimed to determine a specific time frame during which immunity is optimized, and determine whether CarboTaxol mediates normalizing of circulating immune cell frequencies. Eligible patients had advanced, recurrent, or metastatic cervical cancer but were not required to have an HPV16+ tumor. In the first cohort, six patients were given CarboTaxol treatment once every three weeks reaching a total of six CarboTaxol treatments to determine when immunity was optimized. The second cohort collected data from 13 patients and 19 healthy donors. 12 patients from the second cohort received a single vaccination with HPV16-SLP two weeks after their second (n=11) or third (n=1) cycle of chemotherapy. In the first cohort, CarboTaxol treatment was associated with a decrease in myeloid cells, reaching its lowest levels one to two weeks after the second chemotherapy cycle. The decrease in myeloid cells coincided with an increase in the percentage of lymphoid cells, and while the relative number of CD4+ and CD8+ T cells did not change, their function improved. In the second cohort, patients displayed an increased level of circulating myeloid cells before chemotherapy. Throughout CarboTaxol treatment the lymphocyte count remained the same, but the absolute number of circulating leukocytes greatly decreased. Myeloid and lymphoid cells in CarboTaxol treated patients were almost normalized to levels of healthy donors. Furthermore, the 12 patients who received vaccination did not demonstrate a preexisting response to HPV16 E6/E7. HPV16-SLP vaccination induced a proliferative T cell response, which was retained in 11 patients after six cycles of chemotherapy (Welters et al., 2016).

The safety of PepCan, a therapeutic HPV vaccine containing four synthetic peptides covering HPV-16 E6 and Candin, a novel adjuvant, was tested in a phase I clinical trial (Coleman et al., 2016). PepCan was administered at varying doses to six patients and a loop electric excision procedure was performed 12 weeks after the last injection. In the final dose

part of the phase I clinical trial, ten additional patients with biopsy-confirmed HSIL were vaccinated at 50ug per peptide dose, regardless of HPV type. Reported common adverse effects were mild to moderate reactions at the injection site; however, no dose-limiting toxicities were reported. The group that received the 50ug dose showed the best histological regression rate and the most significant virological response. The viral load was decreased in nine patients. The detectability of HPV16 after vaccination may correlate with histological regression.

HPV therapeutic vaccine TA-CIN is a subunit vaccine composed of HPV16 E6E7L2 fusion protein (van der Burg et al., 2001). This vaccine has been proven safe and immunogenic in several phase I/II trials (Davidson et al., 2004; de Jong et al., 2002; Smyth et al., 2004). Additionally, the ability of the TA-CIN vaccine to be administered with imiquimod, a topical immunomodulatory, for the treatment of patients with grade 2 or 3 vulvar intraepithelial neoplasia (VIN) was further tested in a phase II study (Daayana et al., 2010). A total of 19 patients received imiquimod cream in addition to three intramuscular TA-CIN vaccinations at one-month intervals. Adverse effects after imiquimod cream application were observed; however, no side effects or adverse effects associated with TA-CIN vaccination were identified. The ability to elicit antigen-specific immune responses in patients correlated with lesion regression was proven.

GTL001 is a potential therapeutic HPV protein vaccine that targets both HPV 16 and 18. GTL001 consists of equivalent amounts of E7-CyA fusion proteins purified from and expressed in *E. coli*. A phase I clinical trial to examine the safety, tolerability, and immunogenicity of GTL001 was performed in a total of 47 women positive for HPV16 and HPV18 who had normal cervical cytology. The trial was divided into four cohorts, including two placebo sub-cohorts (Van Damme et al., 2016). Each cohort was administered varying amounts (100µg and 600µg) of GTL001 with imiquimod. This study showed that vaccination with GTL001 was considered relatively safe, with participants treated with GTL001 experiencing predominantly injection site reactions including pain, swelling, induration, tenderness, and itching. In congruence with preclinical studies, GTL001 induced a humoral response to CyA in all subjects but did not induce anti-E7 antibodies. Immunogenicity and tolerability were similar among patients receiving different regimen, with patients receiving 600µg GLT001 powder + imiquimod experienced slightly greater injection-site pain and erythema. The highest HPV16/18 clearance rate, and sustained clearance was observed in patients who received GLT001 600µg solution + imiquimod.

4.1.3 Nucleic acid-based—A recent clinical trial was conducted to evaluate the safety, efficacy, and immunogenicity of the DNA plasmid vaccine, pNGVL4a-CRT/E7(detox) (Alvarez et al., 2016). pNGVL4a-CRT/E7(detox) was administered intradermally, intramuscularly, or directly in to the cervical lesion in 32 patients with HPV16 associated CIN2/3. Patients with HPV CIN2/3 were injected three times at weeks 0, 4, and 8. pNGVL4a-CRT/E7(detox) was well tolerated in study patients, and the intralesional administration was shown to elicit the strongest immune response and induce the largest number of CD8+ T cells.

Another recent phase I clinical trial was conducted to evaluate the safety and efficacy of GX-188E, a therapeutic HPV E6 and E7 DNA vaccine, in nine patients with CIN3 (Kim et al., 2014). GX-188E is engineered to express a protein of HPV16 and HPV18 E6 and E7 that is fused to the extracellular domain of Flt3L and signal sequence of plasminogen activator (tpa). Nine patients were vaccinated with GX-188E via intramuscular injection, followed by electroporation to increase immunogenicity. The results of this study indicated that GX-188E was safe and well tolerated by patients and all vaccinated patients showed a statistically significant immune response. Furthermore, the findings in this study may encourage future clinical studies of GX-188E.

A separate clinical study exploring the therapeutic effects of VGX-3100 DNA vaccine also employed electroporation during vaccine administration (Bagarazzi et al., 2012). VGX-3100 is a mixture of two plasmids encoding E6 and E7 antigens of HPV16 and 18 (Yan et al., 2008; Yan et al., 2009). VGX-3100 was administered to 18 patients intramuscularly, who had received previous treatment for CIN2/3 lesions, followed by electroporation. All 18 patients received three rounds of vaccination. The vaccine was well tolerated with no dose-limiting toxicity observed. The study results demonstrated the potential of VGX-3100 to stimulate antigen-specific immune responses and contribute to the eradication of HPV-infected cells. The results of this study encouraged the initiation of a phase IIb trial to further confirm the therapeutic potential of the VGX-3100 DNA vaccine for treatment of CIN2/3 lesions (Trimble et al., 2015). This study also demonstrated that patients who were vaccinated with VGX-3100 showed greater HPV-specific T cell and humoral responses.

4.1.4 Whole cell-based—Dendritic cells play an important regulatory role in the immune system and are known to be the most efficient professional APCs (for review see (Lee et al., 2016)). A phase I dose escalation trial was conducted to evaluate the safety, toxicity, and immunogenicity of a DC-based vaccine in patients with stage Ib or IIa cervical cancer (Santin et al., 2008). There were 10 patients enrolled in the study. Autologous DCs were obtained from patients and pulsed with full-length HPV16/18-E7 and keyhole limpet hemocyanin (KLH), then injected via subcutaneous injection back in to the patient. This study reported that the DC-based vaccine was safe and well tolerated by patients. This vaccine was shown to increase HPV-specific humoral response in patients after vaccination, and an increase in E7-specific CD4+ T cell immune responses was observed.

Another phase I clinical trial evaluated the immunogenicity and toxicity of a DC-based vaccine in patients with HPV+ advanced, recurrent cervical cancer (Ramanathan et al., 2014). Participating patients were separated into three treatment groups: saline only (control), unprimed mature DC, and autologous tumor lysate primed mature DC. DCs were collected from 14 patients and pulsed with or without tumor lysate from the same patient. No statistically significant increase in lymphocyte proliferation was observed in patients from any group.

4.2 T-cell based Vaccines

4.2.1 Tumor-infiltrating lymphocytes—Clinical activity of TIL therapy correlates with the number of CD8+ T cells that are infused into the subject. The clinical activity of TIL

therapy is particularly promising with a response rate of approximately 50% observed in trials at different clinical centers (Kelderman et al., 2016). To increase tumor reactivity of TILs, a greater focus has been placed on defined antigens, coupled with the elimination of counteracting components. This may allow for greater production of T-cell products with greater functionality and therapeutic efficacy (Schober and Busch, 2016).

A phase II clinical trial was performed to determine whether ACT could mediate the regression of metastatic cervical cancer (Stevanovic et al., 2015). This study included nine patients with metastatic cervical cancer (2 with HPV-16, and 7 with HPV-18), who had previously received platinum-based chemotherapy or radiochemotherapy. Treatment consisted of lymphocyte-depleting chemotherapy, followed by administration of aldesleukin, and a single infusion of TILs selected for HPV E6 and E7 reactivity (HPV-TILs). Two patients experienced complete tumor responses that are ongoing at 22 and 15 months, respectively. One patient experienced a partial tumor response that lasted three months. Both HPV reactivity of T cells in infusion product, and frequency of HPV-reactive T cells in peripheral blood one month after treatment were positively associated with the clinical response ($p=0.0238$ for both). Additionally, there were no adverse effects related to cell infusion observed in this study. This study concluded that a single infusion of HPV-TILs could result in durable, complete regression of metastatic cervical cancer.

4.2.2 Engineered T-cell receptor—The number of engineered-TCR clinical trials has remained constant in recent years. Results from these trials have shown that, in some cases, engineered-TCRs can mediate long-term remission of selected hematologic and solid tumors (Klebanoff et al., 2016). A recent Phase I/II clinical study was completed to determine the objective tumor response rate and duration in 12 patients with metastatic or recurrent/refractory HPV-16+ cancers treated with autologous T cells expressing the E6 TCR plus aldesleukin following a lymphocyte-depleting preparative regimen of cyclophosphamide and fludarabine. All participating patients were HLA-A*02:01-positive. The results of this clinical trial have not yet been published (NCT02280811).

4.2.3 Chimeric antigen receptor—To date no clinical trials have been performed using CAR-T cell therapy for HPV-associated malignancies. As mentioned earlier, CAR-T cells mainly recognize antigens present on the cell's surface (Klebanoff et al., 2016); therefore, they may not be the most ideal T-cell based treatment candidates for HPV-associated diseases.

5. Conclusion

The identification and classification of high-risk HPV as a causal agent for many cases of cancer and other diseases offers an essential rationale for the development of therapeutic and T cell-based HPV vaccines. The current advancements in the field provide encouraging strategies for the eventual annihilation of HPV and HPV-related malignancies and diseases. In this review we discuss the various strategies used to target the most relevant antigens, HPV oncoproteins E6 and E7. These tumor-specific antigens serve as ideal targets for both therapeutic HPV vaccines and T cell-based HPV therapies. We conclude that the current therapeutic HPV vaccines including, live vector, protein or peptide, nucleic acid, and cell-

based vaccines have significant advantages and restrictions. In addition, we conclude that T cell-based vaccines including, tumor infiltrating lymphocytes, engineered T cell receptors, and chimeric antigens receptors also have significant advantages and limitations. Many current therapeutic vaccine studies have implemented novel strategies to improve vaccine efficacy and T cell immune response, including the incorporation of adjuvants, prime-boost regimens, and other combination strategies. Strategies to increase TIL therapy efficacy have further improved treatment by enhancing the reactivity toward defined tumor antigens. Several strategies have been developed to optimize expression of engineered-TCR; however, these have led to an increase in tumor toxicity due to on-target/off tumor reactivity. Additionally, co-stimulatory molecules have been incorporated in more recent generations of CARs to increase efficacy. With a greater understanding of the intricate relationship between tumor cells, the tumor microenvironment, and immune cells such as cytotoxic T cells, helper T cells, and regulatory T cell we can continue to create more effective and innovative therapeutic and T cell-based treatments for HPV and HPV-associated malignancies.

With continued effort in the development of therapeutic and T cell-based HPV vaccines and the information accumulated from recent and previous studies, we believe these therapy strategies will continue to gain success. We are confident that in the near future therapeutic and T cell-based vaccines will become clinically available and widely used alongside other therapies including surgery, chemotherapy, and radiation therapy, for the control of HPV and HPV-associated diseases.

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Highlights

- HPV is a causal factor for multiple cancers and diseases
- Preventative HPV vaccine is not effective at clearing preexisting infections
- Therapeutic and T cell based HPV vaccines typically target oncoproteins E6 and E7
- Several clinical trials have been performed to assess the efficacy of the vaccines
- More trials are expected as new strategies to enhance vaccine potency are developed

Figure 1. Immune activation by therapeutic HPV vaccination

Administration of various types of therapeutic HPV vaccines leads to introduction of antigen into the body in different forms. DNA plasmids encoding antigens (HPV oncoprotein E6 and E7) can be introduced into dendritic cells through direct DNA vaccination or through infection of modified live vector vaccine. The DNA encoding antigens will be transcribed into RNA, which can also be introduced into the cell through RNA vaccination. The transcribed RNA will be further translated into antigen proteins, or long peptides, which can also be taken up by the dendritic cells through phagocytosis after protein or peptide-based vaccination. The antigen proteins or long peptides are then processed by the proteasomes into short peptides, loaded onto class one major histocompatibility complex (MHC I) inside the endoplasmic reticulum (ER) to be presented to T cell receptors (TCR) of CD8+ T cells. Alternatively, autologous dendritic or tumor cells can be collected and prepared ex vivo to express target antigen on MHC I molecules as well as the necessary co-stimulatory molecules and be vaccinated back to the body (a process called adoptive transfer) as whole cell-based vaccines for the priming of T cells. (ER – Endoplasmic Reticulum; MHC – Major Histocompatibility Complex; TCR – T Cell Receptor; E6/E7 – Human Papillomavirus E6 and E7 Protein.)

Figure 2. Generation of Passive Immunity via adoptive T cell transfer

A schematic diagram depicting the process of T cell-based vaccination. Briefly, autologous T cells are harvested from patients through PBMC collection. The T cells are non-specifically expanded (typically with CD3 and CD28 agonistic antibodies), followed by several mechanisms to induce antigen-specific T cells: 1) expanded, heterogeneous T cell population can be co-cultured with antigen pulsed feeder cells (typically dendritic cells or irradiated tumor cells) to select for antigen-specific T cells, 2) expanded T cells can be transduced with DNA encoding engineered T cell receptor that is specific to the antigen using viral vectors, and 3) expanded T cells can be transduced with DNA encoding chimeric antigen receptor that is specific to the antigen. The resulted, selected or modified T cells can then be infused back into the patient for the generation of immune responses against antigen-expressing cells.

Table 1

Recent Therapeutic HPV Vaccine Clinical Trials Using Different Forms of Vaccines

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
Bacterial Vector Based							
Lm-LLo-E7 (ADXS 11-001; ADXS-HPV)	HPV-16 E7	prfA-defective <i>Listeria monocytogenes</i> strain transformed with plasmid encoding HPV-16 E7 antigen fused to a fragment of nonhemolytic listeriolysin O (LLO)	Advaxis, Inc.	Phase I in patients with metastatic, refractory, or advanced squamous cell carcinoma of the cervix (15 patients)	Increase in E7-specific T cells detected in PBMCs of three patients. Reduction in tumor size observed in 4 patients.	Pyrexia, vomiting, chills, headache, anemia, nausea, tachycardia, muscle and skeletal pain.	(Maciag et al., 2009)
GLBL101c	HPV16-E7	Recombinant <i>Lactobacillus casei</i> expressing modified version of HPV16-E7	GENOLA C BL Corp	Phase I/IIa in HPV16+ CIN3 patients (17 patients)	Significant increase in E7-CMI in cervical vaginal tract. 9 patients experienced disease regression to CIN2, and 5 further regressed to LSIL	No major side effects observed.	(Kawana et al., 2014)
Viral Vector Based							
TA-HPV	HPV-16/18 E6/E7	Recombinant Vaccinia virus	European Organization for Research and Treatment of Cancer (EORTC)	Phase I/II in patients with advanced stage of cervical cancer (8 patients)	Vaccination induced HPV-specific cytotoxic T lymphocyte immune response in 28% of participants (3 out of 8). 2 patients showed tumor free condition at 15 months and 21 months after vaccination.	Single dose generated mild and tolerable toxicity	(Borysiewicz et al., 1996)
				Phase I in patients with clinical International Federation of Gynecology and Obstetrics (FIGO) stage Ib or IIa cervical	After a single vaccination HPV-specific CTLs were found in 4 patients. 8 patients (28%) developed HPV-specific	Mild to moderate local toxicity	(Kaufmann et al., 2002)

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
				cancer who will undergo radical hysterectomy (29 patients)	serological responses.		
				Phase II in patients ages 42–54 with high-grade HPV-positive vulval or vaginal intraepithelial neoplasia of up to 15 years duration (12 patients)	5 of 12 (42%) patients showed at least 50% reduction in total lesion diameter over 24 weeks with 1 patient showing complete regression of lesion. Overall, 83% of women showed some average decrease in lesion size of 40%. All patients showed an increased IgG titer and T-cell response to the vaccinia virus.	A local reaction at the site of vaccination between day 7–10 was common and 2 patients had temporarily limited arm movement.	(Baldwin et al., 2003)
TG4001	HPV-16 E6/E7	Recombinant Modified Vaccinia Ankara expressing HPV-16 E6, E7, and human IL-2	Transgene/Roche	Phase I in HPV16+ CIN2/3 Patients (21 patients)	10 of 21 (48%) showed disease regression. HPV DNA clearance in 8 patients and mRNA clearance in 7 patients.	Inflammation, pruritus, edema, lymphadenopathy, fever, headache, asthenia, bone pain, vaginal discharge	(Brun et al., 2011)
MVA E2	HPV-16 E2	Recombinant Modified Vaccinia Ankara encoding E2 from BPV	Instituto Mexicano del Seguro Social	Phase III in patients with HPV-induced AGIN (1176 female patients and 180 male patients)	90% lesion clearance in female treated patient 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.	(Rosales et al., 2014)
Peptide/Protein Based							
HPV16 -SLP	HPV-16 E6/E7	Combination of nine HPV-16 E6 and four HPV-16 E7 synthetic peptides with incomplete Freund's adjuvant	ISA Pharmaceuticals	Phase II in patients with HPV16+ VIN3 (20 patients)	15 patients had objective clinical response at 12 months. 9 complete responses and 6 partial response.	Local swelling, redness, increased skin temperature, pain at vaccination site, fever, flu like symptoms, chills, and tiredness.	(Center et al., 2009)

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
					85% with circulating HPV-16 specific T cells, 83% had CMI against HPV-16.		
				Phase II study in patients with HPV16+ HSIL (9 patients)	All vaccinated patients showed strong HPV-specific T cell response after vaccination. Change in patterns of immune infiltrate.	Itching, redness, swelling, and pain at injection site, headache, diarrhea, fatigue/dizziness, nausea, chills, malign, rash, fever, urticarial, edema.	(de Vos van Steenwijk et al., 2012)
				Phase II in patients HPV16+ advanced or recurrent gynecologic al carcinoma (20 patients)	9 patients with HPV-16 specific immune response. Duration of survival correlates with magnitude of T cell response.	Injection site reaction, fever, chills, fatigue, nausea, flue-like symptom	(van Poelgeest et al., 2013)
				Phase II in patients with low-grade abnormalities of the cervix (50 patients)	97% of vaccinated patients generated HPV 16-specific CMI	Flu-like symptom, injection site reaction.	(de Vos van Steenwijk et al., 2014)
				Phase I observation al study in patients with advanced, recurrent or metastatic cervical cancer (18 patients)	11 of 12 vaccinated patients developed proliferative T cell response.	Localized, injection site reaction.	(Welters et al., 2016)
Pepcan + Candin	HPV-16 E6	HPV16 E6 peptides combined with Candida skin testing reagent candin.	University of Arkansas	Phase I study in patients with biopsy-confirmed HSIL (31 patients)	45% patients experienced histological disease regression.	Mild to moderate injection site reaction.	(Coleman et al., 2016)
TA-CIN	HPV-16 E6/E7/L2	HPV16 E6E7L2 fusion protein	Xenova Research Limited	Phase I in healthy patients (40 subjects)	TA-CIN specific IgG in 24 of 32 vaccinated patients. 25 of 32 vaccinated	Injection site reaction, tenderness. Headache and fatigue	(de Jong et al., 2002)

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
				Phase II with VIN2/3 patients (19 patients)	63% lesion response 1 year after vaccination. Significant CMI increase. Significant CMI observed in lesion responders.	Local reaction associated with imiquimod.	(Daayana et al., 2010)
GTL001 (ProCervix)	HPV 16 and 18 E7	2 recombinant adenylate cyclase (CyaA) proteins: CyaA-HPV 16E7 & CyaA-HPV 18E7	Gentical	Phase I with HPV 16 or 18 infected patients with normal cytology (47 patients)	19 vaccinated patients generated cellular immunity against target antigen. No HPV-specific humoral immunity observed.	Mild and moderate transient local reaction	(Van Damme et al., 2016)
Nucleotide Based							
pNGVL 4a-CRT/E 7(detox)	HPV-16 E7	Plasmid encoding mutated form of HPV 16-E7 linked to CRT	Sidney Kimmel Comprehensive Cancer Center	Phase I with HPV 16+ CIN2/3 Patients (32 patients)	30% vaccinated patients experienced histological regression to CIN1 or less. Increase in intraepithelial C8+ T cells infiltrate after vaccination.	Injection site reaction.	(Alvarez et al., 2016)
GX-188E	HPV-16/18 E6/E7	Plasmid encoding fusion protein of HPV 16/18 E6/E7 linked to Flt3L and tpa	Genexine, Inc	Phase I in patients with HPV 16/18+ CIN3 (9 patients)	All patients displayed enhanced HPV-specific CMI. 7 patients demonstrated complete lesion regression by the end of the trial.	Chills, injection site pain, swelling, hypoesthesia, headache, fatigue, rhinitis	(Kim et al., 2014)
VGX-3100	HPV-16/18 E6/E7	Mixture of two plasmids encoding optimized consensus of E6 and E7 antigen of HPV 16 and 18	Inovio Pharmaceuticals	Phase I with HPV 16/18 + CIN2/3 Patients (18 patients)	HPV-specific CMI observed in 78% patients and HPV-specific humoral response observed in all patients.	Injection site reaction, pain, fever, tenderness.	(Bagarazzi et al., 2012)

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
				Phase IIb with HPV16/18 + CIN2/3 Patients (167 patients)	49.5% vaccinated patient demonstrated regression compared to 30.6% in placebo group. Vaccinations enhance T cell and humoral response.	Injection site reaction, fatigue, headache, myalgia, nausea, arthralgia, erythema	(Trimble et al., 2015)
Whole Cell Based							
DC + KLH	HPV-16 and HPV-18 E7	Dendritic Cells pulsed with HPV-16 and HPV-18 E7 and keyhole limpet hemocyanin	National Institutes of Health	Phase I in patients with stage Ib or IIa cervical cancer (10 patients)	Increase in HPV-specific humoral and CD4+ T cell responses observed, but not CD8+ T cell responses.	Local site reaction, erythema, swelling, pruritus	(Santin et al., 2008)
DC	HPV antigens	DC pulsed with HPV+ tumor lysate	Department of Biotechnology (DBT, Govt. of India)	Phase I in patients with HPV+ advanced, recurrent cervical cancer (14 patients)	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.	(Ramanathan et al., 2014)

CIN - Cervical intraepithelial neoplasia; AGIN - Ano Genital Intraepithelial Neoplasia; HSIL - High-grade squamous intraepithelial lesion; VIN - vulvar intraepithelial neoplasia

Table 2**Recent T cell-based HPV Vaccine Clinical Trials**

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Outcome	Side Effects	Reference
TILs	HPV-16 E6/E7	Tumor-infiltrating cells selected for HPV E6, E7 reactivity	National Institutes of Health, National Cancer Institute (NCI)	Phase II in patients with metastatic cervical cancer who had previously received platinum-based chemotherapy or chemo radiotherapy (9 patients)	3 or 9 patients experienced objective tumor responses (2 complete and 1 partial response), 2 complete responses were ongoing 22 and 15 months after treatment, 1 partial response was 3 months in duration.	No adverse events related to cell infusion were observed	(Stevanovic et al., 2015)
Engineered TCR	HPV-16 E6	E6 TCR	National Institutes of Health, National Cancer Institute (NCI)	Phase I/II in patients with metastatic or refractory/recurrent HPV-16+ cancers (cervical, vulva, vaginal, penile, anal, and oropharyngeal) (12 patients)	Not yet published	Not yet published	NCT02280811

HLA – Human Leukocyte Antigen; TILs – Tumor Infiltrating Lymphocytes; TCR – T cell Receptor

Table 3

Currently Ongoing Therapeutic and T cell-based HPV Vaccine Clinical Trials

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Estimated Date of Trial Completion	Clinical Trials.gov Identifier
Persistent HPV Infection and Low-Grade Squamous Intraepithelial Lesion						
PDS0101	HPV-16 E6/E7	R-enantiomer of 1,2-dioleoyl-3-trimethylammonium-propane chloride + Peptides HPV-16 E6 and E7	PDS Biotechnology Corp.	Phase I in female patients with high risk HPV infection or CIN1 (18 estimated patients)	Information not provided	NCT02065973
Cervical Intraepithelial Neoplasia (CIN)/High-Grade Squamous Intraepithelial Lesion						
GX-188E	HPV-16/18 E6/E7	Plasmid encoding fusion protein of HPV 16/18 E6/E7 linked to Flt3L and tpa	Genexine, Inc	Phase II in HPV 16/18+ CIN2, CIN2/3, and CIN3 patients in Eastern Europe (120 estimated patients)	December 2017	NCT02596243
				Phase II in HPV 16/18+ CIN3 patients in South Korea (72 estimated patients)	June 2016	NCT02139267
pNGVL4a-CRT/E7(detox)	HPV-16 E7	Plasmid encoding mutated form of HPV16-E7 linked to calreticulin	Sidney Kimmel Comprehensive Cancer Center	Phase I in patients with HPV16+ CIN2/3 (39 estimated patients)	March 2017	NCT00988559
pNGVL4a-sig/E7(detox)/HSP70 + TA-HPV	HPV-16/18 E6/E7	Plasmid encoding mutated form of HPV16-E7 linked to sig and HSP70 and vaccinia virus with HPV16/18 E6/E7		Phase I in patients with HPV16+ CIN3 in combination with topical imiquimod (48 estimated patients)	December 2016	NCT00788164
TVGV-1 + GPI-0100	HPV-16 E7	Fusion protein of HPV-16 E7 and ER targeting sequence	THEVAX Genetics Vaccine Co.	Phase IIa in patients with HPV induced cervical HSIL (51 estimated patients)	June 2017	NCT02576561

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Estimated Date of Trial Completion	Clinical Trials.gov Identifier
Pepcan + Candin	HPV-16 E6	HPV16 E6 peptides combined with Candida skin testing reagent candin.	University of Arkansas	Phase II in patients with cervical HSIL (125 estimated patients)	August 2020	NCT020481414
Anal Intraepithelial Neoplasia (AIN)						
ISA101 (SLP-HPV-01; HPV16-SLP)	HPV-16 E6/E7	Combination of nine HPV-16 E6 and four HPV-16 E7 synthetic peptides with incomplete Freund's adjuvant	ISA Pharmaceuticals	Phase I/II in HIV+ male patients with HPV-16+ AIN2/3 (45 estimated patients)	February 2018	NCT01923116
HPV-Associated Incurable Solid Tumors						
ISA101 (SLP-HPV-01; HPV16-SLP)	HPV-16 E6/E7	Combination of nine HPV-16 E6 and four HPV-16 E7 synthetic peptides with incomplete Freund's adjuvant	ISA Pharmaceuticals	Phase II in patients with HPV-16+ Incurable solid tumors (oropharyngeal squamous cell carcinoma, cervical, vulvar, vaginal, anal, and penile cancer) as combination therapy with Nivolumab (28 estimated patients)	December 2018	NCT02426892
HPV-Associated Metastatic or Refractory/Recurrent Cancer						
E7 TCR cells	HPV-16 E7	E7 TCR cells with or without PD-1 Blockade (pembrolizumab)	National Institutes of Health, National Cancer Institute (NCI)	Phase I in patients with metastatic or refractory/recurrent HPV-+16 cancers. Patients must be HLA-A*02:01-positive (40 estimated patients)	January 2026	NCT02858310
Head and Neck Cancer						
ADXSI1-001 (Lm-LLo-E7)	HPV-16-E7	prfA-defective <i>Listeria</i>	Advaxis, Inc.	Phase II in patients with	March 2017	NCT02002182

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Estimated Date of Trial Completion	Clinical Trials.gov Identifier
		monocytogenes strain transformed with plasmid encoding HPV-16 E7 antigen fused to a fragment of nonhemolytic listeriolysin O (LLO)		HPV+ Oropharyngeal Squamous Cell Carcinoma before robot-assisted resection (30 estimated patients)		
INO-3112 (VGX-3100 + INO-9012)	HPV-16/18 E6/E7	Mixture of three plasmids encoding optimized consensus of E6 and E7 antigen of HPV 16 and 18 and proprietary and immune activator expressing IL-12	Inovio Pharmaceuticals	Phase I/IIA in patients with HPV associated head and neck squamous cell carcinoma (25 estimated patients)	December 2017	NCT02163057
Cervical Cancer						
ADX511-001 (Lm-LLO-E7)	HPV-16-E7	prfA-defective <i>Listeria</i> monocytogenes strain transformed with plasmid encoding HPV-16 E7 antigen fused to a fragment of nonhemolytic listeriolysin O (LLO)	Advaxis, Inc.	Phase II in patients with persistent or recurrent squamous or non-squamous cell carcinoma of the cervix (67 estimated patients)	October 2018	NCT01266460
INO-3112 (VGX-3100 + INO-9012)	HPV-16/18 E6/E7	Mixture of three plasmids encoding optimized consensus of E6 and E7 antigen of HPV 16 and 18 and proprietary and immune activator expressing IL-12	Inovio Pharmaceuticals	Phase I/IIA in female patients with new, recurrent, or persistent cervical cancer (30 estimated patients) Phase II in patients with locally advanced cervical cancer as combination therapy with chemoradiation (126 estimated patients)	April 2019 May 2021	NCT02172911 NCT02501278

Vaccine	Antigen(s)	Construct	Organization	Trial Design	Estimated Date of Trial Completion	Clinical Trials.gov Identifier
ISA101 (SLP-HPV-01; HPV16-SLP)	HPV-16 E6/E7	Combination of nine HPV-16 E6 and four HPV-16 E7 synthetic peptides with incomplete Freund's adjuvant	ISA Pharmaceuticals	Phase I/II in female patients with HPV-16+ advanced or recurrent cervical cancer (48 estimated patients)	December 2016	NCT02128126
TA-CIN + GPI-0100	HPV-16 E6/E7/L2	HPV16 E6E7L2 fusion protein + GPI-0100 adjuvant	Sidney Kimmel Comprehensive Cancer Center	Phase I in patients with HPV16 associated cervical cancer (30 estimated patients)	May 2020	NCT02405221

AGIN - Ano Genital Intraepithelial Neoplasia; AIN - Anal intraepithelial Neoplasia; ASCUC - atypical squamous cells of undetermined significance; CIN - Cervical intraepithelial neoplasia; ER - Endoplasmic reticulum; HIV - Human immunodeficiency virus; HPV - Human papillomavirus; HSIL - High-grade squamous intraepithelial lesion; LSIL - Low-grade squamous intraepithelial lesion; TILs - Tumor Infiltrating Lymphocytes; TCR - T cell receptor; CAR - Chimeric Antigen Receptor; HPV - Human papillomavirus; PD-1 - Programmed cell death protein 1