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Emerging Therapies in Persistent Human Papillomavirus Infections

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Abstract

Pharmaceutical companies have developed effective vaccines to prevent certain HPV infections and their associated diseases. However, these vaccine products are not for clearing the persistent HPV infections or curing pre-existing conditions. The unmet needs to treat the diseases, especially cancers, caused by persistent HPV infections remain urgent. Thus, the pharmaceutical industry and multiple medical institutions have invested into developing medications of different modalities, such as viruses, stem cells, T-cells, gene therapies, DNAs, antibodies, antigens, and more. These emerging therapies are the focus of this review.

Keywords: Human Papillomavirus; Emerging therapy; Vaccine; Pharmaceuticals

Abbreviations

CD: Cluster Of Differentiation Cell; CIN: Cervical Intraepithelial Neoplasia; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; CyaA: Adenylate Cyclase; EV: Epidermodysplasia Verruciformis; HPV: Human Papillomavirus; HSP: Heat Shock Protein; IFNα: Interferon Alpha; LCR: Long Control Regions; MOA: Mechanism of Action; MVA: Modified Vaccine Ankara; Nd:YAG: Neodymium-doped Yttriumaluminum Garnet; ORF: Open Reading Frames; PBSCT: Peripheral Blood Stem Cell Transplantation; PD-1: Programmed Cell Death-1; PD-L1: Programmed Cell Death-1 Ligand 1; PCR: Polymerase Chain Reaction; SCC: Squamous Cell Cancer; TALEN: Transcription Activator-Like Effector Nucleases; TCR: T-Cell Receptor; VIN: Vulvar Intraepithelial Neoplasia; VLP: Virus Like Particle; ZFN: Zinc Finger Nuclease

Introduction

Human Papillomavirus (HPV) infections are very common world-wide. It is estimated that there are about 3 million cases per year (and 79 million in totals, mostly in their late teens and early 20s) in the United States of America alone. According to the Centers for Disease Control and Prevention's data, many of these cases are undetected and under reported. The viruses can be transmitted sexually and non-sexually. The attention from the medical research and community has largely focused on the sexually transmitted viruses and resulting diseases due to the significant impacts on the society, specifically the sizable medical cost estimated to be $1.7 billion per year [1]. It is known that persistent HPV infections can lead to cancers, such as cervical, vaginal, vulvar, anal, penile, and oropharyngeal cancers [2,3]. Historically, approximately 20,000 cases of HPV-induced cancer, mostly cervical, were detected annually in the United States [2]. In the past decade important progress has been made on preventing a few 'High-Risk' HPV infections (those that have increased likelihood to induce unchecked cellular proliferation that leads to cancer) such as introduction of quadrivalent (Type 6, 11, 16, and 18), bivalent (Type 16 and 18), and 9-valent (Type 6, 11, 16, 18, 31, 33, 45, 52, and 58) vaccines, respectively in 2006, 2009, and 2014. While these prophylactic vaccines are effective in preventing healthy people from contracting certain HPV infections, they are not approved for treating HPV infections or curing the cancers caused by HPV infections. Therefore, there is much need for a treatment of HPV-induced cancers with anti-HPV medicines. The focus of this publication is to review emerging therapies in clinical trials.

HPV Overview

HPV classification

To prevent HPV infections and cure the diseases caused by the them, one ought to understand HPV first. Human Papillomaviruses are nonenveloped small viruses of circular double strand DNA with ~ 8,000 nucleotide base pairs [4]. The classification of HPVs is based on the nucleotide sequences encoding the Late Proteins L1, or Early Proteins E1 and E2. There are more than 200 HPV genotypes that have been reported and over 160 of them sequenced [5-8]. HPV204, a *Mupapillomavirus*, is the latest and only HPV strain reported in the last decade, newly identified in 2017 [9].

HPV types are divided into five genera based on their DNA sequences: *alpha*-, *beta*-, *gamma*-, *nu*-, and *mu*-papillomaviruses. Contained within *beta*-, *gamma*-, *nu*-, and *mu*-genera are all cutaneous viruses [10], whereas *alpha*-papillomaviruses include both cutaneous and mucosal types. The *alpha*- and *beta*-papillomavirus can also be subdivided into high-risk and low risk groups [10,11]. The *gamma*-, *nu*-, and *mu*-papillomaviruses are generally considered to be low risk (causing skin warts as compared to high-risk HPVs which can lead to cancer if unchecked).

HPV molecular structure

Papillomavirus (including HPV) structure can be divided into three major regions: early, late, and long control regions (LCR) [12]. The early region, comprising >50% of the molecule, encodes six common "open reading frames" (ORF) which are translated into individual proteins E1, E2, E4, E5, E6, and E7. The late region, about 40% of the virus genome, encodes L1 and L2 ORFs that are translated into the L1 and L2 proteins. The LCR region does not encode protein [13].

HPV infection life cycle

HPV infection life cycle can be summarized simply: the virus

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initially accesses the host cell and then inserts its viral material into the cell's replicative machinery. This leads to virus genome amplification, maintenance, packaging, and egressing [10].

HPV infection begins when the virus comes in contact with the basal lamina (a layer of extracellular matrix secreted by epithelial cells), interacts with heparin sulphate proteoglycans [14-16], and binds to a secondary receptor on the basal keratinocyte [17]. Other entry pathways have been proposed for different genotypes of HPV [18-23].

Upon entering the host cells, HPV replicates, expressing E1 and E2 proteins which bind to the viral origin of replication and trigger cellular polymerases to replicate the episomal papillomavirus genome [24-26]. It is thought that E1 and E2 proteins are essential for viral replication in the early stage, however the precise roles of E1 and E2 in the infection process needs to be further elucidated. E4 and E5 proteins are thought to help the viral genome amplification indirectly by modifying the cellular environment. For example, E5 protein is believed to be involved in koilocyte formation [27]. Koilocytic change is a pathognomonic histopathologic change of HPV infection that involves nuclear changes including nuclear hyperchromasia and perinuclear haloing. As a transmembrane protein, E5 can form a pore to allow for intracellular trafficking of endocytotic vesicles [28,29]. The exact roles of E6 and E7 proteins are not fully defined, particularly for low risk HPV types. For high-risk types that cause neoplasia, it seems that the E7 protein drives viral proliferation in the upper layers of the epithelium in cooperation with E6 protein [30].

Once the viral genome has been amplified, L1 and L2 protein expression begins. L2 protein is recruited via E2 protein to the region of replication to facilitate the viral genome encapsulation [31,32]. In an oxidative environment, disulfide bonds are formed among L1 proteins to create the pentameric capsomeres which assemble into an icosahedral capsid containing 72 of the pentamers (with a total of 360 L1 proteins). Assembly of this capsule results in very stable infectious virions [33-35]. The precise processes of HPV egression from the host cell is not entirely known. However, E4 protein is thought to contribute to the virus release by binding to keratin filament and disrupting the normal assembly of the confined envelope [36-39].

Cutaneous HPVs

Beta-, *gamma*-, *nu*-, and *mu*-genera are all cutaneous viruses [10] while *alpha*-papillomaviruses include both cutaneous and mucosal types. Cutaneous HPVs can infect skin, induce benign proliferation, and cause warts. Though benign, warts may remain solitary, proliferate, or regress over time depending on the robustness of the host's immune system. Despite their usual benign naturemalignant transformation is reported in patients with Epidermodysplasia Verruciformis (EV) [40-42], which is an extremely rare skin disorder characterized by the growth of scaly tree-bark-like macules and papules mainly on hands and feet, due to HPV infections (particularly HPV Types 5 and 8). Cutaneous lesions can be difficult to manage in patients with severely compromised immunodeficiency systems [43], in organ transplant recipients and in immunosuppressed patients, highlighting the role of a robust immune system in defending against the virus [44]. These patients have not only a higher risk of getting infected with HPV due to their suppressed immune system, but also increased chance of developing Squamous Cell Cancer (SCC) since their depressed immune systems are less effective in responding and clearing the infections. Dozens of studies have been performed on the *beta*-genus for the cause of cutaneous squamous cell cancer based on serology and DNA detection [42] suggesting that elevated *beta*-1, 2, and 3 are responsible for SCC. At the individual HPV level, it is implied that HPV5 (*beta*-1), HPV8 (*beta*-1), HPV15 (*beta*-2), HPV17 (*beta*-2), HPV38 (*beta*-2), HPV49 (*beta*-3) and HPV76 (*beta*-3) are high-risk genotypes as they are associated with skin carcinomas.

Mucosal HPVs

Alpha-papillomaviruses include both cutaneous and mucosal types. The mucosal HPV infection occurs on anogenital or oral mucosa through direct contact. The *alpha*-types can be subdivided into high-risk and low risk groups based on their oncogenic potentials [11]. The HPVs found in anogenital condylomas and warts are low risk, and those detected in cancer cells are of high-risk. Among the *alpha*-types, twelve (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are classified by World Health Organization as being high-risk (Category 1), six (HPV26, 53, 66, 68, 73, and 82) as being "possibly" cancer-causing (Category 2), twelve (HPV6, 11, 40, 42, 43, 44, 54, 55, 61, 72, 81, and 89) as being low risk, and twenty four (HPV2, 3, 7, 10, 27, 28, 29, 30, 32, 34, 57, 62, 67, 69, 71, 74, 77, 83, 84, 85, 86, 87, 90, and 91) as having undetermined risk [45-48].

Warts and cancers caused by HPV infections

HPV infections can cause warts, including common warts usually found on the hands and feet, plantar warts found on the sole of the feet, periungual warts found around fingernails, and flat warts found on the arms and face. It is reported that HPV2, 7, 27, and 57 of the *alpha*-genus, HPV4 and HPV65 of *gamma*-genus, and HPV1 of *mu*-genus were most frequently found in different types of cutaneous warts [49-54]. However, there is no exact correlation between the strain of HPV and the resulting wart.

HPV infection in the genital area can cause venereal warts (genital warts, clinically known ascondylomata acuminate). HPV6 and 11 are responsible for most genital warts [55,56].

HPV infection may result in cancers such as skin cancer [57], head and neck cancer [58], cervical cancer [59], vulva cancer [60,61], vaginal cancer [62,63], and anal cancer [64]. There is international consensus that persistent infections of high-risk HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) can lead to these cutaneous and mucosal cancers.

Prevention of HPV Infections – HPV Vaccine

Some HPV infections are preventable through vaccinations. In the past decades, research and development of HPV vaccines have been largely focused on mucosal types of the *alpha*-genus, specifically high-risk types which are responsible for cervical cancer. A large landmark occurred when the first commercial vaccine, Gardasil, was granted regulatory approval in 2006 for the vaccination of girls and young women. A year later, another vaccine under the trade name of Cervarix was made available on the market in 2007. In 2014, nonavalent Gardasil became available to gradually replace the quadrivalent Gardasil initially introduced to the market. Since then, there have been multiple reviews on the use of the vaccines [2,65-74].

The quadrivalent Gardasil contains Virus Like Particles (VLPs) of the L1 epitope corresponding to HPV6, 11, 16, and 18, whereas nonavalent Gardasil has five additional VLPs for HPV31, 33, 45, 52, and 58, with increased dose of VLPs for HPV6, 16, and 18. Both products have amorphous Aluminum Hydroxyphosphate Sulfate as the adjuvant to induce immunogenicity and Sodium Chloride, L-Histidine, Polysorbate 80, and Sodium Borate as the formulation components for stability. The VLPs are manufactured from Yeast expression system [75].

Cervarix has active components of VLPs for HPV16 and 18 and

3-O-Desacyl-4′-monophosphoryl lipid A adsorbed on Aluminum hydroxide as the adjuvants. It is formulated in Sodium Chloride and Sodium Dihydrogen Phosphate Dihydrate [76], which is thought to directly stimulate antigen presenting cells for pronounced cellular response and humoral immune response, as well as long lasting antibody responses [77]. Since 2006, HPV vaccines have been recommended for girls 11-12 years old in the United States [78,79]. Beginning in 2011, HPV vaccines have also been recommended for boys of the same age group [79-81].

According to the surveillance results published in 2018 by the Centers for Disease Control and Prevention of U.S. Department of Health and Human Services, prevalence of HPV infection and HPV induced cancer decreased significantly from years of 2003-2006 (the pre-vaccine era) to the years of 2011-2014 in specimens collected from girls of 14-24 years old. High-grade Cervical Intraepithelial Neoplasia Grades 2 and 3 (CIN2+) decreased significantly during 2007-2014 among girls aged 15-24, prevalence of anogenital warts decreased significantly during 2009-2014 among females aged 25-29, and anogenital wart prevalence also decreased significantly during 2009-2014 among men aged 20-24. These statistics suggest that the HPV vaccines provide excellent protection against persistent HPV infections.

Screening

HPV infections are preventable through vaccinations, and the associated diseases, before they are formed or in their early stages, can be mitigated by effective screening and treatment. The major tests are highlighted in the following paragraphs.

Pap smear test (cervical cytology)

The Pap smear is a screening test to detect pre-cancerous cells collected from a woman's cervix. It is based on the determination of the color and topology of the cell nuclei and cytoplasm examined under a microscope by a trained cytologist. The cervical cells can be classified as superficial squamous epithelial, intermediate squamous epithelial, columnar epithelial, mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma-in-situ depending on the cell nucleus' properties [82]. The cell size, including cell area, nucleus area, and cytoplasm area, is an important factor to be considered in the test. Additional criteria include cell shape, (nucleus roundness and cytoplasm roundness), cell topology (the distribution of nucleus and its position), and cell color intensity (brightness) [83].

Dysplasia, ranked mild, moderate, or severe, and carcinoma-in-situ, are considered abnormal cervical cells. With mild dysplasia, the nucleus is larger and brighter than that of a normal cell. With moderate dysplasia, the nucleus becomes much larger and darker. With severe dysplasia, the nucleus becomes even larger, darker, and more deformed compared to that of a normal cell, and the cytoplasm becomes smaller and darker than that of normal cells. In carcinoma-in-situ, all the characteristics become even more exacerbated.

If the Pap smear test results show abnormality, subsequent biopsies can be performed to confirm or reject the presence of Cervical Intraepithelial Neoplasia (CIN) by histology. Histology is widely accepted as the gold standard for the determination on clinical significance of the CIN.

The Pap smear test is viewed as effective and is believed to reduce cervical cancer mortality by up to 80% [84].

HPV test

Although the cytology-based Pap smear test is effective, sampling bias could lead to a false negative result due largely to the low sensitivity and varying sampling techniques. For the same reason, women have to have repeated testing performed at regular intervals. In comparison to pap smears, HPV testing is demonstrated to be sensitive and reproducible, with relatively accurate predictions on disease states [85-89].

HPV detection can be DNA or mRNA based. DNA based tests detect the HPV virus genome, and mRNA-based tests detect the expression of the HPV genes. The techniques include Polymerase Chain Reaction (PCR), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), quantitative real-time PCR, and DNA-based in situ hybridization [90-93]. If high-risk HPV genotypes, such as those expressing E6 and E7 proteins, are detected, additional tests are performed to confirm the disease, and appropriate treatment initiated.

Treatment

The prophylactic vaccines on the market are effective in preventing healthy people from being infected by high-risk HPV. However, these vaccines are not approved for clearing established infections or treatment of HPV induced diseases. Developing effective medications to treat HPV associated diseases has been the effort of many medical institutions and pharmaceutical companies. Early efforts to develop therapeutic vaccines have been the subject of several publications [94-99], and this review will focus on the latest studies on various options to treat HPV associated diseases, particularly medicines.

Medicine

Virus based investigational product

Viruses can be modified by removing the genes that cause disease, limiting its ability to replicate, and inserting genes encoding desired proteins. When the modified viruses are given to patients, antibodies are generated which allow for a disease-specific response.

Modified Vaccine Ankara (MVA), which is a highly attenuated vaccinia virus with 10% of its genome lost when produced by more than 500 passages of the virus in chicken embryo fibroblast cells, is considered to be safe and effective as a vaccine platform. It has been tested in numerous animal models including mice, swine, sheep, monkeys, cattle, and horses with no systemic adverse effects. And, more than hundreds of thousands humans have been safely vaccinated with MVA based smallpox vaccine.

An example of an MVA-based recombinant HPV therapeutic is known as TG4001 [100,101]. This product expresses mutation-inactivated HPV 16 E6 and E7 oncoproteins. TG4001 has been tested on patients with cervical intraepithelial neoplasia in randomized placebo-controlled study (Table 1). It is reported in a study on 21 patients with cervical intraepithelial neoplasia 2/3, ten of them responded after 6-month treatments, enabling them to avoid conization (a surgical procedure to remove the lesion). Nine of the patients experienced improvement in their HPV16 infections, and seven had viral RNA clearance along with lesion regression [100].

Axaliogene filolisbac is a live attenuated Listeria monocytogenes strain bioengineered to secrete HPV 16 E7 protein fused with a truncated fragment of listeriolysin O [101,102]. When administered in patients, it is expected to recruit T-cells to attack HPV cancer cells. It also neutralizes regulatory T-cell and myeloid-derived suppressor cells that protect the tumor microenvironment from immunologic attack. The experimental therapeutics is being evaluated for different indications in several clinical studies by a few organizations. The preclinical results

generated on animal models and safety data from early phase I and II clinical studies are summarized in two review articles [103,104]. In one of the Phase I/II studies for anal cancer, it was reported that the product is safe and efficacious: 9 out of 10 patients responded, and 8 of the 9 patients were free of disease progression. However, the studies were terminated for undisclosed reasons. In a separate study [105] systemic listeriosis was reported, causing the clinical study termination early. A few active clinical studies for cervical cancer are still ongoing (Table 1) including a pivotal Phase III clinical study sponsored by the company who discovered the investigational product, yet the results are to be reported.

Cell based investigational product

Stem cell related product

Peripheral Blood Stem Cell Transplantation (PBSCT) is a procedure that infuses healthy blood-forming stem cells from a healthy donor to a patient whose blood-forming stem cells were killed during cancer treatment, for example. To do so, the immature hematopoietic stem cells in the healthy donor's circulation are collected and treated before administered intravenously to the patient. The administered hematopoietic stem cells migrate to the recipient's bone marrow allowing the bone marrow to proliferate healthy blood cells.

A Phase II clinical study was performed to evaluate the efficacy of the peripheral stem cell transplantation, combined with chemoradiotherapy as the conditioning therapy, in treating patients with recurrent metastatic cervical or vaginal cancer caused by HPV infections. The clinical study was completed several years ago whereas the results were not provided at ClinicalTrail.gov. It would be beneficial to have the results published in the future (Table 2).

T-cell related product: In principle, researchers can take a patient's blood, remove white blood cells, and insert genes that proliferate T-Cell Receptors (TCR) targeting the E7 protein found in cancer cells. The genetically engineered T-cells are then given back to the patient to fight against the cancer. It is reported from a Phase I/II study that two of the six patients responded at the maximum tolerated dose; all the patients experienced adverse events (Table 3). There are another five Phase I or combined Phase I/II studies in the recruiting stage. Apparently, there is still much research that needs to be done before any of these investigational therapies becomes a viable treatment option.

B-cell related product: BB-cells express B-Cell Receptors, which can bind antigens from cancer cells, and lock onto them for destruction by T-cells. B-cells sometimes inhibit tumor development or kill tumor cells by producing antibodies that attack cancer cells or oncogenic viruses, such as human papillomavirus.

A planned Phase I/II study using B-cells to treat HPV positive cervical cancer is in the recruiting stage (Table 4). The results are to be reported.

Gene editing based product

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinical trial gov identifier
Virus therapeutics (TG4001) Avelumab	To evaluate the safety and efficacy of the combination therapy of virus vaccine and Avelumab	TG4001 and Avelumab are administered to patients with HPV positive recurrent and metastatic malignancies and in the expansion cohort the squamous cell carcinoma of head and neck A. Ph Ib: dose escalation B. Ph II: determine the recommended Ph II dose RP2D)	Transgene	I/II	Recruiting	To be reported	NCT03260023
Axalimogene Filolisbac (ADXS11-001)	To evaluate the safety and efficacy of therapeutics in treating patients with HPV positive carcinoma of the cervix	The subjects receive 1×1010cfu of the therapeutics in repeating doses	Advaxis	I/II	Active	To be reported	NCT02164461
Axalimogene Filolisbac (ADXS11-001)	To study the efficacy of the therapeutics in treating patients with high-risk locally advanced carcinoma of cervix	A. Subjects receive placebo B. Subjects receive the experimental therapeutics	Advaxis	III	Recruiting	To be reported	NCT02853604
Axalimogene Filolisbac (ADXS11-001)	To evaluate the safety and efficacy of the therapeutics in treating patients with persistent or reocurrent cervical cancer	Patients receive the experimental therapeutics	Gynecologic Oncology Group	II	Active	12 month Overall Survival rate: 38.5% Median Survival Rate: 7.7 months	NCT01266460
Axalimogene Filolisbac (ADXS11-001)	To evaluate the safety and efficacy of the therapeutics in treating patients with anal cancer	Patients receive 1×1010cfu of the therapeutics intravenously once every 28 days for a total of 4 doses. Patients also receive 5-Florouracil, Mitomycin, and Intensity Modulated Radiation Therapy (IMRT)	Brown University	I/II	Terminated	Safety: 10 out of 10 patients Response rate: 9 out of 10 patients Progression-free and overall survival: 8 out 9	NCT01671488
Axalimogene Filolisbac (ADXS11-001)	To evaluate the efficacy of the therapeutics in treating patients with HPV positive oropharyngeal cancer	Patients receive intravenously two doses of the therapeutics at the dose of 1×109 cfu	Andrew Sikora	II	Recruiting	To be reported	NCT02002182

Table 1: Clinical studies on virus based investigational medicine.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinical trial gov identifier
Peripheral stem cell	To study efficacy of peripheral stem cell transplant, when combined with chemoradiotherapy, in treating patients with cancers caused by HPV infections	A. Patients receive conditioning chemoradiotherapy on days -4 and -2 B. Patients receive peripheral stem cell infused on day 0 C.Patients receive Cyclosporine and Mycophenolate (to prevent immune response of the transplanted stem cell against normal cells in the patients) D. In case of disease progression after the above treatments, T cells (nonmobilized donor lymphocyte) infusion will be administered	National Cancer Institute	II	Competed	Not provided	NCT00005941

Table 2: Clinical study on stem cell based investigational therapy.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinical trial gov identifier
T cells with receptor targeting to HPV (not specified)	To evaluate the safety and efficacy of T cell, with receptor targeting HPV, in treating HPV infections	Subjects receive T cells with receptor targeting to HPV	Shenzhen Geno-Immune Medical Institute	I/II	Recruiting	To be reported	NCT03351855
T cells with receptor targeting to HPV E6	To evaluate the safety and efficacy of T cell, with receptor targeting HPVE6, in treating patients with HPV 16 positive head and neck cancer and cervical cancer	Subjects in different groups will receive different doses of the T cells	Xinqiao hospital of Chongqing	I	Not yet recruiting	To be reported	NCT03578406
T Cells with receptor targeting to HPV 16 E6 Fludarabine Cyclophosphamide Aldesleukin (IL-2)	To determine the dose regimen and efficacy of E6 T cell in treating cancers caused by HPV infections	A.Conditioning chemotherapy with Fludarabine and Cyclophosphamide prior to multiple ascending dose with T cell. Subjects will receive Aldesleukin (IL-2) to help the T cell last longer B.Subjects receive fixed dose of T cells determined in the above study	National Cancer Institute	I/II	Completed	1. Maximum tolerated dose (MTD) studied: 2x10^11 cells 2. Tumor complete response: 0. Partial response: 2/6 at the MTD 3. Response duration: 1.5 months at MTD 4. Adverse event: high (100%)	NCT02280811
T Cells with receptor targeting to HPV E6 Aldesleukin (IL-2)	To determine the safe dose of HPV E6 specific T cells in treating patients with vulvar high-grade squamous intraepithelial	Subjects will receive HPV E6 specific T cells followed by a maximum of two doses of Aldesleukin (to help keep the T cell last longer)	National Cancer Institute	I	Recruiting	To be reported	NCT03197025
T Cells with receptor targeting to HPV 16 E7 Aldesleukin Fludarabine Cyclophosphamide	To determine the dose regimen and efficacy of E7 T cell in treating cancers caused by HPV infections	A. Multiple ascending dose with T cell B. Subjects receive fixed dose determined in the above study	National Cancer Institute	I/II	Recruiting	To be reported	NCT02858310
HPV-16 (or 18) E6 and E7 specific T lymphocyte (T cells)	To determine the maximum dose and evaluate the toxicity and efficacy of HPV-16 (or 18) E6 and E7 specific T lymphocyte in treating patients with HPV associated cancers	A. Multiple ascending doses with HPV specific T cells given by IV B. Multiple ascending doses with HPV specific T cells given by IV, plus Cytoxan and Fludarabine (for lymphodepletion) and Nivolumab (or Opdiva, for helping the T cells control the tumer)	Baylor College of Medicine	I	Recruiting	To be reported	NCT02379520

Table 3: Clinical studies on T-cell based therapy.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinical trial gov identifier
B cells	To evaluate the safety and efficacy of B cell and Monocyte based vaccine (known as BVAC-C) in treating patients with HPV 16 and 18 positive cervical cancer	Patients will receive injections of BVAC-C	Cellid Co.	I/II	Recruiting	To be reported	NCT02866006

Table 4: Clinical study on B-cell based therapy.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a family of DNA sequences found in bacteria and archaea that contain DNA segments from viruses that have attacked the prokaryote, which in turn can use these DNA segments to detect and destroy the DNA from similar viruses during subsequent attacks. It is known that small clusters of cas (CRISPR-associated) genes are located next to CRISPR sequences. The CRISPR/Cas combination plays an important role in prokaryotic defense against viruses. A simple version of the CRISPR/Cas, CRISPR/Cas9, has been developed to edit genomes [106,107]. By delivering the Cas9 with a synthetic RNA into a cell, the cell's genome can be edited at a specific position, allowing existing genes to be removed and/or new ones added. CRISPR/Cas9 genome editing techniques have many potential applications, including pharmaceutical development and disease treatment.

Despite the potentials of CRISPR/Cas9 in pharmaceutical development and disease treatment, its applications and clinical studies have been limited due to pattern disputes, ethical concerns, and the variability of regulations from country to country.

Transcription Activator-Like Effector Nucleases (TALEN) are restriction enzymes that can be used to cut a DNA at specific position. The restriction enzymes can be introduced into cells, along with CRISPR/Cas9, for gene editing.

One of the planned clinical studies for the treatment of cervical intraepithelial neoplasia caused by HPV infection is based on the TALEN and CRISPR/Cas9 technologies. The goal is to disrupt HPV 16 and 18 E6/E7 DNA, reduce the expression of E6/E7, and therefore induce cancer cell apoptosis (Table 5). No results have been reported from the study yet.

Gene editing can also be achieved by using Zinc Finger Nuclease (ZFN). ZFNs are endonucleases comprised of a DNA-binding domain which recognizes unique DNA sequences and a DNA-cleaving domain which cleaves the DNA, allowing insertion or deletion of DNA fragment at the cleavage site. Upon binding to DNA, two molecules of ZFN, one on each of the opposite sides of the DNA strand, can cleave it. Subsequently, a cell can activate repair mechanisms to fix the damage. At the non-homologous end of the DNA, insertion or deletion can be introduced, resulting in modification of the gene.

ZFNs known as ZFN-603 and ZFN-758 were under the development as investigational therapeutics to treat CIN by targeting HPV 16 and HPV 18 oncogene E7. Upon transfection of the ZFNs into HPV 16 and HPV 18 positive tumor cells, the ZFNs bind to and cleave the HPV 16 HPV 18 E7 oncogene, resulting in an inhibition of tumor cell replication and induction of apoptosis. It is also expected that inhibition of E7 expression induces the expression of tumor suppressor genes, preventing tumor cell proliferation.

The ZFN-603 and ZFN-758 are being evaluated in a Phase I clinical study to determine whether they are safe and effective in the treatment of HPV16 and HPV18 positive cervical intraepithelial neoplasia (Table 5).

DNA/RNA based investigational products

DNA that encodes HPV E6 and E7 proteins, when administered in humans and taken up in cells, can guide the synthesis of oncogenic proteins in an effort to trigger an immune response. pNGVL4a-Sig/E7(detox)/HSP70 DNA, for example, is a DNA product which encodes the fusion protein of signal peptide pNGVL4a-Sig (a detox form of HPV-16 antigen E7) and the heat shock protein 70 (HSP70). This investigational product is currently being studied by Johns Hopkins University in a Phase I clinical trial. It is expected that the resulting protein, when taken up by dendritic cells, can activate a T-cell specific immune response and stimulate the production of the Cluster of Differentiation cells (CD4 and CD8). CD8+ T-cells are cytotoxic cells that can identify and kill cancerous cells that express HPV E6 and/or E7 proteins. The detox version of the protein refers to mutations at positions 24 (Cysteine to Glycine) and 26 (Glutamic Acid to Glycine).

A different version of a DNA product, known as pNGVL4a-CRT/E7 (detox), was also studied by Johns Hopkins University in a Phase I clinical trial for CIN. The DNA encodes HPV-16 E7 and Calreticulin. Calreticulin is a protein located in the lumen of cell's endoplasmic reticulum and can promote the presentation of HPV-16 E7 to T-cells. Additionally, the DNA has two immunostimulatory sequences in the noncoding region which induce IFN and IL-12 production in transfected keratinocytes and dermal antigen presenting cells, eliciting T-cell responses. It is expected that this DNA product can generate a potent cytotoxic T-lymphocyte response against E7 expressing tumor cells, causing tumor cell death. The Phase I results demonstrated resolution of the clinical lesion for some of the

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinical trial gov identifier
CRISPR/Cas9 TALEN	To evaluate the safety and efficacy	A. Subjects are administered with TALEN (TALEN-HPV 16 E6/E7 or TALEN-HPV 18 E6/E7) plasmid in gel B. Subjects are administered with CRISPR/Cas9 (CRISPR/Cas9-HPV 16 E6/E7 or CRISPR/Cas9-HPV 18 E6/E7) plasmid in gel C. Subjects receive no treatment	First affiliated hospital, Sun Yat-Sen University (China)	I	Not yet recruiting	To be reported	NCT03057912
Zinc Finger Nucleases (ZFN-603 and ZFN-758)	To evaluate the efficacy of the Zinc Finger nucleases	Subjects will receive ZFN-603 and ZFN-758	Huazhong University of Science and Technology	I	Active	To be reported	NCT02800369

Table 5: Clinical studies on gene-editing based investigational product.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
DNA vaccine (pNGVL4a-Sig/ E7(detox)/HSP70 and TA-HPV)	To evaluate the side effects and best dose in treating patients with cervical intraepithelial neoplasia 3	A. Patients receive the DNA vaccines B. Patients receive Imiquimod C. Patients receive the DNA vaccine and Imiquimod	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University	I	Recruiting	To be reported	NCT00788164
DNA vaccine (pNGVL4a-CRT/ E7(detox))	To evaluate the safety and efficacy of the DNA vaccine administered in different routes in treating patients with HPV positive cervical intraepithelial neoplasia 2/3	Subjects will receive the vaccine via A. gene gun B. Intramuscular injection C.Intramucosally D.Intramucosally plus Imiquimod applied	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University	I	Competed	No related serious adverse event Absence of lesion after treatment: 2 of 9, 3 of 9, 3 of 9, and 1 of 6	NCT00988559
DNA therapeutic vaccine (GX-188E)	To evaluate the toxicity and efficacy of DNA vaccine known as GX-188E in treating patients with HPV positive cervical intraepithelial neoplasia 3	A. Patients will receive GX-188E plus intravaginal application known as GX-I7 B. Patients will receive GX-188E plus Imiquimod	Seoul St. Mary's Hospital	N/A	Recruiting	To be reported	NCT03206138
DNA therapeutic vaccine (GX-188E)	To evaluate immune response in subjects who received the DNA vaccine	Subjects will receive GX-188E followed by physical exam, vital signs, ECG, and clinical laboratory tests (by measuring HPV 16/18 E6 and E7 specific T cell response)	Genexine	II	Active	To be reported	NCT02411019
DNA therapeutic vaccine (GX-188E)	To evaluate the safety and efficacy of GX-188E in treating patients with cervical intraepithelial neoplasia 2/3	A. Subjects are administered with GX-188E B. Subjects will receive placebo	Status	Status	Status	To be reported	NCT02596243
DNA therapeutic vaccine (GX-188E)	To determine the optimal dose of GX-188E in treating patients with cervical intraepithelial neoplasia 3	A. Subjects are administered with GX-188E at 1mg level B. Subjects are administered with GX-188E at 4 mg level	Genexine	II	Competed	To be reported	NCT02139267
DNA vaccine (known as VGX-3100)	To evaluate the safety, tolerability, and immunogenicity of the DNA vaccine in treating patients with cervical intraepithelial neoplasia 2/3	A. Subjects administered with VGX-3100 at 0.6mg/dose B. Subjects administered with VGX-3100 at 2 mg/dose C. Subjects administered with VGX-3100 at 6 mg/dose	Inovio Pharmaceuticals	I	Competed	To be reported	NCT00685412
DNA vaccine (known as VGX-3100)	To evaluate the safety and efficacy of the DNA vaccine in treating cervical intraepithelial neoplasia 2/3 caused by HPV infections	A. Intramuscular injection of the DNA vaccine followed by electroporation B. Intramuscular injection of placebo followed by electroporation	Inovio Pharmaceuticals	II	Competed	Results: (Lancet 2015; 386: 2078-88) Histopathological regression: VGX-3100 recipients: 49.5% Placebo recipients: 30.6%	NCT01304524
DNA vaccine (known as VGX-3100)	To evaluate the safety and efficacy of the DNA vaccine in treating vulvar high grade squamous intraepithelial lesion caused by HPV infections	A. Subjects receive the DNA vaccine administered intramuscularly followed by electroporation B. Subjects receive the DNA vaccine administered intramuscularly followed by electroporation plus the application of 5% Imiquimod	Inovio Pharmaceuticals	II	Recruiting	To be reported	NCT03180684
DNA vaccine (known as VGX-3100)	To evaluate the efficacy of the DNA vaccine in treating anal high grade squamous intraepithelial lesion caused by HPV infections	Subjects receive the DNA vaccine administered intramuscularly followed by electroporation	Inovio Pharmaceuticals	II	Recruiting	To be reported	NCT03499795
DNA vaccine (known as VGX-3100)	To determine the efficacy, safety, and tolerability of the DNA vaccine in treating cervical high grade squamous intraepithelial lesion (2/3) caused by HPV infections To establish safe, tolerable, and recommended dose of the RNA vaccine	A. Intramuscular injection of the DNA vaccine followed by electroporation B. Intramuscular injection of placebo followed by electroporation	Inovio Pharmaceuticals	III	Recruiting	To be reported	NCT03185013

Anti-CD40 RNA vaccine	To establish safe, tolerable, and recommended dose of the RNA vaccine	A. Multiple ascending doses of the RNA vaccine in patients with previously treated HPV positive head and neck cancer B. Multiple ascending doses of the RNA vaccine in patients with advanced HPV positive cancers (head and neck, anogenital, penile, or cervical)	University of Southampton	I/II	Recruiting	To be reported	NCT03418480
DNA vaccine (known as MEDI457)	To evaluate the safety, tolerability, activity, and immunogenicity of the DNA vaccine	Patients with HPV positive head and neck squamous cancer will receive the DNA vaccine and Durvalumab (to block the interaction of PD-L1 with PD-1 and CD80)	MedImmune	I/II	Recruiting	To be reported	NCT03162224
DNA therapeutic vaccine (VB10.16)	To evaluate the toxicity and efficacy of DNA vaccine known as VB10.16 in treating patients with HPV positive cervical intraepithelial neoplasia 2/3	Patients in two cohorts will receive same dose in different schedules	Vaccibody	I/II	Active	To be reported	NCT02529930

Table 6: Clinical studies on DNA/RNA based investigational product.

subjects after treatment, with no serious adverse event reported (Table 6).

GX-188E is another DNA based product being studied in clinical trials. Although the molecular structure is not disclosed, it is indicated that the molecule elicits a T-cell response to HPV antigens. The company (Genexine), which developed the product and sponsored the clinical studies, reported on its website that "cervical lesions were completely eradicated in seven responders as determined by cytological, histological, and virological evaluations" after 36 weeks of treatments, and no lesion recurrence was reported a year after the treatments. The Phase II results are to be reported.

VGX-3100 is a DNA product developed by Inovio Pharmaceuticals to treat cancers caused by persistent HPV infections. The dose is delivered by intramuscular injection combined with electroporation. The company reported that the product is well tolerated in Phase I study. Phase II study results indicated that the subjects who received the treatment had 49.5% of lesion regression while those that received the placebo had 30.6% of lesion regression [108].

MEDI457 is a DNA product originally developed by Inovio Pharmaceuticals and later acquired by MedImmune. It contains two DNA plasmids: one encodes E6 and E7, and another IL-12. It is expected that the expression of IL-12 boosts the immune response from T-cells triggered by E6 and E7. MedImmune sponsored a Phase I/II clinical study on head and neck squamous cell carcinoma, which is in the recruiting stage.

Additionally, anti-CD40 RNA is being studied in a Phase I clinical trial for the indication of head and neck cancer, and a DNA based product (known as VB10.16) is being evaluated in a Phase I/II study for the treatment of HPV positive cervical intraepithelial neoplasia 2/3 (Table 6).

Antibody, antigen, and protein based investigational product

Antibody: Specifically designed antibodies can neutralize HPV E6 and E7 proteins and theoretically treat the diseases caused by HPV infections. However, antibody based investigational medicine has rarely been developed and tested in recent years, possibly due to the mechanism of action where the antibody neutralizes E6 and E7 proteins instead of activating a T-cell anti-tumor response. And, the immunogenicity of the antibody is poor, as noted in several comprehensive reviews [94-99]. There is one registered clinical study of an antibody based investigational medicine which is in the recruiting stage (Table 7).

Another study to be initiated as a Phase I/II clinical trial is focused on the evaluation of stereotactic body radiation therapy in combination with the checkpoint inhibitors Durvalumab and Tremelimumab. Durvalumab blocks the interaction between programmed cell death ligand and PD-1 as well as CD80. Tremelimumab is expected to stimulate an immune system attack on tumors by disinhibition of cytotoxic T lymphocyte activities towards cancer cells. Stereotactic body radiation therapy is a specialized radiation therapy that sends x-rays directly to the tumor using smaller doses over several days and may cause less damage to normal tissue. This clinical study is being performed to evaluate the side effects and how well stereotactic body radiation therapy and Durvalumab with or without Tremelimumab work in treating participants with human papillomavirus positive oropharyngeal squamous cell cancer.

Antigen: Peptides derived directly from HPV, or as fusion proteins with human leukocyte antigen epitopes, can be antigenic, producing an HPV specific immune response. When administered in humans and taken up by antigen presenting dendritic cells, these antigens activate a T-cell specific immune response by stimulating the production of CD4+ and CD8+ T-cells. CD8+ cells possess cytotoxic properties and can identify and kill cancerous cells expressing HPV E6 and/or E7 proteins.

DPX-E7 (Table 8) is a synthetic peptide consisting of amino acids 11 - 19 of the HPV 16 E7 (HPV16-E7 11-19). The peptide is formulated into liposomes, the same material as the human cell membrane, to help the bioavailability. It is expected that DPX-E7 will stimulate the host cytotoxic T-lymphocyte to fight against tumor cells expressing the HPV16 E7 protein.

The Dana-Farber Cancer Institute is registered for the Phase I/II clinical studies (NCT02865135) on DPX-E7 as a potential treatment for HPV-positive head and neck cancer, cervical cancer, and anal cancer. Patients will receive DPX-E7 injection and a low dose of Cytoxan (cyclophosphamide, a chemotherapy drug that can slow the growth of cancer cells), and will be monitored for cancer progression for up to two years. The clinical study will track any adverse events related to the treatment and assess clinical benefits through the overall response and survival rate, as well as the progression-free survival rate over the two-year period. It is expected to be completed in May 2023.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial. Gov identifier
Monoclonal antibody HPV19	To evaluate the safety, pharcokinetics, and anti-tumor activity of the IgG	A. Subjects receive the IgG and FOLFOX (5-Florouracil, Oxaliplatin, Leucovorin) B. Subjects receive the IgG and Paclitaxel/Carboplatin C. Subjects receive the IgG and Gemcitabine/Carboplatin D. Subjects receive the IgG and FOLFIRI (5-Florouracil, Irinotecan, Leucovorin)	SSuZhou Stainwei Biotech Inc.	I	Not yet recruiting	To be reported	NCT03503604
Durvalumab Tremelimumab	To study on the efficacy and toxicity of stereotactic body radiation therapy and treatment with Durvalumab plus or minus Tremelimumab on HPV positive oropharyngeal squamous cancer	A. Stereotactic body radiation for 5 days and IV dose of Durvaluman on days 0 and 27, followed by Transoral Robotic Surgery and neck dissection. Start from week 12, dose with Durvalumab every 4 weeks for up to 4 times B. Same as above except for IV dose of Durvaluman plus Tremelimumab on days 0 and 27	Jonsson Comprehensive Cancer Center	I//II	Not yet recruiting	To be reported	NCT03618134

Table 7: Clinical studies on antibody based investigational product.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial. Gov identifier
HPV 16 E7 Peptide 11-19 (also known as DPX-E7)	To evaluate the safety and efficacy of the peptide in treating patients with incurable HPV 16 related oropharyngeal, cervical, and anal cancers	Priming doses followed by booster doses of the drug product, plus low dose of cyclophosphamide (alkylating drug for multiple malignant diseases)	Dana Farber Cancer Institute	I/II	Recruiting	To be reported	NCT02865135
HPV E7 peptide (sequence not disclosed)	To evaluate the effectiveness of the experimental therapeutics when combined with surgery	Patients receive two doses of the therapeutics before and after surgery	European Organization for Research and Treatment of Cancer	II	Completed	No results posted	NCT0002916
Dendritic cell-HPV 16 E7 peptide (sequence not disclosed)	To study the safety and efficacy of the peptide in treating patients with recurrent or persistent cervical cancer	A. Patients undergo leukapheresis to obtain blood mononuclear cells for activation to dendritic cells B. Patients receive the HPV 16 E7 peptide vaccine subcutaneously and the dendritic cell-HPV 16 E7 intravenously	Steward St. Elizabeth's Medical Center of Boston, Inc	I	Completed	No results posted	NCT0003977
HPV 16 E6 and E7 peptide (sequence not disclosed)	To evaluate the efficacy of the therapeutics in treating patients with advanced or recurrent cancer of cervix, vagina, penis, anus, esophagus, or head and neck	A. Peripheral blood mononuclear cell (antigen presenting cell) are harvested and treated in vitro with sargramostim and pulsed with HPV 16 E6 or E7 B. Patients receive HPV 16 E6 or E7 pulsed peripheral blood mononuclear cell	National Cancer Institute	II	Completed	No results posted	NCT00019110
HPV E6 and E7 peptides (sequences not disclosed)	To assess the safety and efficacy of the therapeutics in treating anal intraepithelial neoplasia in HIV positive men	Patients receive the investigational therapeutics with or without interferon-α	Academisch Medisch Centrum, Univeriteit van Amsterdam	I/II	Completed	No results posted	NCT01923116
Peptide P16_37-63	To evaluate the safety and efficacy of the investigational therapeutics in treating patients with HPV induced cancers	Patients receive the investigational therapeutics and Montanide ISA-51 VG	Oryx GmbH & Co. KG	I/II	Completed	No results posted	NCT01462838
HPV 16 E6 peptides (sequence not disclosed)	To evaluate the safety of the investigational therapeutics in treating patients with high grade squamous intraepithelial neoplasia	Subjects receive the therapeutics plus Candin	University of Arkansas	I	Completed	No results posted	NCT01653249
HPV 16 E6/E7 Peptides known as ISA101/ISA101b	To evaluate the safety and tolerability of the investigational therapeutics when combined with carboplatin and paclitaxel, with or without Bevacizumab, in treating patients with advanced or recurrent cervical cancer	Patients receive the investigational therapeutics and standard care (carboplatin and paclitaxel with or without Bevacizumab)	ISA Pharmaceuticals	I/II	Active	To be reported	NCT02128126

ProCervix (containing two recombinant Adenylate Cyclase Proteins (CyaA): CyaA HPV 16 E7 and CyaA HPV 18 E7	To evaluate the efficacy of the therapeutics	A. Patients receive ProCervix and 5% imiquimod as the adjuvant B. Patients receive placebo	Genticel	II	Completed	No results posted	NCT01957878
Hsp E7 fusion protein	To study the efficacy of the protein in treating patients with HPV positive cervical intraepithelial neoplasia	A. Patients receive the therapeutics subcutaneously B. Patients receive standard care	National Cancer Institute	II	Completed	No results posted	NCT00054041
Candida antigen	To evaluate the safety of the common yeast (candida) for treatment of warts	Subjects receive intralesional injections of 0.3mL candida antigen	University of Arkansas	I	Completed	Participants 11 Complete responder 9 Partial responder 1 Non-responder 1	NCT00569231
Candida antigen	To evaluate the safety and efficacy of candida for the treatment of warts	Subjects receive intralesional injections of 0.3mL candida antigen	Neilsen BioSciences, Inc	II	Completed	No results posted	NCT01757392

Table 8: Clinical studies on antigen based investigational product.

There are several other completed Phase I/II studies on single peptides of differing sequence (Table 8), results pending. Nevertheless, peptide based experimental medicines have poor immunogenicity and potency in general, according to a review on clinical studies performed several years ago [94-99]. Therefore, newly designed investigational medicines, such as HspE7 (Table 8), incorporated adjuvants into the molecule to boost immunogenicity.

HspE7 is a fusion protein of heat shock protein 65 (HSP65), HPV E7 antigen, and a Toll-like receptor 3 ligand (so called poly-ICLC, acting as the adjuvant) manufactured using recombinant DNA technology. It is an experimental therapeutic for the treatment of precancerous and cancerous lesions caused by HPV infections. The proposed mode of action is that the E7 antigen elicits a T cell response towards the cancer cell when it is delivered with the help from HSP. The use of the Toll-like receptor 3 as the adjuvant is to improve the efficacy of the therapeutic. The results are to be published.

Another direction in exploring the safety and efficacy of peptide based anti-HPV therapeutics is to include more than one peptide in the experimental medicine. For example, ProCervix, being developed by Genticel in France, has two recombinant Adenylate Cyclase proteins (CyaA): one with HPV 16 E7 antigen and the other HPV 18 E7 antigen. CyaA acts as the antigen carrier. The results from a clinical study, presented at the American Association for Cancer Research Annual Meeting 2015 in Philadelphia titled "Bivalent adenylate cyclase (CyaA)-based therapeutic vaccines: eradication of tumor cells expressing different antigens over time," indicate that "ProCervix has the potential to eradicate on-going HPV 16 infections, while also providing protection against possible future HPV 18 infections, and vice-versa. These data also suggest that it should be possible to protect and treat patients with multiple different antigens for a given cancer".

Another example is ISA101 from ISA Pharmaceuticals consisting of 13 synthetic peptides (with various sequences of 25-35 amino acids) derived from E6 and E7 proteins of HPV 16 virus. The company reported on its website that it has completed a Phase II trial on vulvar intra-epithelial neoplasia and established proof-of-concept. It also has an on-going Phase I/II trial on cervical cancer, where subjects are treated with the experimental medicine in combination with conventional chemotherapy. Conventional chemotherapeutics carboplatin and paclitaxel are thought to exert favorable effects on the tumor micro-environment by interfering with suppressive immune cells and stimulating the release of immune activating molecules by tumor cells, hence working in conjunction with ISA101). The clinical study also included pegylated interferon alpha (IFNα) IIb to further improve the immune response. In a cohort of the study, Bevacizumab (with the trade name Avastin) is included in the treatment to evaluate the clinical performance of the combination therapy.

Candin, with Candida antigens as the active ingredients, is manufactured from cell cultures of two strains of Candida albicans yeast (or fungi). It is used for intradermal injection to assess cellular hypersensitivity to Candida Albicans. As Candida Albicans is an opportunistic pathogenic yeast which does not proliferate outside of human body, and it can become pathogenic in an immunocompromised person under many conditions. It is thought that Candin could be useful in treating patients with reduced cellular immune response due to HPV infections. Using Candidal antigens to treat diseases such as cutaneous warts is not yet approved by the Food and Drug Administration. However, Candidal antigen was tested in a Phase I clinical study to treat warts, with 9 complete responders, 1 partial responder, and 1 non-responder out of 11 participants. An independent Phase II clinical trial was registered to further evaluate the safety and efficacy of Candidal antigen for the treatment of warts, with no results reported yet.

Interferon: Interferon alfa has several subtypes. IFN-α8 enhances the proliferation of B-Cells and activates Natural Killer Cells. The subtypes α10 and α2 also activate Natural Killer Cells. Subtype α1 is believed to enhance the expression of Human Leukocyte Antigen-II (HLA-II) and directly inhibit tumor cell growth, while subtype α2 increases the expression of HLA-I which activates CD8+ T cells against tumor cells. Despite the proposed mode of action is reasonable, the Phase II clinical results (Table 9) using IFN-α to treat oral warts showed no significant difference between the treated group and the placebo group.

Drug	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Interferon-α	To test the efficacy of Interferon-α in treating oral wards in HIV positive patients	A. Subjects will receive Interferon-α B. Subjects will receive placebo	Amarillo Biosciences	II	Completed	Chi-squared statistical analysis results p=0.30, indicating no significant difference between treated group and the placebo group	NCT8072 4181

Table 9: Clinical study on Interferon based investigational product.

Small molecules

Imiquimod, Cisplatin, Carboplatin, Aminolaevulinic Acid and others: Imiquimod (1-isobutyl-1H-imidazo [4,5-c]quinolin-4-amine), as an immune response modifier, is prescribed to treat genital warts (FDA approved in 1997). There were two placebo-controlled Phase III studies registered in ClinicalTrials.gov using Imiquimod to treat genital warts (Table 10). The complete clearance of all warts was reported to be 19%-27% for the treated group vs. 10% for the placebo group in one study, and 25%-29% for the treated group vs. 9% for the placebo group in another study, indicating that Imiquimod is effective in treating genital warts; however, the rate of cure is low. There is a Phase II study investigating Imiquimod in treating Vulvar Intraepithelial Neoplasia (VIN) 2/3 and anogenital warts, with no results reported yet. Two other Phase III studies in the recruiting stage were designed to evaluate the effectiveness of Imiquimod as a replacement for surgery or a combination of both for the treatment of anal lesions or cancer.

Cisplatin (cis-diamminedichloridoplatinum(II)), a chemotherapy medication administered intravenously to treat solid and hematological malignancies (with regulatory approval in 1978/1979), can bind to DNA and inhibit its replication.. Several active, or pending, Phase II/III clinical studies are being designed to evaluate the efficacy of the combination therapy of Cisplatin with Intensity Modulated Radiation Therapy or immunotherapy to treat tumors (mostly oropharynx cancer at different stages), some post-operation therapies (Table 11).

Carboplatin, with similar activity to Cisplatin but reduced side effects, is a chemotherapy medication approved in 1986 to treat a number of cancers. There are three Phase II studies (one completed, one active, and one in recruiting stage) and one Phase III study trying to determine the efficacy of combined radiation therapy of different doses and chemotherapies involving Carboplatin for treating oropharyngeal cancer. A completed Phase II study investigated if chemoradiotherapy with reduced radiation dose would maintain survival outcomes while improving tolerability in patients with oropharyngeal carcinoma caused by HPV infections. In this study, patients with newly diagnosed biopsy-proven stage III or IV squamous-cell carcinoma of the oropharynx received two cycles of induction chemotherapy with Paclitaxel and Carboplatin followed by Intensity-Modulated Radiotherapy. The primary endpoint was progression-free survival at 2 years, and the results were published in Lancet Oncology [109]. The study concluded that induction chemotherapy followed by chemoradiotherapy with reduced radiation by 15-20% from the standard resulted in similar 2-year progression-free and overall survival, compared to standard radiotherapy. It is indicated that a Phase III study is being planned.

A Phase I clinical trial is being conducted to evaluate the dose of Cidofovir, a drug that inhibits viral replication by selectively inhibiting viral DNA polymerases after being metabolized to Cidofovir Phosphate. The drug's uses in conjunction with Carboplatin and radiation therapies for the treatment of cervical cancer is being investigated (Table 12).

Aminolevulinic Acid, a precursor of a potent photosensitizer, can be used as an agent for photodynamic therapy. After it is administered to patients intravenously, orally, or topically, it is incorporated into tumor cells. A light with a specific wavelength is applied to the tumor site thereby activating Aminolevulinic Acid, which reacts with molecular Oxygen to form a highly reactive singlet Oxygen, superoxide radical anion, and highly reactive hydroxy radical. These highly reactive ions are thought to cause targeted cell death within the illuminated area. Two Phase II studies were designed to evaluate the efficacy of the photodynamic therapy in treating HPV positive patients with low grade cervical intraepithelial neoplasia or cervical precancerous lesions with no result made available (Table 13).

Product	Purpose of the study	Trial Design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Imiquimod	To determine the efficacy of Imiquimod in treating genital wards caused by HPV infections	A. Subjects are treated with 2.5% or 3.75% Imiquimod B. Subjects are given placebo	Graceway Pharmaceuticals	III	Completed	No statistical analysis performed. Percentage of subjects with complete clearance of all wards: 19%-27% for treated group vs 10% for placebo group. Adverse event: 1% for treated vs 0% for placebo group	NCT00674739
Imiquimod	To determine the efficacy of Imiquimod in treating external genital wards caused by HPV infections	A. Subjects are treated with 2.5% or 3.75% Imiquimod B. Subjects are given placebo	Graceway Pharmaceuticals	III	Completed	No statistical analysis performed. Percentage of subjects with complete clearance of all wards: 25%-29% for treated group vs 9% for placebo group. Adverse event: 1 – 3% for treated vs 1% for placebo group	NCT00735462

Imiquimod	To evaluate the efficacy of Imiquimod vs surgery for the treatment of vulvar intraepithelial neoplasia	A. Subjects treated with Imiquimod B. Subjects treated with surgery	Medical University of Graz	III	Recruiting	To be reported	NCT01861535
Imiquimod	To compare the effectiveness of Imiquimod treatment and surgery on anal cancer in patients with HIV and High-Gade Squamous Intraepithelial Lesions	A. Patients receive Imiquimo, Fluorouracil, and Trichloroacetic Acid B. Patients receive ablative treatment C. Patients are monitored (only)	AIDS Malignancy Consortium	III	Recruiting	To be reported	NCT02135419
Imiquimod	To evaluate the efficacy of the combination therapy of surgery and Imiquimod treatment on anal lesions due to HPV infection	A. Surgical excision and fulguration of condyloma followed by Imiquimod treatment for 12 weeks B. Surgical excision and fulguration of condyloma followed by giving the patients the placebo for 12 weeks	Medical University Innsbruck (Austria)	III	Not yet recruiting	To be reported	NCT03289260
Imiquimod	To evaluate the efficacy and Mechanism of Action (MOA) of Imiquimod in treating Vulvar Intraepithelial Neoplasia 2/3 and Anogenital Ward	Apply Imiquimod locally three times a week for 16 weeks	Medical University of Vienna	II	Completed	Not provided	NCT00941811

Table 10: Clinical studies on Imiquimod.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Cisplatin	To determine the feasibility of deescalating chemoradiation treatment on tumor (HPV positive oropharyngeal carcinoma)	A. Standard radiation B. Dose-deescalated radiation Good response defined as >40 nodal shrinkage	New York University School of Medicine	II	Recruiting	To be reported	NCT03215719
Cisplatin	To reduce treatment related toxicity while maintaining efficacy (after surgery)	A. Patients with extracapsular extension or positive margin but not clinical or pathologic T4 or clinical N3 disease - 42 Gy radiation therapy in 21 doses and 1 dose of Cisplatin B. Patients with no extracapsular extension and no positive margins - 42 Gy radiation therapy in 21 doses C. Patients with clinical or pathologic T4 or clinical N3 disease - 60 Gy radiation therapy in 30 doses and 3 doses of Cisplatin	Washington University School of Medicine	II	Not yet recruiting	To be reported	NCT03621696
Cisplatin	To study the dose requirement for p16 positive oropharynx cancer patients (after surgery)	A. Radiotherapy (only): 60 Gy/2 Gy IMRT for 5 weeks B. Radiotherapy plus Cisplatin 40 mg/m2 on days 1, 8, 15, 22, 29, and 36	Washington University School of Medicine	II	Active	To be reported	NCT01687413
Cisplatin Docetaxel	To investigate on a less intense radiation treatment following surgery on oropharynx caused by HPV	A. Standard treatment: 60 Gy/2 Gy daily for 40 days, with 40 mg/m2 Cisplatin at days 1, 8, 15, 22, 29, and 36 for high-risk patients B. 30 Gy/1.5 Gy twice daily for 12 days for intermediate risk patients or 36 Gy/1.8 Gy twice daily for 12 days for high-risk patients, plus 15 mg/m2 Docetaxel at days 1 and 8	Mayo Clinic	III	Recruiting	To be reported	NCT02908477
Cisplatin Carboplatin	To study how well transoral surgery followed by low dose or standard dose radiation therapy works in treating patients with HPV positive stage III-IVA oropharyngeal cancer	A. Transoral surgical resection of the oropharyngeal tumor B. Surgery followed by Intensity Modulated Radiation Therapy (IMRT) QD for 5 weeks C. Surgery followed by standard dose IMRT QD for 6 weeks	Eastern Cooperative Oncology Group	II	Active	To be reported	NCT01898494

Cisplatin Cetuximab Carboplatin	To learn about the effectiveness of using low intensity radiation and chemotherapy to treat HPV associated oropharyngeal and/or primary squamous cell carcinomas of the head and neck (followed with or without surgery)	IMRT of 60 Gy/2 Gy plus: A. Cisplatin 30-40 mg/m2, or B. Cetuximab 250 mg/m2, or C. Carboplatin AUC 1.5 and Paclitaxel 45 mg/m2, or D. Carboplatin AUC 3	University of North Carolina Lineberger Comprehensive Cancer Center	II	Active	To be reported	NCT02281955
Cisplatin Cetuximab	To study the efficacy of radiation therapy with Cisplatin vs Cetuximab	A. Patients underdo image-guided intensity-modulated radiation therapy (IMRT) and high dose Cisplatin B. Patients receive Cetuximab and IMRT	Radiation Therapy Oncology Group	III	Active	To be reported	NCT01302834
Cisplatin Durvalumab	To evaluate the efficacy and toxicity of definitive radiotherapy combined with immunotherapy (Durvalumab)	A. Chemoradiotherapy (Cisplatin) B. Radiation therapy plus immunotherapy (Durvalumab)	Institut Claudius Regaud	II	Not yet recruiting	To be reported	NCT03623646

Table 11: Clinical study on Cisplatin.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Nivolumab Carboplatin Nab-paclitaxel Cisplatin Hydroxyurea 5-Flouoroxyurea Dexamethasone Famotidine Diphenhydramine Paclitaxel	To determine radiologic response to induction chemotherapy and effectiveness of locoregional therapy for patients with HPV positive oropharyngeal squamous cell cancer	After induction chemotherapy with Carboplatin, Nab-paclitaxel, and Nivolumab, patient will be treated with: A. Transoral robotic surgery B. De-intensified radiation alone (50 Gy) C. De-intensified radiation (50 Gy) and chemotherapy TFHX 45 Gy (paclitaxel, 5-fluorouracil, hydroxyu hydroxyurea, and twice-daily radiotherapy) D. Radiation with Cisplatin (70 Gy) or TFHX (75 Gy)	University of Chicago	II	Recruiting	To be reported	NCT03107182
Nab-paclitaxel Carboplatin Fluorouracil Hydroxyurea Cisplatin	To study Nab-paclitaxel and Carboplatin followed by locoregional therapy in treating patients with Stage III or IV HPV related oropharyngeal cancer	A. Radiation only B. Low dose radiation plus chemotherapy C. High dose radiation plus chemotherapy	University of Chicago	II	Active	To be reported	NCT02258659
Paclitaxel Carboplatin	To evaluate the impact of the induction dose of Paclitaxel and Carboplatin, followed by radiation therapy and chemotherapy (Paclitaxel), in treating HPV positive patients with oropharynx, hypopharynx, or larynx cancer	A. Induction: with Paclitaxel and Carboplatin B. Radiation and chemotherapy: IMRT plus Paclitaxel	Jonsson Comprehensive Cancer Center	II	Completed	Lancet Oncology 2017 Jun, 18(6): 803-811	NCT02048020
Carboplatin	To compare the outcomes of reduced dose radiation and standard dose radiation for HPV positive oropharynx cancer	After three cycles of induction chemotherapy with Docetaxel, Cisplatin, and 5-Fluorouracil, the patients will be treated with: A. Reduced radiation of 5600cGy with Carboplatin B. Standard radiation of 7000 cGy with Carboplatin	Icahn School of Medicine at Mount Sinai	III	Active	To be reported	NCT01706939
Carboplatin Cidofovir	To evaluate the toxicity and dose of Cidofovir in treating patients with cervical cancer	After two weeks of IV dose of Cidofovir, add radiation therapy and chemotherapy (Cisplatin)	Gustave Roussy, Cancer Campus, Grand Paris	I	Unknown	To be reported	NCT00811408

Table 12: Clinical studies on Carboplatin.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Aminolaevulinic Acid	To study on the efficacy of photodynamic therapy with Aminolaevulinic Acid in treating HPV positive patients with low grade Cervical Intraepithelial Neoplasia	A. Patient will receive Aminolaevulinic Acid of 500 mg strength B. Patient will receive placebo	Shanghai Fudan-Zhengjiang Bio-Pharmaceutical Co.	II	Recruiting	To be reported	NCT02631863
Aminolaevulinic Acid	To study on the efficacy of photodynamic therapy with Aminolaevulinic Acid in treating HPV positive patients with cervical precancerous lesions	A. Aminolaevulinic Acid photodynamic treatment of patients with high-risk HPV infection B. Treatment of patients with CIN1 and HPV infection C. Treatment of patients with CIN 2/3 and HPV infection	Shanghai Fudan-Zhengjiang Bio-Pharmaceutical Co.	II	Completed	Publication not provided	NCT02304770

Table 13: Clinical studies on Aminolaevulinic Acid.

Other small molecules: AMG 319, as an inhibitor of the phosphoinositide 3-kinase enzyme subtype PI3Kδ, is expected to proliferate CD8+ T-cells to kill cancer cells. It is indicated that the molecule has in vitro activity of inhibiting cell proliferation, which might be a candidate for cancer treatment. The molecule was evaluated in a Phase II clinical study for the treatment of head and neck cancer, but the study was terminated. Another terminated study was a Phase IV clinical trial on Zinc Gluconate for the clearance of genital warts.

l-phenylalanine, N, N′-[[[2-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]ethoxy]methyl]phosphinylidene]bis-, bis(2-methylpropyl) ester (known as GS-9191) is a lipophilic molecule. Its antiproliferative effects were evaluated in the treatment of genital warts in a Phase I study, with no results posted yet.

A Phase II study on Dihydroxybenzophenone-2, 4-Dinitrophenyl-hydrazone for the treatment of precancerous cervical dysplasia was concluded with insignificant benefit, and a Phase I/II evaluation on Tetrameprocol for treating cervical intraepithelial neoplasia was completed with no results posted. A few studies on Artesunate, 5-Azacitadine, Zinc Sulfate, Trichloroacetic Acid, and Docetaxel are in recruiting stage (or with no progress indicated). The Phase II trial on Docetaxel is designed to study how well radiation therapy and Docetaxel work in treating patients with HPV-related oropharyngeal cancer when combined. The radiation therapy uses high-energy x-rays to kill tumor cells and the chemotherapy, Docetaxel, is expected to stop the tumor cell growth (Table 14).

Ionic contra-viral therapy

It has been shown that DNA viruses such as HPVs depend on potassium ion influx for replication [110], and the net influx of potassium ions is determined by the cotransporter protein NKCC for influx and NKATPase for efflux [111]. Inhibition of potassium ion influx and controlled depletion of cellular potassium ions could yield antiviral activities. The cardiac glycoside Digoxin and the loop diuretic Furosemide are inhibitors of the transporters. They are expected to have antiviral activity towards HPV, hence their testing in a Phase II clinical study for the treatment of benign and premalignant HPV induced genital lesions. The clinical study is in the recruiting stage (Table 15).

Others: There is a Phase II study on a mixture of natural oils for its efficacy in treating patients with HPV associated precancerous lesions of uterine cervix. It is in the recruiting stage (Table 16).

Surgery

Surgery is a destructive procedure often used as an effective treatment of diseases caused by HPV. The major types of operative procedures include cryotherapy, laser removal, electrosurgery, and excision. These are mature surgical procedures, with few publications on the technical advances or their applications in HPV-induced diseases.

Cryotherapy: Cryotherapy refers to the application of low temperatures, generally with liquid nitrogen, to cause cellular death and destruction of diseased tissue. Liquid nitrogen, with boiling point of -196oC, can cause tissue damage at low temperatures (usually -25oC to -50oC in vivo, depending on the amount of liquid nitrogen applied to the tissue). Tissue destruction is induced by intracellular ice formation, swelling, and lysis. Liquid nitrogen can be applied to diseased tissue in spray form from a cryogun. A tool such as a hemostat may be dipped in the liquid and clamped on to the affected tissue. The area and depth of the frozen tissue can be controlled by the force of application, length of treatment, and the number of treatments. Cryotherapy is a safe treatment option for skin conditions [112]. It can be performed in doctor's office and requires no hospitalization. The potential risks are low but include infection, scarring, dyspigmentation, bleeding and blister formation, and incomplete clearance. For HPV induced disease in particular, several sessions with multiple visits to the doctor's office may be required.

It is reported that cryotherapy can be used for treatment of external genital warts [113-115]. The wart clearance rate was 40% at 3 months and the recurrence rate varied between 38% -73% after 6 months post treatment. Cryotherapy has also been employed to treat high-grade squamous intraepithelial lesions in HIV positive men, with a 60% response rate and 68% recurrence rate over 18 months [116].

Laser: Carbon dioxide lasers produce infrared light mainly at 9.4

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial. Gov identifier
AMG 319	To evaluate the efficacy of the molecule in treating patients with head and neck cancer squamous cell carcinoma	Not disclosed	Cancer research UK	II	Terminated	No results posted	NCT02540928
Zinc Gluconate	To evaluate the effect of Zinc Gluconate on the clearance of genital warts	A. Subjects receive oral Zinc Gluconate 200 mg B. Subjects receive placebo	University of British Clumbia	IV	Terminated	No results posted	NCT01468636
GS-9191 (l -phenylalanine, N,N′-[[[2-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] ethoxy]methyl] phosphinylidene] bis-, bis(2-methylpropyl) ester)	To evaluate the safety and tolerability of the product in the treatment of genital warts	In cohorts 1 – 5: subjects receive GS-9191, GS-9191 ointment of ascending strengths or placebo	Gilead Sciences	I	Completed	No results posted	NCT00499967
Dihydroxybenzophenone-2,4-Dinitrophenyl-hydrazone	To evaluate the safety and efficacy of the compound for the treatment of precancerous cervical dysplasia	A. Subjects are administered with the compound B. Subjects are given with placebo	Tigris Pharmaceuticals	II	Completed	Insignificant benefit: 24 of 77 subjects who received the compound had pathological response 20 of 70 subjects who received placebo had pathological response	NCT00285207
Tetrameprocol	To evaluate the safety and efficacy of the molecule in treating patients with cervical intraepithelial neoplasia	Subjects receive the investigational therapeutics intravaginally	Erimos Pharmaaceuticals	I/II	Completed	No results posted	NCT00154089
Artesunate	To evaluate the safety of the molecule in treating patients with HPV positive anal intraepithelial neoplasia	Subjects receive different/ascending doses of the medicine	Johns Hopkins University	I	Recruiting	To be reported	NCT03100045
5-Azacitadine	To assess the efficacy of 5-Azacitadine in treating patients with head and neck squamous carcinoma	A. HPV positive subjects receive the medicine B. HPV negative patents receive the medicine	Yale University	II	Recruiting	To be reported	NCT02178072
Zinc Sulfate	To determine if oral Zinc Sulfate can improve clearance rate of high-risk HPV	A. Subject receive Zinc Sulfate 220mg twice daily B. Subjects receive placebo	Spectrum Health Hospitals	N/A	Recruiting	To be reported	NCT03404310
Trichloroacetic Acid	To evaluate the efficacy and toxicity of Trichloroacetic Acid treatment (in comparing to surgery)	A. Visible lesions are treated with 85% Trichloroacetic Acid B. Visible lesions are ablated	University Hospital, Essen (Germany)	Unknown	Unknown	No results reported	NCT02615860
Docetaxel	To evaluate the efficacy of radiation and chemotherapy of Docetaxel in treating patients with HPV related oropharyngeal cancer	IV dose of Docetaxel followed by hyperfractionated IMRT	Mayo Clinic	II	Not yet recruiting	To be reported	NCT01932697

Table 14: Clinical studies on small molecule products.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
Ionic Contra-Viral Therapy Digoxin Furosemide	To evaluate the pharmacodynamics and efficacy of the ionic contra-viral therapy in immunocompromised and immunocompetent patients with benign and premalignant HPV induced genital lesions	A. Subjects receive Digoxin and Furosemide in topical formulation B. Subjects receive placebo	Gutanea Life Sciences, Inc	II	Recruiting	To be reported	NCT03334240

Table 15: Clinical study on ionic contra-viral based investigational product.

Product	Purpose of the study	Trial design	Sponsor/ Organization	Phase	Status	Results	Clinicaltrial.Gov identifier
AV2 [a mixture of natural oil (Carvone, Eugenol, Geraniol, Nerolidol) in equal volume of Olive oil	To evaluate the efficacy of the product in treating patients with HPV associated precancerous lesions of uterine cervix	A. Patients receive the topical application B. Patients receive placebo	Jean-Pierre Van geertruyden	III	Recruiting	To be reported	NCT02346227

Table 16: Clinical study on mixture of natural oil.

and 10.6 μm, Diode lasers emit light in the range of 0.8 to 0.98 μm, and Neodymium-doped Yttriumaluminum garnet (Nd:YAG) lasers generate light at 1.064 μm. At these wavelengths, tissue water can absorb energy from the photons of incident laser light, resulting in tissue destruction.

Lasers can be guided precisely to anatomic locations which are otherwise difficult to reach by other cytodestructive operations. Scarring can be avoided, and no additional hemostasis is needed in most cases by precisely controlling the laser's target and strength. However, laser equipment requires specially trained personnel to operate in a safe environment, making it less convenient than cryotherapy. Laser treatment may require local or general anesthesia.

Carbon Dioxide lasers have been used for treating external genital warts [117-119]. The effectiveness and recurrence vary significantly from study to study [120]. It is noted that HPV DNA was found in the smoke of the laser burned tissue, causing concern over the laser operator's health [121]. Studies on Diode lasers for removal of warts are limited. A study of 92 patients suggested a good clearance rate with a small percentage of recurrences [122]. Another study indicated that 60% -80% of patients were free of recurrence three months after the first treatment, with overall success rate of 73% [123].

Nd: YAG lasers can be used for the clearance of verrucae [124]. It canpenetrate deep in the treated tissue while minimizing collateral damage to adjacent tissue [125]. Clinical results from a study of 20 subjects show complete clearance of warts in 56% of patients 24 weeks after initial treatment [126]. Another study of 369 patients reported a 96% clearance rate after four sessions of treatments [127].

Electrosurgery: Electrosurgery is a procedure using heat and electricity to energize water molecules within cells to destroy lesions. A randomized clinical study on external genital warts reported 71% clearance using electrosurgery [117], a better clearance than when using cryotherapy. Another study reported an even better clearance rate of 90% [128]. More studies were reviewed in a publication [115].

It is relatively easy to train personnel in Electrosurgery, bleeding can be controlled while cutting or destroying tissue, and infection is rarely present. However, it may cause electrical shocks and scarring, so a smoke evacuator is recommended for protection of the operator.

Excision: To remove diseased tissue, such as those affected by persistent HPV infection, a scalpel, curettage, or scissors may be employed to excise the tissue. These methods are long-standing practices performed by many doctors. Results of clearance of visible warts have been reported to be 35% to 72% of treated patients, with a recurrence rate of 19% to 29% of patients a year after the operation [115]. Similar results were reported in another study [129].

Conclusion

Viruses have been a plague of humanity for centuries. Among many disease-causing families of viruses identified, Human Papillomavirus is transmitted by direct contact or contact from contaminated fomites, and only causes diseases in humans. With well over 100 genotypes identified, we are learning more about this family of viruses and the diseases they cause. Recognized as causing warts, HPV's oncogenic potential in a variety of cancers is now undisputed. Studying the make-up of these viruses and their mechanism of action in infections can help us develop more specific strategies for disease prevention and treatment.

The vaccines for certain high-risk HPV infections prove to be effective. However, they are not designed to be prophylactic against the broad spectrum of HPVs other than a few selected high-risk genotypes. These products are not for the treatment of diseases caused by HPV infections. Developing clinically efficacious medicines for curing the diseases has been the effort of several medical institutions and pharmaceutical companies. Among the investigational products tested in various clinical trials include different modalities, such as viruses, stem cells, T-cells, B-cells, gene therapies, DNAs, antibodies, and antigens. While each has its own challenges to overcome, each modality has a unique mechanism of action that offers the hope of becoming an effective treatment. It should be noted that many of these investigational products are in their early development stages, and they need the proof of concept as well as a demonstration of efficacy in clinical studies during the late stage development. It becomes obvious that there is still a lot work to be done before an effective medicinal treatment becomes available for curing HPV-infected diseases, especially cancers.

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